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Effect of co-modifiers in cyclodextrin-modified mobile phases on the reversed-phase high-performance liquid chromatographic separation of polyaromatic hydrocarbons

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Abstract

The effect of two co-modifier containing *tert.*-butyl moieties, *tert.*-butyl (N-hydroxy) carbamate and *tert.*-butyl carbazate, on the retention behavior of six polyaromatic hydrocarbons (PAHs) using cyclodextrin-modified mobile phases has been examined. Both modifiers resulted in shorter retention times for all PAHs, when a size-compatible cyclodextrin was present in the eluent. This decrease has been attributed to the formation of a ternary PAH–cyclodextrin–modifier complex. The apparent formation constants calculated for these complexes are of the order of approximately $10^2\ M^{-1}$, with the exception of the β-CD–pyrene complex, which was determined to have a 2:1 stoichiometry. Mixtures of β- and γ-cyclodextrins were used in the mobile phase along with *tert.*-butyl carbazate to achieve a baseline separation of all six PAHs under 50 min.

1. Introduction

The characteristic ability of cyclodextrins to form inclusion complexes with a variety of organic molecules is well known. Cyclodextrins are cyclic oligosaccharides that are soluble in water but contain a hydrophobic cavity which is capable of encapsulating a guest molecule to form inclusion complexes. The three most common cyclodextrins are α-, β-, and γ-, which contain 6, 7 and 8 monomeric glucopyranose units, respectively, and have varying cavity diameters. Cyclodextrin complex formation is influenced in part by the hydrophobicity of the guest as well as its size compatibility with the cavity of the cyclodextrin. An example of the types of compounds that can form a wide variety of cyclodextrin inclusion complexes are polyaromatic hydrocarbons (PAHs), many of which are harmful carcinogens found in the environment. Due to their presence in many oil and petroleum based samples, it is important to effectively characterize the behavior of such compounds in aqueous and non-aqueous media. Complexation of PAHs with cyclodextrins has been the subject of numerous investigations, many of which have used spectroscopic techniques such as absorption and fluorescence [1–4]. In the past decade, high-performance liquid chromatography (HPLC) methods employing cyclodextrins as bonded stationary phases, as well as mobile phase additives have become increasingly popular in the analyses of PAH–cyclodextrin complexes [5–9].

Spectroscopic studies in our laboratory [10]

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have revealed the formation of a strong 2:1 complex between β-cyclodextrin and the PAH pyrene in aqueous media. The addition of a small amount of *tert.*-butyl alcohol to the aqueous solution has been shown to further strengthen and stabilize the complex [10]. This effect has been attributed to the formation of a more stable ternary β-cyclodextrin–pyrene–*tert.*-butyl alcohol complex. Using CPK models, the proposed orientation of the alcohol in this complex was determined to be with its bulky *tert.*-butyl group partially included into the β-cyclodextrin cavity, and its hydroxyl group hydrogen-bonded with the hydroxyls lining the periphery of the cyclodextrin. In addition to spectroscopic measurements, reversed-phase HPLC studies performed in our laboratory indicate that using *tert.*-butyl alcohol as a secondary modifier with mobile phases containing β-cyclodextrin reduces the retention time of the complex, suggesting an enhancement of the β-cyclodextrin–pyrene complexation due to the presence of the alcohol [11,12]. A comparison of apparent formation constants estimated spectroscopically and chromatographically is also discussed in these reports [11,12].

We recently conducted a systematic investigation using a series of modifiers, each possessing a *tert.*-butyl moiety attached to a hydrogen-bonding group that varied in size and polarity [13]. The results indicate that the nature and polarity of the hydrogen-bonding functional group (hydroxyl or other) attached to the *tert.*-butyl moiety of the modifier generally has a significant effect upon the retention behavior of the complex. In particular, secondary modifiers that contain amine groups produce an especially dramatic reduction in the retention times of the β-cyclodextrin–pyrene complex. One possible explanation for this observation is that the formation of hydrogen-bonds between the amine group and the peripheral hydroxyls of the β-cyclodextrin conferred additional stability upon the ternary complex.

In the present study, we report an extensive investigation of the effects of two of these co-modifiers, *tert.*-butyl (N-hydroxy) carbamate and *tert.*-butyl carbazate, upon the size-selective analysis of six PAHs from the EPA's priority pollutant list, using mixtures of β- and γ-cyclodextrins in the mobile phase. In addition to obtaining pertinent information concerning the nature of the interactions between the species involved, our ultimate goal was the development of a method to optimize the separation of the six PAHs in order to provide a model for the possible applications of these modifiers to the analyses of these and other analytes that form complexes with cyclodextrins.

2. Experimental

2.1. Apparatus

The chromatographic system used in this study has been described elsewhere [13].

2.2. Materials

The HPLC grade water and methanol solvents were obtained from Mallinckrodt (Paris, KY, USA). All six PAHs, obtained from Aldrich (Milwaukee, WI, USA) were at least 98% pure and were used as received. The cyclodextrins used in this study were kindly provided by American Maize Products (Hammond, IN, USA) and the β-cyclodextrin was recrystallized twice from deionized water before use. The modifiers, *tert.*-butyl(N-hydroxy)carbamate and *tert.*-butyl carbazate were both purchased from Aldrich and used as received. The potassium nitrite used to determine the void volume of the column was purchased from Mallinckrodt (Paris, KY, USA).

2.3. Methods for sample preparation

PAH sample preparation

A 1.0 m*M* solution of each PAH was prepared by the addition of an appropriate amount of the PAH and ca. 0.5 g of KNO_2 to a volumetric flask, followed by dilution to the mark with methanol. This solution was sonicated for 15 min and stored in the dark. Mixtures of PAHs were prepared in a similar manner, with the con-

centration of each PAH maintained at 1.0 m*M* in methanol.

Mobile phase preparation

Bulk mobile phase solutions were prepared in 4-l quantities by adding the appropriate volumes of methanol and water needed to make the desired methanol–water concentration ratios. A secondary modifier was then added to this bulk solution in quantities required to make the final concentration of the modifier 0.073 *M*, and the resulting solution was allowed to equilibrate at least overnight. The cyclodextrin-modified mobile phases were prepared by weighing out the desired amount of cyclodextrin (or cyclodextrins), and adding 250 ml of the bulk mobile phase solution. This solution was sonicated for 1–2 h, depending on the solubility of the cyclodextrin as well as its concentration, and allowed to stand overnight before use.

2.4. Procedure for the liquid chromatographic runs

All mobile phases were deaerated with helium gas for 15 min before the run. The column was conditioned for 30 min with the mobile phase mixture prior to the injection of the sample. The flow-rate for all the runs was 1.0 ml/min. The column pressure fluctuation was between 1100 and 2300 p.s.i. ($7.6 \cdot 10^6$–$15.8 \cdot 10^6$ Pa), depending upon the concentration of cyclodextrin in the mobile phase. However, the pressure remained constant for the duration of the run using a given mobile phase. The column was cleaned of possible cyclodextrin precipitate as well as residual PAHs by pumping water at 1.0 ml/min for 30 min, followed by methanol at 1.0 ml/min for 20 min after each set of runs.

3. Results and discussion

The retention of a solute in reversed-phase HPLC is in large part dependent upon hydrophobic interactions between the non-polar bonded stationary phase and the non-polar moiety of the solute. These interactions are in turn affected by several factors, such as the chain length and the number of bonded alkyl groups of the stationary phase, as well as the nature and polarity of the mobile phase. Mobile phase additives, such as cyclodextrins, can change the overall polarity of the mobile phase, and can therefore influence the hydrophobic interactions between the solute and the stationary phase. In essence, there is a competitive equilibrium for the non-polar solute between the stationary phase and the mobile phase. In these cases, the addition of a secondary modifier can sometimes serve to enhance cyclodextrin complex formation of the solute and thus affect its retention.

Fig. 1a shows the structures of the two co-modifiers used in this study. The rationale for selection of these two compounds was their structural similarity. Both contain bulky, hydrophobic *tert.*-butyl groups capable of interacting with the cyclodextrin cavity. However, the hydroxyl group in *tert.*-butyl (N-hydroxy) carbamate (A) is replaced by an amine group in *tert.*-butyl carbazate (B). As a result, any variations in the retention behavior of the analytes due to the presence of these modifiers can be understood in terms of their different functional groups.

Fig. 1b lists the six PAHs investigated in this study. Their selection was based mainly upon preliminary experimental data that suggested that these particular PAHs exhibited the most significant and consistent variations in their retention behavior in the presence of modifiers A and B. In addition, each PAH is size-compatible with either β- or γ-cyclodextrin, which was a prerequisite for the application of both of these cyclodextrins to the size-selective separation of the solutes.

Using the conditions previously established for the pyrene–β-cyclodextrin studies [13], which include a 60% MeOH content of the mobile phase containing 0.073 *M* modifier and a C-18 column, the retention times for all the PAHs in the *absence* of co-modifiers A and B were determined to be over 75 min. The addition of α-, β-, or γ-cyclodextrin produced no appreciable reduction of these retention times, suggesting that these PAHs do not form stable

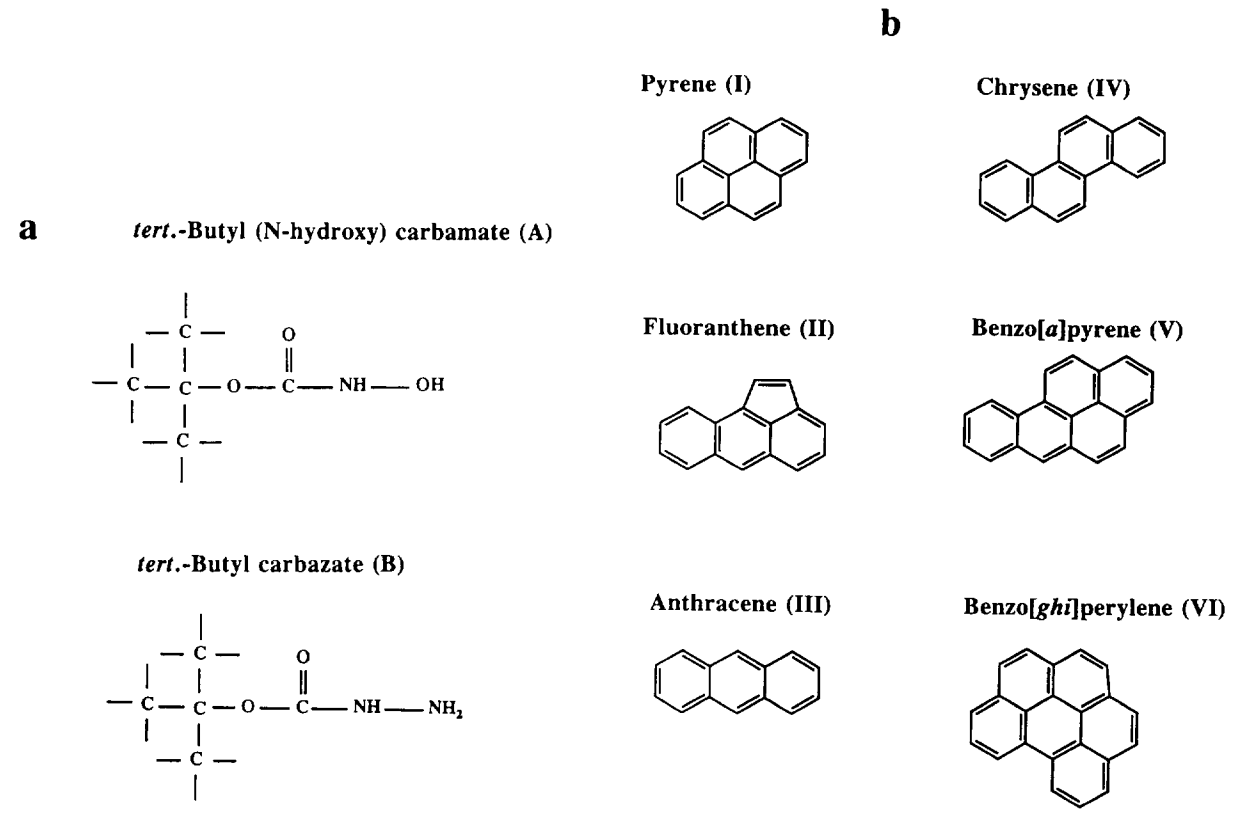

Fig. 1. Structures of (a) the two mobile phase co-modifiers, A and B; (b) the six polyaromatic hydrocarbons, I–VI.

cyclodextrin inclusion complexes under these conditions. In the presence of the co-modifiers, however, significantly different results were obtained. In addition, the co-modifier concentration alone has been shown to have negligible influence upon the retention time of the PAH in the absence of CD [11–13].

3.1. Influence of modifier A

α-Cyclodextrin

Fig. 2a shows the effect of α-cyclodextrin upon the capacity factors of the six PAHs, when modifier A is present in the mobile phase. In the absence of cyclodextrin, the order of elution of the PAHs appears to be dependent on their size, i.e. the smaller ones elute before the larger ones. This can be explained in terms of the enhanced hydrophobic interactions between the C-18 stationary phase and the larger PAHs which possess more non-polar character. The addition of increasing amounts of α-cyclodextrin produces very little change in the retention of any of the six PAHs. This may be attributed to the relatively small size of the α-cyclodextrin cavity, which is too small to accommodate any of the PAHs. As a result, no significant inclusion complex formation, and therefore no reduction in retention times is expected to occur in this case.

β-Cyclodextrin

The influence of β-cyclodextrin on the retention of all the solutes examined is shown in Fig. 2b. Increasing the β-cyclodextrin concentration in the presence of modifier A causes a significant decrease in the capacity factor of pyrene (I), which is consistent with our previous results [13]. Since this decrease is not observed in the absence of modifier A, it may be attributed to the formation of a ternary pyrene–β-

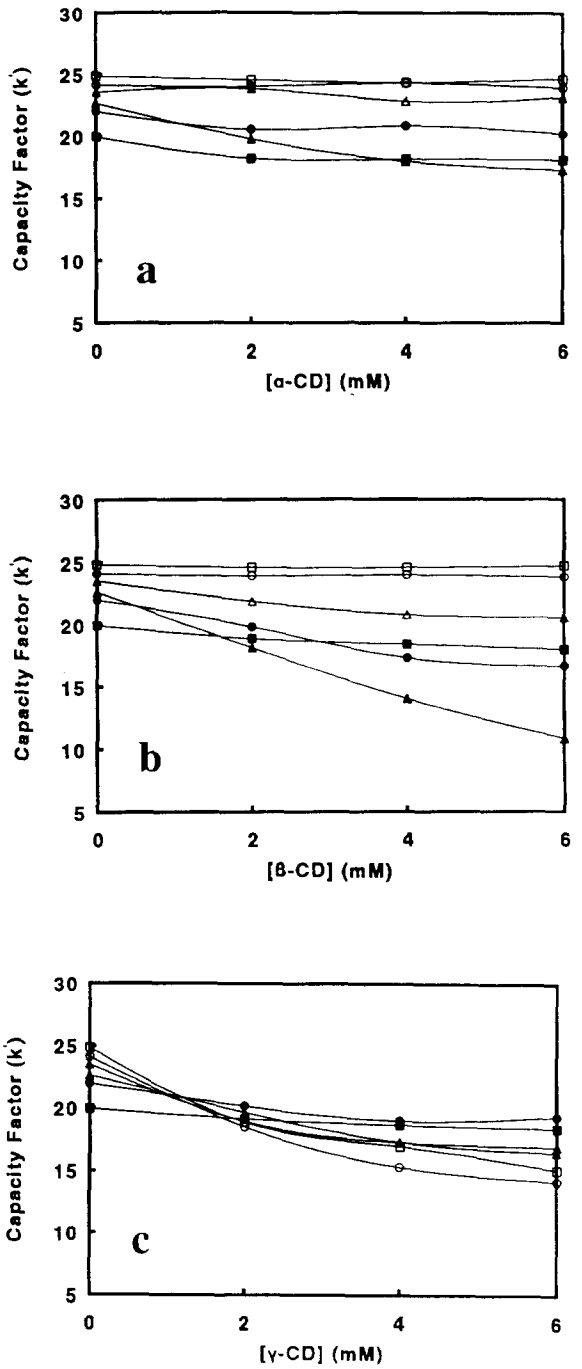

Fig. 2. Influence of modifier A (constant concentration = 0.073 *M*) on the retention of (▲) pyrene (I), (●) fluoranthene (II), (■) anthracene (III), (△) chrysene (IV), (○) benzo[*a*]pyrene (V), (□) benzo[*ghi*]perylene (VI) in the presence of increasing amounts of: (a) α-cyclodextrin; (b) β-cyclodextrin; (c) γ-cyclodextrin.

cyclodextrin–modifier A complex, which results in a weakening of the hydrophobic interaction between the PAH and the stationary phase. In addition to pyrene, fluoranthene (II) shows a slight reduction in its capacity factor upon addition of β-cyclodextrin. This suggests that the fluoranthene–β-cyclodextrin–modifier A complex is less stable than the ternary complex involving pyrene. This observation is supported by the values of the calculated apparent formation constants presented later. The capacity factors of the other solutes remain unchanged in the presence of β-cyclodextrin. Since the larger PAHs (IV, V and VI) are not size compatible with the β-cyclodextrin cavity, and therefore cannot form strong inclusion complexes, the observed lack of variation in their retention times is not unexpected. However, chrysene, the smallest of these, does show a very slight decrease in k'. Then again anthracene (III), which can form a complex with β-cyclodextrin, also does not exhibit a significant change in its retention. This indicates that under the given experimental conditions and in the presence of modifier A, anthracene does not form a stable inclusion complex with β-cyclodextrin. One possible explanation for this may be the number and position of the aromatic rings in anthracene, which are not conducive to maximizing the hydrophobic contact of the guest with the interior surface of the cyclodextrin cavity.

γ-Cyclodextrin

The interaction of γ-cyclodextrin on the capacity factors of the PAHs is depicted in Fig. 2c. In this case, the most significant change in capacity factors is exhibited by benzo[*a*]pyrene (V), although the other PAHs also elute somewhat faster when γ-cyclodextrin is present. It is interesting to note that while the larger PAHs (IV, V, VI) showed no change in retention upon the addition of β-cyclodextrin, the smaller group (I, II, III) do undergo a slight reduction in retention times upon the addition of γ-cyclodextrin. This supports the point mentioned earlier that cyclodextrin complex formation is the primary determinant of the retention of solutes under these conditions. While I, II, and III can

form weak complexes with γ-cyclodextrin, IV, V, and VI are not size compatible with β-cyclodextrin and therefore do not form strong inclusion complexes.

The overall influence of modifier A upon the retention of the PAHs when cyclodextrins are used in the mobile phase is most likely due to the formation of a ternary complex. The structure of this complex is unknown. However, considering the size and polarity of the molecules involved, and with previous data available from CPK models of the pyrene–β-cyclodextrin–*tert.*-butyl alcohol complex [10], a probable structure can be proposed. In this proposed structure, the hydrophobic PAH is encapsulated within the cyclodextrin cavity in a 1:1 (CD:PAH), 2:1, 1:2 or other ratio. The orientation of the modifier is most likely with its bulky, hydrophobic *tert.*-butyl group partially or fully penetrating the cyclodextrin cavity, resulting in more water molecules being eliminated from the cavity and forming a more stable complex. Additional stability may be conferred upon the complex by the interaction (in the form of hydrogen bonds) of the polar functional groups of the modifier with the peripheral hydroxyls of the cyclodextrin. As such, the nature of the polar functional group can affect the stability of the complex and therefore its retention time. In an effort to determine whether this functional group is indeed important, the effect of modifier B, which contains a terminal amine instead of a terminal hydroxyl, on the retention of the PAHs has been investigated.

3.2. Influence of modifier B

α-Cyclodextrin

The influence of α-cyclodextrin on the capacity factors of the six PAHs when *tert.*-butyl carbazate (B) was utilized in the mobile phase is similar to that in the presence of modifier A. In the absence of any cyclodextrin, the capacity factors (k') of each PAH in the presence of B are slightly reduced compared to those observed when A is used as a mobile phase additive. The addition of α-cyclodextrin has very little effect on the value of k', indicating little or no α-cyclodextrin–PAH inclusion complex formation. This may be attributed to the size of the cavity and other considerations discussed earlier.

β-Cyclodextrin

As is evident from Fig. 3a, increasing amounts of β-cyclodextrin result in a dramatic reduction in the capacity factors of both pyrene (I) and fluoranthene (II), whereas anthracene (III) and the other PAHs exhibit very slight decrease in retention. It is apparent that although the trend is similar in the results obtained with both A and B, the magnitude of the decrease in k' values is much larger when B is added to the mobile phase. This suggests that modifier B stabilizes the β-cyclodextrin–PAH complexes to a greater degree than modifier A, possibly by providing the PAH more protection within the cavity. This can then result in decreased hydrophobic interactions between the PAH and the cyclodextrin.

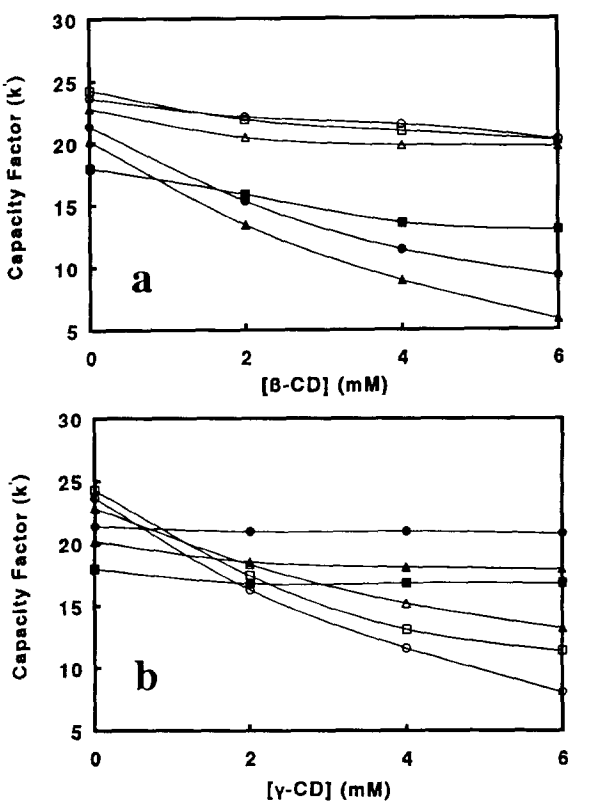

Fig. 3. Influence of modifier B (constant concentration = 0.073 *M*) on the retention of I–VI. Symbols as in Fig. 2.

Since the modifiers A and B are similar in structure except for the amine group in B replacing the hydroxyl in A, the increased stability of the ternary cyclodextrin–PAH–modifier complex may be attributed to the amine group on B.

γ-Cyclodextrin

In the presence of increasing amounts of γ-cyclodextrin (Fig. 3b), modifier B has a much more significant effect than A on the capacity factors of the PAHs under investigation. As with modifier A, benzo[*a*]pyrene (V) exhibits the greatest reduction in its capacity factor in the presence of γ-cyclodextrin. In addition, chrysene (IV) and benzo[*ghi*]perylene (VI) also elute much faster in the mobile phase containing B, suggesting the possibility of the formation of a relatively stable γ-cyclodextrin–PAH–modifier B complex.

3.3. Determination of apparent formation constants

In an effort to determine and compare the stability of the cyclodextrin complexes, previously detailed methods [11–13] have been used to estimate values of their apparent formation constants. Briefly, for a reaction

$$G + n(CD)_m \Leftrightarrow [(CD)_n\text{–}G]_m \quad (1)$$

where G is the PAH, m represents the concentration in the mobile phase, n is the number of cyclodextrins (CDs) associated with each PAH. The relationship of the capacity factor k' of the PAH and the equilibrium concentration of the CD in a water–organic solvent has been determined to be

$$1/k' = 1/k'_0 + K_f([CD]_m)^n / k'_0 \quad (2)$$

where k'_0 is the capacity factor of the PAH in the absence of CD, K_f is the apparent formation constant of the CD–PAH complex, and $[CD]_m$ is the equilibrium concentration of the CD, which can be the same as its initial concentration, assuming negligible interaction between the modifiers and the CD. As such, assuming a correct stoichiometry between the CD and the PAH, a plot of $1/k'$ versus $[CD]^n$ would produce a linear fit.

Figs. 4a and 4b illustrate an example of this type of plot. In the presence of *tert.*-butyl carbazate, a plot of $1/k'$ versus [β-CD] produces a linear fit for all the PAHs except pyrene (Fig. 4a). This indicates that while all the other PAHs form a 1:1 (CD:PAH) complex, pyrene does not. This is confirmed by the plot of $1/k'$ versus $[\beta\text{-CD}]^2$, which assumes a 2:1 stoichiometry of the complex. In this case, only pyrene shows a linear fit while the other PAHs show a curvilinear relationship. The 2:1 stoichiometry found for the β-cyclodextrin–pyrene complex is consistent with previously reported results [13]. The linear fits obtained for each PAH were used to estimate the apparent K_f values for each CD–PAH complex.

The apparent formation constants calculated

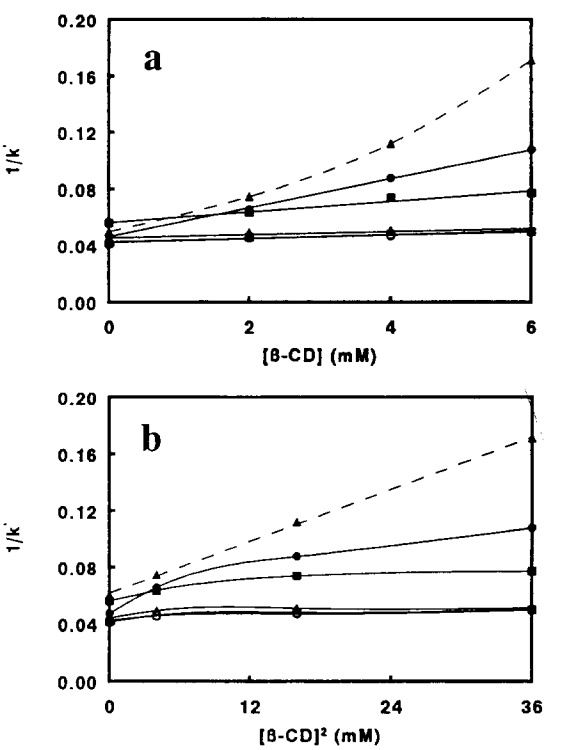

Fig. 4. Plot of $1/k'$ versus $[\beta\text{-CD}]^n$ to determine stoichiometry and formation constants of the CD–PAH complexes of I–VI in the presence of 0.073 *M* modifier B, assuming (a) 1:1 ($n = 1$) stoichiometry; (b) 2:1 ($n = 2$) stoichiometry. Symbols as in Fig. 2.

Table 1
Calculated apparent formation constants (apparent $K_f \times 10^{-2}$) (M^{-1}) for the CD–PAH complexes in the presence of co-modifiers A and B

CD–PAH complex	A		B	
	β-CD	*γ-CD*	*β-CD*	*γ-CD*
I	272.8 (M^{-2})	0.62	500.0 (M^{-2})	0.19
II	0.56	0.23	2.22	0.04
III	0.18	0.14	0.66	0.09
IV	0.24	0.61	0.24	1.21
V	0.02	0.35	0.27	3.26
VI	0.01	0.99	0.32	1.89

for each PAH–CD–modifier system are listed in Table 1. Since cyclodextrin complexation proceeds more appreciably in 100% water, these values in 40% water–60% methanol are somewhat lower than those reported for these PAH–CD complexes in aqueous systems. Considering the fact that the retention times of these solutes in 60% methanol without the modifier are too long to accurately estimate the apparent formation constant, the values in Table 1 emphasize the important role of the modifier in the complexation process.

The apparent K_f values obtained for the β-CD–pyrene complex are the only ones determined from the 2:1 plots, and are several orders of magnitude higher than those calculated for the other systems. The β-CD–pyrene–A and β-CD–pyrene–B values presented here correlate well with those reported in our earlier study [13]. In general, the apparent K_fs for the γ-CD–PAH complexes are higher for analytes IV, V and VI, whereas the β-CD–PAH complex values are higher for I, II and III, indicating the size-selective nature of the complexation by the cyclodextrins. It is important to note, however, that the apparent formation constants for both the γ-CD complexes of IV, V and VI, and the β-CD complexes of I, II, and III are significantly higher in the presence of modifier B than for modifier A. The absence of any possible pH effects was confirmed by measuring the pH of each mobile phase prior to the run. The pH of solvents containing A or B varied no more than by 0.2 pH units for any mobile phase composition. These results suggest that in general, CD–PAH–modifier B complexes are more stable than CD–PAH–modifier A complexes, a point alluded to earlier. Taking into consideration the difference of only one functional group between A and B, it is safe to say that the amine group plays an important role in stabilizing the ternary complex. This is supported by our earlier results [13] obtained for the β-CD–pyrene complex, which exhibited more stability and consequently lower retention times in the presence of *tert.*-butyl modifiers containing amine groups.

It is also important to consider the method of determination of apparent K_f employed here, which involves the division of the slope by the intercept. It is assumed that the intercept (k'_o) remains relatively unchanged while the slope varies according to the experimental conditions. Consequently, apparent formation constant values for systems exhibiting varying k'_o values cannot be compared. In this case, the k'_o values vary between PAHs, but remain constant for a given PAH regardless of the modifier used. As a result, the apparent K_f values for a given PAH with β- or γ-cyclodextrin in the presence of A or B may be compared. However, apparent formation constants of the different PAHs cannot be compared to each other. On the other hand, in cases where the apparent K_f values for different PAH–CD–modifier systems vary significantly, and this variation is large compared to the relatively small magnitude of the difference in their k'_o values, a qualitative comparison of the stability of the complexes may be made.

3.4. Optimization of the separation of the PAHs using mixtures of cyclodextrins in the mobile phase

In addition to determining the effects of the secondary *tert.*-butyl modifiers upon the retention characteristics of the CD–PAH complexes, we have attempted to demonstrate a very simple optimization method for the separation of these PAHs under the conditions described. Since modifier B, *tert.*-butyl carbazate, caused the most significant reduction in the retention times

of all the PAHs, it was selected as the mobile phase modifier in the separation method in order to reduce the analysis time. The most important difference in the method of analysis here compared to the previously described experiments is that in this case, the PAHs are injected simultaneously in a standard mixture instead of individually. The peaks obtained are identified by spiking the sample with the PAH of interest. As expected with excess concentrations of cyclodextrins, the retention times determined in this case are not very different from those obtained when the PAHs are injected individually, as long as the other mobile phase conditions remain constant. Fig. 5 depicts the influence of the concentration of B on the capacity factors of the six compounds. It is apparent that the greatest variation in retention characteristics occurs in the 0.02–0.06 *M* modifier concentration range for each PAH. This suggests that at about 0.06 *M*, all the binding sites for the co-modifier B on the CD–PAH inclusion complex are saturated. Thus, the addition of further increments of B are not used in ternary complex formation, and as a result do not affect the capacity factor. As a result, the 0.073 *M* concentration of B used in all the experiments thus far was considered adequate and this concentration was also used for the optimization method.

Using 0.073 *M* of B in a 60% methanol–40%

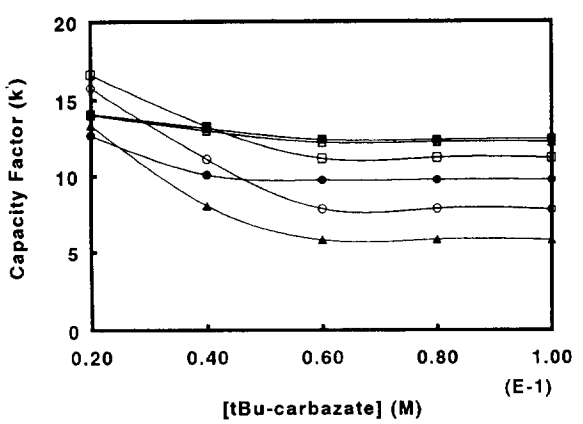

Fig. 5. Influence of the concentration of modifier B on the retention of I–VI, using a constant cyclodextrin concentration. Symbols as in Fig. 2.

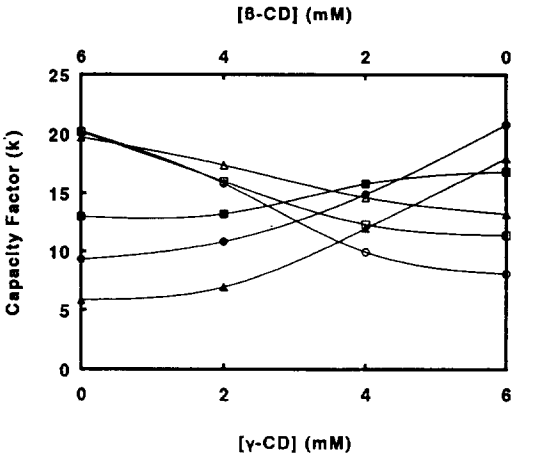

Fig. 6. Influence of the ratio of β- and γ-cyclodextrins (constant total CD (concentration) on the retention of I–VI in the presence of modifier B. Symbols as in Fig. 2.

water mobile phase, the influence of the ratio of the β- and γ-cyclodextrins on the capacity factors of the PAHs is shown in Fig. 6. Note that a constant total concentration of cyclodextrins is maintained. It is evident from this plot, that the selectivity of the separation is greatly influenced by both the size of the guest molecule and the cavity size of the cyclodextrin used. As expected, I, II and III form stronger complexes with β-cyclodextrin and IV, V, and VI with γ-cyclodextrin. As a result, IV, V and VI can only be separated by at least 4 m*M* γ-cyclodextrin, whereas I, II, and III can be selectively separated by either 2 m*M* or 4 m*M* β-cyclodextrins, although the retention times are shorter in the latter case. It is interesting to observe that the *k'* values for a given PAH are somewhat lower in the presence of both cyclodextrins than when the size-compatible cyclodextrin is used individually at the same concentration. This suggests a very complex mechanism for inclusion complex formation; possibly one PAH associates with more than one cyclodextrin molecule, and both β- and γ-cyclodextrin participating in complex formation, resulting in more stable, mixed complexes.

In order to minimize the analysis times for both the smaller (I, II, III) and the larger (IV, V, VI) PAHs, 4 m*M* of each cyclodextrin was used in the eluent. Due to the solubility restrictions of

the cyclodextrins in 60% methanol, a total cyclodextrin concentration of 8 m*M* was the maximum that could be used if both β- and γ-cyclodextrins were being utilized. The solubility of γ-cyclodextrin in methanol is higher than that of β-cyclodextrin, allowing larger total cyclodextrin concentrations to be used when both are dissolved compared to β-cyclodextrin being dissolved individually. Fig. 7 shows the effect of using β- and γ-cyclodextrins together in the mobile phase. It is evident from the definite minimum in capacity factors that the most polar complexes for all six PAHs are formed when 4 m*M* of each cyclodextrin is present. In addition, the selectivity of the separation is optimal under these conditions.

Using 4 m*M* β-CD–4 m*M* γ-CD–0.073 *M* *tert.*-butyl carbazate as additives in a 60% methanol–40% water mobile phase and a C-18 stationary phase, a satisfactory separation was obtained. The overall analysis time was under 50 min, and baseline separation of all six PAHs was achieved. The order of elution corresponds to the magnitude of the apparent formation constant of the PAH with its size-compatible cyclodextrin. The separation is very much improved compared to that in the absence of the modifiers under the same conditions, where the retention times ranged from 75 min to 120 min for the six PAHs, and the selectivity was poor.

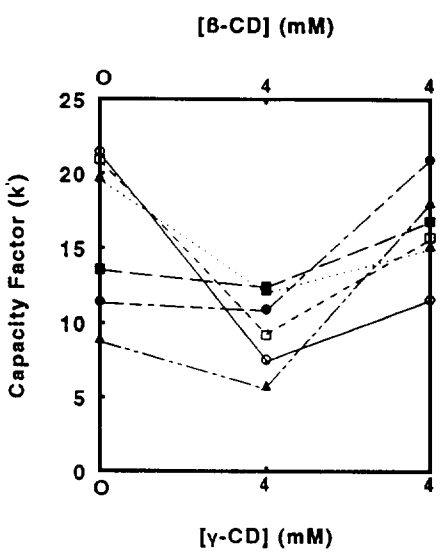

Fig. 7. Effect of using β- and γ-cyclodextrins together in the mobile phase (total CD concentration not constant) on the retention of I–VI in the presence of modifier B. Symbols as in Fig. 2.

4. Conclusion

Our previous studies of the influence of co-modifiers on the retention characteristics of the β-CD–pyrene complex had suggested that these modifiers can dramatically reduce the retention time of the pyrene by the formation of a stable ternary complex. It is clear from the results presented here that these *tert.*-butyl modifiers have more widespread application in the liquid chromatographic analysis of other PAHs using cyclodextrin-modified mobile phases. The amine group on these modifiers also appears to strengthen and stabilize the complex formation by the cyclodextrins, as is evident from the values of the apparent formation constants. In addition, these modifier effects can be used to achieve the size-selective separation of PAHs using mixtures of cyclodextrins in the mobile phase. In general, the addition of a mixture of different cyclodextrins to the mobile phase gives information about the estimated changes in retention. A cyclodextrin that does not react with the guest molecules in the presence of the modifier has no significant impact on the retention, and in the presence of a less active cyclodextrin, the change in retention is small.

Since cyclodextrin–PAH inclusion complexes do not appear to form under these conditions in the absence of secondary modifiers, these compounds can play a potentially important role for cyclodextrin-aided extraction of environmentally significant PAHs from contaminated sites. In addition to facilitating the removal of PAHs, the use of these modifiers with added functionality may reduce analysis time, which would make industrial applications of cyclodextrins more practical.

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References

[1] J. Szejtli, *Cyclodextrins and their Inclusion Complexes*, Akademiai Kiado, Budapest, 1982.
[2] G. Nelson, G. Patonay and I.M. Warner, *J. Incl. Phenom.*, 1988 (6) 277.
[3] T.K. Korpela and J.P. Himanen, *J. Chromatogr.*, 1984 (281) 351.
[4] I. Yamamoto and Y. Katsuda, *Pestic. Sci.*, 1980 (11) 134.
[5] A. Cepada-Saez, P. Prognon, G. Mahuzier and J. Blais, *Anal. Chim. Acta*, 1988 (211) 333.
[6] L.A. Blyshak, K.Y. Dodson, G. Patonay, I.M. Warner and W.E. May, *Anal. Chem.*, 1989 (61) 955.
[7] K. Shimada, O. Tomoyoki, Y. Hirose and Y. Komine, *J. Chromatogr.*, 1989 (478) 339.
[8] D. Sybilska, A. Bieljewski, R. Nowakowski, K. Duszczyk and J. Jurczak, *J. Chromatogr.*, 1992 (625) 349.
[9] J. Boger, R. Corcorn and L.M. Lehn, *J. Chromatogr.*, 1986 (39) 1417.
[10] A. Muñoz de la Peña, T.T. Ndou, J.B. Zung, K.L. Greene, D.H. Live and I.M. Warner, *J. Am. Chem. Soc.*, 1991 (113) 1572.
[11] V.C. Anigbogu, A. Muñoz de la Peña, T.T. Ndou and I.M. Warner, *J. Chromatogr.*, 1992 (594) 37.
[12] V.C. Anigbogu, A. Muñoz de la Peña, T.T. Ndou and I.M. Warner, *Anal. Chem.*, 1992 (64) 484.
[13] N. Husain, V.C. Anigbogu, M.R. Cohen and I.M. Warner, *J. Chromatogr.*, 1993 (635) 211.