

Simon Mwongela¹
Cevdet Akbay²
Xiaofeng Zhu¹
Sibrina Collins³
Isiah M. Warner¹

¹Department of Chemistry,
Louisiana State University,
Baton Rouge, LA, USA

²Department of Chemistry,
Center of Biotechnology and
Drug Design,
Georgia State University,
Atlanta, GA, USA

³Department of Chemistry,
Claflin University,
Orangeburg, SC, USA

Use of poly(sodium oleyl-L-leucylvalinate) surfactant for the separation of chiral compounds in micellar electrokinetic chromatography

A chiral amino acid-based monomeric and polymeric surfactant, sodium oleyl-L-leucylvalinate) (L-SOLV) and poly(sodium oleyl-L-leucylvalinate) (poly-L-SOLV) were synthesized and used for chiral separations in micellar electrokinetic chromatography (MEKC). Poly-L-SOLV was used successfully in the separation of various enantiomers of neutral, acidic, and basic analytes such as 1,1'-bi-2-naphthol, 1,1'-binaphthyl-2,2'-diamine, benzoin, hydrobenzoin, benzoin methylether, warfarin, and coumachlor obtaining well-resolved peaks but with only partial separation of temazepam. In addition, the atropisomer 1,1'-binaphthyl-2, 2'-dihydrogen phosphate was chosen to study the applicability of the polymeric surfactant over a wide range of parameters such as concentration, temperature, voltage, and pH. The most striking characteristic of this new surfactant is its high hydrophobicity. It is favorable to interactions with hydrophobic chiral analytes, and thus may provide better chiral recognition for the compounds.

Keywords: Chiral separation / Micellar electrokinetic chromatography / Poly(sodium oleyl-L-leucylvalinate)
DOI 10.1002/elps.200305521

1 Introduction

Capillary electrophoresis (CE) is a modern analytical technique which has been developed in recent years and has been applied to a wide range of application areas. Thousands of instruments are now in use for the analysis of pharmaceuticals, DNA, proteins, peptides, clinical and forensic samples, agrochemicals, fine chemicals, and natural products. Over the past decade, CE has also become a powerful technique for the separation of enantiomers. The enantioselectivity of chiral compounds is important to the environment and biological fields, as well as to synthetic chemists and the pharmaceutical industry. In CE, various types of chiral selectors have been employed for enantiomeric separations. For example, one type of chiral selector, cyclodextrin (natural and derivatized), has been successfully used for enantiomeric separations [1–4]. Other interesting chiral selectors that have been used

in other investigations include polysaccharides [5, 6], proteins [7, 8], macrocyclic antibiotics [9, 10], crown ethers [11, 12], calixarenes [13], and micelles [14–16].

In recent years, an emerging technique known as micellar electrokinetic chromatography (MEKC), has been employed for chiral separation of various analytes. The main advantage of MEKC as compared to other CE methods, is the ability to separate neutral and charged species in a single run thus, MEKC is a more cost efficient analytical technique. Terabe *et al.* [17, 18] first introduced this technique in 1984 while Cohen *et al.* [19] performed the first successful chiral separation using the MEKC method. In their studies, *N,N*-dodecyl-L-alanine and sodium dodecyl sulfate (SDS) along with a metal ion (Cu^{2+}) was used to form a mixed-micelle chiral ligand. There are many surfactants available for use in separation science but few are chiral [21]. The use of chiral surfactants for enantiomeric separations has recently shown much promise in MEKC [14, 21–32].

In our laboratory, an 11-carbon undecylinic acid chain has primarily been used for the synthesis of amino acid-based surfactants for chiral separation [21–32]. The first polymeric amino acid-based surfactant we synthesized for chiral separation in MEKC was reported in the literature in 1994 [22]. The surfactant synthesized was poly(sodium *N*-undecylenyl-L-valinate) (poly-L-SUV) and used in the separation of the optical isomers of ($\pm$)-1,1'-bi-2-naphthol and laudo-

Correspondence: Dr. Isiah M. Warner, Department of Chemistry, Louisiana State University, 432 Chopin Hall, Baton Rouge, LA 70803, USA

E-mail: iwarner@lsu.edu

Fax: +225-578-3971

Abbreviations: AUC, analytical ultracentrifuge; BNP, ($\pm$)-1,1'-bi-2-naphthyl-2,2'-diyl hydrogen phosphate; L-SOLV, sodium oleyl-L-leucylvalinate; poly-L-SOLV, poly(sodium oleyl-L-leucylvalinate); poly-L-SULV, poly(sodium *N*-undecylenyl-L-leucylvalinate)

nosine. Elsewhere, Hara and Dobashi [33] have also reported the use of polymeric chiral surfactants for enantiomeric separations using MEKC. In subsequent papers, the use of poly-L-SUV for the chiral separation of several other racemic compounds was investigated [24, 25, 33]. Other studies from our group focused on gaining a better mechanistic understanding of chiral interactions with polymeric dipeptide chiral surfactants [20, 26, 27].

Recently, we successfully used 18 monomeric and polymeric chiral surfactants for the MEKC separation of a variety of chiral analytes [31, 32]. The goal of this study was to gain deeper insight into factors governing the enantioselectivity of polymeric amino acid-based surfactants [31]. Among the many polymeric single and dipeptide amino acid surfactants synthesized in our laboratory, poly(sodium *N*-undecylenyl-L-leucylvalinate) (poly-L-SULV) has been found to provide a large number of enantiomeric separations for neutral, acidic, and basic compounds by variation of the background electrolyte (BGE), the pH, concentration, temperature, and voltage [34].

In this study, a novel chiral amino acid-based monomeric surfactant, sodium oleyl-L-leucylvalinate (L-SOLV) and polymeric surfactant, poly(sodium oleyl-L-leucylvalinate) (poly-L-SOLV) were synthesized and used for chiral separations in MEKC. Both the monomer and the polymer were characterized by various techniques including proton nuclear magnetic resonance (^1H NMR) and infrared (IR) spectroscopy for structure elucidation. Surface tensionmetry was used to obtain the CMC, and densitometry and AUC (analytical ultracentrifuge) were employed for determining the partial specific volume and molecular weight of the polymer, respectively. The structural difference between this new surfactant and others synthesized in our laboratory, is that its backbone acid chain contains 18 carbons with the double bond located at the 9,10-position as compared to the previously used 11-carbon chain with the double bond at the 1,2-position. The major advantage of this new surfactant is the low CMC of the monomer and high hydrophobicity of the polymer. Thus, the polymer may be a better chiral selector for some hydrophobic chiral analytes. This surfactant was used in MEKC for chiral separation of several chiral analytes. The determination of molecular weight, applications, and advantages of using poly-L-SOLV in MEKC are discussed in this manuscript.

2 Materials and methods

2.1 Materials

The analytes ($\pm$)-1,1'-bi-2-naphthyl-2,2'-diyl hydrogen phosphate (BNP), warfarin, coumachlor, benzoin, hydrobenzoin, benzoin methyl ether, and temazepam were pur-

chased from Sigma (St. Louis, MO, USA). All analytes were used as received. The structures of the analytes are provided in Fig. 1. The reagents used for synthesis of the surfactant were, oleic acid-*N*-hydroxysuccinimide ester, sodium hydrogen carbonate, and tetrahydrofuran purchased from Sigma while the dipeptide of leucine valine was purchased from BaChem Bioscience (King of Prussia, PA, USA) and used as received.

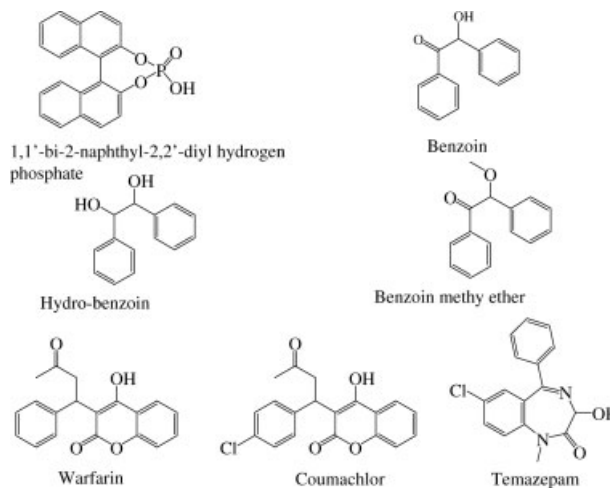


Figure 1. Structure of the chiral analytes separated.

2.2 Synthesis of poly-L-SOLV

The L-SOLV monomer was synthesized using the guidelines of the procedure previously reported by Wang and Warner [22] with some modifications. The synthetic scheme is shown in Fig. 2. The CMC of the L-SOLV surfactant monomer was determined to be 0.8×10^{-3} M using a surface tensionmeter from CSC Scientific Company (Fairfax, VA, USA). A 6×10^{-3} M aqueous solution of

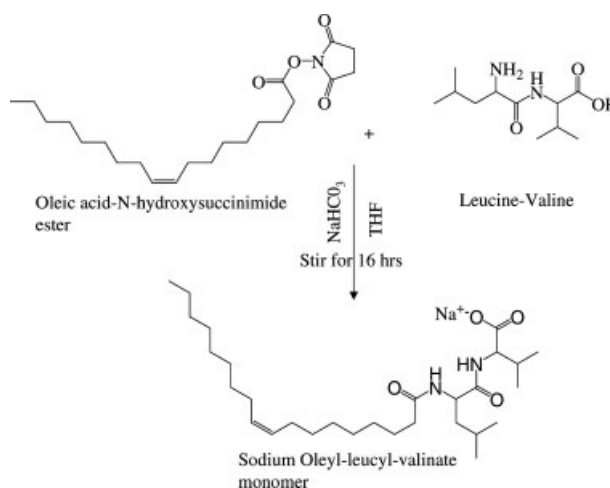


Figure 2. Synthesis scheme of sodium oleyl-L-leucylvalinate monomer.

L-SOLV was exposed to a ^{60}Co γ -ray source (70 krad/h) for a total of seven days for polymerization. After polymerization, the aqueous solution was dialyzed against bulk water using a regenerated cellulose membrane with a 2000 Da molecular weight cutoff. The purified solution was lyophilized under vacuum to obtain the dry product of poly-L-SOLV. ^1H NMR spectroscopy was used to follow the polymerization process.

2.3 Analytical ultracentrifugation

AUC measurements were performed by use of an Optima XLA analytical ultracentrifuge from Beckman Instruments (Palo Alto, CA, USA). The instrument has a high-intensity xenon flash light source and a grating monochromator that scans continuously from 190 to 800 nm. The detection system was set to measure the absorbance at 220 nm. A toroidally curved holographic diffraction grating was used to select the wavelength and to collimate the beam of light. Four sector cells were used in this experiment. Three of the cells have a sample and a solvent chamber. The fourth is a counterbalance cell. The polymer sample concentration was 0.1 g/L in 0.1 M NaCl. Sample volumes in each cell were 110 μL while the solvent volumes were 125 μL . All data were collected at a temperature of 25°C and at a speed of 22 000 rpm. The temperature of the rotor was controlled thermoelectrically to within $\pm 0.5^\circ\text{C}$. The absorbance *versus* the distance from the center of rotation to any position in the sample was collected at 720 min intervals. Successive scans of the cell were compared graphically using the XL-A software to ensure that the samples reached equilibrium. These data were acquired every 10 μm in replicates of 10 and were digitized and displayed as a function of radial distance. Only sedimentation measurements were completed.

2.4 Densitometry

A high-precision densitometer (model DMA58), purchased from Anton Paar USA (League City, TX, USA), was used to perform density measurements. Air and water were used for calibration. The precision of the temperature-controlled system was better than $\pm 0.005^\circ\text{C}$.

2.5 Capillary electrophoresis procedure

The MEKC experiments were performed by use of a Hewlett-Packard 3D CE instrument (Foster City, CA, USA). A bare fused-silica capillary (52 cm effective length, 50 μm ID) was purchased from Polymicro Technologies (Phoenix, AZ, USA). The surfactant was added to the buffer so-

lution and filtered through a 0.45 μm membrane filter. Separations were performed using a voltage of +30 kV, with UV detection at 220 nm. All analytes were prepared in a 50:50 methanol/water mixture with a concentration of 0.1 mg/mL. Samples were injected for 10 s with 10 mbar of pressure. Prior to use, each new capillary was conditioned for 60 min with 1 M NaOH and then for 30 min with 0.1 M NaOH at a temperature of 40°C. Finally, the capillary was rinsed for 15 min with triply distilled deionized water. Prior to each run, the capillary was flushed with the MEKC buffer for 3 min to condition and fill the capillary.

3 Results and discussion

3.1 Determination of the molecular weight of poly-L-SOLV

The exact volume of a substance is a difficult quantity to measure. Therefore, one often uses partial specific volume V (a.k.a $V\text{-bar}$), which is defined as the increase in volume when 1 g of dry solute is dissolved in a large volume of solvent. The V -values are determined by plotting the inverse of the density ($1/\rho$) of the solutions as a function of the weight fraction (W) of the polymers according to the following equation [29, 37, 38], *i.e.*,

$$1/\rho = V + W \frac{\partial(1/\rho)}{\partial W} \quad (1)$$

where ρ is the solution density and W is the solvent weight fraction. The partial specific volume of poly-L-SOLV is then obtained as the y -intercept of the $(1/\rho)$ vs. W plot (Fig. 3). This value was used in the analysis of the polymeric surfactant molecular weight data obtained from the AUC instrument. Analytical ultracentrifugation allows us to determine the molecular weight and sedimentation coefficient of our polymer [36, 37]. Yarabe *et al.* [29]

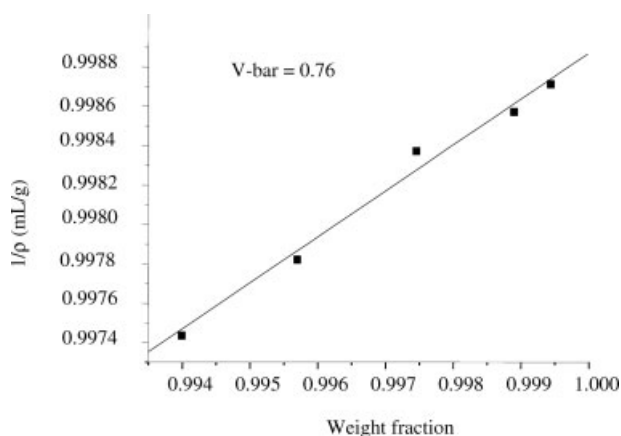


Figure 3. Determination of partial specific volume of poly-L-SOLV. Plot of $1/\rho$ (mL/g) as a function of weight fraction (weight solvent/(weight solvent + weight of solute)).

described the equations relating to the determination of the molecular weight using the AUC and the same equations were used for this study. From the analytical ultracentrifugation measurements, the weight-average molecular weight for the polymeric surfactant poly-L-SOLV, was calculated and found to be $36\,102 \pm 948$. Using this weight-average molecular weight, the aggregation number of the polymer poly-L-SOLV was determined to be 69 which correlates well with the polymer's aggregation number obtained by use of a Spex FluoroMax-3 spectrofluorimeter at room temperature using the fluorescence quenching method described by Billiot *et al.* [39] (data not shown). Figure 4 illustrates the equilibrium distribution of poly-L-SOLV at 25°C. The data points measured around the meniscus and cell bottom were truncated to give a more representative fit. The residuals at the top of the plot indicate how well the data points correlate with the fitting function.

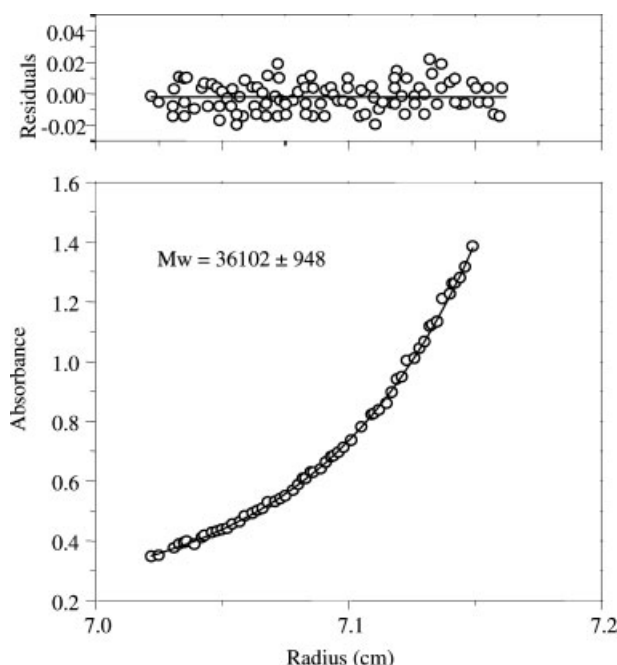


Figure 4. Determination of the molecular weight of poly-L-SOLV. Plot and residual of absorbance vs. radius of poly-L-SOLV in 0.1 M NaCl; wavelength, 220 nm; speed, 22 000 rpm; temperature, 25°C; V-bar, 0.76. All data were truncated to avoid the menisci and to improve the fits.

3.2 Separation of BNP

3.2.1 Effect of poly-L-SOLV concentration on the separation of BNP

Many molecules are chiral even without an asymmetric carbon center. A good example is the atropisomeric binaphthyl compounds such as BNP as shown in Fig. 1.

This compound belongs to a class of molecules that are chiral because they possess an adjacent π system that cannot adopt a coplanar configuration due to steric hindrances and rotational restrictions around a central bond. The ($\pm$)-BNP has been used as a chiral shift reagent [25, 38] for determining the enantiomeric purity of many organic compounds [25, 40, 41]. BNP has also been used as a ligand for dissymmetric catalyst [25, 40] and as building blocks in the synthesis of macrocyclic compounds [25, 42]. In this study, BNP exists in the anionic form due to the basic buffer conditions (pH 10.0) used in our separations. Baseline separation of BNP enantiomers using 25 mM sodium borate buffer and various concentrations of the polymeric pseudostationary phase poly-L-SOLV was achieved (Fig. 5A). A similar study was also completed using the monomer L-SOLV (Fig. 5B). These results demonstrate the chiral recognition ability of the polymeric surfactant as compared to the monomer micelles. The (*R*)-(+)-BNP enantiomer eluted faster than the corresponding (*S*)-form. This suggests that the (*S*)-

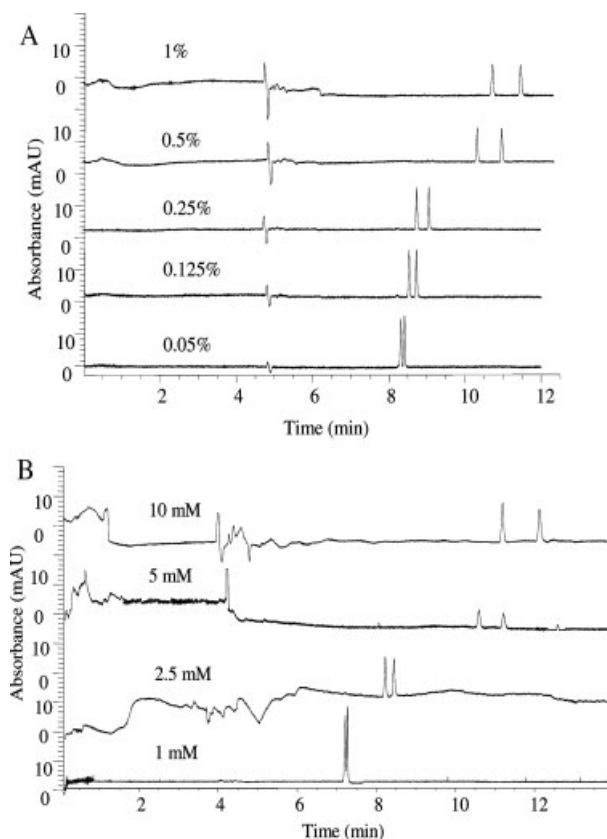


Figure 5. Effect of (A) poly-L-SOLV and (B) SOLV monomer concentration on separation of BNP. Conditions: electrolyte, 25 mM sodium borate; pH 10; temperature, 12°C; voltage, 30 kV; capillary, 52 cm effective length $\times$ 50 μ m ID; pressure injection at 10 mbar for 10 s; UV detection, $\lambda = 220$ nm. Concentrations: (A) 1, 0.5, 0.25, 0.125, and 0.05%; (B) 10, 5, 2.5, and 1 mM.

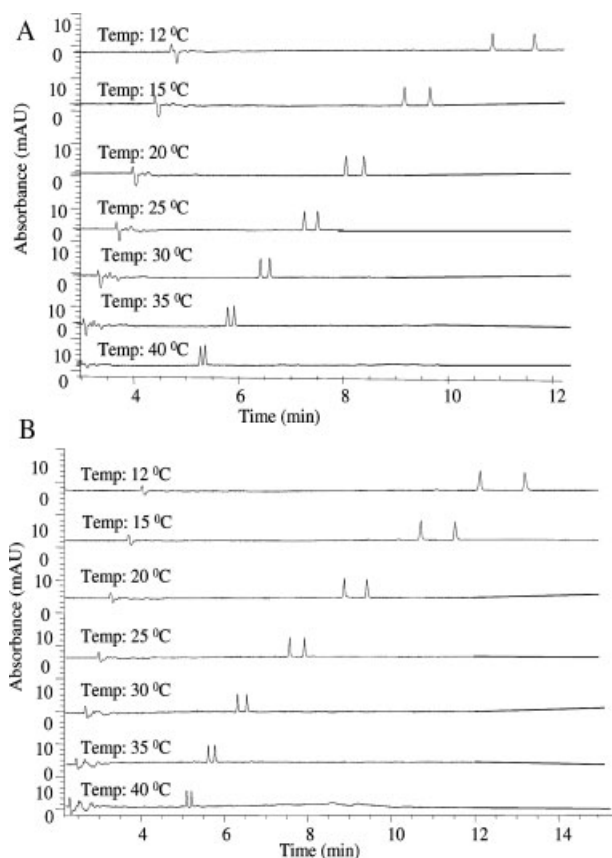


Figure 6. Effect of temperature on separation of BNP using (A) poly-L-SOLV and (B) L-SOLV. Conditions: (A) 0.5% polymer, and (B) 10 mM monomer; other conditions same as Fig. 5, except the temperature was varied.

(–)-BNP has a higher affinity for the chiral polymer. The separation completed using the monomeric form of the surfactant was not as efficient when compared to the polymer for various reasons. First, there was a considerable amount of noise in the baseline and the resolution was far lower as compared to the studies with the polymer at the same concentration (10 mM). Peak broadening was also observed when using the monomeric surfactant regardless of the concentration. Furthermore, when the concentration of the monomer was doubled, no separation was observed. The elution order was the same for both the monomer and the polymer. BNP was chosen to investigate the applicability of the poly-L-SOLV surfactant because previous studies in our laboratory have shown that it is difficult to separate using the 11 carbon amino-based monomeric and polymeric surfactants synthesized in our laboratory [32]. The optimum concentration was determined to be 0.5% (w/v) for the polymer and 10 mM for the monomer and was used to evaluate the effect of temperature and pH on the separation of BNP.

3.2.2 Effect of temperature on separation of BNP

The effect of temperature on the separation of the BNP enantiomers was investigated to determine the thermal stability of both the polymer and the monomer in MEKC. The polymer was found to be more stable and a better chiral selector even at higher temperatures. Better resolution values were noted when using the polymer, while more noise was observed in the baseline when using the monomer. Also, better peak efficiency was observed while using the polymer and it increased with an increase in temperature. Increase in temperature led to shorter retention times as observed in both Figs. 6A and B. This was likely due to an enhancement of the electroosmotic flow. Slightly longer retention times were observed when the monomer was used. The optimum temperature was determined to be 25°C.

3.2.3 Effect of pH on separation of BNP

The pH plays a very important role in the separation of chiral compounds. For the separation of BNP, the pH was varied between 8.5 and 10.0. A decrease in the buffer pH led to an increase in the electroosmotic flow and a shorter retention time for the analyte (Figs. 7A and B). The resolution was reduced as the pH was decreased in

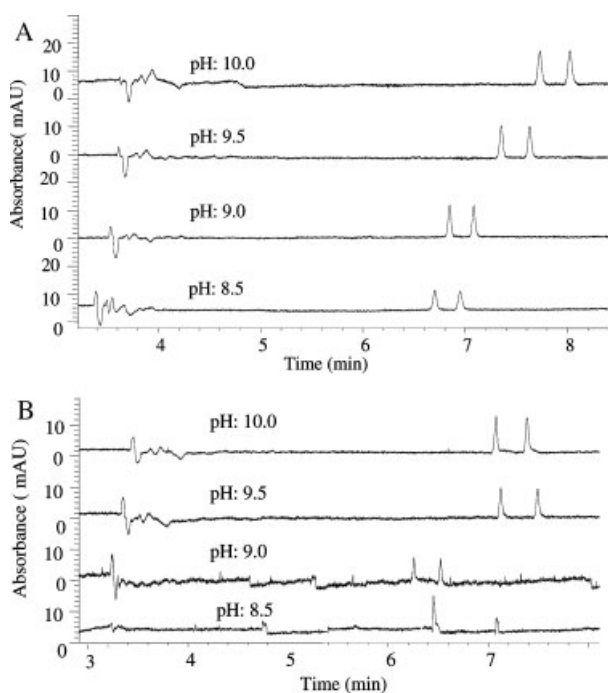


Figure 7. Effect of pH on separation of BNP using (A) poly-L-SOLV and (B) L-SOLV. Conditions same as Figs. 5 and 6, except the pH was varied and the study was done at 25°C.

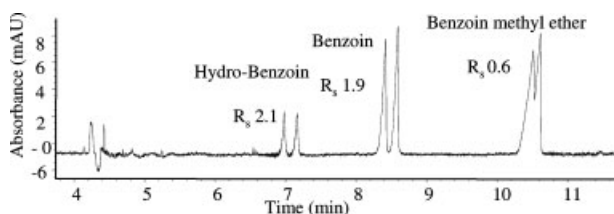


Figure 8. Enantiomeric separation of benzoin derivatives using 1% poly-L-SOLV. Conditions: electrolyte, 20 mM monobasic phosphate +30 mM dibasic phosphate; pH 7.2; temperature, 15°C; voltage, 30 kV; capillary, 52 cm effective length $\times$ 50 μ m ID; UV detection, λ = 254 nm; pressure injection at 10 mbar for 10 s.

both cases. However, there was no enantiomeric separation when the monomer was used in a buffer solution with a pH of 8.5.

3.3 Separation of neutral compounds

Benzoin and its derivatives have been used in the development of antiseptic, astringent, and anti-inflammatory drugs such as Tin-Ben. A mixture of three-benzoin derivatives benzoin, hydrobenzoin, and benzoin methyl ether were separated using the synthesized polymeric surfactant poly-L-SOLV. Baseline resolution was achieved for the separation of benzoin and hydrobenzoin, while only partial enantiomeric separation was observed for benzoin methyl ether as shown in Fig. 8. Other neutral compounds separated were 1,1'-bi-2-naphthol, and 1,1'-binaphthyl-2,2'-diamine with a resolution of 1.7 and 1.9, respectively (data not shown). The resolution (R_s) was calculated using the following equation [17]:

$$R_s = 2 \frac{(t_{r2} - t_{r1})}{w_1 + w_2} \quad (2)$$

where t_{r1} and t_{r2} are the respective migration times of each enantiomer, and w_1 and w_2 are the peak widths at the baseline of each enantiomer. Separations are still being conducted in our laboratory with a goal of expanding the number of analytes that can be separated from this class of compounds.

3.4 Separation of acidic compounds

Warfarin is a coumarin anticoagulant drug frequently used in the treatment of thrombeolic diseases [43]. Although it is sold as a racemic mixture, the (S)-(–) enantiomer is more pharmacologically active than its corresponding (R)-(+)-form [24, 43]. This drug displays a stereoselective metabolism and pharmacokinetics where each enantiomer follows different metabolic pathways [24, 44]. Coumachlor, an analog of warfarin, has been used in HPLC as an inter-

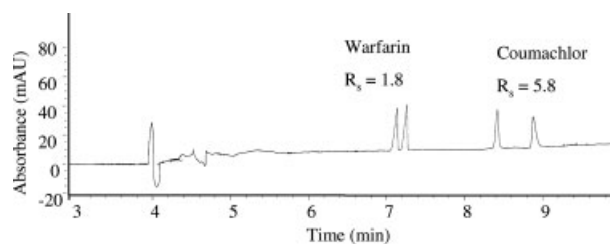


Figure 9. Enantiomeric separation of coumarin drugs, warfarin and coumachlor using 1% poly-L-SOLV. Conditions: electrolyte, 275 mM boric acid +20 mM monobasic phosphate, pH 7.2; other conditions as in Fig. 8, except UV detection, λ = 220 nm and pressure injection at 30 mbar for 3 s.

nal standard. Qualitative and quantitative experiments using both drugs have been documented by use of HPLC and GC [24, 45, 46]. These two drugs are structurally related acidic drugs. They are both electronegative due to their keto-enol groups. The phenolic group on warfarin has a pK_a value of 5.1 [24, 47]. Under neutral and basic pH conditions, both drugs are not expected to complex strongly to the anionic micelle. As noted by Agnew-Heard *et al.* [24], an acidic pH range was better for the separation of these compounds. However, the synthesized poly-L-SOLV tends to precipitate out of solution at a pH lower than 7 due to a decrease in the ionization of its carboxylate functionality. Using poly-L-SOLV in the separation of these two drugs was achieved under basic pH conditions (Fig. 9). When comparing this surfactant with others synthesized in our laboratory, the major advantage has been that lower concentrations of the polymer are required to achieve comparable or better resolution of chiral analytes. For example, for the separation warfarin and coumachlor approximately 2% (50 mM) of poly-L-SULV was needed to achieve a resolution of 1.51 and 3.12, respectively. In contrast, when 1% (20 mM) of poly-L-SOLV was used a resolution of 1.8 for warfarin and 5.8 for coumachlor were obtained. Shorter migration times (less than 10 min) were also recorded when poly-L-SOLV was used compared to (approximately 20 min) when poly-L-SULV was used for the separation of the two analytes under similar separating conditions [34]

3.5 Separation of basic compounds

Temazepam belongs to a class of compounds known as diazepam. These compounds are used as hypotonics, tranquilizers and anticonvulsants [48]. Although these compounds possess similar aromatic skeletons, the difference lies in the number and type of substituents attached to the aromatic ring. The presence of these substituents can make the separation of these compounds

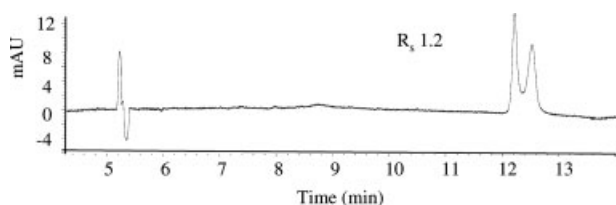


Figure 10. Enantiomeric separation of temazepam using 0.3% poly-L-SOLV. Conditions: electrolyte, 20 mM mono-basic phosphate +30 mM dibasic phosphate, pH 8.0; temperature, 12°C; voltage, 30 kV; capillary, 52 cm effective length +50 μ m ID; UV detection, λ = 220 nm; pressure injection at 10 mbar for 10 s.

difficult. Poly-L-SOLV resolves the enantiomers of temazepam with a resolution of 1.2 and a selectivity of 1.06 as shown in Fig. 10. According to the studies done by Haddadian *et al.* [28], the methyl group of the temazepam may affect chiral selectivity in two ways, namely, blocking the hydrogen-binding site of temazepam or by increasing the steric hindrance.

4 Concluding remarks

Novel monomeric and polymeric surfactants were synthesized and used for chiral separation in MEKC. Both the monomer and the polymer were capable of chiral separation. However, the polymer provided better separation. In this investigation, the poly-L-SOLV surfactant was preferred over the monomer for the separation of neutral, acidic, and basic chiral compounds. When comparing the monomer of L-SOLV to poly-L-SOLV, several advantages are noted for polymeric surfactants over normal micelles in chiral separations. (i) The elimination of the dynamic equilibrium between the monomers and the micelles can enhance chiral recognition of the racemic mixture. Thus, a better resolution is expected. (ii) The lack of a CMC in polymeric surfactants makes them more practical for use in MEKC separations. (iii) The rigidity of the polymeric surfactant improves the mass transfer rate and thus reduces peak broadening.

I. M. W. acknowledges the Philip W. West Endowment, the National Science Foundation and the National Institutes of Health for their support.

Received January 28, 2003

5 References

- [1] Billot, E., Thibodeaux, S., Shamsi, S., Warner, I. M., *Anal. Chem.* 1999, 71, 4044–4049.
- [2] Rawjee, Y. Y., Williams, R. L., Vigh, G., *J. Chromatogr.* 1993, 652, 233–245.
- [3] Biggin, M. E., Williams, R. L., Vigh, G., *J. Chromatogr. A* 1995, 692, 319–325.
- [4] Wang, F., Khaledi, M. G., *Anal. Chem.* 1996, 68, 3460–3467.
- [5] D'Hurst, A., Verbeke, N., *J. Chromatogr.* 1992, 608, 275–287.
- [6] Soini, H., Stefansson, M., Riekkola, M. L., Novotny, M. V., *Anal. Chem.* 1994, 66, 3477–3484.
- [7] Barker, G. E., Russo, P., Harwick, R. A., *Anal. Chem.* 1992, 64, 3024–3028.
- [8] Bucsh, S., Kraak, J. C., Poppe, H., *J. Chromatogr.* 1993, 635, 119–126.
- [9] Gasper, M. P., Berthod, A., Nair, U. B., Armstrong, D. W., *Anal. Chem.* 1996, 66, 2501–2514.
- [10] Rundlett, K. L., Armstrong, D. W., *Anal. Chem.* 1995, 67, 2088–2095.
- [11] Kuhn, R., Erni, F., Bereuter, P., Hausler, J., *Anal. Chem.* 1992, 64, 2815–2820.
- [12] Kuhn, R., Riester, D., Fleckenstein, B., Wiesmüller, K. H., *J. Chromatogr. A* 1995, 716, 371–379.
- [13] Sanchez Pena, M., Zhang, Y., Thibodeaux, S., McLaughlin, M. L., de la Pena, A. M., Warner, I. M., *Tetrahedron Lett.* 1996, 37, 5841–5844.
- [14] Shamsi, S., Warner, I. M., *Electrophoresis* 1997, 18, 853–872.
- [15] Nishi, H., Terabe, S., *J. Chromatogr. A* 1996, 735, 3–27.
- [16] Smith, J. T., Nashabeh, W., El Rassi, Z., *Anal. Chem.* 1994, 66, 1119–1133.
- [17] Terabe, S., Ostuka, K., Ichikawa, K., Ando, T., *Anal. Chem.* 1984, 56, 111–113.
- [18] Terabe, S., Ostuka, K., Ando, T., *Anal. Chem.* 1985, 57, 834–841.
- [19] Cohen, S. A., Paulus, A., Karger, B. L., *Chromatographia* 1997, 24, 15–24.
- [20] Dobashi, A., Hamada, M., Dobashi, Y., *Anal. Chem.* 1995, 67, 3011–3017.
- [21] Williams, C. C., Shamsi, S., Warner, I. M., *Adv. Chromatogr.* 1997, 37, 363–419.
- [22] Wang, J., Warner, I. M., *Anal. Chem.* 1994, 66, 3773–3776.
- [23] Wang, J., Warner, I. M., *J. Chromatogr. A* 1995, 711, 297–304.
- [24] Agnew-Heard, K. A., Sanchez Pena, M., Shamsi, S., Warner, I. M., *Anal. Chem.* 1997, 69, 958–964.
- [25] Shamsi, S. A., Mocossay, J., Warner, I. M., *Anal. Chem.* 1997, 69, 2980–2987.
- [26] Billot, E., Mocossay, J., Thibodeaux, S., Shamsi, S., Warner, I. M., *Anal. Chem.* 1998, 70, 1375–1381.
- [27] Billot, E., Agbaria, R. A., Thibodeaux, S., Shamsi, S., Warner, I. M., *Anal. Chem.* 1999, 71, 1252–1256.
- [28] Haddadian, F., Billot, E., Shamsi, A. S., Warner, I. M., *J. Chromatogr. A* 1999, 858, 219–227.
- [29] Yarabe, H. H., Shamsi, S., Warner, I. M., *Anal. Chem.* 1999, 71, 3992–3999.
- [30] Billot, E., Thibodeaux, S., Shamsi, S., Warner, I. M., *Anal. Chem.* 1999, 71, 4044–4049.
- [31] Billot, E., Warner, I. M., *Anal. Chem.* 2000, 72, 1740–1748.
- [32] Haddadian, F., Billot, E., Warner, I. M., *J. Chromatogr. A* 2001, 922, 329–338.
- [33] Hara, S., Dobashi, A., *Jpn. Patent 04 149 205*, 1993, *Chem. Abstr.* 1993, 118, p39405z.
- [34] Shamsi, S. A., Valle, B. C., Billot, F., Warner, I. M., *Anal. Chem.* 2003, 75, 379–387.

- [35] Durchschlag, H., in: Hinz, H. J. (Ed.) *Thermodynamic Data for Biochemistry and Biotechnology*, Springer-Verlag, New York 1986.
- [36] Fujita, H., in: Elving, P. J., Winefordner, J. D. (Eds.), *Foundations of Ultracentrifugal Analysis*, Wiley and Sons, New York 1975.
- [37] Correia, J. J., Shire, S., Yphantis, D. A., Schuster, T. M., *Biochemistry* 1985, 24, 3292–3297.
- [38] Jacques, J., Fouquey, C., *Tetrahedron Lett.* 1971, 48, 4617–4620.
- [39] Billiot, F. H., McCarroll, M., Billiot, E. J., Rugutt, J. K., Morris, K., Warner, I. M., *Langmuir* 2002, 18, 2993–2997.
- [40] Arnold, W., Daly, J. J., Imhof, R., Kyburz, E., *Tetrahedron Lett.* 1983, 24, 343–346.
- [41] Shao, L., Miyata, S., Muramastu, H., Kaqano, H., Ishii, Y., Saburi, M., Uchida, Y., *J. Chem. Soc., Perkin Trans.* 1990, 1, 1441–1445.
- [42] Castro, P. P., Georgiadis, T. M., Diederich, F., *J. Org. Chem.* 1989, 54, 58835–5839.
- [43] D'Hulst, A., Verbeke, N., *Chirality* 1994, 6, 225–229.
- [44] Lewis, R. J., Tyrager, W. F., Chan, K. K., Breckenridge, A., Orme, M., Roland, M., Schary, W., *J. Clin. Invest.* 1974, 53, 1607–1617.
- [45] Armstrong, D. W., Tang, Y., Ward, T., Nichols, M., *Anal. Chem.* 1993, 65, 1114–1117.
- [46] DeVries, J. X., Schmitz-Kummer, E., *J. Chromatogr. A* 1995, 644, 315–320.
- [47] Hiskey, C. F., Bulloch, E., Whitman, C., *J. Pharm. Sci. A* 1962, 51, 43–46.
- [48] Crossley, R., *Chirality and the Biological Activity of Drugs*, CRC Press, New York 1995.