Anal. Chem. **1995,** *67,* 2026–2029

Capillary Electrochromatography: Analysis of Polycyclic Aromatic Hydrocarbons

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Electrochromatography is utilized to separate a mixture of 16 different polycyclic aromatic hydrocarbons (PAHs). Fused-silica capillary columns ranging in size from 50 to 150 μm i.d. were packed (20–40-cm sections) with 3-μm octadecylsilica particles. A potential of 15–30 kV is applied across the 30–50-cm total length capillary column to generate electroosmotic flow that carries the PAHs through the stationary phase. An intracavity-doubled argon ion laser operating at 257 nm is used to detect the PAHs by laser-induced fluorescence. Efficiencies up to 400 000 theoretical plates/m are obtained when detection is performed within the column packing and up to 150 000 theoretical plates/m when detection is performed following a frit (used to hold the packing). The reproducibility of the peak retention times is better than 2% (RSD). The limits of detection for individual PAHs range between 10^{-17} and 10^{-20} mol (10^{-9}–10^{-11} M), with a linear response spanning 4 orders of magnitude in concentration.

The quest to gain an understanding at the molecular level of various biochemical phenomena and the necessity created by the trend toward strict monitoring of processes that lead to environmental pollution have placed stringent demands on the methods used for such analysis. Because of the complexity of the sample mixtures and often the low levels of compounds present in them, the analytical technique must possess good separation capabilities (high efficiency and high selectivity) and also good detection sensitivity.

Electrokinetic separations performed in capillary columns provide high-resolution and high-efficiency analyses of complex samples.[1] Capillary zone electrophoresis (CZE),[1] in which the capillary column is open and the walls of the capillary serve to establish electroosmotic flow, is useful in separating charged species via their different electrophoretic mobilities but is unable to resolve neutral components. As demonstrated by Terabe et al.,[2] the introduction of micelles into the separation electrolyte (called micellar electrokinetic capillary chromatography (MECC)) results in the separation of some uncharged species. This separation is based on the partitioning of the uncharged analytes between the electrolyte and the micelles that serve as a pseudostationary phase. Although easy to implement, MECC currently lacks selectivity, and it does not offer the choice in stationary phases that is available in high-performance liquid chromatography (HPLC). Consequently, MECC has not yet emerged as a routinely used technique in the laboratory.

As first shown in 1974 by Pretorius, Hopkins, and Schieke,[3] by applying an electric field across columns packed with microparticulates, electroosmotic flow can be made to act as a pump for chromatographic separations. This technique is referred to as capillary electrochromatography (CEC). They demonstrated that the efficiency of a separation obtained by electroosmotic propulsion is superior to that obtained by pressure-driven flow (as is the case in HPLC). CEC has since been applied by Jorgenson and Lukacs[4] in 1981 and by Tsuda, Nomura, and Nakagawa[5] in 1982 to analyze neutral aromatic compounds that could not be separated by CZE. Knox and Grant[6] have pointed out that CEC promises efficiencies comparable to those obtained with capillary gas chromatography if the capillary can be packed with submicrometer-sized particles. They have demonstrated the increase in efficiency that is obtained with particle sizes down to 1.5 μm using a few polycyclic aromatic hydrocarbons (PAHs) as model analytes. Recently, Smith and Evans[7] utilized a pressurized electrochromatographic system to separate certain pharmaceutical compounds. The pressurized system was used to minimize bubble formation in the capillaries that was significantly interfering with the electrochromatographic separations. In spite of these demonstrations, CEC has received relatively little attention because of the difficulty in fabricating and operating the packed capillary columns.

In this study we utilize a methodology developed previously by Yan[8] for the production of packed capillary columns and demonstrate that CEC can be used in a routine manner. In addition, we expand on the high-sensitivity CZE work of Nie, Dadoo, and Zare[9] for the analysis of PAHs by UV laser-excited native fluorescence. We report the use of CEC combined with UV laser-induced fluorescence (LIF) detection for the high-efficiency, high-sensitivity analysis of 16 PAHs classified by the U.S. Environmental Protection Agency (EPA) as priority pollutants. Because capillary separation techniques are so well suited for analyzing ultrasmall volumes (<1 nL), this methodology is expected to be particularly applicable to any study requiring high resolution and high efficiency in the analysis of analytes in a limited volume.

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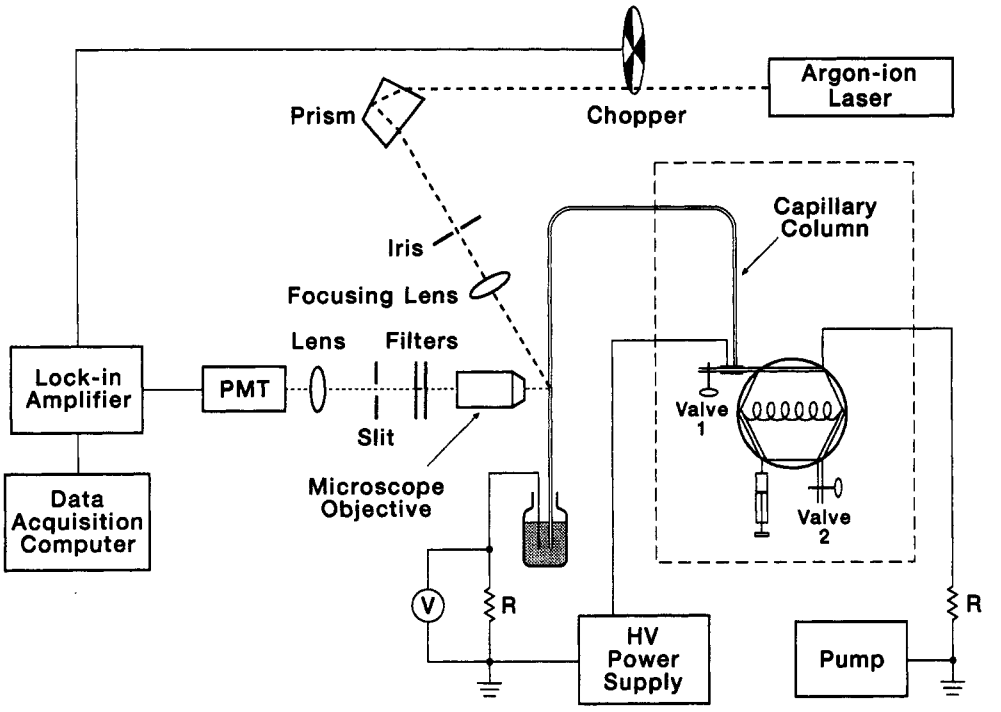

Figure 1. Schematic of the CEC-LIF apparatus.

EXPERIMENTAL SECTION

CEC and Micro-HPLC Apparatus. The apparatus used to perform the separations is similar to that described by Yan, Schaufelberger, and Erni[10] but was modified for our purposes. A schematic of the apparatus is shown in Figure 1. A stainless-steel six-port rotary valve fitted with a 20-μL sample loop (Model 7010, Rheodyne, Inc., Cotati, CA) was used as an injection manifold. The valve was used primarily for hydrodynamic injections with micro-HPLC runs. A pump capable of delivering 0.1–1250 μL/min (Micro-Tech Scientific, Sunnyvale, CA) was connected to the rotary valve and was used for initially filling the capillary column with the mobile phase and for the micro-HPLC experiments. A 0–50-kV high-voltage power supply (Glassman High Voltage, Inc., Whitehouse Station, NJ) provided the electric field for the electrochromatography. A fan was used to assist the heat dissipation of the capillary during the separations.

Detection Apparatus. The confocal-design UV-LIF apparatus described earlier[9] was adapted for electrochromatography. The 257-nm line from an intracavity-doubled argon ion laser (Coherent, Inc., Santa Clara, CA) was used for excitation of the PAHs. A prism was used to separate residual 514-nm light in the laser beam from the 257-nm light. A fused-silica lens (50-mm focal length; Melles Griot, Irvine, CA) focused the laser beam onto the capillary. The fluorescence was collected by using a high-numerical-aperture (NA) microscope objective (40× magnification, NA = 0.85, 0.37-mm working distance; Nikon, Melville, NY) and then sent through a set of filters (a 280-nm longpass in combination with two 600-nm shortpass filters or a 70-nm bandpass filter centered at 400 nm) and a variable slit (Newport Corp., Fountain Valley, CA) onto a photomultiplier tube (PMT) (Model R955, Hamamatsu, San Jose, CA) operated between 500 and 600 V. A lock-in amplifier (Stanford Research Systems, Sunnyvale, CA) was used in conjunction with a chopper (operating at 250 Hz) in the path of the laser beam to measure the output of the PMT. Commercial software (LabCalc, Galactic Industries Corp., Salem, NH) run on an IBM personal computer was used for data acquisition at a rate of 1 Hz.

Preparation of Packed Capillary Columns. Capillary columns with different inside diameters (50–150 μm) were packed using the procedure developed previously.[8] A suspension of 3-μm octyldecylsilica (ODS) particles (90%) and 1-μm silica gel (10%) in methanol containing 2 mM sodium phosphate (pH 4.5) was electrokinetically packed into the column after an initial frit was created by sintering 5-μm silica gel (using a microflame torch). A second frit consisting of fused-ODS particles (~10 cm from the outlet) was made in the column after the packing procedure to hold the particles firmly in place. A thermal wire stripper was used to make the second frit. The wire stripper gently heated the particles in a controlled fashion which minimized disturbance of the adjacent ODS particles. Extraneous particles between the frit and the outlet of the column were then flushed out because passing the laser beam through the particles generated significant background scatter. A narrow window (2–5 mm) for detection was created within 1–2 mm after the packed portion of the column. The column was flushed with the selected mobile phase (by pressurizing the column inlet to 1000 psi) for ~1 h prior to performing the separations.

Materials and Reagents. The fused-silica capillary columns were purchased from Polymicro Technologies, Inc. (Phoenix, AZ). The 3-μm ODS and the 1-μm silica particles were obtained from SynChrom, Inc. (Lafayette, IN) and Phase Separation, Inc. (Norwalk, NJ), respectively. The 5-μm silica gel for creating the frits was graciously provided by John O'Gara from Waters Corp. (Milford, MA). The priority pollutant PAH mixture (standard reference material, SRM 1647c) was a gift from Dr. Lane Sander at the National Institute of Standards and Technology. Sodium tetraborate, acetonitrile (HPLC grade), methanol, and individual PAHs were purchased from Aldrich Chemical Co. (Milwaukee, WI). The mobile phase was prepared by mixing the appropriate percentage of acetonitrile in 4 mM sodium tetraborate solution

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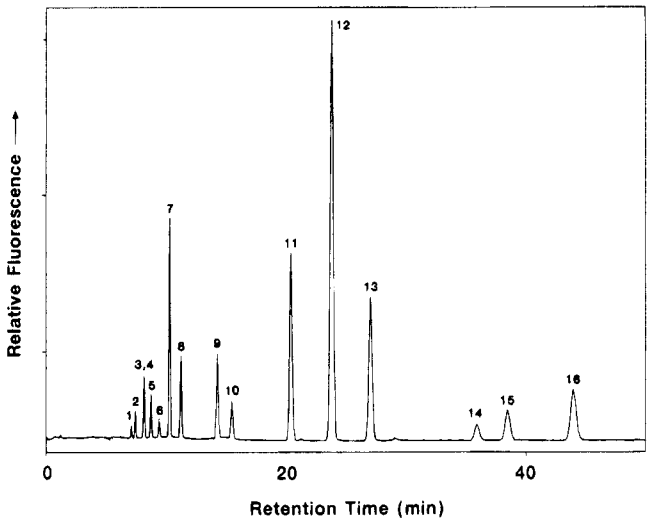

Figure 2. Electrochromatogram showing the capillary electrochromatographic separation of the 16 PAHs. The column dimensions were 75 μm i.d. × 365 μm o.d. (33-cm packed length). The mobile phase consisted of 80% acetonitrile in a 4 mM sodium borate solution. The applied voltage was 15 kV. Injection was performed electrokinetically at 5 kV for 5 s. The peaks are identified as follows (~10^{-6}–10^{-8} M of each compound): (1) naphthalene, (2) acenaphthylene, (3) acenaphthene, (4) fluorene, (5) phenanthrene, (6) anthracene, (7) fluoranthene, (8) pyrene, (9) benz[*a*]anthracene, (10) chrysene, (11) benzo[*b*]fluoranthene, (12) benzo[*k*]fluoranthene, (13) benzo[*a*]pyrene, (14) dibenz[*a,h*]anthracene, (15) benzo[*ghi*]perylene, and (16) indeno-[1,2,3-*cd*]pyrene.

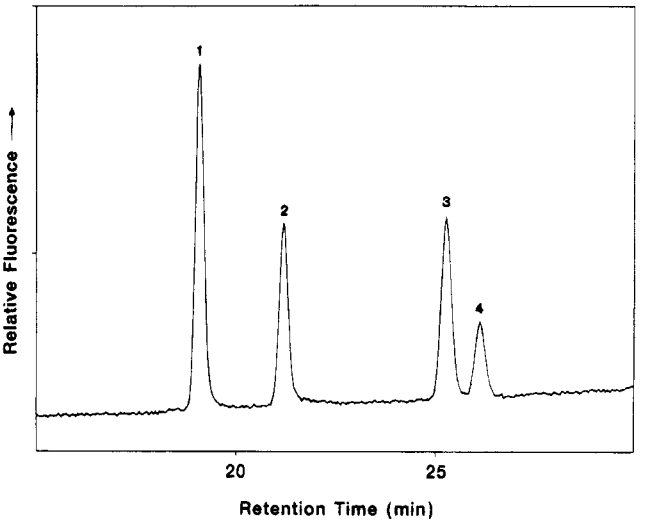

Figure 3. Electrochromatogram showing the separation of the first four PAHs. The conditions are the same as in Figure 2, except that the acetonitrile in the mobile phase has been changed to 60%.

and degassed before use. Water was purified with an Ultra-Pure water system from Millipore (Milford, MA). Stock solutions (1–10 mM) of the individual PAHs were first made in acetonitrile and then diluted to the desired levels in the mobile phase.

RESULTS AND DISCUSSION

Packing Capillary Columns. We found several factors to be important in consistently producing the packed capillary columns. The quality of the frits played a significant role in creating columns that generated reliable results. Overheating the frits caused blockage of the column, resulting in a reduction or elimination of the mobile phase flow, whereas insufficient heating caused the stationary phase particles to migrate through the frits. Initial efforts attempted to create a frit in a second piece of capillary that was attached to the outlet end of the packed column with a Teflon sleeve. Achieving a good connection was tedious and difficult. Therefore, we focused our attention on creating the outlet frit on-column, as described in the Experimental Section. In our experience, packing the columns electrokinetically[8] was more effective than packing the columns with a pump. We also added 1-μm pure silica particles (10%) to the 3-μm ODS particles (90%) in the column packing. This addition may have aided in stabilizing the electroosmotic flow, but no detailed studies were carried out. In addition, particular attention was paid to formulating the mobile phase, degassing it thoroughly to prevent bubble formation, and equilibrating the column before running the samples.

Efficiency and Selectivity. Figure 2 shows a typical electrochromatogram of the 16 PAHs and demonstrates the high efficiency of CEC. (The peaks were identified by comparing their retention times with those of standards run individually.) The numbers of theoretical plates per meter (calculated using the equation $N = 5.54(t_R/w_{0.5})^2$, where N, t_R, and $w_{0.5}$ represent the number of theoretical plates, the retention time of the analyte, and the width at half-height of the peak, respectively, and normalized per meter of column length) for acenaphthalene, fluoranthene, and benzo[*k*]fluoranthene, for example, are 110 000, 120 000, and 150 000, respectively. Because the detection window was created approximately 1–2 mm after the outlet frit, some dispersion of the peaks may result when the solutes pass from the packed section of the column to the unpacked section. Using a 150-μm-i.d. column (20-cm packed length) with a window created immediately prior to the outlet frit, efficiencies up to 400 000 theoretical plates/m were obtained. The higher efficiencies were obtained, however, at the expense of lower detection sensitivities caused by excessive light scattering from the packing particles.

Under the isocratic mobile phase conditions used in the run (80% acetonitrile in the 4 mM sodium tetraborate solution), 15 of the 16 components were baseline resolved. Two of the components, acenaphthene and fluorene, coeluted under these conditions. When the fraction of acetonitrile in the mobile phase was changed to 60%, we were able to separate these two peaks from each other (shown in Figure 3). Using the lower percentage of acetonitrile in the mobile phase, however, resulted in a large increase in the retention times for the later-eluting peaks. It is expected that a gradient elution procedure should allow the separation of acenaphthene and fluorene without significant expense in the total time of analysis.

Sensitivity, Linearity, and Reproducibility. Serial dilutions (10 times to 100 000 times) of the standard reference mixture were run to determine the minimum detection limits of the system. Table 1 lists the values of the detection limits for the PAHs obtained using the UV-LIF detection scheme.[11] The addition of acetonitrile to the mobile phase significantly enhanced the fluorescence intensities observed for the PAHs. Because the fluorescence wavelengths of the different PAHs vary greatly, a broad wavelength window (280–600 nm) was used to detect the emission. A slit was utilized to prevent background luminescence from the capillary walls from reaching the PMT. This luminescence from the fused-silica capillary wall occurs at >600 nm (measurements peformed using a monochromator). Conse-

(11) The values of the mass detection limits of the PAHs were calculated on the assumption that the packing particles occupied 75% of the volume in the packed section of the column.

Table 1. Detection Limits of the 16 PAHs Using UV-LIF[a]

PAH	concn (M × 10^{-10}) I	concn (M × 10^{-10}) II	mass (mol × 10^{-19}) I	mass (mol × 10^{-19}) II
naphthalene	32	64	220	450
acenaphthylene	30	40	210	280
acenaphthene	12	13	84	91
fluorene	5.3	5.8	37	41
phenanthrene	3.5	2.7	25	19
anthracene	1.7	0.73	12	5.1
fluoranthene	1.1	1.4	7.7	9.8
pyrene	3.3	2.0	23	14
benz[*a*]anthracene	1.3	0.50	9.1	3.5
chrysene	2.4	1.5	17	11
benzo[*b*]fluoranthene	0.57	0.53	4.0	3.7
benzo[*k*]fluoranthene	0.20	0.09	1.4	0.63
benzo[*a*]pyrene	0.43	0.27	3.0	3.0
dibenz[*a,h*]anthracene	3.7	2.2	26	15
benzo[*ghi*]perylene	2.0	1.3	14	9.1
indeno[1,2,3-*cd*]pyrene	1.2	15	8.4	110

[a] Two different filter arrangements were used: (I) a 280-nm longpass filter and two 600-nm shortpass filters and (II) a 70-nm (fwhm) bandpass filter centered at 400 nm.

quently, it may be more difficult to detect large PAHs that fluoresce in this region. It was observed that a 70-nm (fwhm) bandpass filter centered at 400 nm gave better limits of detection for most of the PAHs compared to the limits of detection obtained using the broader wavelength range (280–600 nm). The large variation in the limits of detection for the PAHs is caused by the difference in absorption of the PAHs at 257 nm and their different fluorescence quantum efficiencies. We believe that the sensitivity of our detection system was limited by background fluorescence from the mobile phase. This problem may be minimized in the future by using appropriate cleaning procedures (e.g., distillation of solvents) or by photobleaching the mobile phase before use.

The reproducibility in the peak heights of the PAHs and the linearity in the fluorescence response of the detection system were also measured. The variation in the peak heights (n = 3) was less than 5%. To measure the linearity, benzo[*k*]fluoranthene was chosen as a model analyte. The response was found to be linear between 2 × 10^{-7} and 2 × 10^{-11} M, with a correlation coefficient of 0.9995.

The reproducibilty in the retention times of the PAHs is demonstrated in Figure 4. The four electrochromatograms shown were taken over a period of 1 week with the same column. The relative standard deviations for the retention times of the peaks were less than 2%. We have obtained similar results with numerous other columns.

Comparison of CEC with Micro-HPLC and MECC. It is expected that efficiencies obtained in CEC should be higher than those obtained in micro-HPLC because of the pluglike profile of electroosmotic flow.[6] We compared the efficiencies obtained with a column run electrokinetically and the same column run with pressure (75-μm-i.d. column with a 33-cm packed length). The results are shown in Table 2. Under our experimental conditions, up to 75% higher efficiencies were obtained with CEC.

Terabe et al.[12] have also attempted the separation of these 16 PAHs with MECC. By using a high concentration of surfactant (100 mM SDS) in combination with cyclodextrins and urea, partial resolution between the components was achieved. The use of the high concentration of SDS and urea prevented us from taking advantage of LIF detection because of excessive fluorescence background.

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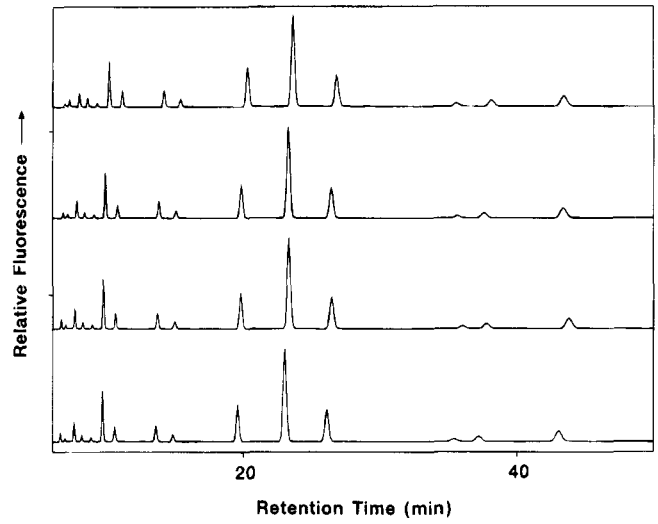

Figure 4. Four different electrochromatograms taken with the same column during the period of 1 week. The separation conditions are similar to those in Figure 2.

Table 2. Comparison of Efficiencies between CEC and Micro-HPLC

PAH	no. of theoretical plates (N)/m CEC	no. of theoretical plates (N)/m micro-HPLC
naphthalene	102 000	67 000
fluoranthene	132 000	85 000
benz[*a*]anthracene	137 000	89 000
benzo[*k*]fluoranthene	138 000	103 000

CONCLUSION

We have demonstrated that CEC can be used effectively in the laboratory to obtain high-efficiency, high-selectivity separations of neutral molecules. It combines the benefits of high-resolution capillary electrokinetic separations with the universality of liquid chromatography. In combination with a LIF detection system, subattomole detection limits can be achieved for PAHs on the basis of their native fluorescence following UV excitation. We believe the production of packed capillary columns can be achieved in a routine manner. This capability might allow CEC to achieve a growth rate that is similar to that which CZE has experienced over the past decade.

ACKNOWLEDGMENT

We would like to thank Gary Hux and Judy Rognlien for technical assistance. R.D. is grateful to Eli Lilly and Co. for sponsoring his ACS Analytical Division Summer Fellowship. This work was supported by a grant from the Defense Programs Technology Transfer Initiative, U.S. Department of Energy.

NOTE ADDED IN PROOF

Since this paper was accepted for publication, Prof. T. Tsuda has kindly informed us of his use of electrokinetic forces in preparing packed capillaries. See: Inagaki, M.; Kitagawa, S.; Tsuda, T. *Chromatography* **1993**, *14*, 55R–60R.

Received for review January 3, 1995. Accepted March 28, 1995.[⊗]
AC9500038

[⊗] Abstract published in *Advance ACS Abstracts*, May 15, 1995.