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Capillary electrochromatography column behavior of butyl and lauryl acrylate porous polymer monoliths

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Abstract

A variety of porous polymer monoliths (PPMs) have been synthesized using the 'conduct-as-cast' format. The resulting polymers have been evaluated for use as separation media in capillary electrochromatography (CEC). The results have shown that substituting a small percentage of the standard polymer formulation with a more hydrophobic monomer produced columns with expected increases in retention for a neutral analyte series. However, substituting larger percentages of a more hydrophobic monomer resulted in columns that exhibited less retention. The unexpected behavior of these hydrophobic columns has been attributed to the non-uniform polymerization of the more hydrophobic monoliths. Van Deemter plots of polyaromatic hydrocarbons have been examined to further analyze the unexpected behavior of these columns. H_{min} values ranged from 8.7 to 9.1 μm for the columns evaluated. The effect of the percentage of organic modifier in the mobile phase on the separation has also been studied. The retention window decreases when altering the ACN concentration in the mobile phase from 50% to 80% (v/v).

Keywords: Monolithic columns; Capillary electrochromatography; Stationary phases; Acrylate-based monoliths

1. Introduction

The use of porous polymer monoliths (PPMs) for chromatographic stationary phases has generated considerable interest over the past several years. PPMs have been used very effectively in capillary electrochromatography (CEC). Recent reviews have discussed the history, polymerization processes, and current developments in the preparation and use of PPMs and their use in CEC [1–7]. These phases differ from other supports in their method of fabrication which results in characteristic structures. PPMs, as used in CEC, consist of a single piece of highly porous material and are typically covalently bonded to the capillary wall; thereby eliminating the need for supporting frits. The pores inside the monolith form an interconnected network of channels that allow the mobile phase to flow through and analytes to interact with the monolith surface. The pore size of the stationary phase can be tailored during the polymerization procedure to obtain optimal separation for desired analytes. This results in separations that are fast and highly efficient. In addition to the chromatographic advantages, the polymerization process of forming PPMs decreases the problems that have plagued particle packed CEC columns for years [8,9].

A variety of polymeric materials for CEC applications have been investigated by numerous research groups. The earliest versions can be traced to work by Hjerten and coworkers [10–12] who introduced and developed acrylamide based systems. Many other groups have contributed to the further development of acrylamide based polymers [13–16]. These investigations have included examples of the incorporation of monomers with long alkyl chains in attempts to alter the polymer properties and increase the hydrophobicity and retention properties of the resulting monoliths [13,15,16]. The field was further advanced with investigations of rigid methacrylate based polymers, first introduced by Svec and coworkers

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[17–22]. Many others have contributed to this area as well [23–26] and additional examples are mentioned in the cited reviews.

Preparation of CEC columns based on methacrylate and acrylate monomer systems is quite simple. To prepare a column, the capillary is filled with the desired monomer and cross-linking agent together with suitable porogenic solvents. Polymerization is initiated with UV light or heat. A number of factors such as varying the amount of monomer to porogen, changing the functionality of the monomer, changing the polymerization temperature, or changing the type and amount of porogen used in the polymerization mixture have been shown to influence the pore size distributions of these and related polymers [27–30].

Merhar et al. investigated the effects of varying the methacrylate monomer on the porous structure of the monolith [31]. In the case of more hydrophobic monoliths, a decrease in solubility of the monomer in the porogenic solvent was found to result in smaller pores. They found that this was especially true when a minimum of 15% of the standard glycidyl methacrylate, used in their studies, was replaced with a more hydrophobic monomer, such as stearyl methacrylate. Hoegger and Freitag compared columns composed of acrylates, methacrylates, and acrylamides [32]. The authors investigated the effects of varying the amount of monomer versus cross-linker using an aqueous solvent, as well as replacing their standard monomer, *N*,*N*-dimethylacrylamide, with more hydrophobic monomers. When hydrophobic monomers, such as butyl acrylate and hexyl acrylate were introduced into the polymer network, the monomers were not soluble in the standard polymerization mixture. Therefore, no attempt was made to investigate the behavior of these rather hydrophobic monomers.

Bedair and El Rassi overcame solubility challenges when incorporating pentaerythritol diacrylate monostearate into monoliths by using a unique casting solvent consisting of cyclohexanol, ethylene glycol and water. Both anionic [33] and cationic [34] polymers were prepared. The polymers were used in CEC separations of both neutral and charged analytes, including proteins [35]. Optimum plate heights in the range of 5–6 μm were achieved.

While there are a number of reports on a variety of polymers that have been investigated and found to be quite useful for CEC, this laboratory has chosen to pursue acrylate based polymers using a 'conduct-as-cast' approach. First reported by Ngola et al. [36] and Shediac et al. [37], the 'conduct-as-cast' method produces a polymer that once formed, allows for electroosmotic flow rather than pressure driven flow for conditioning the column. The polymers yielded highly efficient separations of neutral [36] and charged species [37]. These reports commented on the effects of substituting a more hydrophobic monomer for some of the butyl acrylate. The authors substituted 10% of the butyl monomer with lauryl acrylate. By substituting this small percentage, the structure of the monolith was not significantly altered and the chromatographic performance was maintained. This is consistent with earlier reports on glycidyl methacrylate studies [38] where it was shown that incorporation of up to 10% of a monomer with a more hydrophobic alkyl chain had minimal effect on the polymer properties. Delaunay-Bertoncini et al. also evaluated the effects of substituting a percentage of standard butyl acrylate with the more hydrophobic lauryl acrylate [39] using the 'conduct-as-cast' approach. Efficiencies of 300,000 N/m were obtained for a neutral analyte series. Their studies included altering the organic composition of the mobile phase to confirm the reversed-phase nature of the porous polymer monoliths. However, due to solubility limitations the researchers did not incorporate more than 10% lauryl acrylate into the polymerization mixture. These are convenient polymers to work with as they can be used in the 'conduct-as-cast' approach, and they are easy to prepare and behave reproducibly [40]. In addition, they readily photodepolymerize upon exposure to UV radiation, thereby creating a detection zone immediately adjacent to the monolith.

In this study a number of acrylate monomers were evaluated for use as separation media. Polymers were prepared with the 'conduct-as-cast' method. A small percentage of butyl acrylate was replaced with hexyl or lauryl acrylate monomers to study the change in column behavior. A *tert*-butyl monomer was also studied to evaluate the effect a more compact, branched species would have on the polymer structure and retention of the column. Finally, a larger percentage of a hydrophobic monomer, lauryl acrylate, was substituted for butyl acrylate to determine the effects of dramatically changing the primary monomer. SEM images were used to evaluate the structure of the polymers. The performance characteristics measured by retention factor and efficiency of each of the different columns were evaluated. In addition, the percentage of organic modifier in the mobile phase was varied in order to evaluate the polymer monoliths as reversed-phase separation media.

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 were used for chromatographic data, and 100 μm inner diameter capillaries were used for some SEM image data. Capillary length varied.

The CE instrumentation used was modular in nature and consisted of a Spellman CZE 1000R (Plainview NY, USA) power supply with the maximum current output set to 100 μA, for safety considerations, 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 computer fitted with a multifunction data acquisition board from National Instruments (Austin, TX, USA). Software was written in-house with National Instrument's LabView software.

SEM images of some of the bulk polymers were obtained on an ElectroScan Model E-3 ESEM (Wilmington, MA). A Soxhlet extraction with methanol was performed overnight on the bulk samples prior to analysis to remove residual polymerization material. Samples were then placed into the instrument without further modifications. This allowed images to be taken of the polymers in their 'wet' state. The same instrument was used to image some of the polymers in the capillaries. Columns which had been previously used, and were thus wet with mobile phase, were freshly cut and imaged without further treatment.

Additional SEM images of the polymers in the capillaries were obtained using a JEOL 840A Scanning Electron Microscope with an Oxford Instruments INCA X-Ray Microanalysis Unit (JEOL, Tokyo, Japan). All columns were cut into small pieces and dried in the oven at 50 °C for one week. The columns were then coated with a thin layer of gold for conductive purposes.

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 of thiourea, which was obtained from Fisher. All reagents were used as received. Inhibitors were not removed 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 1 M NaOH. The buffers were then mixed with acetonitrile (ACN) for the various organic-aqueous ratios. Conditioning solution was an ACN:20 mM Tris pH 8.53 (80:20, v/v) mixture. Mobile phases were made by varying the ACN:5 mM Tris ratios pH 8.53 (from 50% to 80% ACN:Tris, v/v). 5 mM stock solutions of fluorene, chrysene, benzo[*a*]pyrene and thiourea were made in the corresponding mobile phases. Analysis samples were made by mixing solutions of fluorene:chrysene:benzo[*a*]pyrene:mobile phase (1:3:3:8, v:v). All samples were filtered with 0.2 μm Acrodisk filters before use. Individual stock solutions of 5 mM toluene, ethylbenzene, *n*-propylbenzene, *n*-butylbenzene, amylbenzene, hexylbenzene, 1-phenylheptane, 1-phenyloctane, and 1-phenylnonane were each made in the mobile phase to be evaluated. Injection samples were made by mixing 200 μL each of the toluene through amylbenzene stock solutions with 400 μL each of the hexylbenzene through 1-phenylnonane stock solutions. All samples contained thiourea as an electroosmotic flow marker.

2.3. Capillary and polymer preparation and run conditions

Fused-silica capillaries were derivatized as previously described [40]. Briefly, the capillaries were rinsed with a series of base, water, and acid solutions. The capillaries were then rinsed with toluene, and 10% 3-methacryloxypropyltrimethoxysilane (MPTS) in toluene followed by other toluene and air rinses. Capillaries were stored in a refrigerator for as long as a week before being filled with a polymerization mixture.

Table 1 shows monomer compositions of each polymer studied. The monomer solution was mixed with 300 μL of 1,3-butanedioldiacrylate (BDDA) as the crosslinker, 5 mg of 2,2′-azobisisobutyronitrile (AIBN) as the free radical polymerization initiator, and 5 mg of acrylamido-2-methyl-1-propanesulfonic acid (AMPS) to support electroosmotic flow. The solubility limitations of adding larger amounts of lauryl acrylate as reported by other groups [36,39], were overcome by sonicating the monomer mixture while heating gently until solution turned from cloudy to clear. It was found that further increases in the lauryl component were not feasible due to solubility regardless of sonicating and heating. A casting solvent was prepared by mixing 200 μL of ethanol, 600 μL of ACN, and 200 μL of 5 mM pH 6.80 phosphate buffer. 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 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.

After polymerization, the capillaries were inspected under a microscope and if they were found to be free of visual

Table 1
Characteristics of different columns

Column/ polymer	Acrylate monomer composition	Monomer (mol%) (butyl acrylate)	Nodule size[a] (nm)	Nodule size[b] (nm)	Nodule size[c] (nm)	EOF mobility[d] ($\times 10^{-4}$ cm^2/V s) (RSD)
A	695 μL butyl acrylate	100	2900–3400	2000–2800	1100–1500	4.1 (1.4%)
B	625 μL butyl acrlyate, 70 μL *t*-butyl acrlyate	90	2900–3400	3400–3900	1500–1800	3.5 (1.6%)
C	627 μL butyl acrylate, 85 μL hexyl acrylate	90	2500–3400	2500–3500	1300–1600	3.6 (0.62%)
D	688 μL butyl acrylate, 132 μL lauryl acrylate	91	2000–2500	1500–2000	1200–1500	3.6 (2.5%)
E	347 μL butyl acrylate, 348 μL lauryl acrylate	65	NA	NA	540–710	2.5 (0.67%)
F	174 μL butyl acrylate, 521 μL lauryl acrylate	39	NA	NA	250–380	2.5 (0.71%)

[a] Nodule size ranges estimated from ElectroScan ESEM images of bulk polymers.
[b] Nodule size ranges estimated from ElectroScan ESEM images of polymer filled columns.
[c] Nodule size ranges obtained from dry samples using JEOL 840A.
[d] *Conditions*: ACN:5 mM Tris pH 8.53 (75:25, v/v), voltage 30 kV ($n = 4$).

defects, they were cut in length, a short section of the UV opaque polyimide coating was removed with a drop of fuming sulfuric acid. The capillary was placed into the CE system with this section in the UV detector path. The capillaries were conditioned prior to use with an ACN:20 mM pH 8.53 Tris mobile phase solution (80:20, v/v) at 7 kV while being exposed to UV radiation at 214 nm. A UV photodepolymerization occurs in the short section of the polymer in the light path. Residual monomeric material is also removed. First reported by Ngola et al. [36], this process can take from 12 to 48 h and has been subsequently reported by others [37,39,40]. The reservoir vials can then be filled with the appropriate mobile phase for analysis. The polyimide coating was removed from the ends of the capillary by shaving with a razor. Column stability, reproducibility, and lifetimes have been previously reported [40].

3. Results

3.1. Physical characteristics

Images of bulk polymers and columns with low levels of monomer substitution were obtained on an environmental SEM and selected images are shown in Fig. 1. Magnification is ×1800 for each case. The polymeric material is believed to be in a wet state. Bulk polymers were Sohxlet extracted with methanol, columns were wet with mobile phase. In each case a range of nodule sizes was estimated from the inset bars, and are thus only approximate. These estimated values are given in Table 1. Mercury injection capillary pressure data (not shown) gives peak pore (throat) diameters centered around 1 and 2 μm for these polymers. This is consistent with other literature reports [20,36].

Additional SEM data for all columns were obtained on material in a dry state and nodule size ranges were obtained with software supplied on the SEM instrument. These values are believed to be more accurate. The images of the polymers in the dry state are shown in Fig. 2. Table 1 lists the nodule size ranges for these dry state polymers as well. This data shows that there is an apparent slight decrease in the size of the nodules when the polymer is in the dry state and gold coated. The differences between the polymer wet with methanol and polymer wet with mobile phase (75:25 ACN:5 mM Tris (pH 8.53) could be attributed to uncertainty in the manual estimation of the nodule size ranges. Others have observed that these and similar polymers do not exhibit shrinkage or swelling with solvent changes [36,39].

As the amount of lauryl acrylate is increased relative to butyl acrylate, phase separation would be expected to occur earlier as the polymer forms and becomes insoluble. The result would be that the number of nucleation sites increases. Since the amount of monomer remains the same in the polymerization mixture, the nodule size must decrease

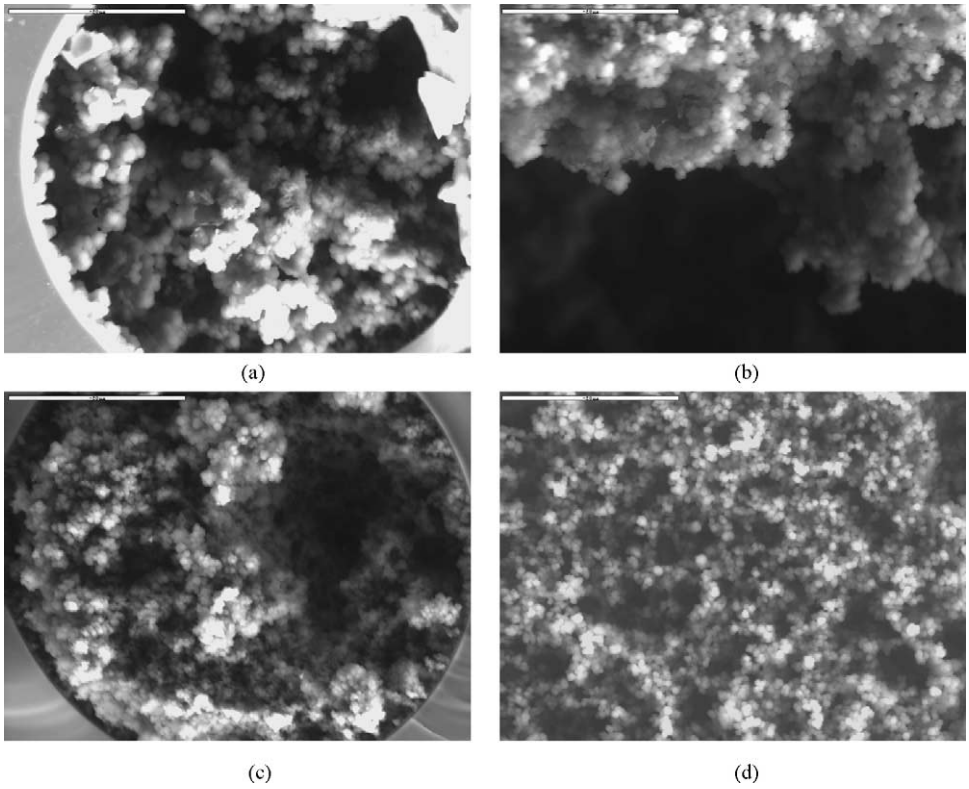

Fig. 1. Environmental SEM images of (a) column C, (b) bulk polymer C, (c) column D, and (d) bulk polymer D. Bar inset for (a–d) is 50 μm. Magnification for all images is 1800. Columns wet with mobile phase, bulk polymers wet with methanol. Column and bulk polymer designations as described in Table 1.

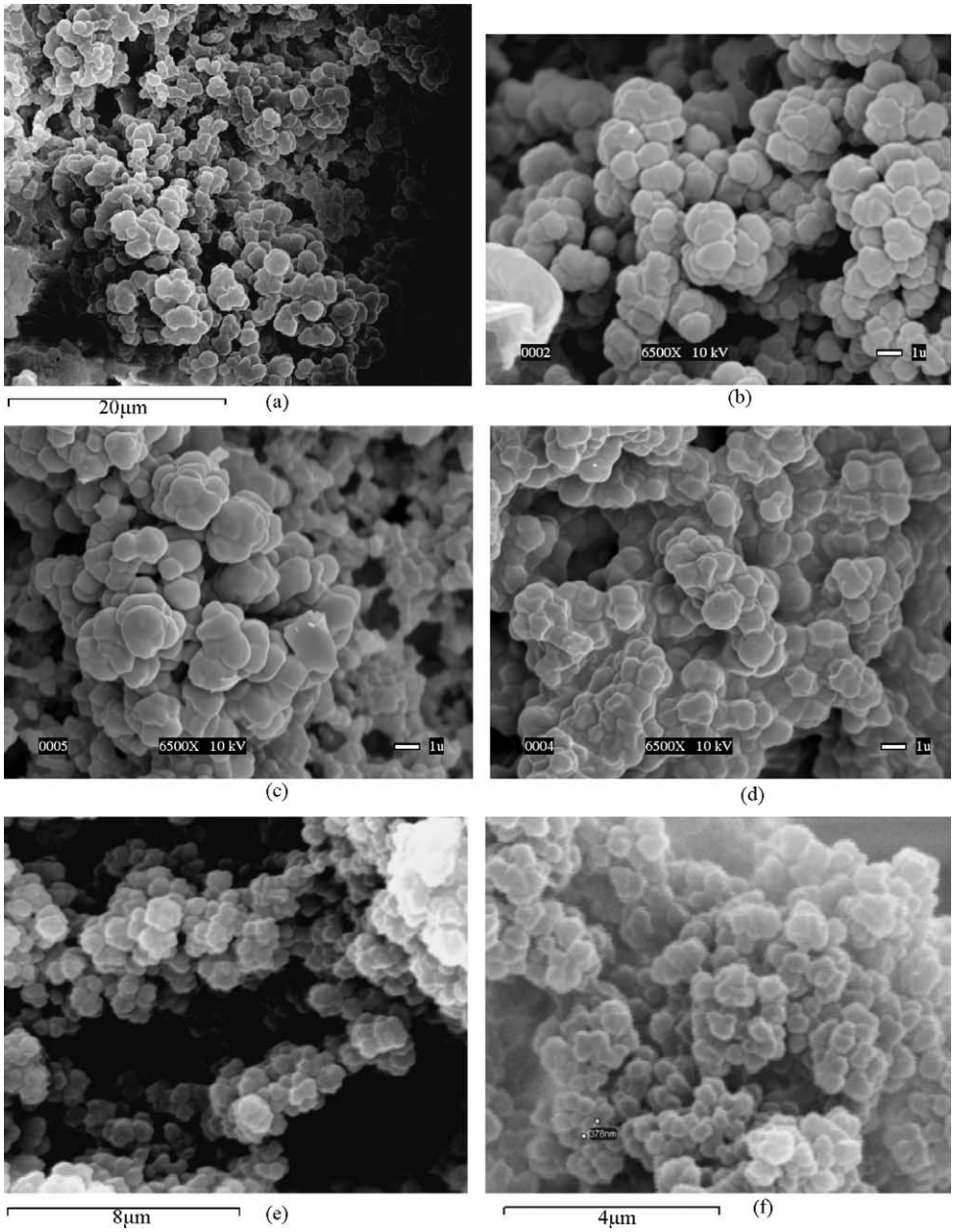

Fig. 2. SEM images of (a) column A, (b) column B, (c) column C, (d) column D, (e) column E, and (f) column F. Bar inset for (a) is 20 μm. Bar inset for (b–d) is 1 μm. Bar inset for (e) and (f) is 8 and 4 μm, respectively. Magnification for images is (a) 5000, (b–d) 6500, (e) 10,000, and (f) 13,000. Column designations as described in Table 1.

and macropore size remains close to the volume of the porogenic solvent. This is seen in Fig. 2 and Table 1. In Table 1 it can be seen that as the degree of substitution increases, the electroosmotic flow decreases. Permeability is related to the overall porosity of the polymer and arises from macropores (diameters over 50 nm), mesopores (diameters from 2 to 50 nm), and micopores (diameters less than 2 nm) [22,28]. The number of mesopores and micropores are more directly related to surface area. As the electroosmotic flow decreases, the total permeability of the columns is also decreasing.

3.2. Selectivity and retention in CEC

The retention mechanism for CEC separations involving neutral analytes is thought to be similar to that of reversed-phase HPLC and has been previously described in the literature [20–22]. It was assumed that substituting the standard butyl formulation with a more hydrophobic monomer would lead to a column exhibiting increased hydrophobic behavior. The hydrophobic selectivity of a series of alkylbenzenes can be calculated from the slope of the log of retention factor versus the carbon number. The intercept of the linear plot is an

Table 2
Information obtained from plot of log retention factor vs. carbon number for the alkylbenzene series[a]

Column	Slope	*y*-intercept	R^2
A	0.095	−0.014	0.999
B	0.095	−0.019	0.999
C	0.098	−0.005	0.998
D	0.111	0.049	0.999
E	0.117	−0.253	0.999
F	0.112	−0.484	0.999

[a] Mobile phase is ACN:5 mM Tris pH 8.53 (75:25, v/v), injection is for 4 s at 8 kV, and run voltage is 30 kV. RSD values ($n = 4$) for each data point are less than 5% for all columns.

indication of the hydrophobicity of the column. Table 2 lists the slopes and *y*-intercepts for plots of log of retention factor versus carbon number for the alkylbenzene series, toluene through 1-phenylnonane, for all columns tested. A minimum of four determinations per data point was obtained on each column, and RSD values for retention factors were less than 5% for each analyte. The large *y*-intercept shows column D to be the most hydrophobic column and column F to be the least hydrophobic. Despite the broad range of hydrophobicities, the three columns containing the lauryl acrylate polymer have close to the same selectivity, while the columns with the shorter alkyl monomers are only slightly less selective as is indicated by the slopes.

To further evaluate the retention of the columns, a more hydrophobic polycyclic aromatic series was studied. Fig. 3 shows the retention factors for fluorene, chrysene, and benzo[*a*]pyrene on all of the columns. Polymer incorporating the bulkier *tert*-butyl material (column B) results in essentially the same retention as compared to column A. A column containing equal volumes of butyl and *tert*-butyl acrylate (column G), also presented in Fig. 3, shows a decrease in retention for this analyte series as would be expected. Comparative retention increases for columns C and D, relative to column A. Since the monomers become slightly more hydrophobic in these two cases, this is also in line with expectations. The retention trends for columns B–D are consistent with the nodule size changes shown in Table 1 and *y*-intercept changes shown in Table 2.

Expecting to see a further increase in retention, the amount of lauryl acrylate was increased in columns E and F. The decrease in retention on columns E and F is not what would be expected based on the nodule size changes. It is consistent with the changes in *y*-intercept shown in Table 2. Raising the

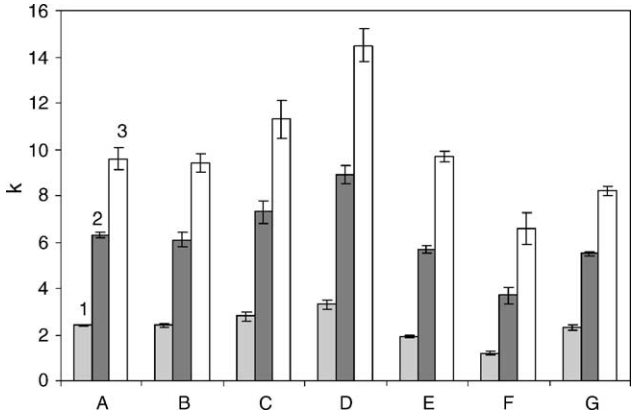

Fig. 3. Bar graph representation of retention factors for the polyaromatic hydrocarbon series on the different polymers: (1) fluorene, (2) chrysene, and (3) benzo[*a*]pyrene. Column designation is as shown in Table 1 and column G monomer composition is 347 μL butyl acrylate and 348 μL *t*-butyl acrylate. Error bars are ± 1SD. Mobile phase: ACN:5 mM Tris pH 8.53 (75:25, v/v).

amount of lauryl acrylate relative to butyl acrylate would normally be expected to increase the overall hydrophobicity of the polymer, even if the relative increase in hydrophobicity were not proportional to the larger amount of lauryl acrylate. The distribution of the two monomers in the resultant polymer however, is unlikely to be random. Copolymerization of monomers with different reactivities will give rise to polymers where there are sections of significant length which are enriched with one of the monomers. The more reactive monomer is incorporated into the polymer preferentially to the other at early stages of the polymerization. Another possible explanation is when the hydrophobicity of the monomer components is increased, the decreased solubility in the casting solvent results in earlier nodule formation, which yields more and smaller nodules. The more hydrophobic monomer components can then show greater solubility within the growing nodules [30,41]. The lauryl acrylate is then not distributed uniformly throughout the polymer, but some is sequestered within the nodule, unavailable to analytes, and thus does not impart an overall increase in hydrophobicity as is expected. If the lauryl acrylate monomer preferentially polymerizes within the growing nodules, this would in turn decrease the overall volume of the mesopores and micropores. The result would be a polymer with less surface area, and thus columns with less capacity.

Table 3
Comparison of efficiencies of select columns[a]

Analyte	Polymer A			Polymer E			Polymer F		
	H (μm)	*N* (plates/m)	*k*′	*H* (μm)	*N* (plates/m)	*k*′	*H* (μm)	*N* (plates/m)	*k*′
Fluorene	8.7	160,000	1.3	9.1	110,000	1.2	8.7	160,000	0.91
Chrysene	10.0	130,000	3.1	10.1	100,000	3.4	13.3	100,000	2.7
Benzo[*a*]pyrene	10.9	120,000	4.7	10.2	100,000	5.7	13.1	100,000	4.6

[a] Mobile phase is ACN:5 mM Tris pH 8.53 (75:25, v/v).

3.3. Column performance

Electroosmotic flow (EOF) in CEC is monitored by introducing a neutral marker. It has been reported that the mobility of the mobile phase through a capillary is related to the size of the pores formed during the polymerization process [20]. Larger pores allow greater linear flow velocities to be attained. By comparing the EOF mobility for each of the columns studied, information on their relative pore sizes can be deduced. The EOF mobilities for the columns are listed

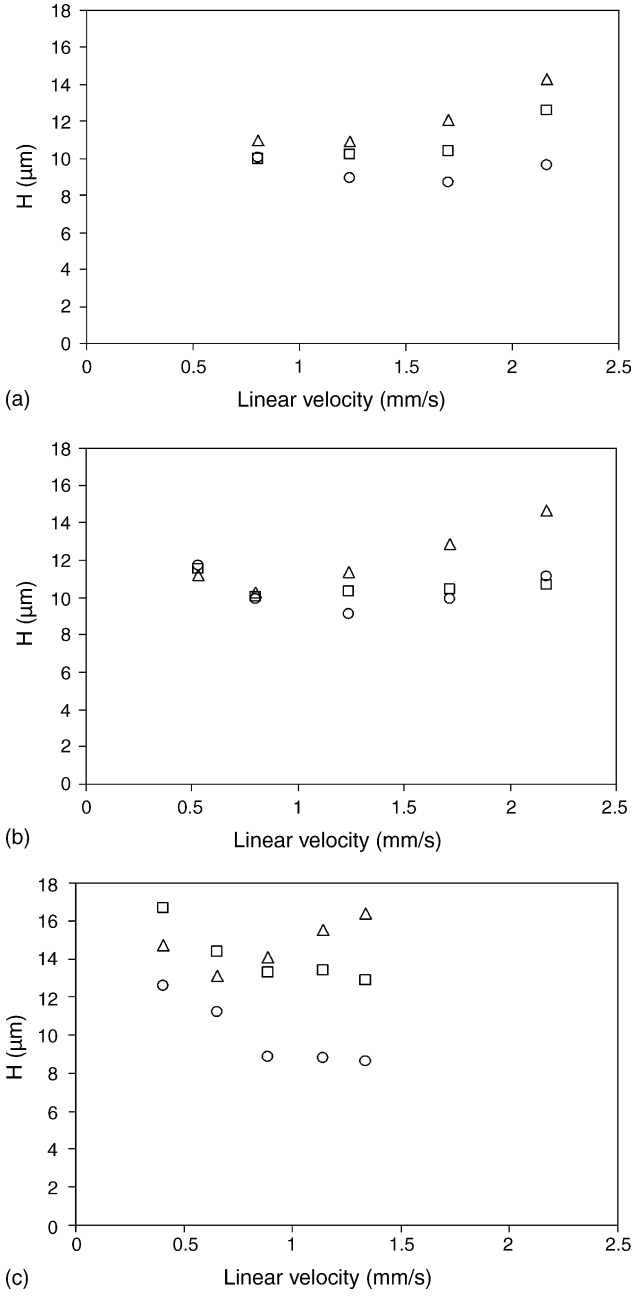

Fig. 4. Van Deemter curves for polycyclic aromatic hydrocarbons on (a) column A, (b) column E, and (c) column F. (◯) fluorene, (□) chrysene, and (△) benzo[*a*]pyrene. Mobile phase is 75/25 ACN:5 mM Tris pH 8.53 (v/v) (RSD <5% for each point).

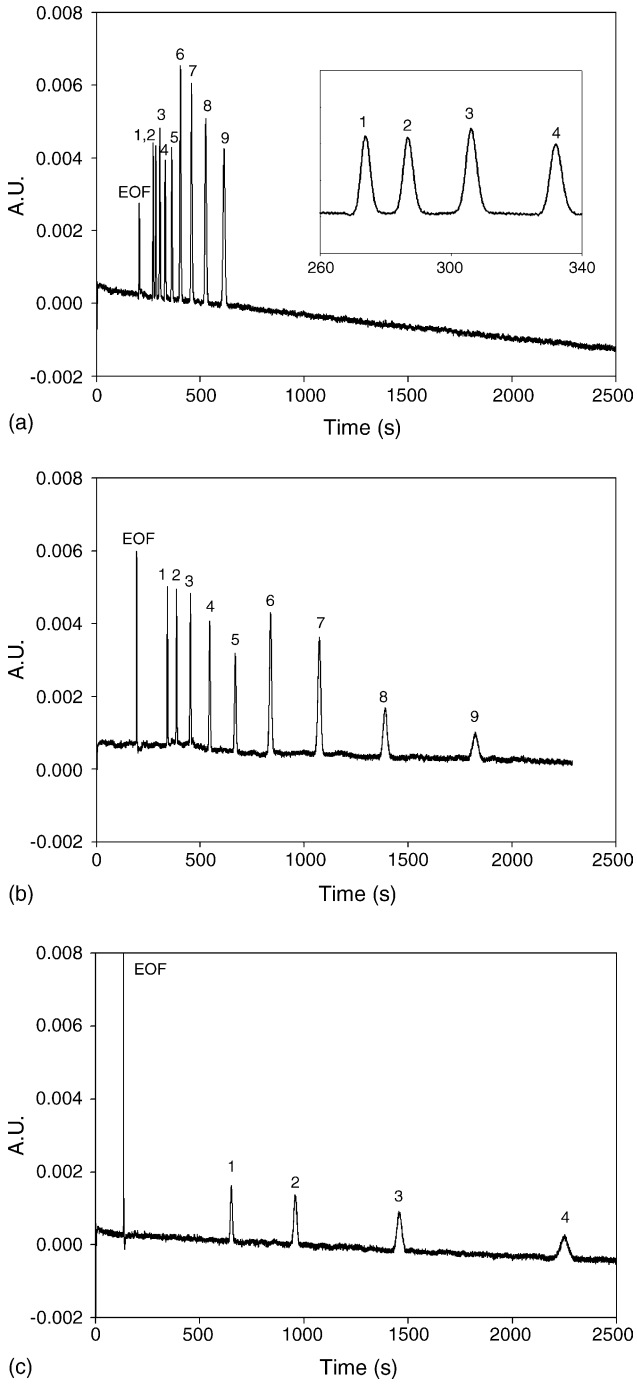

Fig. 5. Chromatograms for alkyl benzene series on column F. Mobile phase conditions: (a) 80:20 ACN:5 mM Tris (v/v), (b) 65:35 ACN:5 mM Tris (v/v), (c) 50:50 ACN:5 mM Tris (v/v). Run voltage was 30 kV and sample was injected for 4 s at 8 kV. Total column length was 48.5 cm and the migration length was 35.5 cm. Peak identifications: (EOF) thiourea, (1) toluene, (2) ethylbenzene, (3) *n*-propylbenzene, (4) *n*-butylbenzene, (5) amylbenzene, (6) hexylbenzene, (7) 1-phenylheptane, (8) 1-phenyloctane, and (9) 1-phenylnonane. Inset on (a) shows baseline resolution for peaks (1)–(4). Amylbenzene and larger analytes are insoluble in mobile phase used in part (c) and are thus not included.

in Table 1. Column A has the greatest linear velocity implying a greater pore size. As the pore size increases, the surface area should decrease causing less retention of analytes [20], if all other factors were held constant. The decreased EOF of columns C and D are supportive of their slightly increased retention relative to column A. However, columns E and F have a smaller EOF but also exhibit less retention relative to column A. This further supports the idea that the hydrophobic lauryl acrylate is not evenly distributed through the polymer.

The plate heights (*H*) for the PAHs tested on each column are presented in Table 3. The efficiencies of columns A, E, and F are similar to columns A and F, producing plate heights equivalent to 160,000 theoretical plates per meter for the fluorene, while column E produces plate heights equivalent to only 110,000 N/m (retention factor approx. 1).

Fig. 4 shows the Van Deemter plots for these three columns using the PAH analytes. Columns A and F exhibit a minimum plate height of approximately 8 μm for the earliest eluting peak (fluorene) with optimum linear velocities between 0.5 and 1.5 mm/s for all compounds. The minimum plate height value of 8 μm is comparable to the 6 μm minimum obtained by Ngola et al. for the butyl column for the least retained analyte [36]. Note that these minima are below the value of ∼18 μm reported previously [40]. Removing the polyimide coating with a razor at the injection end is less damaging to the polymer than the previously reported method of removal with a flame. Therefore, the injection end of the capillary is less affected resulting in less broadening. The highly retained benzo[*a*]pyrene demonstrates C term effects at fast flow rates on all three columns, but only column A has sufficient retention to yield noticeable C term effects at the higher flow rates for the two earlier eluting peaks.

A brief comparison to previous reports is warranted. Both Ngola et al. [36], and Delaunay-Bertoncini et al. [39] have investigated the effects of adding small amounts (10%) of a more hydrophobic monomer to the monomer mixture. In the first case, slight improvements in resolution were seen with the more hydrophobic monomers. Van Deemter curve comparisons showed similar minimum flow rates and only small variations in plate heights. Changes in retention were not discussed. In the more recent report, a slight decrease in retention was observed with addition of 10% lauryl acrylate and a slight increase in efficiency was observed. Our results differ in several ways. Our plate heights are slightly greater than both of these previous reports, but that is partially explained by the greater retention observed for these test compounds as our results have retention factors of greater than two for fluorene and greater than six for chrysene. In both previous results these values were close to one and four, respectively. Our increased retention is despite a larger EOF than was found in either of these previous reports. The optimum velocity shifts to lower values as the amount of lauryl acrylate increases (see Fig. 4), comparable to what would indicate an increase in the effective particle size for particle packed columns, but overall efficiency is not greatly impacted.

There are several possible explanations for these differences. The two previous reports both used UV polymerization. We have used thermal polymerization. Changes in polymer behavior have been reported for mixtures polymerized under different conditions [42]. Also, in each case inhibitors were not removed before polymerization. While this step may not be necessary, it may also give rise to slight changes in the polymerization process. Finally, solubility limits for lauryl acrylate mean that slight variations in solution treatment, may result in different polymers. We both heated and sonicated polymerization solutions, increasing the solubility of the lauryl acrylate which allowed for use of higher relative concentrations.

3.4. Effect of acetonitrile composition

In CEC, the separation of neutral analytes is based on chromatographic partitioning. The effect of varying the acetonitrile concentration of the mobile phase on the separation of alkyl benzenes was investigated. The percent of acetonitrile in the mobile phase was varied from 50% (v/v) to 80% (v/v), while maintaining a background electrolyte concentration of 5 mM Tris, pH 8.53, and operating at ambient temperature and 30 kV. Only columns A, E, and F were used for this study due to their unexpected performance.

Fig. 5 shows chromatograms for three different ACN concentrations on column F for the alkyl benzene series. The inset on Fig. 5a shows that baseline resolution is achieved for the early eluting analytes. As the amount of ACN is decreased, the retention of the analytes increases. Also, it should

Table 4
Effect of varying the acetonitrile concentration in the mobile phase[a]

Percent CAN	Column A			Column E			Column F		
	Slope	*y*-intercept	R^2	Slope	*y*-intercept	R^2	Slope	*y*-intercept	R^2
50	0.169	0.521	1.000	0.189	0.469	1.000	0.197	0.369	1.000
55	0.145	0.354	0.999	0.164	0.141	0.999	0.158	−0.061	0.999
60	0.133	0.140	0.999	0.149	0.014	0.999	0.145	−0.109	0.999
65	0.124	0.013	0.999	0.133	−0.110	0.999	0.130	−0.188	0.999
70	0.107	−0.075	0.999	0.124	−0.195	0.999	0.120	−0.359	0.999
75	0.094	−0.208	0.999	0.117	−0.253	0.999	0.112	−0.484	0.999
80	0.088	−0.292	0.999	0.110	−0.321	0.999	0.099	−0.617	0.999

[a] Background electrolyte was 5 mM Tris pH 8.53.

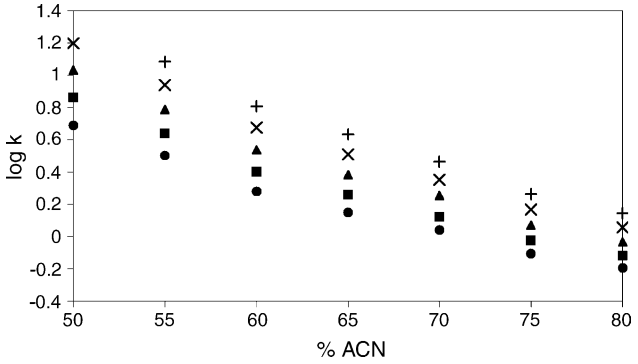

Fig. 6. Plot of log of retention factor vs. percent acetonitrile in mobile phase for column A. Aqueous portion of mobile phase is 5 mM Tris pH 8.53. All runs done at 30 kV. (●) toluene, (■) ethylbenzene, (▲) *n*-propylbenzene, (×) *n*-butylbenzene, and (+) amylbenzene. Amylbenzene is not soluble in 50% ACN:50% Tris buffer.

be noted that Fig. 5c only contains the first four of the alkyl benzene analytes due to the low solubility of the larger analytes in mobile phases with lower organic concentrations. For all columns, decreasing the ACN concentration in the mobile phase results in a wider separation window, but also leads to longer runs and greater peak broadening.

Table 4 shows the slope, *y*-intercept, and R^2 value for plots of log retention factor versus carbon number as a function of altering the percent acetonitrile (v/v) in the mobile phase. Column A shows the least selectivity as demonstrated by the consistently lower slope values, while columns E and F show essentially the same selectivity as each other. Trends in the *y*-intercept versus ACN concentration plots show that the hydrophobicity is again inversely dependent on the amount of lauryl acrylate in the polymer and inversely dependent on the ACN concentration. Fig. 6 shows the plot of log *k* versus percent acetonitrile in the mobile phase for select alkyl benzene analytes for column A. These retention data are indicative of reversed-phase behavior towards neutral solutes. As expected the retention increases with a decrease in acetonitrile concentration in the mobile phase, as does the retention window. This trend is seen for all three columns evaluated. While many researchers show linear trends for plots of log *k* versus organic concentration [43–45], our results show deviation from linearity. Studies are often done for lower or more restricted changes in the percentage of organic mobile phase components. At lower concentrations, linear behavior is to be expected, but deviations from linearity are expected at higher concentrations and over wider concentration ranges. These deviations also occur more readily with acetonitrile than with other organic solvents such as alcohols. This is due to the hydrogen donating/accepting characteristics of ACN relative to water [46].

4. Conclusions

A variety of porous polymer monoliths have been fabricated and evaluated as reversed-phase separation media in capillary electrochromatography. The columns were evaluated by altering the concentration of the organic modifier, acetonitrile, in the mobile phase, and demonstrated the expected reversed phase behavior. Butyl acrylate monomer was replaced with varying amounts of other monomers in the standard polymerization solution. The results show that substituting small percentages of the monomer component had the expected effect on the retention factors of the analytes tested. The substitution of a small, branched chain monomer resulted in a decrease in retention, while substitution with a more hydrophobic monomer led to an increase in retention factor. But further increases in the percentage of lauryl acrylate relative to butyl acrylate resulted in polymers that demonstrated less retention for nonpolar analytes than the polymers formed from less hydrophobic monomers. These results are opposite of what might be expected and can be attributed to nonuniform polymerization of the more hydrophobic monomer. Without compensating for monomer solubility or reactivity, simply increasing the amount of more hydrophobic monomer components will not necessarily result in CEC columns yielding greater retention of hydrophobic analytes.

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