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Effect of cetyltrimethylammonium bromide on the migration of polyaromatic hydrocarbons in capillary electrokinetic chromatography

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Abstract

The separation of different ring numbered polyaromatic hydrocarbons (PAHs) was accomplished by using cetyltrimethylammonium bromide (CTAB) in capillary electrokinetic chromatography. In order to increase the solubilities and selectivities of PAHs, acetonitrile (ACN) was used as an organic modifier. Under the optimised conditions, 11 aromatic compounds were separated within 14.5 min in a running electrolyte containing 10 mM phosphate, 30 mM CTAB, and 40% ACN at pH 6.0. The effects of CTAB and ACN concentrations, voltage and pH on the resolution were investigated. Reproducibilities of migration times range between 0.55 and 1.27 R.S.D.% and peak areas between 1.02 and 7.23 R.S.D.%. Limit of detections (LODs) range between 0.09 and 2.24 μg ml^{-1}. This new and fast separation method of PAHs was applied to cooked oil sample.

Keywords: Capillary electrophoresis; PAHs; Cationic surfactant; Cetyltrimethylammonium bromide; Cooked oil

1. Introduction

Polyaromatic hydrocarbons (PAHs) are characterized by their fused-ring structures consisting of between two and seven aromatic rings. The conjugated π electron systems of these aromatic compounds determine their physical and chemical properties. Recently, much attention has been focused on their possible carcinogenic effects on human health. PAHs are generally formed by incomplete combustion and high temperature pyrolysis reactions. Major sources of PAHs can be associated with transportation vehicles, coke ovens, metal smelters, industrial wastes, petroleum cracking, and domestic heating. Due to the widespread distribution of PAHs in the environment and their lipophilic nature, most types of food contain measurable levels of PAHs, generally in the parts per billion (ppb) and parts per million (ppm) range. Certain technological processes and cooking procedures can also cause important levels of PAHs in some foods. Especially, determination of PAHs from the oil samples has been attractive since their legal limits are not completely defined for the vegetable oils and fats. It is indicated that burning and cooking processes can increase the PAH content by destroying about ≥70% of which is originally present in the oils [1].

Due to their unique toxic effects, it is important to develop new analysis methods for the simultaneous identification and determination of PAH mixtures. More recently, CE has become more popular and a complementary technique to the other chromatographic methods. The main advantage is the considerable diminution in the sample preparation and analysis time, as well as in the reagent consumption. Moreover, fused silica capillaries are much less expensive than chromatographic columns, easily washed between runs, and free of irreversible contamination of the matrix, unlike the packed columns. Micellar electrokinetic chromatography (MEKC) is a widely used CE technique based on differential partitioning between aqueous and micellar pseudo-stationary phase [2]. Retention of solutes in MEKC depends mainly on the hydrophobicity of the solute. In order to provide a participation of highly hydrophobic PAHs from the hydrophobic core of micelles to solvent phase, high concentration of organic solvents are needed. However, in organic-rich media, surfactants lack the capability of aggregating to form micelles [3]. Recently, neutral analytes were separated interacting with ionic surfactant monomers added to the buffer in the presence of high concentration of organic solvents. This spe-

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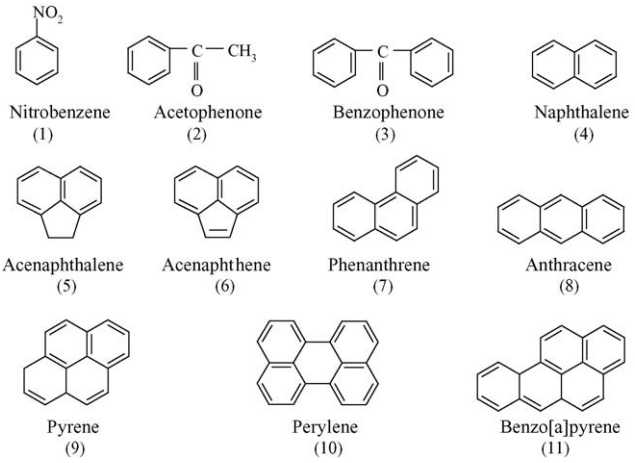

Fig. 1. Molecular structure of investigated PAHs.

cial CE technique is generally called capillary electrokinetic chromatography (CEKC). In the literature tetraalkylammonium [4–7], dioctylsulfosuccinate (DOSS) [8], sulfonated Brij-30 [9], dodecylbenzene sulfonate (SDBS) [10] and cetylpyridinium ions [11] have been reported for the electrokinetic separations of PAHs.

In this work, the separation and determination of three aromatic and eight polyaromatic compounds (Fig. 1) were achieved using the selective interaction of cetyltrimethylammonium bromide (CTAB) monomers in 40% ACN containing aqueous solution by CE. The effects of CTAB and acetonitrile (ACN) concentrations, voltage and pH on electroosmotic mobility (EOM), i.e., the mobility of electroosmotic flow and electrophoretic mobilities (EPM) of PAHs were investigated. Linear concentration range, reproducibilities, limit of detection (LOD) and limit of quantification (LOQ) values of investigated analytes were defined and developed electrophoretic conditions were finally applied to the analysis of PAHs in cooked soybean oil sample.

1.1. Legal Limits

German Society for Fat Science has fixed the legal limits at 25 ppb for total PAHs and 5 ppb for the heavy fraction [12]. For benzo[a]pyrene (BaP), this limit is 1 ppb in smoked foodstuff [13], but there are no legal limits for PAH content in oils and fats, one of the major sources of PAHs in the diet because of their lipophilic nature. Especially BaP has been reported in fumes from refined vegetable, soybean, and vegetable oils [14–18] but the concentration in the oil was not reported. To our knowledge, there are a few studies in the literature reporting the PAHs amount of cooked oil [19–21].

1.2. Extraction

Liquid–liquid partition, caffeine complexation and saponification are mostly used extraction methods of PAHs from oil samples. In the liquid–liquid partition method, the oil sample is dissolved in an organic solvent such as hexane, and PAHs are mostly extracted with DMF-water or DMSO [22,23]. Comparing these three extraction methods for an olive oil sample, it has been noted that the saponification method gave highly enough squalene residue which co-elutes with PAHs. This method needed an additional purification step [24], but the liquid–liquid partition method showed high purification power without an important amount of squalene in the samples. However, the described methods takes more time because of many clean-up steps used in order to remove the co-extracts before the detection step. Moreover, usage of the high amount of toxic organic solvents and their costs are the other disadvantages of these methods. In this work, we used ACN for the extraction of oil sample since it is easier to remove and reaches lower quantification limits than the described methods [25].

1.3. Clean-Up

The extract obtained with one of these extraction methods contains some impurities other than PAHs and they may interfere with the main analyses. For the purification, different clean-up procedures such as thin layer chromatography (TLC) and column chromatography on different adsorbents are widely applied [26]. For this work, silica gel column (30 cm × 1 cm i.d.) was packed with 20 g of silica gel 60 (70–230 mesh) and deactivated with 5% distilled water for the clean-up of PAHs extract. After loading the sample onto the column, extract was fractionated into aliphatic and aromatic hydrocarbons using *n*-hexane and *n*-hexane:dichloromethane (4:1), respectively.

2. Experimental

2.1. Instrumentation

The electrokinetic measurements were performed on Beckman P/ACE MDQ electrophoretic system (Fullerton, CA, USA) equipped with an on-column UV absorbance detector (230 nm) and system 32 Karat software. An uncoated fused silica capillary (50 μm i.d. × 375 μm o.d., Agilent, Switzerland) with total and effective lengths of 57 and 50 cm, respectively, was used. Temperature was maintained at 25 °C. Centrifugation was performed on Heraeus Biofuge Primo (Osterode, Germany) system. For heating the oil sample, a commercial deep fryer (230 V, 900 W) was used.

2.2. Reagents and analytes

Nitrobenzene, acetophenone, naphthalene, acenaphthene, acenaphthylene, phenanthrene, anthracene, pyrene, perylene, benzo[a]pyrene, acetonitrile, hexane, cetyltrimethylammonium bromide, and sodium dihydrogen phosphate (NaH_2PO_4) were obtained from Fluka (Buchs, Switzerland). Benzophenone was from Aldrich (Milwaukee, WI, USA) and silica gel 60 (70–230 mesh) was from Merck (Darmstadt, Germany). Soybean oil sample was obtained from local markets in Switzerland.

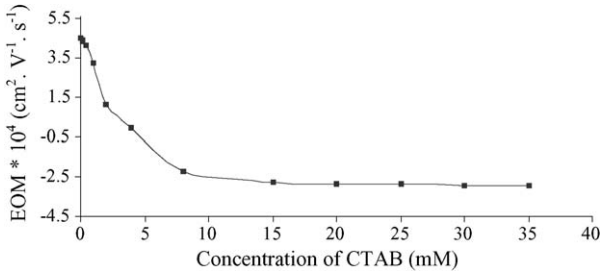

Fig. 2. Changing of EOM with CTAB concentration. Electrophoretic conditions: 10 mM phosphate, 40%ACN, pH 6.0. *V*: −25 kV. Injection: 5 s at 0.5 psi. Detection: 230 nm.

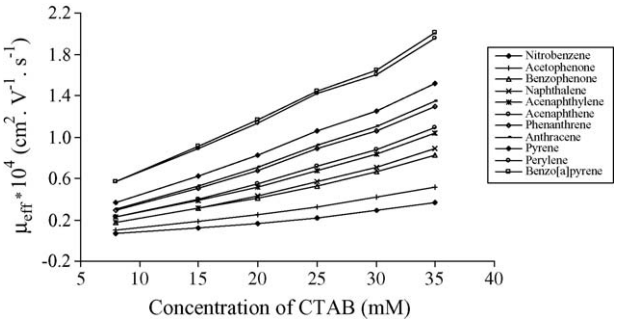

Fig. 3. Changing of EPM with CTAB concentration. Electrophoretic conditions are the same as in Fig. 2.

2.3. Procedure

2.3.1. Capillary column

New capillary column was initially treated with 1 M, 0.1 M NaOH, deionised water and running electrolyte for 30, 10, 5, and 10 min, respectively. Injections were performed hydrodynamically for 5 s at 0.5 psi (1 psi = 6894.76 Pa). Between runs, capillary column was washed with running electrolyte for 2 min.

2.3.2. Sample preparation

Stock solutions of investigated PAHs were prepared in ACN. Sample solutions of PAH standards were diluted with water:ACN (3:2) and 30 mM CTAB for the injected concentrations. The soybean oil sample (500 g) was cooked at 190 °C for 45 min using a commercial deep fryer (230 V, 900 W). Organic content of cooked oil was extracted with three portions (500 ml) of ACN addition [25]. The mixture was agitated manually for 5 min at each addition and centrifuged for 10 min at 6000 rpm. Upper layers (ACN phase) were collected and the solvent was evaporated. The sample was redissolved in *n*-hexane and cleaned-up by silica gel column chromatography as described above. In this procedure, aliphatic and aromatic fractions were eluted with *n*-hexane (200 ml) and *n*-hexane:dichloromethane (4:1, 400 ml), respectively. After the solvent evaporation of aromatic fraction, sample was redissolved in buffer solution (500 μl) and filtered before the analysis.

3. Results and discussion

3.1. Influence of additive concentration on the separation

Separation in CEKC is based on the partitioning of the analyte between the running electrolyte and the pseudo-stationary phase. In this work, CTAB was used as pseudo-stationary phase. A 0.0–35.0 mM concentration range of CTAB was prepared in a buffer consisting of 10 mM phosphate and 40% ACN at pH 6.0. Between the 0.0–4.0 mM concentration ranges, with the addition of CTAB, EOM decreases, changes direction, and subsequently increases in the reversed direction. The reason for this is that the positively charged surfactant molecules coat the capillary walls. After 8 mM CTAB, EOM remains constant. Fig. 2 shows how EOM changes by increasing CTAB concentration. Before the addition of CTAB all analytes were detected as a single peak migrating with EOM. When 8 mM CTAB was added, 11 analytes splited into seven peaks as shown in Fig. 3. Since the electroosmotic flow in the capillary is from the negative electrode to positive, PAH compounds interacting with the positively charged surfactant gains positive charge and migrate after the electroosmotic flow marker (here the negative water peak). The more tightly bonded larger nonpolar solutes gain more positive charge and their electrophoretic mobility to the negative electrode is faster than the smaller ones, so the larger PAH are swept to the positive electrode, i.e., to the detector side later compared to the smaller solutes. The separation difference between the first and last peaks continued to increase with higher concentrations of CTAB. With the addition of 30 mM CTAB, baseline resolution of all analytes was achieved. Effective mobilities of each analyte were calculated according to Eq.

$$\mu_{\text{eff}} = \mu_0 - \mu_{\text{app}} = \frac{LL_{\text{d}}}{V}\left(\frac{1}{t_0} - \frac{1}{t}\right) \quad (1)$$

Here, μ_0 shows the EOM, μ_{app} the apparent mobility, L the total length of capillary (cm), L_{d} the capillary length to the detector (cm), V the applied voltage (V), t_0 the migration time of the neutral marker (s), and t is the migration time of the analyte (s).

3.2. Influence of organic solvent concentration on the separation

PAHs are highly hydrophobic compounds and without organic solvent in running buffer it is impossible to achieve any partition of analytes between running buffer and pseudo-stationary phase. Therefore, ACN was used as organic modifier to increase the solubility of PAHs in this work. Forty percent ACN containing running buffer solution was found as optimal for the separation. Decreasing ACN content to 30% causes precipitation of analytes in capillary column and the current decreases. On the other hand, when ACN content is increased to 50%, electrophoretic mobilities of the analytes and resolution between them decreases. This is the indication of less interaction between PAHs and CTAB because solvophobic interactions are weaker in organic solvents compared to in aqueous solution. If CTAB micelles were present, we would expect that especially hydrophobic analytes having isomeric rings reside in the inner regions of the micelle and elute together.

Table 1
Optimum separation conditions of PAH standards

Running electrolyte	10 mM phosphate, 40% ACN, 30 mM CTAB, pH 6.0
Injection	0.5 psi, 5 s, hydrodynamically
Applied voltage and current	−25 kV, −18 μA
Wavelength	230 nm
Capillary column	50/57 cm
Capillary diameter	50/365 μm

3.3. Influence of voltage and pH on the separation

Optimal separation of PAHs was performed at −25 kV. At −20 kV, the separation window enlarged and longer analysis time was observed while the efficiencies of the last peaks decreased. At −28 kV, separation window became narrow and the resolution of peaks especially between isomers of phenanthrene–anthracene and perylene–BaP was lost. At pH 6, EOM change in the capillary is minimal and in that pH the best separation of 11 analytes was obtained. At lower pH values EOM increased and resolutions of analytes were lost. At the higher pH values mobilities decreased and the analysis time increased. Moreover, no better separation was observed especially between the isomer pairs. Optimum separation conditions of PAHs were shown in Table 1 and the electropherogram was given in Fig. 4(A).

3.4. Quantitation

The limit of detection of each PAH was obtained as PAH concentration that caused a peak with a height three times the baseline noise level. For the limit of quantification values S/N ratio was taken as 10. LOD and LOQ values were given in Table 2. The precision of method was calculated as R.S.D.% of migration times and corrected peak areas for six successive injections. Results are shown in Table 2. The linearity range of each PAH was found and calibration curves were plotted between these concentration intervals. The linearity range and regression equations of PAHs are given in Table 3.

3.5. Application

The analysis method for PAHs was applied to the determination of PAH content in the cooked soybean oil. After the extraction and purification steps, cooked soybean sample was injected to the capillary. Naphthalene, acenaphthene, phenanthrene, pyrene and BaP were detected in the oil between 2.1 and 23.3 ppb range. Fig. 4(B) shows the electropherogram of the oil sample and the PAH levels in the soybean oil are given as μg PAH/kg oil in Table 4. Recoveries were determined by spiking the oil sample with a known amount of PAHs with the same sample preparation procedure. Results were shown in Table 4.

Table 2
Analytical characteristics of the method

Analytes	LOD (μg/ml) 230 nm (S/N = 3)	LOQ (μg/ml) 230 nm (S/N = 10)	R.S.D.% (for migration time)	R.S.D.% (for normalised peak areas)	Injected concentrations (mM)
Nitrobenzene	1.27	3.17	0.55	1.02	0.75
Acetophenone	0.66	1.98	0.61	2.24	0.30
Benzophenone	0.96	2.69	0.72	5.38	0.30
Naphthalene	0.58	1.17	0.73	1.13	0.50
Acenaphthalene	0.25	0.57	0.80	1.80	0.12
Acenaphthene	0.09	0.22	0.82	1.80	0.10
Phenanthrene	0.69	1.74	0.95	4.12	0.20
Anthracene	0.90	1.98	0.90	4.69	0.20
Pyrene	0.25	0.53	1.01	7.23	0.10
Perylene	2.24	4.70	1.18	2.03	0.10
Benzo[a]pyrene	0.93	1.86	1.27	6.72	0.10

Table 3
The results of regression analysis on calibration

Analytes	Regression equation ($y = ax + b$)	Correlation coefficient (R^2)	Linear range (mM)
Nitrobenzene	$y = 1.78 \times 10^4 x + 5.23 \times 10^2$	0.9997	0.100–5.000
Acetophenone	$y = 3.27 \times 10^4 x + 1.72 \times 10^2$	0.9999	0.014–5.500
Benzophenone	$y = 0.32 \times 10^4 x - 2.92 \times 10^2$	0.9990	0.053–3.975
Naphthalene	$y = 1.94 \times 10^4 x - 4.45 \times 10^2$	0.9996	0.014–4.600
Acenaphthalene	$y = 10.1 \times 10^4 x - 0.96 \times 10^2$	0.9995	0.002–1.600
Acenaphthene	$y = 91.1 \times 10^4 x - 30.6 \times 10^2$	1.0000	0.002–4.800
Phenanthrene	$y = 14.1 \times 10^4 x - 25.6 \times 10^2$	0.9993	0.006–1.950
Anthracene	$y = 3.05 \times 10^4 x - 3.46 \times 10^2$	0.9998	0.008–1.250
Pyrene	$y = 23.9 \times 10^4 x - 23.9 \times 10^2$	0.9979	0.004–1.260
Perylene	$y = 2.55 \times 10^4 x + 7.53 \times 10^2$	0.9993	0.009–0.220
Benzo[a]pyrene	$y = 12.5 \times 10^4 x + 1.25 \times 10^2$	0.9989	0.011–0.185

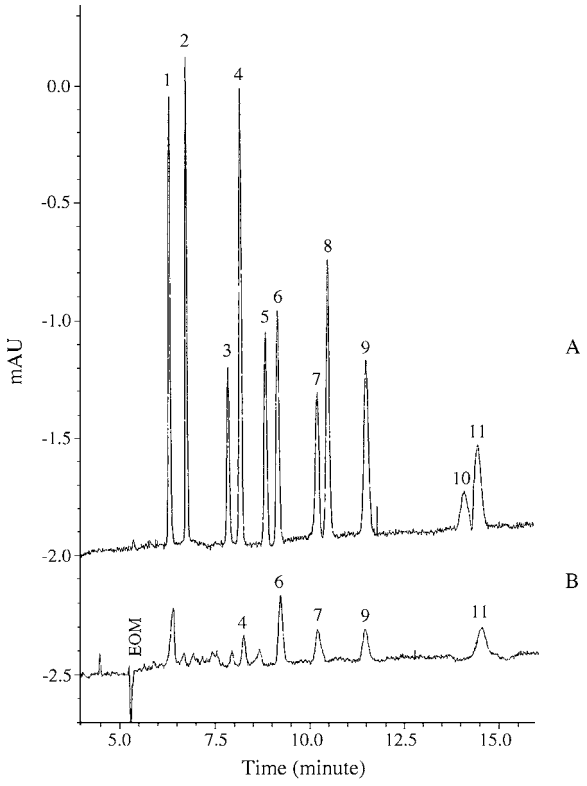

Fig. 4. (A) Separation electropherogram of PAH standards. (B) Separation electropherogram of oil sample. Running electrolyte: 10 mM phosphate, 40% ACN, 30 mM CTAB, pH 6.0. Applied voltage = −25 kV. Injection: 0.5 psi, 5 s, hydrodynamically. Wavelength = 230 nm. Peak identifications are the same as Fig. 1.

Table 4
Quantitative results of sample analysis

Analytes	Concentration (μg PAH/kg oil)	Recovery%
Naphthalene	5.3	92
Acenaphthene	2.2	96
Phenanthrene	3.5	97
Pyrene	2.1	94
Benzo[a]pyrene	23.3	91

4. Conclusion

In this study, we performed a new and rapid CE method for PAH analysis with the addition of positively charged CTAB into the separation electrolyte containing phosphate and ACN at pH 6.0. The addition of CTAB both creates an electrically driven counter-flow in the capillary and selective interactions of PAHs with this pseudo-stationary phase cause their retardation and separation. The developed method was applied to a cooked soybean oil sample. Results show that PAH contents of oil increase by heating and home use cooking oils can be significant source of PAHs.

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