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Copolymerized polymeric surfactants: Characterization and application in micellar electrokinetic chromatography

An achiral monomeric surfactant (sodium 10-undecenyl sulfate, SUS) and a chiral surfactant (sodium 10-undecenoyl L-leucinate, SUL) were synthesized and polymerized individually to form poly-SUS and poly-SUL. These surfactants were then copolymerized at various molar ratios to produce a variety of copolymerized surfactants (CoPSs), possessing both achiral (sulfate) and chiral (leucinate) head groups. The CoPSs, poly-SUS, poly-SUL, and sodium dodecyl sulfate were characterized using several analytical techniques. The aggregation numbers of the polymeric surfactants and the partial specific volumes were determined by the use of fluorescence quenching and density measurements, respectively. These polymeric surfactants were investigated as novel pseudostationary phases in micellar electrokinetic chromatography (MEKC) for the separation of chiral and achiral solutes. Solute hydrophobicity was found to have major influence on the MEKC retention of alkyl phenyl ketones. In contrast, hydrogen-bonding ability of benzodiazepines is the major factor that governs their retention, but hydrophobicity has an insignificant effect on MEKC retention of benzodiazepines.

Keywords: Aggregation number / Benzodiazepines / Chiral separation / Copolymerized surfactants / Partial specific volume
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1 Introduction

As an alternative to conventional micelles, polymeric surfactants have been used as pseudostationary phases in micellar electrokinetic chromatography (MEKC). Some advantages of polymeric surfactants over conventional micelles include: (i) they do not have a critical micelle concentration (CMC) and they may be used at low concentrations below the normal CMC of the monomer; (ii) they are stable in the presence of a high content of organic solvents; (iii) they can be used in conjunction with inclusion molecules (e.g., cyclodextrins) without significant interference resulting from the formation of host complexes between the solutes and the host molecule.

Poly (sodium 10-undecylenate) (poly-SUA) was the first successful anionic polymeric surfactant used as a pseudostationary phase in MEKC [1, 2], separating a wide range of neutral compounds. However, due to the ionization of the carboxylated head groups, poly-SUA resulted

in nonreproducible analysis times and limited solubility (not soluble below pH 7.0). To overcome these problems, Palmer and Terabe [3, 4] and Shamsi *et al.* [5] synthesized poly(sodium 10-undecenyl sulfate) (poly-SUS), which has a sulfate head group. Alternatively, chiral polymeric surfactants have been prepared and used as pseudostationary phases in MEKC [6, 7] where the functionality of the head group was modified. Benzodiazepines are a class of compounds that have been widely used in psychotherapy as anticonvulsants, sedatives, muscle relaxants, and hypnotics [8]. Side effects include dizziness, interaction with alcohol, and their abuse can produce a drug-dependence. Due to the potential dependence and hazard of abuse, a variety of capillary electrophoresis (CE) modes exist in the literature for separation of benzodiazepines [9, 10] including capillary electrochromatography (CEC) [11–13], open-tubular CEC (OT-CEC) [14, 15], and MEKC [16–20].

Providing distinctive selectivity, mixed normal micelles have been shown to be advantageous in MEKC separations [21–23]. However, a major drawback of conventional micelles is that several parameters such as ionic strength, organic solvents, and temperature may have a significant effect on the CMC and the aggregation number of the

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Abbreviations: CoPS, copolymerized surfactant; HBA, hydrogen bond accepting; HBD, hydrogen bond donating; poly-SUL, poly(sodium 10-undecenoyl L-leucinate); poly-SUS, poly(sodium 10-undecenyl sulfate)

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micelle, which may affect the quality of the separation in MEKC. It is also important to realize that micellization is a dynamic equilibrium and thus conventional micelles, unlike polymeric micelles, have finite lifetimes [24]. This, in turn, may cause significant irreproducibility in MEKC separations. Thus, control or elimination of the dynamic equilibrium may result in improved separation reproducibility in MEKC. In the study reported here, sodium 10-undecenyl sulfate (SUS), an achiral surfactant, and sodium *N*-undecanoyl L-leucinate (SUL), a chiral surfactant, were synthesized. These two surfactants were then polymerized separately to form poly(sodium 10-undecenyl sulfate) (poly-SUS) and poly(sodium *N*-undecanoyl L-leucinate) (poly-SUL). In addition, SUS and SUL were polymerized together at various molar fractions to produce a variety of copolymerized surfactants (CoPSs) possessing both chiral (*i.e.*, leucinate) and achiral (*i.e.*, sulfate) head groups. Finally, the CoPSs, poly-SUL, and poly-SUS were used as novel pseudostationary phases in MEKC to separate both chiral and achiral molecules. The separation results obtained with CoPSs, poly-SUS, and poly-SUL, were then compared to the separation results using SDS as a pseudostationary phase.

2 Materials and methods

2.1 Materials

The seven benzodiazepines (flunitrazepam, nitrazepam, clonazepam, temazepam, diazepam, oxazepam, lorazepam) (Fig. 1) and L-leucine were obtained from Sigma (St. Louis, MO, USA). The reagents for the monomeric surfactants *N*-hydroxysuccinimide, undecylenic acid, and dicyclohexylcarbodiimide (DCC) were purchased from Aldrich (Milwaukee, WI, USA). The alkyl phenyl ketone homologues series (*i.e.*, acetophenone, propiophenone, butyrophenone, valerophenone, hexanophenone, heptanophenone, and octanophenone), HPLC-grade ethyl acetate, sodium bicarbonate, disodium hydrogen phosphate, and sodium carbonate were all reagent grade and obtained from Aldrich. The undecylenyl alcohol, chlorosulfonic acid, sodium dodecyl sulfate (SDS), and pyridine (PY) were of analytical reagent grade and were also purchased from Aldrich. All chemicals were used as received.

2.2 Synthesis of SUS and SUL

Details of the synthesis of the achiral surfactant with a sulfated head group, SUS [5], and the chiral surfactant with a leucinate head group, SUL [25], are available in the literature, thus are not repeated in this report.

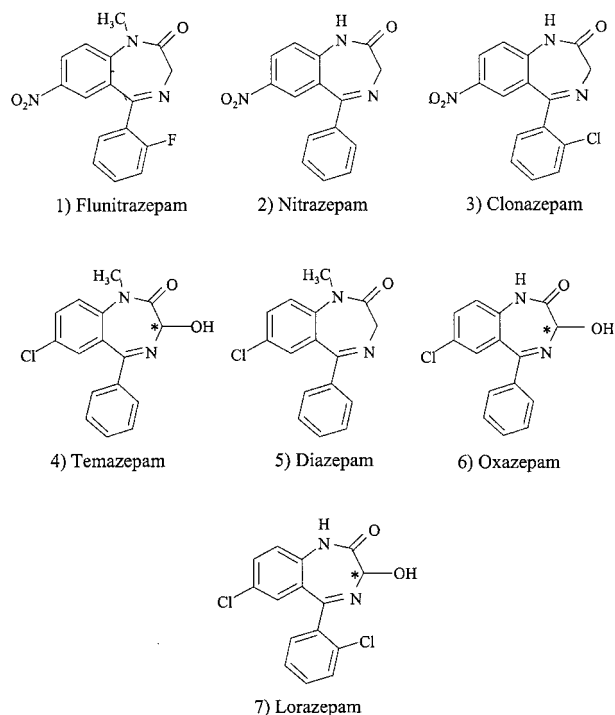


Figure 1. Chemical structures and numerical designations for each of the seven benzodiazepines examined in this study. Temazepam, oxazepam, and lorazepam are chiral while the rest are achiral. The asterisk (*) represents the stereogenic center of the chiral molecules.

2.3 Polymerization of the surfactants

Polymerization of SUS and SUL was achieved by preparing a 100 mM solution of each surfactant in triply deionized water. The copolymerized polymeric surfactants were prepared in 100:0; 80:20; 60:40; 40:60; 20:80; and 0:100 molar ratios of monomeric SUL:SUS surfactants where the total concentration of both monomers was set at 100 mM. For example, to prepare a poly(L₈S₂) CoPS, equal volumes of 160 mM aqueous solution of SUL and 40 mM SUS were mixed together. Similarly, all six surfactant solutions were prepared and exposed to a ⁶⁰Co-ray source (680 rad/h) for seven days. After irradiation, the CoPSs solutions were filtered and then lyophilized to yield the final products.

2.4 Preparation of separation buffers and analyte solution

A 100 mM stock solution of phosphate buffer (pH 8.0) was prepared by dissolving the appropriate amount of sodium dihydrogenphosphate in triply deionized water. The solution of each pseudostationary phase was prepared by

first dissolving 0.1 g of the surfactant in 5.0 mL of deionized water and then adding 2.0 mL of the 100 mM phosphate stock buffer. The pH of the surfactant solutions were adjusted to a pH of 8.0 by use of either dilute phosphoric acid or dilute sodium hydroxide solutions, and the total volume was adjusted to 10.0 mL with deionized water. Solution of each pseudostationary phase was sonicated for 10 min, filtered through a 0.45 μm syringe filter (Nalgene, Rochester, NY, USA), and then sonicated for 3 additional minutes before use in MEKC. All stock solute solutions (*i.e.*, the benzodiazepines and the alkyl phenyl ketone homologues) were prepared in methanol with the concentrations of the solutes ranging from 0.15 to 0.30 mM.

2.5 Characterization of polymeric surfactants

2.5.1 Determination of the aggregation number

The aggregation numbers of the surfactants were determined at ambient temperature ($\sim 25^\circ\text{C}$) by use of the fluorescence quenching method proposed by Turro and Yekta [26]. Fluorescence measurements were performed on a SPEX model F2T211 spectrophotometer. Pyrene and cetylpyridinium chloride were used as fluorescent probe and quencher, respectively. A 1.0×10^{-3} M stock solution of pyrene was prepared in methanol. A 2.0×10^{-3} M stock solution of the quencher and a 2.0% w/v of each of CoPS, poly-SUL, poly-SUS, as well as SDS stock solutions were prepared separately in triply deionized water. A known volume of the pyrene stock solution was pipetted into a clean volumetric flask. Methanol was then evaporated under a gentle flow of nitrogen gas and then an appropriate volume of aqueous surfactant solution was added. The final concentrations of pyrene and the surfactant were 2.0×10^{-6} M and 2.0% w/v, respectively (probe solution 1). The solution was sonicated (90 min) and stored overnight in the dark. Probe solution 1 was then divided in half with one-half being diluted with triply deionized water to give a 1.0×10^{-6} M pyrene and 1.0% w/v surfactant (probe solution 2). The other half was mixed with quencher stock solution to produce a solution of 1.0×10^{-3} M quencher, 1.0×10^{-6} M probe, and 1.0% w/v surfactant (quencher solution). The quencher solution was added to probe solution 2 in increasing increments of 25 μL and equilibrated for 20 min before obtaining the fluorescence spectrum at ambient temperature. The decrease in emission intensity of the probe was recorded at 393 nm with the excitation at 335 nm after each aliquot of the quencher solution was added. The logarithm of the fluorescence intensity ratio I_0/I was plotted against the quencher concentration $[Q]$. The aggregation number, N , was then obtained from a slope of this plot.

2.5.2 Determination of partial specific volume

The partial specific volume, \bar{v} , is defined as an increase in volume upon dissolving 1 g of a dry material (*e.g.*, surfactant) in a large volume of a solvent (*e.g.*, water) at constant temperature and pressure. The \bar{v} can be determined using Eq. (1) [27]:

$$\bar{v} = \left(\frac{1}{\rho}\right)_{\text{app}} - \frac{\partial \left(\frac{1}{\rho}\right)_{\text{app}}}{\partial W} \times W \quad (1)$$

where the value of W is defined as the weight fraction of the surfactant and ρ is the density of polymeric surfactant solution. The term $(1/\rho)_{\text{app}}$ is the apparent specific volume of the surfactant. A graph of $1/\rho$ against W allows the determination of \bar{v} from the y -intercept. In order to measure the density for each surfactant solution (poly-SUS, poly-SUL, the CoPSs, and SDS), varying weight percents of each surfactant (*i.e.*, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, and 0.7% w/v) in 20 mM phosphate buffer (pH 8) were prepared. The density for each surfactant system was measured using a high-precision Anton Paar DMA 58 digital density meter (Anton Paar USA, League City, TX, USA) at 15, 20, 25, 30, 35, and 40°C . The precision of the temperature-controlled system used in this study was better than $\pm 0.005^\circ\text{C}$.

2.6 Capillary electrophoretic separations

2.6.1 Instrumentation

A Beckman P/ACE model 5510 capillary electrophoresis (CE) instrument (Fullerton, CA, USA) was used for the MEKC separations. The MEKC separations were performed in a 57 cm total length (50 cm effective length) \times 50 μm ID (367 μm OD) fused-silica capillary (Polymicro Technologies, Tucson, AZ, USA). The temperature of the capillary was controlled by use of a fluoro-organic fluid. The Beckman System Gold software was used for system control and data handling.

2.6.2 Separation of benzodiazepines

Each new capillary was activated with 1 M NaOH (1 h) and deionized water (30 min) before use. For a given pseudostationary phase, the capillary was rinsed for 5 min with deionized water, 3 min with 0.1 M NaOH, and 3 min with the separation buffer between injections. Each day, the capillary was reactivated by rinsing with 1 M NaOH (15 min), triply deionized water (2 min), and the running buffer (5 min). When the pseudostationary phase was changed, the capillary was reconditioned with triply deionized water (15 min), 0.1 M NaOH

(10 min), and with the separation buffer (5 min). Unless otherwise noted, the time for pressure injection was 0.5 psi for 2 s.

2.6.3 Calculations

The capacity factor, k' , of neutral solutes was measured by use of the following formula [28]:

$$k' = \frac{t_R - t_{eo}}{t_{eo} \left[1 - \left(\frac{t_R}{t_{psp}} \right) \right]} \quad (2)$$

where t_R , t_{eo} , and t_{psp} are the migration times of the retained solute, the electroosmotic flow (EOF), and the pseudostationary phase, respectively. Methanol was used as the t_{eo} marker and was measured from the time of injection to the first signal deviation of the baseline. Decanophenone was used as a t_{psp} tracer. By graphing $\log k'$ versus carbon number for the alkyl phenyl ketone homolog series, the methylene selectivity (also known as hydrophobic selectivity), α_{CH_2} , was calculated from the antilogarithm of the slope of the regression line.

The electroosmotic mobility, μ_{eo} , was calculated at six different temperatures (15, 20, 25, 30, 35, and 40°C) by use of Eq. (3):

$$\mu_{eo} = \frac{l_t l_d}{V t_{eo}} \quad (3)$$

where l_t is the total length of the capillary (cm), l_d is the length of the capillary from injector to detector (cm), V is the applied voltage (V), and the retention times were measured in seconds (s).

The effective electrophoretic mobility of the pseudostationary phase (μ_{ep}) was calculated using Eq. (4). The apparent electrophoretic mobility, μ_{app} , can be calculated by replacing t_{psp} with t_{eo} , in Eq. (3).

$$\mu_{ep} = \mu_{app} - \mu_{eo} \quad (4)$$

Finally, the elution window was calculated using the ratio of t_{psp}/t_{eo} .

The distribution coefficient, K , is related to the capacity factor, k' , (Eq. 2), by Eq. (5):

$$K = \frac{k'}{\beta} \quad (5)$$

where β is known as the phase ratio of pseudostationary phase over aqueous phase (V_{psp}/V_{aq}), which can be determined using following relationship:

$$\beta = \frac{\bar{v}(C_{psp} - CMC)}{1 - \bar{v}(C_{psp} - CMC)} \quad (6)$$

where \bar{v} is the partial specific volume of the pseudostationary phase, C_{psp} is the concentration of the pseudostationary phase (28). Polymeric surfactants do not have a CMC value, thus the CMC term in Eq. (6) is omitted for polymeric surfactants.

3 Results and discussion

3.1 Partial specific volumes of pseudostationary phases

The partial specific volume, \bar{v} , was used to measure the approximate volume of the polymeric surfactants. Figure 2 shows a representative plot of $1/\rho$ versus $W\%$ at 25°C in 20 mM phosphate buffer. The \bar{v} values were obtained from the y -intercepts of the $1/\rho$ versus $W\%$ plots. From the data in Table 1 it is noted that the \bar{v} value of poly-SUL is the lowest, suggesting that this polymeric surfactant has a relatively more compact structure than the other polymeric surfactants. Poly-SUL has a carbonyl group in the polar head and the low \bar{v} value is likely due to hydrogen bonding between the carbonyl group and the amide proton of the poly-SUL. In contrast SDS, has the highest \bar{v} value. This is not surprising because SDS has a longer hydrophobic carbon tail (C12) than poly-SUS (C11). Poly-SUS has the second highest partial specific volume. This shows that SDS and poly-SUS have more open and flexible structures than poly-SUL and the four CoPSs. The \bar{v} values of CoPSs increase at all temperature with increasing the mole fraction of sulfated surfactant. This indicates that sulfate head group provides the CoPS a more flexible character. It should be noted that the temperature dependence of the \bar{v} values is negligible (Table 1). The change in \bar{v} value is only 3.0, 2.2,

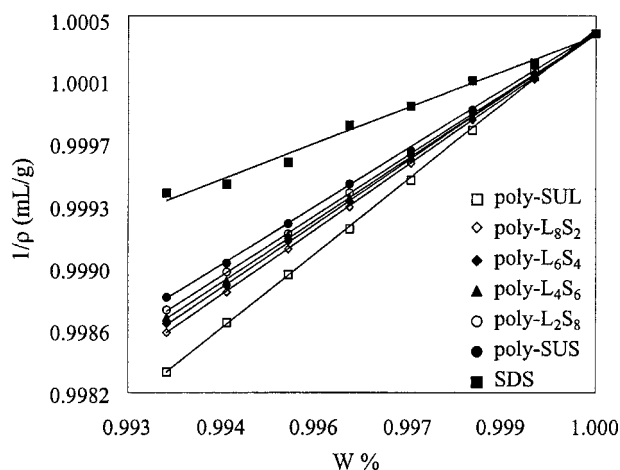


Figure 2. Representative plot of $1/\rho$ as a function of $W\%$ for all pseudostationary phases in 20 mM phosphate buffer at 25°C. Legends are shown in the plot.

Table 1. Partial specific volume^{a)}, \bar{v} , methylene-group selectivity^{b)}, α_{CH_2} , and aggregation number^{c)}, N , of pseudostationary phases as a function of temperature

Pseudostationary phases		Temperature (°C)					
		15	20	25	30	35	40
Poly-SUL	\bar{v}	0.693	0.707	0.701	0.702	0.709	0.714
	α_{CH_2}	2.14	2.15	2.13	2.20	2.15	2.14
	N			61			
Poly-L ₈ S ₂	\bar{v}	0.727	0.732	0.736	0.738	0.742	0.743
	α_{CH_2}	2.26	2.18	2.18	2.16	2.17	2.13
	N			61			
Poly-L ₆ S ₄	\bar{v}	0.736	0.739	0.742	0.746	0.748	0.748
	α_{CH_2}	2.20	2.17	2.16	2.16	2.17	2.14
	N			62			
Poly-L ₄ S ₆	\bar{v}	0.743	0.748	0.749	0.754	0.756	0.760
	α_{CH_2}	2.22	2.16	2.18	2.15	2.15	2.14
	N			49			
Poly-L ₂ S ₈	\bar{v}	0.747	0.752	0.756	0.757	0.760	0.754
	α_{CH_2}	2.22	2.19	2.15	2.17	2.17	2.14
	N			68			
Poly-SUS	\bar{v}	0.763	0.768	0.768	0.762	0.771	0.777
	α_{CH_2}	2.32	2.31	2.25	2.26	2.21	2.19
	N			21			
SDS	\bar{v}	0.844	0.850	0.854	0.857	0.865	0.869
	α_{CH_2}	2.53	2.60	2.57	2.55	2.53	2.50
	N			62			

a) mL/g

b) Calculated from the antilogarithm of the slope of the regression line of $\log k'$ versus carbon number of alkyl phenyl ketones (C8–C14)c) Determined using the fluorescence quenching method at ambient temperature ($\sim 25^\circ\text{C}$). The N values are not available at all temperatures.

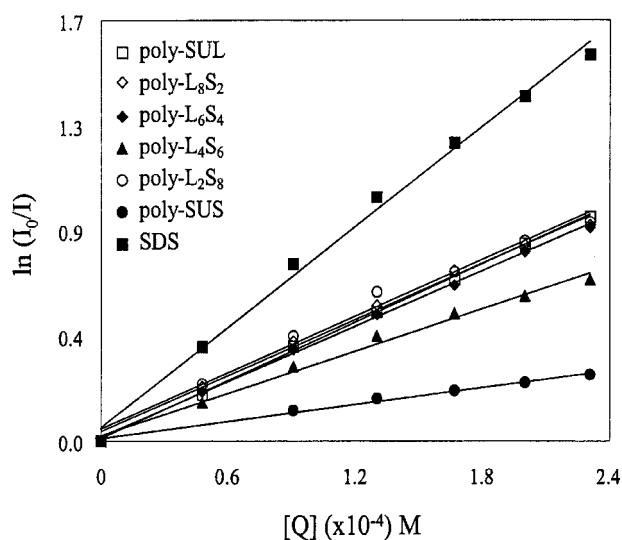
1.6, 2.3, 0.9, 1.8, 3.0% for poly-SUL, Poly-L₈S₂, Poly-L₆S₄, Poly-L₄S₆, Poly-L₂S₈, Poly-SUS, and SDS, respectively, as temperature elevated from 15°C to 40°C.

3.2 Aggregation number of the pseudostationary phases

The aggregation number, N , represents the number of surfactant molecules taking part in micelle formation. The N value of each polymeric surfactant and SDS was obtained from the slope of the $\ln(I_0/I)$ versus $[Q]$ plot (Fig. 3). Knowing the slope of $\ln(I_0/I)$ versus $[Q]$ plot, total surfactant concentration, and the CMC of the surfactants, N can be calculated using Eq. (7).

$$N = \text{slope} \times ([S_{\text{tot}}] - \text{CMC}) \quad (7)$$

The CMC values of the surfactants are needed to determine the N values using Eq. (7). Polymeric surfactants do not have a CMC value; in other words, the CMC

**Figure 3.** Aggregation number measurement plots for the seven pseudostationary phases used in this study.

value for polymeric surfactant is considered to be zero in Eq. (7). The CMC of SDS is 8.2 mM [29]. Evaluation of the N values (Table 1) suggests that there is no distinguishable relationship between the molar fraction of the SUL or SUS and the aggregation numbers of the polymeric surfactant. There is an excellent agreement between N value of SDS determined in this study (62) and those reported in literature (62 and 60) [30, 31]. This agreement indicates that the fluorescence quenching technique is a reliable tool for determination of N values. Poly-SUL, Poly-L₈S₂, poly-L₆S₄, and SDS appear to have very similar N values (~62). However, poly-SUS shows a significant decrease in its N value (21). In contrast, there is a striking increase in the N value of poly-L₂S₈ (68). These irregularities require additional studies in order to completely understand the aggregation process of these mixed polymeric surfactants. However, it should be noted that poly-L₄S₆ appears to be the only mixed polymeric surfactant with an intermediate value of N (49).

3.3 Methylene-group selectivity of pseudostationary phases

The methylene selectivities, α_{CH_2} , of poly-SUS, poly-SUL, CoPSs, and SDS were calculated from the antilogarithm of the slope of the regression line of $\log k'$ versus carbon number of the alkyl phenyl ketone homologous series. Figure 4 is a representative plot of $\log k'$ versus carbon

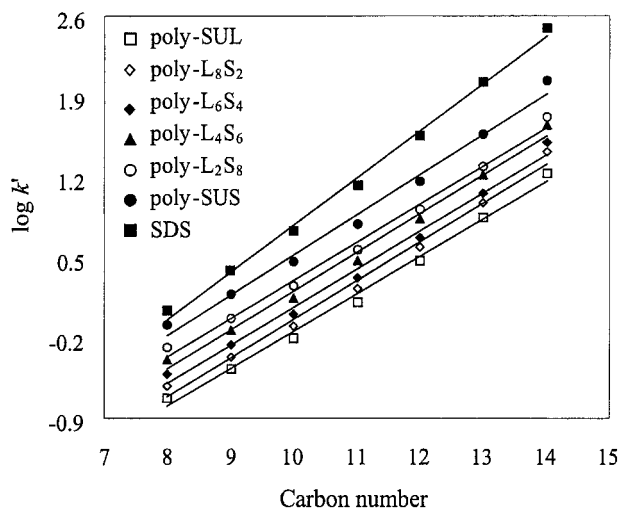


Figure 4. Linear relationship between $\log k'$ versus carbon number of alkyl phenyl ketone homologous series. MEKC separation conditions: pseudostationary phase concentration, 1.0% w/v each in 20 mM phosphate buffer (pH 8.0); applied voltage, +25 kV; temperature, 25°C; UV detection, 254 nm. Alkyl phenyl ketones: acetophenone (C8), propiophenone (C9), butyrophenone (C10), valerophenone (C11), hexanophenone (C12), heptanophenone (C13), and octanophenone (C14).

number of alkyl phenyl ketones. The α_{CH_2} values for the seven pseudostationary phases at six different temperatures between 15°C and 40°C are listed in Table 1. There is no general trend of the α_{CH_2} as a function of temperature. As expected, SDS provided the most hydrophobic environment (highest α_{CH_2} value) due to its relatively longer hydrocarbon tail. Similarly, poly-SUS, structurally similar to SDS, provided the second highest α_{CH_2} value. Poly-SUL, in contrast, provided the least hydrophobic environment for the alkyl phenyl ketones due probably to leucinate head group. The hydrophobicities of all CoPSs are very similar despite their different SUL:SUS molar ratios.

3.4 Mobilities and migration-time window of pseudostationary phases

The electroosmotic mobility, μ_{eo} , effective electrophoretic mobility, μ_{ep} , and the migration-time window, $t_{\text{psp}}/t_{\text{eo}}$, values of pseudostationary phases are listed in Table 2. Surfactant systems with sulfate head groups, *i.e.*, poly-SUS and SDS, have the largest μ_{eo} values (4.61×10^{-4} and $4.47 \times 10^{-4} \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$, respectively). In contrast, poly-SUL provides the smallest μ_{eo} ($3.95 \times 10^{-4} \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$). The μ_{eo} increases as the mole fraction of sulfate head group in the CoPSs is increased. The variations in μ_{eo} for different surfactant systems can be attributed to a variety of parameters including viscosity of the surfactant solution, zeta potential of both capillary walls and pseudostationary phases, and the charge density on the capillary wall. An increase in temperature produced an increase in the μ_{eo} of all polymeric surfactants. This can be attributed to a decrease in the viscosity of buffer solutions.

The μ_{ep} values for anionic pseudostationary phases are negative because the pseudostationary phases are attracted to the anode (the opposite direction of EOF movement) (Table 2). However, the net mobility of the pseudostationary phase is positive because the μ_{eo} is larger than the μ_{ep} . Thus, the stronger EOF drags the polymeric surfactants toward the cathode. It is noted that the μ_{ep} of poly-SUS is the largest of all the pseudostationary phases used in this study. Also, due to the larger μ_{eo} the migration-time window of poly-SUS is relatively smaller than those of the CoPS systems, but larger than that of SDS (Table 2). At higher temperatures, μ_{ep} tends to increase for all pseudostationary phases, mostly due to the viscosity of the buffer system. The migration-time window, generally, decreased with increasing temperature. Poly-SUL had the largest migration window, which allows the analysis of a larger number of solutes. In comparison, SDS exhibited the smallest migration-time window (Table 2).

Table 2. Effect of temperature on electroosmotic mobility^{a)}, μ_{eo} , electrophoretic mobility^{b)}, μ_{ep} , and migration-time window, t_{psp}/t_{eo} , of seven MEKC systems in 20 mM phosphate buffer at pH 8.0

Pseudostationary phases		Temperature (°C)					
		15	20	25	30	35	40
Poly-SUL	μ_{eo}	3.95	4.38	4.96	5.55	6.20	6.79
	μ_{ep}	-3.13	-3.48	-3.91	-4.32	-4.62	-5.22
	t_{psp}/t_{eo}	4.84	4.87	4.72	4.49	3.93	4.34
Poly-L ₈ S ₂	μ_{eo}	4.04	4.49	5.11	5.71	6.32	6.94
	μ_{ep}	-3.07	-3.42	-3.87	-4.25	-4.72	-5.20
	t_{psp}/t_{eo}	4.18	4.18	4.14	3.91	3.94	3.99
Poly-L ₆ S ₄	μ_{eo}	3.94	4.52	5.14	5.75	6.35	6.97
	μ_{ep}	-3.01	-3.50	-3.92	-4.40	-4.81	-5.25
	t_{psp}/t_{eo}	4.24	4.43	4.22	4.25	4.12	4.05
Poly-L ₄ S ₆	μ_{eo}	4.06	4.70	5.33	5.96	6.56	7.18
	μ_{ep}	-3.09	-3.62	-4.05	-4.47	-4.87	-5.35
	t_{psp}/t_{eo}	4.19	4.35	4.16	3.99	3.88	3.92
Poly-L ₂ S ₈	μ_{eo}	4.14	4.79	5.40	6.01	6.62	7.24
	μ_{ep}	-3.18	-3.66	-4.10	-4.56	-4.97	-5.41
	t_{psp}/t_{eo}	4.30	4.27	4.17	4.15	4.02	3.97
Poly-SUS	μ_{eo}	4.61	5.05	5.71	6.36	7.00	7.61
	μ_{ep}	-3.43	-3.81	-4.23	-4.69	-5.10	-5.48
	t_{psp}/t_{eo}	3.90	4.06	3.88	3.81	3.68	3.58
SDS	μ_{eo}	4.47	5.06	5.69	6.33	6.96	7.58
	μ_{ep}	-3.08	-3.60	-3.96	-4.36	-4.79	-5.18
	t_{psp}/t_{eo}	3.22	3.48	3.28	3.22	3.20	3.16

a) $\times 10^{-4} \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$; calculated from Eq. (3)b) $\times 10^{-4} \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$; calculated from Eq. (4)

3.5 Separation of benzodiazepines

Chemical structures of the seven benzodiazepines used in this study are shown in Fig. 1. The pK_a [18, 32], $\log P$, number of hydrogen-bond accepting and hydrogen-bond donating atoms on each benzodiazepine and alkyl phenyl

ketone molecule are listed in Table 3. Based on their pK_a values, all benzodiazepines and alkyl phenyl ketones are considered to be neutral at pH 8.0. Electropherograms of the seven benzodiazepines using the seven pseudostationary phases are compared in Fig. 5. Among all the pseudostationary phases, poly-L₆S₄ and SDS provided

Table 3. The pK_a , $\log P$, HBA, and HBD values of benzodiazepines and alkyl phenyl ketones

Benzodiazepines	pK_a ^{a)}	$\log P$ ^{b)}	HBA ^{b)}	HBD ^{b)}	Alkyl phenyl ketones	$\log P$	HBA	HBD
Flunitrazepam	1.4	3.02	6	0	Acetophenone	1.67	1	0
Nitrazepam	3.2, 10.8	2.84	6	1	Propiophenone	2.20	1	0
Clonazepam	1.5, 10.5	3.02	6	1	Butyrophenone	2.73	1	0
Temazepam	1.6, 11.8	3.10	4	1	Valerophenone	3.26	1	0
Diazepam	3.3	3.86	3	0	Hexanophenone	3.79	1	0
Oxazepam	1.7	2.31	4	2	Heptanophenone	4.32	1	0
Lorazepam	1.3, 11.5	2.48	4	2	Octanophenone	4.85	1	0

a) pK_a values are taken from [18, 32]b) $\log P$, HBA, and HBD values are taken from SciFinder Scholar (2002), American Chemical Society. HBA, number of hydrogen-bond accepting atom(s) on the molecule; HBD, number of hydrogen-bond donating atom(s) on the molecule

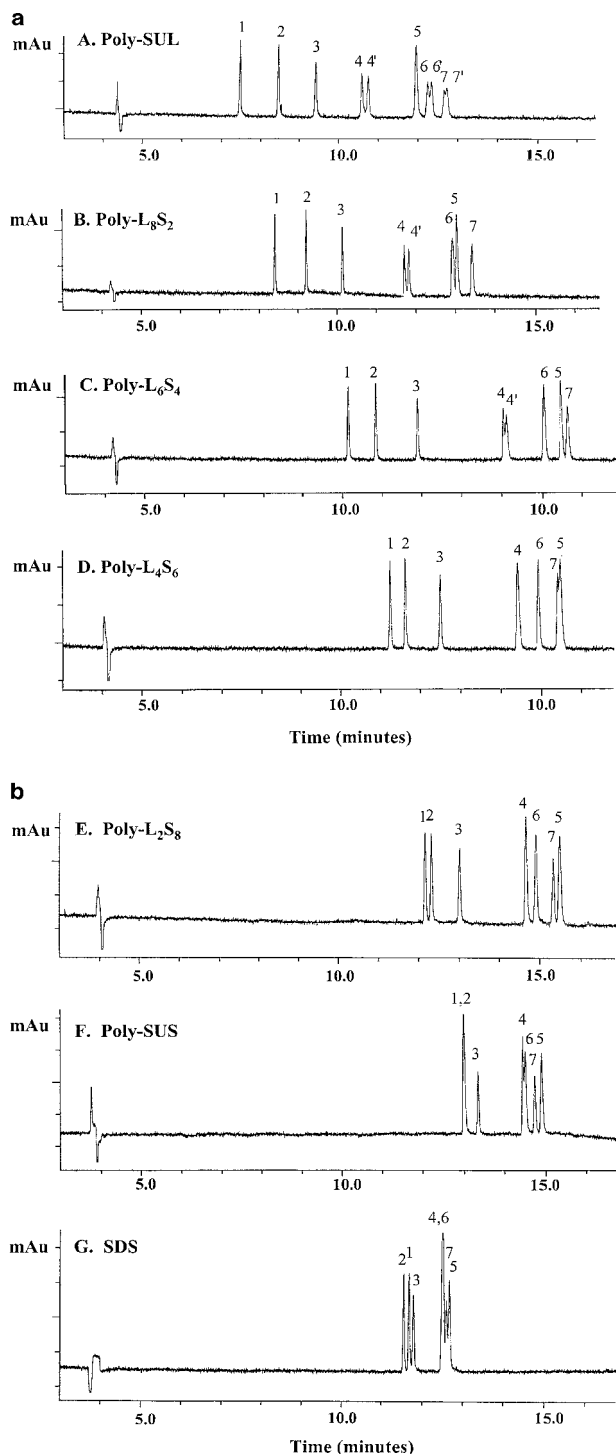


Figure 5. (a) Comparison of (A) poly-SUL, (B) poly-L₈S₂, (C) poly-L₆S₄, (D) poly-L₄S₆ for the separation of seven benzodiazepines. The MEKC separation conditions are the same as in Fig. 4 except a temperature of 20°C was used. Peak identifications are the same as in Fig. 1. (b) Comparison of (E) poly-L₂S₈, (F) poly-SUS, and (D) SDS for the separation of seven benzodiazepines. The MEKC separation conditions are the same as in (a). Peak identifications are same as in Fig. 1.

the largest (ca. 5.45 min) and the smallest (ca. 1.14 min) elution windows between the first (t_F) and the last (t_L) eluting benzodiazepines ($t_L - t_F$). The $t_L - t_F$ values of poly-SUL, poly-L₈S₂, poly-L₄S₆, poly-L₂S₈, and poly-SUS are 5.22, 5.09, 4.24, 3.43, and 1.89 min, respectively. Faster analysis times were obtained with poly-SUL, poly-L₈S₂, and SDS; however, poly-SUL provides a better separation of the benzodiazepines than SDS (Figs. 5A, B and G). Possessing the same head group, poly-SUS and SDS separated six out of seven benzodiazepines. However, poly-SUS provided a better resolution between adjacent peaks with slightly longer migration times than SDS (Figs. 5F and G).

Distinct selectivity differences were observed between pseudostationary phases as seen in Figs. 5A–G. All seven benzodiazepines were separated with poly-SUL and the four CoPSs (Figs. 5A–E). The elution order of the seven solutes using poly-SUL is flunitrazepam, nitrazepam, clonazepam, temazepam, diazepam, oxazepam, and lorazepam (peaks 1–7 in Fig. 5A). The elution order of the last three benzodiazepines, *i.e.*, diazepam, oxazepam, and lorazepam, is shifted as SUS ratio is increased in CoPS. Among all benzodiazepines studied, diazepam appeared to interact relatively stronger with high sulfate containing pseudostationary phases (Figs. 5D–G). The surfactants with higher SUL fraction are observed to interact relatively stronger with lorazepam (Figs. 5A–C) interacted relatively stronger with lorazepam. It can be seen from the electropherograms that selectivity of the surfactant can be successfully manipulated by simply changing the SUS:SUL ratio.

A closer examination of the interaction between benzodiazepines-surfactant is necessary to better understand the selectivity differences between the surfactant systems. Solute interactions with the pseudostationary phases occur *via* a variety of mechanisms such as surface adsorption, comicellization, or partitioning into the hydrophobic core of the micelles. Depending upon their nature, the analytes may reside in several regions on and/or within the micelles [33]. For example, nonpolar analytes (*e.g.*, aromatic hydrocarbons) with polarizable electrons reside near polar head group rather than deep into the core of the micelle [34]. Hydrophobic alkanes are believed to penetrate into the hydrophobic micellar core [35]. Solute with amphiphilic character have special interaction with the micelle and align themselves with the nonpolar part of the analyte directed toward the hydrophobic core and the polar part directed to the bulk aqueous phase [36].

Although the hydrophobic interaction between the solute and the pseudostationary phase is the major driving force in chromatographic separations, the elution order of the

benzodiazepines in this study does not seem to be governed entirely by the hydrophobicity. For example, less hydrophobic solutes, such as oxazepam ($\log P = 2.31$) and lorazepam ($\log P = 2.48$) retain longer than solutes with highly hydrophobic character, e.g., flunitrazepam ($\log P = 3.02$). To verify the factors that control the elution

of benzodiazepines in the pseudostationary phases, the partition coefficients, $\log K$, are compared with the $\log P$, the number of hydrogen bond accepting (HBA) and hydrogen bond donating (HBD) atoms on each solute. As seen in Fig. 6, the correlation coefficient, R^2 , of $\log K$ versus $\log P$ plots of benzodiazepines in pseudostationary

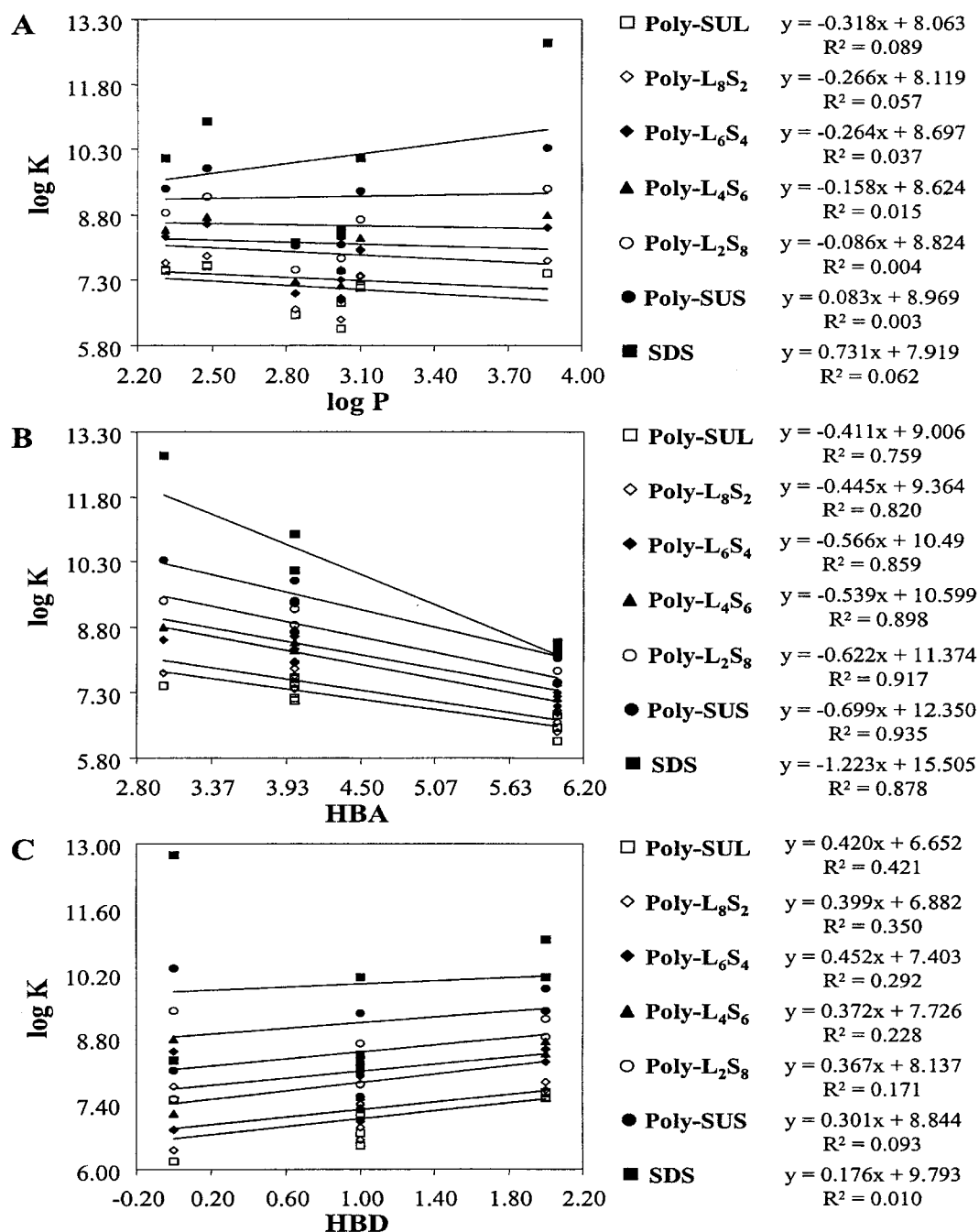


Figure 6. Plots of partition coefficient ($\log K$) of benzodiazepines against (A) analyte hydrophobicity ($\log P$), (B) number of hydrogen bond accepting atoms (HBA), and (C) number of hydrogen bond donating atoms (HBD), on benzodiazepine molecules. MEKC experimental conditions are the same as in Fig. 4. Linear regression equations and legends are shown in the plots.

phases ranges from 0.004 to 0.089 and clearly is insignificant (Fig. 6A). Figures 6B–C reveal that higher correlations are observed between $\log K$ and hydrogen bonding ability of the benzodiazepines. The $\log K$ found to be directly related to the HBD ($R^2 = 0.01$ – 0.42), but inversely related to the HBA ($R^2 = 0.76$ – 0.94) of the solutes. It is important to notice that the R^2 value of $\log K$ vs. HBA plots is highest in poly-SUS (0.94), lowest in poly-SUL (0.76), and increases gradually with an increase in SUS fraction of CoPS (e.g., 0.82 and 0.92 in poly- L_8S_2 and poly- L_2S_8 , respectively). The benzodiazepines with a high HBA and a low HBD characters (e.g., flunitrazepam, nitrazepam, and clonazepam) elute first, while those with high HBD and low HBA characters (e.g., diazepam, oxazepam, and lorazepam) elute late in all surfactant systems. It should be noted that the surfactants with high sulfate fraction tend to interact strongly with the benzodiazepines that show low both HBA and HBD characters (e.g., diazepam, oxazepam, and lorazepam). Therefore, the HBA and/or HBD character of the benzodiazepines have a significant effect on their retention. Based on the obtained data, it is assumed that benzodiazepines do not penetrate into the hydrophobic core of the micelles rather interact with the palisade and/or Stern layers of the polymeric micelles. The R^2 values of $\log K$ vs. $\log P$ and $\log K$ vs. HBD plots decrease, whereas those of $\log K$ vs. HBA plots increase linearly with increasing temperature from 15 to 40°C (data not shown).

Alkyl phenyl ketones, in contrast, possess a single hydrogen bond accepting atom but does not have any hydrogen bond donating atoms. Thus, HBA and/or HBD character does not have a significant effect on their retention

in MEKC. As seen in Fig. 7, in general, the hydrophobic character (i.e., $\log P$) governs the retention of alkyl phenyl ketones. The correlation between the $\log K$ and the $\log P$ of the alkyl phenyl ketones is better than 0.99 in all surfactant systems. No linear dependence was observed in the R^2 of $\log K$ vs. $\log P$ plots as a function of temperature (data not shown). Based on the data presented here, it is proposed that unlike benzodiazepines the alkyl phenyl ketones align themselves with their nonpolar part (i.e., alkyl hydrocarbon tail) directed toward the hydrophobic core of the micelle and the polar part (i.e., benzene) directed to the bulk aqueous phase.

The selectivity differences between surfactant systems are expected due to the chemical differences (e.g., SUS fraction) in their head groups. However, the selectivity difference between poly-SUS and SDS is surprising because both possess the same head group, i.e., sulfate. The only differences are the hydrocarbon chain length (C11 in poly-SUS, and C12 in SDS) and that poly-SUS micelles are formed from covalently linked SUS monomers while SDS micelles are formed from free SDS monomers. It is believed that it is actually the water molecules that reside in the palisade and the Stern layer that are responsible for the hydrogen bonding and hence different selectivity between poly-SUS and SDS as well as other CoPS systems. It should also be mentioned that sulfate head group has HBA atoms (oxygen) but does not hold any HBD atoms. Leucinate head group, on the other hand, possess both HBA (oxygen and nitrogen) and HBD (hydrogen of amid group) atoms. This difference may also be responsible for hydration of the micellar outer layers and thus for the selectivity differences.

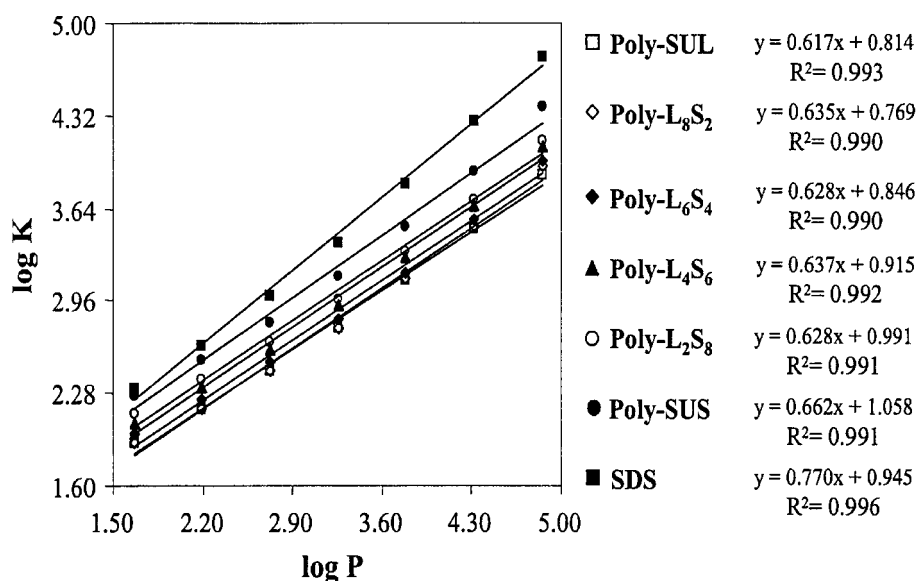


Figure 7. Plot of partition coefficient ($\log K$) of alkyl phenyl ketones against analyte hydrophobicity ($\log P$). MEKC experimental conditions are the same as in Fig. 4. Linear regression equations and legends are shown in the plots.

3.6 Enantioseparation of temazepam, oxazepam, and lorazepam

Among the seven benzodiazepines examined in this study, only temazepam, oxazepam, and lorazepam have an asymmetric carbon (Fig. 1). Although these three benzodiazepines possess similar aromatic skeletons, the major difference is the number and the type of the substituents attached to the aromatic ring. The only difference between temazepam and the other two molecules is the methyl group located on the nitrogen on the seven-member ring of temazepam and the chlorine on the *ortho*-position of the lower benzene ring of lorazepam. Of the five chiral polymeric surfactants, *i.e.*, poly-SUL, poly-L₈S₂, poly-L₆S₄, poly-L₄S₆, and poly-L₂S₈, only the first three provided enantioseparation of temazepam. However, the enantiomers of oxazepam and lorazepam were only partially resolved using poly-SUL (Fig. 5A). By lowering the temperature to 15°C, better enantiomeric separations were achieved using poly-SUL. Resolution values for the enantiomers of temazepam at 15°C were 2.74, 1.92, and 1.21, by use of poly-SUL, poly-L₈S₂, and poly-L₆S₄, respectively. The resolution of enantiomers deteriorated when the temperature was increased partly due to racemization of the solutes. No enantiomeric separation of temazepam was observed at 40°C. No chiral separation was successful above 25°C for oxazepam and lorazepam.

4 Concluding remarks

In this study, we have synthesized an achiral monomeric surfactant, SUS, and a chiral surfactant, SUL. These two surfactants were then polymerized separately to form poly-SUS and poly-SUL and together at various molar ratios to produce a variety of CoPSs possessing both chiral (*i.e.*, leucinate) and achiral (*i.e.*, sulfate) head groups. These CoPSs, poly-SUS, poly-SUL, and SDS were characterized using several analytical techniques. Fluorescence quenching was used for determination of the micellar aggregation number. Density measurements were used to determine the partial specific volumes of the polymeric surfactants. Finally, MEKC was used to examine these polymeric surfactants as novel pseudostationary phases for the separation of chiral and achiral molecules.

To test the applicability of our polymers as potential chiral selectors, several chiral solutes such as temazepam, oxazepam, and lorazepam were tested. In addition to chiral benzodiazepines, four additional achiral benzodiazepines (*i.e.*, flunitrazepam, nitrazepam, clonazepam, and diazepam) and seven alkyl phenyl ketones (*i.e.*, acetophenone,

propiophenone, butyrophenone, valerophenone, hexanophenone, heptanophenone, and octanophenone) were also separated using the seven pseudostationary phases. Each surfactant system was found to have different selectivity toward the test solutes used. Thus, this approach proved to be a viable method for improving the solubility of our amino acid based polymeric surfactants, which are not soluble below pH 7.0 as well as enhancing the selectivity for specific analytes. It appears from the obtained data that the interaction of benzodiazepines and alkyl phenyl ketones with the pseudostationary phases occurs through different mechanisms. It is assumed that benzodiazepines interact with the outer parts of the micelles (*i.e.*, the palisade and the Stern layers), while alkyl phenyl ketones tend to partition into the hydrophobic core of the micelles.

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5 References

- [1] Palmer, P. C., Khaled, M. Y., McNair, H. M., *J. High Resolut. Chromatogr.* 1992, 15, 756–762.
- [2] Palmer, P. C., McNair, H. M., *J. Microcol. Sep.* 1992, 4, 509–514.
- [3] Palmer, C. P., Terabe, S., *Anal. Chem.* 1997, 69, 1852–1860.
- [4] Palmer, C. P., Terabe, S. J., *Microcol. Sep.* 1998, 8, 115–121.
- [5] Shamsi, S. A., Akbay, C., Warner, I. M., *Anal. Chem.* 1998, 70, 3078–3083.
- [6] Wang, J., Warner, I. M., *Anal. Chem.* 1994, 66, 3773–3776.
- [7] Dobashi, A., Hamada, M., Dobashi, Y., Yamaguchi, J., *Anal. Chem.* 1995, 67, 3011–3017.
- [8] Sternbach, L. H., Randall, L. O., Lehr, H., Burger, A. (Eds.), *Drugs Affecting the Central Nervous System*, Vol. 2, Marcel Dekker, New York 1968.
- [9] Drummer, O. H., *J. Chromatogr. B* 1998, 713, 201–225.
- [10] Rizzo, M., *J. Chromatogr. B* 2000, 747, 203–216.
- [11] Catabay, A. P., Okumura, C., Saito, Y., Jinno, K., *J. Microcol. Sep.* 1997, 9, 81–85.
- [12] Catabay, A. P., Okumura, C., Jinno, K., Pesek, J. J., Williamsen, E., Fetzer, J. C., Biggs, W. R., *Chromatographia* 1998, 47, 13–20.
- [13] Cahours, X., Morin, P., Dreux, M., *J. Chromatogr. A* 1999, 845, 203–216.
- [14] Kapnissi, C. P., Akbay, C., Schlenoff, J. B., Warner, I. M., *Anal. Chem.* 2002, 74, 2328–2335.
- [15] Kamande, M. W., Kapnissi, C. P., Zhu, X., Akbay, C., Warner, I. M., *Electrophoresis* 2003, 24, 945–951.
- [16] Wernly, P., Thormann, W., *Anal. Chem.* 1992, 64, 2155–2159.
- [17] Tomita, M., Okuyama, T., Sato, S., Ishizu, H., *J. Chromatogr.* 1993, 621, 249–255.
- [18] Renou-Gonnord, M. F., David, K., *J. Chromatogr. A* 1996, 735, 249–261.

- [19] McGrath, G., McClean, S., Okane, E., Smyth, W. F., Tagliaro, F., *J. Chromatogr. A* 1996, 735, 237–247.
- [20] Boonkerd, S., Detaevernier, M. T., Vindevogel, J., Michotte, Y., *J. Chromatogr. A* 1996, 756, 279–286.
- [21] Wallingford, R. A., Curry, P. D. Jr., Ewing, A. G., *J. Microcol. Sep.* 1989, 1, 23–31.
- [22] Rasmussen, H. T., Goebel, L. K., McNair, H. M., *J. Chromatogr. A* 1990, 517, 549–555.
- [23] Ahuja, E. S., Preston, B. P., Foley, J. P., *J. Chromatogr. B* 1994, 657, 271–284.
- [24] Almgren, M., Grieser, F., Thomas, J. K., *J. Am. Chem. Soc.* 1979, 101, 279–291.
- [25] Wang, J., Warner, I. M., *Anal. Chem.* 1994, 66, 3773–3776.
- [26] Turro, N. J., Yekta, A., *J. Am. Chem. Soc.* 1978, 100, 5951–5952.
- [27] Lüscher-Mattli, M., in: Hinz, H.-J. (Ed.), *Thermodynamic Data for Biochemistry and Biotechnology*, Springer-Verlag, New York 1986.
- [28] Terabe, S., Otsuka, K., Ando, T., *Anal. Chem.* 1985, 57, 834–841.
- [29] Shinoda, K., Tamamushi, B., Nagakawa, T., Isemura, T., *Colloidal Surfactants*, Academic Press, New York 1963.
- [30] Terabe, S., *Micellar Electrokinetic Chromatography*, Beckman Instruments, Fullerton, CA 1993.
- [31] Kerker, M., *The Scattering of Light and Other Electromagnetic Radiation*, Academic Press, New York 1969.
- [32] Boonkerd, S., Detaevernier, M. T., Vindevogel, J., Michotte, Y., *J. Chromatogr. A* 1996, 756, 279–286.
- [33] McIntre, G. L., *Crit. Rev. Anal. Chem.* 1990, 21, 257.
- [34] Mousty, C., Pouoillen, P., Martre, A.-M., Mousset, G., *J. Colloid Interface Sci.* 1986, 113, 521.
- [35] Cline-Love, L. J., Habarta, J. G., Dorsey, J. G., *Anal. Chem.* 1984, 56, 1132–1135A.
- [36] Brugger, P.-A., Infeltra, P. P., Braun, A. M., Gratzel, M. J., *J. Am. Chem. Soc.* 1981, 103, 320–326.