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Butyl acrylate porous polymer monoliths in fused-silica capillaries for use in capillary electrochromatography

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Abstract

Capillary electrochromatography incorporates features of both capillary electrophoresis and liquid chromatography. Butyl acrylate polymers, cast in-situ with heat initiated polymerization and no retaining frits have been made. Van Deemter plots of chrysene have been examined at a variety of operating temperatures to examine column behavior. H_{min} moves to faster flow-rates and increases slightly in magnitude as temperature is increased. The longevity and reproducibility of the columns have been examined with a homologous series. Performance is very reproducible between two different columns of different diameters, operated on different systems and prepared from the same polymeriation batch. The relative standard deviation of retention factors is a maximum of 3.1% with most values calculated at less than 1%. The uniformity of the polymers as a function of length has also been studied with a series of polycyclic aromatic hydrocarbons, and the columns have proved to be very uniform across their length as measured by the consistency of retention factors with a maximum relative standard deviation of 3.4% and most values calculated between 1 and 2%. Plate numbers of between 65 000 and 80 000 plates/m have been attained for compounds with retention factors of 3 to 12. These columns have proved easy to make, are quite reproducible, and long lived.

Keywords: Monolithic columns; Electrochromatography; Stationary phases, electrochromatography; Butyl acrylate; Polynuclear aromatic hydrocarbons

1. Introduction

Capillary electrochromatography (CEC) is a blending of liquid chromatography and CE. The technique utilizes a stationary phase as in high-performance liquid chromatography (HPLC), but unlike HPLC which utilizes pressure driven flow, transport through the stationary phase is achieved as it is in CE with electroosmotic flow. The advantages of this transport method have been known for some time [1–3]. Separation is based on interactions of the analyte with the phase in the case of neutral analytes, or in the case of charged analytes, on a combination of electrophoretic and chromatographic processes.

There are several approaches to making CEC columns. A number of review articles and books have appeared in print [4–6] describing these methods. The first attempts at CEC involved packing small diameter capillaries with chromatographic particles. This technique requires that retaining frits be used to keep the stationary phase material in place in the column. Frits can be problematic to construct reproducibly, and can contribute to zone broadening and analyte adsorption. Frits can be the site of mobile phase degassing. Detection is also by necessity post column in these approaches and this can

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further contribute to zone broadening. Packing irregularities can contribute to the reduced performance of these systems. And finally, the resulting ''duplex'' nature of the columns, where a portion is packed and a portion is unpacked, complicates the characterization of electroosmotic flow and can have deleterious effects on separations [7,8].

Despite these drawbacks, these approaches are often superior to traditional HPLC. Unlike pressure driven flow, which has a parabolic flow profile, electroosmotic flow has a flat flow profile and does not contribute to zone broadening [1]. CEC thus has the potential to yield separations with higher efficiencies than HPLC. The combination of chromatographic and electrophoretic separation mechanisms provide for different and unique selectivity over either of the individual techniques. This promise of great efficiency and multifunction separation mechanisms has prompted continued interest in the development of CEC approaches, and has led to the development of another column construction method, the sol–gel approach. Here a silica monolith with a uniform structure is formed as a result of hydrolytic polycondensation of alkoxysilanes. The silica structure can later be silyated for added functionality [5–9].

Yet a third, and newest method of column preparation involves the in-situ polymerization of an organic polymer monolith. Acrylate [10–13], styrene [14], and acrylamide [15] are among the most popular materials used. Reviews devoted to this particular type of column preparation have appeared in the literature [16–18]. The capillary is filled with an unpolymerized solution. Polymerization is initiated with UV light or heat. Chemical initiators have also been used [19]. Following polymerization the columns are typically flushed and treated to remove unreacted components. The result is a porous polymer monolith (PPM) with a narrow distribution of pore sizes. Analytes are transported through the column by electroosmotic flow or a combination of electroosmotic flow and electrophoretic mobility. Packing irregularities are reduced or eliminated. Frits are unnecessary. Detection can be accomplished on-column or immediately adjacent to the column, thereby eliminating much of the post column broadening seen in earlier methods. Variation in monomers, ratios of monomers to cross linkers, and choice of porogens all alter the structure and performance of the resulting monoliths.

The in-situ polymerization method has numerous advantages over earlier approaches. The polymerization mixture can be altered to add additional functionality for selectivity [20] to control and adjust the magnitude and direction of the electroosmotic flow [21,22] to adjust the resulting pore size of the polymer [23,24]. Chiral separations can be achieved either by using chiral monomers [25,26] or incorporating cyclodextrins [27]. Polymerized systems are easily constructed both in capillary columns and on chips thereby increasing the portability of separation systems as well as their ''disposability'' [28].

The further development and characterization of CEC will open whole new areas of investigation and utility that previously were impossible, difficult, or limited with chromatographic and CE systems. As researchers are just beginning to investigate this area, much about CEC systems remains unknown. Basic performance characteristics are only beginning to be investigated. The extent to which a monomer solution can be altered and still yield useful columns, the degree and variety to which selectivity enhancers can be included has not been fully investigated. Only a limited number and types of CEC in-situ polymerized systems have been tested to date.

The work described here for producing PPMs is based on that of Ngola et al. [28] of Sandia National Laboratories. This is a ''conduct-as-cast'' polymer, meaning once polymerization is complete, electroosmotic flow only is used to move new mobile phases through the column. Pressure is not used. A key difference in the approach described here is that the published method relies on initiating the polymerization with UV irradiation. While this has advantages for chip based systems, it requires the use of Teflon coated capillaries when producing columns. Teflon coated capillaries have decreased flexibility compared to polyimide coated fused-silica. This makes them very difficult to load into commercial instrumentation, and capillary lifetime is decreased due to the fragility of the capillaries. This method has been adapted by initiating the polymerization with heat. This allows for the use of polyimide coated capillaries. These capillaries are durable and perform well. It has been reported that polymerizations initiated by heat yield columns with smaller pores and greater selectivity [26]. Thus the same chromatographic conditions may not be achievable with the two different polymerization proce-

dures. The UV initiated polymerization method previously described [28] also makes use of an ''adhesion promoter'' to anchor the polymer to the chip or capillary material. In the method described here, the polymer is chemically bonded to the capillary wall with a silane. In addition, the columns used in this study were used for many analyses over several weeks. Reproducibility information is provided for different polymer preparations and for different columns made from the same polymer batch and used on different instrumental systems.

2. Experimental

2.1. Apparatus and reagents

Fused-silica capillaries were purchased from Polymicro Technologies (Phoenix, AZ, USA). Capillaries with inner diameters of 75 μm, and 100 μm were used. Capillary length varied.

Two different CE systems were used. One was modular in nature and consisted of Spellman CZE 100R (Plainview NY, USA) power supply with the maximum current output set to 100 μA, and a Linear Instruments UVIS 200 detector (Reno, NV, USA). The system included an interlock system for operator safety. Detection was at 214 nm. The system was controlled by a Dell Optiplex GX1P (Austin, TX, USA) fitted with a multi-function data acquisition board from National Instruments (Austin, TX, USA). Software was written in-house with National Instrument's LabView software. Capillary length and homologous series studies were performed on this instrument. The second system was a Beckman-Coulter (Fullerton, CA, USA) P/ACE MDQ with version 1.6 software. Temperature and homologous series studies were performed on this instrument.

A Barnstead Nanopure system (Fisher Scientific, Austin, TX, USA) provided deionized water for all experiments. All reagents were purchased from Sigma–Aldrich (St. Louis, MO, USA) with the exception that thiourea and naphthalene were obtained from Fisher, and a US Environmental Protection Agency (EPA) standard of 16 polycylic aromatic hydrocarbons (PAHs) from the US National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) was generously provided by Yolanda Fintschenko of Sandia National Laboratories, Livermore. All reagents were used as received. It was not found necessary to remove inhibitors prior to polymerization.

2.2. Buffer and sample preparation

Aqueous Tris buffers were diluted to volume and the pH adjusted to the desired value with 5 *M* NaOH. The buffers were then mixed with acetonitrile for the various organic–aqueous ratios. Conditioning buffer was an acetonitrile–20 m*M* Tris (80:20, v/v) mixture. Running buffer was an acetonitrile–5 m*M* Tris (75:25, v/v) mixture. Analyte stock solutions of fluorene, chrysene, and benzo[a]pyrene were made at 5 m*M* in an acetonitrile–5 m*M* Tris (75:25, v/v) solution. Analysis samples were made by mixing fluorene–chrysene–benzo[a]pyrene–running buffer (1:3:3:8). Thiourea at 0.0005 g per sample was added to mark the electroosmotic flow. All samples were filtered with 0.2 μm Acrodisk filters before use. For the PAH study, 5 m*M* stock solutions of napthalene, phenanthrene, anthracene, and fluoranthene were prepared in the 75:25 solution. Various ratios of these solutions were used to spike the NIST PAH sample for purposes of peak identification. Stock solutions of 5 m*M* of toluene, propylbenzene, butylbenzene, amylbenzene, 1-phenylhexane, 1-phenylheptane, 1-phenyloctane, and 1-phenylnonane were each made in the 75:25 solution. A 10 m*M* solution of ethylbenzene was made. Injections samples were made by mixing 100 μl of each stock solution (50 μl of ethylbenzene) with 850 μl of the 75:25 solution. Thiourea at 0.0005 g was added to each injection sample.

2.3. Capillary and polymer preparation and run conditions

Fused-silica capillaries were initially derivatized as follows: 1.0 *M* NaOH for 5 min, stand in 1.0 *M* NaOH for 10 min, water rinse, air rinse, water rinse, air rinse, 1.0 *M* HCl rinse each for 2 min, air rinse for 5 min, toluene for 10 min, 10% 3-methacryloxypropyltrimethoxysilane (MPTS) in toluene for 8 min, stand in 10% MPTS in toluene for 2 h, rinse with toluene for 5 min, air rinse for 5 min. Solutions were aspirated through the capillaries with Nalgene hand pumps (Fisher, Austin, TX, USA). Capillaries de-

rivatized in this manner were stored in a refrigerator for typically 1 day but occasionally for as long as 1 week before being filled with a polymerization mixture. This derivatization procedure was based on a literature report [29].

Polymers were cast in derivatized capillaries. A casting solvent was prepared by mixing 200 μl ethanol, 600 μl methylenechloride, and 200 μl of 5 m*M* pH 6.8 phosphate buffer. The monomer solution was prepared by mixing 300 μl of 1,3-butanedioldiacrylate (BDDA) as the crosslinker, 695 μl butyl acrylate as the monomer, 5 mg of 2,2′-azobisisobutyronitrile as the free radical polymerization initiator, and 5 mg of acrylamido-2-methyl-1-propanesulfonic acid to support electroosmotic flow. A 67:33 (v/v) solution of casting solvent to monomer solution was mixed and sonicated for about 8 min. The solution was pushed through the capillaries with nitrogen pressure. The use of an aspirator in filling the capillaries with the polymerization solution created problems with gas bubble formation. The capillary ends were sealed with rubber stoppers. The capillary and remaining polymer solution (in a sealed glass vial) were placed in a 60 °C water bath for 20 h. Inspection of the glass vial helped to confirm that polymerization was complete and satisfactory. This polymerization procedure is based in part on a literature report [28].

After polymerization, the capillaries were inspected under a microscope and if they were found to be free of air bubbles, they were cut in length, a short section of the polyimide coating was removed with a drop of fuming sulfuric acid and the capillary was placed into the CE system. The capillaries were further conditioned prior to use by placing the ends of the capillary in an acetonitrile–20 m*M* pH 8.5 Tris buffer (80:20, v/v). The capillary was illuminated at 214 nm, and voltage was applied at 200 V/cm until a detection window was noted. Under these conditions a short section of the polymer depolymerizes to create a stable polymer-free detection zone. Residual monomeric material is also removed during this step and the capillary solvent system is exchanged for that in the end vials. This process can take from 12 to 48 h. The end vials can then be filled with the solution used for analysis. A stable baseline is typically obtained after one or two conditioning analyses. Columns produced in this manner were stable for at least 1 month and provided reproducible data for over 70 injections. The ends of columns were stored in water between uses. Capillaries have been routinely stored for at least 1 week between uses. Degradation of the polyimide capillary coating has not been an issue with this procedure.

3. Results and discussion

SEM images of columns are shown in Fig. 1 for several magnifications. The 225 power magnification (Fig. 1A) shows the relative size of the 75 μm I.D. column to its 365 μm O.D. polyimide coating. The 850 power magnification (Fig. 1B) shows that the polymer is bonded to the capillary wall. This column has an inner diameter of 100 μm. The 1800 power magnification (Fig. 1C) was used to estimate the polymer nodule diameter at 3 μm. It is an image of the same capillary as is shown in Fig. 1B. The column in Fig. 1A was prepared from a different polymerization batch from that shown in Fig. 1B and C.

An image of the UV depolymerized detection window is shown in Fig. 2. The diameter of the capillary is 100 μm and the image shows the sharp transition between polymer and polymer-free region. Previously we have simply filled the capillary to the desired length with the polymerization mixture as others have done [30]. However, that method sometimes results in polymers with tapered ends. This small detection window, typically about 250–300 μm long, is stable for the duration of the column lifetime. Windows were never observed to change in size or location, indicating that the polymer is well bonded to the capillary wall and that the polymer itself is stable.

Fig. 3 shows a chromatogram of fluorene, chrysene, and benzo[a]pyrene. Thiourea is the electroosmotic flow marker. This data was obtained on the P/ACE MDQ instrument and the capillary length from injection to detection is 40 cm. The applied run voltage is 20 kV. Other run conditions are given in the figure caption. The results shown in Fig. 3 were obtained as part of the study on operating temperature. Analysis time is short and peak shape is excellent.

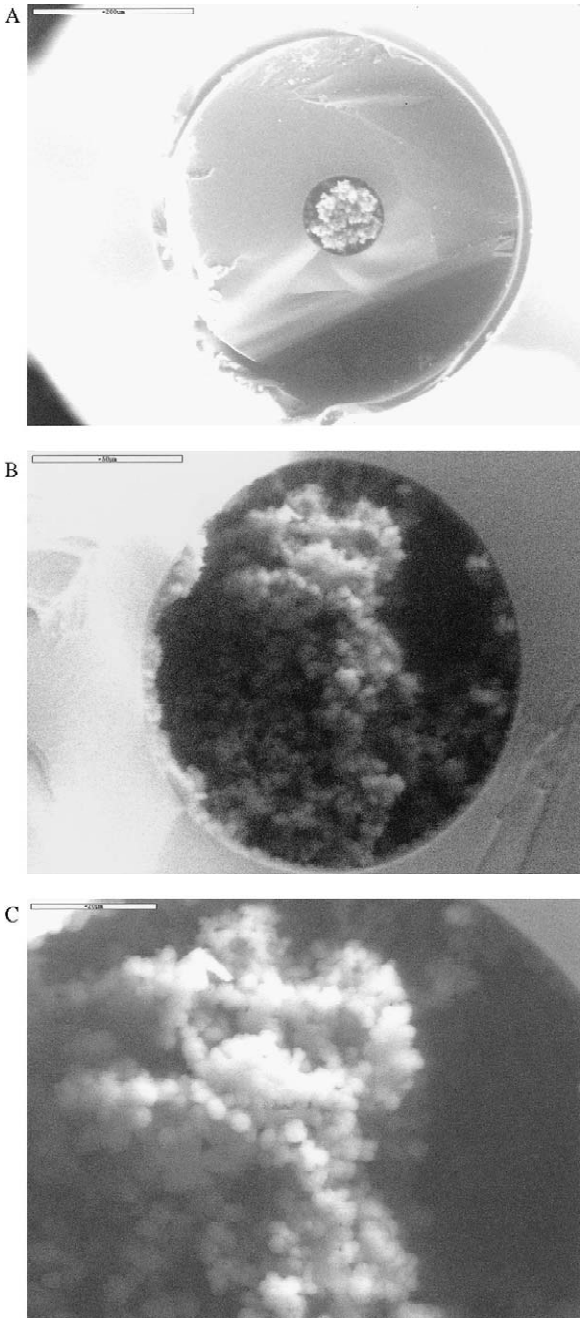

Fig. 1. (A) SEM of 75 μm I.D. capillary, ×225 magnification. Bar inset is 200 μm. (B) SEM of 100 μm capillary at ×850 magnification. Bar inset is 50 μm. Note the appearance of the polymer on the right side of the image where the polymer is chemically bonded to the fused-silica wall. (C) SEM of same 100 μm capillary shown in (B) at ×1800 magnification. Bar inset is 20 μm.

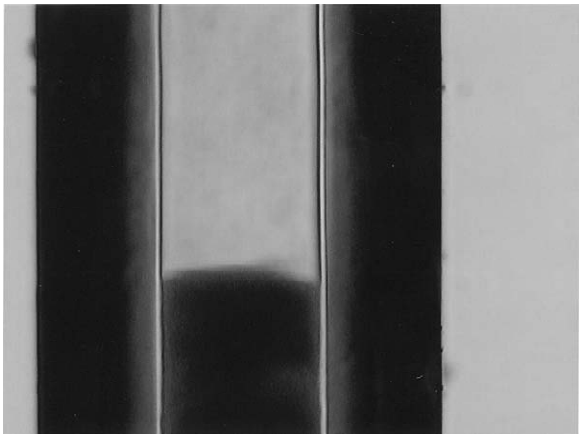

Fig. 2. Image of 100 μm capillary showing the transition from polymer to polymer-free region in the detection window following UV depolymerization.

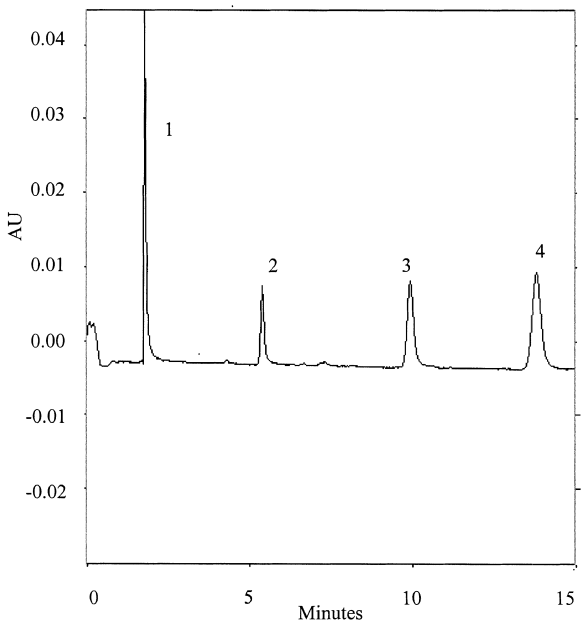

Fig. 3. Chromatogram of (1) thiourea (electroosmotic flow marker), (2) fluorene, (3) chrysene and (4) benzo[*a*]pyrene on a butyl acrylate column. Injection: 6 kV for 3 s 214 nm detection, 100 μm I.D., 20 kV run voltage at 55 °C. Column length 40 cm to detector, 50 cm overall. Buffer acetonitrile–5 m*M* Tris, pH 8.57 (75:25, v/v). 25 °C. Obtained on P/ACE MDQ.

Fig. 4A is a chromatogram of 16 PAHs. These PAHs were provided by Sandia National Laboratories and are the same test analytes shown in a recent

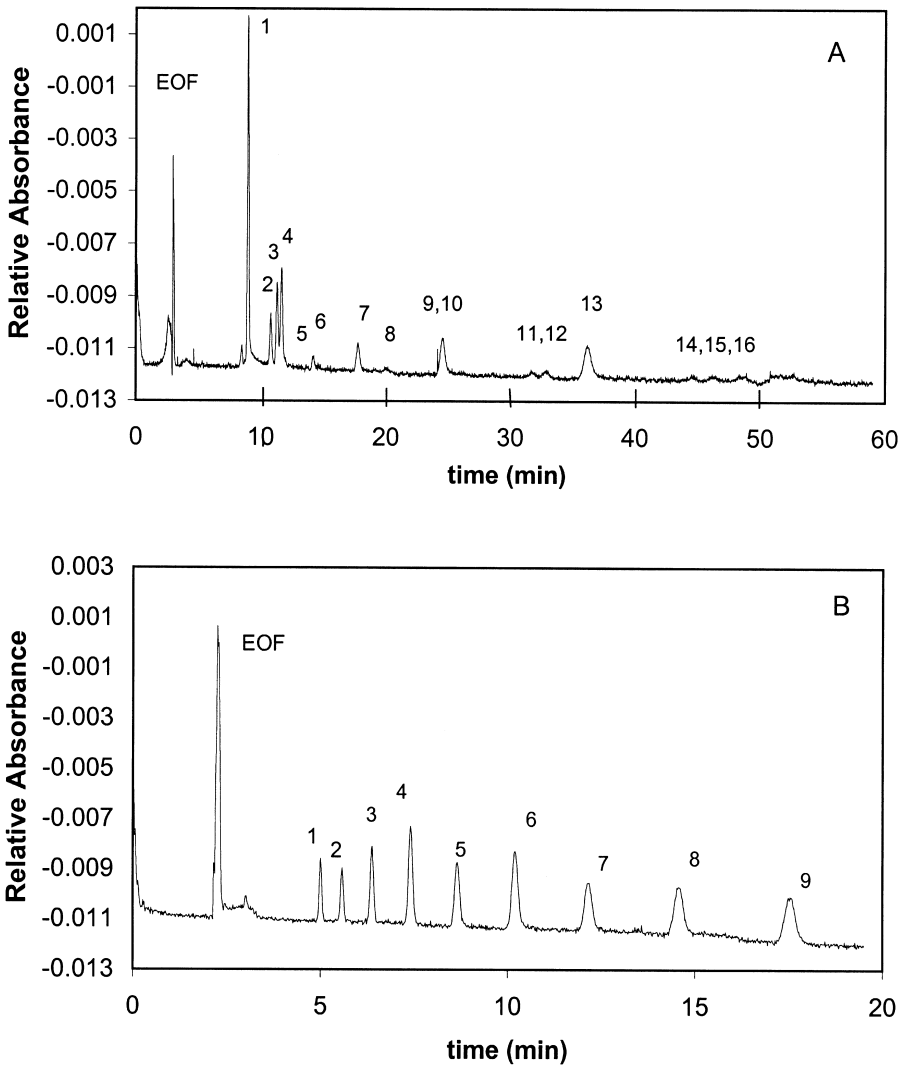

Fig. 4. (A) Chromatogram of 16 PAHs. (1) Naphthalene, (2) acenaphthalene, (3) acenaphthene, (4) *fluorene*, (5) phenanthrene, (6) anthracene, (7) fluoranthene, (8) pyrene, (9) benzo[*a*]anthracene, (10) *chrysene*, (11) benzo[*b*]fluoranthene, (12) benzo[*k*]fluoranthene, (13) *benzo[a]pyrene*, (14) dibenz[*a,h*]anthracene, (15) benzo[*ghi*]perylene, and (16) indeno[1,2,3-*cd*]pyrene. Injection: 15 kV for 7 s 214 nm detection, 100 μm I.D. 30 kV run voltage at ambient temperature. Column length: 37 cm to detector, 54 cm overall. Buffer as in Fig. 3. Obtained on the modular instrument. B. Chromatogram of homologous series (1) toluene, (2) ethylbenzene, (3) propylbenzene, (4) butylbenzene, (5) amylbenzene, (6) 1-phenylhexane, (7) 1-phenylheptane, (8) 1-phenyloctane, (9) 1-phenylnonane. Injection: 6 kV for 3 s, 16 kV run voltage. Column length 25 cm to detector, 39 cm overall. Other conditions as in (A).

literature report [28]. The peaks were identified in part by spiking the sample (peaks 1, 4, 5, 6, 7, 10, 13), and in part by comparing these results to those reported previously by the Sandia researchers. It should be noted that the results obtained previously were done so with fluorescence detection whereas the data presented here was obtained with single wavelength UV detection. Thus some peaks are barely visible above the background. Retention factors and RSD's for peaks that were clearly visible in at least three runs are given in Table 1. While the resolution of peaks 2, 3, and 4 is slightly decreased

Table 1
Retention factors of PAHs: experimental conditions as described in Fig. 4A caption ($n=3$)

	Peak identification number									
	1	2	3	4	6	7	9, 10	11	12	13
Retention factor	2.0	2.7	2.8	3.0	3.9	5.1	7.4	9.9	10.3	11.4
RSD (%)	0.9	1.1	1.3	1.2	3.4	1.5	1.6	1.8	1.5	1.4

over that obtained with the UV polymerization, the retention factors are nearly doubled those reported earlier [28]. Fig. 4B shows a chromatogram of the homologous series. These results were also obtained on the modular instrument. The figure caption summarizes the run conditions.

A study of performance as a function of column length was undertaken. Results from that study are shown in Fig. 5A, a graph of retention factor of the three analytes, fluorene, chrysene, and benzo[a]pyrene, as a function of capillary length. This study was designed to test the uniformity of the polymer throughout the length of the column. All runs were done on a single column. Data for lengths 14, 15, and 16 cm were obtained by reversing the power supply polarity and injecting the samples on the short end of the same capillary. As can be seen in the figure, the retention factors of the analytes are constant with respect to migration length. A single run at constant voltage (30 kV) and a single run at constant field strength (500 V/cm) were done at each length. The data corresponding to constant field strength are shown. There is a deviation in the data occurring at approximately 35 cm. Upon visual inspection of the column it was clear that the injection end of the polymer was damaged, perhaps partially dried out, as gaps in the polymer were apparent for a short length. This section of the column was cut off, and results showed that a damaged portion of the column remained. This was evidenced by decreased flow rates and higher retention factors for both the constant voltage and constant field strength data. Upon further cutting, the column returned to its prior level of performance.

Fig. 5B shows the plot of theoretical plates versus column length. Theoretical plates were calculated by $5.54(t_r^2/w_{1/2}^2)$. A straight line response should be expected for this data. The linear fits shown for the three analytes only include the data obtained over the long end of the column and also do not include the data obtained at 31 and 33 cm which included the damaged portion of the column described above. The R^2 values for the fits change from 0.91 to 0.98, 0.92 to 0.93, and 0.91 to 0.95 for fluorene, chrysene, and benzo[a]pyrene respectively when the data at 31 and 33 cm are excluded. The data obtained over the short end of the column also do not fit on the lines. That end of the column provides results with higher theoretical plates than are expected. There are several explanations for this. It should be noted that while the system used to obtain this data is computer controlled, samples and buffer vials must be manipulated manually. The short end of the column is less flexible than the longer end, it so it is moved less when making injections. In addition, far fewer injections were made on the short end as compared to the long end. The overall result may be that the short of the column is showing less signs of aging and general usage than the longer end. For these three PAHs, the plate count/m is between 65 000 and 80 000. The RSD for theoretical plates for this manually operated system is calculated to be 10%. This number is based on theoretical plate calculations for compounds 1, 5, and 9 of the homologous series (Fig. 4B, $n=3$).

A Van Deemter plot for chrysene as a function of column temperature is shown in Fig. 6A. These data were collected on the P/ACE instrument. Data from four different temperatures (25, 35, 45 and 55 °C) are shown. Data were also obtained at 15 °C but it proved to be unreliable as these analysis times were greatly lengthened and the reproducibility of the data may have been compromised. Since run times were exceptionally long, and runs were more likely to fail, it is assumed that this low temperature is below the reliable operating temperature for this system. This could be an instrumental limitation or a limitation of the polymer at this low temperature, or a limitation

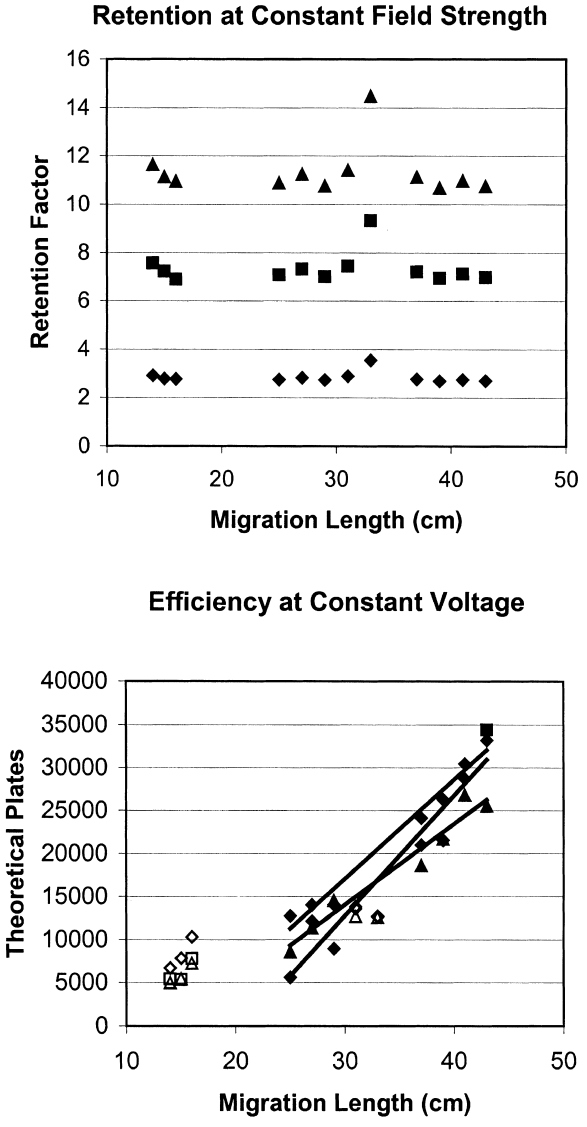

Fig. 5. (A) Plot of retention factors as a function of column length for ♦ fluorene, ■ chrysene, and ▲ benzo[*a*]pyrene on a 100 μm column. All runs done at 500 V/cm field strength. (B) Plot of theoretical plates vs. column length for ♦ fluorene, ■ chrysene, and ▲ benzo[*a*]pyrene. All runs done at 30 kV. First three data points on each plot obtained 'short end of capillary' with reversed polarity. Data points not used to generate linear fits shown by open symbols.

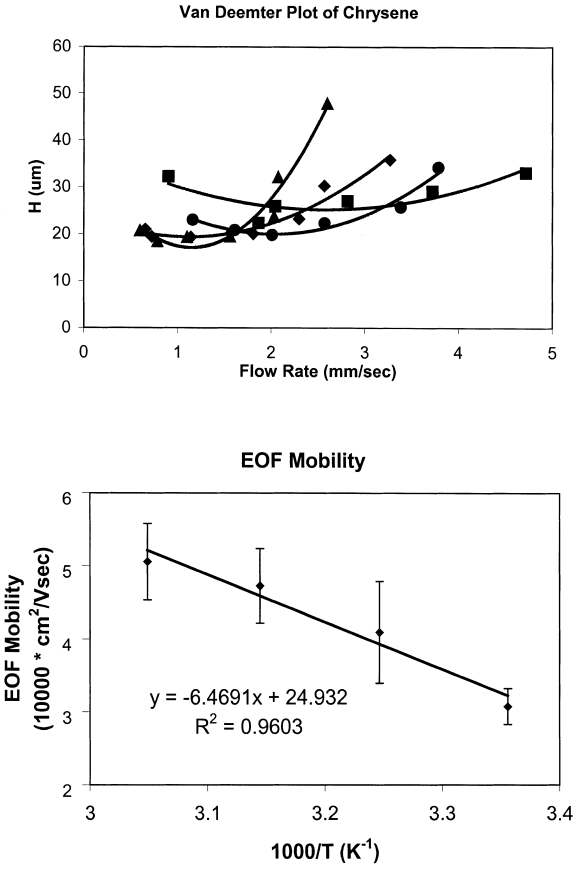

Fig. 6. (A) Van Deemter plot for chrysene at four different analysis temperatures. Column length 50 cm (effective length 40 cm), 100 μm I.D. 214 nm detection. Injection: 6 kV for 3 s. Buffer acetonitrile–5 m*M* pH 8.57 Tris (75:25, v/v). Data for each temperature is fitted with a polynomial curve only to aid visualization. ▲ 25 °C, ♦ 35 °C, ● 45 °C, ■ 55 °C. (B) EOF mobility vs. $1000 \cdot 1/T$ in Kelvin. One standard deviation above and below each data point is shown. *n* ranges from 5 to 8 for each temperature.

of the mobile phase (which is 75% acetonitrile). In any case runs were more likely to fail at this low temperature and so the 15 °C data is not presented.

CEC is a blending of chromatographic and electrophoretic processes. For the analytes investigated in this study, only chromatographic processes contribute to obtaining the separation, but the driving force for the mobile phase is of course electroosmotic flow. Thus, it is unclear if CEC systems will behave similarly to HPLC systems regarding temperature changes. The flow-rates of this system obtained at the elevated temperatures were markedly faster than those obtained at lower temperatures. This is due to decreased mobile phase viscosity at higher temperatures. H_{min} moves to faster flow-rates (from roughly

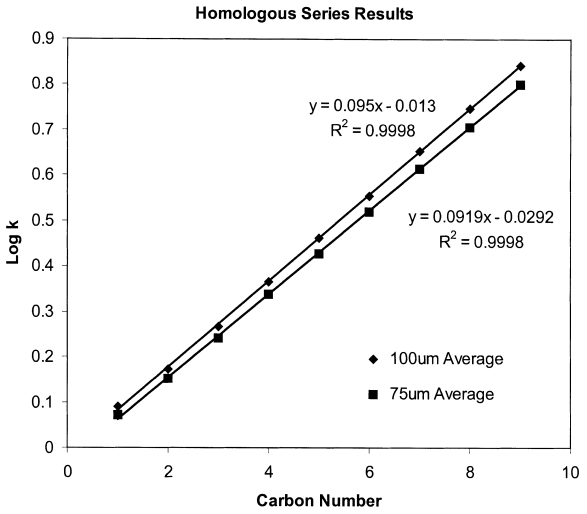

Fig. 7. Plot of log retention factor vs. carbon number on columns with two different diameters. Series consists of: toluene, ethylbenzene, propylbenzene, butylbenzene, amylbenzene, 1-phenylhexane, 1-phenylheptane, 1-phenyloctane, 1-phenylnonane. R^2 values for the linear fits are provided on the figure. 75 μm capillary data was obtained on the P/ACE MDQ instrument at 25 °C and 400 V/cm, 40 cm to detector, 50 cm overall. 100 μm capillary data was obtained on the modular instrument at ambient temperature, 400 V/cm, 25 cm to detector, 39 cm overall. 6 kV, 3 s injections for both cases.

1.2 to 2.6 mm/s over the 30 °C range) as the temperature increases. H_{min} also increases in value at higher temperatures (from roughly 18 to 25 μm over the 30 °C range) but the response does ''level off'', showing an overall decreased response to changes in flow-rate at the higher temperatures, thus providing a wider window of effective operating flow-rates.

Fig. 6B shows the change in electroosmotic flow as a function of operating temperature. The increase in electroosmotic flow-rate at higher temperatures is evident. For each data point, there were five to eight determinations obtained over a variety of applied voltages. The change in mobility from $3.1 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1} to $5.1 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1} is very close to the same magnitude of change demonstrated by other researchers in different columns [31].

A plot of log retention factor vs. carbon number is shown in Fig. 7 for a homologous series on columns with two different diameters on the two different instrument systems. The 100 μm capillary was used on the modular instrument without temperature control, and the 75 μm capillary was used on the P/ACE MDQ at 25 °C. Both capillaries were prepared at the same time from the same polymerization batch. The homologous series was analyzed three times on both columns at identical field strengths. Other conditions are given in the figure caption. These results show that system performance is quite reproducible from column to column, even when column diameters, lengths, and instrument systems are varied. Retention factors and RSDs are given in Table 2. It is interesting to note that up through carbon number 7, RSD values are actually smaller on the modular instrument operated under ambient conditions.

Data shown in Fig. 6 were obtained on a column prepared from a different polymerization batch than that shown in Figs. 5 and 7. The column producing the data in Fig. 6 was subjected to a variety of voltages and temperatures over the course of three weeks provided an electroosmotic flow value of $2.7 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1} with an RSD of 26% ($n = 19$) at 25 °C. For the temperature study (the portion of

Table 2
Retention factors for homologous series analytes on two columns prepared from the same polymerization batch: experimental conditions as described in Fig. 7 caption ($n = 3$)

	Carbon number								
	1	2	3	4	5	6	7	8	9
100 μm I.D. column									
Retention factor	1.2	1.5	1.8	2.3	2.9	3.6	4.5	5.6	7.0
RSD (%)	1.2	0.86	0.79	0.48	0.57	0.42	0.36	0.40	0.40
75 μm I.D. column									
Retention factor	1.2	1.4	1.7	2.2	2.7	3.3	4.1	5.1	6.3
RSD (%)	3.1	2.7	2.2	1.8	1.3	0.95	0.65	0.35	0.28

the analyses included in this paper) the calculated electroosmotic flow is $3.3 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1} with a RSD of 18% ($n = 8$) at 25 °C. The same polymerization batch was used to produce the columns generating data in Figs. 5 and 7. The 75 μm column, again used in the Beckman P/ACE instrument provided an electroosmotic flow value of $3.7 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1} with a RSD of 7.6% at 25 °C ($n = 7$). This column had been subjected to over 70 injections and was used for 3 weeks. The 100 μm column used in the homologous series study on the manual instrument was also used for 3 weeks and was subjected to over 50 injections under ambient conditions. The electroosmotic flow was $3.7 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1} with a RSD of 3.7% when used in the normal polarity mode ($n = 31$) and $3.7 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1} with a RSD of 5.4% when both the normal and reversed polarity voltages runs were included ($n = 36$). Given that these columns were used under very different conditions, thermostated and ambient temperatures, a manual system and fully automated system, the agreement in electroosmotic flow rates and retention factors (as shown in Table 2) is reasonable if not very good.

4. Conclusions

The results show that the PPMs produced in the manner described in this report, are durable, easy to make, and reproducible. No retaining frits are needed as the polymer is chemically bonded to the capillary wall. High pressure is never applied to the columns once polymerization is completed. These porous polymer monoliths are soft and spongy. It is likely that high pressure would deform the structure. While preparation of a column can be time consuming, the columns are very long lasting and provide consistency in performance as measured by electroosmotic flow and retention factors. The UV depolymerization of a detection window further means that the power supply polarity can be reversed, and analysis can be performed over the ''short end'' of the column, increasing the versatility of the columns. Furthermore, as discussed in the introduction, since the entire column is filled with the polymer, with the exception of the short detection window, problems associated with ''duplex'' columns are avoided. Reproducibility in terms of electroosmotic flow and retention factors is comparable between different columns operated on very different instrument systems. The study on temperature effects was done with material from a different polymerization batch from that of the homologous series and length studies. Thus, the reproducibility from different polymerization batches is also demonstrated.

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