Anal. Chem. **2006,** *78,* 7061−7069

Synthesis, Characterization, and Application of Chiral Ionic Liquids and Their Polymers in Micellar Electrokinetic Chromatography

Syed Asad Ali Rizvi and Shahab A. Shamsi*

Department of Chemistry, Center of Biotechnology and Drug Design, Georgia State University, Atlanta, Georgia 30303

Two amino acid-derived (leucinol and *N*-methylpyrrolidinol) chiral ionic liquids are synthesized and characterized in both monomeric and polymeric forms. Leucinol-based chiral cationic surfactant is a room-temperature ionic liquid, and pyrrolidinol-based chiral cationic surfactant melts at 30−35 °C to form an ionic liquid (IL). The monomeric and polymeric ILs are thoroughly characterized to determine critical micelle concentration, aggregation number, polarity, optical rotation, and partial specific volume. Herein, we present the first enantioseparation using chiral IL as a pseudostationary phase in capillary electrophoresis. Chiral separation of two acidic analytes, (±)-α-bromophenylacetic acid and (±)-2-(2-chlorophenoxy)propanoic acid (±)-(2-PPA) can be achieved with both monomers and polymers of undecenoxycarbonyl-L-pryrrolidinol bromide (L-UCPB) and undecenoxycarbonyl-L-leucinol bromide (L-UCLB) at 25 mM surfactant concentration using phosphate buffer at pH 7.50. The chiral recognition seems to be facilitated by the extent of interaction of the acidic analytes with the cationic headgroup of chiral selectors. Polysodium *N*-undecenoxycarbonyl-L-leucine sulfate (poly-L-SUCLS) and polysodium *N*-undecenoxycarbonyl-L-leucinate (poly-L-SUCL) were compared at high and low pH for the enantioseparation of (±)-(2-PPA). At pH 7.5, poly-L-SUCLS, poly-L-SUCL, and (±)-(2-PPA) are negatively charged resulting in no enantioseparation. However, chiral separation was observed for (±)-(2-PPA) using poly-L-SUCLS at low pH (pH 2.00) at which the analyte is neutral. The comparison of chiral separation of anionic and cationic surfactants demonstrates that the electrostatic interaction between the acidic analyte and cationic micelle plays a profound role in enantioseparation.

The separation of chiral compounds is currently the center of great interest.[1] This interest can be attributed largely to a heightened awareness that enantiomers of a racemic drug usually display markedly different pharmacological activities.[2,3] The human body metabolizes individual enantiomers by separate pathways to produce different pharmacological effects. Presently, a majority of commercially available drugs are synthetic and chiral. Most of these chiral drugs are obtained as a mixture of two enantiomers during synthesis.[4] To avoid the possible undesirable effects of enantiomeric impurity in chiral drug, it is inevitable that only a therapeutically active form be marketed. Hence, there is a continuous need to develop technologies that have the ability to separate enantiomers.

Very recently, ionic liquids (ILs) have found great applications in efficient and environmentally benign chemical processing and chemical analysis.[5,6] By definition, the ILs are organic salts with melting points (mp) below 100 °C or more often even lower than room temperature.[7−11] These compounds possess the dual capability of dissolving both polar and nonpolar species, and the most useful feature is that they do not evaporate even at high temperatures.[12−15] Most commonly, ILs are based on nitrogen-rich, alkyl-substituted heterocyclic cations, with a variety of anions (e.g., 1-ethyl-3-methylimidazolium tetrafluoroborate). Although, the reasons for low melting points of ILs are not clear, it is stated that ILs consist of bulky inorganic anions with delocalized charged organic cations, which prevents the formation of a stable crystal lattice or random molecular packing resulting in lower melting points.[16] Due to these remarkable characteristics, ILs have been used as medium for liquid−liquid extractions,[17−19] mobile-phase

* Corresponding author. Phone: 404-651-1297. Fax: 404-651-2751. E-mail: chesas@langate.gsu.edu.

(1) Bladon, C. *Pharmaceutical Chemistry: Therapeutic Aspects of Biomacromolecules*, 5th ed.; John Wiley & Sons: New York, 2002.
(2) Coleman, M. *Human Drug Metabolism: An Introduction*; John Wiley & Sons: Chichester, England, 2005.
(3) Williams, D. A.; Lamke, T. L.; Foye, W. O. *Foye's Principles of Medicinal Chemistry*; Lippincott Williams & Wilkins: Philadelphia, 2002.
(4) Silverman, R. B. *The Organic Chemistry of Drug Design and Drug Action*; Academic Press: San Diego, 2004.
(5) Blanchard, L. A.; Hancu, D.; Beckman, E. J.; Brennecke, J. F. *Nature* **1999**, *399*, 28.
(6) Stalcup, A. M.; Cabovska, B. *J. Liq. Chromatogr Relat. Technol.* **2004**, *27*, 7−9.
(7) Wasserscheid, P.; Keim, W. *Angew. Chem., Int. Ed.* **2000**, *39*, 3772−3789.
(8) Sheldon, R. *Chem. Commun.* **2001**, 2399−2407.
(9) Dupont, J.; de Souza, R. F.; Saurez, P. A. Z. *Chem. Rev.* **2002**, *102*, 3667−3692.
(10) Wasserscheid, P.; Welton, T. *Ionic Liquids in Synthesis*; Wiley-VCH: Weinheim, Germany, 2003.
(11) Anderson, J. L.; Armstrong, D. W.; Wei, G. T. *Anal. Chem.* **2006**, *78*, 2893−2902.
(12) Brennecke, J. F.; Maginn, E. J. *AIChE J.* **2001**, *47* (11), 2384−2389.
(13) Chauvin, Y.; Olivier-Bourbigou, H. *Chemtech* **1995**, *25*, 26−30.
(14) Earle, N. J.; Seddon, K. R. *Pure Appl. Chem.* **2000**, *72*, 1391−1398.
(15) Welton, T. *Chem. Rev.* **1999**, *99*, 2071−2083.
(16) Del Po'polo, M. G.; Voth, G. A. *J. Phys. Chem. B* **2004**, *108,* 1744−1752.
(17) Carda-Broch, S.; Berthod, A.; Armstrong, D. W. *Anal. Bioanal. Chem.* **2003**, *375*, 191−199.
(18) Holbrey, J. H.; Seddon, K. R. *J. Chem. Soc., Dalton Trans.* **1999**, 2133-2139.

10.1021/ac060878u CCC: $33.50
Published on Web 09/07/2006

additives in high-performance liquid chromatography (HPLC),[20,21] electrolytes in capillary electrophoresis (CE),[22−26] matrixes for matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS),[27,28] stationary phases for gas chromatography[29−32] and as modifiers in micellar electrokinetic chromatography (MEKC).[33,34] However, there is no report in the literature about the use of ILs as chiral selector in CE.

Cationic surfactants are referred to as compounds containing at least one long hydrophobic chain attached to a positively charged nitrogen. These quaternary ammonium group-containing surfactants are well known for displaying emulsifying properties, antimicrobial activity, components in cosmetic formulations, anticorrosive effects, and phase-transfer catalyst and as a chiral induction medium (if chiral cationic surfactant) in organic reactions.[35−41] As with the case of chiral anionic surfactants, amino acid-based (both monomeric and polymeric) and ephedrine-based (monomeric) chiral cationic surfactants have been used as chiral selectors in MEKC.[42,43] However, unlike chiral anionic polymeric surfactants, chiral cationic polymeric surfactants have not found great application so far, and only one report of chiral cationic polymeric surfactants as pseudostationary phase in MEKC is reported.[42]

In this study, we report the synthesis, characterization, and application of novel IL-type surfactants and their polymers for chiral separation of acidic analytes in MEKC. Acidic analytes due to inherent negative charge poorly interact with most commonly employed chiral anionic surfactants at basic pH. As a result, still a large number of acidic analytes could not be resolved by MEKC. The cationic surfactant undecenoxycarbonyl-L-leucinol bromide (L-UCLB) is an ionic liquid at room temperature, while undecenoxycarbonyl-L-pyrrolidinol bromide (L-UCPB) is a greasy solid that melts to form an ionic liquid at 30−35 °C. In our case, quaternized nitrogen (chiral headgroup) is surrounded by a hydrophobic tail and leucinol or pyrrolidinol side chain, which presumably prevent the proper packing of the cations and anions in regular three-dimensional patterns to form ionic liquids.

The current report is the first demonstration of MEKC chiral separation of several anionic compounds such as phenoxypropionic acid herbicide, (±)-(2-PPA), and a very useful synthetic intermediate ±-α-bromophenylacetic acid, (±)-(α-BP-AA),[44,45] using two synthetic chiral ionic liquids, L-UCLB and L-UCPB, as well their polymers. Chiral separation of acidic analyte is compared using polymeric anionic surfactants containing similar headgroups under both acidic and basic pH conditions.

MATERIALS AND METHODS

Standards and Chemicals. The analytes (±)-(α-BP-AA) and (±)-(2-PPA) were obtained as a racemic mixture from Sigma Chemical Co. (St. Louis, MO) and Aldrich (Milwaukee, WI), respectively. Chemicals used for the synthesis of surfactants included *ω*-undecylenyl alcohol, triphosgene, pyridine, dichloromethane, 2-bromoethylamine hydrobromide, L-leucinol, *N*-methylpryrrolidinol, 96% formic acid, 37% formaldehyde, and 2-propanol (HPLC grade), were also obtained from Aldrich, and were used as received.

Synthesis and Characterization of Monomeric Surfactants and Micelle Polymers. Choloroformate has been synthesized as reported earlier[46] by reacting triphosgene with unsaturated alcohol (step 1, Figure 1). The carbamate-functionalized alkenyl bromide (step 2, Figure 1) was synthesized by dropwise addition of (10 mmol) choloroformate over an equimolar aqueous solution of 2-bromoethylamine hydrobromide and Na_2CO_3 and the resultant mixture was stirred for 2 h. The resulting solution was extracted twice with dichloromethane, which then was washed three times with H_2O, dried over Na_2SO_4, and concentrated by evaporating solvent to yield product 1 (89−93%). The *N,N*-dimethylleucinol (product 2, step B, Figure 1) was synthesized by reductive alkylation of primary amine of leucinol using the well-known Eschweiler−Clark reaction (yield 55−70%).[47−49] The chiral ionic liquids were synthesized by refluxing the carbamate-functionalized alkenyl bromide (product 1) with *N,N*-dimethylleucinol or *N*-methylpryrrolidinol for 48 h in 2-propanol (IPA). After 48 h, the reaction mixture was concentrated by evaporating IPA, and the resulting fluid was dissolved in water and extracted with ethyl acetate. The aqueous solution of ionic liquids (products 3 and 4, Figure 2) was lyophilized (yield 40−55%) at −50 °C collector temperature and 0.05 mbar pressure for 14 days (to ensure

(19) Carmichael, A. J.; Earle, M. J.; Holbrey, J. D.; McCormac, P. B.; Seddon, K. R. *Org. Lett.* **1999**, *1*, 997−1000.
(20) Poole, C. F.; Kersten, B. R.; Ho, S. S. J.; Coddens, M. E.; Furton, K. J. *J. Chromatogr.* **1986**, *352*, 407−425.
(21) Poole, S. K.; Shetty, P. H.; Poole, C. F. *Anal. Chim. Acta* **1989**, *218*, 241−264.
(22) Huang, X.; Luckey, J. A.; Gordon, M. J.; Zare, R. N. *Anal. Chem.* **1989**, *61*, 766−770.
(23) Harrold, M. P.; Wojtusik, M. J.; Riviello, J.; Henson, P. *J. Chromatrogr.* **1993**, *640*, 463−471.
(24) Quang, C.; Khaledi, M. G. *Anal. Chem.* **1993**, *65*, 3354−3358.
(25) Yanes, E. G.: Gratz, S. R.: Stalcup, A. M. *Analyst* **2000**, *125*, 1919−1923.
(26) Yanes, E. G.; Gratz, S. R.; Baldwin, M. J.; Robinson, S. E.; Stalcup, A. M. *Anal. Chem.* **2001**, *73*, 3838−3844.
(27) Armstrong, D. W.; Zhang, L.; He, L.; Gross, M. L. *Anal. Chem.* **2001**, *73*, 3679−3686.
(28) Carda-Broch, S.; Berthod, A.; Armstrong, D. W. *Rapid Commun. Mass Spectrom.* **2003**, *17*, 553−560.
(29) Armstrong, D. W.; He, L.; Liu, Y. S. *Anal. Chem.* **1999**, *71*, 3873−3876.
(30) Berthod, A.; He, L.; Armstrong, D. W. *Chromatographia* **2001**, *53*, 63−68.
(31) Anderson, J. L.; Armstrong, D. W. *Anal. Chem.* **2003**, *75*, 4851−4858.
(32) Ding, J.; Welton, T.; Armstrong, D. W. *Anal. Chem.* **2004**, *76*, 6819−6822.
(33) Mwonngela, S. M.; Numan, A.; Gill, N.; Agbaria, R. A.; Warner, I. M. *Anal. Chem.* 2003, *75*, 6089−6096.
(34) Laamanen, P. L.; Lahtinen, S. B. M.; Matilainen, R. *J. Chromatogr., A* **2005**, *1095*, 164−171.
(35) Cross, J., Singer, E. J., Eds. *Cationic Surfactants*; Marcel Dekker Inc.: New York, 1994.
(36) Dwars, T.; Paetzold, E.; Oehme, G. *Angew. Chem., Int. Ed.* **2005**, *44*, 7174−7199.
(37) Goldberg, S. I.; Baba, N.; Green, R. L. *J. Am. Chem. Soc.* **1978**, *100*, 6768−6769.
(38) Borocci, S.; Ceccacci, F.; Galantini, L.; Mancini, G.; Monti, D.; Scipioni, A.; Venanzi, M. *Chirality.* **2003**, *15*, 441−447.
(39) Davidson, T. A.; Mondal, K.; Yang, X. *J. Colloid Interface Sci.* **2004**, *276*, 468−502.
(40) Diego-Castro, M. J.; Hailes, H. C.; Lawrence, M. J. *J. Colloid Interface Sci.* **2001**, *234*, 122−126.
(41) Diego-Castro, M. J.; Hailes, H. C. *J. Chem. Soc., Chem. Commun.* **1998,** *15*, 1549−1550.
(42) Dobashi, A.; Hamada, M.; Yamaguchi, J. *Electrophoresis* **2001**, *22*, 88−96.
(43) Dey, J.; Mohanty, A.; Roy, S.; Khatua, D. *J. Chromatogr., A* **2004**, *1048*, 127−132.
(44) Hamdouchi, C.; Martinez, C. S.; Gruber, J.; Prado, M. D.; Lopez, J.; Rubio, A.; Heinz, B. A. *J. Med. Chem.* **2003**, *46* (20), 4333−4341.
(45) Procopio, A.; Alcaro, S.; Cundari, S.; De Nino, A.; Ortuso, F.; Sacchetta, P.; Pennelli, A.; Sindona, G. *J. Med. Chem.* **2005**, *48* (19), 6084−6089.
(46) Rizvi, S. A. A.; Shamsi, S. A. *Electrophoresis* **2003**, *24*, 2514−2526.
(47) Eschweiler, W. *Ber.* Dtsch. Chem. Ges. **1905**, *38*, 880.
(48) Clarke, H. T.; Gillespie, H. B.; Weisshaus, S. Z. *J. Am. Chem. Soc.* **1933**, *55*, 4571−4587.
(49) Farkas, E.; Sunman, C. J. *J. Org. Chem.* **1985**, *50*, 1110−1112.

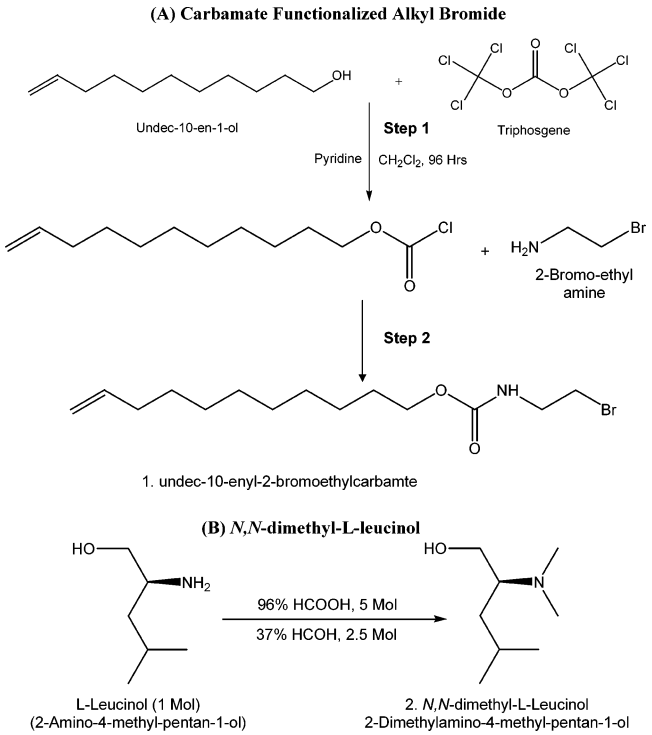

Figure 1. Synthesis of the carbamate-functionalized (A) alkyl bromide and (B) *N,N*-dimethyl-L-leucinol.

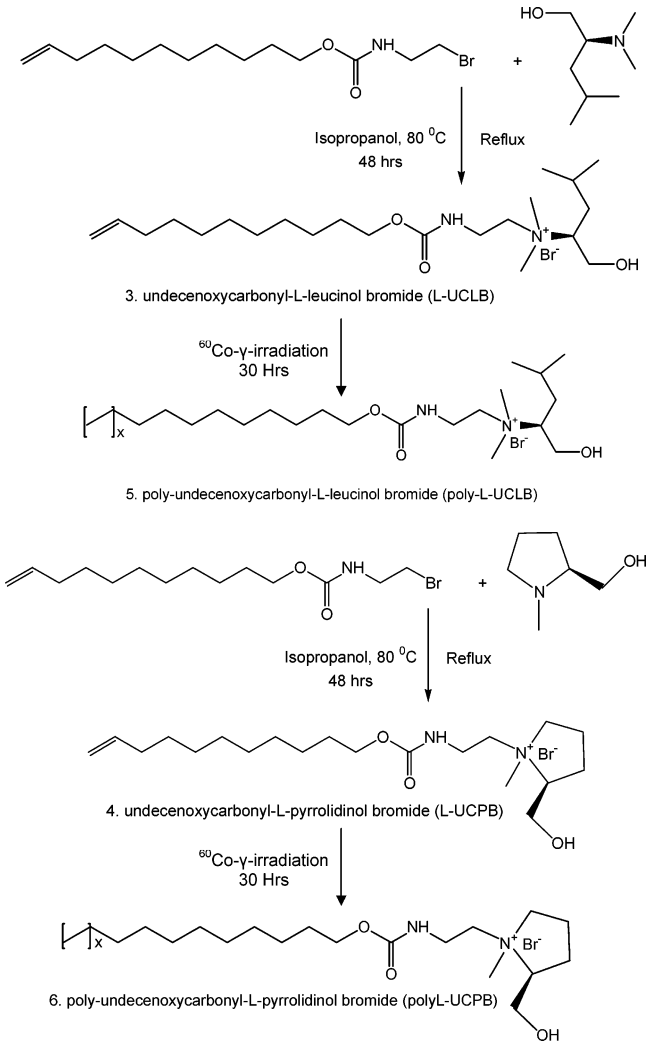

Figure 2. Synthesis and polymerization of leucinol- and pryrrolidinol-derived ionic liquid and their polymers.

complete removal of water from both products). ^{1}H NMR spectra of L-UCPB, L-UCLB and their polymers were recorded on a Varian Unity+ 300-MHz spectrometer using D_2O as the solvent. The surfactants were characterized and checked for purity by MALDI-TOF MS (Figure 3, ^{1}H NMR, and elemental analysis. **L-UCPB.** ^{1}H NMR: δ 0.759−0.893 (b, 6H), 1.170 (m, 12H), 1.471 (m, 2H), 1.767 (m, 2H), 1.883 (b, 1H), 2.085 (m, 2H), 3.06 (b, 2H), 3.239−3.613 (b, 8H), 3.777−3.844 (m, 1H), 4.052 (d, J = 14.7, 2H), 4.379 (b, 2H), 4.789 (m, 2H), 5.626 (m, 1H). Anal. Calcd for $C_{20}H_{39}N_2O_3Br + 2H_2O$: C, 50.95; H, 9.19; N, 5.94; Found: C, 51.56; H, 10.07; N, 5.88. **L-UCLB.** ^{1}H NMR: δ 1.170 (b, 12H), 1.468 (b, 2H), 1.766−1.992 (b, 4H), 1.992−2.164 (b, 2H), 3.032 (b, 2H), 3.147 (b, 2H), 3.472 (b, 3H), 3.506−3.619 (b, 3H), 3.830 (b, 2H), 4.376 (b, 2H), 4.804 (m, 2H), 5.658 (m, 1H). Anal. Calcd for $C_{22}H_{45}N_2O_3Br + 2H_2O$: C, 52.68; H, 9.85; N, 5.59; Found: C, 52.14; H, 9.00; N, 6.87.

The critical micelle concentration (cmc) was determined using a Sigma 7^{03} digital tensiometer (KVS Instruments USA, Monroe, CT), by the Du NoÜy ring method at room temperature. Complete olymerization of the synthesized ionic liquids was achieved by continuous ^{60}Co γ-irradiation (8 Mrad/h) of a 100 mM aqueous solution for 30 h. The ^{1}H NMR indicated the disappearance of double bond protons signal in the region of 4.8−5.0 and 5.7−5.9 ppm. After irradiation, the polymeric surfactant solutions were filtered and dialyzed against triply deionized water using a regenerated cellulose dialysis membrane (Spectrum Laboratories, Inc., Rancho Dominguez, CA) with a 1000-Da molecular mass cutoff for 24 h. Finally, the dialyzed solutions were lyophilized to obtain the dried polymeric surfactants. Further characterization, such as aggregation number and polarity of the amphiphilic ionic liquids (monomers and polymers), was determined by using the pyrene emission vibronic fine structure method.[46] The partial specific volume of both monomer and polymer was determined using previously reported procedure.[46] The optical rotation of monomeric and the polymeric surfactants was obtained by an Autopol III automatic polarimeter (Rudolph Research Analytical, Flanders, NJ) by measuring the optical rotation at 589 nm of a 10 mg/mL solution of each monomer and polymer in triply deionized water at 25 °C.

MEKC Instrumentation. All experiments were performed using an Agilent CE system (Agilent Technologies, Palo Alto, CA) equipped with 0−30-kV high-voltage power supply, a diode array detector for UV detection, and Chemstation software (V 9.0) for system control and data acquisition. The fused-silica capillary was obtained from Polymicro Technologies (Phoenix, AZ). The total length of the capillary used with an Agilent CE system was 64.5 cm (56.0 cm from inlet to detector, 50-μm i.d., 350-μm o.d.), prepared by burning ~3-mm polyimide coating to create a detection window.

Capillary Electrophoresis Procedures and Calculations. The capillaries for all MEKC experiments were prepared by flushing with 1 M NH_4OH for 60 min at 50 °C followed by a 30-min rinse with triply deionized water at 20 °C. Between each injection, the capillary was flushed with 0.1 M NH_4OH and H_2O for 3 min each. Separations began after a 2-min rinse with the running buffer, followed by a 5-min flush with the running buffer

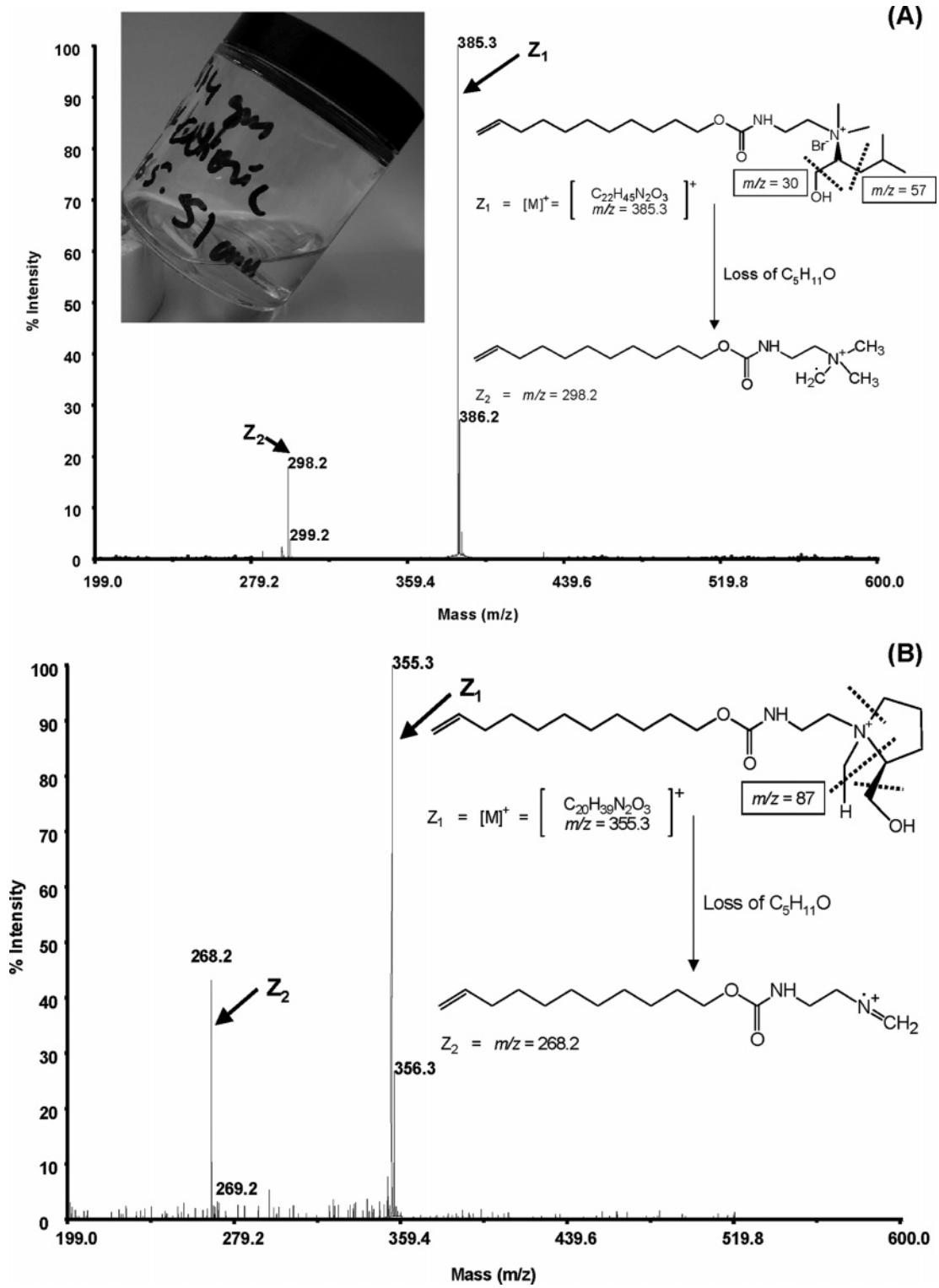

Figure 3. MALDI-TOF mass spectra (positive mode) with proposed cleavages and the corresponding fragment masses for IL-type surfactants (A) undecenoxycarbonyl-L-leucinol bromide (L-UCLB) and (B) undecenoxycarbonyl-L- pyrrolidinol bromide (L-UCPB) after freeze-drying on a lyophilizer at −50 °C collector temperature and 0.05 mbar pressure for 14 days. The mass spectra were obtained using α-cyano-4-hydroxycinnamic acid as MALDI matrix.

containing ionic liquids. All separations were performed at −20 kV and at 20 °C. All surfactants (both monomers and polymers) were run with a new capillary (cut to the same length from the same capillary bundle) and were preconditioned using the identical flushing procedure as mentioned above. Chiral resolution (R_s) of acidic analytes (±)-(2-PPA) and (±)-(α-BP-AA) were calculated by Chemstation software using the peak width at half-height method:

$$R_s = \frac{(2.35/2)(t_{r_2} - t_{r_1})}{W_{50(1)} + W_{50(2)}}$$

$W_{50(1)}$ and $W_{50(2)}$ are the widths at 50% height for peaks 1 and 2, respectively. The selectivity (α) is defined as t_2/t_1, where t_1 and t_2 are the migration times of the first- and second-eluting enantiomers. Methanol was used as the t_0 marker and was measured from the time of injection to the first deviation from the baseline. Dodecanophenone was used as tracer for t_{mc} at 100 mM surfactant concentration of each monomer and polymer. The effective electrophoretic mobility of the monomers and polymers of ionic liquids was calculated by the following equation:

$$\mu_{ep} = -\mu_{app} - (-\mu_{eof})$$

where μ_{ep}, μ_{eof}, and μ_{app} are effective electrophoretic mobility, electroosmotic mobility, and apparent electrophoretic mobility, respectively. The negative sign of μ_{app} and μ_{eof} is due to the fact that monomeric and polymeric ionic liquids coat the capillary wall and result in anodic electroosmotic flow; therefore, negative voltage (−20 kV) has to be applied for separation.

Preparation of MEKC Buffers and Analyte Solutions. For all MEKC experiments, the final background electrolyte (BGE) consisted of 25 mM each of Na_2HPO_4/NaH_2PO_4 buffered at pH 7.5. The desired pH value was obtained by using 1 M NaOH. The pH of BGE was adjusted before the addition of ILs (monomers and polymers). This BGE solution is finally filtered through a 0.45-μm Nalgene syringe filter (Rochester, NY). The running MEKC buffer solution was prepared by addition of 25 mM IL-type surfactants to the BGE, followed by ultrasonication for about 25−30 min. The analytes prepared in 50/50 (v/v) of MeOH/H_2O at various concentrations were injected at a pressure of 50 mbar for 1−5 s. The dodecanophenone was prepared in 100% MeOH at 3 mg/mL (stock solution), diluted to 1.8 mg/mL in 60:40 MeOH/H_2O, and injected at a pressure of 50 mbar for 10 s.

RESULTS AND DISCUSSION

Physicochemical Properties. Table 1 represents the physicochemical properties of the synthesized enantiomerically pure chiral surfactants L-UCLB (room-temperature ionic liquid) and L-UCPB (IL) (mp 30−35 °C) as well as their polymers, poly-L-UCLB and poly-L-UCPB. The L-UCLB exhibited higher polarity, lower cmc, partial specific volume ($\bar{V}$), significantly higher optical rotation, but similar aggregation number (A) compared to L-UCPB. A similar trend was also observed for the poly-L-UCLB and poly-L-UCPB, except that the A value was higher for the former polymer. Comparing physicochemical properties of monomeric and polymeric cationic surfactants, it can be noticed that A is always lower for the polymers than monomers, while polarity and $\bar{V}$ is always higher for polymeric surfactants.

Figure 3 shows the MALDI-TOF MS of both L-UCLB (A) and L-UCPB (B) in positive mode. Both L-UCLB and L-UCPB surfactants showed the molecular ion peak (base peak) at mass-to-charge ratios (m/z) of 385.3 and 355.3, respectively along with a fragment generated by the loss $C_5H_{11}O$. For L-UCLB, the masses at 386.2 and 299.2 m/z, and for L-UCPB, the masses at 356.3 and 268.2 m/z, are generated due to the ^{13}C isotope related to the molecular ion and the generated fragment ion, respectively. The generation of the cationic fragments (Z_2) for both ionic liquids as shown in Figure 3 is in accord with the previous observations that most of the fragments generated from cationic surfactants bear preformed cations (contain quaternary nitrogen of the cationic surfactants).[50,51]

Table 1. Physicochemical Properties of the Monomers and Polymers of Chiral Amino Acid-Derived Cationic Surfactants L-UCLB and L-UCPB

characteristic of the IL-type monomeric surfactants	L-UCPB	L-UCLB
cmc[a] (mM)	1.15 ± (0.01)[g]	0.84 ± (0.05)
aggregation number[b]	95 ± (0.09)	97 ± 0.04
polarity (I_1/I_3) ratio[c]	1.095 ± (0.001)	1.180 ± 0.040
optical rotation[d]	−2.35 ± (0.02)	+21.67 ± 0.03
partial specific volume[e]	0.8281 ± (0.0036)	0.7185 ± 0.00
electroosmotic mobility	-2.83×10^{-4}	-2.42×10^{-4}
μ_{eof} ($cm^2 V^{-1} s^{-1}$)[f]	($\pm 1.56 \times 10^{-5}$)	($\pm 5.31 \times 10^{-6}$)
effective electrophoretic mobility	2.08×10^{-4}	1.94×10^{-4}
μ_{ep} ($cm^2 V^{-1} s^{-1}$)[f]	($\pm 1.54 \times 10^{-5}$)	($\pm 7.49 \times 10^{-7}$)
migration time window (t_{mc}/t_o)[f]	3.79 (± 0.20)	5.09 (± 0.38)

characteristic of the polymeric surfactants	poly-L-UCPB	poly-L-UCLB
aggregation number[b]	34 ± (0.780)	25 ± (0.034)
polarity (I_1/I_3) ratio[c]	1.219 ± (0.001)	1.22 ± (0.007)
optical rotation[d]	−7.84 ± (0.04)	+17.45 ± (0.64)
partial specific volume[e]	0.8408 ± (0.0075)	0.7634 ± (0.0008)
electroosmotic mobility	-2.54×10^{-4}	-2.34×10^{-4}
μ_{eof} ($cm^2 V^{-1} s^{-1}$)[f]	($\pm 3.67 \times 10^{-6}$)	($\pm 3.12 \times 10^{-6}$)
effective electrophoretic mobility	2.02×10^{-4}	1.91×10^{-4}
μ_{ep} ($cm^2 V^{-1} s^{-1}$)[f]	($\pm 2.96 \times 10^{-6}$)	($\pm 3.52 \times 10^{-6}$)*
migration time window (t_{mc}/t_o)[f]	4.87 (± 0.16)	5.38 (± 0.53)

[a] Critical micelle concentration is determined by the surface tension measurements. [b] Aggregation number is determined by the florescence quenching experiment using pyrene as a probe and cetylpyridinium chloride as a quencher. [c] Polarities of the surfactants are determined using ratio of the fluorescence intensity (I_1/I_3) of pyrene. [d] Optical rotations of 10 mg/mL monomer and micelle polymers were determined in triply deionized water snd were obtained at 589 nm (sodium D line). [e] Partial specific volumes were determined by the density measurements at different surfactant concentrations. [f] The μ_{ep} values for all monomer and polymeric ionic liquids were determined using methanol as t_o marker and dodecanophenone as t_{mc} tracer. Experimental conditions: 64.5 cm (56-cm effective length) × 50 μm i.d. capillary with an applied voltage of −20 kV at 25 °C using a running buffer of 25 mM each of NaH_2PO_4 and Na_2HPO_4, 100 mM monomer and polymeric ionic liquids; dodecanophenone introduction, 50 mbar for 10 s (1.5 mg/mL in 50:50 MeOH/H_2O). [g] Standard deviations are given in parentheses.

The electrophoretic parameters of monomeric and polymeric ionic liquids were also examined (Table 1) at 100 mM surfactant concentrations (at lower surfactant concentration, t_{mc} marker was not observed even after 3 h). The reversed electroosmotic flow ($-\mu_{eof}$) and effective electrophoretic (μ_{ep}) mobilities of both poly-L-UCPB and poly-L-UCLB were slightly lower, while the migration time window (t_{mc}/t_0) was greater compared to their respective monomers. In addition, the monomer and polymer of L-UCLB compared to the monomer and polymer of L-UCPB have lower μ_{ep} and provided larger t_{mc}/t_0.

Enantioseparation of Acidic Analytes. The optimization of chiral resolution of (±)-(α-BP-AA) and (±)-(2-PPA) was performed

(50) Morrow, A. P.; Kassim, O. O.; Ayorinde, F. O. *Rapid Commun. Mass Spectrom.* **2001**, *15*, 767−770.
(51) Tuiman, A. A.; Cook, K. D.; Magid, L. J. *J. Am. Soc. Mass Spectrom.* **1990**, *1*, 85−91.

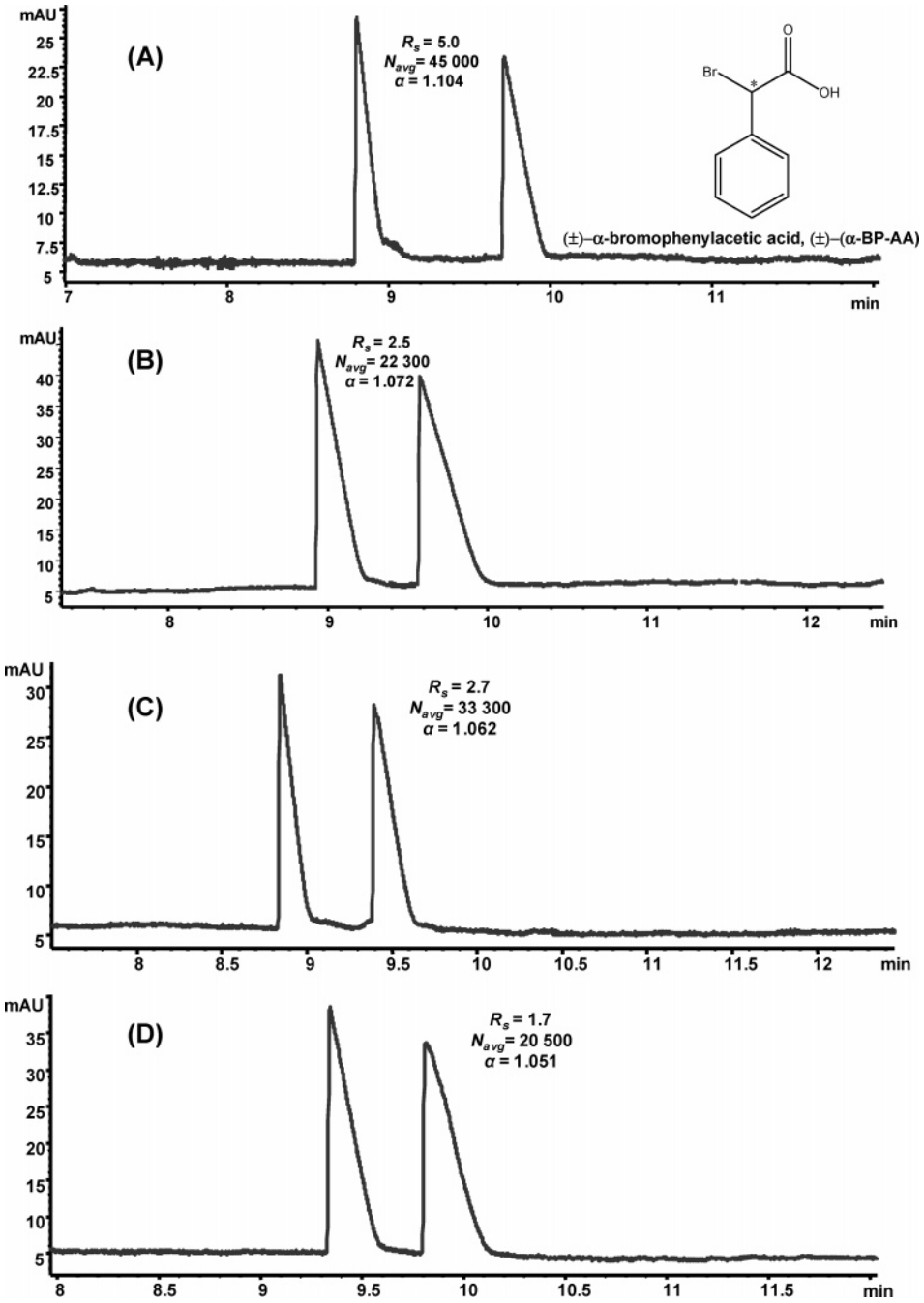

Figure 4. Comparison of 25 mM L-UCPB (A), poly-L-UCPB (B), 25 mM L-UCLB (C), and poly-L-UCLB (D) for enantioseparation of (±)-(α-BP-AA) (2.5 mg/mL in MeOH/H_2O). MEKC conditions: 50 mM NaH_2PO_4/Na_2HPO_4, pH 7.5, pressure injection 50 mbar 5 s, −20 kV, 20 °C, and UV detection at 214 nm.

by studying pH of the BGE, type and concentration of BGE, organic modifiers, and surfactant concentration. After optimizing the chiral MEKC conditions, chiral separation of (±)-(α-BP-AA) and (±)-(2-PPA) were compared using L-UCPB, L-UCLB, and their respective polymers (poly-L-UCPB and poly-L-UCLB) to get insight on the factors affecting analyte−micelle interactions and ultimately chiral separation.

Enantioseparation of (±)-α-Bromophenylacetic Acid. Panels A and C in Figure 4 show the chiral separation of (±)-(α-BP-AA) at optimum separation conditions with L-UCPB and L-UCLB, respectively. Since (±)-(α-BP-AA) has a dissociable carboxylic acid group with pK_a = 2.40 (±0.10), the effect of pH on enantioseparation was evaluated from pH 2.00 to 8.50 (data not shown). Although chiral resolution (R_s) at a lower pH range (4.00−6.00) do not differ drastically, maximum R_s was obtained at pH 7.5, but no R_s at pH 2.00, and at pH > 7.5, R_s deteriorates (data not shown). One plausible explanation of R_s deterioration at a pH of >7.5 could be the excess hydroxide ions (originated from the use of NaOH to adjust the BGE pH), which competes with the anionic chiral analyte for the positively charged ionic liquid at basic pH. The absence of any R_s at pH 2.00 and lower R_s at intermediate pH suggest that electrostatic interaction indeed contributes significantly to chiral recognition. It has been reported in the literature that, in the presence of certain organized media (e.g., micelles), the pK_a of the organic acid is altered up to more than 4 pH units.[43,52] Therefore, it is reasonable to believe that the amphiphilic

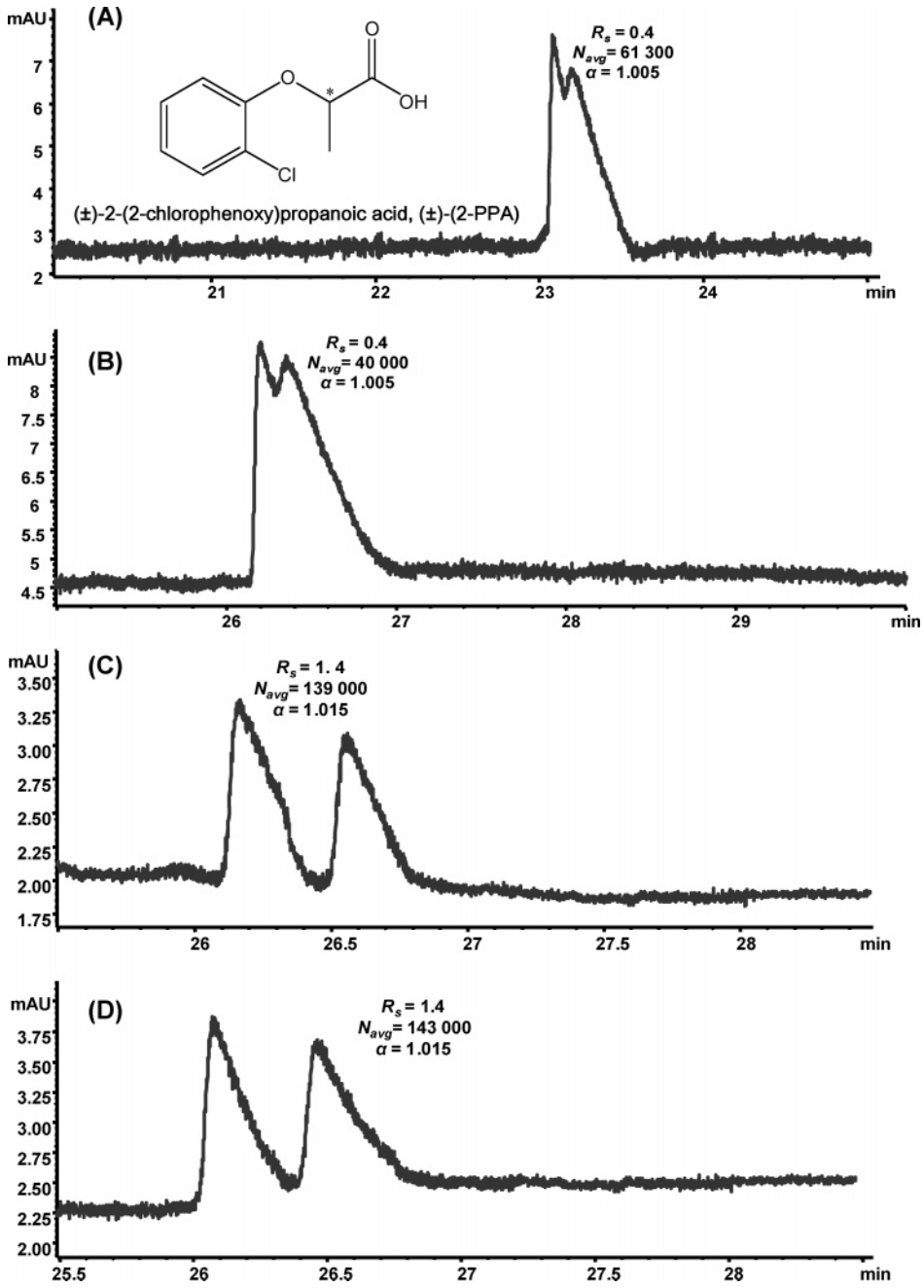

Figure 5. Comparison of 25 mM L-UCPB (A), poly-L-UCPB (B), 25 mM L-UCLB (C), and poly-L-UCLB (D) for enantioseparation of (±)-(2-PPA) (0.5 mg/mL in MeOH/H_2O). MEKC conditions are the same as in Figure 4.

ionic liquids might have increased the pK_a of (±)-(α-BP-AA) such that maximum ionization occurs around pH 7.50. Hence, greater electrostatic interaction with the positively charged ionic liquids provided maximum chiral R_s at pH 7.50. On the other hand, L-UCPB and L-UCLB concentrations, as well as the use of organic modifiers (e.g., methanol, acetonitrile) did not show any significant variations in R_s.

As depicted, L-UCPB provided almost twice as high chiral R_s for (±)-(α-BP-AA) compared to L-UCLB (Figure 4A vs C). One possible explanation for enhanced chiral resolution provided by L-UCPB over L-UCLB could be due to the rigid ring system of L-UCPB, which apparently allows maximum interaction via three-point interaction with (±)-α-Br-Ph-AA.[53] The R_s trend is consistent with the findings of Thiobodeaux et al.,[54] who observed that surfactants derived from L-proline (a rigid amino acid) provided better chiral separation for rigid chiral molecules (e.g., BNP). The analyte (±)-(α-BP-AA) has a chiral center, which is adjacent to a bromo group and a carboxylate group. Thus, it appears that the chiral recognition was greatly facilitated not only by electrostatic interactions between carboxylate group of the analyte and the positively charge nitrogen but the presence of a bromo group

(52) Bertschinger, A. T.; Perry, C. S.; Galland, A.; Prannkerd, R. J.; Charman, W. N. *J. Pharm. Sci.* **2003**, *92*, 2217−2228.

(53) Davankov, V. A. *Pure Appl. Chem.* **1997**, *69*, 1469−1474.

(54) Thibodeaux, S. J.; Billiot, E.; Warner, I. M. *J. Chromatogr., A* **2002**, *966*, 179−186.

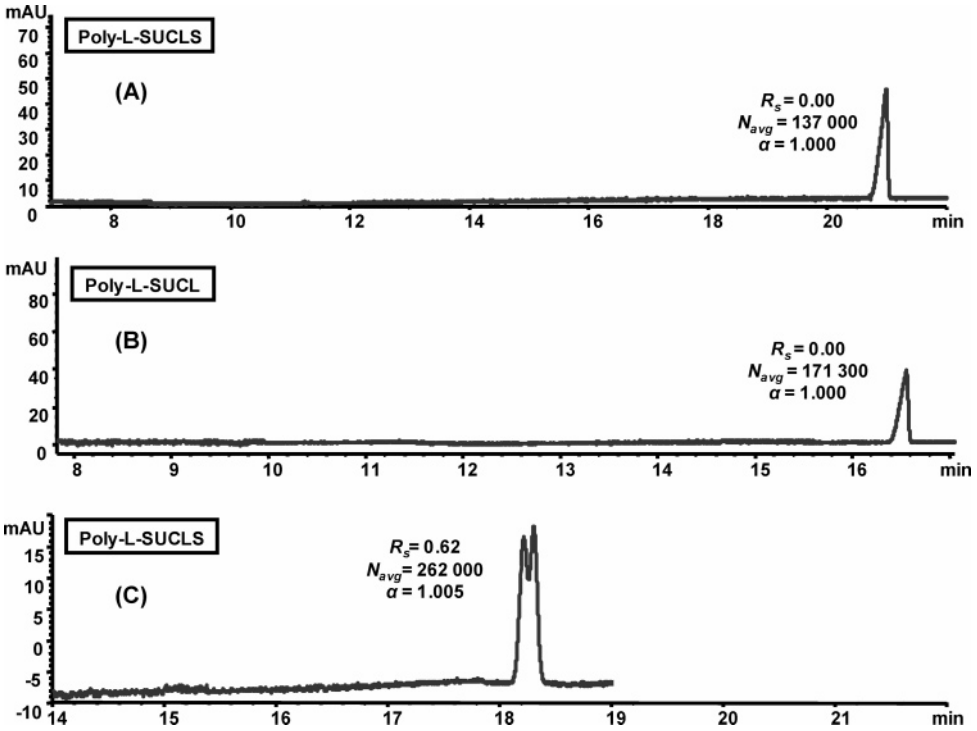

Figure 6. Comparison of 25 mM poly-L-SUCLS (A), 25 mM poly-L-SUCL (B), and 50 mM poly-L-SUCLS (C) for enantioseparation of chiral (±)-(2-PPA) (0.5 mg/mL in MeOH/H_2O). MEKC conditions (A, B): pH 8.00, 25 mM NH_4OAc/25 mM TEA, 15 °C, pressure injection 50 mbar s, +20 kV applied for separations, and UV detection at 200 nm. (C) MEKC conditions same as Figure 5A except pH 2.00, 25 mM NaH_2PO_4/25 mM CH_3COONa, and −20 kV applied for separations.

adjacent to the chiral center might also hydrogen bond with the OH group of the ionic liquids (Figure 2).

Comparing the electropherograms in Figure 4A versus (C) and (B) versus (D), it is obvious that monomers of both L-UCPB and L-UCLB provided better chiral resolution, selectivity, and efficiency compared to the corresponding polymers. The probable reason behind this observation could be the polydispersity of the polymers,[55] which usually is the case when surfactants are polymerized at a concentration higher than the cmc.[55,56]

Enantioseparation of (±)-2-(2-Chlorophenoxy)propanoic Acid. As discussed above, in the case of (±)-(α-BP-AA), maximum chiral R_s was obtained at pH 7.50 and no R_s at pH 2.00. O'Keeffe et al.[57] and Haynes et al.[58] have reported the separation of (±)-(2-PPA) at pH 5.00−6.00 with a cationic substituted β-cyclodextrin. Similar to the case of (±)-(α-BP-AA) separation, the variation in surfactant concentration and addition of organic modifier showed no significant effects on chiral R_s of (±)-(2-PPA).

Panels A and C in Figure 5 show the chiral separation of (±)-(2-PPA) at optimum MEKC parameters using L-UCPB and L-UCLB, respectively. The nonrigid leucine-based (L-L-UCLB) chiral selector (Figure 5C) provided significantly higher chiral R_s of (±)-(2-PPA) than L-UCPB. This resolution trend is opposite to the separation of (±)-(α-BP-AA) (Figure 4A, C). As stated, the proximity of the bromo and carboxylate group to the chiral center of (±)-(α-BP-AA) as well as the rigidity of the chiral selector was thought to be the key factors ensuring maximum enantioselectivity. However, in case of (±)-(2-PPA), the chloro group on the benzene ring is farther away from the chiral center. Furthermore, the nonrigidity of L-UCLB might have resulted in favorable hydrogen-bonding interactions between the chloro group on the benzene ring and the primary alcohol of the L-leucinol. Comparing Figure 5 panels (A) versus (B) and (C) versus (D), it is clear that monomers and polymers of L-UCPB and L-UCLB show very similar stereoselective interactions with (±)-(2-PPA) as evident from the R_s and α values.

It is interesting to compare the enantioseparation capability between two polymeric chiral anionic surfactants [polysodium *N*-undecenoxycarbonyl-L-leucine sulfate (poly-L-SUCLS) and polysodium *N*-undecenoxycarbonyl-L-leucinate (poly-L-SUCL)] with the chiral cationic surfactants discussed earlier for racemic anionic analyte. The chiral separation of (±)-(2-PPA) with both sulfated and carboxylated headgroup polymeric surfactants was investigated at basic pH (Figure 6A and B). As we have mentioned earlier, anionic compounds are usually difficult to separate with anionic surfactant due to the electrostatic repulsion between similar charges. Hence, as expected, no chiral resolution was obtained for (±)-(2-PPA) at pH 8.00. Since poly-L-SUCLS has a sulfated headgroup, it can be used at any pH without any solubility problem. Therefore, we performed MEKC at pH 2.00 (Figure 6C) in order to minimize dissociation of the carboxylic acid group of (±)-(2-PPA) (pK_a 3.11 ± 0.10). As can be seen in Figure 6C, partial chiral separation of (±)-(2-PPA) was achieved at pH 2.00. However, we could not improve this chiral R_s any further even after fine-tuning of the MEKC parameters (data not shown). Hence, comparing the chiral separation of (±)-(2-PPA) with poly-L-UCLB (Figure 5D), poly-L-SUCLS (Figure 6A, C), and poly-L-SUCL

(55) Tarus, J.; Agbaria, R. A.; Morris, K.; Mwongela, S.; Numan, A.; Simuli, L.; Fletcher, K. A.; Warner, I. M. *Langmuir* **2004**, *20*, 6887−6895.
(56) Mileva, E. *J. Colloid Interface Sci.* **2000**, *232*, 211−218.
(57) O'Keeffe, F.; Shamsi, S. A.; Darcy, R.; Schwinte, P.; Warner, I. M. *Anal. Chem.* **1997**, *69*, 4773−4782.
(58) Hanes, J. L., III; Shamsi, S. A.; O'Keeffe, F.; Darcy, R.; Warner, I. M, III *J. Chromatogr., A* **1998**, *803*, 261−271.

(Figure 6B), it is clear that indeed electrostatic attraction interaction plays a dominant role in chiral recognition. In addition, structural features (e.g., rigidity and charges) of both analyte and chiral selector also seem to affect the chiral recognition.

CONCLUSIONS

This paper is the first demonstration of successful chiral separation of acidic analytes with synthetic chiral ILs in CE. Both L-UCLB and L-UCPB ionic liquid-type surfactants were thoroughly characterized before and after the polymerization. It was found that chiral separation of the acidic analytes with the chiral ILs and their polymers is strongly dependent on the presence of opposite charge as well as the structural compatibility between chiral selector and the analyte. Even though we did not demonstrate the enantioseparation of a large number of acidic analytes, we still believe that our findings will guide the future research in MEKC separation of acidic analytes with intelligently designed synthetic chiral ionic liquids.

ACKNOWLEDGMENT

This work was supported by a grant from the National Institutes of Health (Grant GM 62314).

Received for review May 12, 2006. Accepted August 16, 2006.

AC060878U