ELSEVIER

Journal of Chromatography A, 924 (2001) 123–135

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Effect of pendant chain lengths and backbone functionalities on the chemical selectivity of sulfonated amphiphilic copolymers as pseudo-stationary phases in electrokinetic chromatography

Wei Shi, Dominic S. Peterson, Christopher P. Palmer*

Department of Chemistry, New Mexico Institute of Mining and Technology, 801 Leroy Place, Socorro, NM 87801, USA

Abstract

Amphiphilic copolymers of AMPS (2-acrylamido-2-methyl-1-propanesulfonic acid) and hydrophobic monomers with various chemical structures were synthesized, characterized and used as novel electrokinetic chromatography polymeric pseudo-stationary phases, showing significant chemical selectivity differences from that of the conventional monomeric pseudo-stationary phase, sodium lauryl sulphate. Copolymers of AMPS and methacrylates with different pendant chain lengths (C_8, C_{12} and C_{18}) were investigated and no significant difference in chemical selectivity was observed among them. However, the spacer bonding chemistry was shown to contribute to significant chemical selectivity difference, e.g. poly(AMPS–lauryl methacrylate) showed different chemical selectivity from poly(AMPS–lauryl methacrylamide). Linear solvation energy relationship analysis of 20 solutes by eight different polymeric pseudo-stationary phases was employed to investigate the solute molecule structural contributions to the retention. Hydrogen-bonding properties (described by system constants *b* and *a*) of poly(AMPS–alkyl methacrylamide) were found stronger than those of poly(AMPS–alkyl methacrylate).

Keywords: Pseudo-stationary phases; Electrokinetic chromatography; Linear solvation energy relationships; Methacrylamide; Methacrylate

1. Introduction

Micellar electrokinetic chromatography (micellar EKC), one of the modes of capillary electrophoresis (CE), was introduced by Terabe et al. in 1984 [1]. Addition of low-molecular-mass surfactants to the buffer as pseudo-stationary phases enables neutral solutes, as well as charged solutes with the same charge-to-mass ratio, to be separated [2,3]. In the past decade, there have been significant efforts investigating the use of high-molecular-mass surfactants (polymeric surfactants) as the pseudo-stationary phases, because they show higher stability with regard to the variations in analytical conditions, such as the pH, ionic strength, and organic solvent strength [4–8]. Polymer surfactants also facilitate EKC–MS coupling [9,10].

Different polymeric surfactants have been used as pseudo-stationary phases [11–13], including modified acrylate copolymers [14,15], polySUS [poly(sodium 10-undecenyl sulfate)] [16–18], polySUA [poly(sodium 10-undecylenate)] [19,4,20], polySUT [poly(sodium *N*-undec-10-ene-1-oyl-taurate)] [21], polyallylamine-supported phases [22,23], silicone-based polymers [24,25], polyethyleneimine [26], poly(diallyldimethylammonium) [27], poly(Na 11-

*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(01)00760-9

AAU) [poly(sodium 11-acrylamidoundecanoate)] [28], dendrimers [29,30], and also many chiral selective polymers [31,32], such as poly-L-SUV [poly(sodium undecanoyl-L-valinate)] [33], polymeric dipeptide surfactant [34], etc. Seeking polymeric surfactants with significantly different chemical selectivities is of great interest in this field. In order to accomplish this, it is important to investigate fully the molecule structural factors that affect chemical selectivity.

The effect of alkyl chain length of low-molecular-mass surfactants on chemical selectivity has been studied. Sodium *N*-acyl sarcosinates were studied by Takeda et al. [35], who observed slightly different separation selectivity (without any change of the peak order) for hydrophobic solutes. With the increase of alkyl chain length, the krafft point increased, and the critical micellar concentration (CMC) decreased [35]. Homologues of sodium lauryl sulphate (SDS) with alkyl tail lengths from C_{10} to C_{16} have also been investigated [36]. The surfactants with alkyl tails of less than eight carbons did not generally form micelles [36], and those with chains longer than 14 carbons displayed limited solubility in water, and decyl sulfate showed poor reproducibility [37]. Compared with micelles formed by low-molecular-mass surfactants, polymeric surfactants are more stable with respect to solubility and CMC. Polymeric surfactants with different pendant chain lengths can thus be conveniently employed to change the migration window, and to adjust the separation selectivity of complex samples with a wide distribution of hydrophobicity [22,23]. In this work, the effect of backbone chemistry and pendant alkyl chain length on the chromatographic selectivity and performance of 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) copolymers has been investigated.

Copolymers of AMPS with LMAm (lauryl methacrylamide) or LMAt (lauryl methacrylate) are an interesting family of copolymers to study because the monomer unit ratio can be easily controlled [38] and because both the backbone and pendant group chemistries can be systematically varied. Poly(AMPS–*tert*.-butylacrylamide) has recently been employed to coat the capillary wall in open-tubular capillary electrochromatography (CEC) [39]. We have previously described the use of poly(AMPS–LMAm) as the pseudo-stationary phase for EKC [40]. Sulfonated amphiphilic copolymers have great aqueous solubility even under acidic conditions, and are soluble in organic–aqueous binary solvents.

Copolymers of AMPS with acrylamide or acrylate co-monomers have been found to have different association properties in aqueous solution [41]. Noda and Morishima have shown that poly(AMPS–LMAm) (LMAm mole percentage $f_{\mathrm{LMAm}}\%$ ~10%) form ''unimer'' micelles (single-polymer micelles) in aqueous solutions even at very high polymer concentrations, arising from the predominant intra-polymer association of pendant hydrophobes (lauryl groups). However, the same authors proposed a different bridged ''poly-core multi-polymer micelles'' model for poly(AMPS–LMAt) with LMAt mole percentage $f_{\mathrm{LMAt}}\%=15\%$, and an ''unicore multi-polymer micelle'' model for poly(AMPS–LMAt) with $f_{\mathrm{LMAt}}\%=9\%$ [41,42]. Based on the different association behavior, we chose to compare the EKC performance of poly(AMPS–LMAt) with that of poly(AMPS–LMAm) as pseudo-stationary phases.

LSER (linear solvation energy relationship) analysis is a very useful method to investigate the difference of solubility properties in light of the solute chemical structures [43–54]. Solutes are described by solvation descriptors V_x, π_2^{H}, R^2, $\Sigma\beta_2^{\mathrm{H}}$ and $\Sigma\alpha_2^{\mathrm{H}}$, which describe the characteristic volume, polarity/polarizability, excess molar refractive index, hydrogen-bond accepting ability and hydrogen-bond donating ability of the solutes, respectively. The log of the retention factors for a diverse collection of solutes is then fit to Eq. (1) using multiple linear regression:

$$\log k' = c + mV_x + s\sum \pi_2^{\mathrm{H}} + rR_2 + b\sum \beta_2^{\mathrm{H}} + a\sum \alpha_2^{\mathrm{H}} \quad (1)$$

The *c*-term in Eq. (1) shows the phase ratio contribution. The system constants describe the relative strengths of various chemical interactions with the pseudo-stationary phase: *m*, *s*, *r*, *b* and *a* describe the energy of cavity formation/dispersive interaction, polarity/polarizability, ability to interact

with the n- and π-electrons of the solutes, hydrogen-bond donating and hydrogen-bond accepting ability of phases relative to the aqueous buffer, respectively. *c*-, *m*- and *b*-terms make major contributions to the retention in EKC, while *s*-, *r*-, *b*-, and *a*-terms make major contributions to the chemical selectivity difference [43,45,55,56]. *c*-, and *m*-terms generally do not explain the chemical selectivity differences between pseudo-stationary phases [51]. The contributions to retention from polar interactions for various pseudo-stationary phases are the easiest to compare by normalizing the system constants for polar interactions by division with the constant *m*. This resulted ratio represents the capacity of the pseudo-stationary phases for polar interactions independent of solute size [57]. LSER analysis using the solvation-descriptor model and/or the solvatochromic-descriptor model has been employed to investigate the characteristics of polymeric pseudo-stationary phases [24,21,27]. LSER analysis using the solvation-descriptor model was employed in this work to investigate the distribution of solutes between sulfonated copolymer phases and the background solvent phase.

To our knowledge, poly(AMPS–alkyl methacrylate), poly(AMPS–alkyl acrylate) and poly(AMPS–alkyl acrylamide) have not been reported being used as EKC pseudo-stationary phases before. Four comparisons of chromatographic performance and chemical selectivity are made in this work among copolymers with different chemical structures: (a) between SDS micelles and AMPS copolymers; (b) between poly(AMPS–alkyl methacrylate) and poly(AMPS–alkyl methacrylamide); (c) among poly(AMPS–alkyl methacrylate)s with different pendant chain lengths; and (d) between copolymers with different α-methyl group percentages on the backbone, e.g. between poly(AMPS–alkyl methacrylamide) and poly(AMPS–alkyl acrylamide), or between poly(AMPS–lauryl methacrylate) and poly(AMPS–lauryl acrylate). Plots of $\log k'$ for multiple analytes on a given phase versus these on another phase are compared to see the overall chemical selectivity difference. *s*-, *r*-, *b*-, and *a*-terms of LSER analysis are compared to see the source causing the overall chemical selectivity differences among polymers. In an attempt to compare the system constants (*m*, *s*, *r*, *b*, and *a*) among the eight polymeric pseudo-stationary phases investigated, a multiple correlation equation between the system constants and structural factors of the polymeric pseudo-stationary phases was constructed.

2. Experimental

2.1. Chemicals

n-Octyl methacrylate (OMAt, C_8), lauryl methacrylate (LMAt, C_{12}), stearyl methacrylate (SMAt, C_{18}), lauryl acrylate (LAt), lauryl methacrylamide (LMAm) and stearyl acrylamide (SAm) were purchased from Polysciences (Warrington, PA, USA). AMPS was from Aldrich (Milwaukee, WI, USA). 2,2′-Azobis(2-methylpropionitrile) (AIBN) was from Dionex (Sunnyvale, CA, USA). Ketone homologues and sodium tetraborate were from Aldrich. HPLC-grade tetrahydrofuran (THF) and phosphoric acid were from Acros (NJ, USA). Deionized water was obtained by a water purification system (Millipore, Bedford, MA, USA). The rest of the LSER solutes were from Fisher (NJ, USA). All reactants and solvents were used as received from the manufacturers without further purification.

2.2. Synthesis and characterization

Six types of copolymers, poly(AMPS–OMAt), poly(AMPS–LMAt), poly(AMPS–SMAt), poly(AMPS–LAt), poly(AMPS–LMAm), and poly(AMPS–SAm) were synthesized. These are abbreviated in the following text as pOMAt-*f*, pLMAt-*f*, pSMAt-*f*, pLAt-*f*, pLMAm-*f*, and pSAm-*f*, respectively, where *f* represents the hydrophobe mole percentage (*f*%). The chemical structures are shown in Fig. 1. The pendant hydrophobes are C_8, C_{12}, or C_{18}, the spacer bonding between the main and pendant chains are via amide (NH) or ester (O), and the α-substitution on the backbone neighboring to C=O is either H or methyl group. The synthesis and purification procedures were similar to our previous work [40] and other reports [58]. A 3-mmol amount of the monomers in the desired ratio was dissolved in 70 ml THF–water (80:20, v/v) solution and placed in a flask. AIBN (0.2 mol% based on total moles of

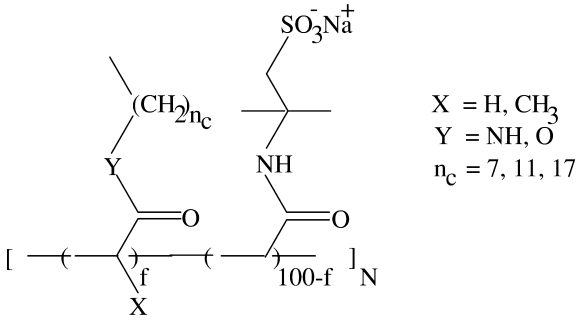

Fig. 1. Illustration of chemical structures of six types of copolymers: (1) pOMAt: nc=7, x=CH_3, Y=O; (2) pLMAt: nc=11, x=CH_3, Y=O; (3) pSMAt: nc=17, x=CH_3, Y=O; (4) pLAt: nc=11, x=H, Y=O; (5) pLMAm: nc=11, x=CH_3, Y=NH; (6) pSAm: nc=17, x=H, Y=NH.

monomers) was used as the initiator. The solution was flushed by nitrogen, then heated to and maintained at 62°C by a bench-top temperature controller (ThermoWorks, Alpine, UT, USA) for 24 h. The pH of the solution after polymerization was adjusted to about pH 9 using NaOH. The products were then purified by dialysis, with molecular mass cut-off (MWCO) 500 or 2000 dialysis tubing (this difference in the MWCO of the dialysis tubing will not affect the performance or the chemical selectivity of the polymers, as shown by our unpublished experiments), followed by filtering through a 0.45-μm syringe filter (Whatman, Clifton, NJ, USA), and freeze drying. A JEOL Eclipse 300+ NMR with a Silicon Graphics workstation was used to characterize the copolymers. Single pulse ^{1}H NMR experiments were performed. The resonances ranging from 3.27 to 3.43 ppm were indicative of the CH_2SO_3 group on the AMPS portion of the copolymer, and the resonance located at 0.87 ppm showed the presence of the terminal methyl groups on the pendant hydrophobes. The numbers following the polymer name abbreviations in the text represent the hydrophobe percentage, *f*%, obtained from the ratio of the integrals of the resonance peak at 3.4 ppm to that at 0.9 ppm [40,41].

The lower limit of the molecular mass (M_r) of the polymers used in this work is set by the MWCO of the dialysis tubing used (MWCO=500 or 2000). Static light scattering (SLS) was employed to characterize the molecular mass of one of the copolymers, pLMAm-9 (*f*%=9%). A water–ethanol (50:50, v/v) mixture was chosen as the solvent to minimize the interpolymer aggregation of this type of polyelectrolyte, and a weight-average M_r (M_w) of $3\cdot10^5$ was obtained. Noda and Morishima fully characterized similar copolymers synthesized by a similar method and found the molecular mass to be rather independent of structure (4.2 to $5.4\cdot10^4$ for pLMAt with *f*% ranging from 1 to 15% by size-exclusion chromatography (SEC) [41], and 3.7 to $6.2\cdot10^4$ for pLMAm with *f*% ranging from 2.5 to 10.0%) [59]. Fujimoto et al. also got a very high molecular mass ($M_w=3\cdot10^6$) for poly(Na 11-AAU) using SEC–multiangle laser light scattering detection [28].

2.3. EKC conditions

2.3.1. EKC condition 1

A Hewlett-Packard (Palo Alto, CA, USA) ^{3D}CE capillary electrophoresis instrument with Chemstation software was used to perform the EKC experiments. 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*, pH 9.2) and filtered through a 0.45-μm syringe filter (Whatman). Stock sample solutions were prepared in acetone at a concentration of ~1000 ppm. Before each run, 10 μl of the stock sample solutions were dissolved in 100 μl polymer buffer solution, resulting in sample concentrations of ~100 ppm (unless stated otherwise). Injections were performed at 5000 Pa for 3 s, unless stated otherwise. Separations were performed at 20 kV. The capillary cartridge temperature was maintained at 25.0°C. The UV detector was set at 214 and 254 nm. The capillary was flushed with background buffer for 3 min between runs. Each set of separations was run at least twice. The electroosmotic flow (EOF) marker was acetone, and the migration times of the pseudo-stationary phases were obtained by the iteration method [60] using homologues of alkyl phenyl ketones (from acetophenone to heptanophenone). The methylene selectivity α ($\alpha=k_2'/k_1'$, where k_2' and k_1' are the retention factors of two adjacent compounds in the homologous

series) were calculated by 10^k, where k is the slope of log k' versus carbon number plot. The current was ~27–33 μA.

2.3.2. EKC condition 2 (for all LSER experiments in Section 3.3)

For an LSER study on the nine pseudo-stationary phases, the capillaries used had an effective length of 25.0 cm and a total length of 33.3 cm. The separation buffer used was 20.0 m*M* Tris adjusted to pH 7.0 with phosphoric acid. Pseudo-stationary phases were dissolved in the buffer solutions at concentrations of 1.0% (w/v). The RSD of the migration times of acetone was typically 0.45% for 38 continuous injections throughout one set of experiments. Each separation was run in duplicate. At the end of the investigation of one pseudo-stationary phase, the capillary was flushed by acetone–water (50:50, v/v) for 30 min. Before investigating a new pseudo-stationary phase, the capillary was flushed by 0.1 *M* NaOH for 10 min, then water for 20 min. The current was ~48–50 μA. Other conditions were the same as those in Section 2.3.1.

3. Results and discussion

3.1. EKC performance

Six polymer chemistries (ten different copolymers) with three different pendant chain lengths and three different backbone/spacer chemistries (pOMAt-*f*, pLMAt-*f*, pSMAt-*f*, pLAt-*f*, pLMAm-*f*, and pSAm-*f*) were synthesized in different batches with differing hydrophobe percentages, and characterized by electrokinetic chromatography. Representative separations of a homologous series of six ketones using pLMAm-19 and pLMAt-15 are shown in Fig. 2. The methylene selectivity and electrophoretic mobility for the pseudo-stationary phases were calculated from the homologous series separations under two sets of separation conditions, and are listed in Table 1. Most of the mobilities and methylene selectivities of those copolymers were greater than or similar to those of SDS. Trends of the methylene selectivity and electrophoretic mobility are very difficult to ascertain, because several variables of polymer structures are often varied simultaneously. Nevertheless,

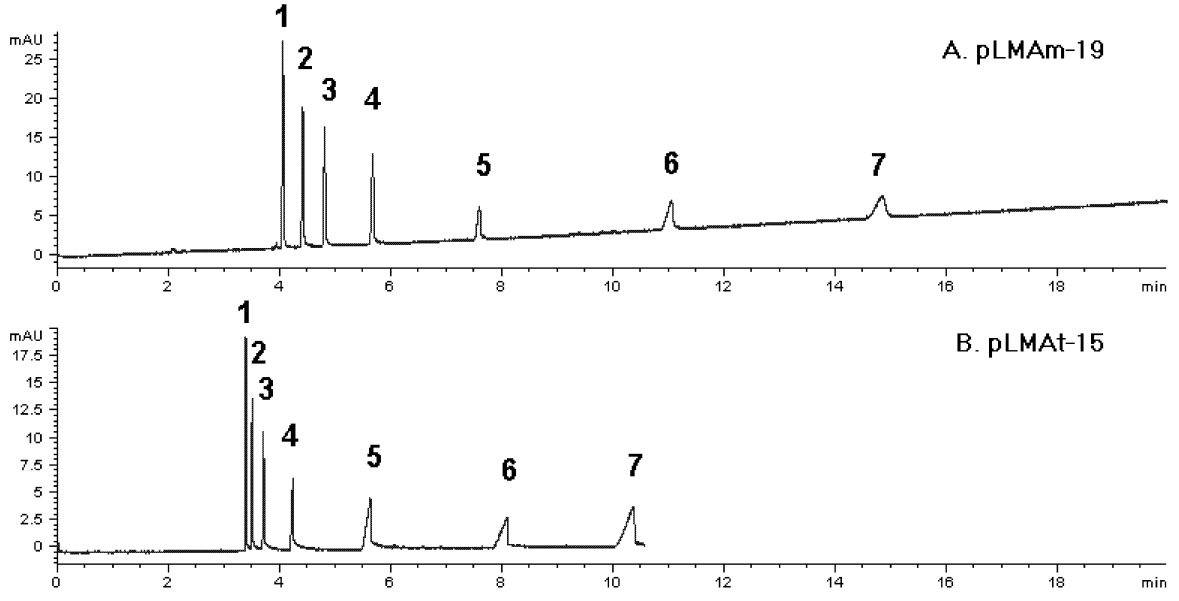

Fig. 2. Separation of ketone homologues by two copolymers: (A) pLMAm-19, (B) pLMAt-15; copolymer concentration, 1% (w/v); UV detection, 254 nm; injection for 3 s at 5000 Pa (for other EKC conditions see Section 2.3.1). Samples: (1) acetone; (2) acetophenone; (3) propiophenone; (4) butyrophenone; (5) valerophenone; (6) hexanophenone; (7) heptanophenone.

Table 1
EKC performance of different pseudo-stationary phases

Pseudo-stationary phase				Mobility ($\times 10^{-4}$ cm^2 V^{-1} s^{-1})	Methylene selectivity
Abbreviation	Synth. yield (%)	Hydrophobe mole percentage (f%)[a]	Concentration		
EKC condition 1: pH 9.2, 50 m*M* borate (buffer 1)					
SDS	N/A	50	30 m*M*	−3.97±0.00	2.42±0.04
pOMAt-21	46.0	20.6	1.0% w/v	−4.03±0.009	3.09±0.002
pLMAt-22	10.8	22.2	1.0% w/v	−4.00±0.003	3.19±0.009
pSMAt-13	19.2	13.1	1.0% w/v	−3.96±0.017	2.94±0.0002
pLMAm-19	15.6	19.0	1.0%w/v	−4.10±0.009	2.34±0.007
pSAm-17	11.8	16.6	1.1% w/v	−3.88±0.005	2.73±0.04
EKC condition 2: pH 7.0, 20 m*M* Tris (buffer 2)					
SDS	N/A	50	1.0% w/v	−4.58±0.017	2.34±0.0004
pOMAt-21	46.0	20.6	1.0% w/v	−4.67±0.048	3.04±0.0049
pLMAt-15	54.5	14.6	1.0% w/v	−5.01±0.011	3.00±0.018
pSMAt-13	19.2	13.1	1.0% w/v	−4.80±0.001	3.02±0.007
pLAt-9	21.2	9.0	1.0%w/v	−7.23±0.056	2.31±0.020
pLMAm-19	15.6	19.0	1.0% w/v	−4.89±0.020	2.26±0.003
pSAm-28	41.0	27.8	1.0% w/v	−4.47±0.017	2.65±0.008
pLAt-13	13.2	12.8	1.0% w/v	−5.34±0.04	2.88±0.136
pSMAt-16	13.25	15.8	1.0% w/v	−4.91±0.01	3.25±0.021

[a] The actual f% were calculated by NMR spectra.

different spacer bonding (via CONH– vs. COO–) between the main and pendant chains has an effect on the methylene selectivity (lower for acrylamide copolymers), but no effect on the electrophoretic mobility of the copolymers.

The two EKC conditions also had significant effects on the performance of the copolymers. The electrical field strength (E) was higher and the separations were faster under condition 2 (Section 2.3.2). Methylene selectivities differed by less than 4% between the two conditions, while ~10% higher differences were observed in mobility under condition 2, which are most likely caused by Joule heating due to the higher E employed. A 10% lower mobility ($4.48 \cdot 10^{-4}$ cm^2 V^{-1} s^{-}1) was obtained for pLMAt-15 when using a longer capillary with an effective length of 45 cm under EKC condition 2 (data not shown in Table 1). The average column efficiency of the six ketones by each copolymer under EKC condition 1 ranged from 101 000 to 242 000 plates/m. The average column efficiency under EKC condition 2 was reduced to ~40 000 plates/m. This was also due to the higher E employed. Higher column efficiency of 148 000±133 000 plates/m was obtained by pLMAt-15 when a longer capillary with an effective length of 45 cm was used under EKC condition 2 (chromatogram not shown).

The separations of nine aromatic solutes with different functional groups by pSAm-17 and pLMAt-15 are shown in Fig. 3. The elution orders of naphthalenemethanol/naphthylamine and naphthaleneethanol/*p*-xylene were reversed by these two copolymers.

3.2. Effect of hydrophobe percentage on chemical selectivity

Former research on pLMAm has shown that variation of f% from 5 to 25% did not have a significant effect on the chemical selectivity [40,61]. However, it was pointed out by Morishima that the association behavior of the pLMAt was dramatically different from that of pLMAm [42]. To test whether the conclusion from our former work with pLMAm is also applicable for pLMAt, the effect of f% on the chemical selectivity of pLMAt was investigated. A plot of $\log k'$ using pLMAt-22 versus those using

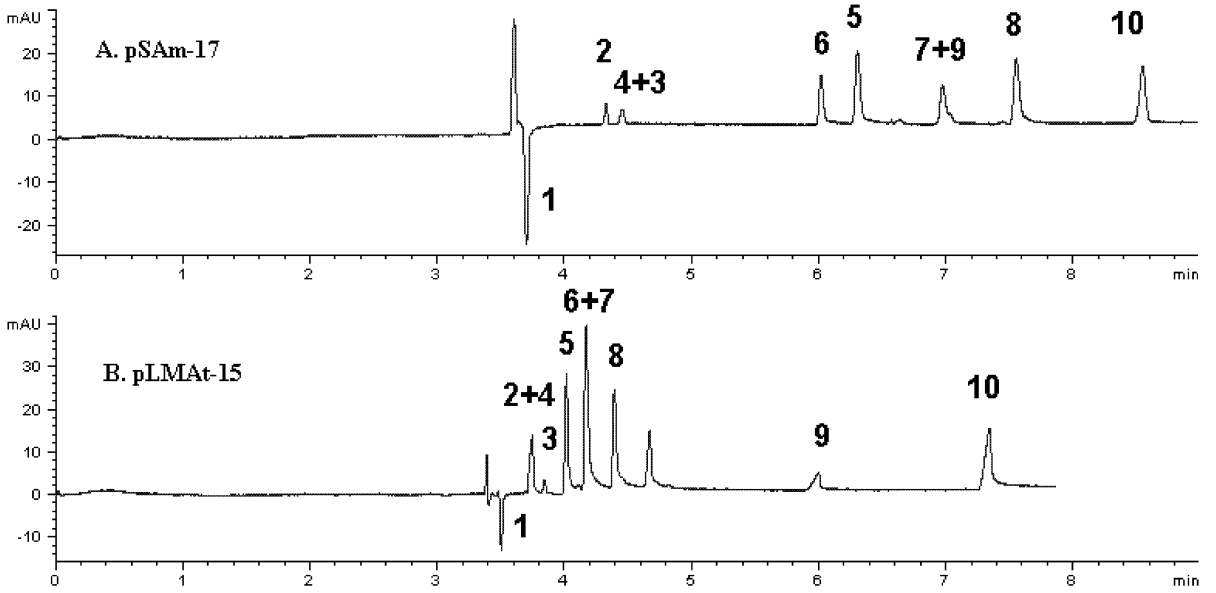

Fig. 3. Separation of nine benzene derivatives by two copolymers: (A) pSAm-17, (B) pLMAt-15; copolymer concentration, 1.0% (w/v); UV detection, 214 nm; sample concentration, 50 ppm; injection time: (A) 1 s, (B) 3 s at 5000 Pa (for other conditions see Section 2.3.1). Samples: (1) acetone, (2) nitrobenzene, (3) anisole, (4) *p*-nitroaniline, (5) naphthalenemethanol, (6) naphthylamine, (7) acenaphthenol, (8) naphthaleneethanol, (9) *p*-xylene, (10) naphthalene.

pOMAt-21 for nine solutes in borate buffer under EKC condition 1 gave an R^2 of 0.997, and a similar plot using pLMAt-15 versus pOMAt-21 for 20 solutes in Tris buffer under EKC condition 2 also gave an R^2 of 0.997 (Table 2). It can thus be safely concluded that, like pLMAm, the variation of f% from 15 to 22% does not significantly affect the chemical selectivity of pLMAt. Therefore, the effect of small differences in f% is ignored in the discussion of the chemical selectivity in Section 3.3.

However, because the hydrogen bonding ability of the CONH– group of AMPS and the COO– group of LMAt was quite different (unlike the case of AMPS vs. LMAm), different polymeric micelle structure models have been proposed for pLMAt with $f\% = 9\%$ and f% greater than 15% [41]. Large differences in AMPS percentages in pLMAt may theoretically cause a significant difference in chemical selectivity, thus we should be careful when comparing the chemical selectivity of pLMAt with very low or very high f% (e.g. 5 and 40%).

3.3. Investigation on the retention mechanism by LSER

A total of 20 aromatic solutes with different

Table 2
R^2 of $\log k'$ plot (by 20 solutes)

	SDS	pOMAt-21	pLMAt-15	pSMAt-13	pLAt-9	pLMAm-19	pSAm-28
SDS	1	0.924	0.924	0.916	0.927	0.899	0.922
pOMAt-21		1	0.997	0.995	0.952	0.872	0.937
pLMAt-15			1	0.997	0.961	0.887	0.950
pSMAt-13				1	0.960	0.893	0.955
pLAt-9					1	0.956	0.982
pLMAm-19						1	0.983
pSAm-28							1

Table 3
Test solutes and their solvation parameters [62]

	Solute	V_x	π_2^H	R_2	$\Sigma\beta_2^H$	$\Sigma\alpha_2^H$
1	Benzene	0.716	0.52	0.61	0.14	0
2	Toluene	0.857	0.52	0.601	0.14	0
3	Ethylbenzene	0.998	0.51	0.613	0.15	0
4	Propylbenzene	1.139	0.5	0.604	0.15	0
5	*p*-Xylene	0.998	0.52	0.613	0.16	0
6	Chlorobenzene	0.839	0.65	0.718	0.07	0
7	Iodobenzene	0.975	0.82	1.188	0.12	0
8	Naphthalene	1.085	0.92	1.36	0.2	0
9	1-Methylnaphthalene	1.226	0.9	1.344	0.2	0
10	Acetophenone	1.014	1.01	0.818	0.48	0
11	Propiophenone	1.155	0.95	0.804	0.51	0
12	Benzonitrile	0.871	1.11	0.742	0.33	0
13	Ethylbenzoate	1.214	0.85	0.689	0.46	0
14	4-Chloroanisole	1.038	0.86	0.838	0.24	0
15	Phenol	0.775	0.89	0.805	0.3	0.6
16	4-Methylphenol	0.916	0.87	0.82	0.31	0.57
17	4-Fluorophenol	0.793	0.97	0.67	0.23	0.63
18	4-Chloroaniline	0.939	1.13	1.06	0.31	0.3
19	3-Bromophenol	0.95	1.15	1.06	0.16	0.7
20	2-Naphthol	1.144	1.08	1.52	0.4	0.61

chemical functional groups and a wide range of solvation parameters were chosen as the target solutes for LSER study (Table 3). To keep them electrically neutral, the pH value of the background buffer was adjusted to 7.0. A total of nine pseudo-stationary phases (SDS, pOMAt-21, pLMAt-15, pSMAt-13, pSMAt-16, pLAt-9, pLAt-13, pLMAm-19 and pSAm-28) were investigated by LSER, all with the same counterion (Na^+). Most of the co-polymers were of similar f% (17±5%), except pLAt-9 and pSAm-28. The system constants (x) and normalized system constants (x/m) for the nine phases are listed in Table 4. The greater a system constant, the stronger the corresponding interaction between solutes and pseudo-stationary phase relative to the buffer [51]. The LSER results obtained for SDS in this work are similar to those reported elsewhere by other authors [57].

3.3.1. Comparison between copolymers and SDS

As shown in Table 4, the main contributors to retention for SDS and all of the polymeric pseudo-stationary phases are the *m*- and *b*-terms. This has been noted earlier by Trone and Khaledi for low-molecular-mass pseudo-stationary phases [51].

Comparing SDS and the copolymers, the intercept-term of SDS is the highest, showing the phase ratio of SDS is the highest among the pseudo-stationary phases. This was not only related to the molar concentration of the pseudo-stationary phases, but also to the hydrophobe percentages. The intercept generally increases with f% (plot not shown). The intercepts of the copolymers (except pLAt-9) were distributed closely around −2.7, because their f% and copolymer mole concentrations were similar. The intercept of pLAt-9 was extremely low, −3.2, because f% was as low as 9.0%. However, the intercept of SDS was −2.164, much higher than those of the copolymers, because SDS had the highest ''hydrophobe percentage'' (f%=50%) among these nine pseudo-stationary phases.

The *m* values increased in the order of pLAt-9, pLMAm-19, SDS, pSAm-28, pOMAt-21, pLAt-13, pLMAt-15, pSMAt-13 and pSMAt-16, indicating that the cohesiveness of those pseudo-stationary phases decreased in this order. The *m* values of pLMAm-19 and pSAm-28 are smaller than pOMAt-21, pLMAt-15, pSMAt-13 and pSMAt-16, while closer to that of SDS, suggesting that the micelle structure of acrylamide copolymers (uni-core single-polymer micelle) is closer to that of SDS (uni-core micelle), while different from that of poly(AMPS–

Table 4
LSER results (solute number $n=20$; for other conditions see Section 2.3.2)

Pseudo-stationary phase		Intercept	m	s	r	b	a	Statistics[b]
SDS	x[a]	−2.164	3.165	−0.321	0.3653	−2.193	−0.310	$R^2=0.995$
	SD	(±0.0938)	(±0.107)	(±0.0915)	(±0.0625)	(±0.131)	(±0.0491)	$F=520.2$
	x/m	−0.6838	1	−0.1013	0.1154	−0.6929	−0.09781	SE=0.046
pOMAt-21	x	−2.662	3.559	−0.595	0.4697	−3.747	−0.407	$R^2=0.993$
	SD	(±0.14)	(±0.16)	(±0.137)	(±0.094)	(±0.196)	(±0.074)	$F=396.4$
	x/m	−0.7480	1	−0.1672	0.1320	−1.053	−0.1144	SE=0.069
pLMAt-15	x	−2.842	3.654	−0.672	0.435	−3.704	−0.274	$R^2=0.991$
	SD	(±0.159)	(±0.181)	(±0.155)	(±0.106)	(±0.222)	(±0.0832)	$F=303.1$
	x/m	−0.7778	1	−0.1839	0.1190	−1.0137	−0.07499	SE=0.078
pSMAt-13	x	−2.733	3.764	−0.693	0.422	−3.873	−0.223	$R^2=0.994$
	SD	(±0.135)	(±0.154)	(±0.132)	(±0.0899)	(±0.188)	(±0.0706)	$F=439.0$
	x/m	−0.7260	1	−0.1840	0.1120	−1.0290	−0.05931	SE=0.066
pSMAt-16	x	−2.729	3.782	−0.846	0.653	−3.831	−0.495	$R^2=0.990$
	SD	(±0.187)	(±0.214)	(±0.183)	(±0.125)	(±0.262)	(±0.0981)	$F=273.8$
	x/m	−0.7215	1	−0.2236	0.1726	−1.013	−0.1308	SE=0.092
pLAt-9	x	−3.250	2.841	−0.3166	0.3430	−2.754	0.001	$R^2=0.971$
	SD	(±0.202)	(±0.231)	(±0.198)	(±0.135)	(±0.283)	(±0.106)	$F=93.0$
	x/m	−1.144	1	−0.1115	0.1207	−0.9694	0.0004	SE=0.099
pLAt-13	x	−2.962	3.582	−0.3950	0.3896	−3.5239	−0.0242	$R^2=0.977$
	SD	(±0.228)	(±0.260)	(±0.222)	(±0.152)	(±0.318)	(±0.119)	$F=117.0$
	x/m	−0.827	1	−0.110	0.109	−0.984	−0.00676	SE=0.112
pLMAm-19	x	−2.690	2.880	−0.322	0.374	−2.453	0.254	$R^2=0.990$
	SD	(±0.113)	(±0.129)	(±0.110)	(±0.0752)	(±0.158)	(±0.0591)	$F=265.5$
	x/m	−0.9341	1	−0.1117	0.1299	−0.8516	0.08824	SE=0.055
pSAm-28	x	−2.566	3.385	−0.534	0.421	−3.053	0.187	$R^2=0.993$
	SD	(±0.115)	(±0.131)	(±0.112)	(±0.0764)	(±0.160)	(±0.0600)	$F=387.4$
	x/m	−0.7580	1	−0.1579	0.1245	−0.9020	0.05534	SE=0.056

[a] System constant x=intercept, m, s, b, a, and r.
[b] F, Fisher F-test statistics, at significance 0.001, $F(5,14)=7.92$; R, correlation coefficient; SE, standard error of the predicted $\log k'$ values.

alkyl methacrylate) (poly-core multi-polymer micelle) [41]. The difference in m values between pLAt-9 ($f\%=9.0\%$) and pLAt-13 ($f\%=12.8\%$) was probably due to the polymer micelle structure change, because the critical $f\%$ value for structure change from uni-core multi-polymer micelle to poly-core multi-polymer micelle is reported to be somewhere between 9 and 15% [41].

The b and b/m of SDS were obviously the highest. The a value of SDS is similar to those of the methacrylate copolymers, while much smaller than those of acrylamide copolymers. The s of SDS is close to those of pLMAm-19 and pLAt-9, but much higher than those of pOMAt-21, pLMAt-15, pSMAt-13 and pSMAt-16. All these suggest different chemical selectivities between SDS and the copolymers. The $\log k'$ of 20 solutes separated by six copolymers were plotted against SDS, and the square of the

correlation coefficient (R^2) are shown in Table 2, e.g. R^2 for pLMAt-15 versus SDS and pLMAm-19 versus SDS were 0.924 and 0.899, respectively.

3.3.2. Effect of polymer structure on the chemical selectivity

Investigation of the system constants for the polymers presented in Table 4 leads to several significant conclusions. The backbone spacer bonding, hydrophobe percentage and pendant alkyl chain length have significant effects on the system constants *m*, *s*, *r*, *b* and *a*. The presence or absence of α-methyl substitution on the backbone has some effect on the system constants, especially on *s* and *a*.

The polymers with amide spacers show significantly different system constants from polymers with ester spacers. The differences in the system constants are evident by comparison of pLMAm-19 and pLMAt-15. The acrylamide copolymer is more cohesive, more polar, more acidic, and significantly more basic than the methacrylate copolymer. The same results are obtained with pSAm-28 and pSMAt-*f* (f=13, 16). A dramatic contrast between the chemistries is observed for the basicity term, *a*. This term is negative for all methacrylate copolymers investigated, and positive for the two acrylamide copolymers. However, the basicity of pLAt-9 (f%=9.0%) and pLAt-13 (f%=12.8%) is not significantly different from that of water, and significantly higher than that of pLMAt-15, showing that the absence of α-methyl groups on the backbone of acrylate copolymers could result in increased hydrogen-bonding basicity.

The differences in selectivity caused by the differences in backbone spacer chemistry are also apparent in the overall chemical selectivity of the copolymers shown by $\log k'$ plots. Plots of the $\log k'$ of the 20 solutes separated by pLMAm-19 versus pLMAt-15, pOMAt-21 and pSMAt-13, yielded R^2 values of 0.887, 0.872 and 0.893, respectively (Table 2). The small change of the f% should not affect the overall chemical selectivity, hence the source of the selectivity change must be due to the spacer bonding chemistry difference.

With the increases of the pendant hydrophobe chain lengths, the *m* value increased. Polymers with longer pendant chains are less cohesive. This difference in *m* will not affect the overall chemical selectivity. The normalized system constants, x/m, generally changed only slightly, and probably counteract with each other affecting the chemical selectivity. Actually, plots of the $\log k'$ of 20 solutes separated by pSMAt-13 and pLMAt-15 versus pOMAt-21, yielded R^2 values higher than 0.995, as shown in Fig. 4, suggesting no significant difference of chemical selectivities among the copolymers with different pendant chain lengths. The conclusion was further proved by the $\log k'$ plot between pSAm-28 and pLMAm-19, with an R^2 of 0.983 (Table 2).

It is difficult with the limited data set to determine the effects of any individual structure factor alone, without the influence of other factors. In an attempt to determine what effect, if any, each of the structural factors has on the system constants, a model was developed to evaluate four independent copolymer chemical structure factors, and the data were fit to the following linear equation:

$$Y = c + x_1\mathrm{f(NH)} + x_2\mathrm{f(SO_3)} + x_3\mathrm{f(CH_3)} + x_4\mathrm{f(C)} \quad (2)$$

where Y is one of the system parameters (*m*, *s*, *r*, *b* or *a*) shown in Table 4. c is the intercept, f(NH), $\mathrm{f(SO_3)}$ and $\mathrm{f(CH_3)}$ are the amide fraction, AMPS

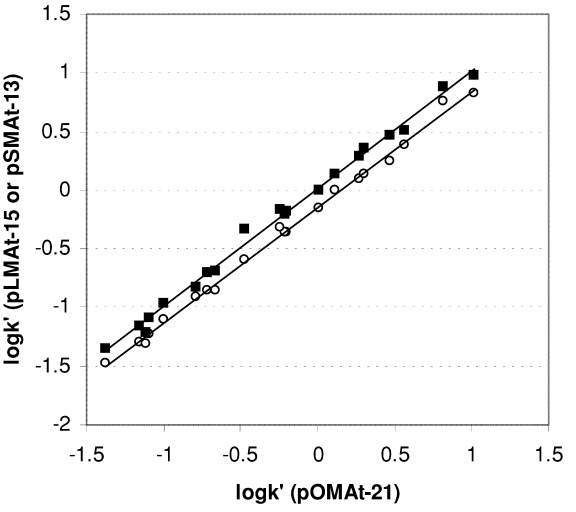

Fig. 4. Plot of $\log k'$ values using pLMAt-15 and pSMAt-13 versus pOMAt-21 (for EKC conditions see Section 2.3.2). ○, pLMAt-15 versus pOMAt-21: $y=0.9885x-0.1519$, $R^2=0.997$; ■, pSMAt-13 versus pOMAt-21: $y=1.0076x-0.0079$, $R^2=0.995$.

Table 5
Chemical structure factors of eight copolymers (as explained in Eq. (2))

	f(NH)	f(SO_3)	f(CH_3)	f(C)
pOMAt-21	0.794	0.794	0.206	0.165
pLMAt-15	0.854	0.854	0.146	0.175
pSMAt-13	0.869	0.869	0.131	0.236
pSMAt-16	0.842	0.842	0.158	0.285
pLAt-9	0.910	0.910	0	0.108
pLAt-13	0.872	0.872	0	0.154
pLMAm-19	1.00	0.810	0.190	0.228
pSAm-28	1.00	0.722	0	0.500

fraction and α-methyl content on the copolymer backbone, respectively, as listed in Table 5. f(C) is the overall copolymer sidechain hydrophobicity, which was calculated by hydrophobe content (f%) multiplied by the pendant chain length (8, 12, or 18), and then divided by 10 to keep it of the same scale as that of f(NH), f(SO_3) and f(CH_3). The parameters x_1, x_2, x_3 and x_4 show the contribution of those four structure factors.

The results of these fits are shown in Table 6. The R^2 values of the fits were good for $Y=s$, b and a, in excess of 0.91, and F-tests show the correlations of s, b and a with the copolymer structures were significant. However, the F-tests show that the correlation of m and r with polymer structure factors were not significant. The polymer cohesiveness may well be affected by polymer aggregation behavior and different polymer micelle structure, which is not included in this basic model. Nevertheless, this model could help to explain the contributions of each structural factor to the retention mechanism.

The results indicate that the fraction of amide groups has a significant effect on the system constants s, b and a. The polymers become more polar, significantly more acidic and more basic as the fraction of amide, f(NH), is increased. When acrylate spacers are used, the reduction in the fraction of amide groups results in reduced acidity and basicity. This helps to explain the significant reduction of the retention of several solutes with hydroxyl/amino group (e.g. naphthalenemethanol, naphthylamine, acenaphthenol and naphthaleneethanol) separated by pLMAt-15, compared with pSAm-17 (Fig. 3). The polymers also become more cohesive with the increase of f(NH), because of increased hydrogen bonding between neighboring NH groups on the copolymer backbone [41,42].

Increases in the polymer overall pendant-chain hydrophobicity, f(C), reduce the cohesiveness, polarity, basicity and acidity of the copolymers. It can also be seen that increases in α-methyl substitution percentage on the backbone, f(CH_3), reduce the polarity and hydrogen-bonding acidity/basicity of the copolymers, hence causing some difference in overall copolymer chemical selectivity, e.g. between pLMAt-15 and pLAt-9 (Table 2; $R^2=0.961$).

4. Conclusions

A total of ten sulfonated amide or ester containing polymeric surfactants were synthesized, characterized and used as pseudo-stationary phases for EKC. An LSER study and a log k' plot study of 20 neutral

Table 6
Linear multiple regression results using model $Y=c+x_1\text{f(NH)}+x_2\text{f}(SO_3)+x_3\text{f}(CH_3)+x_4\text{f(C)}$

	c	x_1	x_2	x_3	x_4	R^2	SE	F
m	5.014	−5.101	2.620	0.4075	3.206	0.816	0.2444	3.34
	(±3.306)	(±1.573)	(±3.061)	(±1.245)	(±1.508)			
s	0.2270	2.177	−2.511	−1.012	−2.233	0.972	0.04887	26.0
	(±0.6612)	(±0.3146)	(±0.6121)	(±0.2490)	(±0.3015)			
r	0.2116	−0.9108	0.9327	0.4774	0.9172	0.735	0.07475	2.08
	(±1.011)	(±0.4813)	(±0.9363)	(±0.3810)	(±0.4612)			
b	−6.830	8.073	−3.420	−0.4034	−3.668	0.911	0.2472	7.69
	(±3.345)	(±1.592)	(±3.096)	(±1.260)	(±1.525)			
a	−1.493	3.654	−1.790	−0.8260	−1.354	0.970	0.07118	24.7
	(±0.9631)	(±0.4583)	(±0.8915)	(±0.3627)	(±0.4392)			

The numbers in the parenthesis show SD. Number of observations is 8. See Table 4 for meanings of R^2, SE, and F. At significance of 0.100, $F(4,3)=5.34$.

solutes provided several significant results. Significant differences between the chemical selectivities of SDS and sulfonated acrylamide/acrylate copolymers were found. A small range of variation of hydrophobe percentages from 15 to 22% had no significant effect on the chemical selectivities of poly(AMPS–alkyl methacrylate)s. Methacrylate copolymers have significantly different solvation characteristics from acrylamide copolymers, with weaker hydrogen-bonding ability and less cohesiveness. These differences are thought to be caused by the differences in polymer structure and aggregation for acrylamide and methacrylate copolymers, as reported by Morishima. The length of the pendant alkyl chains (C_8, C_{12} and C_{18}) has significant effect on the cohesiveness of the copolymers, and slight effect on other normalized system constants (s/m, r/m, b/m and a/m), but does not have a major effect on the overall chemical selectivity as shown by $\log k'$ plots. The presence or absence of α-methyl groups on the copolymer backbone had some effect on the polarity and hydrogen-bonding basicity of the copolymers. Overall, the major chemical selectivity differences among the copolymers resulted from the differences between amide and/or ester spacer bonding chemistries, and not from differences in pendant chain length or fraction of pendant hydrophobic groups, as shown by $\log k'$ plots.

Acknowledgements

The authors would like to acknowledge major funding for this work provided by the Department of Energy (Sandia National Laboratories) through a Presidential Early Career Award. Thanks also go to Professor Fuierer and Lee Benysek of the Department of Material Sciences for assistance with light scattering experiments.

References

[1] S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya, T. Ando, Anal. Chem. 56 (1984) 111.
[2] H. Ozaki, A. Ichihara, S. Terabe, J. Chromatogr. A 709 (1995) 3.
[3] E.S. Ahuja, E.S. Little, K.R. Nielson, J.P. Foley, Anal. Chem. 67 (1995) 26.
[4] C.P. Palmer, M.Y. Khaled, H.M. McNair, J. High Resolut. Chromatogr. 15 (1992) 756.
[5] C.P. Palmer, J. Chromatogr. A 780 (1997) 75.
[6] C.P. Palmer, N. Tanaka, J. Chromatogr. A 792 (1997) 105.
[7] M.L. Riekkola, S.K. Wiedmer, I.E. Valko, H. Siren, J. Chromatogr. A 792 (1997) 13.
[8] S.A. Shamsi, I.M. Warner, Electrophoresis 18 (1997) 853.
[9] H. Ozaki, N. Itou, S. Terabe, Y. Takada, M. Sakairi, H. Koizumi, J. Chromatogr. A 716 (1995) 69.
[10] W.Z. Lu, S.A. Shamsi, T.D. McCarley, I.M. Warner, Electrophoresis 19 (1998) 2193.
[11] J.P. Quirino, S. Terabe, J. Chromatogr. A 856 (1999) 465.
[12] B. Maichel, E. Kenndler, Electrophoresis 21 (2000) 3160.
[13] H.H. Yarabe, E. Billiot, I.M. Warner, J. Chromatogr. A 875 (2000) 179.
[14] H. Ozaki, S. Terabe, A. Ichihara, J. Chromatogr. A 680 (1994) 117.
[15] S. Yang, J.G. Bumgarner, M.G. Khaledi, J. High Resolut. Chromatogr. 18 (1995) 443.
[16] C.P. Palmer, S. Terabe, J. Microcol. Sep. 8 (1996) 115.
[17] C.P. Palmer, S. Terabe, Anal. Chem. 69 (1997) 1852.
[18] S.A. Shamsi, C. Akbay, I.M. Warner, Anal. Chem. 70 (1998) 3078.
[19] C.P. Palmer, H.M. McNair, J. Microcol. Sep. 4 (1992) 509.
[20] C.P. Palmer, K.T. Tellman, J. Microcol. Sep. 11 (1999) 185.
[21] K.T. Tellman, C.P. Palmer, Electrophoresis 20 (1999) 152.
[22] N. Tanaka, K. Nakagawa, H. Iwasaki, K. Hosoya, K. Kimata, T. Araki, D.G. Patterson Jr., J. Chromatogr. A 781 (1997) 139.
[23] N. Tanaka, K. Nakagawa, K. Hosoya, C.P. Palmer, S. Kunugi, J. Chromatogr. A 802 (1998) 23.
[24] T. Chen, C.P. Palmer, Electrophoresis 20 (1999) 2412.
[25] D.S. Peterson, C.P. Palmer, Electrophoresis 21 (2000) 3174.
[26] A. Cifuentes, H. Poppe, J.C. Kraak, F.B. Erim, J. Chromatogr. B 681 (1996) 21.
[27] B. Potocek, E. Chmela, B. Maichel, E. Tesarova, E. Kenndler, B. Gas, Anal. Chem. 72 (2000) 74.
[28] C. Fujimoto, Y. Fujise, S. Kawaguchi, J. Chromatogr. A 871 (2000) 415.
[29] N. Tanaka, T. Fukutome, T. Tanigawa, K. Hosoya, K. Kimata, T. Araki, K.K. Unger, J. Chromatogr. A 699 (1995) 331.
[30] J.L. Haynes III, S.A. Shamsi, J. Dey, I.M. Warner, J. Liq. Chromatogr. 21 (1998) 611.
[31] S. Hara, A. Dobashi, Chem. Abstr. 118 (1993) p39405z, Jpn. Pat. 04-149 205.
[32] J. Wang, I.M. Warner, Anal. Chem. 66 (1994) 3773.
[33] S.A. Shamsi, J. Macossay, I.M. Warner, Anal. Chem. 69 (1997) 2980.
[34] H.H. Yarabe, S.A. Shamsi, I.M. Warner, Anal. Chem. 71 (1999) 3992.
[35] S. Takeda, S. Wakida, M. Yamane, K. Higashi, S. Terabe, J. Chromatogr. A 744 (1996) 135.
[36] B. Lindman, H. Wennerstrom, Top. Curr. Chem. 87 (1980) 3.
[37] D.E. Burton, M.J. Sepaniak, M.P. Maskarinec, J. Chromatogr. Sci. 25 (1987) 514.

[38] Y. Morishima, Y. Tominaga, M. Kamachi, T. Okada, Y. Hirata, N. Mataga, J. Phys. Chem. 95 (1991) 6027.
[39] H. Sawada, K. Jinno, Electrophoresis 20 (1999) 24.
[40] W. Shi, C.J. Watson, C.P. Palmer, J. Chromatogr. A 905 (2001) 281.
[41] T. Noda, Y. Morishima, Macromolecules 32 (1999) 4631.
[42] Y. Morishima, Chin. J. Polym. Sci. 18 (2000) 323.
[43] S. Yang, M.G. Khaledi, Anal. Chem. 67 (1995) 499.
[44] S. Yang, J.G. Bumgarner, L.F. Kruk, M.G. Khaledi, J. Chromatogr. A 738 (1996) 265.
[45] P.G. Muijselaar, H.A. Claessens, C.A. Cramers, Anal. Chem. 69 (1997) 1184.
[46] M.H. Abraham, J. Phys. Org. Chem. 6 (1993) 660.
[47] C.F. Poole, S.K. Poole, J. Chromatogr. A 792 (1997) 89.
[48] S.K. Poole, C.F. Poole, Anal. Commun. 34 (1997) 57.
[49] S.K. Poole, C.F. Poole, J. High Resolut. Chromatogr. 20 (1997) 174.
[50] M.H. Abraham, Chem. Soc. Rev. 22 (1993) 73.
[51] M.D. Trone, M.G. Khaledi, Electrophoresis 21 (2000) 2390.
[52] N. Chen, Y.K. Zhang, S. Terabe, T. Nakagawa, J. Chromatogr. A 678 (1994) 327.
[53] Z. Liu, H.F. Zou, M.L. Ye, J.Y. Ni, Y.K. Zhang, J. Chromatogr. A 863 (1999) 69.
[54] Z. Liu, H.F. Zou, M.L. Ye, J.Y. Ni, Y.K. Zhang, Electrophoresis 20 (1999) 2898.
[55] S.K. Poole, C.F. Poole, Analyst 122 (1997) 267.
[56] M.D. Trone, M.G. Khaledi, Anal. Chem. 70 (1999) 1270.
[57] C.F. Poole, S.K. Poole, M.H. Abraham, J. Chromatogr. A 798 (1998) 207.
[58] N. Tetsuya, Y. Morishima, Macromolecules 32 (1999) 4631.
[59] A. Hashidzume, H. Yamamoto, M. Mizusaki, Y. Morishima, Polym. J. 31 (1999) 1009.
[60] M.M. Bushy, J.W. Jorgenson, Anal. Chem. 61 (1989) 491.
[61] C.P. Palmer, Electrophoresis 21 (2000) 4054.
[62] M.H. Abraham, H.S. Chadha, G.S. Whiting, R.C. Mitcthell, J. Pharm. Sci. 83 (1994) 1085.