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Capillary electrochromatography of methylated benzo[*a*]pyrene isomers
I. Effect of mobile phase tuning

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Abstract

It is of increasing importance that chromatographic methods be developed for the separation and identification of biological and environmentally harmful compounds such as methylated polycyclic aromatic hydrocarbons (PAH). Capillary electrochromatography (CEC) is fast becoming a useful technique for analysis of PAH, as it offers both high efficiency and superior resolution. The separation of 12 methylated benzo[*a*]pyrene (MBAP) isomers is a challenge due to the extreme hydrophobicity and structural similarity of these compounds. In this work, we present Part I of our ongoing study, a method for the systematic mobile phase tuning for CEC separation of the 12 MBAP isomers. The CEC experiments were conducted utilizing a CEC-octadecylsilica (ODS) stationary phase and fused-silica capillary [(75 μm I.D., 363 μm O.D.) 36.5 cm total length, 25.0 cm effective length] which was slurry pressure packed in our laboratory. Several mobile phase parameters were manipulated to provide optimum separation. These included acetonitrile (ACN) concentration, tris(hydroxymethyl) aminomethane (Tris) concentration, pH, and addition of a tertiary buffer constituent such as tetrahydrofuran (THF) and isopropanol (IPA) to ACN–aqueous buffer mixtures. Optimum CEC separation conditions were achieved using 75% (v/v) ACN–25% (v/v) 12.5 m*M* Tris, pH 8.0, and 30 kV at 25 °C. These mobile phase conditions were then utilized for Part II of our study, the CEC stationary phase optimization for the separation of 12 MBAP isomers.
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Keywords: Mobile phase composition; Electrochromatography; Methylbenzo[*a*]pyrene isomers; Polynuclear aromatic hydrocarbons

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are harmful to both man and the environment. Benzo[*a*]pyrene (BaP) is one of the most carcinogenic PAH, and is classified as one of the Environmental Protection Agency (EPA) 16 priority pollutants. The methyl group can be substituted to the 12 different positions of the ring structure of the BaP molecule. Therefore, 12 geometrical isomers of methyl benzo[*a*]pyrene (MBAP) are possible (Fig. 1). The MBAP isomers have been detected in automobile exhaust, cigarette smoke, and forest fire smoke [1]. In addition, methylation of BaP has been shown to occur in vivo also, thus presenting biological impor-

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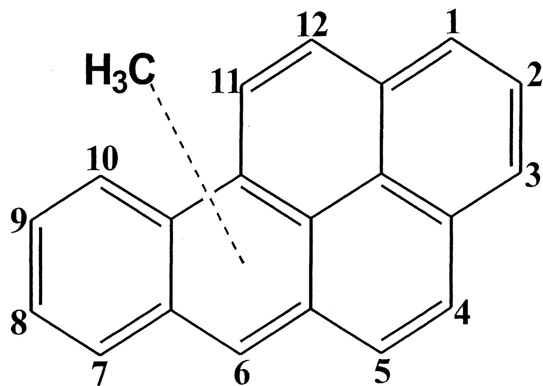

Fig. 1. The chemical structure representing the 12 methylated isomers of benzo[*a*]pyrene.

tance for identification of MBAP isomers [2,3]. The position of the methyl substituent around the outer BaP ring determines the overall carcinogenicity of the compound, hence tumor initiating capability is greater for some isomers than others [4–6]. Therefore, there is a need for the development of chromatographic methods for the resolution of MBAP isomers.

Gas chromatography (GC) [7] and high-performance liquid chromatography (HPLC) [8] have been used for the separation of MBAP isomers. There are several limitations of GC and HPLC for separation of MBAP isomers. In the case of GC, there are several problems inherent to the analysis of high-molecular mass MBAP isomers (MW=266). For example, a high-temperature (e.g., 270 °C) is required to elute MBAP isomers from the liquid crystalline stationary phase because of their low volatility. This high-temperature is very close to the bleeding temperature of the liquid crystalline phase which can result in decreased longevity and loss of liquid crystalline properties of the column. Reversed phase HPLC using polymeric C_{18} also is also reported [8], but offers lower peak capacity than GC. Although separation of MBAP isomers is possible in capillary electrophoresis (CE) using either a combination of γ-cyclodextrins (CDs) and sodium dodecyl sulfate [9] or molecular micelles [10], only a few isomers could be separated simultaneously. A very attractive alternative to micellar electrokinetic chromatography (MEKC) or cyclodextrin modified micellar electrokinetic chromatography (CD-MEKC) is capillary electrochromatography (CEC) using packed columns. Therefore, the development of CEC as an alternative technique for this difficult separation of 12 MBAP isomers has been undertaken in our laboratory.

Since CEC is considered a hybrid technique of HPLC and CE, it combines both chromatographic and electrophoretic principles. The use of a packed stationary bed in CEC allows for high selectivity like that of HPLC, while the application of a high electric field and laminar flow profile allows for high separation efficiency similar to that of CE. In addition, the improved efficiency reported with the use of submicron particles in CEC enables faster separations over HPLC [11]. For the analysis of neutral and water-insoluble compounds such as MBAP isomers, CEC is an attractive technique as high percentages of organic solvents are routinely used. Therefore, the application of CEC for MBAP separation is a logical choice.

The present study is an initial attempt to explore the CEC separation of geometrical isomers of MBAP by examining the effect of various mobile phase parameters. We present a systematic approach for the mobile phase tuning utilizing a monomeric C_{18} stationary phase. The column performance of the packed stationary phase was first evaluated using a standard test mixture, and the effect of various mobile phase parameters were manipulated to provide optimum separation of MBAP isomers. These included the effect of acetonitrile concentration, Tris buffer concentration, pH, and addition of a tertiary mobile phase constituent. Therefore, the optimization of the mobile phase comprises Part I of our ongoing study. The following paper (Part II) utilizes the optimum mobile phase conditions from Part I as a guide for the stationary phase optimization of MBAP isomers. The data collected in this study will aid in the development of CEC as a practical and useful analytical tool for analysis of MBAP and other similar methylated PAH isomers. To our knowledge, this paper is the first report utilizing CEC for separation of the geometrical isomers of MBAP.

2. Experimental

2.1. Reagents and chemicals

Twelve methylated benzo[*a*]pyrene (MBAP) isomers were kindly donated by Dr. Harold E. Seifred at the Division of Cancer Prevention, National Cancer Institute (Rockville, MD, USA). The molecular structure of the MBAP is given in Fig. 1. Acetonitrile (ACN) and isopropanol (IPA) both of HPLC grade were purchased from Burdick and Jackson (Muskegon, MI, USA). Tetrahydrofuran (THF) of HPLC-grade, tris(hydroxymethyl) aminomethane (Tris) (99.9+%), thiourea (99%), biphenyl (99.5%), *o*-terphenyl (99%), thalic acid diethyl ester (99%), and dimethyl ester (99+%) were purchased from Sigma (St. Louis, MO, USA). Hydrochloric acid was purchased from Fisher Scientific (Springfield, NJ, USA). Acetone of HPLC grade was purchased from EM Science (Gibbstown, NJ, USA).

2.2. Column fabrication

Reliasil monomeric C_{18} (100 Å, 3 μm) stationary phase was purchased from Column Engineering (Ontario, CA, USA). The stationary phase was packed into fused-silica capillary (75 μm I.D.×363 μm O.D.) which was purchased from Polymicro Technologies (Phoenix, AZ, USA). Packed columns were fabricated using the slurry packing technique as reported in the literature [12], but with some important modification as detailed below. A brief explanation of the packing procedure is as follows: first, a fused-silica capillary of 50 cm total length was fitted at the inlet and outlet end to a Valco nut (Houston, TX, USA)/PEEK (Upchurch Scientific, Oak Harbor, WA, USA) tubing. The outlet end was equipped with a 0.5-μm fine screen. Stationary phase was then slurried in acetone at a concentration of 13 mg/350 μl, sonicated for 6 min and then quickly transferred with a micropipette into a stainless steel reservoir loop (60 cm×0.50 mm I.D.×1.60 mm O.D.). Next, the inlet of the reservoir loop was quickly connected to a Knauer Wellchrom K-1900 pneumatic pump (Berlin, Germany), followed by connection of the outlet end of the reservoir loop to the inlet of the capillary. The pump was then turned on and the capillary was high pressure packed at 300 bar. Acetonitrile (ACN) was used as a packing solvent due to its low viscosity and ability to solubilize the C_{18} packing material. To ensure a homogeneous packing, the capillary was immersed in a sonication bath for 25 min and visually inspected using a microscope. The capillary was finally left to pack overnight for 8 h. For retaining frit fabrication, as recommended by Henry et al. [13], the packed capillary column was first flushed with 10 m*M* sodium chloride (NaCl) for 3 h prior to heating the frits. This procedure promoted the formation of more stable and durable sodium silicate bonds. Following this, the outlet frit was prepared approximately 13 cm from the PEEK end fitting by applying heat with a homemade burner for 15 s and maintaining 300 bar pressure. While still under pressure, the excess stationary phase behind the outlet frit was flushed out of the column. A detection window was then fabricated 0.5 cm just after the outlet frit. Next, a 25-cm packed bed length was measured from the outlet frit up the column, whereupon the inlet frit was burned. The pump was then turned off allowing the pressure to bleed slowly so as not to create any backpressure. This is followed by reversal of the capillary column and removal of the excess stationary phase particles located behind the inlet frit. Finally, ACN was allowed to flush through the column for about 30 min to clean the column prior to installing into the Agilent capillary cassette. While not in use, care was taken to avoid any drying of the packed bed by placing the ends of the packed column in triply deionized water containing microvials.

2.3. CEC instrumentation

Capillary electrochromatography experiments were conducted using an Agilent model HP 3D-CE (Agilent Technologies, Palo Alto, CA, USA) equipped with a diode array detector. Data were collected using ChemStation software. The new packed capillary column was installed in the Agilent cassette and then preconditioned with 80:20% (v/v) (ACN/5 mmol Tris, pH 8). The column was conditioned by applying successively increasing voltages in 5-kV increments for 10 min each up to 30 kV

using 12 bar on the inlet vial. Finally, the capillary was conditioned for 30 min using 12 bar pressure at 30 kV on both inlet and outlet vials. The temperature was maintained at 25 °C and all CEC separations were performed with 12 bar pressure applied to both vials to prevent bubble formation. Both the test mixture and the MBAP analyte solutions were electrokinetically injected for 5 s, 5 kV followed by a 3-s, 5-kV running buffer injection.

2.4. Buffer preparation

All mobile phases were obtained by first preparing the stock background electrolyte (BGE) which was prepared by weighing and dissolving 7.57 g Tris in triply deionized water (ca. 60 ml) in a 100-ml beaker. The desired pH of Tris in the range of 7.0–9.0 was achieved by adjusting with HCl. The pH adjusted solution was then transferred to a 100-ml volumetric flask and filled to just below the mark. This solution was then sonicated for 15 min and finally filled to the mark with triply deionized water. The BGE was filtered through a 0.45-μm syringe filter, and then thoroughly degassed for 6 min. A small aliquot of the stock (625 m*M*) Tris BGE was then added to the appropriate amount of filtered organic solvent, followed by addition of triply deionized water in appropriate ratio in order to prepare a mobile phase of constant ionic strength for all CEC experiments. The final mobile phase was lastly sonicated for 7 min and thoroughly degassed for 6 min prior to use.

2.5. Sample preparation

Stock solutions of the individual MBAP isomers were prepared by dissolving 3 mg of each isomer in 1 ml of ACN. The stock mixture of MBAP isomers was prepared by sonicating the individual isomers for 5–10 min and then taking an equal aliquot (50–100 μl) of each isomer. The mixture was then concentrated by evaporating the ACN to half the total volume. A typical injection aliquot (~40 μl) was prepared by taking ~25 μl of MBAP stock mixture and then diluting with 15 μl of triply deionized water. Since MBAPs are carcinogens, stock solutions were handled in a ventilated hood and stored in a closed container in the freezer. Disposable latex gloves were worn during MBAP standard preparation and care was taken to dispose of the MBAP waste appropriately.

3. Results and discussion

All CEC separations were performed on the same C_{18} reversed-phase column to avoid any influence of differences in column packing or frit stability. The influence of several mobile phase parameters were studied for the effect on CEC separation of MBAP isomers. These included the effect of ACN concentration, Tris concentration, pH, and addition of a third organic modifier such as THF and IPA. A monomeric, non-endcapped CEC-C_{18} packing material was chosen as the stationary phase due to its durability under different mobile phase conditions, and its ability to maintain a robust EOF. It is well known that monomeric phases usually exhibit lower selectivity as compared to polymeric phases for the separation of PAH isomers in HPLC [8]. However, the choice of the former phase was based on its compatibility with a variety of organic solvent and superior EOF promoting capability. In fact, the column selectivity was optimized in Part II under the optimum mobile phase conditions discussed in this article. The performance of the newly packed C_{18} column was first evaluated with a model test mixture containing thiourea, biphenyl, *o*-terphenyl, thalic acid dimethyl and diethyl ester. The separation was accomplished in less than 5 min with an average total column efficiency in the range of 35 100 plates (ca. 140 300 plates/m) (data not shown).

3.1. Effect of acetonitrile content

Acetonitrile (ACN) was selected as the organic constituent in the running buffer due to its superior EOF promoting ability in CEC work [14], its UV transparency, and also the suitability of ACN to promote the solubility of the hydrophobic MBAP isomers in the running buffer. Furthermore, the use of ACN in CEC has been proven to offer higher selectivity as compared to methanol for certain three ring containing analytes [15]. The effect of ACN on the separation of MBAP isomers was investigated with various fractions of ACN added to the running

buffer in the range of 70–80% (v/v), while maintaining a BGE of 5 m*M* Tris, pH 8, and operating conditions of 25 °C and 30 kV. Figure 2 shows that at 70% (v/v) ACN, the MBAP isomers are retained longer which results in a wider separation window. This volume fraction of ACN provides good separation selectivity, but is offset by peak broadening and lower detectability. Slightly higher ACN fraction of 75% (v/v) affords shorter retention of all isomers with increased efficiency, as indicated by sharper peaks. Further increasing the ACN content to 80% (v/v) provided the sharpest peaks, but is offset by a narrow elution window and loss in resolution of several MBAP isomers.

Another purpose of organic solvents such as ACN in CEC is to reduce the k' value of highly hydrophobic solutes such as MBAP isomers [16]. Dependency of the k' value of MBAP isomers on ACN (v/v) fraction is shown in Fig. 3. The k' values for all MBAP isomers decreased as the ACN fraction was raised from 70 to 75% (v/v). Further increasing the ACN fraction from 75 to 80% (v/v) resulted in the narrowest k' window. It is clear that the elution of all MBAP isomers is controlled by a reversed-phase mechanism. Since the mobile phase becomes more non-polar, the hydrophobic interactions between the RP-C_{18} column and the MBAPs are decreased in favor of growing affinity of MBAPs for the mobile phase. The EOF can be also affected by the ACN fraction, due to changes in the dielectric constant and viscosity of running buffer. However, the inset linear velocity plot of Fig. 3 shows no significant change in the EOF over the range of ACN fraction studied. Overall, a 75% (v/v) fraction of ACN provided the best trade off between resolution and analysis time.

3.2. *Effect of Tris concentration*

Next, the effect of Tris concentration on the CEC

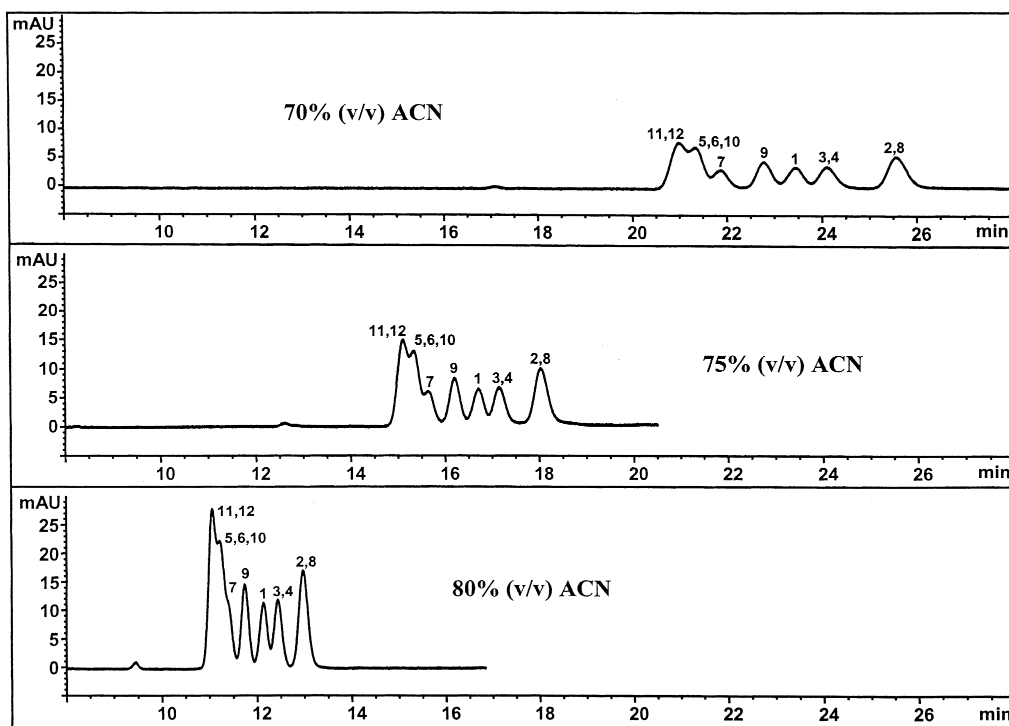

Fig. 2. Electrochromatograms showing the effect of ACN volume fraction on the separation of 12 MBAP isomers. Conditions: mobile phase, volume fraction [% (v/v)] of ACN varied/5 m*M* Tris buffer; pH 8.0; 25 °C; separation voltage, 30 kV; electrokinetic sample injection 5 s, 5 kV, followed by a 3-s, 5-kV run buffer injection; UV detection at 254 nm.

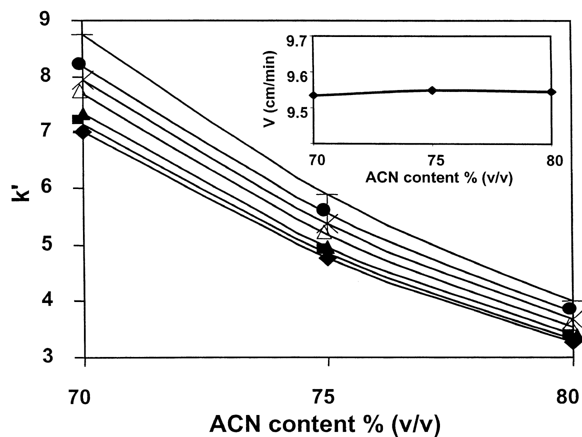

Fig. 3. The effect of ACN volume fraction on the capacity factor, k', for the separation of 12 MBAP isomers. Conditions are the same as Fig. 2. The inset plot shows the trend in linear velocity upon varying the volume fraction of ACN.

separation of MBAP isomers was studied using 2.5, 5.0, 12.5, 20.0, and 25.0 m*M* of Tris buffered at pH 8.0 and previously optimized 75% (v/v) ACN with operating conditions of 25 °C and 30 kV. As shown in Fig. 4, the retention time increases as Tris concentration increased from 2.5 to 25.0 m*M*. This increase in retention is due to the interactions of Tris with the silica surface [17] which decreased the thickness of the electrical double layer and zeta potential, thus causing a decrease in EOF with increasing Tris concentration. This is supported by the inset plot of Fig. 4, which shows a corresponding decrease in linear velocity at higher ionic strengths. The ionic strength of the buffer affects the linear velocity and also plate height [18], which in turn can influence the resolution. Going from 2.5 to 12.5 m*M* Tris shows a gradual improvement in the resolution of the earlier eluting isomers, but then resolution

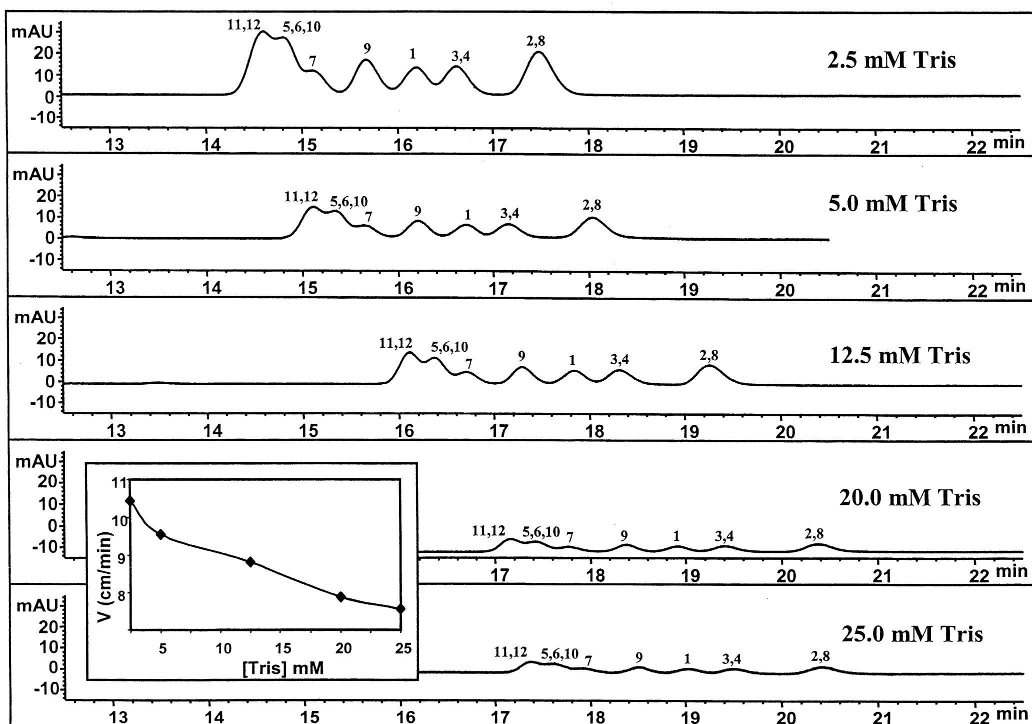

Fig. 4. Electrochromatograms showing the effect of Tris concentration on the separation of 12 MBAP isomers. The inset shows the linear velocity upon varying the Tris concentration. Conditions are the same as Fig. 2 except ACN was 75% (v/v) and [Tris] was varied.

deteriorates going from 12.5 to 25 m*M* Tris. A similar trend was observed by other investigators [15,18]. Furthermore, a decrease in peak height of MBAP isomers leads to poor detectability when increasing Tris concentration over the same range due to the increased background absorbance of the Tris buffer at high concentration. Thus, a mobile phase of 12.5 m*M* Tris was chosen for subsequent experiments as it offered the best compromise between chromatographic resolution and UV detectability of MBAP isomers.

3.3. Effect of pH

The effect of pH on the EOF and separation of MBAP isomers was investigated by varying the mobile phase pH from 7.0 to 9.0 with previously optimized 75% (v/v) ACN in 12.5 m*M* Tris buffer at operating conditions of 25 °C and 30 kV. Generally, the EOF decreases with pH for CEC packings because of decreased ionization of residual silanols at low pH [19]. The natural pH value of 12.5 m*M* Tris solution was measured to be pH 10.4, therefore the addition of concentrated HCl in order to lower the pH resulted in an increased ionic strength and protonation of Tris amine moiety ($pK_a = 8.08$). At a pH of 9.0, a lower linear velocity (see Fig. 5 inset) produced the longest retention times and peak broadening which in turn reduced both efficiency and peak detectability (Fig. 5, top panel). Decreasing the pH to 8.0 increases the EOF and linear velocity significantly (see Fig. 5, inset). This is because decreasing the pH promoted the ionization of Tris from essentially neutral to partially protonated amine form [20]. Since mobility depends on charge/size ratio, Tris should acquire partial positive charge. Thus, faster cathodic EOF and hence shorter migration times for the MBAP isomers were observed (Fig. 5, middle panel). The trend observed in this study is in accordance with others who report that

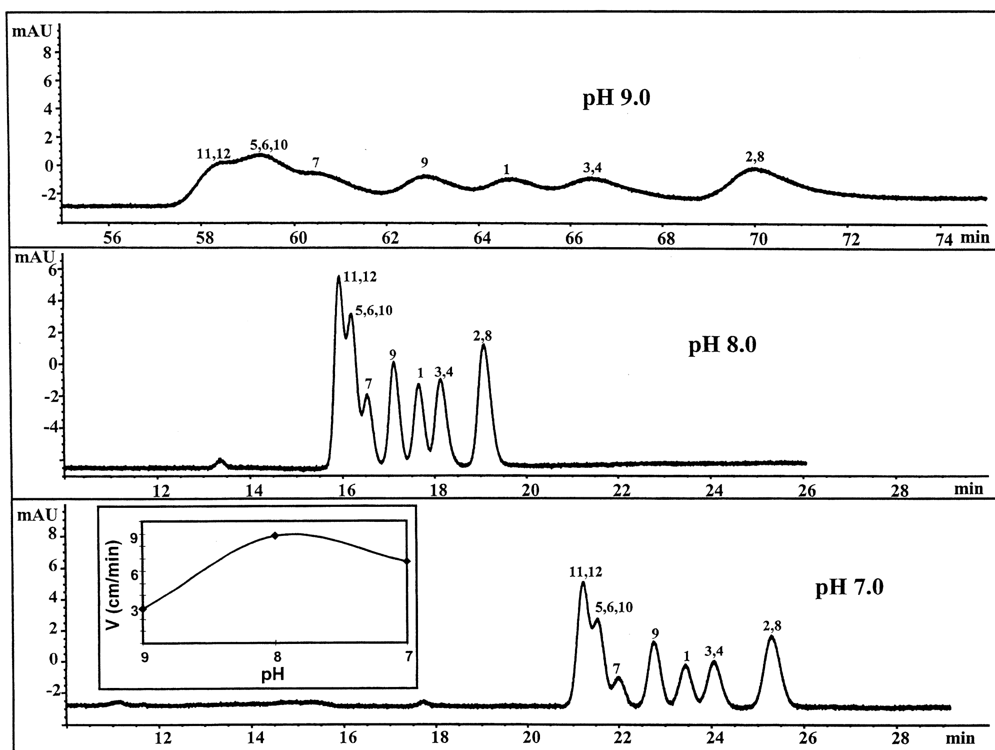

Fig. 5. Electrochromatograms showing the effect of pH on the separation of 12 MBAP isomers. The inset shows the linear velocity upon varying the pH of the background electrolyte. Conditions are the same as Fig. 4 except [Tris] was 12.5 m*M* and pH was varied.

Tris–HCl buffer has a maximum buffering capacity around pH 8.0, thus permitting a very stable electroosmotic flow [21]. As the pH is decreased further to 7.0, the EOF was slightly lowered due to increased ionic strength and protonation of exposed silanols. These effects are demonstrated in Fig. 5 (bottom panel), where at pH 7.0 the retention time of all isomers is slightly increased, resulting in some loss of chromatographic efficiency. Although the elution window is slightly narrower at pH 8.0 than at pH 7.0, a pH of 8.0 provided overall the best separation of MBAP isomers.

3.4. Effect of a ternary mobile phase

Finally, the addition of a third organic solvent to the running buffer was investigated with hope of expanding the migration time window and improving resolution and selectivity of MBAP isomers. While maintaining previously optimized conditions of 75% (v/v) ACN in 12.5 m*M* Tris, pH 8.0, and operating conditions of 25 °C and 30 kV, various fractions of tetrahydrofuran (THF) in the range of 0–10% (v/v) and isopropanol (IPA) in the range of 0–20% (v/v) were substituted for ACN in the mobile phase.

The use of THF as a mobile phase constituent in CEC has been previously reported [15,21–25]. A recent report by Banholcer and Pyell showed that resolution of neutral compounds could be improved in CEC with modification in selectivity by the addition of THF to the running buffer [26]. Furthermore, the replacement of the organic modifier ACN with THF also extensively reduces the electroosmotic mobility and the velocity of the mobile phase [27]. Fig. 6 shows the effect of THF on the EOF and separation of MBAP isomers. Substitution of 5% (v/v) ACN with THF provides a slightly faster separation and sharpens the solute peak shape, but resolution of the MBAP isomers is decreased. The EOF is lowered, as indicated by the inset linear

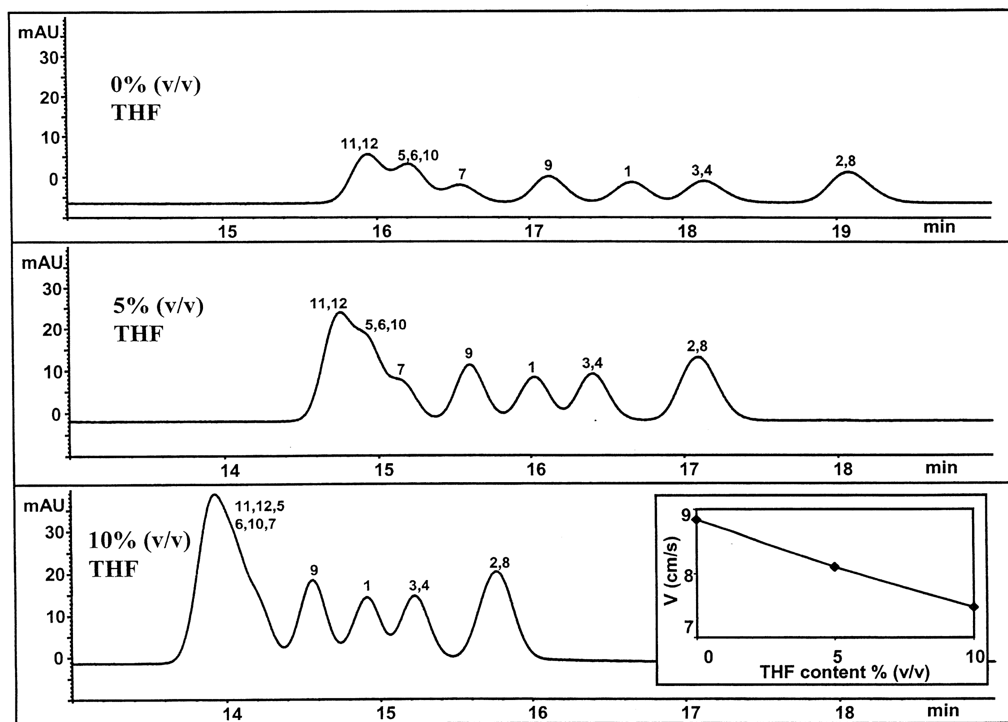

Fig. 6. Electrochromatograms showing the effect of THF on the separation of 12 MBAP isomers. The inset shows the linear velocity upon replacement of volume fraction of ACN with THF. Conditions are the same as Fig. 5 except pH 8.0 and THF was substituted into running buffer for ACN.

velocity plot of Fig. 6, due to the decreased dielectric constant–viscosity ratio (ε_D/η) which in turn reduces the zeta potential [15]. However it is noticeable that retention time of all MBAP isomers is shorter due to the lower polarity and higher elutropic strength of THF over ACN, which is in accordance to the Snyder polarity index [27]. Increased substitution of ACN by THF to 10% (v/v) [65% (v/v) ACN, 10% (v/v) THF, 25% (v/v) aqueous buffer] further reduces the separation window and sharpens the peak shape, but the resolution of the early eluting isomers is completely lost with lower overall selectivity. The retention time is also lowered and a further decrease in linear velocity and EOF is shown (Fig. 6 inset). The increased peak height from 0 to 10% (v/v) THF is most likely due to the increased solubility of MBAP isomers in less polar THF, thus providing better detectability. This trend shows the stronger eluting power of THF over ACN for highly hydrophobic compounds such as MBAP isomers in reversed-phase CEC using C_{18} stationary phase. However, the partial substitution of ACN by THF in the mobile phase offered no significant advantage as slight addition resulted in a loss in resolution of the MBAP isomers and shortening of the elution window.

The addition of isopropanol (IPA) to the running buffer was lastly investigated for its effect on EOF and separation of MBAP isomers. While maintaining previously optimized conditions, various volume fractions of IPA were substituted for ACN in the range of 0–20% (v/v) IPA. The use of IPA as a tertiary mobile phase constituent has previously been reported in a MEKC separation of various test analytes by Liu et al. [28]. Fig. 7 shows that going from 0 to 5% (v/v) IPA [70% (v/v) ACN, 25% (v/v) aqueous buffer] slightly lowers the resolution of the first few eluting MBAP isomers but increases the

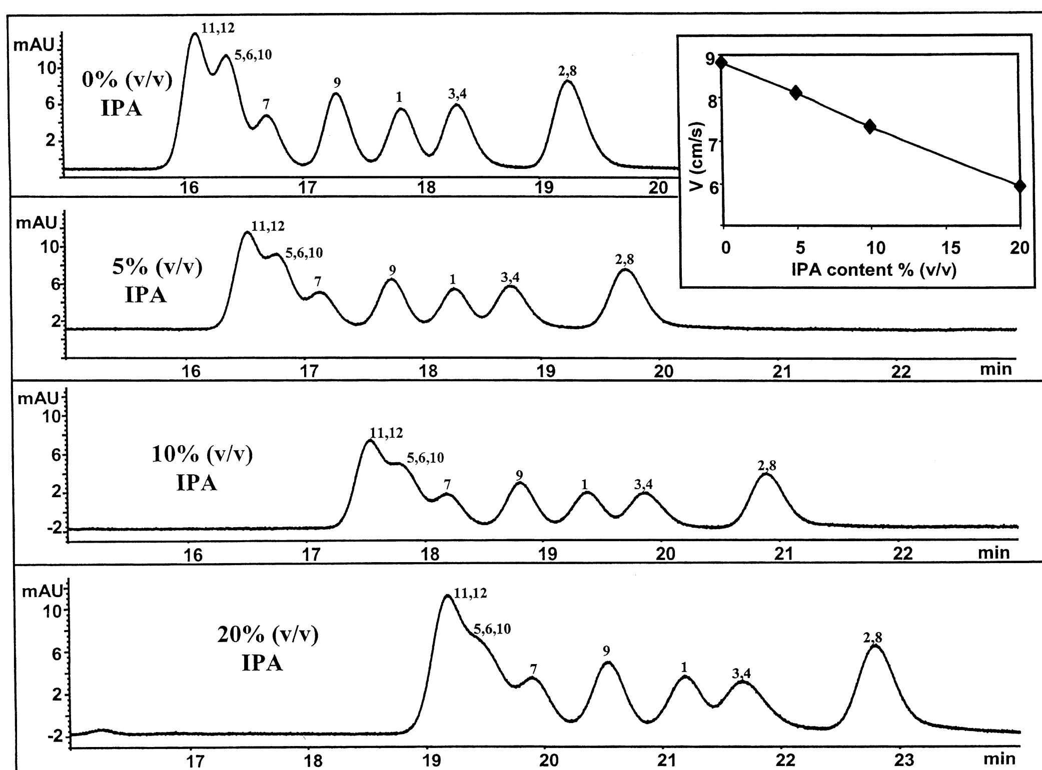

Fig. 7. Electrochromatograms showing the effect of IPA on the separation of 12 MBAP isomers. The inset shows the linear velocity upon replacement of volume fraction of ACN with IPA. Conditions are the same as Fig. 6 except IPA was substituted into running buffer for ACN.

retention time of all isomers. The increase in retention is most likely caused by a decrease in electroosmotic mobility, indicated by the inset linear velocity plot in Fig. 7. This is due to the decreased ε_D/η of IPA over ACN, and hydrogen bonding interaction between IPA and the silica capillary wall. According to the Snyder polarity index, IPA is a relatively less polar solvent compared to ACN and THF, and should provide a higher elutropic strength for non-polar compounds on C_{18} stationary phases. Therefore, one would expect to observe a trend similar to that of THF (i.e., a shorter retention of MBAP isomers). However, in contrast, an actual increase in retention time of MBAP isomers was observed with increasing IPA content. This trend is likely explained by the increased viscosity of IPA, resulting in lower electroosmotic mobility that ultimately overrides the elutropic strength effects of IPA. Going from 5 to 20% (v/v) IPA provided no significant expansion of the migration window. A slight increase in the retention and deterioration of the resolution between the first two peaks was observed.

The influence of THF and IPA on the capacity factor, k', is shown in Fig. 8. As expected, a general decrease in k' values for all MBAP isomers is demonstrated by replacement of either solvent for ACN. However, a more pronounced drop in k' values were observed when substitution of ACN by THF as compared to equal volume fraction of IPA. When ACN is replaced by 10% (v/v) THF, the change in k' window ($\Delta k'$) (defined as the difference in k' of the highest retained MBAP and the least retained MBAP isomers) decreased from 1.11 to 0.54, a significant decrease of ~51% in $\Delta k'$. In contrast, substitution of ACN by the same volume fraction of IPA decreases the $\Delta k'$ from 1.11 to 0.98, which is only a 12% decrease of $\Delta k'$. Furthermore, only a 32.5% overall decrease of $\Delta k'$ was observed when going from 0% (v/v) to 20% (v/v) of IPA.

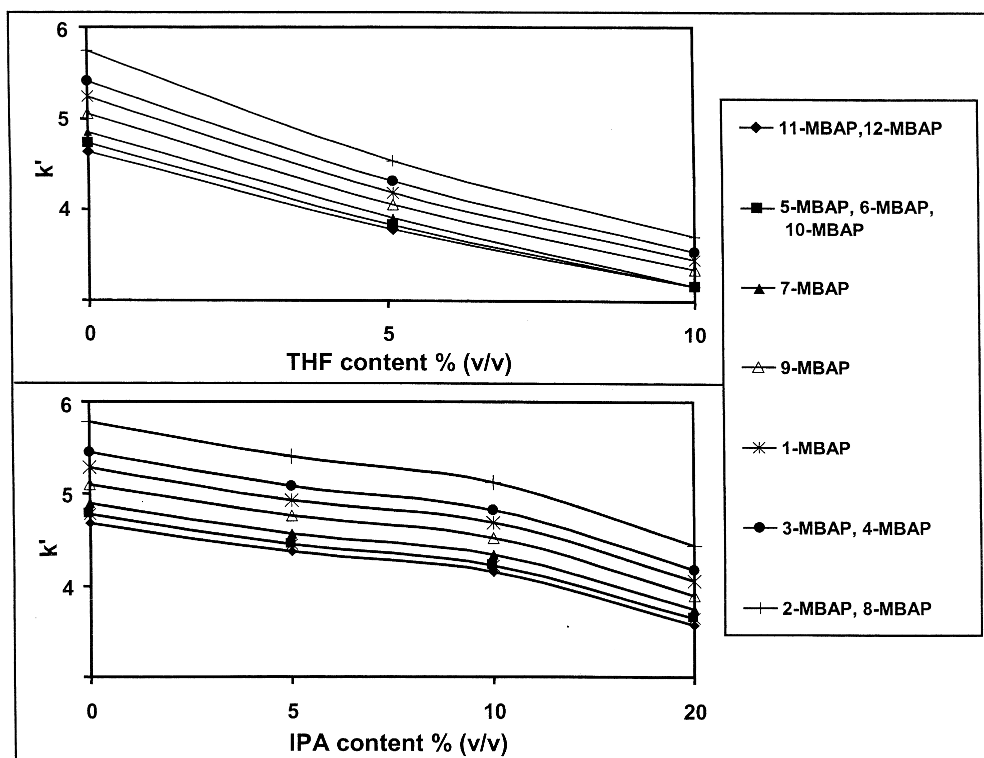

Fig. 8. Retention factors of the 12 MBAP isomers as a function of increased volume fraction of THF or IPA in ACN/H20 mobile phase. Conditions are the same as Fig. 6.

Therefore, it is concluded that IPA has a significantly less deleterious effect on selectivity of MBAP isomers as compared to THF when used as a ternary mobile phase substituent.

4. Conclusions

This work has demonstrated the capability of CEC as a useful separation technique for the analysis of very hydrophobic MBAP isomers. We have presented a method for the systematic mobile phase optimization for CEC separation of MBAP isomers using a monomeric CEC-C_{18} stationary phase. Optimum separation conditions of 75% (v/v) ACN, 12.5 m*M* Tris, and pH 8.0, provide separation of seven out of 12 MBAP isomers. The substitution of ACN by THF or IPA decreases both k' and linear velocity, however, the use of these tertiary buffer constituents had no impact in improving the selectivity of MBAP isomers. This lack of change of selectivity and incomplete resolution of all 12 isomers of MBAP points out that for such rigid solute, the evaluation of stationary phase parameters is warranted. The following paper (Part II) expands this optimization by variation in stationary phase properties such as surface coverage, pore size, particle size and type of alkyl substituent.

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References

[1] R. Santella, T. Kinoshita, A. Jeffrey, Mutat. Res. 104 (1982) 209.
[2] J.W. Flesher, S. Myers, K. Stansbury, Carcinogenesis 11 (3) (1990) 493.
[3] J.W. Flesher, S. Myers, G. Lovelace, in: M. Cooke, A.J. Dennis (Eds.), Polynuclear Aromatic Hydrocarbons: Mechanics, Methods, and Metabolism, Battelle Press, Columbus, OH, 1995, p. 423.
[4] B.D. Silverman, Cancer Biochem. Biophys. 5 (1981) 207.
[5] D. Utesch, H. Glatt, F. Oesh, Cancer Res. 47 (1987) 1512.
[6] R.P. Lyer, J.W. Lyga, J.A. Secrist III, G.H. Daub, T.J. Slaga, Cancer Res. 40 (1980) 1073.
[7] L.C. Sander, M. Schneider, S.A. Wise, J. Microcol. Sep. 6 (1994) 115.
[8] L.C. Sander, S.A. Wise, in: K. Jinno (Ed.), Chromatographic Separations Based on Molecular Recognition, Wiley, New York, 1997, p. 30.
[9] L. Copper, J. Sepaniak, Anal. Chem. 66 (1994) 147.
[10] D. Norton, S.A. Shamsi, Anal. Chim. Acta (in press).
[11] K.D. Altria, N.W. Smith, C.H. Turnbull, J. Chromatogr. B 717 (1998) 341.
[12] H. Wikström, L.A. Svensson, A. Torstensson, P.K. Owens, J. Chromaotgr. A 869 (2000) 395.
[13] C.W. Henry III, C. Fortier, I.M. Warner, Anal. Chem. 73 (24) (2001) 6077.
[14] M.G. Cikalo, K.D. Bartle, P. Myers, J. Chromatogr. A 836 (1999) 35.
[15] X. Cahours, Ph. Morin, M. Dreux, J. Chromatogr. A 845 (1999) 203.
[16] S.A. Shamsi, C. Akbay, I.M. Warner, Anal. Chem. 70 (1998) 3078.
[17] S. Thiam, S.A. Shamsi, C.W. Henry III, J. Robinson, I.M. Warner, Anal. Chem. 72 (2000) 2541.
[18] B. Chankvetadze, I. Kartozia, Y. Okamoto, G. Blaschke, J. Sep. Sci. 24 (2001) 635.
[19] K.D. Bartle, P. Myers, J. Chromatogr. A 916 (2001) 3.
[20] R. Weinberger, in: Practical Capillary Electrophoresis, Academic Press, San Diego, CA, 2000, p. 74.
[21] A. Banholzer, U. Pyell, J. Chromatogr. A 869 (2000) 363.
[22] M.M. Dittmann, G.P. Rozing, J. Chromatogr. A 744 (1996) 63.
[23] M.M. Dittmann, G.P. Rozing, J. Microcol. Sep. 9 (1997) 399.
[24] C.W. Henry III, M.E. McCarroll, I.M. Warner, J. Chromatogr. A 905 (2001) 319.
[25] R.M. Seifar, J.C. Kraak, H. Poppe, W.Th. Kok, J. Chromatogr. A 832 (1999) 133.
[26] A. Banholczer, U. Pyell, J. Sep. Sci. 24 (2001) 736.
[27] L.R. Snyder, J. Chromatogr. A 92 (1974) 223.
[28] Z. Liu, H. Zou, M. Ye, J. Ni, Y. Zhang, Electrophoresis 20 (1999) 2898.