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Retention behavior of β-cyclodextrin complexes of anthracene and pyrene using reversed-phase liquid chromatography

The effects of *tert.*-butanol and cyclopentanol

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ABSTRACT

The effects of *tert.*-butanol and cyclopentanol on the formation of β-cyclodextrin (β-CD)–anthracene and β-CD–pyrene complexes have been studied using reversed-phase liquid chromatography. The retention times of anthracene and pyrene were monitored when eluted in a C_{18} column with mobile phase mixtures consisting of methanol, water, β-CD, and a small amount of *tert.*-butanol or cyclopentanol. Plots of capacity factor of anthracene *versus* concentration of β-CD in the mobile phase in methanol–water mixtures and in mixtures containing 1% (v/v) cyclopentanol or *tert.*-butanol as secondary modifiers showed very similar trends. This suggests that anthracene forms weak complexes with β-CD, and that the presence of these secondary mobile phase modifiers have little effect on the β-CD–anthracene inclusion reaction. In contrast, pyrene showed no discernable change in retention time with increases in β-CD concentration. However, in the presence of 1% (v/v) *tert.*-butanol or cyclopentanol, capacity factor of pyrene decreased drastically, more in 1% (v/v) cyclopentanol than in 1% (v/v) *tert.*-butanol. Formation of a ternary β-CD–pyrene–alcohol complex has been proposed to explain the effects.

INTRODUCTION

It is well established that α-, β- and γ-cyclodextrins (CDs) readily form stable inclusion complexes with a variety of organic and inorganic molecules and ions [1]. The CD-inclusion phenomenon has found applications in pharmaceutics [2] and chromatography [3–9]. Applications in chromatography have mostly centered on the improved resolution of isomers [3–5]. Improvement in resolution results from differences in the retention behavior of the free and complexed solute species in the column. The degree of inclusion of the guest is determined by several factors including cavity size of the CD, the polarity of the solute, the nature and position of substituents on the solute, and the nature and concentration of concomitants in the solution medium.

The effects of concomitant species on the inclusion phenomenon of β-CD have been reported. Hamai [10] has reported on the quenching of fluorescence signal of pyrene- and 1-pyrenesulfonate complexes of β-CD by anilines. Others [11,12] have also shown that there are changes in the chemical behavior of inclusion complexes of CDs in

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the presence of surfactants as third components. Ueno *et al.* [13] and Nelson and Warner [14] reported on the concomitant effect of alcohols on the inclusion complexes of cyclodextrins with some guest molecules. Fluorescence studies in our laboratory of the effects of alcohol size on the stoichiometry and formation constants of β-CD and γ-CD complexes of pyrene were recently published [15–167]. The results show that in the presence of small amounts of alcohols as modifiers, ternary complexes of stoichiometry 2:1:2 are formed for β-CD–pyrene–alcohol and 1:1:1 (depending on the size of the alcohol) for γ-CD–pyrene–alcohol, respectively. In the case of β-CD–pyrene–alcohol complexes, the explanation for the observed results is that the *tert.*-butanol or cyclopentanol molecules are positioned at the open ends of the cyclodextrin cavities [15]. In this case, the geometry and molecular volume of the alcohols play important roles in the formation of the ternary complexes with β-CD. In contrast, there does not appear to be a correlation between the size and volume of the alcohols in the formation of γ-CD–pyrene–alcohol complexes. The alcohols are postulated to fill the void inside the γ-CD cavity; thus, the alcohols act as space regulators inside the larger γ-CD cavity.

The use of spectroscopic techniques to study the mechanisms of the inclusion complexes of the CDs has its advantages and disadvantages. The major advantages includes high sensitivity and short analysis time. A major disadvantage is the fact that not all CD inclusion phenomenon would be accompanied by significant changes in spectroscopic signals. Several reports have appeared in the literature on the use of liquid chromatography to study the concomitant effects of alcohols and other compounds on the formation of β-CD complexes. Tarr *et al.* [18] investigated the influence of 1% (v/v) *tert.*-butanol as a secondary organic mobile phase modifier on the retention behavior of 1-methyl-, 2-methyl-, 1,3-dimethyl-, 1,4-dimethylnaphthalenes, and acenaphthylene in a β-CD bonded column. Lamparczyk *et al.* [19] and others [7,8] have examined the effects of molecular structure and the nature and position of substituents on the formation of β-CD complexes in methanol–water mixtures. This paper re-examines fluorescence studies of the effects of *tert.*-butanol and cyclopentanol on the formation of anthracene–β-CD–pyrene complexes using reversed-phase high-performance liquid chromatography (RP-HPLC). Pyrene and anthracene were used as probe molecules in keeping with the experimental conditions of the fluorescence studies [15–17]. The enhanced β-CD solubility in aqueous solutions of methanol and other analytical implications of these effects are discussed.

EXPERIMENTAL

Apparatus

The liquid chromatographic system consisted of a Beckman Model 114 solvent delivery system equipped with a Rheodyne Model 7010 sample injector with a 20-μl sample loop (Rheodyne, Berkeley, CA, USA) and a Beckman Model 160 variable-wavelength UV absorbance detector set at 255 nm. The column used was a Bondclone C_{18} column (300 mm × 3.9 mm) purchased from Phenomenex (Torrance, CA, USA). The column temperature was maintained at 22 ± 1°C using a Brinkman (Westbury, NY, USA) Model Lauda RM3 constant-temperature bath connected to an Alltech (Deerfield, IL, USA) column water jacket. The Hewlett-Packard (Avondale, PA, USA) Model 3399A integrator was used to acquire and analyze the chromatograms.

Reagents

The primary organic modifier (methanol) and water used were HPLC grade purchased from B&J (Baxter, McGraw Park, IL, USA) and Fisher (Fair Lawn, NJ, USA), respectively. The *tert.*-butanol and the cyclopentanol (secondary organic modifiers), the pyrene (99 + %) and anthracene (99 + %), were all purchased from Aldrich (Milwaukee, WI. USA). These reagents were used as received. The β-cyclodextrin was obtained from American Maize-Products (Hammond, IN, USA) and was also used as received. The column void volume was determined using reagent-grade potassium nitrite purchased from Mallinckrodt (Paris, KY, USA).

Procedure for the preparation of samples

Appropriate amounts of anthracene and pyrene required to make about 0.10 m*M* solution of each were accurately weighed (to the nearest 0.1 mg) into a 250-ml volumetric flask. About 200 ml of methanol were added to the flask; the contents were

sonicated for 10 min and *ca.* 0.5 g of KNO_2 added to the flask before diluting to the mark with methanol. Different mobile phase mixtures were prepared by initially making about 4 l of the desired methanol–modifier–water mixtures, which were allowed to equilibrate overnight. A series of solutions from each mixture, containing from 0 to 6 m*M* β-CD, were prepared by weighing appropriate amounts of β-CD into a 1 l HPLC solvent reservoir, adding 500 ml of the solvent mixture and sonicating the mixture at 40°C for 1–3 h. The dissolution time depended on the amount of β-CD and the solution mixture. For example, mixtures with low methanol concentration (<60% (v/v) methanol in water) and those with 1% (v/v) *tert.*-butanol or 1% (v/v) cyclopentanol, dissolved readily within an hour. The mixture solutions containing various β-CD concentrations were allowed to sit overnight before use. It was noticed that higher concentrations of β-CD (>4 m*M*) in methanol–water mixtures, though fully dissolved during the preparation step and stable many hours after, showed some precipitation after they were allowed to stand overnight. Consequently the solutions containing 5 m*M* and 6 m*M* β-CD in methanol–water mobile phase mixtures were discarded. On the other hand, β-CD solutions in mobile phase mixtures containing 1–2% (v/v) *tert.*-butanol or cyclopentanol were stable for several days.

Procedure for the liquid chromatographic runs

Mobile phase mixtures were purged with helium for *ca.* 10 min prior to being used to elute the solutes at 1 ml/min. Whenever the mobile phase solution was changed, the column was conditioned for at least 30 min with the new mixture. The solutions containing different concentrations of β-CD were run randomly in an attempt to minimize the effect of drift. For runs with reasonable retention times (less than 45 min), two runs were averaged. Otherwise, one run was made. However, preliminary evaluation showed that retention times were reproducible within 2% in the best and 5% in the worst cases. The column pressure varied from 800 to 2300 p.s.i. and depended on the mobile phase mixture; for a given mixture, it remained constant throughout the run time. This suggests that β-CD was not precipitating in the column as was initially feared, particularly for the methanol–water mixtures containing high concentrations of β-CD. At the end of each set of runs, which usually lasted over 8 h, water was pumped through the column for about 1 h, methanol–water (55:45, v/v) for 10 min, and methanol overnight at a flow-rate of 0.3 ml/min.

RESULTS AND DISCUSSION

Effects of organic modifier and β-CD on retention behavior of pyrene and anthracene

The choice of primary organic modifier and its concentration in the mobile phase mixtures was governed by several factors including short analysis time, adequate solubility of β-CD in the resultant mobile phase mixture, and low formation constant for the β-CD–primary organic modifier in order to minimize competition with the solute molecules for the β-CD cavity. Of the most commonly used polar organic solvents in RP-HPLC, namely acetonitrile, methanol and tetrahydrofuran, methanol forms the weakest complex with β-CD [8]; hence, it was chosen as the primary organic modifier.

As would be expected, the retention times of anthracene and pyrene are very sensitive to the concentration of methanol in the mobile phase. Fig. 1 shows the typical linear relationship between log k' and methanol concentration in the mobile phase, suggestive of a hydrophobic-type interaction between the solute molecules and the stationary phase. The concentration of methanol in the mobile phase is very critical if the interaction between β-CD, the secondary organic modifier, and the solute mole-

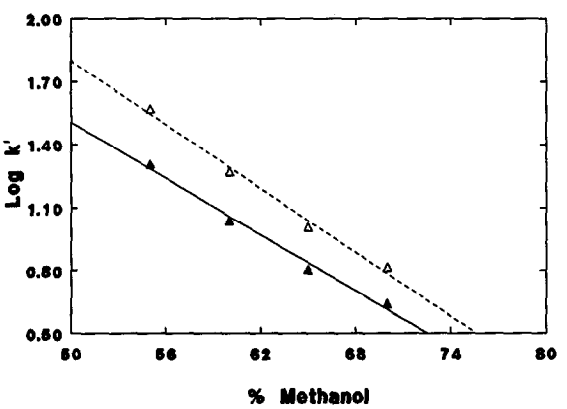

Fig. 1. The effect of volume percent methanol on the capacity factors (k'), of anthracene (▲) and pyrene (△) in a solution containing 3.0 m*M* β-CD.

TABLE I

RETENTION TIMES OF ANTHRACENE AND PYRENE IN METHANOL–WATER MOBILE PHASE MIXTURES

Methanol (%)	Retention time (min)	
	Anthracene	Pyrene
80	7.79	9.66
75	12.91	18.03
70	18.46	25.49
65	24.76	36.39
60	43.42	65.53
55	66.12	125

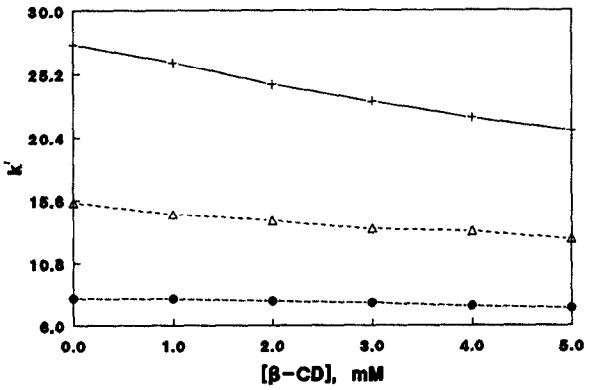

Fig. 2. Plots of k' *vs.* [β-CD] for anthracene in mobile phase mixtures containing 55% (v/v) methanol (+), 60% (v/v) methanol (△) or 65% (v/v) methanol (●).

cules is to be observed at reasonable retention times. For example, Table I shows that the retention times of anthracene and pyrene were about 66 and 125 min, respectively, when eluted with methanol–water (55:45, v/v) mixture, and about 18 and 25 min, respectively, with methanol–water (70:30, v/v) mixture. At methanol–water (55-45, v/v), the retention time of pyrene is over 2 h resulting in severely broadened chromatographic peaks. At high concentration of methanol, not only does the solubility of β-CD decreases drastically, but the competitive equilibria between methanol and β-CD molecules are dramatically enhanced. Matsui and Mochida [23] and Buvari *et al.* [24] have independently estimated the apparent formation constants of the β-CD–methanol complex to be 0.32 and 0.40 M^{-1}, respectively. Although these numbers suggest that methanol forms very weak complexes with β-CD, it effectively impairs or even precludes the inclusion of many solutes such as pyrene at high concentrations. Consequently, 55, 60 and 65% (v/v) methanol concentrations in water were chosen, in keeping with the three foregoing considerations, as the initial test solutions. Moreover, extreme sensitivity of the retention times of anthracene and pyrene to the methanol concentration in the mobile phase required that extra care be exercised in preparing the mobile phase mixtures. The approach described in the *Procedure for the preparation of samples* section was found satisfactory in maintaining a uniform methanol concentration for a given set of runs.

The effects of β-CD concentration on the capacity factors of anthracene when eluted with different methanol–water mobile phase mixtures are shown in Fig. 2. Anthracene exhibited the expected decrease in capacity factor with increasing β-CD concentration as the associated molecules interact less with the stationary phase than the free molecules in solution. Also, for a given β-CD concentration, the capacity factor of anthracene decreased with increasing methanol concentration from 55 to 60% (v/v). In contrast, the capacity factors of pyrene (Fig. 3) did not show the expected trend in all methanol–water mixtures (in the absence of co-modifiers). It should be mentioned that both anthracene and pyrene include readily in β-CD in highly aqueous systems [15,16]. A possible explanation for minimal inclu-

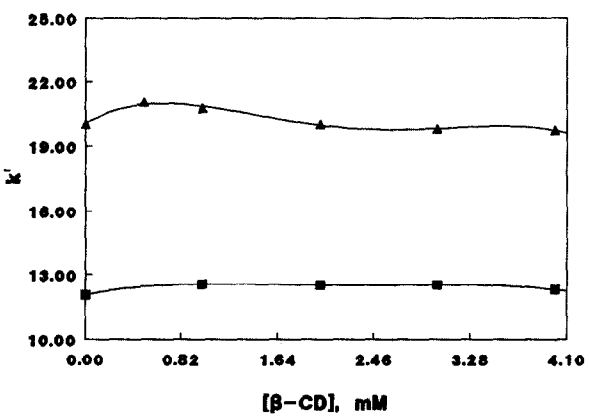

Fig. 3. Plots of k' *vs.* [β-CD] for pyrene in mobile phase mixtures containing 60% (v/v) methanol (▲) and 65% (v/v) methanol (■).

sion of these solutes in these high methanol (> 55% v/v) solutions is the competitive equilibria between the methanol an the solute molecules for the concentration-limited β-CD. It should be reiterated that methanol–water mixtures did not sustain β-CD concentrations above 4 m*M*.

The effects of secondary organic modifier in the mobile phase on the capacity factor of anthracene and pyrene

Warner and co-workers [15,16] recently reported their extensive studies of β-CD– and γ-CD–pyrene complexes, in the presence of various alcohols using steady-state fluorescence spectroscopy. In these studies, the apparent formation constants of β- and γ-CD–pyrene complexes were determined by monitoring the variations in the I/III fluorescence vibronic band ratio of pyrene [20–22] with increasing β-CD concentration. In the case of β-CD–pyrene complexes, and 1% (v/v) alcohol concentration in water, all the alcohols investigated showed an enhancement effect (increase in apparent formation constant, K_f). The largest increases in β-CD–pyrene K_f values were observed in the presence of *tert.*-butanol (for the straight chain) and cyclopentanol (for the cyclic chain). This observation was attributed to the formation of a ternary complex of β-CD–pyrene–alcohol. The influence of 1% (v/v) *tert.*-butanol as a secondary organic mobile phase modifier on the retention behavior of 1-methyl-, 2-methyl-, 1,3-dimethyl- and 1,4-dimethylnaphthalenes, and acenaphthylene using a bonded β-CD stationary phase has been reported by Tarr *et al.* [18]. The results show that the 1-methyl-, 1,4-dimethylnaphthalenes and acenaphthylene exhibited the largest increases in retention times in the presence of 1% (v/v) *tert.*-butanol. We investigated the effect of the addition of 1% (v/v) *tert.*-butanol and cyclopentanol, as secondary modifiers, on the formation of β-CD–pyrene and β-CD–anthracene complexes especially since we did not discern any complexation of pyrene in mobile phase mixtures of 55, 60 and 65% (v/v) methanol in water mixtures. These effects, if any, would be manifested as decreases in the retention times of these solutes with increasing amounts of β-CD concentration in the mobile phase.

The effects of the secondary modifier on the capacity factor of anthracene, eluted with methanol–

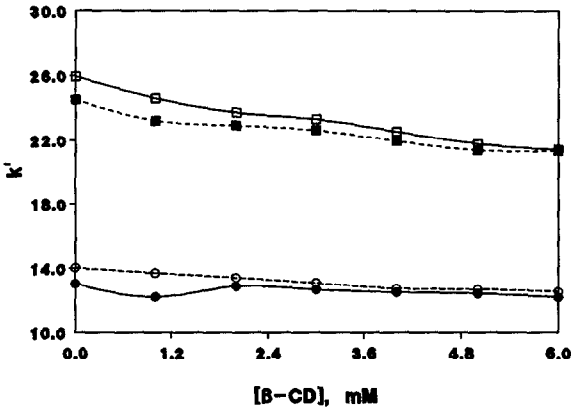

Fig. 4. Plots of *k'* *vs.* [β-CD] for anthracene in mobile phase mixtures methanol–*tert.*-butanol–water (54:1:45, v/v/v) (□), methanol–cyclopentanol–water (54:1:45, v/v/v) (■), methanol–*tert.*-butanol–water (59:1:40, v/v/v) (○) and methanol–cyclopentanol–water (59:1:40, v/v/v) (●).

modifier–water (54:1:45, v/v/v) and methanol–modifier–water (59:1:40, v/v/v), are shown in Fig. 4. The secondary modifiers seem to have little or no influence on the β-CD–anthracene complex formation. However, as Fig. 5 shows, the capacity factor of pyrene decreased remarkably in the presence of 1% (v/v) *tert.*-butanol or cyclopentanol in the mobile phase. This clearly suggests that these alcohols participate, directly or indirectly, in the formation of the inclusion complexes of pyrene. It should

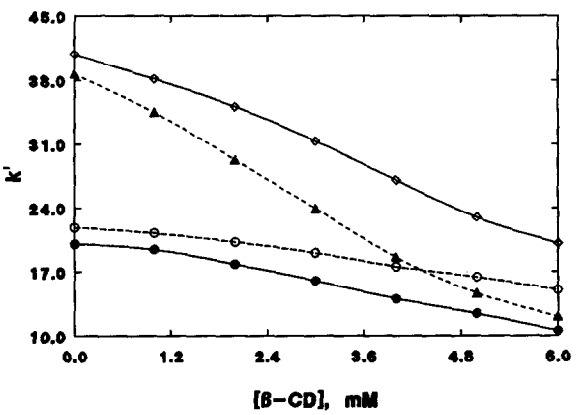

Fig. 5. Plots of *k'* *vs.* [β-CD] for pyrene in mobile phase mixtures methanol–*tert.*-butanol–water (54:1:45, v/v/v) (◇), methanol–cyclopentanol–water (54:1:45, v/v/v) (▲), methanol–*tert.*-butanol–water (59:1:40, v/v/v) (○) and methanol–cyclopentanol–water (59:1:40, v/v/v) (●).

be noted that the effects are more pronounced in aqueous solutions than in the mixtures examined here [9,15,16]. Again, the formation of a ternary complex of β-CD–pyrene–cyclopentanol of 2:1:2 stoichiometric ratio has been postulated to explain these effects [15].

The effects of secondary modifiers on the selectivity factor

The decrease in capacity factor of pyrene with increasing β-CD concentration imply that a mobile phase comprised of appropriate concentrations of β-CD and organic modifiers, could be used to enhance both the selectivity and the efficiency of separation by reversed-phase liquid chromatography. For example, the selectivity enhancement is illustrated in Fig. 6 where the separation factor, α, is plotted against total β-CD concentration for different mobile phase mixtures. In the absence of β-CD, the retention time of pyrene is about 1.5 times that of anthracene in all mobile phase mixtures. However, as the concentration of β-CD increases, the retention time of pyrene decreases faster than that of anthracene. This effect is so profound in mixtures containing cyclopentanol that, at 6.0 m*M* β-CD, the retention time of pyrene in methanol–water–cyclopentanol (54:45:1, v/v/v) is about 1.5 times less than that of anthracene. This is a complete reversal of their initial retention times, reducing the separation time by one-third. This observation predicts that at even lower methanol concentration than 55% (v/v), the retention time of pyrene would be further reduced at 6 m*M* β-CD. Another potential application of the modifier effects is their use to improve the solubility and stability of β-cyclodextrin in solutions of high methanol composition. As previously stated in the *Procedure for the preparation of samples* section, high concentrations of β-CD (>4 m*M*) solutions were stable in the presence of *tert.*-butanol or cyclopentanol but not in methanol–water mixtures.

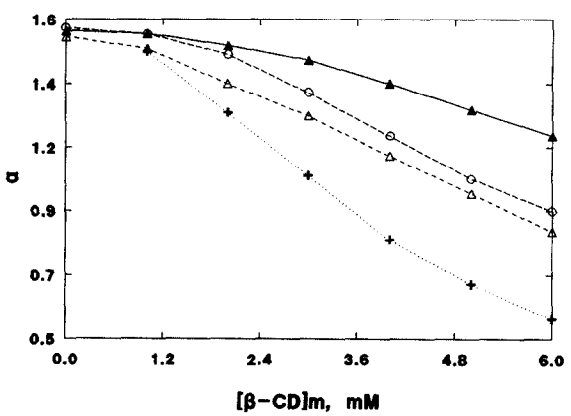

Fig. 6. Plots of selectivity factors, α, as the ratio of *k'* of pyrene to anthracene, *vs.* [β-CD] in mobile phase mixtures methanol–*tert.*-butanol–water (59:1:40, v/v/v) (▲), methanol–cyclopentanol–water (59:1:40, v/v/v) (○), methanol–*tert.*-butanol–water (54:1:45, v/v/v) (△) and methanol–cyclopentanol–water (54:1:45, v/v/v) (+).

CONCLUSIONS

The results presented show that the addition of 1% (v/v) *tert.*-butanol or cyclopentanol in methanol–water mobile phase mixtures enhances the complexation of pyrene molecules by β-CD which is in keeping with fluorescence results [9]. In the case of cyclopentanol–methanol–water (1:55:45, v/v/v) this resulted in almost 70% reduction in the retention time of pyrene as its capacity factor changed from 38 to 12, and β-CD concentration changed from 0 to 6.0 m*M*, respectively. The modifier effects could also be used to improve solubility of β-CD in aqueous solutions of polar organic solvents. For example we have been able to prepare 10 m*M* β-CD in methanol–water–cyclopentanol (62:35:3, v/v/v). A full scale study of the solubility effect is already in effect. Another potential use of the ternary forming concomitants is to improve resolution in liquid chromatography or selective extraction of ternary-forming solutes in a mixture. These latter techniques would be applicable to environmental analysis.

It is highly unlikely that pyrene would be the only species that forms ternary complex with β-CD. Other large molecules can be encapsulated partly or fully by one or more β-CD molecules, and the process can further be enhanced by a third component such as alcohols. As previously discussed, both molecular geometry, volume, and polarity influence the degree of β-CD–guest formation. Molecules such as perylene, 1,2,3,6,7,8-hexahydropyrene and 4H-cyclopenta[*def*]phenanthrene, have similar molecular volume and geometry comparable to pyrene, and are being studied by RP-HPLC in an attempt to understand structural relationships, if any, to the ternary complex formation. Obviously,

full exploitation of the β-CD inclusion phenomenon in developing pharmaceutical, spectroscopic, chromatographic and other methodologies depends on some or full knowledge of these kinds of interactions. Our results suggests that liquid chromatography will continue to play a major role in comparative studies of cyclodextrin complexes under various conditions.

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