ELSEVIER

Journal of Chromatography A, 905 (2001) 281–290

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Sulfonated acrylamide copolymers as pseudo-stationary phases in electrokinetic chromatography

Wei Shi, Christopher J. Watson, Christopher P. Palmer*

New Mexico Institute of Mining and Technology, Socorro, NM 87801, USA

Received 6 June 2000; received in revised form 31 August 2000; accepted 21 September 2000

Abstract

Sulfonated copolymers were synthesized, characterized and used as separation media in electrokinetic chromatography. The polymers used were synthesized from AMPS (2-acrylamido-2-methyl-1-propanesulfonic acid) and LMAm (lauryl methacrylamide) in different mole ratios (from 100:0 to 60:40). Electrophoretic mobilities and methylene selectivities were calculated, which showed the expected correlation with the monomer ratios. The chemical selectivities for the separation of nine solutes by the copolymers were compared with that of sodium lauryl sulfate micelles, showing significant differences. No significant difference in chemical selectivities was observed for copolymers with different monomer ratios. No significant change of hydrophobic microdomain of copolymers was found in background buffers with different ionic strength values, based on the investigation of the retention factors, methylene selectivities and polymer effective mobilities. No change of hydrophobic microdomain of the copolymer solutions was found at copolymer concentrations from 0.17 to 3% (w/v), however, plots of k' versus polymer concentration suggested a different copolymer phase at lower concentrations (from 0 to 0.1%, w/v) from that at higher concentrations (from 0.17 to 3%, w/v). The copolymer with AMPS–LMAm (80:20) could be chosen as optimum copolymer as far as the methylene selectivity, peak symmetry and polymer mobility were concerned.

Keywords: Pseudo-stationary phases; Electrokinetic chromatography; Polymers; Surfactants; Lauryl methacrylamide; Acrylamidomethylpropanesulfonic acid

1. Introduction

Electrokinetic chromatography (EKC) was introduced by Terabe et al. in 1984 [1]. The separation mechanism is based on the differential partitioning of the solutes between a pseudo-stationary phase and the background electrolyte. Commonly used pseudo-stationary phases are micelles, formed dynamically from low-molecular-mass surfactants such as SDS (sodium lauryl sulfate). SDS provides high efficiency separations of complex mixtures. However, the critical micelle concentration (CMC) of such conventional surfactants varies with the concentration of the surfactant [2], ionic strength, pH, temperature [3,4], etc. Under high organic solvent concentration, the micelle structure could be changed dramatically [5,6], making separation impossible.

Polymeric surfactants have been shown to provide high-efficiency separations [7–10], while providing a stable chemical structure under different analysis

*Corresponding author. Tel.: +1-505-835-5263; fax: +1-505-835-5364.

E-mail address: palmer@nmt.edu (C.P. Palmer).

0021-9673/01/$ – see front matter
PII: S0021-9673(00)00984-5

conditions. The methods for synthesizing these polymers have been reported elsewhere in the literature [11,12]. These polymers have been named micellar polymers by Anton et al. [11]. The micellar polymers have a much higher stability because the hydrophobic tails are covalently bonded along the backbone of the polymer. The stabilized backbone also drastically reduces or eliminates the CMC that is found in conventional micelles. Polymeric surfactants can even be employed with very high organic modifier concentrations, due to the stable chemical bonding [7,8,13–18]. Due to these advantages, a recent study has shown oligomers of sodium undecylenic acid to be the most effective phase for the separation of polycyclic aromatic hydrocarbons (PAHs) among several pseudo-stationary phases investigated [19]. Polymeric surfactants could also make EKC–MS hyphenation easier [20,21].

During the past 20 years, much attention was focused on the investigation of behavior of amphiphilic polyelectrolytes in aqueous solutions because significant kinetic effects were observed due to the existence of the polymer microphase structures [22]. It was proved that repulsive Coulombic interactions among charged segments competed with hydrophobic interactions among hydrophobic functionalities on an amphiphilic polyelectrolyte chain, and the self-organization of hydrophobic functionalities to form a micelle-like microphase structure could occur when the hydrophobic interactions were stronger than the segmental electrostatic repulsions. It was observed that the consequence of the competition between the hydrophobic attraction and the Coulombic repulsion strongly depends on the content of the hydrophobic functionalities in the polymers [23]. Copolymer concentration and the ionic strength of the solution can both affect the polymer structure in aqueous solutions [24–26].

Polymer surfactants that have been used for EKC include: poly(sodium 10-undecylenate) [14,18,27], BBMA (butyl acrylate–butyl methacrylate–methacrylic acid [28,29], poly(sodium 10-undecenylsulfate) [16,17,21,30–32], poly(sodium-*N*-undec-10-ene-1-oyl-taurate) [15], poly(sodium-*N*-undec-10-ene-1-oyl aminoethyl-2-phosphonate) [15], silica-based polymers [13], polyallylamine-supported surfactants [33–35], polymeric dye such as poly(vinylamine) sulfonate anthrapyridone [36], dendrimers [32], polyethyleneimine [37] and surfactants with chiral selectivity, such as poly(sodium undecenoyl-L-valine) [38–44], and dipeptide polymeric surfactants [45], etc. Among the general observations that we have made in our work are that polymers with strongly acidic head groups provide superior electrophoretic mobility and that the possibility of using these polymers at low pH extends their utility (e.g., reversed-flow EKC, preconcentration by sweeping, and selectivity adjustment for ionizable compounds) [14,15]. It has also been observed that copolymers with varied side-group chemistry can be used to provide unique and predictable selectivity [46,34].

In this study, a new kind of ionic copolymer with sodium sulfonate head groups is introduced. Copolymers of 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) and lauryl methacrylamide (LMAm) have been synthesized with varied mole ratios of monomers. The copolymers have been characterized by NMR spectroscopy and electrokinetic chromatography. The sodium sulfonate head group provides the copolymer with high electrophoretic mobility and utility in low pH buffers. By controlling the AMPS–LMAm ratio, the copolymer could be synthesized with different electrophoretic mobilities, which could be used to adjust the resolution power of solutes with different hydrophobicity. Given sufficient substitution with LMAm, the new pseudo-stationary phases provide excellent chromatographic performance. This family of copolymers shows significant promise for use as pseudo-stationary phases in EKC.

2. Experimental

2.1. Chemicals

LMAm was purchased from Polysciences (Warrington, PA, USA). AMPS, TEA (triethylamine), and 2-mercaptoethanol were purchased from Aldrich (Milwaukee, WI, USA). HPLC-grade THF (tetrahydrofuran) was from Acros (NJ, USA). Deionized water was obtained by a water purification system (Millipore, Bedford, MA, USA). 2,2′-Azobis(2-methylpropionitrile) (AIBN) was from Dionex. Ketone homologues, benzene derivatives and sodium

tetraborate were from Aldrich. All reactants and solvents were used as received from the manufacturer without further purification.

2.2. Synthesis

AMPS–LMAm random copolymers were synthesized by a free radical polymerization (Fig. 1). Two different kinds of solvent systems were used for the polymerization: solvent 1, THF–TEA (equimolar AMPS) and solvent 2, THF–water (80:20). A 3-mmol amount of monomer was used for each reaction. The AMPS and LMAm were added to a three-necked round-bottom flask in a known ratio. Monomers may be added in one to five batches over 4 h, as stated in the text. A 70-ml volume of solvent was added to the flask to completely dissolve the AMPS and the LMAm. AIBN (0.2 mol% based on total moles of monomers) was then added to the flask. The reaction vessel was then flushed with nitrogen for 8 h to remove any oxygen in the flask and maintain a nitrogen atmosphere inside the reaction system. Mercaptoethanol (chain transfer reagent) was added (1 mol% based on total moles of monomers) just before heating (chain transfer reagent was not employed in solvent 2 system in this study). The reaction vessel was placed on a heating mantle and heated to a temperature of 60°C for 24 h, while stirring by an electromagnetic stir bar. In this study all the EKC performance data are for the polymers synthesized in solvent 2 system, unless stated otherwise in the text.

2.3. Purification

The solvent was evaporated using a rotary evaporator (Rotavapor R110, Brinkmann, Wesibuky, NY, USA). The polymer was then dissolved in water, and pH was raised by adding an excess (3 ml) of 5% (w/w) NaOH to neutralize the AMPS acid group. In solvent 1 system, the solution was filtered to remove any insoluble compounds, and then extracted multiple times with 25 ml ether or isooctane (Fisher chemical) with the aqueous phase adjusted to pH 12.0 to remove TEA. The excess NaOH and other low-molecular-mass compounds were then removed by dialysis using a Spectra/Por Cellulose Ester Membrane. In this study, dialysis membranes with a MWCO (molecular-mass cut-off) of either 500 for solvent 1 system, or 2000 for solvent 2 system were employed. The polymer solution was filtered and freeze-dried to remove any water. The resulting white solid polymers were then stored in a desiccator. The synthetic yield of copolymer products were generally greater than 7%. For polymers with an AMPS–LMAm ratio greater than 70:30, the yield could reach 30–40%.

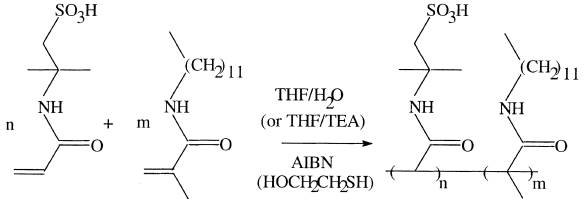

Fig. 1. Polymerization scheme for AMPS–LMAm copolymers.

2.4. NMR characterization

A Jeol Eclipse 300+ NMR with a Silicon Graphics workstation was used to characterize the copolymer. Approximately 3000 ppm polymers were dissolved in $^{2}H_2O$. Single pulse ^{1}H NMR experiments were performed. Thirty-two scans were completed for each sample. Resonances ranging from 3.27 to 3.43 ppm are indicative of the CH_2SO_3 group on the AMPS portion of the polymer, the resonances ranging from 3.17 to 3.24 ppm and 1.21 to 1.28 ppm show the definite presence of TEA in the sample (if solvent 1 was used). The broad peak ranging from 1.92 to 2.22 ppm is indicative of the polymer backbone. Another region that is indicative of the backbone ranges from 1.62 to 1.80 ppm. The peak located at 0.87 ppm shows the presence of the terminal methyl group on the alkyl chain of the LMAm. The AMPS–LMAm ratio in the polymer was obtained by calculating the ratio of the integral of the resonance peak at 3.4 ppm to that at 0.9 ppm. For the copolymers with AMPS–LMAm $<$60:40, the resonance peak at around 1.3 ppm (CH_2 group on LMAm chain) is so large and tailing, that nearly half of the 0.9 ppm resonance peak was covered by the tail of the 1.3-ppm resonance peak. The corrected integration of the 0.9-ppm resonance peak was

estimated by weighing the spectrum paper on an electronic balance.

2.5. EKC

A Hewlett-Packard (Palo Alto, CA, USA) ^{3D}CE capillary electrophoresis instrument with Chemstation software was used to perform the EKC experiment. Fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) of 50 μm I.D. were used, with an effective length of 45.00 cm and a total length of 53.55 cm. Polymers were dissolved in borate buffers (50 m*M*, unless stated otherwise) and filtered through a 0.45-μm syringe filter (Whatman, Clifton, NJ, USA) before the EKC experiments.

Stock sample solutions were prepared in acetone at a concentration of around 1000 ppm. Ten μl of the stock sample solutions were dissolved in 100 μl polymer buffer solution before each run, resulting in a sample concentration of approximately 100 ppm (unless stated otherwise). Injections were performed at 5000 Pa for 3 s, unless stated otherwise. Separations were performed at 20 kV unless stated otherwise. The capillary cartridge temperature was maintained at 25.0°C. The UV detector was set at 214 and 254 nm. Each analyte was identified by matching the UV spectra to a known UV spectra on file. For ambiguous UV spectra matching, the spiking technique was used to identify the peaks. Between runs, the capillary was flushed by background buffer for 2 min. Each set of separations was run at least twice.

3. Results and discussion

3.1. Synthesis

We first tried THF–TEA (solvent 1) as the polymerization solvent system according to the literature [24]. A stoichiometric ratio of TEA was added into the THF solution to increase the solubility of AMPS. NMR studies showed traces of TEA remaining in the product even after repeated (8 times) extraction with ether at pH 12.0. The remaining TEA might affect the mobility and methylene selectivity of the polymers in the EKC system (see also Section 3.2), and the presence of TEA signals in NMR spectrum affected the integration of the peaks at around 3.4 ppm.

Therefore, a THF–water solvent system (solvent 2), without TEA, was developed. AMPS dissolved well in this solvent system without the need for TEA. Using this solvent system, we were able to synthesize copolymers with different monomer ratios by changing the ratio of the feed stock.

The molecular mass and molecular mass distribution of this kind of polymer is difficult to determine because of the lack of suitable molecular mass standards, and because of the tendency of the polymers to form intra- and inter-molecular aggregates in solution. Mass spectrometry by electrospray ionization and matrix-assisted laser desorption ionization do not provide useful information because of the extremely complicated spectra obtained. Purification of our products by dialysis allows us to set a minimum molecular mass of 2000 g/mol. Preliminary results with static light scattering in 50 m*M* borate buffer solutions gave a molecular mass of 1.38×10^6 g/mol, which most likely represents the apparent molecular mass of intermolecular aggregates. Morishima and co-workers have reported a value of 4.8×10^5 g/mol for similar AMPS–LMAm polymers in distilled water, but 4.0×10^4 g/mol for the same polymers in ethanol [47].

3.2. EKC performance

3.2.1. Mobility and methylene selectivity

A mixture of six alkyl phenyl ketone homologues were separated by the copolymer surfactants to characterize the EKC performance. Representative separations are shown in Fig. 2. Acetone was used as the EOF marker. The electrophoretic mobilities of the copolymers were calculated by the homologue iteration method [48], and the methylene selectivities α ($\alpha = k_2'/k_1'$, where k_2' and k_1' are the retention factors of two adjacent compounds among the homologous series) were calculated from the slope of the log k' versus carbon number plot. The results are presented in Table 1. Generally, with the decrease of AMPS content in the copolymer, the polymer electrophoretic mobilities decreased accordingly, because the charge to mass ratios of the polymers decreased. It should be noted, however, that the calculated value for the mobility using the homologue iteration

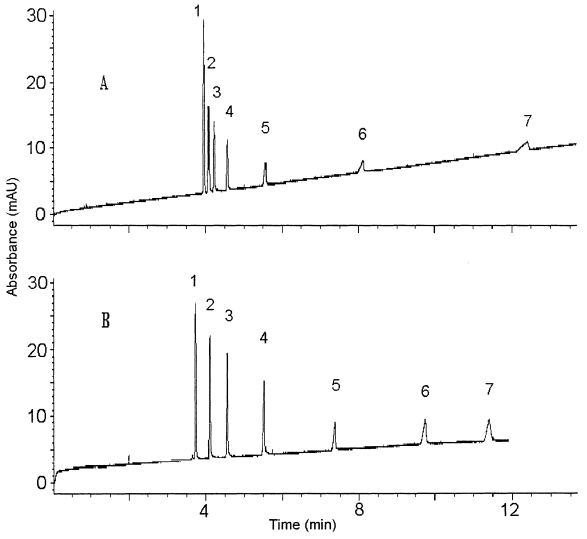

Fig. 2. Chromatograms of six ketones. Injection time, 3 s at 5000 Pa; polymer concentration, 1% (w/v); UV detection, 254 nm; applied *V*, 20 kV. (A) AMPS–LMAm (91.2:8.8), (B). AMPS–LMAm (75.1:24.9) (1) Acetone; (2) acetophenone; (3) propiophenone; (4) butyrophenone; (5) valerophenone; (6) hexanophenone; (7) heptanophenone.

method becomes less reliable when the homologues are not equally divided in the migration window, as is the case with the copolymer with AMPS–LMAm (96.6:3.4) in Table 1, and for low phase ratios. At the same time, the methylene selectivity increased, because the hydrophobicity of the polymers increased with increasing content of LMAm. The polymers with an AMPS–LMAm ratio less than 91.2:8.8 showed higher methylene selectivities than 30 m*M* SDS. The polymers with an AMPS–LMAm ratio greater than 75.2:24.8 showed the same or higher electrophoretic mobilities than 30 m*M* SDS. The efficiency of the separations were comparable to SDS. (Note: to compare these theoretical plates in the same conditions, all samples were injected for 3 s, though 1-s injection could have provided higher plates.) It was noted, however, that the polymers with higher percentages of AMPS showed significant fronting for the more hydrophobic analogues. This is evident in Fig. 2. Copolymers with an AMPS–LMAm ratio of approximately 85:15 to 75:25 could be chosen as the optimum copolymer as far as the methylene selectivity, polymer mobility, and peak symmetry were concerned.

The above data were from the copolymers synthesized in THF:water system (no TEA added). These results (the relationship between monomer ratios and EKC performance) were consistent, as opposed to the results of the polymers synthesized in the THF–TEA system. Methylene selectivity results for copolymers synthesized in the THF–TEA system showed that the lower the LMAm percentage, the more hydrophobic the polymer was, and there was no dependence of the electrophoretic mobilities of

Table 1
EKC performance of different pseudo-stationary phases: EKC conditions are stated in Section 2.5

Pseudo-stationary Phase				Mobility ($\times 10^{-4}$ cm^2 v^{-1} s^{-1})	Methylene selectivity	Average theoretical plates of six solutes/m[d]
Monomer feeding ratio (AMPS–LMAm)	Yield	Copolymer component ratio[b] (AMPS–LMAm)	Concentration			
SDS		N/A	30 m*M*	−3.97±0.00	2.42±0.04	149 000±11 000
100:0	85.0%	100:0	1.0% (w/v)	N/A	N/A	N/A
95:5	15.8%	96.6:3.4	1.0% (w/v)	−20.1±0.58[c]	2.26±0.01	N/A
90:10	41.3%	91.2:8.8	1.02% (w/v)	−4.60±0.06	2.50±0.01	172 000±16 000
80:20	9.2%	84.9:15.1	0.99% (w/v)	−3.99±0.01	2.60±0.00	130 000±6000
70:30	33.0%	75.1:24.9	1.00% (w/v)	−3.91±0.03	2.54±0.01	232 000±5000
70:30[a]	7.0%	75.2:24.8	1.00% (w/v)	−3.97±0.05	2.59±0.02	163 000±13000
50:50	7.3%	58.7:41.3	1.03% (w/v)	−3.77±0.01	2.96±0.02	132 000±4000

[a] AMPS was fed in four batches. Others: fed in one batch.
[b] Actual AMPS–LMAm ratios were calculated by NMR spectrums.
[c] Higher error due to the homologue iteration of the non-baseline separated homologue peaks.
[d] Data were from the average plates of the six peaks of ketone homologues. (standard deviation of plates was calculated from two repeating chromatograms).

the polymer on the AMPS percentages (data not shown). These quite unexpected results are probably due to the effect of the TEA counter ion on the performance of the polymer. The inability to remove TEA from the copolymers synthesized in this manner, and the effect of the TEA on the chromatographic results, makes this solvent system unsuitable for the synthesis of copolymers to be used for EKC.

3.2.2. Peak shape

As noted above, significant fronting was observed with the more hydrophobic homologues (heptanophenone, hexanophenone) using the polymer AMPS–LMAm (91.2:8.8) at a concentration of 1% (w/v), as shown in Fig. 2a. The fronting of the later eluting compounds is an indication that the polymers appear to have a low solvation capacity for the more hydrophobic analytes. The sample matrix (10% acetone) may play a role in determining the peak shape, but this was not observed when SDS was employed as the pseudo-stationary phase. More likely, the more hydrophilic polymers were not able to solvate these compounds efficiently, causing an apparent sample overloading effect. Furthermore, when the polymer AMPS–LMAm (96.6:3.4) was used at 1% (w/v), the peaks of less hydrophobic solutes (e.g., butyrophenone) also showed significant fronting (chromatogram not shown). However, when the polymer AMPS–LMAm ratio (75.1:24.9) was used at 1% (w/v), the peak fronting problem was improved, as shown in Fig. 2b. Additionally, the last two peaks became more symmetric at higher polymer concentrations (up to 3.05%, w/v), as shown in Fig. 3. In 3.05% (w/v) of this copolymer solution the symmetry factors of hexanophenone and heptanophenone were 1.02 and 1.07, respectively, the peak shapes were very symmetric. In general, low solvation capacity of the polymer phase, caused by either low polymer concentrations or low LMAm percentage, produced an apparent sample overloading effect and peak fronting for very hydrophobic solutes.

3.2.3. Separation by poly(100% AMPS)

PolyAMPS showed poor methylene selectivities for the ketone homologues in EKC. At lower concentration (1%, w/v), poly AMPS showed no separation power at all with all of the ketones eluting at the same time as that of acetone. However, at higher concentration (3.0%) of polyAMPS, acetone and butyrophenone can be just baseline resolved. Acetone and acetophenone still cannot be separated at this concentration. Higher concentrations of the AMPS copolymer could not be tested because of excessive current. This result proves that the methylene selectivity observed for the copolymers is entirely due to the presence of the lauryl side chains, and indicates that changing the property of the side group may have a significant impact on the selectivity of the polymers.

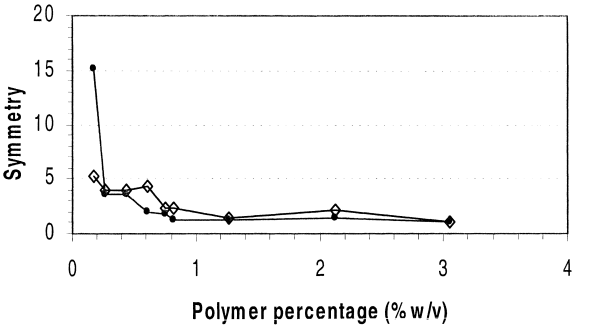

Fig. 3. Dependence of peak symmetry on the polymer concentration. AMPS–LMAm (75.1:24.9). (◇) Hexanophenone; (●) heptanophenone. Symmetry factor was calculated by A/B (A is the distance from the peak front to the center at 1/10 peak height; B is the distance from the peak center to the tail at 1/10 height).

3.3. Chemical selectivity

Nine nonionic compounds were separated by EKC using the various AMPS copolymers. A representative separation is shown in Fig. 4. The $\log k'$ values of these nine compounds using the AMPS–LMAm (84.9:15.1) copolymer are plotted versus those using 30 m*M* SDS in Fig. 5. The square of the correlation coefficient of the plot is around 0.88, showing a significant difference between the chemical selectivity of the copolymer and SDS. The migration order was reversed for a few pairs of solutes: *p*-nitroaniline and nitrobenzene, naphthyl amine and naphthalene methanol, and *p*-xylene and acenaphthenol.

In Fig. 6, the $\log k'$ values of the same nine solutes using the copolymers with different monomer ratios (AMPS–LMAm, 96.6:3.4, 91.2:8.8, 84.9:15.1 and 75.1:24.9) were plotted versus those using the

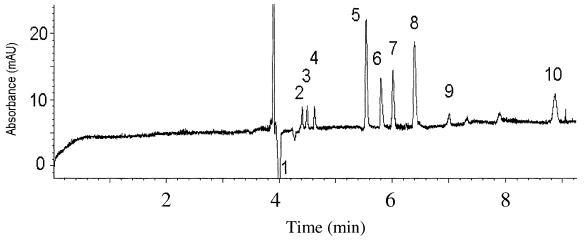

Fig. 4. Chromatogram of nine benzene derivatives. Capillary, 53.55 cm (effective length 45.00 cm)×I.D. 50 μm; column temperature, 25°C. Injection time, 1 s at 5000 Pa; UV detection at 214 nm; $V=20$ kV; $I=29.0$ μA. Polymer, AMPS–LMAm (84.9:15.1); polymer concentration, 0.99% (w/v). Sample concentration, 50 ppm. (1) Acetone; (2) nitrobenzene; (3) anisole; (4) *p*-nitroaniline; (5) naphthalenemethanol; (6) naphthyl amine; (7) acenaphthenol; (8) naphthaleneethanol; (9) *p*-xylene; (10) naphthalene.

copolymer with AMPS–LMAm (75.2:24.8). The square of the correlation coefficients of the plots were greater than 0.97. The polymers with similar mole ratios showed very high correlation ($r^2>0.99$). There was no change of the migration order. These results show that the chemical selectivities of the copolymers is only very weakly affected by the percentage of hydrophobic functionality (LMAm percentage). This suggests that we have to change the monomer chemical functional group to get copolymers with different chemical selectivities under the same buffer condition. Investigation of the effect of different side carbon chain length on chemical selectivity is underway.

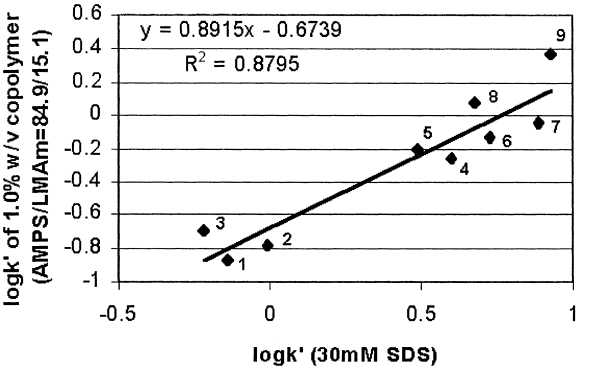

Fig. 5. Comparison of log k' for nine solutes using 1.0% AMPS–LMAm (84.9:15.1) and 30 m*M* SDS. (1) Nitrobenzene; (2) anisole; (3) *p*-nitroaniline; (4) naphthalenemethanol; (5) naphthylamine; (6) acenaphthenol; (7) naphthaleneethanol; (8) *p*-xylene; (9) naphthalene.

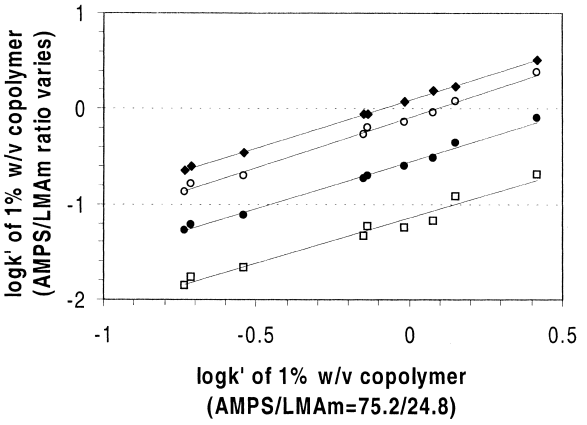

Fig. 6. Plot of log k' of nine solutes for five different copolymers with various AMPS–LMAm ratios. *x*-axis: log k' values with AMPS–LMAm (75.2:24.8). (♦) AMPS–LMAm (75.1:24.9); equation: $y=1.002x+0.095$, $R^2=0.999$. (○) AMPS–LMAm (84.9/15.1); equation: $y=1.0481x-0.0918$, $R^2=0.994$. (●) AMPS–LMAm (91.2:8.8); equation: $y=1.0003x-0.5523$, $R^2=0.992$. (□) AMPS–LMAm (96.6:3.4); equation: $y=0.9553x-1.1462$, $R^2=0.970$.

3.4. Effect of polymer concentration on k'

A plot of the k' values for the six ketone homologues versus the polymer concentration is shown in Fig. 7. Within the range of 0.17–3%, the plots are linear, suggesting a singular pseudo-stationary phase structure within this range. Surprisingly, the lines have non-zero *x*-intercepts around 0.1% (w/v). Similar results were also obtained by copolymers synthesized in the THF–TEA solvent system, with an AMPS–LMAm ratio of approximately 80:20 (data not shown).

To determine whether the k' values were truly zero at copolymer concentrations less than 0.17%, a copolymer concentration (AMPS–LMAm, 75.1:24.9) of 0.05% was employed. Even at such a low copolymer concentration, butyrophenone, valerophenone, hexanophenone and heptanophenone were well resolved from acetone, while acetophenone and propiophenone could not be separated from acetone. While the k' values cannot be calculated from the iteration method, they were obviously higher than

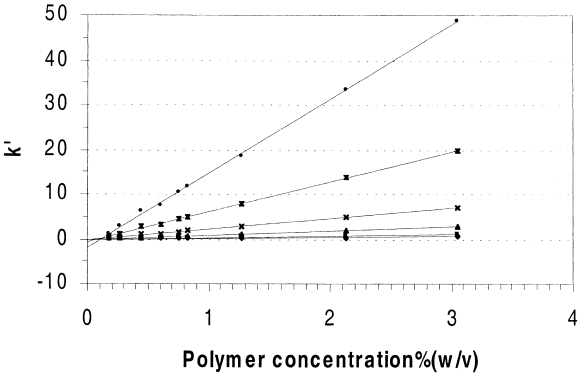

Fig. 7. Effect of polymer concentration on k'. AMPS–LMAm (75.1:24.9). (●) Heptanophenone: $y = 16.56x - 1.8936$, $R^2 = 0.9992$, x-intercept = 0.114. (*) Hexanophenone: $y = 6.75x - 0.6847$, $R^2 = 0.9994$, x-intercept = 0.101. (×) Valerophenone: $y = 2.4447x - 0.25$, $R^2 = 0.9994$, x-intercept = 0.102. (▲) Butyrophenone: $y = 0.9007x - 0.0791$, $R^2 = 0.9996$, x-intercept = 0.0878. (■) Propylphenone: $y = 0.3652x - 0.0287$, $R^2 = 0.9995$, x-intercept = 0.0786. (♦) Acetophenone: $y = 0.1519x - 0.0101$, $R^2 = 0.9994$, x-intercept = 0.0665.

zero. This suggests that the k' versus concentration plots might simply have lower slopes in the low concentration region. The plots between 0 and 0.17% (w/v) could be either linear with smaller slopes, or non-linear.

The results suggest that there exist two different kinds of polymer hydrophobic microdomains, one is less retentive at polymer percentages less than 0.17% (w/v), and the other shows greater retention at polymer percentages from 0.17 to 3% (w/v). Conformational changes of the polymer phase at various concentrations were also reported by other authors [35,24], which is a typical behavior of ionized polyelectrolytes, showing a balance of the intermolecular hydrophobic interactions and the segmental electrostatic repulsion [49].

To further investigate this concentration effect, the effect of the polymer concentration on the electrophoretic mobility was investigated, and is plotted in Fig. 8. As copolymer concentrations were decreased from 0.8 to 0.2%, the electroosmotic mobility only changed slightly, while the copolymer mobility increased greatly as the copolymer concentration decreased from 0.4 to 0.17% (w/v). This is a further evidence of change in the structure of copolymer at concentrations below 0.4% (w/v). The copolymer

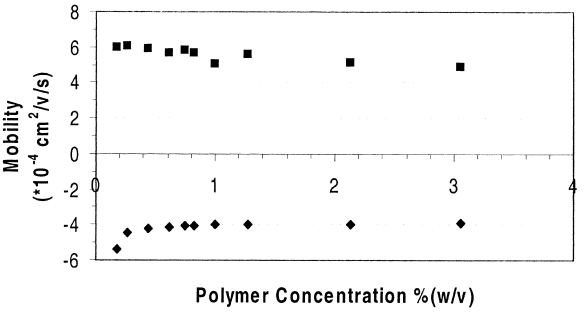

Fig. 8. Effect of polymer concentration on electroosmotic and electrophoretic mobility. AMPS–LMAm (75.1:24.9). (■) Electroosmotic mobility; (♦) electrophoretic mobility of copolymer.

electrophoretic mobility did not change significantly when copolymer concentrations were increased from 0.4 to 3.05% (w/v). The copolymer should be a singular-phase without micro-domain change in solutions with concentrations from 0.4 to 3.0% (w/v).

3.5. Effect of background buffer concentration

It has been observed that the conformation of ionic polymers in solution is affected greatly by the ionic strength of the solution [26]. To investigate the importance of this for the AMPS–LMAm copolymers, retention factors (k') of the ketone homologues were measured in borate buffers ranging in concentration from 5 to 100 m*M*. The results are plotted in Fig. 9, and show no significant change in retention within this concentration region. This shows that there was no significant hydrophobic microdomain change of the polymer as the ionic strength was changed. The methylene selectivities of the polymer in 5, 25, 50, 100 m*M* borate buffer are 2.526 ± 0.004, 2.541 ± 0.005, 2.542 ± 0.003, 2.572 ± 0.010, respectively. The slight differences (less than 2%) among the methylene selectivities are not significant enough to prove the existence of any hydrophobic microdomain change of the polymer due to the buffer concentration change.

The analysis time increased dramatically with the increase of the concentration of background buffer, due to a significant decrease of the electroosmotic mobility. The electroosmotic mobility, polymer electrophoretic mobility, and effective polymer mobility are plotted in Fig. 10. The electroosmotic mobility is

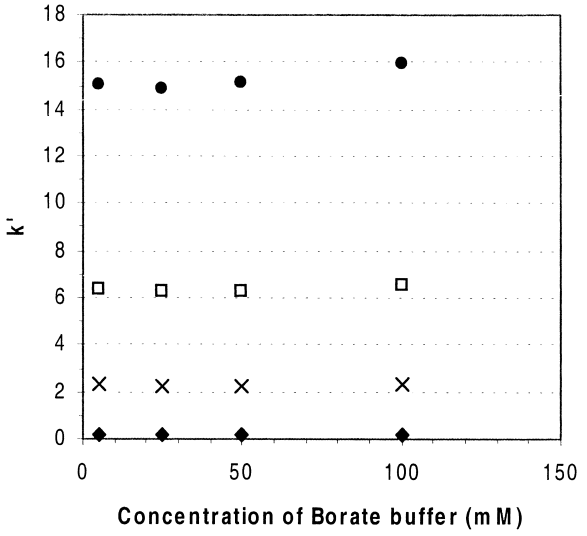

Fig. 9. Dependence of k' of ketones on the concentration of borate buffer. Polymer, AMPS–LMAm (75.1:24.9); polymer concentration, 1% (w/v). (♦) Acetophenone; (×) valerophenone; (□) hexanophenone; (●) heptanophenone.

reduced substantially due to the change of the zeta potential of the double layer on the capillary wall, and the change of the viscosity of the buffer solution. However, the electrophoretic mobility of the polymer does not change significantly above 5 m*M* borate buffer. The combination of reduced electroosmotic mobility and constant polymer electrophoretic mobility results in greatly reduced effective polymer mobility, and a greatly extended migration range.

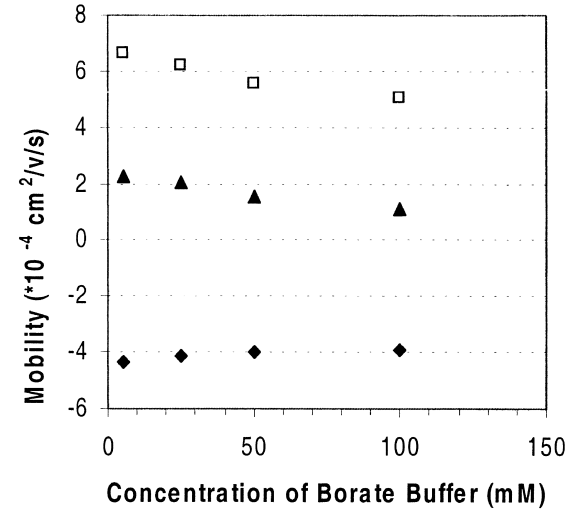

Fig. 10. Effect of buffer concentration on mobilities. Polymer, AMPS–LMAm (75.1:24.9); polymer concentration, 1% (w/v). (□) Electroosmotic mobility; (▲) polymer effective mobility; (♦) polymer electrophoretic mobility.

4. Conclusions

Sulfonated copolymers with different AMPS–LMAm mole ratios (from 100:0 to 60:40) were synthesized, characterized and successfully used as pseudo-stationary phases in EKC. Polymer electrophoretic mobilities and methylene selectivities showed good correlation with the monomer ratios. The chemical selectivities of the polymers for the separation of nine solutes showed a significant difference compared with that of SDS micelles. No significant change of chemical selectivities was found between copolymers with different monomer ratios. Thus monomers with different chemical functionalities should be used to synthesize polymers with different chemical selectivities. No significant change of microstructure of copolymers was found either in background buffers with different ionic strengths or in copolymer solutions at concentrations from 0.17 to 3% (w/v), based on retention factors, methylene selectivities and polymer electrophoretic mobilities. However, plots of k' versus polymer concentration suggested a different copolymer phase exists at lower concentrations (from 0 to 0.1%, w/v) from that at higher concentrations (from 0.17 to 3%, w/v). The copolymer AMPS–LMAm (80:20) could be chosen as optimum copolymer considering the methylene selectivity, peak symmetry and polymer mobility.

Acknowledgements

The authors would like to acknowledge a 1998 Department of Energy Presidential Early Career Award and the Department of Chemistry at New Mexico Tech for financial support. The authors are grateful to Dr. Fuierer and Lee Benysek of Materials Engineering Department at New Mexico Tech for assistance in the laser light scattering experiments.

References

[1] S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya, T. Ando, Anal. Chem. 56 (1984) 111.
[2] B.L. Bales, M. Almgren, J. Phys. Chem. 99 (1995) 15153.
[3] S. Terabe, T. Katsura, Y. Akada, Y. Ishihama, K. Otsuka, J. Microcol. Sep. 5 (1993) 23.
[4] J.H. Knox, K.A. McCormack, Chromatographia 38 (1994) 207.
[5] M.F. Emerson, A. Holtzer, J. Phys. Chem. 71 (1967) 3220.
[6] L. Magid, in: K. Mittal (Ed.), Solution Chemistry of Surfactants, Vol. 1, Plenum, New York, 1979, p. 427.
[7] C.P. Palmer, N. Tanaka, J. Chromatogr. A 792 (1997) 105.
[8] C.P. Palmer, J. Chromatogr. A 780 (1997) 75.
[9] J.L. Haynes, I.M. Warner, Rev. Anal. Chem. 18 (1999) 317.
[10] S.A. Shamsi, I.M. Warner, Electrophoresis 18 (1997) 853.
[11] P. Anton, P. Koberle, A. Laschewsky, Makromol Chem. 194 (1993) 1.
[12] A. Laschewsky, Adv. Polym. Sci. 124 (1995) 1.
[13] T. Chen, C.P. Palmer, Electrophoresis 20 (1999) 2412.
[14] C.P. Palmer, K.T. Tellman, J. Microcol. Sep. 11 (1999) 185.
[15] K.T. Tellman, C.P. Palmer, Electrophoresis 20 (1999) 152.
[16] C.P. Palmer, S. Terabe, Anal. Chem. 69 (1997) 1852.
[17] C.P. Palmer, S. Terabe, J. Microcol. Sep. 8 (1996) 115.
[18] C.P. Palmer, M.Y. Khaled, H.M. McNair, J. High Resolut. Chromatogr. 15 (1992) 756.
[19] T.W. Moy, P.L. Ferguson, A.H. Grange, W.H. Matchett, V.A. Kelliher, W.C. Brumley, J. Glassman, J.W. Farley, Electrophoresis 19 (1998) 2090.
[20] H. Ozaki, N. Itou, S. Terabe, Y. Takada, M. Sakairi, H. Koizumi, J. Chromatogr. A 716 (1995) 69.
[21] W.Z. Lu, S.A. Shamsi, T.D. McCarley, I.M. Warner, Electrophoresis 19 (1998) 2193.
[22] Y. Morishima, Y. Itoh, T. Hashimoto, S. Nozakura, J. Polym. Sci., Polym. Chem. Ed. 20 (1982) 2007.
[23] Y. Morishima, Y. Itoh, S. Nozakura, Makromol. Chem. 182 (1981) 3135.
[24] Y. Morishima, T. Kobayashi, S. Nozakura, Polym. J. 21 (1989) 267.
[25] M.G. Neumann, G.L. de Sena, Colloid Polym. Sci. 277 (1999) 414.
[26] M. Hara, in: Polyelectrolytes: Science and Technology, Marcel Dekker, New York, 1993.
[27] C.P. Palmer, H.M. McNair, J. Microcol. Sep. 4 (1992) 509.
[28] S. Terabe, H. Ozaki, Y. Tanaka, J. Chin. Chem. Soc. 41 (1994) 251.
[29] H. Ozaki, S. Terabe, A. Ichihara, J. Chromatogr. A 680 (1994) 117.
[30] S.A. Shamsi, S.M. Mathison, S. Dewees, J. Wang, I.M. Warner, presented at the Pittcon 96 Conference, Chicago, IL, 1996, Poster 84P.
[31] S.A. Shamsi, C. Akbay, I.M. Warner, Anal. Chem. 70 (1998) 3078.
[32] N. Tanaka, T. Tanigawa, K. Hosoya, K. Kimata, T. Araki, S. Terabe, Chem. Lett. 6 (1992) 959.
[33] N. Tanaka, K. Nakagawa, H. Iwasaki, K. Hosoya, K. Kimata, T. Araki, D.G. Patterson, J. Chromatogr. A 781 (1997) 139.
[34] N. Tanaka, K. Nakagawa, K. Hosoya, C.P. Palmer, S. Kunugi, J. Chromatogr. A 802 (1998) 23.
[35] N. Tanaka, K. Nakagawa, H. Nagayama, K. Hosoya, T. Ikegami, A. Itaya, M. Shibayama, J. Chromatogr. A 836 (1999) 295.
[36] S. Kolb, J.P. Kutter, T. Welsch, J. Chromatogr. A 792 (1997) 151.
[37] B. Maichel, B. Potocek, B. Gas, E. Kenndler, J. Chromatogr. A 853 (1999) 121.
[38] J. Wang, I.M. Warner, Anal. Chem. 66 (1994) 3773.
[39] J. Wang, I.M. Warner, J. Chromatogr. A 711 (1995) 297.
[40] A. Dobashi, M. Hamada, Y. Dobashi, Anal. Chem. 67 (1995) 3011.
[41] K.A. AgnewHeard, M.S. Pena, S.A. Shamsi, I.M. Warner, Anal. Chem. 69 (1997) 958.
[42] C.C. Williams, S.A. Shamsi, I.M. Warner, Adv. Chromatogr. 36 (1996) 363.
[43] S. Hara, A. Dobashi, Jpn Kokai Tokkyo Koho, JP 92 149 (1992) 205.
[44] S. Hara, A. Dobashi, Jpn Kokai Tokkyo Koho, JP 92 149 (1992) 206.
[45] F. Haddadian, S.A. Shamsi, I.M. Warner, Electrophoresis 20 (1999) 3011.
[46] C.P. Palmer, Proceedings of the 21st International Symposium on Capillary Chromatography and Electrophoresis, Park City, UT, 20–24 June, 1999, p. 79.
[47] A. Kobayashi, F. Matsuzaki, T. Yanaki, Y. Morishima, J. Appl. Polym. Sci. 73 (1999) 2447.
[48] M.M. Bushy, J.W. Jorgenson, Anal. Chem. 61 (1989) 491.
[49] H. Morawetz, in: Macromolecules in Solution, 2nd Edition, Interscience, New York, 1975, p. 359.