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Advances in Capillary Electrochromatography: Rapid and High-Efficiency Separations of PAHs

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Capillary columns packed electrokinetically with 1.5-μm nonporous octadecylsilica particles are used to achieve rapid separations with high efficiencies. A mixture containing five polycyclic aromatic hydrocarbons (PAHs) as test compounds is separated in less than 5 s with 28 kV applied to a column with a packed length of 6.5 cm (10 cm total length). A sample containing 16 PAHs (classified as priority pollutants by the U.S. Environmental Protection Agency) is isocratically separated in under 10 min by using longer columns (15−30 cm). Separation efficiencies greater than 700 000 theoretical plates/m are obtained when detection (via UV-excited laser-induced fluorescence) is performed on the packed portion of the capillary. The outlet frit is observed to play an important role in determining column efficiency. The optimum flow rate for best separation efficiency occurs at ~2 mm/s. The run-to-run reproducibility of the peak retention times on a single column is better than 1% (relative standard deviation).

Electrokinetic separations performed in capillary columns provide high-resolution and high-efficiency analyses of complex samples.[1,2] These separations include various formats such as capillary zone electrophoresis (CZE), capillary gel electrophoresis, and micellar electrokinetic capillary chromatography. A particularly powerful variation of this type of technology, capillary electrochromatography (CEC), involves packing the capillary column with chromatographic particles. This technique is of considerable interest because it combines the high efficiency of electrokinetic separations with the universality of liquid chromatography. Electrochromatography uses electroosmotic flow to pump the mobile phase through the chromatographic column and was initially demonstrated by Mould and Synge[3] and Pretorius and co-workers.[4] It was subsequently adapted to capillaries by Jorgenson and Lukacs.[5]

The early work in CEC utilized columns packed with particles that were 5−10 μm in size. Chromatographic theory predicts the efficiency to increase as the size of the particles used for the separation is decreased. This increase in efficiency is a result of reduced eddy diffusion and resistance to mass transfer as the particle size decreases. Hence, there has been a trend toward developing the ability to perform separations with smaller particles. However, the use of traditional pressure-based pumps to drive the mobile phase through columns packed with small (less than 3 μm) particles becomes increasingly difficult because of the high back pressures generated. As a result, by taking advantage of electroosmotic pumping to propel the mobile phase and the resulting high efficiency, CEC has been gaining momentum in recent years.

Although a variety of research has been previously reported in CEC,[5−29] of particular relevance to this work are reports of separations performed in columns packed with small (<3 μm)

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particles because of their superior performance. For example, Knox and Grant[8] first reported the benefits of CEC using columns packed with particle sizes down to 1.5 μm. They achieved a plate height of 2 μm using frontal analysis for an unretained solute in a column packed with 1.5-μm bare silica particles and envisioned columns producing separation efficiencies of 500 000 theoretical plates/m. Yamamoto et al.[11] utilized 1.6-μm octadecylsilica (ODS) particles for the separation of small organic compounds. They obtained a peak efficiency of 240 000 plates for thiourea migrating through a 68-cm-long column, corresponding to 353 000 plates/m. Smith and Evans[14] used a modified CEC apparatus with an external pressure applied to both the inlet and outlet reservoirs to minimize bubble formation. Using a column packed with 1.8-μm C8 particles, they obtained efficiencies of ~300 000−400 000 plates/m. They observed that the efficiencies obtained with their 1.8-μm particles were not significantly better than those they had obtained with 3-μm ODS particles. By using a combination of electric field and pressure applied to the column, Behnke et al.[21] demonstrated efficiencies of 50 000 plates for a 15-cm-long column packed with 1.5-μm ODS particles. This value corresponds to ~330 000 plates/m. Also, Schmeer et al.[22] used 1.5-μm particles in a CEC apparatus coupled to a mass spectrometer. However, column efficiencies were not reported in this case. Recently, Seifar et al.[29] performed CEC separations using 1.5-μm ODS particles and achieved efficiencies of 500 000 plates/m in columns having packed lengths of 24 cm. In their study, pressurization of the buffer vials and addition of a surfactant to the mobile phase were required to prevent drying of the columns.

In this study, we expand on our earlier work in CEC[16] and demonstrate that fast separations and high efficiencies are achievable with columns packed electrokinetically with 1.5-μm nonporous ODS particles. We have not found it necessary to either pressurize the buffer vials or add surfactant to the mobile phase. A sample mixture containing 5 of the 16 PAHs classified by the U.S. Environmental Protection Agency as priority pollutants is separated in 5 s. All of the 16 PAHs are resolved isocratically in less than 10 min, a feat that has required gradient elution and longer run times in the past.[25] Separation efficiencies up to 750 000 theoretical plates/m are obtained for the PAHs detected on-packing (prior to the outlet frit) and 300 000−400 000 plates/m for detection performed after the outlet frit. We observe that for these columns the optimal flow rate for best separation efficiencies is ~2 mm/s. Because the high-resolution nature of this technique allows the possibility of using short columns that lead to high-speed analyses, we expect CEC to be well suited for portable instrumentation and on-line process monitoring applications.

EXPERIMENTAL SECTION

CEC Apparatus. The apparatus used to perform the CEC separations is similar to that described previously.[16] Either a 0−60-kV (Glassman High Voltage, Inc., Whitehouse Station, NJ) or a 0−30-kV (Bertan High Voltage, Hicksville, NY) high-voltage power supply was used to provide the electric field for the separations. A manual syringe pump (Unimicro Technologies, Inc., Pleasanton, CA) was used for initially filling the capillary column with the mobile phase and for flushing columns. All injections of sample into the packed columns were performed electrokinetically. In the case of the faster separations, separate power supplies were used for electrokinetic injections and for providing the running voltage. No cooling system was used to dissipate heat from the column during runs, and the buffer vials were not pressurized in any of the experiments. The apparatus was enclosed in an interlocked clear plastic fixture for safety purposes.

UV-LIF Detection Apparatus. The apparatus used for detection of the PAHs is also similar to one described previously.[16,30] In brief, an intracavity-doubled argon ion laser (Coherent, Inc., Santa Clara, CA) operating at 257 nm was used for excitation of the PAHs. A high numerical aperture (NA) quartz microscope objective (40× magnification, NA = 0.85) in a confocal design was used for fluorescence collection from the capillary. A variable slit and a set of filters transmitting wavelengths between ~280 and 600 nm were placed between the objective and a photomultiplier tube (PMT) to minimize interference to the fluorescence signal from the capillary wall fluorescence and scattered light. 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 1−120 Hz.

Preparation of Packed Capillary Columns. Electrokinetically packed capillary columns with different inside diameters (75−100 μm) were either packed in-house using the packing procedure developed previously[16,31] or provided by Unimicro Technologies, Inc. (Pleasanton, CA). To make the columns in-house, an initial frit was formed by sintering a silica gel plug in the end of the column. A mixed suspension of the 1.5-μm ODS particles (90%) and 1-μm silica gel (10%) in 2-propanol was then packed electrokinetically into the column. A second frit consisting of fused ODS particles was made in the column after the packing procedure to hold the particles firmly in place. Either a resistively heated thermal wire stripper or a fiber-optic splicer (Fiberline IFS-2001, Preformed Line Products, Mayfield Village, OH) was used to make the second frit at the required distance from the inlet frit. Extraneous particles between the second frit and the outlet of the column were then flushed out, typically using a pressure of ~500 psi. A narrow window (~1 mm) for detection was created generally within a few millimeters after the packed portion of the column by burning off the polyimide coating. The flushing process was continued until bubbles that were generated during the making of the window and extraneous particles exited from the end of the capillary. For some experiments involving on-packing detection, the window was created just prior to the frit on the packed section by dripping hot sulfuric acid on the polyimide coating. The column was flushed with the selected mobile phase before performing the separations using electro-

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osmotic flow. The flushing process was considered complete when the column current and fluorescent background became constant, usually after ~1 h.

Materials and Reagents. The fused-silica capillaries were purchased from Polymicro Technologies, Inc. (Phoenix, AZ). The 1.5-μm nonporous ODS particles were provided by Micra Scientific, Inc. (Northbrook, IL) and the 1-μm silica gel was obtained from Phase Separation, Inc. (Norwalk, NJ). 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 (NIST). Sodium tetraborate, TRIS, acetonitrile (HPLC grade), 2-propanol, and individual PAHs were purchased from Aldrich Chemical Co. (Milwaukee, WI). The mobile phase was prepared by mixing the appropriate percentage of acetonitrile (by volume) in 2−4 mM (pH 9) sodium tetraborate or TRIS solution and thoroughly degassed by ultrasonication and vacuum before use. Typically, a vacuum of 50 Torr was applied during ultrasonication until the initial frothing due to degassing subsided (usually less than 1 min). Then ultrasonication continued for 10 min without application of vacuum. Water was purified with an Ultrapure 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

The packed capillary columns were evaluated for several performance characteristics. We were particularly interested in two factors: (1) the ability to perform fast separations because of the nonporous nature of the particles resulting in high flow rates and (2) the ability to obtain high separation efficiencies because of the small particle size. The retention time reproducibility of the columns and the loading capacity in the columns were also evaluated. The results are discussed below.

Speed. Separations were performed using a short column packed with the 1.5-μm particles (6.5-cm packed length and 10-cm total length) under different electric fields with a sample containing five PAHs. The resulting electrochromatograms are shown in Figure 1 for applied voltages ranging from 5 to 25 kV. Even though these electric fields (greater than 2500 V/cm in the packed part of the column) are higher than those typically used in CZE or previously used in CEC, we were able to obtain reproducible separations under these conditions and did not have problems caused by bubble formation or drying of the column.

Figure 2 shows a separation of the PAHs under an applied voltage of 28 kV. Under these conditions, the linear velocity of the mobile phase through the packed part of the column is 18 mm/s. The five PAHs are resolved in less than 5 s. This time scale for a separation is the shortest that we are aware of for packed capillary columns. However, it should be noted that even faster separations for a few charged components have been reported using open columns in CZE.[32−34]

Although we were successful in running the columns at voltages up to 30 kV, we began to experience occasional arcing

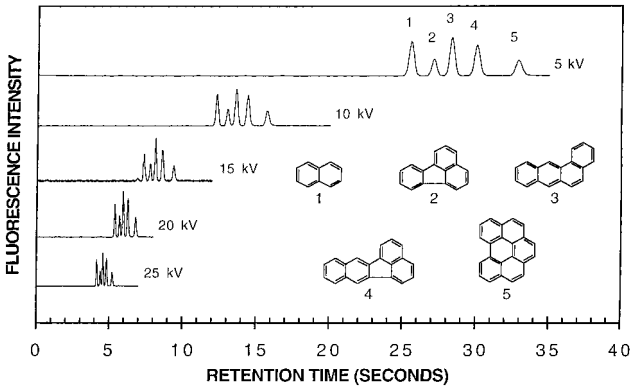

Figure 1. Electrochromatograms showing the CEC separation of five PAHs using 1.5-μm nonporous ODS particles. The column dimensions were 100-μm i.d. × 6.5-cm packed length (10-cm total length). The mobile phase consisted of 70% acetonitrile in a 2 mM TRIS solution. The applied voltage for the separation was varied for the different runs. Injection was performed electrokinetically at 5 kV for 2 s. Peaks: (1) naphthalene, (2) fluoranthene, (3) benz[*a*]-anthracene, (4) benzo[*k*]fluoranthene, and (5) benzo[*ghi*]perylene.

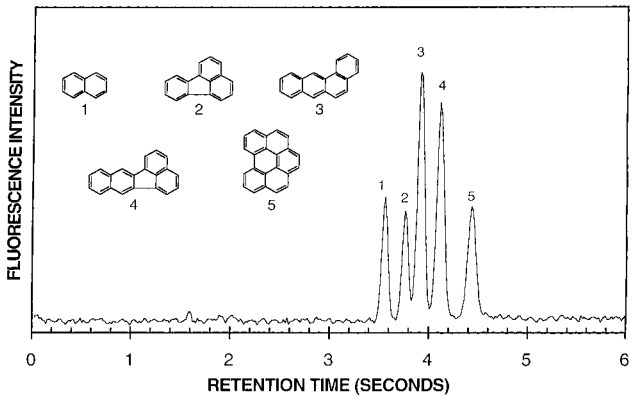

Figure 2. Electrochromatogram showing the rapid CEC separation of five PAHs using 1.5-μm nonporous ODS particles. The run conditions are the same as in Figure 1. The applied voltage for the separation was 28 kV. Note that the linear velocity of the mobile phase was ~20 mm/s.

caused by the proximity of the high-voltage electrode to the capillary holder. The arcing led to inconsistent results and interfered with detection. The column, however, was still running well and continued to operate when the voltage was lowered again below the threshold for arcing.

One factor that allowed the operation at high electric field was the use of mobile phases with low conductivity (acetonitrile mixed with 2 mM TRIS in this case). At 30 kV, the current through the column was 6.7 μA. A plot of current versus voltage (5−30 kV) showed a positive deviation from linearity amounting to ~20% at 30 kV. At this voltage, 0.20 W was dissipated in the column due to Joule heating. This power corresponds to more than 20 mW/cm in the packed portion of the column. We attribute successful operation at these power levels, in part, to the thorough degassing of the mobile phases before use.

Calculation of the theoretical plate numbers (and corresponding plate heights) for the peaks in Figure 1 showed that this column was reasonably efficient at the high mobile-phase linear velocities used for these separations. The equation $N = 5.54(t_r/w_{0.5})^2$ was used, where N, t_r, and $w_{0.5}$ represent the theoretical

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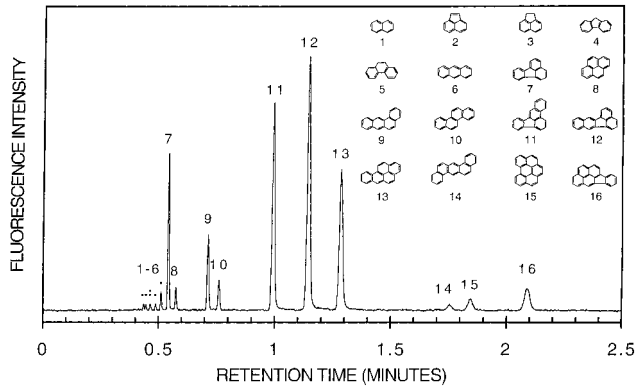

Figure 3. Electrochromatogram showing the CEC separation of 16 PAHs using 1.5-μm nonporous ODS particles. The column dimensions were 100-μm i.d. $\times$ 20-cm packed length (30-cm total length). The mobile phase consisted of 70% acetonitrile in a 2 mM TRIS solution. The applied voltage for the separation was 55 kV. Peaks ($\sim 10^{-6}$–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. Note that peaks 3 and 4 coelute.

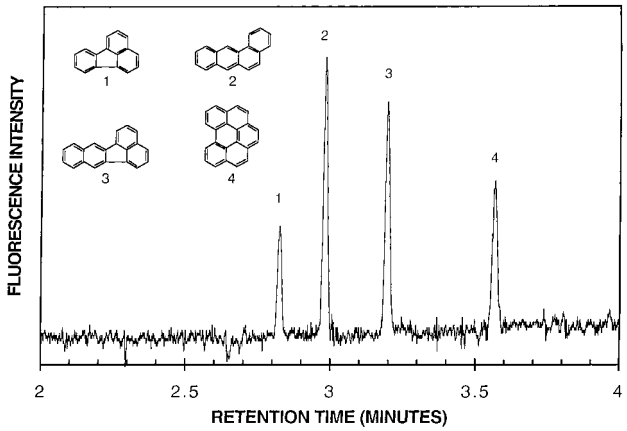

Figure 4. Electrochromatogram showing the high-efficiency CEC separation of four PAHs using 1.5-μm nonporous ODS particles. The column dimensions were 100-μm i.d. $\times$ 28-cm packed length (36-cm total length). The mobile phase consisted of 70% acetonitrile in a 4 mM sodium tetraborate solution. The applied voltage for the separation was 20 kV. Injection was performed electrokinetically at 5 kV for 2 s. Peaks: (1) fluoranthene, (2) benz[*a*]anthracene, (3) benzo[*k*]fluoranthene, and (4) benzo[*ghi*]perylene.

plate number, the retention time of the analyte, and the width of the peak at half-height, respectively. The theoretical plate number was divided by the column length to obtain the number of theoretical plates per meter. At 5 kV, where the linear velocity was 2.5 mm/s, the plate heights for the five peaks ranged from 2.4 to 3.0 μm (reduced plate height of 1.6 to 2.0). The plate heights increased with increasing linear velocity. At 25 kV they ranged from 3.9 to 5.1 μm (reduced plate height of 2.6 to 3.4) at a linear velocity of 16 mm/s.

The peak widths (full width at half-height) in Figure 1 range from 80 to 500 ms. They are not limited by the duration the electrokinetic injections, which were performed by momentary application of 1 kV to the sample vial. For all the peaks in Figure 1, an injection time of 2−3 s at 1 kV would have been required to account for the total peak width. (This calculation is done by scaling the observed width of the peak by the ratio of the running voltage to the injection voltage.) Since 2−3 s is significantly longer (more than 10-fold) than the injection time used in these separations, the peak width arises from other sources.

With such narrow peaks, it is necessary to adjust correctly the conditions for data acquisition. For the fastest runs, the lock-in amplifier time constant was set to 3 ms and the software sampling period was set to 8.3 ms. This allowed for sufficiently fast response to the peaks in the electrochromatogram and adequate sampling to characterize them.

Efficiency and Selectivity. For the analysis of more complex samples, longer columns are required. A column packed with the 1.5-μm particles and having a 20-cm packed length (30-cm total length) was used to obtain the electrochromatogram of the priority pollutant mixture containing the 16 PAHs shown in Figure 3 (two of the PAHs, acenaphthene and fluorene, coelute under these separation conditions). The peaks were identified by comparing their retention times with those of standards run individually. The theoretical plate numbers for the peaks in this separation range between 300 000 and 400 000 plates/m. This electrochromatogram was obtained by performing the detection approximately 0.5−1 mm after the outlet frit. As we[16] and others[10,28] have observed previously, the outlet frit can be a source of dispersion of the analyte bands as they migrate through the frit. By performing detection just prior to the frit on the packing material itself, we were able to obtain efficiencies of 600 000−750 000 plates/m for the PAHs. This is shown in the electrochromatogram obtained using on-packing detection in Figure 4. As expected, there is significantly increased background and noise resulting in poorer signal-to-noise ratio in the electrochromatogram. This increase arises from scatter of the excitation laser light by the particles and fluorescence of the packing material.

The loss of separation efficiency caused by the frit is not clearly understood. Two possible causes are nonuniformities in the frit or adsorption of analyte molecules on the surface of the particles in the frit, both resulting from the sintering process used in creating the frit. It is possible to avoid this problem by using fritless columns for CEC produced by other methodologies.[35,36] However, these types of columns have not demonstrated to date the separation selectivity that is obtained with columns packed with chromatographic particles, such as those used in this study. A third possible contribution to broadening after the outlet frit arises from mismatches in the volumetric flow of the mobile phase between the packed part of the column and the open part. For example in flow visualization experiments, Paul et al. have observed a pressure-driven component of the fluid flow immediately after the outlet frit in a packed CEC column.[37,38]

Figure 3 demonstrates the high resolving power of CEC using such columns resulting in short analysis times. A previous study[16] by us utilized porous 3-μm ODS particles to analyze the same 16 PAHs. The CEC separation under those circumstances took ~45

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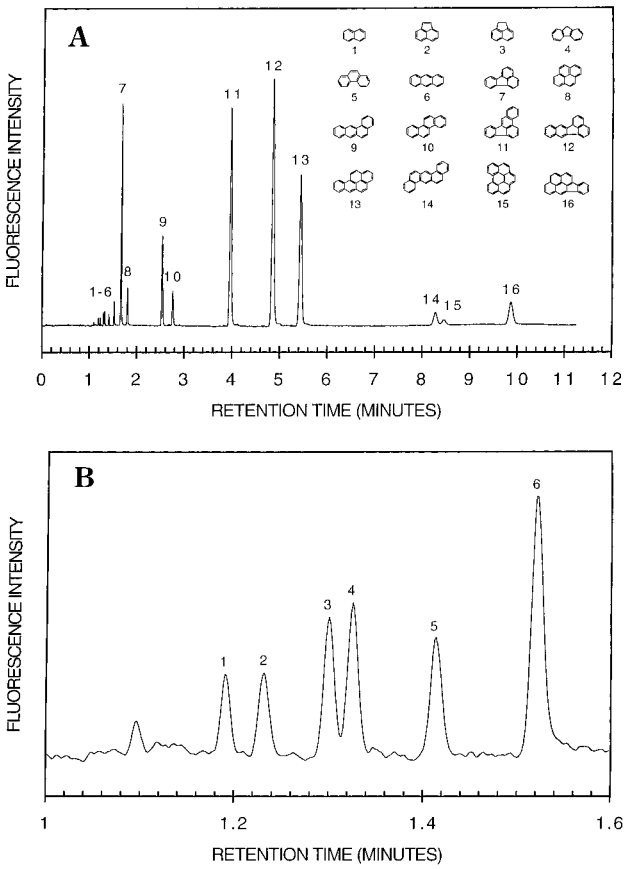

Figure 5. (A) Electrochromatogram showing the CEC separation of 16 PAHs using 1.5-μm nonporous ODS particles. The column dimensions were 100-μm i.d. × 20-cm packed length (30-cm total length). The mobile phase consisted of 65% acetonitrile in a 2 mM TRIS solution. The applied voltage for the separation was 29 kV. The PAHs are the same as in Figure 3. (B) Expanded version of part A showing the peaks between 1.0 and 1.6 min in the electrochromatogram. Note that peaks 3 and 4 are resolved.

min, with acenaphthene and fluorene coeluting also. A further study by us[25] utilized an electroosmotically driven gradient elution system to separate all of the 16 PAHs in a single run, with the analysis taking ~100 min. We show in Figure 5A an isocratic separation of the 16 PAHs with all of the peaks resolved in less than 10 min. Figure 5B shows a magnified view of the separation between 1.0 and 1.6 min. This characteristic of higher efficiency (and higher resolution) obtained with the 1.5-μm ODS packed columns may circumvent the need for gradient elution in certain applications.

The effect of the separation voltage and, thereby, the mobile-phase flow rate on the separation efficiency was determined. The results are shown in Figure 6 for a few test PAHs. It was observed that the best efficiencies for these columns under our separation conditions were obtained for flow rates of ~2 mm/s. The downward trend in the plate heights for flow rates up to 2 mm/s is in agreement with previous studies performed by others.[10,11,29] The previous studies, however, did not make observations at flow rates greater than 2.5 mm/s. Even under the extreme conditions used to obtain the electrochromatogram in Figure 2 (corresponding to a flow rate of 18 mm/s), efficiencies of 200 000−250 000 plates/m are achieved in the separation.

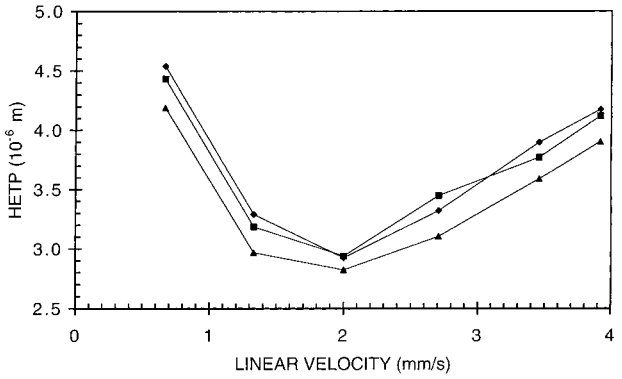

Figure 6. Effect of the linear velocity (electroosmotic flow rate) on the height equivalent to a theoretical plate (HETP) for three PAHs.

Reproducibility. A series of 13 electrochromatograms was taken with the same column. The relative standard deviations for the retention times of the peaks were less than 0.8%. Other columns we have tested performed in a similar manner. We expect that some variability is introduced by the manual column manipulation and that automation of CEC will result in better reproducibility.

Sample Loading Capacity. Because of the nonporous nature of these particles, the amount of sample that can be loaded onto the column was of concern to us. We did observe some broadening of the peaks when sample concentrations greater than 10^{-3} M were injected. This issue necessitates the use of detectors that have limits of detection well below 10^{-3} M for use with these type of particles in CEC. The highly sensitive laser-induced fluorescence detection used in this work, which has detection limits ranging from 3.2×10^{-9} to 2.0×10^{-11} M for PAHs[16] alleviates this problem.

CONCLUSIONS

We have demonstrated that columns packed with 1.5-μm particles can be used effectively in CEC to obtain fast and high-efficiency separations. When these particles are used in short columns, extremely fast separations can be performed. This rapidity provides sensorlike characteristics (fast response time) while retaining the benefits of a chromatographic method (ability to analyze simultaneously multiple components). In the current arrangement, sample throughput is severely limited by the time required to perform manual injections. These columns would benefit from automated injections to fully exploit the speed of the separation. Cross-type injectors similar to those used in micromachined separation schemes,[39] flow-gated approaches,[40] or microcross injections[41] would enable rapid sampling and provide nearly real-time monitoring of liquid samples.

In longer columns, high-efficiency separations of complex samples may be performed on the time scale of several minutes. The utility of using electroosmosis as a means for driving the mobile phase in chromatography will become increasingly important as even smaller particle sizes and longer columns are used.

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