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Novel anionic copolymerized surfactants of mixed achiral and chiral surfactants as pseudostationary phases for micellar electrokinetic chromatography

One disadvantage of amino acid-based chiral selectors for micellar electrokinetic chromatography (MEKC) is that either they have very low solubility or are insoluble at acidic pHs. In order to increase solubilities at lower pHs, we have synthesized a highly water-soluble achiral surfactant and copolymerized it with an amino acid-based chiral surfactant. These two surfactants were polymerized either separately or at various molar ratios of binary solutions, yielding pure molecular or copolymerized surfactant (CoPS), respectively. All surfactants were characterized by use of several analytical techniques prior to using them as novel pseudostationary phases in MEKC. The chromatographic performance of the CoPS in MEKC was tested with chiral and achiral analytes. The highly soluble sulfate head group significantly increased the solubility of amino acid-based CoPS over a wide range of pH. Three chiral binaphthyl derivatives were tested and each surfactant system was found to have different selectivity.

Keywords: Binaphthyl derivatives / Chiral separation / Copolymerized polymeric surfactants / Micellar electrokinetic chromatography / Polymeric surfactants DOI 10.1002/elps.200305712

1 Introduction

Capillary electrophoresis (CE) is a powerful technique for separation of charged molecules. However, the applicability of electrophoretic methods for simultaneously separating both charged and neutral molecules was achieved only after introducing micellar electrokinetic chromatography (MEKC). MEKC uses surfactants as pseudostationary phases, and was first introduced by Terabe and co-workers in the early 1980s [1]. The separation principle is based on the partitioning of analytes between the mobile and the pseudostationary phases. Consequently, sodium dodecyl sulfate (SDS) has been extensively used as a pseudostationary phase in MEKC applications.

Micelles have successfully been used as carriers in many separation applications. Yet, conventional micelles have some drawbacks as pseudostationary phases in MEKC. (i) Conventional micelles require high surfactant concentrations (at least 2–10 times the critical micelle concentra-

tion, CMC) for effective separations. As a result, the ionic strength of the solution increases enormously. This causes Joule heating and thus, the temperature inside the capillary rises. Consequently, the CMC as well as the viscosity of the mobile phase are affected and serious problems with poor reproducibility are introduced. (ii) The CMC is influenced by the surfactant concentration, the pH, and the ionic strength of the running buffer [2–4]. All of these factors may induce changes in the micellar structure, and therefore, reduce the reproducibility in MEKC. (iii) The separation of highly hydrophobic compounds requires a high content of an organic co-solvent, which tends to shift the dynamic equilibria of micelles [5–8]. (iv) The presence of low-molecular-weight surfactants in the running buffer makes mass spectrometric detection difficult. In particular, large signals from monomers will interfere with most MEKC solutes in the low-molecular-mass region. In addition, accumulation of surfactants can cause fouling of the ion source, and may limit the sensitivity in electrospray ionization-mass spectrometry [9–11]. (v) Surfactant monomers in the running buffer will more likely form inclusion complexes with host molecules [12]. Therefore, competitive binding of free surfactant monomers with host molecules will interfere with host-guest interaction of the analyte, and may result in poor separations.

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Abbreviations: **BNA**, (\pm)-1,1'-binaphthyl-2,2'-diamine; **BNP**, (\pm)-1,1'-binaphthyl-2,2'-dihydrogen phosphate; **BOH**, (\pm)-1,1'-bi-2-naphthol; **CoPS**, copolymerized surfactants; **poly-SUL**, poly(sodium 10-undecenyl L-leucinate); **poly-SUS**, poly(sodium 10-undecenyl sulfate)

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Several types of pseudostationary phases have been developed as alternatives to conventional surfactant micelles. These include, but are not limited to, neutral pseudostationary phases such as cyclodextrin (CD) polymers [13–15] and polyvinylpyrrolidone [6–18], ionic pseudostationary phases such as anionic [19–21] and cationic [22–25] polymers, proteins [26–28], charged CDs [29–31], calixarenes [33–34], dendrimers [35–37], siloxane polymers [38–40], and achiral [41–44] as well as chiral [45–49] polymeric surfactants. Polymeric surfactants, another term used for molecular micelles, have drawn considerable attention as potential pseudostationary phases in MEKC. This is largely due to their distinct advantages over conventional micelles. First, they can be purified from their free monomers, and they have no CMC. Thus, they can be effective pseudostationary phases over a wide range of concentrations. Second, polymeric surfactants are stable in the presence of high content of organic co-solvents or host molecules. Thus, polymeric surfactants can be used in conjunction with host molecules such as CDs. Finally, polymeric surfactants can be modified to obtain desired properties either through synthesis or polymerization processes.

The first successful application of an anionic polymeric surfactant, poly(sodium 10-undecylenate) (poly-SUA), as a pseudostationary phase in MEKC for the separation of alkyl phthalates and some polycyclic aromatic hydrocarbons (PAHs) was reported by Palmer *et al.* [41]. The same pseudostationary phase was also used for the successful separation of EPA's 16 priority pollutant PAHs by using THF as an organic modifier [50], which provided high-performance separation for a wide range of neutral compounds. However, the electrophoretic mobility of this polymer was influenced by the ionization of the carboxylated head groups, resulting in poor reproducible analyses times. In addition, this polymeric surfactant was not soluble below pH 7.0 due to the carboxylated head group. In order to overcome these problems, the Warner group [51] and Palmer and Terabe [7] synthesized polymerized surfactants with a sulfate head group, poly(sodium 10-undecenyl sulfate) (poly-SUS). Palmer and Terabe [52] used potassium persulfate as a free radical initiator for the polymerization process, which resulted in contamination of the product with sodium sulfate. In contrast, the Warner group [43, 44, 51] used ^{60}Co -irradiation for the polymerization of the surfactants.

Single amino acid-based polymeric surfactants as chiral selectors for MEKC were first introduced by Wang and Warner [45] and Dobashi *et al.* [46]. A major advantage of this approach is that different functionalities, such as a variety of chiral head groups, can be integrated into the polymeric surfactants. This introduces an effective way of manipulating selectivities of the pseudostationary

phase. In addition, the availability of both D and L optical configurations of amino acid-based pseudostationary phases is particularly advantageous to more accurately determine enantiomeric impurities by reversal of the migration order of the two enantiomers. The main disadvantage of polymeric surfactants is that they are not yet commercially available; therefore, they must be synthesized. However, the synthetic procedure is straightforward. Another drawback of pseudostationary phases with carboxylated head groups (*e.g.*, amino acids) is their poor solubility below pH 7.0.

In this study, we attempted to incorporate a highly soluble achiral surfactant in combination with a less soluble chiral surfactant within the same polymeric surfactant. We expect that this approach may circumvent the solubility limitations at low pH, while maintaining the chiral selectivity character of the novel polymeric surfactants. Therefore, sodium 10-undecenyl sulfate (SUS), an achiral surfactant, and sodium *N*-undecenoyl L-leucinate (SUL), a chiral surfactant were synthesized. These two surfactants were then polymerized separately to form poly-SUS and poly-SUL, or copolymerized at various molar ratios to form novel copolymerized surfactants (CoPS). Then poly-SUS, poly-SUL, and the four CoPS were applied as pseudostationary phases in MEKC for the separation of chiral binaphthyl derivatives and achiral alkyl phenyl ketone molecules.

2 Materials and methods

2.1 Apparatus

A Beckman P/ACE model 5510 capillary electrophoresis (CE) instrument (Fullerton, CA, USA) was employed for MEKC separations. This CE instrument was equipped with two sample carousels (a 21-position inlet and 10-position outlet) for automatic sample/buffer change, a 0–30 kV high-voltage power supply, 200, 214, 254, and 280 nm selectable wavelength filters for UV detection, a liquid thermostated capillary cartridge, and a System Gold software for system control and data handling. MEKC separations were performed in a 57 cm in length (50 cm effective length) and 50 μm ID (367 μm OD) fused-silica capillaries obtained from Polymicro Technologies (Phoenix, AZ, USA). The capillary in the CE instrument was thermostated using a fluoroorganic fluid. The detector time constant was 0.2 s in all experiments.

2.2 Materials

The racemates of (\pm)-1,1'-binaphthyl-2,2'-diamine (BNA), (\pm)-1,1'-bi-2-naphthol (BOH), (\pm)-1,1'-binaphthyl-2,2'-dihydrogen phosphate (BNP), and L-leucine were obtained

from Sigma (St. Louis, MO, USA). *N*-Hydroxysuccinimide, undecylenic acid, dicyclohexylcarbodiimide (DCC), HPLC-grade ethyl acetate, disodium hydrogen phosphate, sodium bicarbonate, and sodium carbonate were all reagent-grade and obtained from Aldrich (Milwaukee, WI, USA). Analytical reagent grade undecylenyl alcohol, alkyl aryl ketone homologues, pyrene, chlorosulfonic acid, and pyridine (PY) were also purchased from Aldrich. All chemicals were used as received.

2.3 Synthesis of SUS and SUL

Mono-SUS and poly-SUS were synthesized according to previously described procedure [43]. Mono-SUL, an amino acid-based chiral surfactant with a leucinate head group, was synthesized from the *N*-hydroxysuccinimide ester of undecylenic acid and the synthesis of amino acid-based chiral surfactant is detailed elsewhere [45].

2.4 Preparation of polymeric surfactants

Monomers of SUL and SUS were polymerized or copolymerized in pure or mixed solutions of the surfactants to yield poly-SUL, poly-SUS, or CoPS. Four different molar ratios of SUL/SUS surfactant solutions were prepared: 80/20, 60/40, 40/60, and 20/80 (Fig. 1). The six molar fractions of each surfactant were adjusted so that the overall concentration of surfactants is 100 mM. Preparation of CoPS and proposed names for each CoPS are summarized in Table 1. All surfactant solutions were polymerized by exposing the solutions to a ^{60}Co - γ ray source (~ 680 rad/h) for seven days followed by filtration and lyophilization. All CoPS were applied as pseudostationary phases in MEKC without any further purification or dialysis.

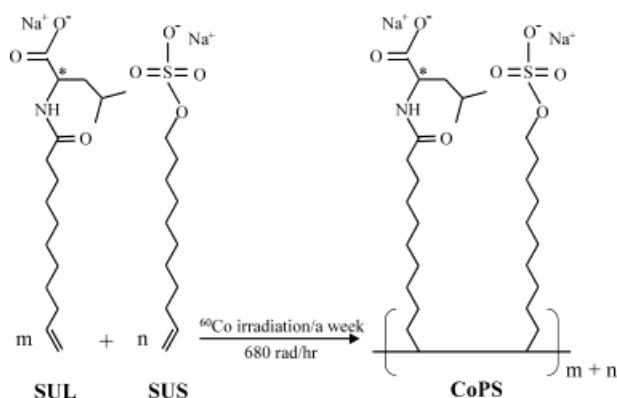


Figure 1. Scheme for copolymerization of SUL and SUS surfactants. The asterisk represents the chiral center of the surfactant. The *m* and *n* represent the mole fractions of SUL and SUS, respectively, in the mixture.

Table 1. Preparation of polymerized and copolymerized surfactants from SUL and SUS surfactants

	Proposed acronyms for the surfactants					
	poly-SUL	poly-L ₈ S ₂	poly-L ₆ S ₄	poly-L ₄ S ₆	poly-L ₂ S ₈	poly-SUS
M_{SUL}	100	80	60	40	20	0
M_{SUS}	0	20	40	60	80	100

M_{SUL} and M_{SUS} are the molar fractions of SUL and SUS in copolymerized surfactants.

2.5 Characterization of polymeric surfactants

The aggregation numbers (*N*) of the surfactants were determined by a fluorescence quenching method proposed by Turro and Yekta [53]. Fluorescence measurements were performed using a SPEX model F2T211 spectrophotometer. Pyrene was used as the fluorescent probe and cetylpyridinium chloride (CPyCl) was used as the quencher. The partial specific volume (\bar{v}) of the polymeric surfactants was determined by inverse of density ($1/\rho$) versus weight fraction of the solvent (*i.e.*, water) of surfactant solutions at 20°C in 20 mM phosphate buffer [54]. A high-precision model DMA 58 digital densitometer (Anton Paar USA, League City, TX, USA) was used to perform density measurements. Details of the characterization are discussed elsewhere [55].

2.6 Capillary electrophoresis

New capillaries were prepared by a standard wash cycle of 1 M NaOH (1 h) and triply deionized water (20 min) before use. Prior to each separation with the same surfactant, the capillaries were rinsed with triply deionized water (5 min), 0.1 M NaOH (3 min), and separation buffer (3 min). The capillaries were reactivated each day by rinsing with 1 M NaOH (15 min), triply deionized water (2 min), and the running buffer (10 min). When the pseudostationary phase was changed, the capillaries were reconditioned with deionized water (15 min), 0.1 M NaOH (10 min), and separation buffer (5 min). Unless otherwise noted, the time for pressure injection was 2 s. The cartridge temperature was maintained at 20°C for the separation of BNA, BNP, and BOH.

2.7 Preparation of separation buffers and standard solutions

Four 100 mM stock solutions of phosphate buffer (pH 3.0, 7.0, 8.0, and 9.0) were prepared by dissolution of an appropriate amount of disodium hydrogenphosphate or

sodium dihydrogenphosphate. Solutions were adjusted to the desired pH values using solutions of phosphoric acid or sodium hydroxide. All solutions were refrigerated after each use. The solution of each pseudostationary phase was prepared by first dissolving 0.1 g of the surfactant in 5.0 mL of deionized water. Two mL of the appropriate buffer was then added to this solution and the final volume was adjusted to 10.0 mL with deionized water. After thoroughly mixing in a sonicator for 10 min, the final running buffers were filtered through a 0.45 μm syringe filter (Nalgene, Rochester, NY, USA) and then sonicated for an additional 3 min before running capillary electrophoretic experiments. All stock analyte solutions were prepared in methanol:deionized water (1:1) at concentrations of ca. 0.15–0.30 mM each.

2.8 Calculations

The capacity factor, k' , of the solutes was measured using the following equation [56]:

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

where t_R , t_{eo} and t_{psp} are the migration times of the retained analyte, 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 deviation from the baseline. Decanophenone was used as the tracer for t_{psp} . The elution window is defined as t_{psp}/t_{eo} . The apparent electrophoretic mobility of pseudostationary phase was calculated according to Eq. (2):

$$\mu_{app} = \frac{l_t l_d}{V t_{psp}} \quad (2)$$

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 retention times are in second (s). Calculation of the electroosmotic mobility of the buffer solution required replacing the t_{psp} term in Eq. (2) with t_{eo} . Finally, the methylene selectivity, α_{CH_2} (also called hydrophobic selectivity) was calculated by use of the antilogarithm of the slope of the regression line of $\log k'$ vs. carbon number of alkyl phenyl ketone homologous series (Fig. 2).

3 Results and discussion

3.1 Characterization of polymeric surfactants

The \bar{v} values for each surfactant system are listed in Table 2. The \bar{v} value of poly-SUL is the lowest among all of the surfactants, indicating that poly-SUL has a relatively compact

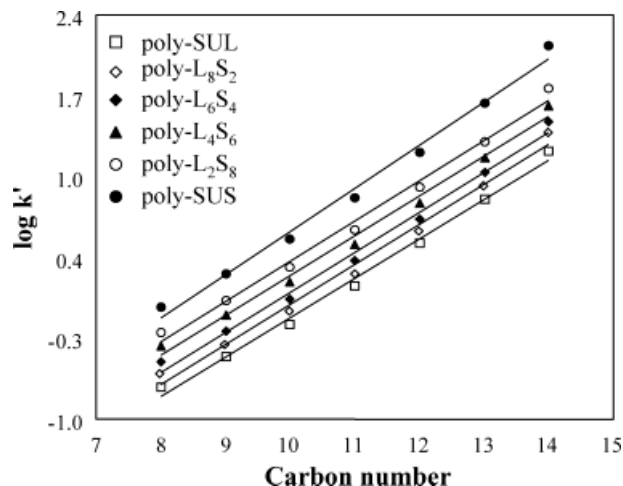


Figure 2. Linear relationship between $\log k'$ versus carbon number of alkyl phenyl ketone homologous series. MEKC conditions: pseudostationary phase concentration 1.0% w/v each; 20 mM phosphate buffer (pH 8.0); +25 kV applied voltage; temperature, 20°C for separation; UV detection at 254 nm. Alkyl phenyl ketones: acetophenone (C8), propiophenone (C9), butyrophenone (C10), valero-phenone (C11), hexanophenone (C12), heptanophenone (C13), and octanophenone (C14). Legends are shown in the plot.

structure. In contrast, poly-SUS has the highest \bar{v} value suggesting that poly-SUS has a bulkier structure as compared to poly-SUL and all other CoPS. As the molar ratio of SUS increased, the \bar{v} values of the CoPS become larger. The N values of the six pseudostationary phases are also listed in Table 2. The N values for poly-SUL, poly-L₈S₂, and poly-L₆S₄ are found very similar. Poly-SUS and poly-L₂S₈ have the lowest and highest aggregation numbers, respectively. There is no apparent relationship between the molar fraction of SUL or SUS and the aggregation numbers of the polymeric surfactants. According to methylene selectivity (α_{CH_2}) data in Table 2, poly-SUS provides the most hydrophobic environment (*i.e.*, highest α_{CH_2} value) and poly-SUL provides the least (*i.e.*, lowest value) for alkyl phenyl ketones under the experimental conditions studied. The hydrophobicity values (*i.e.*, α_{CH_2} values) of all CoPS are very similar despite the different SUL and SUS molar ratios.

The electroosmotic (μ_{eo}), apparent electrophoretic (μ_{app}), and effective electrophoretic (μ_{ep}) mobilities of the six pseudostationary phases are listed in Table 2. Poly-SUS has the largest while poly-SUL has the smallest μ_{eo} value. For the CoPS, the value of μ_{eo} increased with an increase in the mole fraction of SUS. The variations in μ_{eo} for different surfactant systems can be attributed to a variety of parameters including the change in the viscosity of the surfactant solution, the change in the zeta potential of

Table 2. Physicochemical properties of the six pseudostationary phases used in this study

Physicochemical property	Pseudostationary phase					
	Poly-SUL	Poly-L ₈ S ₂	Poly-L ₆ S ₄	Poly-L ₄ S ₆	Poly-L ₂ S ₈	Poly-SUS
Critical micelle concentration ^{a)} (mM)	0	0	0	0	0	0
Aggregation number ^{b)} (<i>N</i>)	61	61	62	49	68	21
Partial specific volume ^{c)} \bar{v} (mL · g ⁻¹)	0.707	0.732	0.739	0.748	0.752	0.768
Methylene-group selectivity ^{d), e)} α_{CH_2}	2.15	2.18	2.17	2.16	2.19	2.31
Electroosmotic mobility ^{d), f)} μ_{eo} (10 ⁻⁴ cm ² V ⁻¹ s ⁻¹)	4.38	4.49	4.52	4.70	4.79	5.05
Apparent electrophoretic mobility ^{d), g)} μ_{app} (10 ⁻⁴ cm ² V ⁻¹ s ⁻¹)	0.90	1.07	1.02	1.08	1.12	1.24
Effective electrophoretic mobility ^{d), h)} μ_{ep} (10 ⁻⁴ cm ² V ⁻¹ s ⁻¹)	-3.48	-3.42	-3.50	-3.62	-3.66	-3.81
Migration-time window ^{d)} $t_{\text{psp}}/t_{\text{eo}}$	4.87	4.18	4.43	4.35	4.27	4.06

a) Critical micelle concentration of the polymerized surfactant is assumed to be zero.

b) Determined in water by fluorescence quenching method at room temperature

c) Determined from density measurements

d) Data were collected with 57 cm (50 cm effective length) × 50 μm ID capillary with an applied voltage of +25 kV using a 20 mM phosphate buffer at pH of 8.0; temperature, 20°C; final surfactant concentration, 1.0% w/v.

e) Calculated from the antilogarithm of the slope of the regression line of log *k'* vs. carbon number of alkyl phenyl ketones (C8–C14)

f) Calculated from Eq. (2) where t_{psp} was replaced with t_{eo}

g) Calculated from Eq. (2)

h) Calculated from $\mu_{\text{ep}} = \mu_{\text{app}} - \mu_{\text{eo}}$

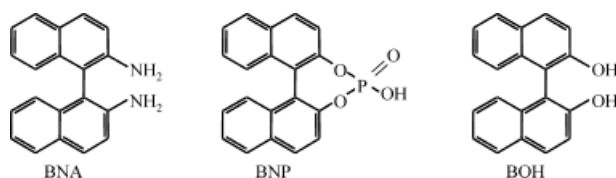
The relative standard deviations (RSDs) of each physicochemical property determination were less than 1.5%.

both the capillary walls and the pseudostationary phase, and the change in the charge density on the capillary wall. As expected, the μ_{ep} values of the anionic pseudostationary phases are negative because the polymeric surfactants move toward the anode (Table 2). However, the net

mobility of polymeric surfactants is positive because the mobility of the EOF (*i.e.*, μ_{eo}) is larger than the μ_{ep} of polymeric surfactants. Thus, a stronger EOF drags the polymeric surfactants toward the negative electrode faster. Poly-SUS has the largest μ_{ep} among all pseudostationary phases used in this study. It should be noted that the μ_{app} value increased gradually as the ratio of SUS/SUL is increased in the CoPS. Due to a larger μ_{eo} value, poly-SUS had a relatively smaller migration-time window as compared to that of poly-SUL and the four CoPS. Among all polymeric surfactant systems, poly-SUL provides the widest migration window, which allows the analysis of a larger number of solutes.

3.2 Enantiomeric separation of binaphthyl derivatives

The chemical structures of binaphthyl derivatives are provided in Fig. 3. The enantiomeric separation of binaphthyl derivatives (BNA, BNP, and BOH) using poly-SUL and the four CoPS are shown in Fig. 4. Poly-SUS is an achiral surfactant and thus not used for separation of binaphthyl derivatives. All three binaphthyl derivatives were baseline-resolved using poly-SUL and poly-L₈S₂ polymeric surfactants. When poly-SUL was used as a chiral selector, the resolution values of BNA, BNP, and BOH at pH 7.0 were found to be 2.02, 2.05, and 1.59, respectively (Fig. 4A). It is interesting to note that poly-L₈S₂ provided better resolutions for BNP (2.21) and BOH (1.76) but gave a slightly lower resolution for BNA (1.88) as compared to poly-SUL (Fig. 4B). As the SUL/SUS ratio of the CoPS is decreased, the resolution of the binaphthyl derivatives generally decreased. This can be attributed to a decrease in the number of chiral sites available within the CoPS for the interaction with the chiral solutes. For example, the resolution of BNP decreased from 1.95 (poly-L₆S₄) to 1.30 (poly-L₄S₆) and 0.74 (poly-L₂S₈) (Figs. 4C–E). Poly-L₆S₄ provided a partial resolution of BNA (0.89) and BOH (0.96). However, these two binaphthyl derivatives could not be resolved by either poly-L₄S₆ or poly-L₂S₈. Comigration of BNA and BOH was observed in poly-L₆S₄ (Fig. 4D), while migration order reversal of these two solutes was observed with poly-L₂S₈ (Fig. 4E).

**Figure 3.** Chemical structures of binaphthyl derivatives.

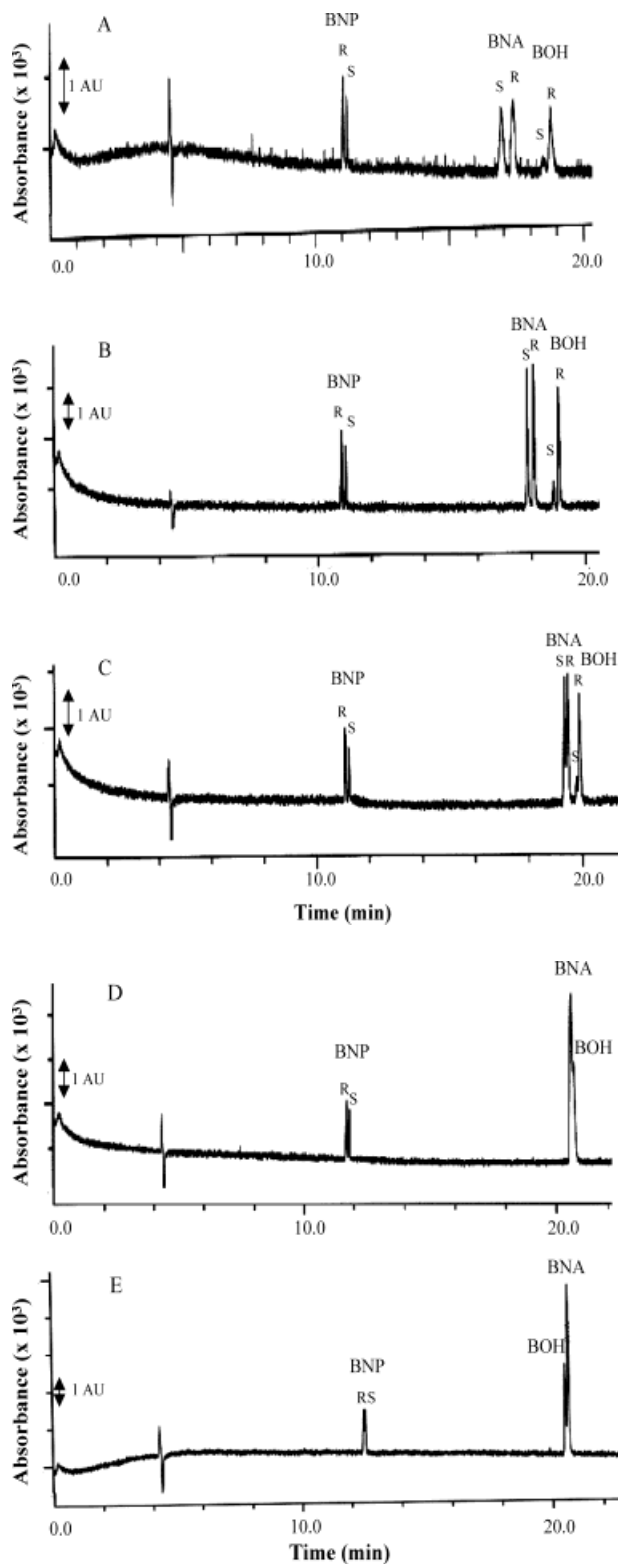


Figure 4. Chiral separation of BNA, BNP, and BOH using (A) poly-SUL, (B) poly-L₈S₂, (C) poly-L₆S₄, (D) poly-L₄S₆, and (E) poly-L₂S₈. Conditions: 20 mM phosphate buffer (pH 7.0); 25 kV applied voltage; temperature, 20°C; current, 30–43 μ A.

3.3 Effect of pH on enantiomeric separation of binaphthyl derivatives

One of the objectives of this study was to increase the solubility of the amino acid-based chiral surfactants over a wide pH range, especially acidic pHs. The solubilities of poly-SUL, poly-SUS, and the four CoPS were tested at acidic pH values. Poly-SUL was not soluble below pH \sim 6.9. In contrast, poly-SUS and poly-L₂S₈ were soluble at all acidic pHs. Poly-L₈S₂, poly-L₆S₄, and poly-L₄S₆ were insoluble at pHs lower than 4.0, 1.7, and 1.6, respectively. The effect of pH on the separation of binaphthyl derivatives is shown in Fig. 5. At pH 9.0, poly-SUL sepa-

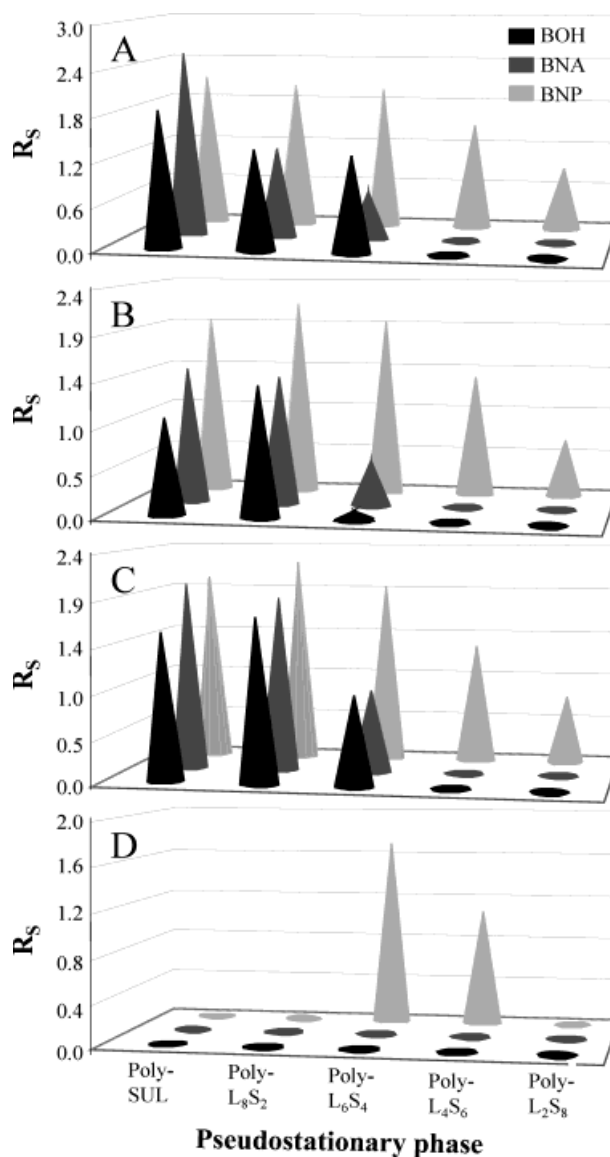


Figure 5. Comparison of resolution values in five pseudostationary phases at pH (A) 9.0, (B) 8.0, (C) 7.0, and (D) 3.0. Conditions: 20 mM phosphate buffer; applied voltage of +25 (A–C) or (D) –25 kV; temperature, 20°C.

rated BNA, BNP, and BOH successfully with resolution values of 2.54, 2.12, and 1.86, respectively (Fig. 5A). Poly-L₈S₂ separated the analytes best at pH 7.0. Comparison of poly-L₈S₂ and poly-SUL at pH 7.0 and 8.0 revealed that poly-L₈S₂ provided relatively better enantiomeric separations of BNP (2.21 vs. 2.05 at pH 7.0 and 2.14 vs. 1.97 at pH 8.0) and BOH (pH 7.0: 1.76 vs. 1.59 and pH 8.0: 1.41 vs. 1.05), while poly-SUL separated BNA slightly better at both pHs (pH 7.0: 2.02 vs. 1.88 and pH 8.0: 1.48 vs. 1.40). Poly-L₆S₄ resulted poorer enantiomeric separations of BNA and BOH as compared to poly-SUL and poly-L₈S₂ at all pH values studied. However, the resolution of BNP in poly-L₆S₄ (pH 7.0: 1.95, pH 8.0: 1.96, and pH 9.0: 1.98) was comparable with that in poly-SUL and poly-L₈S₂. Further increase in sulfate head group in the CoPS pseudostationary phases resulted in poorer enantioseparations. This is particularly observed with poly-L₄S₆ and poly-L₂S₈ where the molar fraction of sulfate is more than that of leucinate. Poly-L₄S₆ and poly-L₂S₈ could not separate either BNA or BOH, but did separate BNP with reasonable resolutions (Figs. 5A–D). The electropherograms in Fig. 4 show that BOH and BNA interact stronger with pseudostationary phases than BNP. Our current observations are in agreement with previous observations reported by Billiot *et al.* [57].

The presence of the sulfate head group along with the chiral leucinate increases not only the solubility of the surfactants but also improves (in the case of poly-L₈S₂) the

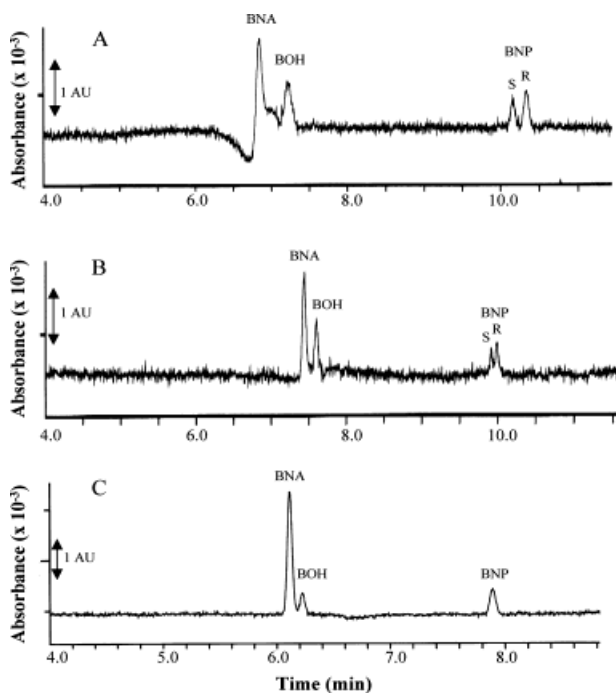


Figure 6. Chiral separation of BNA, BNP, and BOH using (A) poly-L₆S₄, (B) poly-L₄S₆, and (C) poly-L₂S₈. Conditions: 20 mM phosphate buffer (pH 3.0); –25 kV applied voltage; temperature, 20°C; current, 24–26 μ A.

resolution of the chiral analytes. Of all the CoPS, poly-L₈S₂ showed the best resolution of the analytes. Higher concentrations of sulfate head groups, however, diminished chiral separations due mainly to steric hindrance of sulfate head group and lower numbers of chiral sites on the CoPS for chiral interactions. Figures 5D and 6 show the enantiomeric separation of BNA, BNP, and BOH at pH 3.0. Only poly-L₆S₄ and poly-L₄S₆ provided separation of BNP but were not successful in separating BNA and BOH. Poly-SUL and poly-L₈S₂ were not soluble, while poly-L₂S₈ was not successful at pH 3.0. This anomalous behavior may be attributed to less chiral sites on the surfactant as well as the carboxylate group of leucinate being less ionized at lower pHs and thus may effect the solutes' ability to interact with the chiral center of the CoPS. This preliminary separation of BNP in acidic pH using CoPS shows that these types of novel pseudostationary phases may prove to be effective, particularly in the acidic pH range of 2–5 where most of the cationic drugs are usually separated.

4 Concluding remarks

In this study, an achiral monomeric surfactant (SUS) and a chiral surfactant (SUL) were synthesized. These two surfactants were then polymerized separately or together at various molar ratios to give a variety of CoPS possessing both chiral (leucinate) and achiral (sulfate) head groups. These polymeric surfactants were characterized by use of several analytical techniques. Fluorescence quenching was used for determining the aggregation number; density measurements were used to estimate partial specific volume, and MEKC was used to evaluate their chromatographic performance as novel pseudostationary phases for separation of chiral and achiral molecules. The addition of highly soluble sulfate head group into the polymeric surfactant structure significantly improved the solubility of the amino acid-based CoPS over a wide range of pH.

To test the applicability of these polymers as potential chiral selectors, three chiral test solutes (*i.e.*, binaphthyl derivatives, BNA, BNP, and BOH) were used. All three binaphthyl derivatives were baseline-resolved using poly-SUL and poly-L₈S₂. At high pH values (*e.g.*, 7.0, 8.0, and 9.0) BNA, BNP, and BOH were baseline-resolved using poly-SUL and poly-L₈S₂. As the SUL/SUS ratio in CoPS decreased, the enantioseparation ability of the surfactants as chiral selectors also decreased. Thus, poly-L₆S₄, poly-L₄S₆, and poly-L₂S₈ gave relatively poor chiral separations for the test analytes. At pH 3.0, poly-L₆S₄ and poly-L₄S₆ provided good chiral separation of BNP. However, poly-SUL and poly-L₈S₂ were not soluble while poly-L₂S₈ did not give any chiral separation at pH 3.0. Each surfactant system was found to have different selectivities

toward the test solutes used in this study as observed in Figs. 4D, E, where the migration order of BNA and BOH is altered in different CoPS systems.

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