ELSEVIER

Journal of Chromatography A, 829 (1999) 289–299

JOURNAL OF
CHROMATOGRAPHY A

Influence of organic solvents in the capillary zone electrophoresis of polycyclic aromatic hydrocarbon metabolites

Xin Xu, R.J. Hurtubise*

Department of Chemistry, University of Wyoming, Laramie, WY 82071, USA

Received 28 April 1998; received in revised form 29 September 1998; accepted 2 October 1998

Abstract

A rapid capillary zone electrophoresis (CZE) method was developed using a 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS) buffer for the compound-class separation of nine polycyclic aromatic hydrocarbon (PAH) metabolites including tetrols, benzo[*a*]pyrene diols, and hydroxyl aromatics. In addition, the effects of two organic solvents (methanol and acetonitrile) on the electroosmotic flow and electrophoretic mobilities of the analytes were studied. Separation of nine PAH metabolites was ultimately achieved within 24 min with a 100 m*M* CAPS buffer with 40% (v/v) MeOH at an apparent pH of 10.4. In addition, it was possible to partially resolve the parent PAH compounds from the metabolites with the previous buffer system. The simple CZE methods developed would be useful for the rapid separation and characterization of several important biomarkers. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Buffer composition; Polycyclic aromatic hydrocarbons

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are an important class of pollutants that exist in air, waterways and oceans, soils and foodstuffs. They are mainly formed by the incomplete combustion of organic material containing carbon and hydrogen [1]. Examples of the sources of PAHs include automotive exhaust, heating, refuse burning and industrial processes [2]. Some PAHs show both mutagenic and carcinogenic properties and are a threat to human health. Therefore, the control of the level of PAHs is receiving more and more attention. Benzo[*a*]pyrene (B[*a*]P) is one of the most extensively studied PAH compounds. It is often used as a biomarker for PAH exposure because of its potential carcinogenicity [3]. In vivo B[*a*]P is metabolized to epoxides, hydroxyl aromatics, quinones, dihydrodiols, dioepoxides, tetrols and water-soluble conjugates [4]. The potential toxicity of B[*a*]P depends largely on its metabolic fate [5]. Pyrene and benz[*a*]anthracene (B[*a*]A) are also environmentally important PAH compounds. Some of the metabolites of pyrene, B[*a*]A and B[*a*]P are 1-hydroxypyrene (1-OH-Py), 3-hydroxybenz[*a*]anthracene (3-OH-B[*a*]A) and 3-hydroxybenzo[*a*]pyrene (3-OH-B[*a*]P), respectively. These hydroxyl derivatives have been used as biomarkers for environmental and occupational PAH exposures [6].

A number of analytical approaches, including high-performance liquid chromatography (HPLC) [7–15] and gas chromatography [16] have been used to isolate and separate B[*a*]P metabolites. Capillary

*Corresponding author. Tel.: +1-307-7662363; fax: +1-307-7662807; e-mail: hurtubis@uwyo.edu

0021-9673/99/$ – see front matter © 1999 Elsevier Science B.V. All rights reserved.
PII: S0021-9673(98)00794-8

electrophoresis (CE) has been used extensively for the efficient separation of a wide range of analytes. Owing to the fast separations, extremely high efficiency and minute sample size, CE has become a very important technique in the area of liquid-phase separation. Until now, very little work has been done in the separation of PAH metabolites with CE. Cyclodextrin (CD)-modified micellar electrokinetic capillary chromatography (CD-MECC) was recently used for the separation of PAH metabolites [17,18]. Even though CD-MECC can give high resolution, in some cases, the addition of a micelle and CD to the buffer can result in poor reproducibility [19]. On the other hand, capillary zone electrophoresis (CZE) is the simplest and most commonly used technique in CE. It is characterized by high efficiency, high resolving power and good reproducibility [20]. It is traditionally thought that CZE is limited to the analysis of water-soluble, charged species and therefore is not applicable for the analysis of neutral and water-insoluble PAH metabolites [18–20]. However, several research results have shown that, by using organic modifiers, the applicability of CZE could be broadened to a wide range of slightly polar and non-polar compounds [21–32].

The addition of organic solvents to electrolyte systems can have many useful effects on the separation of organic compounds. As early as 1984, Walbroehl and Jorgenson used acetonitrile as a nonaqueous CZE medium in the separation of organic bases (quinoline and isoquinoline) [21]. Since then, more and more attention has been paid to the expanded separation applicability of CZE in nonaqueous systems or binary aqueous–organic solvent systems. Aqueous–organic buffer electrolytes are used, e.g., to increase analyte solubility [21], to lower the electroosmotic flow [22,23], to change the electrophoretic mobility of analyte ions [23–25], to affect the acid–base properties of the sample components [26], or to adjust the selectivity in order to improve resolution [26–28]. Furthermore, the influence of organic solvents on the electroosmotic mobility and the zeta (ζ) potential have been studied in detail by Kenndler and co-workers [29,30]. Recently, Masselter and Zemann investigated the effects of organic solvents in coelectroosmotic capillary electrophoresis of substituted phenols [31]. Carabias-Martinez et al. successfully separated a mixture of chlorotriazines and methylthiotriazines by using nonaqueous solvents and ionic additives in CZE [32]. Most recently, a review on the topic of the influence of organic solvents on the separation selectivity in CE has been published [33].

In this work, first, a simple and rapid CZE method is presented for compound-class separation of the PAH metabolites. Then, the effects of organic modifiers on improving the CZE separation are studied. The results obtained show great improvement in overall resolution, and all the PAH metabolites could be separated within 24 min.

2. Experimental

2.1. Apparatus

All electropherograms were obtained from a P/ACE 5000 (Beckman, Fullerton, CA, USA) instrument equipped with a UV detector. A fused-silica capillary of 57 cm (50 cm from inlet to detector)×75 μm I.D.×375 μm O.D. was used.

All fluorescence excitation and emission spectra were taken with a Perkin-Elmer LS-5 (Norwalk, CT, USA) fluorescence spectrometer.

2.2. Reagents

The benzo[*a*]pyrene metabolites and the benz[*a*]anthracene metabolite were purchased from the National Cancer Institute (NCI) repository at Midwest Research Institute (Kansas City, MO, USA) and used without further purification. Pyrene, benzo[*a*]pyrene, benz[*a*]anthracene and 1-OH-Py were obtained from Aldrich (Milwaukee, WI, USA). All buffer reagents were purchased from Aldrich. Methanol (MeOH), acetonitrile (ACN) and water were HPLC grade and were purchased from J.T. Baker (Phillipsburg, NJ, USA).

2.3. Buffers

The aqueous buffers were prepared by dissolving an individual buffer reagent in water. In the experiments with organic solvents, buffer electrolytes were prepared in binary water–organic solvent systems consisting of different volume percents of the or-

ganic component. The pH was adjusted with 1 *M* NaOH solution. Before use, all buffer solutions were either filtered through a 0.45-μm Supor Acrodisc filter (for aqueous buffers) or a 0.45-μm GHP Acrodisc filter (for buffers with organic solvents) (Gelman, Ann Arbor, MI, USA) and sonicated 10 min to degas.

2.4. Procedures

The CZE system was operated in a conventional mode with the cathode at the detector end. Hydrodynamic injection was used and set for 2 s. The temperature of the capillary was maintained by means of a liquid coolant in the capillary cartridge. Ultraviolet absorbance detection was used at 254 nm. Since the methanol content in the sample solution was relatively high, a linear ramp of 2.5 min was applied to increase the voltage from 0 to 25 kV in order to ensure a more gradual mixing of the sample plug with the running buffer and minimize the possibility of zone spreading.

At the beginning of each working day, and whenever necessary, the capillary was rinsed sequentially with 1 *M* NaOH, 0.1 *M* NaOH, water, MeOH, water and then running buffer. Between each analysis, the capillary was washed with 0.1 *M* NaOH and buffer. MeOH in the sample solution was used as the neutral marker for the measurement of electroosmotic flow (EOF). Electropherograms were recorded and analyzed on a IBM 350-P90 computer using Beckman's System Gold electrophoresis software (Beckman). Peak identification was done by comparing the migration time of each peak with that of the individual standard injected under the same experimental conditions.

Stock solutions containing 48 μg/ml of PAH metabolites were prepared in MeOH–water (7:3, v/v). Working solutions for CZE separation were prepared by diluting these stock solutions. Stock solutions for the parent PAH compounds B[*a*]A and B[*a*]P were prepared in MeOH and further diluted with MeOH–water (7:3, v/v). Pyrene was first dissolved in distilled ethanol and then diluted with HPLC water for a working solution. The final concentration of each component in the standard mixture was about 4 μg/ml. Working solutions for fluorescence excitation and emission spectra were diluted from the stock solution with 50 m*M* 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS) buffer (pH 10.4). The final sample concentration was 0.2 μg/ml. All samples were stored in the dark at 4°C. The glassware was wrapped in aluminum foil to protect sample solutions from light.

3. Results and discussion

3.1. Buffer selection

In CZE separation, the running buffer is one of the most important parameters since it controls the pH and hence determines the extent of ionization and mobility of each analyte. Buffer selection was critical in our study. The pK_a values of the four hydroxyl B[*a*]Ps used in our study were determined previously to be around 9 by fluorescence spectroscopy [34]. Thus, a suitable pH for the buffer should be somewhat greater than 9 so that a reasonably large fraction of the solute will be ionic. The buffer most often used with a pK_a greater than 9 is borate. Therefore, initially, the individual migration characteristics of each PAH metabolite was investigated using borate buffer at pH 10.0. However, except for 1-OH-Py, borate buffer turned out not to be suitable for the PAH metabolites since it gave very broad bands. This could have been due to the large number of aromatic rings in our compounds, which would cause a mismatch of the buffer ion mobility with the solute mobility [35]. After an extensive study of various buffer systems, e.g., CAPS (3-[cyclohexylamino]-1-propanesulfonic acid), CAPSO (3-[cyclohexylamino]-2-hydroxy-1-propanesulfonic acid), AMPSO (3-[(1,1-dimethyl-2-hydroxyethyl)-amino]-2-hydroxy-1-propanesulfonic acid) and CHES (2-[cyclohexylamino]ethanesulfonic acid), CAPS was found to be an appropriate buffer. The bands obtained with CAPS buffer for all nine PAH metabolites at pH 10.4 were very sharp. Also, the signal-to-noise ratio obtained with CAPS buffer was much higher than that obtained with borate buffer.

3.2. Effects of experimental parameters in aqueous medium

Using aqueous CAPS buffer, the effects of several

experimental parameters on the separation of the metabolites were examined. These included: buffer pH, temperature, buffer concentration and separation voltage. Four compounds, 1-OH-Py, 3-OH-B[*a*]A, 3-OH-B[*a*]P, and 9-OH-B[*a*]P were chosen as model compounds for this part of the study because they contained different structural features for the model compounds.

First, a standard sample mixture containing the four compounds was separated using 50 m*M* CAPS as a running buffer in the pH range of 10.0 to 11.6. For all four compounds, migration times increased as the pH increased, but the resolution of 3-OH-B[*a*]P and 9-OH-B[*a*]P was lost at a pH value higher than 10.4. In order to achieve a high buffer capacity, we chose a pH that was equal to the pK_a value of CAPS, namely, pH 10.4 [36].

The effect of temperature was investigated by using 20, 25 and 35°C. The migration times decreased as temperature was increased mainly because higher temperatures decrease the viscosity of the buffer. While the migration time differences between the four-ring metabolites (1-OH-Py and 3-OH-B[*a*]A) and the five-ring metabolites (3-OH-B[*a*]P and 9-OH-B[*a*]P) remained almost the same, the migration time difference between these two B[*a*]P metabolites increased with increasing temperature. It was found that the best resolution was achieved for these two compounds at 35°C (R_s=0.9). This increased resolution at high temperature could be related to several factors such as the changes in buffer viscosity, buffer pH, and the changes in the pK_a values of the analytes. Assuming that changes in buffer viscosity and buffer pH would affect the solutes to approximately the same extent, then the relative change in the pK_a values of the analytes with temperature could be responsible for the enhanced resolution.

The influence of buffer concentration was also studied. Migration times increased as buffer concentration increased because a more concentrated buffer lowers the ζ potential [37]. When the buffer concentration was increased from 10 to 50 m*M*, the migration time difference between the two B[*a*]P metabolites increased. Further increase of the buffer concentration to 100 m*M* did not have any obvious impact on the resolution of these four compounds. Finally, 100 m*M* CAPS was chosen because experimental results showed that 100 m*M* CAPS helped resolve a pair of neutral compounds (tetrol I-1 and benzo[*a*]pyrene–*trans*-7,8-dihydrodiol), while 50 m*M* CAPS did not.

Applied voltage turned out to have little effect on the resolution of these four compounds and 25 kV was chosen as the running voltage.

Based on the study of the experimental parameters, it was possible to group the compounds into compound classes (see next section). The optimum conditions with aqueous CAPS buffer for the CZE separation of the metabolites into compound classes were: 100 m*M* CAPS buffer at pH 10.4 with an applied voltage of 25 kV and a temperature of 35°C.

3.3. Compound-class separation with aqueous CAPS buffer

Fig. 1 presents the electropherogram of the nine PAH metabolites using the optimized experimental conditions. It is clear from the electropherogram that tetrol I-1 and the two diols migrate the fastest and the more acidic compounds appear later, because the more acidic compounds carry negative charges at pH 10.4. This was verified by the fluorescence emission spectra of the anions of the six more acidic PAH metabolites in CAPS buffer at pH 10.4. All six fluorescence emission spectra of the metabolites were shifted to much longer wavelengths relative to the fluorescence emission spectra of the metabolites in neutral solutions. The spectra recorded at pH 10.4 gave no indication of the neutral forms of the metabolites. Also, from the fact that tetrol I-1 and the two diols do not co-migrate with the neutral marker, it can be concluded that these compounds also carry negative charges under the present experimental conditions. The pK_a values of benzo[*a*]pyrene–*trans*-7,8-dihydrodiol and benzo[*a*]pyrene–*trans*-9,10-dihydrodiol were reported as 11.8 and 13.2, respectively [38]. Therefore, at pH 10.4, these two diol compounds and possibly tetrol I-1 may possess a small amount of negative charges. This would explain their migration behavior. In order to distinguish these three earlier-migrating compounds from the other six more acidic ones, we still call them neutral compounds. With the experimental conditions, the general migration order followed tetrol I-1 < dihydrodiol < monohydroxybenz[*a*]anthracene <

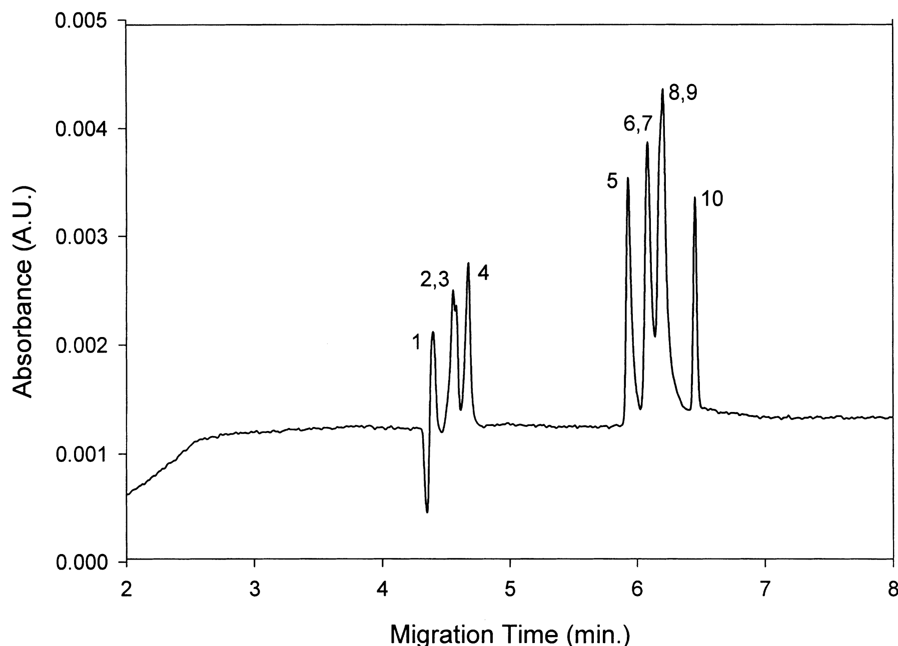

Fig. 1. Electropherogram of a mixture of nine PAH metabolites. Buffer, 100 m*M* CAPS, apparent pH 10.40; voltage, 25 kV; temperature, 35°C. Peak identification: (1) EOF marker (MeOH); (2) benzo[*a*]pyrene-*r*-7-*trans*-8,9-*cis*-10-tetrahydrotetrol (I-1); (3) benzo[*a*]pyrene-*trans*-9,10-dihydrodiol; (4) benzo[*a*]pyrene-*trans*-7,8-dihydrodiol; (5) 3-hydroxybenz[*a*]anthracene; (6) 7-hydroxybenzo[*a*]pyrene; (7) 9-hydroxybenzo[*a*]pyrene; (8) 12-hydroxybenzo[*a*]pyrene; (9) 3-hydroxybenzo[*a*]pyrene; (10) 1-hydroxypyrene.

monohydroxybenzo[*a*]pyrene$<$monohydroxypyrene.

Experiments were performed to determine if two of the parent compounds (benz[*a*]anthracene and benzo[*a*]pyrene) could be separated from the metabolites. No separation was achieved for the parent compounds with CAPS buffer. B[*a*]P was essentially not detectable in aqueous CAPS buffer. Although B[*a*]A appeared as a broad band after the neutral marker, it could not be separated from the metabolites. Therefore, we concluded that the parent compounds could not be separated from the metabolites in aqueous CAPS buffer.

Due to the extreme similarity of the structures of some of the compounds, complete separation of all the PAH metabolites under the conditions in Fig. 1 was not possible. That is why two pairs of hydroxyl benzo[*a*]pyrenes, namely, 7-OH-B[*a*]P and 9-OH-B[*a*]P, 3-OH-B[*a*]P and 12-OH-B[*a*]P and a pair of 'neutral' metabolites, namely, tetrol I-1 and benzo[*a*]pyrene–*trans*-9,10-dihydrodiol were not separated in the aqueous CAPS buffer. In order to achieve complete separation of all nine PAH metabolites, some modification of the buffer system was necessary.

3.4. CZE separation with binary water–organic solvents

In this part of the work, two different organic solvents, namely ACN and MeOH were studied as buffer additives in 100 m*M* CAPS. To our knowledge there has been no detailed study of the separation of these compounds in which only organic solvents were added to the running buffer. The effects of ACN and MeOH on the separation of PAH metabolites (μ_{ep}) are shown in Figs. 2 and 3, respectively. In general, the migration times of both the neutral marker (MeOH) and the PAH metabolites increased steadily with increasing proportion of organic modifiers. In Fig. 2, 3-OH-B[*a*]P and 12-OH-B[*a*]P began to show improved separation with 10% (v/v) ACN. Complete separation of these two compounds was achieved with 20% (v/v) ACN (R_s= 1.80) and greater percentages of ACN. However, the

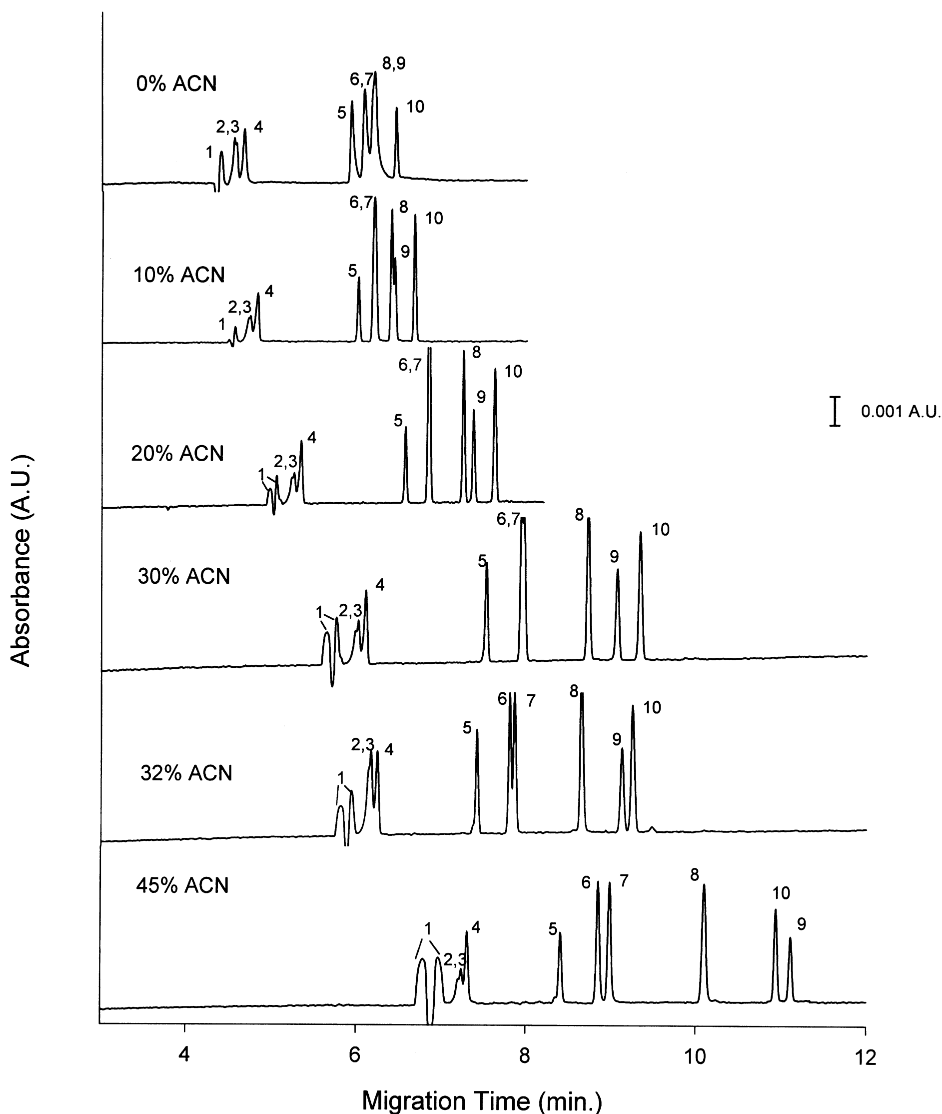

Fig. 2. Electropherograms of a mixture of nine PAH metabolites with addition of acetonitrile in 100 m*M* CAPS at an apparent pH of 10.4. Peak identification and operating conditions are the same as for Fig. 1.

resolution of 7-OH-B[*a*]P and 9-OH-B[*a*]P required at least 32% (v/v) ACN. Separation of these two compounds was obtained with 40% (R_s=1.40) (result not shown) and 45% (v/v) ACN (R_s=1.50). In the case of MeOH acting as organic modifier, solute migration times were longer than that with ACN (Fig. 3). Also, a higher percentage of MeOH in the buffer was necessary than for ACN for an effective separation of the two pairs of hydroxyl benzo[*a*]pyrenes: 30% for 3-OH-B[*a*]P and 12-OH-B[*a*]P (R_s=1.46); 45% for 7-OH-B[*a*]P and 9-OH-B[*a*]P (R_s=1.73). No major improvement of the resolution of 7-OH-B[*a*]P and 9-OH-B[*a*]P was obtained with higher MeOH content. It was also noticed that the addition of 45% ACN reversed the migration order of 3-OH-B[*a*]P and 1-OH-Py (Fig. 2), while MeOH did not give this effect over the concentration range studied.

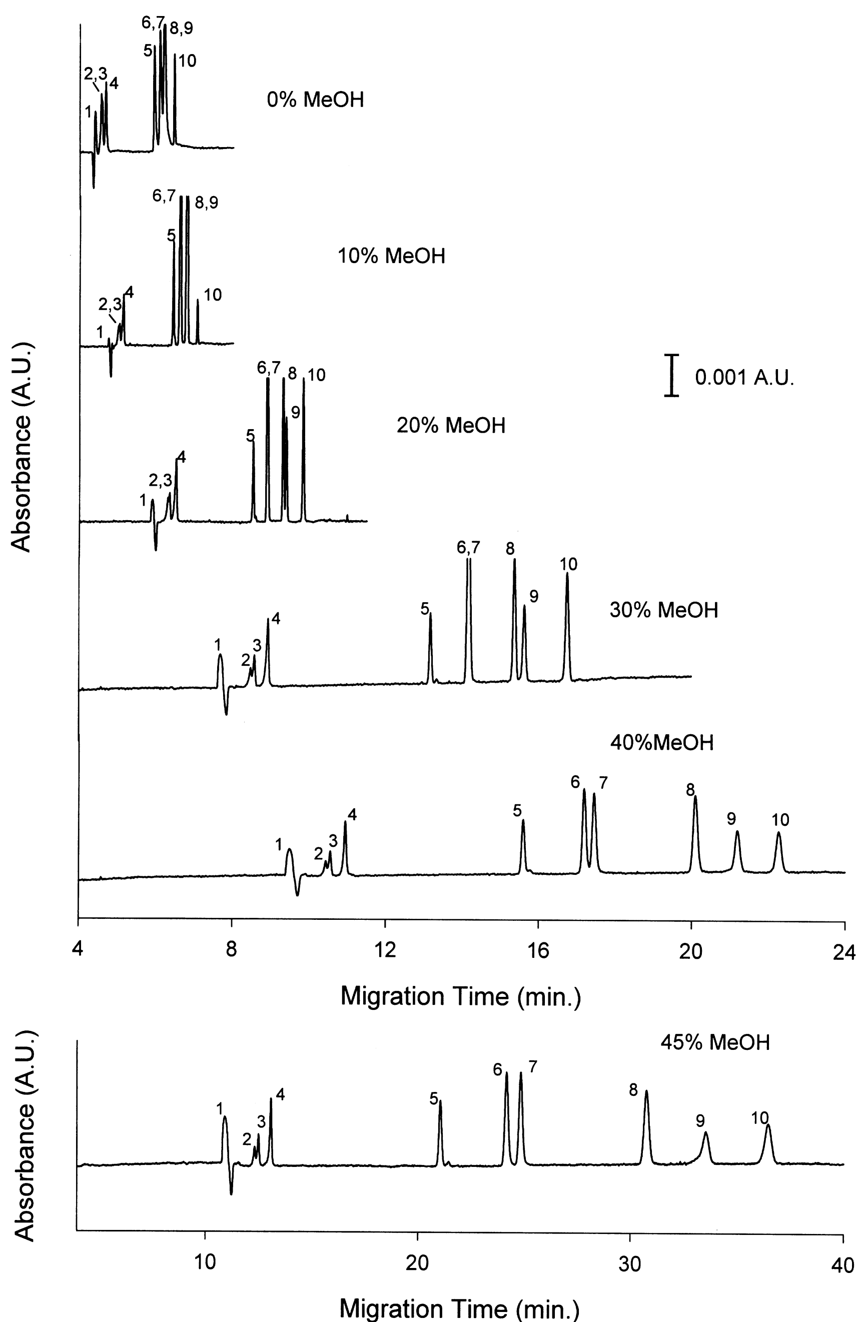

Fig. 3. Electropherograms of a mixture of nine PAH metabolites with addition of methanol in 100 m*M* CAPS at an apparent pH of 10.4. Peak identification and operating conditions are the same as for Fig. 1.

The electropherograms in Figs. 2 and 3 were very reproducible. The typical standard deviation for migration time was 0.026 (n=5). The good reproducibility of the migration time facilitates peak identification. The typical standard deviation for peak area ranged from 0.006 to 0.035 (n=5). The

reproducibility in peak area indicates that the present method would be easily applied to quantitative analysis of PAH metabolites.

The theoretical plates and resolution values were calculated for the solutes in Figs. 2 and 3 with 45% ACN and 40% MeOH, respectively. With 45% ACN, solutes 2 and 3 gave theoretical plates of 2.32×10^4. The other solutes in Fig. 2 gave theoretical plate values that ranged from 1.07×10^5 to 2.08×10^5. The resolution for solutes 2 and 3 and 3 and 4 were 0.54 and 0.78, respectively. For the other pairs of solutes in Fig. 2, the resolution values ranged from 1.50 (solutes 6 and 7) to 11.2 (solutes 4 and 5). For 40% MeOH, solutes 2 and 3 resulted in theoretical plates of 7.94×10^4 and 1.59×10^5, respectively (Fig. 3). For the other solutes in Fig. 3, the theoretical plates were from 8.62×10^4 (solute 4) to 1.45×10^5 (solute 5). The resolution for solutes 2 and 3 was 0.80, and for the other pairs of solutes in Fig. 3, the resolution ranged from 1.09 (solutes 6 and 7) to 25.3 (solutes 4 and 5). Generally, the addition of organic modifiers increased efficiency, greatly improved resolution, and helped achieve the separation of all nine PAH metabolites.

Both ACN and MeOH have their advantages and drawbacks. For overall separation, MeOH was a better choice because it gave improved resolution for the neutral compounds (tetrol I-1, benzo[*a*]pyrene–*trans*-9,10-dihydrodiol and benzo[*a*]pyrene–*trans*-7,8-dihydrodiol) in 40% and 45% MeOH. However, this was achieved at the expense of longer migration time and some band broadening for the compounds that migrated later in the electropherogram (45% MeOH). In terms of the separation of the six acidic compounds, using 45% ACN was advantageous for the short analysis time (less than 12 min) and the good resolution. For overall separation of the nine PAH metabolites and a reasonable analysis time, the optimum separation medium was: 100 m*M* CAPS with 40% (v/v) MeOH at an apparent pH of 10.4.

Additionally, an investigation of the separation of the parent PAH compounds (pyrene, benz[*a*]anthracene, and benzo[*a*]pyrene) from these metabolites was carried out in the binary water–organic solvent systems. For human samples, the parent PAHs would most likely not be present with the metabolites. However, in some cases, there is the possibility that some environmental samples could consist of both PAHs and their metabolites. Fig. 4 shows the separation of the three parent PAHs and nine PAH metabolites with 40% MeOH in 100 m*M* CAPS. All 12 compounds were separated within 35 min. Although the resolution in the early part of the electropherogram was not as good as that for the acidic metabolites in the later part of the electropherogram, Fig. 4 still demonstrates the versatility of the present buffer system. CAPS is a zwitterionic buffer [39]. At pH 10.4, which is equal to the pK_a value of CAPS, both the zwitterionic form and the anionic form of CAPS are present in buffer solution. The positive charge on the amino group of CAPS could interact with the π electrons in the PAH aromatic ring system and form a charge-transfer complex. The use of charge-transfer complexes to separated PAH using CZE have been discussed in the literature [40,41]. Due to the negative charge on the sulphonic group of CAPS, the complex would be anionic in character and migrate in an electric field. In Fig. 4, the separation of pyrene, B[*a*]A and B[*a*]P is most likely due to the differences in the strength of the complexes with CAPS. The same argument could also be applied to the three 'neutral' compounds to partially explain their migration behaviors in the separation. The organic modifier MeOH also played an important role in resolving the three parent PAHs because good separation of the PAHs with 40% (v/v) MeOH in 100 m*M* CAPS was obtained. However, no separation of the parent PAHs was achieved using 45% (v/v) ACN in 100 m*M* CAPS buffer.

3.5. Influence of organic modifier on electroosmotic mobility and electrophoretic migration

Since the primary interest in this work was on PAH metabolites, and not the parent compounds, the following discussion focuses on the PAH metabolites. Fig. 5 presents the effects of the addition of organic solvents on electroosmotic mobility (μ_{eo}). Increasing proportions of either ACN or MeOH resulted in a steady decrease in μ_{eo}. At equal organic content, the buffer with ACN always had a higher electroosmotic mobility than the buffer with MeOH. According to Janini et al. [42], there is universal agreement that μ_{eo} decreases with increasing MeOH content, but the same relationship for ACN has not

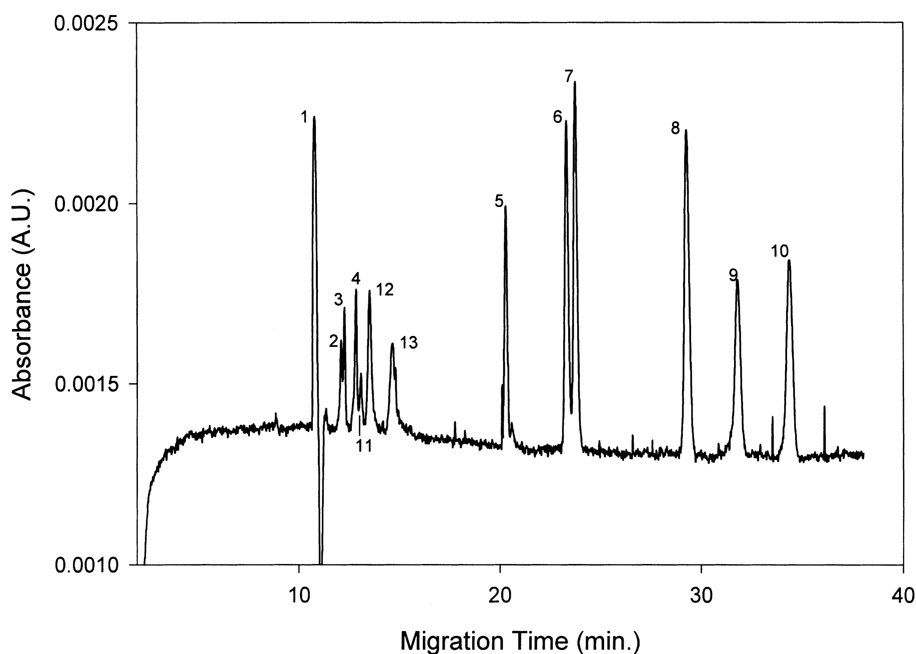

Fig. 4. Electropherogram of a mixture of the three parent compounds and the nine PAH metabolites with 40% MeOH in 100 m*M* CAPS at an apparent pH of 10.4. Peak identification for the metabolites and operating conditions are the same as for Fig. 1. Parent compounds: (11) pyrene; (12) benz[*a*]anthrancene (B[*a*]A); (13) benzo[*a*]pyrene (B[*a*]P).

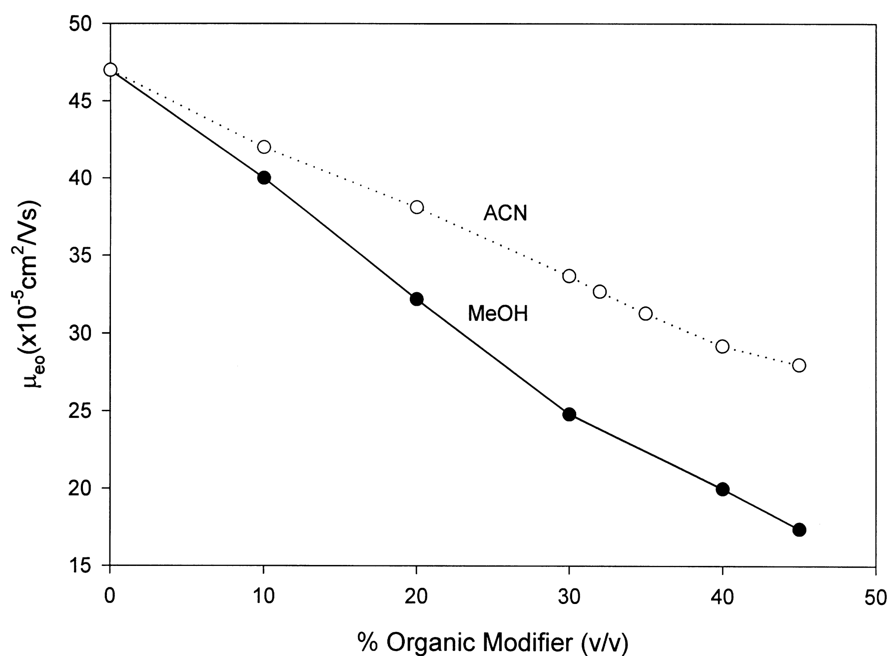

Fig. 5. Dependence of electroosmotic mobility on the percentage of organic solvents in water. Operating conditions are the same as for Fig. 1.

been unequivocally established. However, the steady reduction of EOF with ACN was discussed in various reports [21,22,29]. Our results in Fig. 5 were consistent with those reports.

The effects of ACN and MeOH on the electrophoretic mobilities (μ_{ep}) of the PAH metabolites were investigated from 0 to 45% (v/v). In both cases, the electrophoretic mobilities of the three 'neutral' metabolites (2, 3 and 4) remained more or less constant over the entire range of ACN or MeOH investigated. However, the electrophoretic mobilities of the six acidic metabolites (5–10) showed several changes upon the addition of organic modifiers. Upon the addition of 10% organic solvent, the μ_{ep} values of all six acidic compounds were increased somewhat. In the range of 10 to 30% ACN, the plot of μ_{ep} showed either a plateau (last three compounds in the electropherogram) or a steadily decreasing pattern. However, changing from 30 to 32% ACN, all six compounds showed a major reduction of their μ_{ep} values. The decrease in μ_{ep} continued throughout the rest of the ACN concentration range. The change of μ_{ep} with the addition of MeOH was different from that of ACN. The values of μ_{ep} stayed approximately constant to about 30% MeOH, but a major decrease of μ_{ep} occurred beyond 30% MeOH as they did with ACN. Beyond 40% MeOH, the μ_{ep} values increased somewhat. This is most likely related to the fact that the viscosity of MeOH–water mixtures decrease beyond 40% MeOH [43]. Because μ_{ep} is inversely proportional to viscosity, as viscosity decreases μ_{ep} would increase. However, additional experimental data would be needed to fully substantiate the previous statement.

4. Conclusions

Capillary zone electrophoresis without organic modifiers was very effective in separating the PAH metabolites into compound classes. The metabolites are important in cancer research. With MeOH and ACN as buffer modifiers, excellent separation was achieved for the metabolites, and the parent compounds were separated from the metabolites with 40% (v/v) MeOH. The binary water–organic solvent systems helped to achieve the resolution that would not be possible in an aqueous buffer by altering the electroosmotic mobility, electrophoretic mobility, and the acid–base characteristics of the analytes. Also, additional work is needed in which parameters such as viscosity and ζ potential are measured and then correlated with electroosmotic flow and electrophoretic mobilities of the analytes to expand the results reported here.

To the best of our knowledge, this is the first time that these PAH metabolites have been separated into compound classes by CZE. In addition, highly efficient separation of the metabolites was achieved with organic modifiers. The methods developed avoid the use of micelles and cyclodextrins, making the approach very simple and easy to use. Finally, the concepts and methodology developed should find a wider range of application for similar compound types.

Acknowledgements

The authors thank the United States Environmental Protection Agency for financial support of this project under Grant No. R824100.

References

[1] A. Bjorseth, G. Becher, PAH in Work Atmospheres: Occurrence and Determination, CRC Press, Boca Raton, FL, 1986, pp. 35–55.

[2] G. Grimmer, F. Pott, in: G. Grimmer (Ed.), Environmental Carcinogens: Polycyclic Aromatic Hydrocarbons, CRC Press, Boca Raton, FL, 1983, pp. 61–128.

[3] G.H. Phillips, Nature 33 (1983) 468–472.

[4] J.C. Gautier, P. Urban, P. Beaune, D. Pompon, Chem. Res. Toxicol. 9 (1996) 418–425.

[5] A.R. Steward, J. Zaleski, H.C. Sikka, Chem. Biol. Interact. 74 (1990) 119–138.

[6] R.S. Whiton, C.L. Witherspoon, T.J. Buckley, J. Chromatogr. B 665 (1995) 390–394.

[7] S.K. Yang, P.P. Roller, H.V. Gelboin, Biochemistry 16 (1977) 3680–3687.

[8] S.K. Yang, H.B. Weems, M. Mushtaq, J. Chromatogr. 316 (1984) 569–584.

[9] R. Wang, J.W. O'Laughlin, Environ. Sci. Technol. 26 (1992) 2294–2297.

[10] S.J. Kok, R. Posthumus, I. Bakker, C. Gooijer, U.A.Th. Brinkman, N.H. Velthorst, Anal. Chim. Acta 303 (1995) 3–10.

[11] M. Rozbeh, R.J. Hurtubise, J. Liq. Chromatogr. 18 (1995) 1909–1931.
[12] M. Rozbeh, R.J. Hurtubise, J. Liq. Chromatogr. 18 (1995) 17–37.
[13] J. Zaleski, A.R. Steward, H.C. Sikka, Carcinogenesis 12 (1991) 167–174.
[14] J. Lintelmann, C. Hellemann, A. Kettrup, J. Chromatogr. B 660 (1994) 67–73.
[15] Sugiyanto, G.M. Holder, A.J. Ryan, J. Chromatogr. 530 (1990) 337–346.
[16] B.W. Day, S. Naylor, L.S. Gan, Y. Sahali, T.T. Nguyen, P.L. Skipper, J.S. Wishnox, S.R. Tannenbaum, J. Chromatogr. 562 (1991) 563–571.
[17] C.J. Smith, J. Grainger, D.G. Patterson, J. Chromatogr. A 803 (1998) 241–247.
[18] U. Krismann, W. Kleibohmer, J. Chromatogr. A 774 (1997) 193–201.
[19] R.S. Brown, J.H.T. Luong, O.H.J. Szolar, A. Halasz, J. Hawari, Anal. Chem. 68 (1996) 287–292.
[20] P.D. Grossman, in: P.D. Grossman, J.C. Colburn (Eds.), Capillary Electrophoresis, Theory and Practice, Academic Press, San Diego, CA, 1992, pp. 111–132.
[21] Y. Walbroehl, J.W. Jorgenson, J. Chromatogr. 315 (1984) 135–143.
[22] K.J. Potter, R.J.B. Allington, J. Algaier, J. Chromatogr. A 652 (1993) 427–429.
[23] Y. Shi, J.S. Fritz, Anal. Chem. 67 (1995) 3023–3027.
[24] Y. Shi, J.S. Fritz, J. High Resolut. Chromatogr. 17 (1994) 713–718.
[25] S. Fujiwara, S. Honda, Anal. Chem. 59 (1987) 487–490.
[26] M.P. Harrold, M.J. Wojtusik, J. Riviello, P. Henson, J. Chromatogr. 640 (1993) 463–471.
[27] G.M. McLaughlin, J.A. Nolan, J.L. Lindahl, R.H. Palmieri, K.W. Anderson, S.C. Morris, J.A. Morrison, T.J. Bronzert, J. Liq. Chromatogr. 15 (1992) 961–1021.
[28] W.C. Lin, C.E. Lin, E.C. Lin, J. Chromatogr. A 755 (1996) 142–146.
[29] C. Schwer, E. Kenndler, Anal. Chem. 63 (1991) 1801–1807.
[30] W. Schutzner, E. Kenndler, Anal. Chem. 64 (1992) 1991–1995.
[31] S.M. Masselter, A.J. Zemann, Anal. Chem. 67 (1995) 1047–1053.
[32] R. Carabias-Martinez, E. Rodriguez-Gonzalo, J. Dominguez-Alvarez, J. Hernandez-Mendez, Anal. Chem. 69 (1997) 4437–4444.
[33] K. Sarmini, E. Kenndler, J. Chromatogr. A 792 (1997) 3–11.
[34] A.C. Capomacchia, V. Kumar, C. Brazzel, Talanta 29 (1982) 65–69.
[35] D.R. Baker, Capillary Electrophoresis, Wiley, New York, 1995, pp. 179–183.
[36] D. Heiger, R.E. Majors, R.A. Lombardi, LC·GC 15 (1997) 14–23.
[37] D.R. Baker, Capillary Electrophoresis, Wiley, New York, 1995, p. 27.
[38] A.C. Capomacchia, F.L. White, Anal. Chim. Acta 120 (1980) 313–320.
[39] R.J. Beynon, J.S. Easterby, Buffer Solutions The Basics, IRL Press, New York, 1996, p. 74.
[40] Y. Walbroehl, J.W. Jorgenson, Anal. Chem. 58 (1986) 479–481.
[41] J.L. Miller, M.G. Khaledi, D. Shea, Anal. Chem. 69 (1997) 1223–1229.
[42] G.M. Janini, K.C. Chan, J.A. Barnes, G.M. Muschik, H.J. Issaq, Chromatographia 35 (1993) 497–502.
[43] W.R. Melander, Cs. Horváth, in: Cs. Horváth (Ed.), High-Performance Liquid Chromatography: Advances and Perspectives, vol. 2, Academic Press, New York, 1980, p. 168.