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Separation of polycyclic aromatic hydrocarbons by micellar electrokinetic chromatography with cyclodextrins as modifiers

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ABSTRACT

The use of α-, β- and γ-cyclodextrins as modifiers to the electrophoretic medium containing sodium dodecyl sulphate and a phosphate–borate buffer in the micellar electrokinetic chromatography of polycyclic aromatic hydrocarbons (PAHs) was investigated. The results showed that the migration behaviour of PAHs could be related to their sizes and the cavity sizes of the cyclodextrins. With the addition of γ-cyclodextrin higher selectivity could be achieved, which consequently resulted in better separation of the seven PAHs investigated.

INTRODUCTION

Micellar electrokinetic chromatography (MEKC) is a separation technique of relatively recent origin. It was developed from capillary zone electrophoresis (CZE), which is based on the differential migration of solutes when a potential field is applied. MEKC employs the same principle as CZE, except that an ionic surfactant is added to the buffer mobile phase. MEKC is able to effect the separation of neutral compounds [1,2] based on the differential distribution of solutes between an electroosmotically pumped aqueous mobile phase and a slower moving electrophoretically retarded micellar phase.

Polycyclic aromatic hydrocarbons (PAHs) are of great environmental concern owing to their carcinogenicity. As they are all neutral, non-ionizable and of similar hydrophobicity, one would not expect a good separation by using conventional CZE or MEKC. Hence it is necessary to extend the concept of employing a mobile phase and a pseudostationary phase to the utilization of buffer modifiers [3,4]. Some common buffer modifiers that have been used are derivatized cyclodextrins [5], tetrahexylammonium perchlorate [3], copper(II)–L-histidine complex [6], copper(II)–aspartame complex [4] and organic modifiers [7].

One of the limitations of MEKC is the need for samples to be reasonably soluble in an aqueous mobile phase. Hydrophobic compounds, such as the PAHs, tend to be completely solubilized by the micelles and co-elute with migration times near that of the micelles. This problem is exacerbated by the limited elution range observed in MEKC. These problems can be alleviated by silanizing the capillary column walls [8], coating the walls with polymers [9] or adding modifiers to the electrophoretic buffer [10–13]. Terabe *et al.* [13] reported that PAHs were so hydrophobic that they were almost totally incorporated into the micelle. Although the addition of aqueous organic solvents increased the distribution of PAHs to the aqueous phase, satisfactory resolution was not achieved by this method because of peak tailing. They suggested that the use of γ-cyclodextrin would improve the resolution of PAHs in MEKC. However, only preliminary results were reported [13]. In this work, the use of α-, β-

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and γ-cyclodextrins as modifiers in the MEKC of PAHs was studied. Further, the migration behaviour of PAHs at different concentrations of cyclodextrins (CDs) as modifiers in the electrophoretic media was investigated.

EXPERIMENTAL

A fused-silica capillary tube (Polymicro Technologies, AZ, USA) 580 mm × 0.05 mm I.D. (500-mm effective length) was used as a separation column. On-column UV detection was carried out with a MicroUVis20 spectrophotometric detector (Carlo Erba, Milan, Italy) at 254 nm. Data were recorded using a Model 252A/MM chart recorder (Linear Instruments, CA, USA).

The chromatographic solutions were prepared by dissolving the modifiers and sodium dodecyl sulphate (SDS) in 0.05 *M* phosphate–0.1 *M* borate buffer solution (pH 7.0). All the solutes were first dissolved in the minimum amount of dimethylformamide (DMF) before diluting further with methanol. In our system, sample introduction was carried out via gravitational feed: sample solution was introduced into one end of the capillary tube by siphoning from the sample solution at a higher level than the electrophoretic solution in which the other end of the tube was immersed. A sample mixture consisting of seven PAHs at a concentration of 100 ppm was injected. The injection time and height were 3 s and 50 mm, respectively.

All reagents were of the purest grade available. The seven PAHs studied were acenaphthene, benz[*a*]anthracene, benzo[*a*]pyrene, chrysene, fluoranthene, perylene and phenanthrene. All the PAHs except benz[*a*]anthracene were obtained from Aldrich (Milwaukee, WI, USA). Benz[*a*]anthracene, α-, β- and γ-cyclodextrins, Sudan III and SDS were purchased from Fluka (Buchs, Switzerland). Methanol was obtained from Carlo Erba and dimethylformamide from Merck (Darmstadt, Germany). The buffer solution was prepared by dissolving sodium dihydrogenphosphate and sodium tetraborate (Fluka) in water purified with a Millipore Alpha-Q system.

RESULTS AND DISCUSSION

Previous work by Terabe and co-workers [14,15] has demonstrated that the selectivity can be improved in open-tubular high-performance capillary electrophoresis (HPCE) by including ionic surfactants in the electrophoretic medium. The effects of varying the SDS concentration on the separation of the seven PAHs are depicted in Fig. 1 and the migration times are given in Table I. It was observed that, in general, as the concentration of SDS increased, the migration times of the solutes increased. This can be attributed to the increase in the micelle concentration, which consequently retains the electrically neutral PAHs longer. The plot shows that 10 m*M* SDS provides the optimum se-

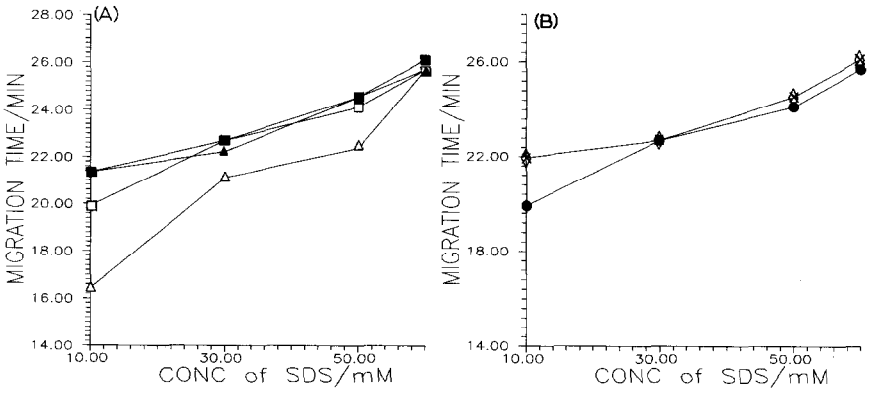

Fig. 1. Plots of migration time *vs.* concentration of SDS. Electrophoretic solution, SDS in 0.05 *M* phosphate–0.1 *M* borate buffer (pH 7.0); separation tube, 500 mm × 0.05 mm I.D. fused-silica capillary; voltage, 15 kV; detection wavelength, 254 nm. (A) △ = Acenaphthene; ▲ = phenanthrene; □ = perylene; ■ = benzo[*a*]pyrene. (B) ◇ = Chrysene; × = benz[*a*]anthracene; ● = fluoranthene.

TABLE I

MIGRATION TIME (min) OF PAHs OBTAINED USING SDS AND CD

PAH	SDS (m*M*)[a]				α-CD(m*M*)[b]		β-CD (m*M*)[c]			γ-CD (m*M*)[d]		
	10	30	50	60	2	5	1	2	5	1	2	3
Acenaphthene	16.44	21.10	22.37	25.69	12.90	12.36	14.04	12.30	8.13	12.63	9.60	9.00
Phenanthrene	21.35	22.22	24.54	25.69	16.80	15.90	17.70	17.55	15.18	16.77	12.68	11.10
Perylene	19.92	22.70	24.11	25.69	21.18	19.65	19.74	19.80	19.95	18.54	14.78	12.21
Benzo[*a*]pyrene	21.35	22.70	24.54	26.10	21.00	19.65	19.74	19.68	19.44	18.77	15.30	12.30
Chrysene	21.95	22.70	24.54	26.10	19.80	18.30	19.20	19.50	19.05	18.41	15.90	12.60
Benz[*a*]anthracene	21.95	22.70	24.54	26.10	19.80	18.30	19.20	19.65	19.44	19.26	16.44	13.05
Fluoranthene	19.92	22.70	24.11	25.69	19.20	18.30	19.20	18.60	18.18	18.80	16.80	13.20

[a] Operating buffer contains SDS only.
[b] Operating buffer contains 10 m*M* SDS and α-CD as modifier.
[c] Operating buffer contains 10 m*M* SDS and β-CD as modifier.
[d] Operating buffer contains 10 m*M* SDS and γ-CD as modifier.

lectivity. However, satisfactory separation of all the PAHs could not be achieved by varying the SDS concentration alone.

Cyclodextrins are neutral oligomers with different units of D-(+)-glucopyranose and the most commonly used CDs in chromatographic separation techniques [16] are α-, β- and γ-CD with six, seven and eight glucose units, respectively. The structure of CDs forms a cavity and hence can offer the possibility of behaving similarly to SDS, *i.e.*, providing the partition between CD and solutes.

The effects of the addition of various concentrations of the three different types of CDs to 10 m*M* SDS buffer were investigated at pH 7.0. The migration times of the compounds studied are given in Table I. Plots of migration time *vs.* concentration of CD are depicted in Figs. 2–4.

The general trend observed is that as the number of D-(+)-glucopyranose units of the CD decreased, the migration times increased. This is a consequence of the competition between the CD cavity and SDS micelles for PAH solutes. As the size of the CD cavity decreases, the larger PAH solutes would tend to be solubilized by SDS rather than entering the CD cavity. Hence longer migration times were observed.

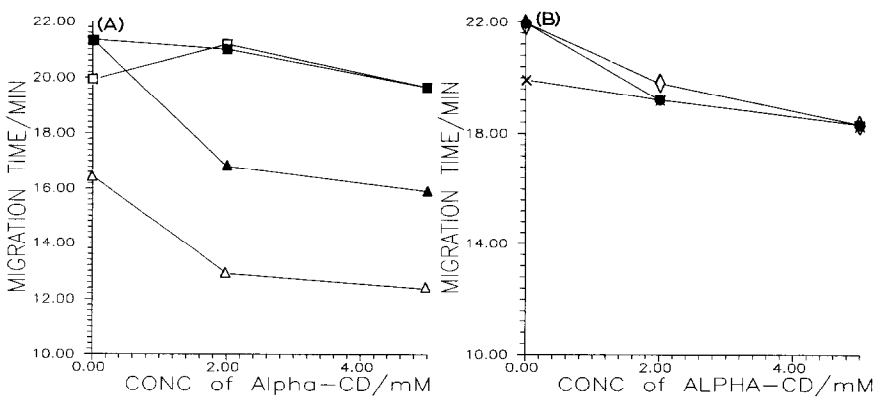

Fig. 2. Plots of migration time *vs.* concentration of α-CD with 10 m*M* SDS in 0.05 *M* phosphate–0.1 *M* borate buffer (pH 7.0). Separation tube, 500 mm × 0.05 mm I.D. fused-silica capillary; voltage, 15 kV; detection wavelength, 254 nm. (A) Symbols as in Fig. 1. (B) ◇ = Chrysene; ● = benz[*a*]anthracene; × = fluoranthene.

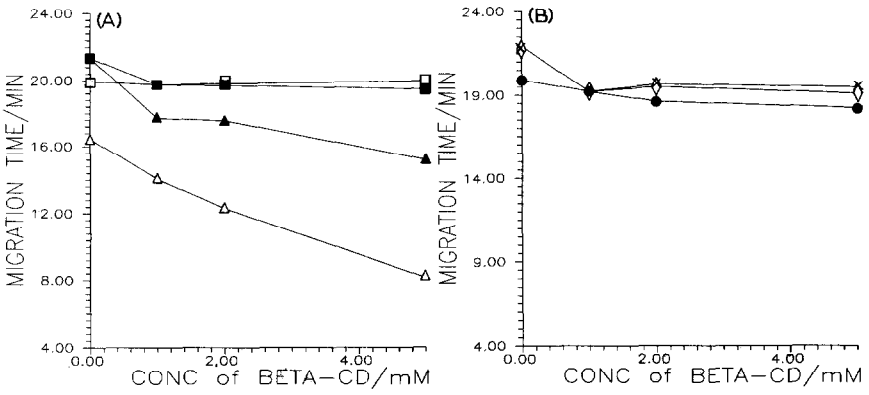

Fig. 3. Plots of migration time *vs.* concentration of β-CD with 10 m*M* SDS in 0.05 *M* phosphate–0.1 *M* borate buffer (pH 7.0). Other conditions as in Fig. 2. Symbols as in Fig. 1.

Fig. 2 depicts the effects of changing the concentration of α-CD on the separation of the seven PAHs. The general trend observed was that when the concentration of CD increased, the migration times of the PAHs decreased. This behaviour may be attributed to the nature of the CD. Unlike SDS micelles, CD is neutral, and hence it is expected to have no electrophoretic velocity and to migrate faster than SDS. Further, at higher concentrations of CD, the tendency of these PAHs to be solubilized in the cavity increases. Hence, as the concentration of CD increases, solubilization increases and consequently a decrease in migration time would be expected. An exception to this trend was observed for perylene, which showed a slight increase in migration time at low α-CD concentration (0–2 m*M*). The difference in behaviour can be attributed to the fact that perylene is the largest among the PAHs investigated, and α-CD has a relatively small cavity size consisting of only six D-(+)-glucopyranose units.

A similar investigation was carried out on β-CD, which has a cavity size intermediate between those of α-CD and γ-CD. The migration times obtained for the PAHs at different concentrations of β-CD are shown in Fig. 3. Six peaks were observed at a concentration of 5 m*M* β-CD. A further increase in β-CD concentration resulted in a decrease in selectivity. Fig. 3 depicts a trend of decreasing migration times with increasing concentration of β-CD for the same reason as cited earlier for α-CD. However, the decrease in migration times is not very prominent for the larger PAHs such as benz[*a*]anthracene,

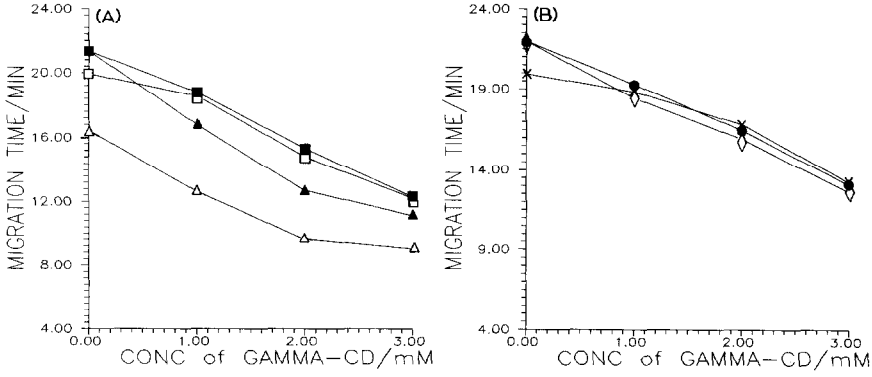

Fig. 4. Plots of migration time *vs.* concentration of γ-CD with 10 m*M* of SDS in 0.05 *M* phosphate–0.1 *M* borate buffer (pH 7.0). Other conditions and symbols as in Fig. 2.

chrysene, benzo[*a*]pyrene and perylene. This could be due to the difficulty in fitting these large solutes into the cavity of β-CD.

Results of experiments on varying the concentration of γ-CD are shown in Fig. 4. A similar trend to that for β-CD was observed for γ-CD, except that the decrease in migration times is now greater. The shorter migration times observed at higher concentrations of CD could be explained by the tendency of the PAH molecules to stay out of the micelle under these conditions. With increase in the concentration of γ-CD, a decrease in selectivity was also observed. As the concentration increased to 3 m*M* γ-CD, the selectivity was found to decrease to an unacceptable level, the last two peaks corresponding to benz[*a*]anthracene and fluoranthene were not being completely resolved.

Nevertheless, a concentration of 2 m*M* γ-CD and 10 m*M* SDS gives a satisfactory resolution of the seven PAHs, as shown in Fig. 5. The analysis was carried out in only 18 min. The improved selectivity compared with α- and β-CD could be attributed to the more effective interaction of the larger cavity size provided by γ-CD, which consists of eight glucopyranose units. The larger PAHs were therefore able to fit into the cavity more readily.

Solute migration in this investigation is dependent on a number of factors, the most important of which are probably hydrophobic retention and partitioning in the CD cavity. The principle of separation is based on the host–guest interaction between the SDS, γ-CD and solutes. The solutes partition between the pseudo-stationary phase (SDS) and the mobile phase (phosphate–borate buffer and γ-CD). This interaction operates as the distribution process. Here, selectivity towards the shape and size of the solutes plays an important role. The usual trends of increasing retention as a function of size are observed for PAHs. Exceptions to these trends are not uncommon, however, and it is clear that solute shape also influences migration behaviour.

With regard to the migration order in Fig. 5, it was noted that fluoranthene, although small, migrated last. There is reason to believe that the rigidity of the cyclopentane ring of fluoranthene caused a poor interaction with the CD cavity, whereas the other PAH solutes possess the ability to "bend" to suit the cavity. Hence the compromising effects of fluoranthene solubilizing into the SDS micelles, which is the dominating factor, and partitioning into the CD cavity explain its migration order.

Finally, it is worth noting that among the seven PAHs, perylene and benzo[*a*]pyrene and also chrysene and benz[*a*]anthracene are two pairs of structural isomers. As complete separation is achieved using γ-CD as modifier in the electrophoretic medium, we can conclude that this technique is very promising for resolving mixtures of PAH isomers.

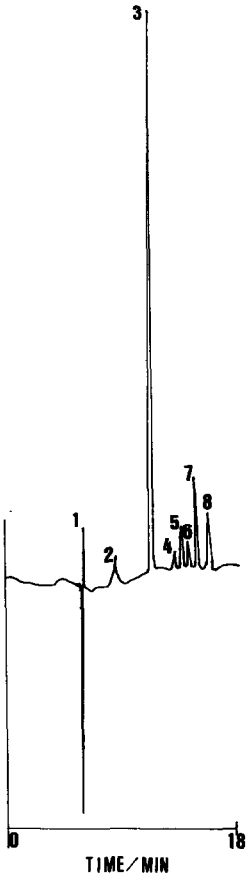

Fig. 5. Electropherogram showing the resolution of (1) methanol, (2) acenaphthene, (3) phenanthrene, (4) perylene, (5) benzo[*a*]pyrene, (6) chrysene, (7) benz[*a*]anthracene and (8) fluoranthene. Electrophoretic solution, 10 m*M* SDS and 2 m*M* γ-CD in 0.05 *M* phosphate–0.1 *M* borate buffer (pH 7.0); separation tube, 500 mm × 0.05 mm I.D. fused-silica capillary; voltage, 15 kV; current, 36 μA; detection wavelength, 254 nm.

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