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Original Paper

Comparison of two capillary electrophoresis online stacking modes by analysis of polycyclic aromatic hydrocarbons in airborne particulates

Naphthalene, fluorene, pyrene, anthracene, phenanthrene, and chrysene were successfully separated by CD-modified MEKC (CD-MEKC) using 20 mM borate (pH 9.0) containing 90 mM SDS and 75 mM β -CD. Two online stacking methods, *i.e.*, sweeping and field-enhanced sample injection (FESI), were explored to enhance the detection sensitivity. The influences of some crucial parameters in sweeping and FESI procedures were investigated. For FESI method, a plug of water and low-conductivity sample matrix was used to increase the stacking efficiency. Compared with the sweeping method, FESI can increase the sensitivity in the range of 10–20-fold. The proposed method was used for the analysis of polycyclic aromatic hydrocarbons in airborne particulates.

Keywords: Cyclodextrin / Field-enhanced sample injections / Micellar electrokinetic chromatography / Polycyclic aromatic hydrocarbons / Sweeping

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1 Introduction

Polycyclic aromatic hydrocarbons (PAHs), containing two or more benzene rings, are a group of ubiquitous organic pollutants. They are produced *via* natural and anthropogenic combustion processes. As PAHs exhibit toxic, mutagenic, and carcinogenic properties and cause adverse health effects, such as the endocrine-disrupting activities and lung cancer, there is a serious concern about their environmental presence [1]. PAHs exist in the environment at very low concentration because of their poor water solubility and strong hydrophobicity; therefore, it is necessary to develop sensitive and rapid PAHs analytical techniques [2].

GC/MS is widely used for PAHs' determination because PAHs are easily volatile [3]. Nevertheless, GC/MS being an expensive technique is not available for all analytical laboratories. The conventional HPLC separation of PAHs still exhibits several disadvantages such as long analytical time, large organic solvent consumption, and expensive separation column [4]. In recent years, CE has been

developed quickly as a high-efficiency separation technique [5]. MEKC is one of the most effective CE modes due to its highly efficient separation for neutral analytes [6]. Its principle is based on the partition of the neutral analytes between the electrolyte and the micelles in the buffer. However, because PAHs are highly hydrophobic compounds and strongly associated with the hydrocarbon phase, they are difficult to be separated by some common surfactants [7]. Therefore, Koch *et al.* [8] increased the solubility of PAHs by the addition of organic solvents, but satisfactory resolution was not achieved because of peak tailing. Warner and coworkers [10] have successfully separated 16 PAHs [9] and 12 monomethyl-benz[a]anthracene isomers by exploring a high-purity T-type polymerized surfactant with an undecenyl (C_{11}) and a sulfate group. Moreover, several other additives were reported, and they included a bile salt surfactant [11], a high-concentration ionic surfactant [12], and a neutral and anionic CD mixture [13]. Among these techniques, CD-modified MEKC (CD-MEKC) has been demonstrated to be an efficient separation method for PAHs [14].

Nevertheless, CD-MEKC suffers from poor detection sensitivity due to the short optical path length and low injected sample volume (nL) [15]. Therefore, the direct analysis of trace amounts of analytes in the real samples with CD-MEKC is difficult. In order to solve the problem, several online stacking methods have been developed for the preconcentration of neutral analytes before separation [16, 17]. The concentration effect is based on the sud-

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Abbreviations: FESI, field-enhanced sample injection; FNF, 2-fluoro-7-nitrofluorene; PAHs, polycyclic aromatic hydrocarbons

den change of the analyte's electrophoretic velocity between the sample region and the separation region. In the present work, two sample stacking methods were compared. One is the sweeping method [18], which is based on the accumulation of analyte molecules using an additive owning considerable affinity for analytes. The sweeping method requires that the sample matrix is devoid of micelles. The extent of focusing is determined by the interaction between the analytes and the micelles in the buffer. The other is field-enhanced sample injection (FESI) [19]. A plug of water is introduced to the capillary, resulting in the increase in the effective electrophoretic velocity of the analytes. This focus is initiated at the water–buffer solution boundary.

In this work, we studied the separation, identification, and quantification of PAHs by the use of CD-MEKC with diode array detection (DAD). Moreover, the different pre-concentration procedures were combined with CD-MEKC to analyze the low-level PAHs. The reliability of this approach was validated through determining the PAHs recoveries in airborne particulates.

2 Experimental

2.1 Chemicals

All the reagents were of analytical grade and used without further purification. The six PAHs studied, including naphthalene, fluorene, pyrene, anthracene, phenanthrene, and chrysene, were purchased from Aldrich (Milwaukee, WI, USA). α -, β -, and γ -CDs were obtained from Fluka (Buchs, Switzerland). SDS was obtained from Wako Pure Chemical (Japan); and 2-fluoro-7-nitrofluorene (FNF) was purchased from Aldrich. Sodium borate, methanol, benzene, ethanol, sodium hydroxide, sulfuric acid, and ACN were purchased from Beijing Chemical Reagent (Beijing, China). Water for preparation of sample and buffer solutions was deionized by a easypureTMLF purification system with a 0.2 μ m fiber filter (Barnstead, USA).

2.2 Apparatus

All experiments were performed on a Beckman P/ACETM MDQ CE system (Beckman, Fullerton, CA, USA) equipped with a photodiode array detection system. The electropherograms were recorded and integrated by an IBM PC with 32 KaratTM software version 4.0 (Beckman). A fused-silica capillary (Yongnian Optical Fiber, Hebei, China) with a total length of 57 cm and an id of 75 μ m was used. A detection window was created at 50 cm from the capillary inlet by removing the polyimide coating. Direct UV detection was performed at a single wavelength of 254 nm. Kimotodenshi (Osaka, Japan) HV-120 high-volume air sampler and 2500 QAT-UP filter were used for the collection of airborne particulates.

2.3 CD-MEKC separation conditions

A new capillary was initially washed with methanol for 5 min, followed by deionized water for 2 min, 1 M HCl for 5 min, deionized water for 2 min, 1 M NaOH for 10 min, and deionized water for 2 min. To achieve reproducible separations, all experiments were performed at 25°C and were run in triplicate. Before each separation, the capillary was rinsed with methanol for 2 min, deionized water for 1 min, 1 M NaOH for 2 min, deionized water for 1 min, and run buffer for 2 min.

The optimal electrophoretic run buffer was 20 mM borate containing 90 mM SDS and 75 mM β -CD. Appropriate amount of boric acid was used to adjust buffer pH to 9.0. The separation voltage was 25 kV, and the injection was performed at 0.5 psi for 5 s. Stock solutions of PAHs were obtained by dissolving the PAHs in ACN, and the concentration of all stock PAHs solutions was 250 μ g/mL. Standard solutions were prepared by dilution of stock solutions.

2.4 Sample treatment

Sample was collected at the top of the environmental building of the Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences. The sample treatment procedures were according to the method reported by Hayakawa *et al.* [20]. The filters were cut and an adequate area (10–20 cm²) was thoroughly cut into small pieces. After the pieces were placed in a flask and FNF was added as an internal standard, the filter sample was extracted with 30 mL of benzene–ethanol (3:1), washed successively with 60 mL of 5% sodium hydroxide, 60 mL of 20% sulfuric acid, and 60 mL of water. After the benzene phase was evaporated to dryness, the residue was dissolved in 1 mL of ethanol and refluxed with 1 mL of 7% sodium hydrosulfide for 1.5 h. PAHs were extracted into 1.5 mL of benzene. After the benzene phase was evaporated to dryness, the residue was dissolved into 0.2 mL of ACN. An aliquot of the solution was injected into the CE system.

3 Results and discussion

3.1 Separation of PAHs

CDs are neutral oligomers with different numbers of D-(+)-glucopyranose units. Among them, the behavior patterns of α -, β -, and γ -CDs are similar to SDS; therefore, they are often used in CE separation [21]. In the present MEKC separation, α -, β -, and γ -CDs were studied as modifiers. The results indicated that α -CD with the smaller size of the hydrophobic cavity limited the partition of the larger PAH molecules to the α -CD cavity, while smaller PAH molecules easily entered the larger size γ -CD cavity. Therefore, the distribution was close to two extremes and the separation was unsuccessful with α - and γ -CD. In

Table 1. Effect of the concentration of SDS and β -CD on the resolution

PAHs	SDS (mM) ^{a)}				β -CD (mM) ^{b)}			
	80	90	100	110	70	75	80	90
Naphthalene	2.27	2.97	2.72	2.12	2.3	2.6	2.55	2.48
Fluorene	0.71	1.64	1.46	0.78	1.53	1.71	1.66	1.07
Pyrene	0.9	1.34	1.26	0.71	1.33	1.6	1.48	0.87
Anthracene	1.0	2.02	1.71	0.9	2.69	2.98	2.97	2.67
Phenanthrene	1.58	2.44	2.02	1.05	1.43	1.86	1.73	1.19
Chrysene	2.18	2.87	2.64	2.24	2.46	2.77	2.72	2.4

a) Operating buffer of 75 mM β -CD

b) Operating buffer of 90 mM SDS.

this study, β -CD was finally selected as the additive in the buffer.

The concentrations of SDS and β -CD are the most important factors for the resolution of PAHs. If major fractions of PAHs are incorporated into SDS or β -CD, PAHs cannot be separated completely. As shown in Table 1, when the concentrations of SDS and β -CD were 90 mM and 75 mM, respectively, the resolution reached an optimum.

Higher concentration of electrophoretic buffer reduces the ζ potential and the electroosmotic velocity, resulting in the increase in the separation efficiency and resolution of PAHs. However, the higher concentration of electrophoretic buffer can lead to a larger current, which broadens the peaks and decreases separation efficiency. In this study, 20 mM borate was selected as an optimum buffer concentration. Moreover, the pH value of the buffer solution has little effect on the resolution. When pH is 9.0, the peak shapes appeared symmetrical and sharpest.

3.2 Online preconcentration of PAHs

To improve the detection limits, the sweeping and FESI were evaluated as a way of stacking modes for PAHs focusing on CD-MEKC.

3.2.1 Sweeping mode

Sweeping in MEKC is defined as the accumulation of analytes by the charged micelle that fills or penetrates the sample zone during application of a voltage [18]. The online preconcentration procedures were as follows: The capillary was initially conditioned with micellar buffer solution; then, a long plug of sample diluted with buffer without addition of SDS was introduced at 0.5 psi for 90 s. When 25 kV voltage was applied, PAHs at the front end of the sample plug were trapped by the more slowly moving micelles; hence, the migration velocities of PAHs were reduced. PAHs at the rear end travel more quickly until they are incorporated into the micelles, and the whole sample region was compressed.

The concentration extent is affected by the swept zone length (l_{sweep}) [18]. The shorter the swept zone length is, the more efficiently the analytes can be focused. According to [15], l_{sweep} was determined by two factors: the injected sample plug length and the retention factor. Figure 1 shows the effect of the sample plug injection time on the focused efficiency. Peak areas increased with an increase in the sample injection time until a maximum was reached at 90 s. Above this period, excess organic solvent was introduced, resulting in the breakdown of the separation current. Therefore, 90 s was selected as the optimum injection time.

3.2.2 FESI stacking mode

The steps and mechanism involved in FESI stacking are depicted in Fig. 2. A long water plug was injected for 40 s into the capillary previously filled with the micellar buffer solution, and then the samples, diluted with a low-conductivity micellar buffer, were injected hydrodynamically for 80 s (Fig. 2A). The voltage was applied at negative polarity (Fig. 2B). Due to the enhanced field in the water plug and the sample zone, electrophoretic velocities of micelles in the sample zone were greater than that of the bulk EOF. Therefore, the micelles in the sample zone will carry PAHs toward the anode. Meanwhile, the water plug and sample matrix were pumped out by the bulk EOF. Removal of the sample matrix lessened the dispersive effects by the mismatch of local electrophoretic velocities of the low- and high-conductivity zones [22]. When the current reached 97–99% of the separation current at negative polarity, the voltage was switched to positive polarity to facilitate separation and detection (Fig. 2C and D).

The choice of the sample matrix was very important for the focus of PAHs. The effect of the sample matrix on the focusing efficiency was examined. When the buffer solution or high-conductivity micellar matrices were used, the improvements of peak responses were lower and the peak shapes were distorted for the less-retained compounds. Sample was prepared in low-conductivity

Figure 1. Investigation of the effect of the sample injection time on the focused efficiency using the sweeping method. The sample was prepared in nonmicellar buffer solution. Separation conditions were as follows: buffer solution, 20 mM borate (pH 9.0) containing 90 mM SDS and 75 mM β -CD; capillary, fused-silica capillary of 75 μ m id with the total length of 57 cm and effective length 50 cm; separation voltage, 25 kV; UV detection wavelength, 254 nm; temperature, 25°C. 1. naphthalene; 2. fluorene; 3. pyrene; and 4. phenanthrene.

Figure 2. Mechanism for FESI-focused method. (A) Introduction of water plug and sample plug. (B) Negative voltage was applied and the sample was focused at the boundary between water plug and buffer solution. (C) The sample matrix and water plug were pumped out of the capillary and the focus was completed. (D) The voltage was switched and electrophoretic separation of PAHs started.

matrices so that most PAHs in the sample zone can reach the water zone faster. Also, a more effective focus can be reached since most PAHs will reach the water zone with very small time differences. Furthermore, we studied the effect of the concentration of SDS in the sample matrix. No improvement in peak heights was observed and peak shapes were heavily distorted when the concentration was above 90 mM. This result was in accordance with

[23], in which it was reported that micelles in the sample zone are not necessary for the stacking. Therefore, the low-conductivity micellar solution with 5 mM borate containing 90 mM SDS and 75 mM β -CD was utilized as sample matrix.

To provide a zone inside the capillary with higher electric field, a water plug was injected before injection of

Figure 3. Investigation of the effect of injection time on the peak area: a, Water plug injection: dynamic injection for sample plug, 0.5 psi $\times$ 80 s. b, Sample plug injection: dynamic injection for water plug, 0.5 psi $\times$ 40 s. The sample was prepared in 5 mM borate containing 90 mM SDS and 75 mM β -CD; negative voltage, 10 kV. Separation conditions were the same as in Fig. 1.

sample plug. The water plug acted like a fast freeway for the micelles that carry PAHs. Also, the extra-band broadening effect due to the presence of micelles in the sample zone during stacking can be eliminated [17]. Figure 3a and b shows the effects of the water plug and sample plug injection time on the peak area. With the increase of the fraction of capillary filled with low-conductivity zone, the peak areas increase to a maximum value. This phenomenon can be explained by $\kappa_{\max}^*$, which is obtained from Eqs. (1), (2) [24].

$$\kappa_{\max}^* = \kappa_{\max} \frac{K_s}{K_s + 1} \quad (1)$$

$$\kappa_{\max} = \frac{\mu_{\text{ep}}(mc)}{\mu_{\text{eof}}(S)} \quad (2)$$

where K_s is the retention factor of analyte in the sample zone; $\mu_{\text{ep}}(mc)$ is the electrophoretic mobility of the

Table 2. Calibration curves for six PAHs and linear range

PAHs	<i>a</i>	<i>b</i>	<i>r</i>	Linear range (ng/mL)
Naphthalene	3956.3	16 557	0.9977	4–100
Fluorene	27 234	20 126	0.9983	2–100
Pyrene	27 890	23 456	0.9983	2–100
Anthracene	69 342	18 030	0.9970	1–100
Phenanthrene	80 607	60 776	0.9951	1–100
Chrysene	3455.9	2231.3	0.9973	10–1000

Calibration curves are expressed as regression lines ($y = ax + b$), where y is integrated peak area, x is concentration of PAHs (ng/mL), a is slope, b is intercept, and r is relative coefficient.

micelle and $m_{\text{eof}}(S)$ is the electroosmotic mobility. The value of $\kappa_{\max}^*$ denotes the maximum fraction of the capillary filled with low-conductivity zones. $\kappa_{\max}^*$ increases with the increase in K_s . When the fraction of the capillary injected with the low-conductivity zones exceeded $\kappa_{\max}^*$, the analytes with low retention factor were probably lost to the cathodic vial and the analytes with high retention factor were stacked. Furthermore, when the injection time increased, the time of removing the water plug and sample matrix was longer, resulting in long analytical time. Therefore, the time of 40 s for the water plug and 80 s for the sample plug were used in the FESI method. Figure 4a shows the standard. After the standard solution was diluted ten-fold, the chromatogram by FESI was obtained under the optimal focused conditions as shown in Fig. 4b. From Fig. 4b, although the resolution decreased slightly and the peaks exhibited some small tailing, the quantitative results and application of the FESI method had not been affected.

3.3 Validation

As shown in Table 2, the calibration curves for five PAHs show linear dynamic ranging from 1 to 100 ng/mL and from 10 to 1000 ng/mL for chrysene. The correlation coefficients of all PAHs were higher than 0.995. RSD of peak area was calculated on the basis of five duplicate injections of a standard sample, and the values are given in Table 3. Table 3 also gives the LODs ($S/N = 3$) of the sweeping and FESI stacking methods. Compared with the sweeping method, the FESI method resulted in 10-fold reduction in detection limits for naphthalene, 20-fold reduction for pyrene, phenanthrene, and fluorine. Also, the detection limit of the present FESI method is almost at the same level or lower than those reported in many papers [14, 25].

4 Application

The optimized FESI-CE method was used to analyze the PAHs in airborne particulates. The airborne particulates

Figure 4. Electropherograms of six PAHs. a: β -CD-MEKC separation: dynamic injection, $0.5 \text{ psi} \times 5 \text{ s}$. The concentrations of six PAHs were $0.1 \text{ } \mu\text{g/mL}$. Other conditions were the same as in Fig. 1. b: FESI stacking: injection time, 40 s for the water plug and 80 s for the sample plug; negative voltage, 10 kV. Separation conditions were the same as in (a). 1. naphthalene; 2. fluorene; 3. pyrene; 4. anthracene; 5. phenanthrene; and 6. chrysene.

Table 3. LODs ($S/N = 3$) and RSD using sweeping and FESI

PAHs	Sweeping		FESI	
	LOD (ng/mL)	RSD (%)	LOD (ng/mL)	RSD (%)
Naphthalene	22	3.5	2.0	1.4
Fluorene	18	3.1	1.0	0.8
Pyrene	18	2.2	1.0	1.0
Anthracene	–	–	0.50	0.4
Phenanthrene	10	1.7	0.50	0.6
Chrysene	–	–	5.0	1.5

RSD is relative standard deviation ($n = 5$).

were ultrasonically extracted by benzene–ethanol with sodium hydroxide solution, sulfuric acid solution, and water. That procedure was verified effectively for the reduction of interferences usually present in exhaust particulates [26]. The filter on which airborne particulates were collected for 24 h was cut into small sections of equal area ($2 \times 5 \text{ cm}$). Each section, containing about 3.15 mg of particulates, was thoroughly cut into small

pieces in a flask. In the flask, aliquots of standard solution of PAHs and 0.2 mL of FNF solution ($1 \times 10^{-6} \text{ M}$ in ethanol) were added.

As reported in [27], the actual amount of PAHs in the airborne particulates in Beijing was 0.075–0.44 ng/L, which was lower than the detection limit of our present method. Therefore, the reliability of this method was demonstrated by the recoveries. As shown in Table 4, all samples analyzed provided satisfactory recoveries in the range between 94 and 104%.

5 Concluding remarks

In this work, we successfully separated six PAHs based on CD-MEKC and online preconcentration procedures. The comparative study between sweeping and FESI stacking methods has shown that FESI can provide the best results in terms of sensitivity, efficiency, and reproducibility. The detection limits reached 0.5–5.0 ng/mL, which was a 10- to 20-fold reduction compared with the sweeping method. The applicability of the method was demonstrated by the analysis of PAHs in airborne particulates.

Table 4. Results for the determination of PAHs in airborne particulates

PAHs	Naphthalene	Fluorene	Pyrene	Anthracene	Phenanthrene	Chrysene
Added ($\mu\text{g/mL}$)	0.05	0.05	0.05	0.02	0.02	0.05
Found ($\mu\text{g/mL}$)	0.052	0.0475	0.0485	0.021	0.0196	0.047
Recovery (%)	104	95	97	101	98	94

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6 References

- [1] Larsen, R. K., Baker, J. E., *Environ. Sci. Technol.* 2003, 37, 1873–1881.
- [2] Boer, J. D., Law, R. J., *J. Chromatogr. A* 2003, 1000, 223–251.
- [3] Fang, G. C., Wu, Y. S., Chen, J. C., Fu, P. P. C., *et al.*, *Chemosphere* 2005, 60, 427–433.
- [4] Oliferova, L., Statkus, M., Tsysin, G., Shpigun, O., Zolotov, Y., *Anal. Chim. Acta* 2005, 538, 35–40.
- [5] Perl, I. M., Füzfa, Z., *J. Chromatogr. A* 2005, 1073, 201–227.
- [6] Terabe, S., *Anal. Chem.* 2004, 77, 240A–246A.
- [7] Kavran, G., Erim, F. B., *J. Chromatogr. A* 2002, 949, 301–305.
- [8] Koch, J. T., Beam, B., Phillips, K. S., Wheeler, J. F., *J. Chromatogr. A* 2001, 914, 223–231.
- [9] Shamsi, S. A., Akbay, C., Warner, I. M., *Anal. Chem.* 1998, 70, 3078–3083.
- [10] Akbay, C., Shamsi, S. A., Warner, I. M., *J. Chromatogr. A* 2001, 910, 147–155.
- [11] Cole, R. O., Sepaniak, M. J., Hinze, W. L., Gorse, J., Oldiges, K., *J. Chromatogr.* 1991, 557, 113–123.
- [12] Lin, J.-M., Nakagawa, M., Uchiyama, K., Hobo, T., *Electrophoresis* 2002, 23, 421–425.
- [13] Nguyen, A. L., Luong, J. H. T., *Anal. Chem.* 1997, 69, 1726–1731.
- [14] Brown, R. S., Luong, J. H. T., Szolar, O. H. J., Halasz, A., Hawari, J., *Anal. Chem.* 1996, 68, 287–292.
- [15] Quirino, J. P., Kim, J. B., Terabe, S., *J. Chromatogr. A* 2002, 965, 357–373.
- [16] Palmer, J., Burgi, D. S., Munro, N. J., Landers, J. P., *Anal. Chem.* 2001, 73, 725–731.
- [17] Quirino, J. P., Otsuka, K., Terabe, S., *J. Chromatogr. B* 1998, 714, 29–38.
- [18] Quirino, J. P., Terabe, S., *Science* 1998, 282, 465–468.
- [19] Quirino, J. P., Terabe, S., *J. Chromatogr. A* 1998, 798, 251–257.
- [20] Hayakawa, K., Tang, N., Akutsu, K., Murahashi, T., *et al.*, *Atmos. Environ.* 2002, 36, 5535–5541.
- [21] Schneiderman, E., Stalcup, A. M., *J. Chromatogr. B* 2000, 745, 83–102.
- [22] Chien, R. L., Burgi, D. S., *Anal. Chem.* 1992, 64, 1046–1050.
- [23] Quirino, J. P., Terabe, S., *Anal. Chem.* 1998, 70, 149–157.
- [24] Quirino, J. P., Terabe, S., *J. Chromatogr. A* 1997, 791, 255–267.
- [25] Kavran, G., Erim, F. B., *J. Chromatogr. A* 2002, 949, 301–305.
- [26] Hayakawa, K., Butoh, M., Miyazaki, M., *Anal. Chim. Acta* 1992, 266, 251–256.
- [27] Zhang, Y., Chen, Y. Q., Zhang, X. S., Zhou, J. B., Wang, T. G., *Environ. Chem.* 2004, 23, 681–685.