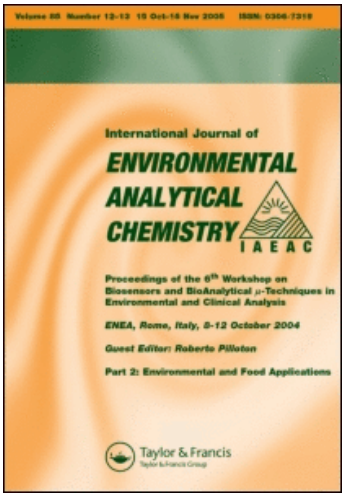


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# Analysis of Polycyclic Aromatic Hydrocarbons by Reversed Phase Capillary Electrochromatography

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# ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBONS BY REVERSED PHASE CAPILLARY ELECTROCHROMATOGRAPHY

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Reversed phase capillary electrochromatography (RP-CEC) was used to achieve the rapid analysis of six polycyclic aromatic hydrocarbons (PAHs). Capillary column packed with 5 μm ODS stationary phase in the 10 cm packed length (31 cm total length) and UV detector was used. The effect of the separation voltage, organic modifier on the electroosmotic flow (EOF) and column performance was investigated. Baseline separation could be reached in the 90% acetonitrile content and 10 kV-separation voltage, and theoretical plate numbers per meter for six PAHs were all above 70,000. The total analytical time was less than 4 min with RSD $\leq$ 0.60% (in retention factor) and 9.83% (in peak area) under the optimized experimental conditions. When the solute strength of the sample solution was less than the elution strength of the mobile phase, on-column concentration of the analytes could be occurred.

*Keywords:* Capillary electrochromatography; Polycyclic aromatic hydrocarbons; On-column concentration

## INTRODUCTION

Capillary electrochromatography (CEC) is a relatively recent electrokinetic separation technique, which combines the advantages of capillary zone

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electrophoresis (CZE) and micro-high performance liquid chromatography (μ-HPLC). The first application of an electrical field to column chromatography was reported in 1974 by Pretorious *et al.* [1] but only in the past few years, when Knox and Grant [2] developed the theory of CEC in 1990, there had been a significantly increased interest in CEC and related studies. The separation mechanism in CEC is based on both the partitioning of the solutes between mobile phase and stationary phase and on the electrophoretic mobility of charged solutes. Neutral compounds are separated in CEC due to the differences in partitioning between mobile and stationary phases, while ionic compounds are separated due to both partitioning and electrophoresis mechanism. Compared to the hydraulic pressure motility in HPLC, an electric field is used to propel the mobile phase through the packed bed in CEC, so it is possible to use smaller diameter packings and thereby achieve very high efficiency [3]. Furthermore, the flow profile in a pressure driven system is parabolic, whereas in an electrically driven system it is flatter profile, which gives rise to more efficiency.

The range of possible application of CEC is likely to be similar to that of both CE and HPLC, including impurity analysis, chiral separations, protein, steroid and amino acids analysis [4–8]. But to date, most of the reported applications of CEC have been focused on the separation of neutral compounds of widely different structure such as aromatic compounds and neutral drugs [9–11]. Because of the complexity of the real samples and absence of more sensitive detection mode, there are seldom reports on the analysis of real environmental samples using CEC method.

The purpose of this study is to get rapid separation of mixed PAHs standards using reversed phase CEC. Various parameters that affect the separation and the column performance in the RP-CEC had been investigated. On-column concentration method was used to improve the sensitivity of the method.

## EXPERIMENTAL

### Material and Reagents

All reagents were analytical reagent grade, except acetonitrile that used as organic modifier was of HPLC grade. Buffer solutions of trihydroxymethylaminomethane (Tris) were prepared by dissolving Tris in ultra-pure water, then adjusting to pH 8.5 by 1 M HCl. Ultra-pure water for preparing the buffer was purified with a Milli-Q water system (Millipore Corp.,

MA, USA). Mobile phases were prepared by mixing appropriate volume of Tris–HCl buffer and ultra-pure water. Before running, the mobile phase was degassed in an ultrasonic bath for 10 min.

The individual PAH standards, including naphthalene, phenanthrene, fluoranthene, pyrene, chrysene and benzo[k]fluoranthene were obtained from ChemService Inc. (West Chester, PA, USA). They were dissolved in methanol and mixed together to obtain the stock standard solution. The solutes were purged by high pure nitrogen to nearly dry then dissolved in the mobile phase to get proper concentration before use. The final standard concentration for each compound was 5 μg/ml.

### Instrumentation

All the CEC experiments were performed on a P/ACE system MDQ (Beckman, Fullerton, CA, USA) capillary electrophoresis instrument; data collection and instrument controlling were conducted with the software provided by Beckman installed in a personal computer. Electro-kinetic injection voltage is 10 kV; the pH of the mobile phase is adjusted at 8.5. Wavelength of ultraviolet detector is maintained at 214 nm. The separation temperature of the cartridge was maintained at 25°C during the analysis.



### Column Preparation

Fused silica capillary (75 μm I.D. 365 μm O.D.) used in this report was obtained from Yongnian optic fiber plant (Hebei, China). Five micrometre Spherisorb-ODS I and 3 μm Hypersil-ODS were purchased from Waters Phase Separation (Milford, MA, USA). The CEC columns were packed by a Spectra-Physics pump (Spectra-Physics Inc., San Jose, CA, USA), using slurry packing method as described in the previous literature [12]. The total column length was 31 cm and packed length was 10 cm. Before an experiment, the column was flushed with the mobile phase for 20 min to extrude air bubbles that might exist in the column, then conditioned on the instrument for another 20 min until the current became stable.

## RESULTS AND DISCUSSION

### Separation Efficiency and Repeatability of the CEC Columns

In this paper, the efficiency of CEC column is evaluated with thiousea and PAHs. A typical chromatogram is shown in Fig. 1. The highest

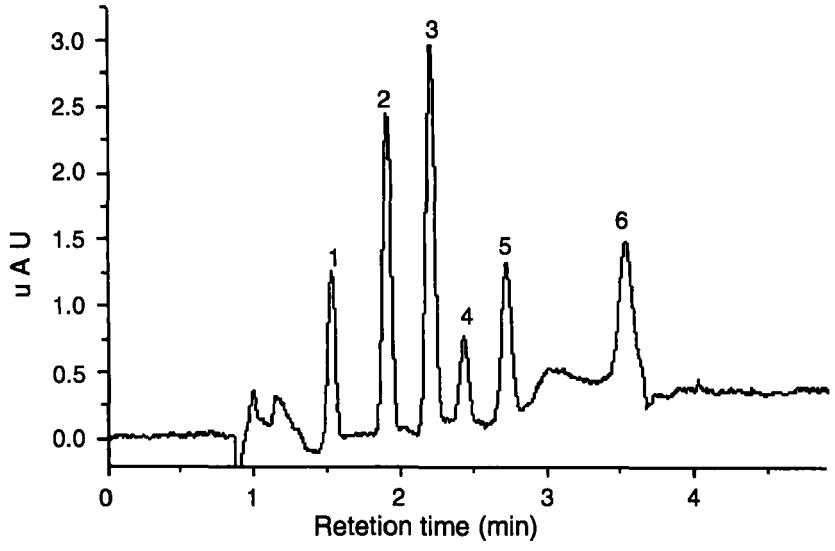


FIGURE 1 CEC separation of PAHs mixture standard. Conditions: mobile phase, acetonitrile–3 mM Tris (90:10, v/v), pH 8.5; electrokinetic injection, 5 s ×5 kV; applied voltage, 10 kV; UV detection wavelength, 214 nm; capillary column, 75 μm I.D. × 365 μm O.D.; packing material, 5 μm Sphrisorb-ODS I, 10 cm packed (total length 31 cm). Peaks: (1) naphthalene, (2) phenanthrene, (3) fluoranthene, (4) pyrene, (5) chrysene, (6) benzo[k]fluoranthene



theoretical plate number obtained is about 80,000, which may be not very good for CEC column that packed with 5 μm ODS stationary. But we can see from the chromatogram that the six PAHs can be baseline separated in less than 4 min and this time range is enough for the environmental analysis. When the ODS stationary phase changed to 3 μm and the packed column length changed to 21 cm (total 31 cm), the column efficiency can be improved to 310,000 theoretical plates/m. Yan *et al.* [10] used 3 μm porous ODS packing materials to analysis PAHs, where the samples were detected using a laser induced fluorescence detector and the column efficiencies approaching 180,000 plates/m, and separation efficiency up to 700,000 with 1.5 μm nonporous ODS packing also has been reported [13]. But smaller stationary phase diameter and longer packed column usually cause difficulty in the column packing procedure, which may deteriorate reproducibility among different columns.

The long lifetime and stable performance of a packed column is essential for the use of CEC in environmental analysis. Stability of a column can be evaluated by three parameters: chromatographic retention factor ($k'$),

theoretical plate number per meter ($N$) and peak area of the solutes. $k'$ of neutral compounds in RP-CEC can be calculated as: $k' = (t_r - t_0)/t_0$; where $t_0$ is void time, $t_r$ is the apparent migration time of the solutes. $N$ is calculated using equation: $N = 5.54(t_r/\Delta t_{1/2})^2$, where $\Delta t_{1/2}$ is peak width in the half height of the peak. All of these experiments are performed with the same column in six days (5 times per day). From the data (Table I), we can see that the relative standard deviation (RSD) of $k'$ ranges from 0.12% (for chrysene) to 0.60% (for naphthalene). The theoretical plate numbers ranges from 30,000 (for thiousea) to 79,000 (for pyrene) and theoretical plate numbers of PAHs are all above 70,000. These results indicate the repeatability of retention time of different PAHs compounds is suitable for qualification, and packed column has relative long lifetime without efficiency loss.



## Effect of the Applied Voltage on the EOF Velocity

Electroosmotic flow (EOF), the driving force in CEC is highly dependent on the buffer concentration, pH, the organic modifier and the type of stationary phase. For conventional silica based stationary phases, EOF drops off almost linearly between pH 10 and pH 2 with a factor 3 [14], thus most CEC for neutral compounds are performed at above pH 8.0. So, pH 8.5 was chosen and maintained constant during this study.

Because the tested compounds in this study are all neutral, only partition mobility occurs in the RP-CEC and their migration wholly depend on the EOF. The influence of applied voltage on EOF is investigated by varying voltage from 3 kV to 15 kV while keeping other conditions constant. The variation of linear velocity of mobile phase, estimated from the retention time of unretained marker – thiousea, with the applied electric field strength

TABLE I Average and RSD ($n = 30$) of chromatographic retention factor ($k'$), theoretical plate numbers and peak area

| *Solute* | *k'* | | *Theoretical plate* | | *Area* | |
|---|---|---|---|---|---|---|
| | *AVG* | *RSD* (%) | *AVG* | *RSD* (%) | *AVG* | *RSD* (%) |
| Thiourea | – | – | 29923 | 4.13 | 2627 | 5.95 |
| Naphthalene | 0.578 | 0.60 | 70012 | 3.84 | 972 | 9.83 |
| Phenanthrene | 0.978 | 0.36 | 77790 | 3.28 | 3602 | 6.97 |
| Fluoranthene | 1.294 | 0.27 | 77835 | 2.31 | 5202 | 5.95 |
| Pyrene | 1.533 | 0.21 | 79208 | 4.70 | 1154 | 7.16 |
| Chrysene | 1.840 | 0.12 | 77333 | 2.72 | 2810 | 7.37 |
| Benzo[k]fluoranthene | 2.708 | 0.19 | 73008 | 6.13 | 2965 | 8.26 |

is investigated in this study. When field strength (V/cm) increases, the EOF velocity also increase linearly, from 0.5 to 2.5 mm/s, and the following linear regression equation is obtained:

$$y = 0.0054x + 0.0311 \qquad r^2 = 0.9972.$$

This result means that there is no significant Joule heating effect under the maximum applied voltage (15 kV). Previous reports [14,15] have reported that the best efficiency of different columns and packings was obtained for the flow velocity of 1.0 to 2.0 mm/s. So, separation voltage of 10 kV (EOF velocity $\sim 1.8$ mm/s) is chosen in our experiment. When the applied voltage increases up to 20 kV or above, bubbles begin to form more easily in the packed column, which may result in decreasing current, increasing retention time and reduction of efficiency.



**Effect of the Organic Modifiers Content on EOF and Separation**

The analysis time can be dramatically improved by increasing both the organic solvent concentration and the pH whilst trying to maintain a reasonable electrolyte concentration in order to improve column efficiency. It has been shown [9,16,17] that with acetonitrile as the organic modifier, the EOF increases almost linearly with increasing acetonitrile concentration.

We change the acetonitrile concentration in the mobile phase while the ionic strength is kept constant (Tris was maintained at 3 mm and pH at 8.5). The current becomes unstable when the acetonitrile content is decreased to 60%, so only 70% to 90% acetonitrile is investigated. In 70% acetonitrile content, the electroosmotic flow (EOF) is 1.37 mm/s, when acetonitrile increases to 85%, the EOF also increases, but from 80% to 85% acetonitrile, the EOF has a slight decrease (from 1.50 to 1.47 mm/s), and rises again. The EOF velocity increases about 14% when acetonitrile content increases from 70% to 90%. This result is less than the other reports [8].

Retention mechanisms of neutral compounds like PAHs in the RP-CEC are similar to that in RP-HPLC, so the retention equation in RP-HPLC can be used as a reference to CEC. In RP-HPLC, the relationship between retention factor ($k'$) and organic solvent concentration ($\varphi$) can be described as the following equation:

$$\log k' = \log k'_w - S\varphi,$$

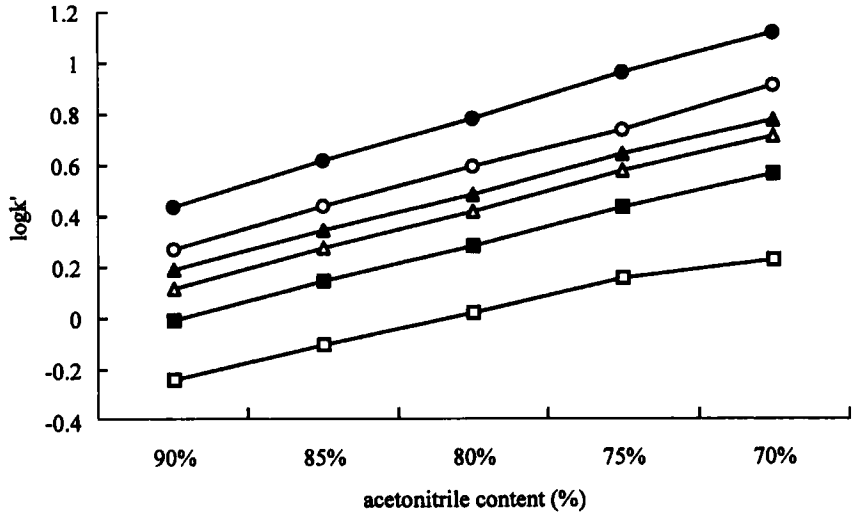


FIGURE 2 Influence of acetonitrile content on retention factor ($k'$). Conditions: electrokinetic injection, 5 s × 5 kV; applied voltage, 10 kV; detection wavelength, 214 nm; capillary column, 75 μm I.D. × 365 μm O.D.; packing material, 5 μm Sphrisorb-ODS I, 10 cm packed (total length 31 cm); organic modifier content changes from 70% to 90%, pH 8.5. naphthalene (●), phenanthrene (○), fluoranthene (▲), pyrene (△), chrysene (■) and benzo[k]fluoranthene (□).



where $k'_w$ is theoretical capacity factor of the analyte in the pure aqueous mobile phase; $\varphi$ represents the volume percentage of the organic in binary-phase mobile phase system; $S$ is the negative value of the slope.

Figure 2 shows the dependency of $\log k'$ on the percentage of acetonitrile in buffer at a constant ionic strength of 3 mM Tris at pH 8.5. When the acetonitrile content increases from 70% to 90%, the total analysis time lasts longer. Linear relationship between $\log k'$ and $\varphi$ is obvious with $r^2 > 0.9970$.

## On-Column Concentrations and Real Sample Analysis

Pyell *et al.* [18] reported "zone sharping" effect in RP-CEC in 1997. According to their opinion, if the elution strength of the sample solvent is comparable to the elution strength of the mobile phase, or even lower, zone sharping effects can be used for improvement of the detection sensitivity. Ding and Vouros [19] used such effect to get 10-fold preconcentration factor but did not go on to study in detail. Stead *et al.* [14] used non-eluting solution to separate steroids that provide 17-fold increase in sensitivity.

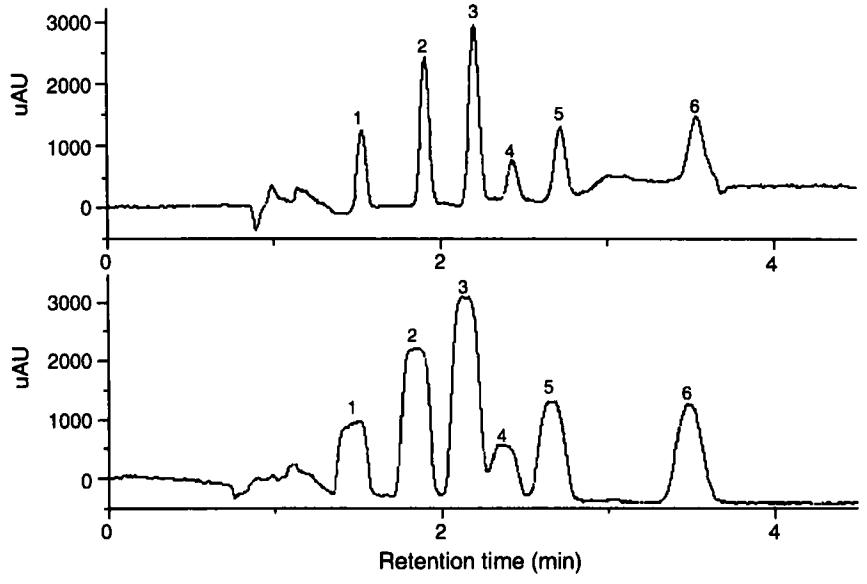


FIGURE 3 Influence of injection time on separation and column efficiency. Conditions: mobile phase, acetonitrile–3 mM Tris (90 : 10, v/v), pH 8.5; applied voltage, 10 kV; UV detection wavelength, 214 nm; electrokinetic injection, 5 kV, PAHs standards are dissolved in 90% acetonitrile solution; peaks are the same as those in Fig. 1. Injection time: (a) 5 s (b) 30 s.

We carried out a series PAHs standard prepared with different solvent content. Figure 3 shows the effect of variation of the injection time (with a constant injection voltage 10 kV) on the separation of PAHs. When PAHs standard is dissolved with the mobile phase (90% acetonitrile solution–3 mM Tris). Figure 3(a) is the chromatogram with normal injection time (5 s) and Fig. 3(b) is the one with 30 s injection time. When the injection time prolonged to 30 s, the peak height almost not increased but the width of peaks became wider and displayed obvious sample overloading characters, which means the loss of column efficiency and resolution.

When the PAHs standard was dissolved in 60% acetonitrile–3 mM Tris buffer and the mobile phase kept in 90% acetonitrile–3 mM Tris, the injection time could increase to a relative longer time. Figure 4 is the chromatogram of PAHs separated by CEC through different injection time. Each chromatogram is recorded during the injection time; injection time should be subtracted from the apparent retention time. With the injection time increasing from 5 s to 240 s, the height for most analytes appears linear increase as shown in Fig. 4(d), meanwhile no obvious loss of column performance can be observed. Through this on-column concentration

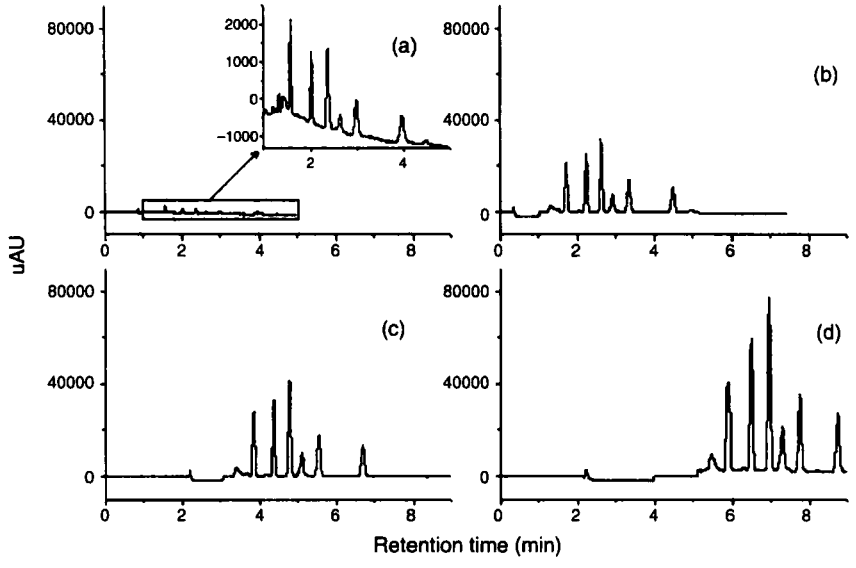


FIGURE 4 On-column concentration of PAHs. Conditions: mobile phase, acetonitrile–3 mM Tris (90 : 10, v/v), pH 8.5; applied voltage, 10 kV, UV detection wavelength, 214 nm; electrokinetic injection, 5 kV; PAHs standards are dissolved in 60% acetonitrile solution; peaks are the same as those in Fig. 1. Injection time: (a) 5 s (b) 60 s (c) 120 s (d) 240 s.

effect, 10 to 50-fold concentration can be obtained for different compounds being analyzed.

## CONCLUSIONS

RP-CEC was useful tool for the separation and quick analysis of PAHs. Baseline resolution of six PAHs was obtained with 5 μm ODS packings and relative short column (10 cm) in less than 4 min. Ninty percent acetonitrile content and 10 kV-separation voltage were used in optimized condition. The separation efficiency and repeatability of the method were also evaluated in the same packed capillary column. On-column concentration effect can improve the sensitivity in order of 10 to 50 times.

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