Simultaneous Enantioseparation and Tandem UV–MS Detection of Eight β-Blockers in Micellar Electrokinetic Chromatography Using a Chiral Molecular Micelle

ARTICLE *in* ANALYTICAL CHEMISTRY · APRIL 2005

Impact Factor: 5.64 · DOI: 10.1021/ac0401422 · Source: PubMed

3 AUTHORS:

Cevdet Akbay
Fayetteville State University
31 PUBLICATIONS **641** CITATIONS
SEE PROFILE

Syed A. A. Rizvi
Nova Southeastern University
48 PUBLICATIONS **860** CITATIONS
SEE PROFILE

Shahab A Shamsi
Georgia State University
130 PUBLICATIONS **3,468** CITATIONS
SEE PROFILE

Anal. Chem. **2005,** *77,* 1672−1683

Simultaneous Enantioseparation and Tandem UV−MS Detection of Eight β-Blockers in Micellar Electrokinetic Chromatography Using a Chiral Molecular Micelle

Cevdet Akbay,[†] Syed A. A. Rizvi,[‡] and Shahab A. Shamsi*[,‡]

Department of Chemistry, Center of Biotechnology and Drug Design, P.O. Box 4098, Georgia State University, Atlanta, Georgia 30302-4098, and Department of Natural Sciences, Fayetteville State University, Fayetteville, North Carolina 28301

The feasibility of using a new and more versatile polymeric chiral surfactant, i.e., poly(sodium *N*-undecenoxy carbonyl-L-leucinate (poly-L-SUCL) is investigated for simultaneous enantioseparation and detection of eight structurally similar β-blockers with tandem UV and MS detection. Three optimization approaches, i.e., direct infusion-MS, capillary zone electrophoresis-MS, and chiral micellar electrokinetic chromatography−mass spectrometry (CMEKC−MS), were investigated to optimize sheath liquid parameters, spray chamber parameters, and CMEKC separation parameters for maximum sensitivity and chiral resolution. Compared to unpolymerized micelle of L-SUCL, the use of micelle polymer (i.e., poly-L-SUCL) provided significantly higher separation efficiency, lower separation current, and higher detection sensitivity for CMEKC−ESI-MS of β-blockers. It was also observed that, unlike monomeric L-SUCL, polymeric L-SUCL provided enantioseparation of all β-blockers even at the lowest surfactant concentration (i.e., 5 mM poly-L-SUCL). Under optimum CMEKC and ESI-MS conditions (15 mM poly-L-SUCL, 25 mM each of NH_4OAc and TEA (pH 8.0); 80% (v/v) methanol sheath liquid containing 40 mM NH_4OAc (pH 8.0); sheath liquid flow rate, 5.0 μL/min; drying gas flow rate, 5 L/min; drying gas temperature, 200 °C; nebulizing pressure, 6 psi (0.414 bar); capillary voltage, +2.5 kV; fragmentor voltage, 85 V), baseline enantioseparation of eight β-blockers was achieved by tandem UV (in ∼30 min) and MS (in ∼60 min) detection. Calibration curves for all β-blockers were linear in the range of 0.01−0.6 mM for both CMEKC−UV and CMEKC−MS methods, but the later method provided better concentration limit of detection with similar RSD for migration time and peak areas. The CMEKC−ESI-MS method appears suitable for use as a routine procedure for high-throughput separation of β-blockers with high sensitivity.

Surfactant forming micelles are widely used in micellar electrokinetic chromatography (MEKC) as pseudostationary phases for separation of neutral as well as ionic solutes. Although conventional micelles are now routinely used in MEKC with ultraviolet (UV) detection, the coupling of MEKC with mass spectrometry (MS) using conventional micelles is desired. This is because detection in MEKC−UV remains a primary challenge because of very low sample size (picomoles or lower) injected in the small-diameter capillaries. In addition, the tiny flow cell (i.e., the use of small-diameter capillary) limits the detection sensitivity of even UV-absorbing analytes. Being accepted as more universal than conventional detection modes (e.g., UV absorbance, laser-induced fluorescence, or electrochemical detection), MS detection for CE has been actively utilized in recent years. In fact, the use of MS detection not only provides molecular mass and structural information but also single ion monitoring (SIM) adds a new dimension in separation selectivity for coeluting solutes of different molecular masses. Furthermore, variations in the analysis times, a problem frequently encountered in CE, can be compromised by the selectivity and specificity of MS detection. In general, MS improves detection limits as compared to UV detection and simplifies the data interpretation.[1]

Combining chiral separations with electrospray ionization (ESI)-MS detection is not simple because low-volatility chiral selectors (e.g., cyclodextrins,[2] crown ethers[3]) are typically not compatible with MS instrumentation. In addition, the use of low molecular weight surfactant monomers in MEKC makes mass spectrometric detection difficult due to the fact that large background signal is generated from the dissociation of unpolymerized micelle into surfactant monomers that interfere with most solutes under study in the low molecular mass region (Figure 1A). In general, accumulation of nonvolatile surfactants or chiral selectors can cause fouling of the ion source, and may limit the sensitivity in ESI-MS.[4−7] Although some researchers have coupled

* To whom correspondence should be addressed. Phone: (404) 651-1297. Fax: (404) 651-1416. E-mail: chesas@langate.gsu.edu.
[†] Fayetteville State University.
[‡] Georgia State University.

(1) Barrachina, E. B.; Moyano, E.; Galceran, M. T. *Electrophoresis* **2004**, *25*, 1927−1948.
(2) Cherkaoui, S.; Rudaz, S.; Varesio, E.; Veuthey, J.-L. *Electrophoresis* **2001**, *22*, 3308−3315.
(3) Tanaka, Y.; Otsuka, K.; Terabe, S. *J. Chromatogr., A* **2000**, *875*, 323−330.
(4) Shamsi, S. A. *Anal. Chem.* **2001**, *73*, 5103−5108.
(5) Varghese, J.; Cole, R. B. *J. Chromatogr., A* **1993**, *652*, 369−376.

10.1021/ac0401422 CCC: $30.25
Published on Web 02/09/2005

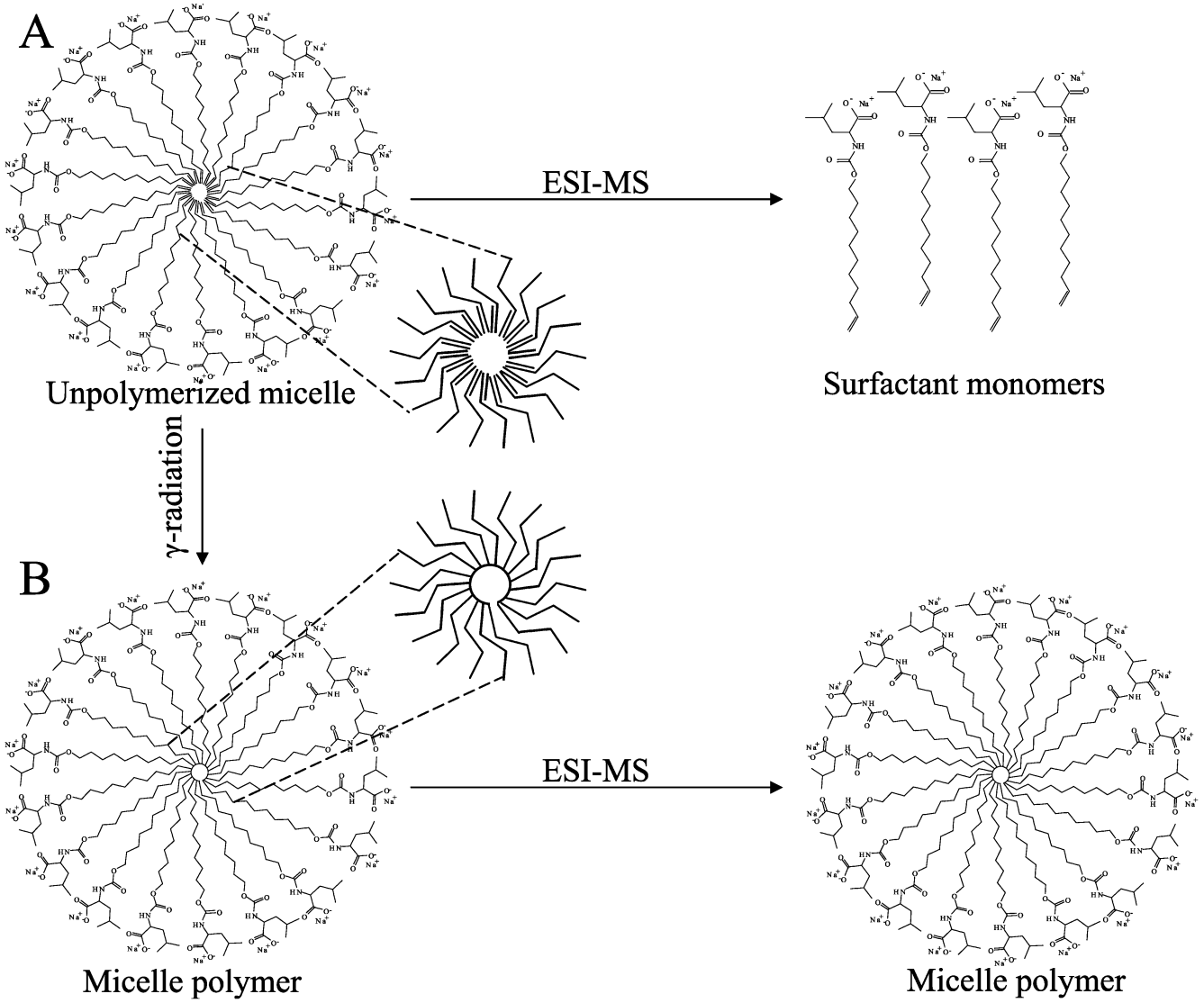

Figure 1. Comparison of (A) low molecular mass monomeric (unpolymerized) micelles and (B) high molecular mass micelle polymer introduced to ESI-MS.

chiral electrokinetic chromatography using partial filling technique with MS compatible volatile background electrolyte (BGE), both chiral resolution (Rs) and sensitivity are significantly compromised.[2,3] To overcome these drawbacks, molecular micelles (also called polymeric surfactants, micelle polymers,[4−8] or high molecular mass surfactants[9]) have been introduced as alternative pseudostationary phases to conventional micelles in MEKC−MS.

Molecular micelles provide several advantages over conventional micelles in MEKC−MS applications. First, they have zero cmc;[10,11] thus, they may be used at concentrations well below the cmc of the unpolymerized surfactants.[11−13] Second, molecular micelles are stable in the presence of a high content of organic solvents due to the covalent bond between surfactant monomers. Hence, organic additives do not disrupt the primary covalent structure of the micelle polymer.[11] One should keep in mind that most biological samples typically comprise polar compounds that may also contain hydrophobic moieties. Thus, the use of organic solvents in combination with micelles is often required for the analysis of such compounds. In addition, the fixed micellar structure prevents dissociation of surfactant molecules during the electrospray process[4] (Figure 1B) in MS.[11] Third, due to their high molecular weight, molecular micelles can be conveniently used in MEKC−MS applications without background interference from surfactant monomers of low molecular weights. Fourth, lower surface activity and low volatility of molecular micelles provide a stable electrospray and hence less suppression of analyte signal in MEKC−MS.[7]

In recent years, there has been increased interest in the development of novel polymeric chiral surfactants for separation of chiral molecules using CMEKC with UV detection.[14−21] However, there is only one report on the use of polymeric chiral

(6) Lu, W.; Poon, G. K.; Carmichael, P. L.; Cole, R. B. *Anal. Chem.* **1996**, *68*, 668−674.
(7) Rundlett, K. L.; Armstrong, D. W. *Anal. Chem.* **1996**, *68*, 3493−3497.
(8) Lu, W.; Shamsi, S. A.; McCarley, T. D.; Warner, I. M. *Electrophoresis* **1998**, *19*, 2193-2199.
(9) Ozaki, H.; Itou, N.; Terabe, S.; Takada, Y.; Sakairi, M.; Koizumi, H. *J. Chromatogr., A* **1995**, *716*, 69−79.
(10) Palmer, C. P.; Terabe, S. *J. Microcolumn Sep.* **1996**, *8*, 115−121.
(11) Shamsi, S. A.; Akbay, C.; Warner. I. M. *Anal. Chem.* **1998**, *70*, 3078−3083.
(12) Akbay, C.; Shamsi, S. A. *Electrophoresis* **2004**, *25*, 622−634.
(13) Akbay, C.; Shamsi, S. A. *Electrophoresis* **2004**, *25*, 635−644.

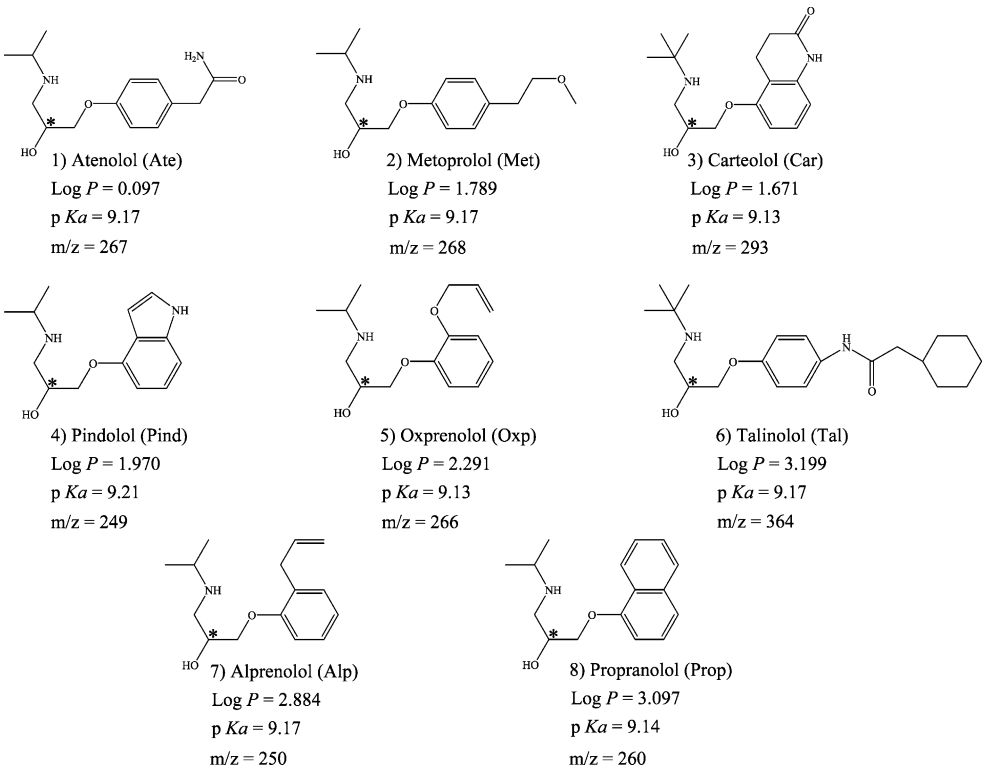

Figure 2. Chemical structures, hydrophobicity (log *P*), pK_a, and *m*/*z* values of selected β-blockers used in present study. Asterisk represents the chiral center.

surfactant using CMEKC−ESI-MS.[4] In this first report, Shamsi has demonstrated the feasibility of a polymeric chiral surfactant, i.e., poly(sodium *N*-undecanoyl-L-valinate (poly-L-SUV), as pseudostationary phase in CMEKC−ESI-MS for enantioseparation of (±)-1,1′-bi-2-naphthol [(±)BOH]. Although very high concentration (e.g., 1% w/v) poly-L-SUV was found to somewhat lower the signal-to-noise (S/N) ratio, an adequate separation with sensitive ESI-MS detection was still achieved.

In the present study, a new and more versatile alkenoxy amino acid molecular micelle, i.e., poly(sodium *N*-undecenoxy carbonyl-L-leucinate (poly-L-SUCL) was synthesized and applied as chiral selector for simultaneous enantioseparation of eight structurally similar β-blockers (Figure 2). The use of poly-L-SUCL was found to be very beneficial for CMEKC−MS as both chiral and achiral resolutions between structurally similar β-blockers were essentially unchanged when BGE containing 100 mM 2-(*N*-cyclohexylamino)-ethanesulfonic acid (CHES) and 10 mM triethylamine (TEA) previously reported for CMEKC−UV[19] was replaced by 25 mM each of ammonium acetate (NH_4OAc) and TEA as MS-compatible BGE for chiral separations. However, as expected, the concentration of both BGE and poly-L-SUCL affected the S/N ratio.

A systematic chiral method development was carried out in this work to obtain optimum system parameters for maximum sensitivity and chiral separation. First, direct infusion-MS (DI-MS) and capillary zone electrophoresis-MS (CZE-MS) experiments were conducted to optimize sheath liquid parameters (i.e., sheath liquid methanol (MeOH) composition, sheath liquid pH, sheath liquid ionic strength, and sheath liquid flow rate) and MS spray chamber parameters (i.e., fragmentor voltage, drying gas flow rate, and drying gas temperature). Second, under optimum sheath liquid and spray chamber conditions, CMEKC separation parameters (i.e., run buffer pH, run buffer concentration, and monomeric and polymeric surfactant concentration) as well as nebulizing pressure were evaluated. Since poly-L-SUCL provided a wider chiral window and lower separation current, high-throughput separation of structurally similar chiral drugs was possible in a single run with tandem UV and MS detection.

EXPERIMENTAL SECTION

Instrumentation. All CMEKC−ESI-MS experiments were conducted using an Agilent CE system interfaced to an Agilent 1100 series MSD quadrupole mass spectrometer and Agilent 1100 series isocratic HPLC pump, which was equipped with 1:100 splitter (Agilent Technologies, Palo Alto, CA) as described elsewhere.[4] Nitrogen was used as both nebulizing and drying gas and was produced in-laboratory using a nitrogen generator.

CE−ESI-MS Conditions and Procedures. The fused-silica capillary (Polymicro Technologies, Phoenix, AZ) with total length of 120 cm was prepared by burning a ~3-mm segment of polyimide coating to create a UV detection window (60 cm from capillary inlet). The capillary was then installed in an Agilent cassette and conditioned for 30 min with 1 N NH_4OH at 40 °C

(14) Wang, J.; Warner, I. M. *Anal. Chem*. **1994**, *66*, 3773−3776.
(15) Wang, J.; Warner, I. M. *J. Chromatogr*. **1995**, *711*, 297−304.
(16) Shamsi, S. A.; Macossay, J.; Warner, I. M. *Anal. Chem*. **1997**, *69*, 2980−2987.
(17) Billiot, E.; Agbaria, R. A.; Shamsi, S. A.; Warner, I. M. *Anal. Chem*. **1999**, *71*, 1252−1256.
(18) Yarabe, H. H.; Shamsi, S. A.; Warner, I. M. *Anal. Chem*. **1999**, *71*, 3992−3999.
(19) Rizvi, S. A. A.; Shamsi, S. A. *Electrophoresis* **2003**, *24*, 2514−2526.
(20) Rizvi, S. A. A.; Akbay, C.; Shamsi, S. A. *Electrophoresis* **2004**, *25*, 853−860.
(21) Rizvi, S. A. A.; Simons, D. N.; Shamsi, S. A. *Electrophoresis* **2004**, *25*, 712−722.

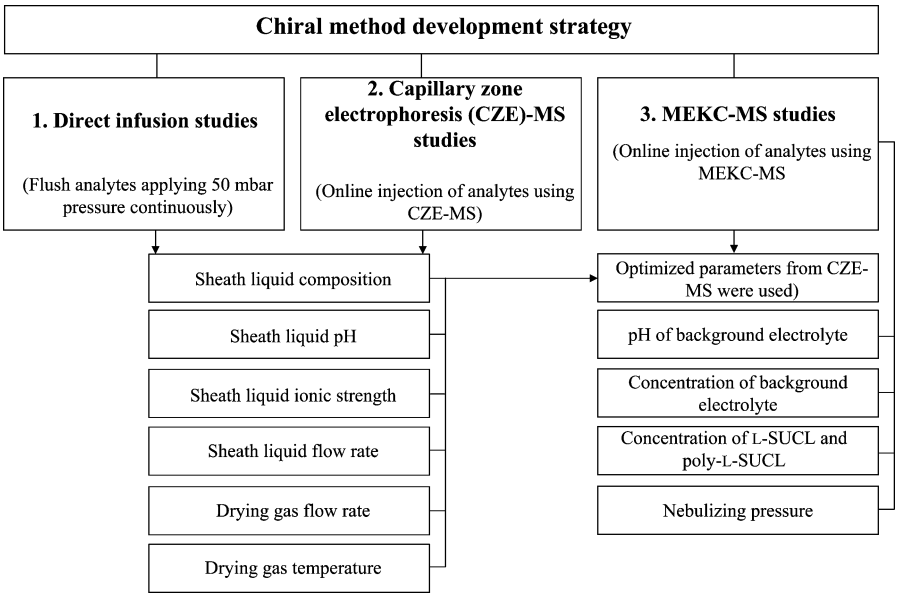

Figure 3. Chiral method development chart for CMEKC and MS parameter optimization.

followed by 10 min with triply deionized water and 10 min with run buffer prior to the insertion into the sprayer needle.[11] After the installation of the capillary into the sprayer, the capillary was flushed with 1 N NH_4OH for 1 min, with triply deionized water for 1 min, and with run buffer for 3 min before each CMEKC−MS run. The mixture of eight β-blockers was injected with pressure injection of 50 mbar for 1 s.[11] All CMEKC separations were performed at a constant voltage of +30 kV, and the capillary temperature was fixed at 20 °C.

DI-MS, CZE-MS, and CMEKC−MS experiments were conducted to optimize sheath liquid parameters (i.e., sheath liquid MeOH composition, sheath liquid pH, sheath liquid ionic strength, and sheath liquid flow rate), MS spray chamber parameters (i.e., fragmentor voltage, drying gas flow rate, drying gas temperature, and nebulizing pressure), and CMEKC separation parameters (i.e., buffer pH, buffer concentration, and surfactant concentration). In all experiments, a potential of +2.5 kV was applied to the sprayer tip for optimum electrospray performance. The SIM mode was performed for MS detection. Since all eight β-blockers exist as cations under experimental conditions, positive $[M + H]^+$ ions were monitored at corresponding m/z for each β-blocker (m/z values are reported in Figure 2).

A manual tuning on the mass spectrometer was performed to optimize the fragmentor voltage for each β-blocker. In this DI-MS study, a 3 mM solution of each β-blocker dissolved in MeOH was continuously introduced for 5−10 min from the CE inlet vial using a 50-mbar pressure. From the manual tune procedure, fragmentor voltage values ranging from 70 to 110 V were obtained for the β-blockers. However, a fragmentor voltage of 85 V was used throughout the experiment as this was found to be a good compromise for all β-blockers.

Materials. The β-blockers (±)-atenolol (Ate), (±)-metoprolol (Met), (±)-pindolol (Pin), (±)-oxprenolol (Oxp), (±)-alprenolol (Alp), and (±)-propranolol (Pro) were purchased from Sigma (St. Louis, MO) or Aldrich (Milwaukee, WI). The racemic mixture of carteolol (Car) hydrochloride was kindly donated by BetaChem (New Jersey, NY), and (±)-talinolol (Tal) was kindly provided by Dr. Bittes of AWD Pharma (Dresden, Germany). The HPLC-grade MeOH was purchased from EM Science (Gibbstown, NJ). The SigmaUltra grade (99%) NH_4OAc was obtained as a 7.5 M solution from Sigma. Ammonium hydroxide and acetic acid were purchased from Fisher Scientific (Springfield, NJ). The poly-L-SUCL was synthesized according to a procedure reported previously.[19]

Preparation of Analytes and Electrolyte Solution. The BGE was prepared by diluting the stock 7.5 M NH_4OAc solution and then adjusting to various pH values (6−10) with NH_4OH or acetic acid. Run buffers were prepared by addition of various amount of surfactant to the BGE. Sheath liquid solutions were prepared by simply diluting the 7.5 M stock solution of NH_4OAc to the desired concentration in varied amounts of MeOH. The pH of NH_4OAc solution was adjusted to the preferred value before addition of MeOH. All run buffers and sheath liquids were filtered through a 0.45-μm syringe filter (Nalgene, Rochester, NY) followed by degassing using ultrasonication for 5 and 20 min, respectively. Stock solutions of β-blockers (40 mM) were prepared in MeOH. A mixture of eight β-blockers was obtained at 3 mM each in pure MeOH for DI-MS and CZE-MS optimization studies. For CMEKC−MS study, however, the concentration of each β-blocker in a mixture was 0.8 mM prepared in 1:2 MeOH/water.

RESULTS AND DISCUSSION

The β-blockers are weakly basic compounds having similar pK_a values but different hydrophobic characters and m/z values (Figure 2). The chart in Figure 3 outlines the method development strategy for simultaneous enantioseparation and detection of β-blockers. Three systematic approaches were followed. In the first DI-MS approach, a mixture of eight β-blockers was dissolved in MeOH and continuously flushed from the CE inlet vial using a 50-mbar pressure. The continuously pumped mixture of β-blockers combines with the sheath liquid and the nebulizing gas, which is then introduced into the ionization region of the mass spectrometer. The DI-MS approach does not involve the use of separation buffer and no real CE separation conditions, and hence, no baseline noise can be determined. Therefore, in the second CZE-MS approach, a mixture of β-blockers was injected on-line using a 50 mbar·s injection. This is followed by applying a

separation voltage of 30 kV for achiral CZE separation. Using the two aforementioned approaches, the sheath liquid parameters and spray chamber parameters were compared and optimized. In the third CMEKC−MS approach, the mixture of eight β-blockers was injected on-line and CMEKC−MS was conducted at the optimum sheath liquid and spray chamber conditions (obtained from the first two approaches).

Optimization of the Sheath Liquid Parameters. Sheath liquid in MEKC−MS establishes an electrical connection between the outlet of CE capillary and electrospray, assists the electrospray ion source, and serves as outlet BGE reservoir.[22,23] Therefore, the optimization of the sheath liquid parameters is critical to obtain high ESI-MS sensitivity. In this section, the sheath liquid parameters were compared using DI-MS and achiral CZE-MS approaches. As mentioned earlier, DI-MS approach does not provide S/N ratio. Thus, only signal intensity was compared when the two aforementioned approaches were evaluated. First, the sheath liquid composition was studied, followed by sheath liquid pH, sheath liquid ionic strength, and sheath liquid flow rate. As expected, none of the aforementioned parameters has an effect on CZE or CMEKC separations (data not shown). Their effects on signal intensity and S/N ratio (normalized abundance) are discussed as follows.

Effect of the Sheath Liquid MeOH Composition. Both DI-MS and achiral CZE-MS approaches were applied to optimize the content of MeOH (40−90% v/v) in the sheath liquid at a fixed concentration of 5 mM NH_4OAc (pH 6.8). As seen in Figure 4, a gradual increase in signal intensity was observed in both approaches as the content of MeOH was increased from 40 to 90% (v/v). In the CZE-MS approach, increase in signal intensity is more profound than DI-MS between 40 and 70% MeOH and then stays somehow constant up to 90% MeOH (Figure 4B inset). However, an increase in normalized abundance was observed upon increasing MeOH content from 40 to 80%, and then a decrease was observed at 90% v/v MeOH (Figure 4B). Thus, 80% MeOH in sheath liquid provided a maximum abundance. The increase in peak intensity with increasing MeOH is probably due to the decrease in the surface tension of the sheath liquid, which, in turn, enhances the stability of the electrospray.[24] The decrease in normalized abundance at 90% MeOH may be explained by high background noise, which possibly resulted from unstable spray at such high MeOH concentration in the sheath liquid.[25] Thus, sheath liquid containing 80% (v/v) MeOH was chosen as optimum for further investigation.

Effect of the Sheath Liquid pH. The β-blockers used in this study are weakly basic compounds with similar pK_a values ranging from 9.13 to 9.21 (Figure 2). Thus, the ESI analysis of these analytes is expected to work best at low pHs. The signal intensity was found to decrease slightly as pH is decreased from 6.8 to 4.0 or increased from 6.8 to 10.0 with both DI-MS and CZE-MS approaches (data not shown). However, the normalized abundance in the CZE-MS approach was found to increase only slightly as pH of the sheath liquid was increased from 4.0 to 8.0, but

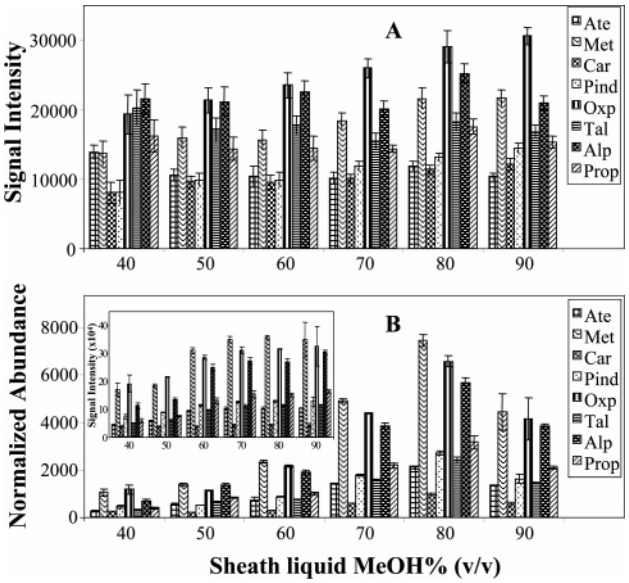

Figure 4. Influence of sheath liquid MeOH content on (A) signal intensity using the DI-MS approach and (B) normalized abundance (S/N) ratio using the CZE-MS approach. Experimental conditions: concentration of each β-blocker in the mixture, 3 mM; capillary length, 120 cm; capillary temperature, 20 °C; sheath liquid contained 5 mM ammonium acetate (apparent natural pH 6.8). Sheath liquid flow rate, 5 μL/min; drying gas (nitrogen) flow rate, 8 L/min; drying gas temperature, 250 °C; nebulizing pressure, 4 psi; acquisition in positive mode, V_{cap} +2.5 kV, fragmentor voltage 100 V, SIM at eight different m/z. (A) β-blocker mixture was introduced to MS using 50-mbar pressure for 10 min. (B) All β-blocker mixture was injected to CE column using 50 mbar for 1 s; applied voltage, 25 kV; background electrolyte contained 20 mM each of NH_4OAc and TEA (pH 8.8). Inset in (B) represents the signal intensity of β-blocker.

decreased substantially as pH was further increased up to 10.0 (data not shown). It appears that, at higher pH, there is an excess of random background noise that resulted in lower normalized abundance. One potential source of random noise could be because the clusters of analytes and solvents may impact the deflector plate on the detector and create a number of fragments with various m/z values.[25] Since the maximum abundance of the majority of β-blockers was obtained at pH 8.0, this was chosen as the optimum sheath liquid pH for further experiments.

Effect of the Sheath Liquid Ionic Strength. The conductivity of sheath liquid in CE−MS is generally tuned with a significant concentration of some ionic species. Varied concentrations of NH_4OAc ranging from 0.0 to 50.0 mM were added to the sheath liquid containing 80% v/v MeOH at pH 8.0. In the DI-MS approach, signal intensity was decreased gradually as NH_4OAc concentration was increased from 1.0 to 50.0 mM (data not shown). However, higher fluctuations (large error bars) in signal intensity were observed with lower NH_4OAc concentrations in the sheath liquid. A different trend was detected with the CZE-MS approach (data not shown); that is, signal intensity increased with increased NH_4OAc concentration to 5 mM and then leveled off. Unlike abundance, the normalized abundance was found to increase with increasing NH_4OAc up to 40 mM and then decreased substantially at 50 mM. Higher concentrations of NH_4OAc (except 50 mM) seemed to provide more stable electrospray. At 50 mM, however, baseline noise increased; thus, a decrease in normalized abundance was observed. Since 40 mM NH_4OAc provided the maximum abun-

(22) Choudhary, G.; Apffel, A.; Yin, H.; Hancock, W. *J. Chromatogr., A* **2000**, *887*, 85−101.
(23) Choudhary, G.; Horváth, C.; Banks, J. F. *J. Chromatogr., A* **1998**, *828*, 469−480.
(24) Foret, F.; Thompson, T. J.; Vouros, P.; Karger, B. L.; Gebauer, P.; Boček, P. *Anal. Chem.* **1994**, *66*, 4450−4458.
(25) Cech, N. B.; Enke, C. G. *Mass Spectrom. Rev.* **2001**, *20*, 362−387.

Table 1. Optimum Sheath Liquid and Spray Chamber Paramters for Simultaneous Analysis of (±)β-Blockers in the SIM Mode

(a) Sheath Liquid Parameters	
methanol composition	80/20% (v/v) methanol/water
pH	8.0
NH_4OAc concentration	40 mM
flow rate	5 μL/min
(b) Spray Chamber Parameters	
drying gas flow rate	5.0 L/min
drying gas temperature	200 °C
fragmentor voltage	100 V
peak width	0.15 min
electrospray voltage	+2.5 kV

dance, it was chosen as the optimum electrolyte concentration in the presence of 80% MeOH in sheath liquid (pH 8.0) to optimize sheath liquid flow rate.

Effect of the Sheath Liquid Flow Rate. For a stable ESI flow, the sheath liquid is usually introduced to MS at a greater flow rate than the CE flow rate (several μL/min). Signal intensity was observed to decrease only for longer retained β-blockers (e.g., Alp and Prop) with increasing flow rate from 2.5 to 5.0 μL/min using the DI-MS approach (data not shown). Further increase in flow rate up to 10 μL/min showed no substantial change in signal intensity. On the other hand, in the CZE-MS approach, signal intensity remained constant between 2.5 and 5.0 μL/min and then decreased only slightly between 7.5 and 10.0 μL/min due to the dilution of β-blockers at higher flow rates. It should be noted that larger fluctuations in signal intensities were observed at 2.5 μL/min in both approaches due to unstable ESI at lower sheath liquid flow rates. No correlation was observed between flow rate and normalized abundance. However, 5.0 μL/min provided relatively higher normalized abundance for the majority of β-blockers. Thus, this sheath liquid flow rate was chosen as the optimum flow rate for optimization of spray chamber parameters.

Optimization of Spray Chamber Parameters. Although nebulizing gas pressure can influence signal intensity, it also affects separation in open tubular CE or CMEKC. Therefore, the impact of nebulizing gas pressure was studied only after optimizing the sheath liquid and spray chamber parameters and will be discussed in the later sections. For the next step in CMEKC−MS method development, two spray chamber parameters, drying gas flow rate, and drying gas temperature were optimized using both DI-MS and CZE-MS approaches.

Effect of Drying Gas Flow Rate and Drying Gas Temperature. Drying gas flow rate and drying gas temperature mostly affected the stability and intensity of the ESI-MS signals. No change in CE retention time of the compounds was observed (data not shown). Under optimum sheath liquid conditions (see Table 1), signal intensity of β-blockers was increased only slightly in both DI-MS and CZE-MS modes as drying gas flow rate was elevated from 2.5 to 5.0 L/min. This can be explained by an increase in desolvation velocity by a moderate drying gas flow rate (e.g., 5 L/min), which is believed to increase the population of charged solutes in the gas phase.[26] A further increase in flow rate from 5 to 10.0 L/min caused a gradual decrease in signal intensity in both DI-MS and CZE-MS approaches (data not shown). The results were in accordance with that obtained by Zheng and Shamsi using capillary electrochromatography−MS.[27] Therefore, a drying gas flow rate of 5.0 L/min was found to be appropriate.

The influence of drying gas temperature on signal efficiency was also evaluated. No noteworthy difference was observed in signal intensities as temperature was gradually increased from 150 to 350 °C in 50 °C intervals using the DI-MS approach (data not shown). However, with the CZE-MS approach, signal intensity increased as temperature was increased from 100 to 200 °C. Signal intensity then tend to decrease as temperature was further increased to 350 °C. When background noise is taken account, a significant decrease in normalized abundance is observed at lower (e.g., 100 °C) and higher (250−350 °C) temperatures. Similar trends in normalized abundance as a function of drying gas temperature were observed in CMEKC−ESI-MS of 1,1′-binaphthol that was published recently.[4] It can be speculated that low drying gas temperature was not significant for solvent evaporation in ESI. Although drying gas temperatures above 200 °C enhanced the solvent evaporation, considerable background noise is produced probably due to small m/z particles formed at higher drying gas temperatures. Thus, a drying gas temperature of 200 °C appears to be a good compromise.

Optimization of CMEKC Conditions. To transfer a CMEKC−UV method to a CMEKC−ESI-MS method, nonvolatile CHES buffer[19] was replaced by a volatile buffer mixture of NH_4OAc and TEA. Under optimum sheath liquid and spray chamber conditions (Table 1), the effect of CMEKC parameters (i.e., pH, concentration of background electrolyte poly-L-SUCL concentration, and nebulizing pressure) was studied to find a good compromise between CMEKC separation and MS sensitivity.

Effect of CMEKC Background Electrolyte pH and Concentration. First, a running CMEKC buffer containing 25 mM poly-L-SUCL, 20 mM each of NH_4OAc, and TEA was studied over the pH range of 6.0−10.0. Figure 5A shows that the chiral resolution of all β-blockers was found to decrease with increased pH of BGE from 6.0 to 10.0. There seem to be a noticeable decrease in retention time of β-blockers from pH 6.0 to 8.0, a slight increase at pH 8.8, and a final drop at pH 10.0 (Figure 5A, inset A1). Trends in retention time can be explained by decreased (at low pHs) and increased (at high pHs) electroosmotic flow. A slight increase in retention time at pH 8.8 compared to pH 8.0 was observed. However, it should be noted that the increase is profound only in the longer eluting β-blockers (e.g., Tal, Alp, and Prop) while the retention time of the remaining β-blockers is very similar to that at pH 8.0. At pH 6.0, the S/N ratio values of β-blockers were significantly lower than that observed at pH 7.0 with the exception of Ate, which was more or less the same (Figure 5A, inset A2). Further increase in pH to 8.0 produced a similar S/N ratio with an interesting drop at pH 8.8. Finally, at pH 10.0, the S/N was very similar to 8.0 but a substantial drop in chiral Rs of all β-blockers was observed. Therefore, pH 8.0 was found as a good compromise between chiral resolution and analysis time as well as S/N ratio.

Similar to CMEKC−UV, high chiral resolutions in CMEKC−ESI-MS can be achieved by increasing volatile BGE concentration.

(26) Sjoeberg, P. J. R.; Boekman, C. F.; Bylund, D.; Markides, K. E. *J. Am. Soc. Mass Spectrom.* **2001**, *12*, 1002−1010.

(27) Zheng, J.; Shamsi, S. A. *Anal. Chem.* **2003**, *75*, 6295−6305.

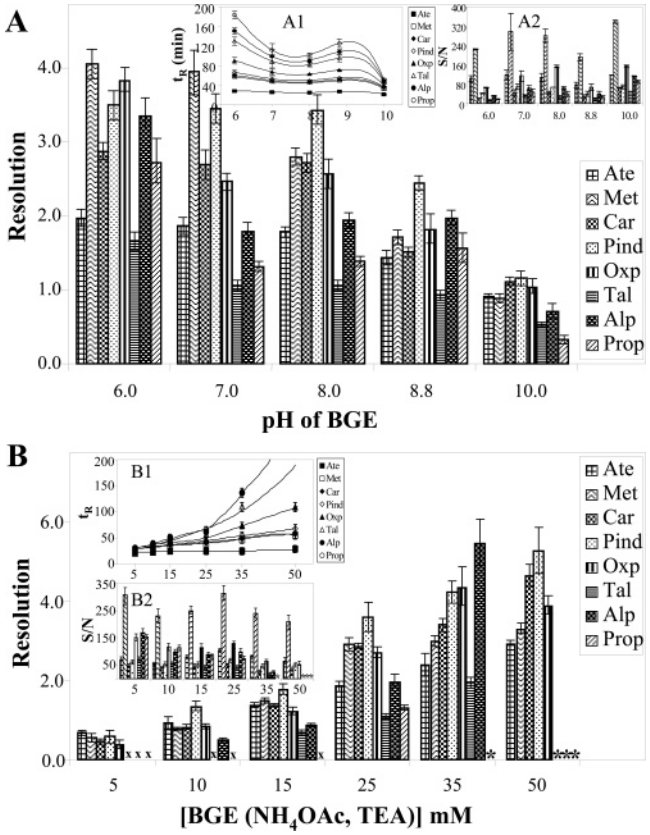

Figure 5. Influence of (A) pH of BGE and (B) concentration of background electrolyte on enantiomeric Rs of β-blockers using CMEKC−MS mode. Experimental conditions: (A) run buffer, 25 mM poly-L-SUCL, 20 mM each of NH_4OAc and TEA (pH 8.8); capillary, fused silica, total length 120 cm, 50-μm i.d.; capillary temperature, 20 °C; CMEKC separation voltage, +30 kV; sample introduction, 50 mbar for 1 s; β-blocker concentration, 0.8 mM each; sheath liquid composition, 40 mM NH_4OAc in 80% (v/v) methanol (pH 8.0); sheath liquid flow rate, 5 μL/min; drying gas flow rate, 5 L/min; drying gas temperature, 200 °C; acquisition, positive mode; V_{cap} +2.5 kV; fragmentor voltage, 85 V; SIM 8 ions at eight different *m*/*z* (see Figure 2 for *m*/*z* values). (B) same as Figure 5A, except pH of background electrolyte was fixed to 8.0. The effect of pH and concentration of BGE on retention time and S/N ratio is shown in the insets A1, B1 and A2, B2, respectively. The symbols (x) and (*) represent zero chiral Rs and uncalculated chiral Rs (due to very long migration times), respectively.

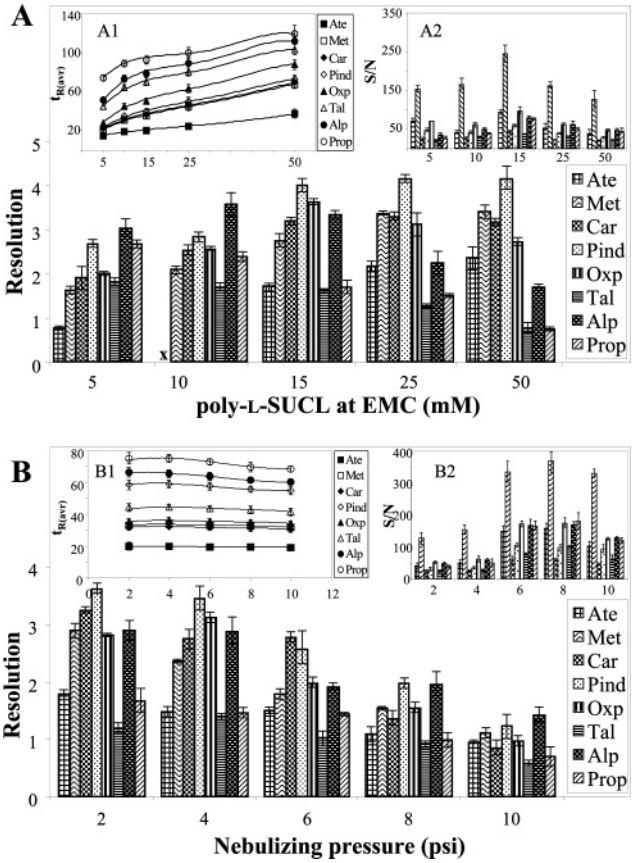

Figure 6. Influence of (A) poly-L-SUCL at EMC and (B) nebulizing pressure on enantiomeric Rs of β-blockers using CMEKC−MS mode. Experimental conditions in (A) are the same as in Figure 5B, except background electrolyte concentration is fixed at 25 mM. All conditions in (B) are the same as in Figure 6A, except poly-L-SUCL concentration is fixed at 15 mM. The effect of poly-L-SUCL concentration and nebulizing pressure on t_R and S/N ratio is shown in the insets A1, B1 and A2, B2, respectively.

This is shown in Figure 5B where chiral resolutions were increased as BGE (i.e., NH_4OAc and TEA) concentration was gradually increased in the range of 5−50 mM at pH 8.0. However, increase in chiral Rs was accompanied by an increase in analysis time (Figure 5B, inset B1) and decreased S/N ratios (with exceptions of a few analytes, Figure 5B, inset B2). It should be mentioned that, due to the excessively long analysis time at 35 and 50 mM BGE, the Rs and t_R values for some β-blockers (i.e., Prop at 35 mM; Tal, Alp, and Prop at 50 mM) are not calculated as shown by the asterisk (*) in Figure 5B. In addition, no chiral Rs (shown by x) was observed for Tal, Alp, and Prop (5 mM), Tal and Prop (10 mM), and Prop (15 mM). As a result, a conclusive compromise of the BGE concentration was found to be ~25 mM each NH_4OAc and TEA.

Effect of Polymeric Surfactant Concentration. Figure 6A shows the bar plots for Rs values of β-blockers at various equivalent monomer concentrations (EMC) of poly-L-SUCL on chiral Rs. Although migration times continue to increase with increase in EMC of poly-L-SUCL (inset A1), it appears that the chiral Rs is analyte dependent. In general, chiral Rs values of hydrophilic β-blockers (i.e., Ate, Met, and Car) were increased, while those of hydrophobic β-blockers (i.e., Tal, Alp, and Pro) were decreased with increasing surfactant concentration. This indicates that, at low surfactant concentrations, chiral interaction between hydrophobic β-blockers and surfactant micelles is more favorable as the hydrophobic analytes do not penetrate deep into the micellar core. In addition, it is also possible that, at low surfactant concentrations, the molecular micelle of poly-L-SUCL is more flexible in such way that chiral interaction between molecular micelle and hydrophobic β-blockers is more favorable. At high surfactant concentration, however, the hydrophobic nonchiral interactions are believed to be more favorable than chiral interaction resulting in decreased chiral Rs values for hydrophobic analytes. On the other hand, the reverse is observed for hydrophilic analytes. Unlike hydrophobic β-blockers, hydrophilic β-blockers (e.g., Ate) do not penetrate deep into the hydrophobic core of the micelle, but they localize close to the chiral center bearing headgroup of the poly-L-SUCL micelles. With an increase in surfactant concentration chiral interaction between the hydrophilic β-blockers and the micelles increased resulting in increased chiral

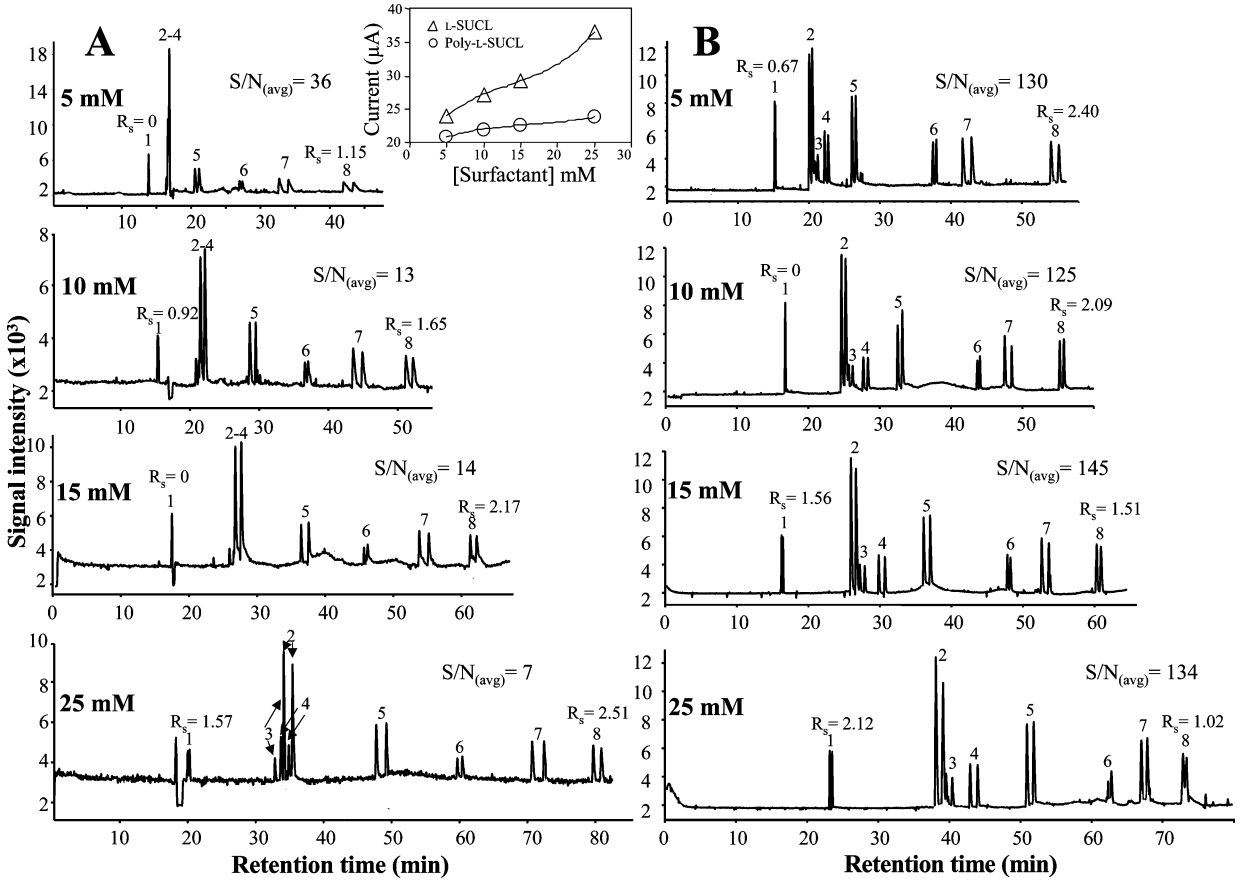

Figure 7. CMEKC−ESI-MS electropherograms showing comparison of (A) L-SUCL and (B) poly-L-SUCL at various concentrations on Rs and average S/N of eight β-blockers. Experimental conditions are same as Figure 6B, except nebulizing pressure is fixed at 6 psi, SIM positive ion (8 ions). Inset represents the separation current as a function of L-SUCL or poly-L-SUCL EMCs.

Rs values. However, at certain surfactant concentrations, the chiral interaction levels off (around 25 mM surfactant concentration in this study). This trend in Rs as a factor of surfactant concentration is consistent with our recent study using the same leucine-based polymeric surfactant but with different chain length.[28] In this study, we observe similar k' but the resolutions of hydrophobic β-blockers were significantly better using shorter chain length (e.g., C8-leucinate) compared to longer chain length (e.g,, C11-leucinate) polymeric surfactant at the same molar concentrations. In addition, studies by Foley also pointed out that in most cases the best Rs is predicted at capacity factor ranging from 1.2 to 2.[29] Further increase in surfactant concentration does not increase the Rs once the capacity factor exceeds ~2. The S/N ratio for all the β-blockers first increased up to at least 15 mM (Figure 6A, inset A2), with the S/N ratio being reduced at higher poly-L-SUCL concentration, ≥25 mM. Therefore, 15 mM poly-L-SUCL was found to be optimum, which generally provides a reasonable tradeoff between chiral Rs and S/N ratio.

Effect of Nebulizing Gas Pressure. To study the effect of nebulizing gas pressure, both separation parameters (Rs, t_R) and detection parameters (MS signal intensity, S/N) were measured. The results are shown in Figure 6B. Note that the chiral Rs was decreased gradually by increasing the nebulizing gas pressure. This suggests that at higher nebulizing pressures the nebulizer is generating a suction resulting in a laminar flow inside the CE separation capillary. This, in turn, causes a band broadening, which results in a decreased chiral Rs. In addition, as seen in inset B1 of Figure 6B, the migration time of all β-blockers is also decreased by nebulizing gas pressure. Moreover, it is interesting to note that the effect of nebulization on t_R is more critical for relatively high hydrophobic β-blockers (e.g., Prop and Alp), while t_R of hydrophilic β-blockers (e.g., Ate) remained almost unchanged. As shown in Figure 6B (inset B2), S/N is increased as nebulizing gas pressure was increased from 2 to 8 psi and then decreased at 10 psi probably due to band broadening, which decreased the peak intensity of the β-blockers. Thus, nebulizing gas pressure of 6 psi is enough to ensure baseline Rs of all chiral β-blockers without causing significant increase and decrease in t_R and S/N, respectively.

Comparison of Monomeric and Polymeric Surfactants. Figure 7 represents the simultaneous enantioseparation of the eight β-blockers using varied concentrations of both monomeric and polymeric L-SUCL surfactants. Several trends are evident. First, the average S/N ratio is ~5-fold higher with polymeric surfactant than with monomeric surfactant at the lowest concentration (i.e., 5 mM). However, the difference in S/N ratios between monomers and polymers increases rapidly with the increase in their respective surfactant concentration. For example, under

(28) Rizvi, S. A., Shamsi, S. A. Polymeric Alkenoxy Amino Acid Surfactants: V. Effect of Hydrophobic Chain Length of Molecular Micelles on Chiral Separation. Presented at Pittsburgh Conference, Chicago, IL, 2004; March 11, Paper 22900−500.

(29) Foley, J. P. *Anal. Chem.* **1990**, *62*, 1302−1308.

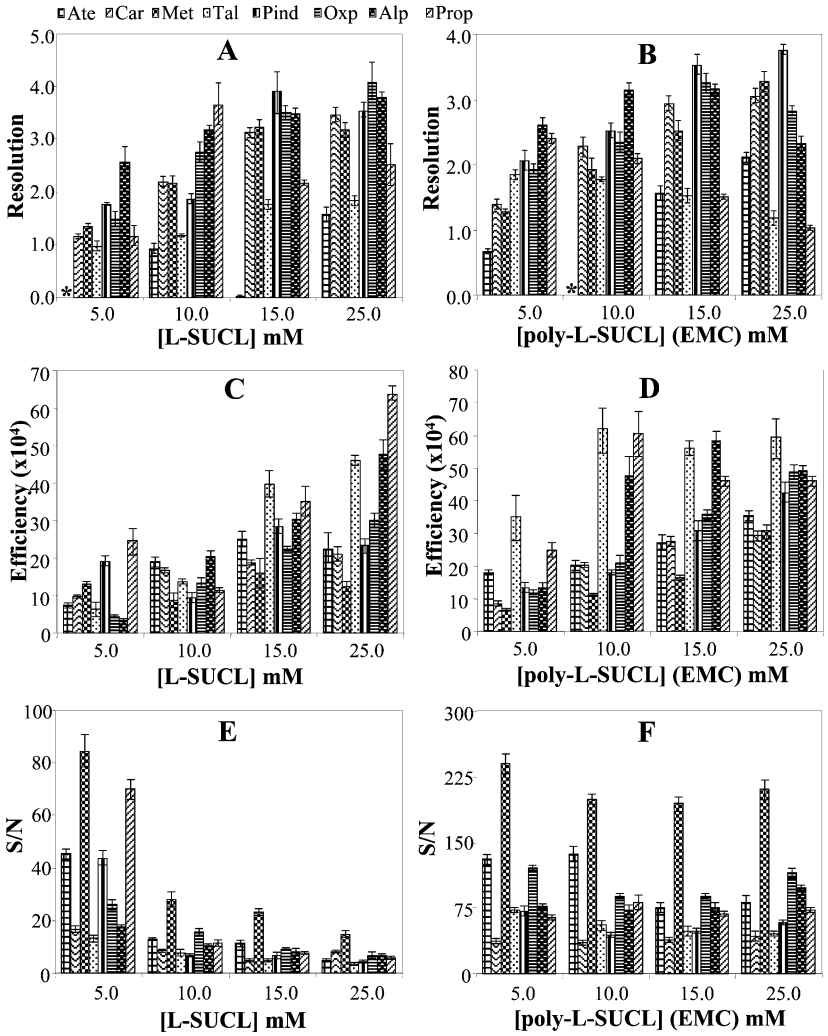

Figure 8. Comparison of L-SUCL (left) and poly-L-SUCL (right) bar plots on (A, B) chiral Rs, (C, D) peak efficiency, and (E, F) S/N ratio. Experimental conditions are the same as in Figure 7.

optimum condition (i.e., 15 mM poly-L-SUCL) the S/N is ~1 order of magnitude higher with the polymer than the monomer. One explanation is that unpolymerized micelles of monomeric surfactants break down once introduced to the ESI chamber, thus resulting in unstable electrospray which in turn increases background noise of the mass spectrometer (see Figure 1A).[30] Fouling of the ionization source in the ESI interface was observed by several researchers when conventional micelles (i.e., micelles of monomeric surfactants) were used.[31−34] For instance, the use of a high concentration of sodium dodecyl sulfate was found to produce strong signals in both negative and positive ion modes and to suppress the ionization efficiency of the source resulting in poor S/N ratios for the cationic analyte.[32] Second, it should be noted that polymeric surfactant provided separation of all β-blockers even at the lowest surfactant concentration studied (i.e., 5 mM poly-L-SUCL). However, at the same concentration (i.e., 5 mM) of monomeric L-SUCL, chiral resolution of four (i.e., Ate, Met, Car, and Pind) out of eight β-blockers was not possible. Chiral resolution of all β-blockers was achieved by increasing monomeric L-SUCL concentration to 25 mM at the expense of very low S/N ratios. Third, the elution order of enantiomers of three aforementioned β-blockers are different in 25 mM monomeric (Figure 7A, bottom electropherogram) and polymeric (Figure 7B, bottom electropherogram) surfactants. It is worth noting that the separation current was increased from ~24 to ~38 μA using 5−25 mM L-SUCL whereas it increased only slightly from ~21 to 24 μA using poly-L-SUCL over the same concentration range (Figure 7, inset). Since most of the dedicated CE−MS instruments have an upper separation current limit of 50 μA, the use of unpolymerized surfactant at higher concentration provides a major limitation for reliable CE−MS operation.

The monomer and polymer of L-SUCL were also compared in terms of Rs, efficiency (*N*), and S/N (Figure 8). Again, several trends are evident. The chiral Rs of both hydrophilic and hydrophobic β-blockers continue to increase with increase in monomeric surfactant concentration (Figure 8A). As discussed

(30) Shamsi, S. A. *Electrophoresis* **2002**, *23*, 4036−4051.
(31) Cole, B. R.; Varghese, J. *J. Chromatogr.* **1993**, *652*, 369−376.
(32) Kirby, D.; Greve, K. F.; Foret, F.; Vourous, P.; Karger, B. L. Capillary Electrophoresis Electrospray Ionization Mass Spectrometry Utilizing Electrolytes Containing Surfactants. Presented at the 42nd ASMS Conference on Mass Spectrometry; Chicago, IL, May 29−June 3, 1994; pp 1014−1015.
(33) Dekkers, S. E. G.; Tjaden, U. R.; Greef, J. V. D. *J. Chromatogr., A* **1995**, *712*, 201−209.
(34) Lu, W.; Poon, G. K.; Carmicheal, P. L.; Cole, B. R. *Anal. Chem.* **1996**, *68*, 668−674.

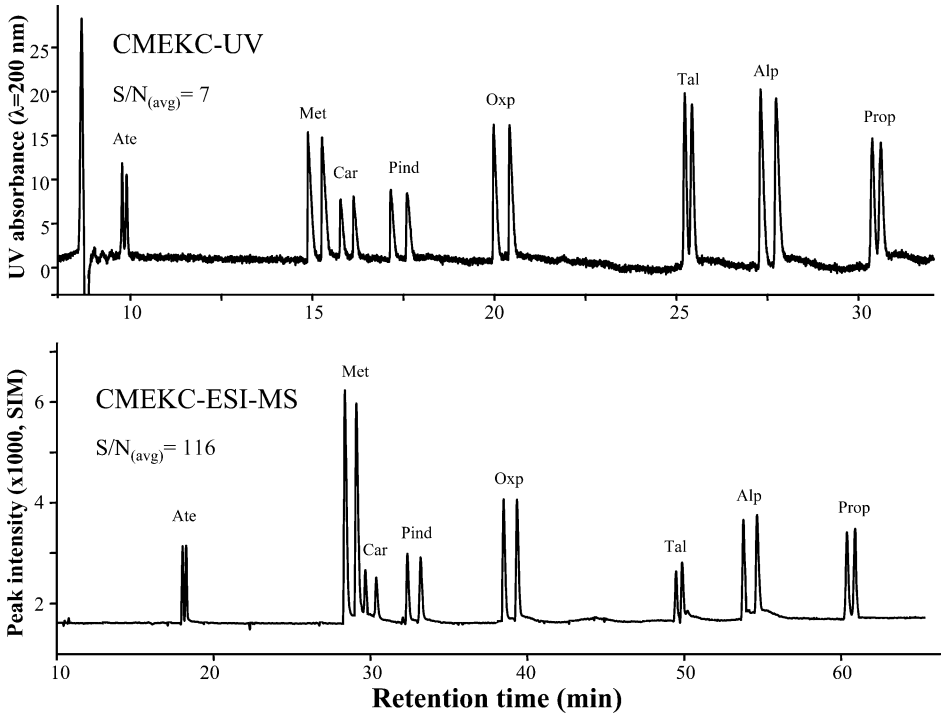

Figure 9. Electropherograms illustrating simultaneous UV (top) and MS (bottom) detection of β-blockers. Experimental conditions are the same as in Figure 7.

earlier, with polymeric surfactant, chiral Rs of hydrophilic β-blockers (e.g., Ate) was increased, while that of hydrophobic β-blockers (e.g., Alp and Prop) was decreased with increased surfactant concentration (Figure 8B). The efficiency of each β-blocker was also compared in both monomeric and polymeric surfactants (Figure 8C, D). It should be noted that *N* values were increased with increasing surfactant concentration in both surfactant types due probably to the sample stacking effect. Furthermore, it was observed that poly-L-SUCL provided an overall better *N* on the order of 2−5-fold higher compared to L-SUCL. The exceptions were Alp and Prop, which showed lower *N* only at 25 mM. Finally, S/N ratios of the β-blockers were compared. As seen in Figure 8E and F, S/N ratios of β-blockers obtained from polymeric surfactant were always higher than those obtained from monomeric surfactant. For instance, comparison of the S/N ratio of Met, which has the highest S/N ratio in both surfactant systems, reveals that this β-blocker has ∼3 times larger S/N ratio in polymeric surfactant than in monomeric surfactant. When surfactant concentration was further increased to 25 mM, the difference in S/N ratio increased to 15 times higher using polymeric surfactant rather than monomeric surfactant. A similar trend was observed for all other β-blockers. In general, it was noted that S/N ratio remained more or less similar in the range of 15−25 mM poly-L-SUCL, while a significant decrease in the S/N ratio of all β-blockers was observed over the same concentration range using L-SUCL.

Comparison of UV and MS Detection. In this study, CMEKC with tandem UV and MS detections was also compared. The β-blockers were first detected by UV detection by placing the UV window at 60 cm from the inlet end of the separation capillary, and then ESI-MS detection was performed at the outlet end (∼120 cm) from the inlet side of the capillary. Figure 9 shows the CMEKC−UV and CMEKC−ESI-MS electropherograms of the eight β-blockers. As can be seen, CMEKC−ESI-MS on average provides ∼16 times better S/N ratio than CMEKC−UV. All β-blockers have the same migration order in both detection modes. However, it should be noted that the achiral Rs between the second peak of Met and the first peak of Car was slightly decreased with ESI-MS detection as compared to UV detection. In addition, it is worth mentioning that even though all β-blockers were injected at equimolar concentration (i.e., 0.8 mM in 1:2 MeOH/water), the MS signal intensities differ considerably. This can be explained by the fact that each β-blocker has a different affinity to acquire a proton in the gas phase. Thus, some β-blockers (e.g., Met) have the highest ionization efficiency as compared to other β-blockers (e.g., Car), which showed the lowest ionization. The two detection modes were also compared with regard to Rs, *N*, and S/N ratio (Figure 10). As seen in Figure 10A and B, chiral Rs and *N* of each β-blocker is always higher in MS than in UV due to longer capillary length in MS detection (120 cm) as opposed to a shorter capillary length (60 cm) in UV detection. Finally, when the S/N ratio of each β-blocker was compared, MS detection provided 7−32 times higher values than UV detection (Figure 10C).

Finally, the reproducibility of migration time, peak area, linearity, and concentration detection limit for all eight β-blockers was compared in both detection modes, and the results are listed in Table 2. Satisfactory reproducibilities were obtained for all β-blockers with RSD values ($n = 3$) for migration times better than 0.4 and 1.2% with UV and MS mode, respectively. Due to the shorter distance between inlet and UV window, the β-blockers elute faster in UV compared to MS detection. Hence, relatively smaller RSD values were obtained with UV detection. Peak area, which is important in quantitation of compounds, was also compared. Always higher peak areas ranging from 16 to 70 times higher were obtained in MS than in UV detection. The reproduc-

Table 2. Reproducibility, Linearity, and Sensitivity of β-Blockers by MEKC−UV and MEKC−MS

	migration time[a] (% RSD)		peak area (% RSD)		linearity (0.01−0.6 mM)[b] correl coeff		concn limit of detection (μmol/L) (S/N = 3)	
β-blockers	VU	MS	UV	MS	UV	MS	UV	MS
Ate	9.8 (0.1)	19.8 (0.2)	530 (1)	11160 (8.3)	0.986	0.997	2.0	0.9
Met	15.2 (0.1)	30.8 (0.2)	870 (1)	60850 (0.5)	0.955	0.996	2.5	1.1
Car	16.1 (0.2)	32.1 (0.4)	780 (9)	11940 (6.1)	0.996	0.962	11	7.5
Pind	17.5 (0.2)	35.1 (0.5)	780 (2)	15180 (1.2)	0.958	0.995	10	5.0
Oxp	20.4 (0.3)	41.5 (0.5)	890 (14)	28760 (5.7)	0.990	0.996	2.5	1.0
Tal	25.6 (0.3)	52.9 (0.7)	700 (2)	11400 (4.7)	0.984	0.985	13	5.0
Alp	27.9 (0.2)	57.7 (0./9)	930 (10)	25540 (2.5)	0.970	0.986	9.2	7.5
Prop	31.1 (0.4)	65.4 (1.2)	660 (3)	20570 (13.1)	0.957	0.996	13	3.8

[a] Experimental conditions are the same as in Figure 9 ($n = 3$). [b] 0.6, 0.3, 0.1, 0.05, and 0.01 mM (at least two injections for each level).

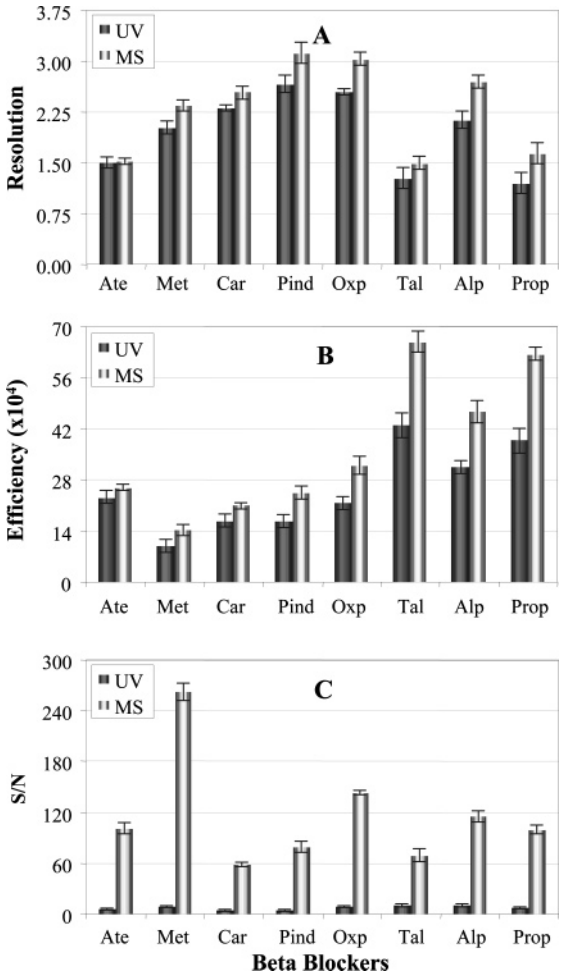

Figure 10. Comparison of CMEKC−MS and CMEKC−UV for (A) chiral Rs, (B) efficiency, and (C) S/N. Experimental conditions are the same as in Figure 7.

ibility of peak areas in both UV and MS detection seems to be analyte dependent. In general, reproducibility ranged from 1.0 to 13.9% for UV and 0.5 to 13.1% for MS. To set up the calibration curve for eight β-blockers, a mixture of each β-blocker was prepared at concentrations of 0.6, 0.3, 0.1, 0.05, and 0.01 mM using (−)-Alp as an internal standard at a concentration of 0.8 mM. All of five standard mixtures were injected in a tandem CMEKC−UV-MS fashion and peak area ratios of each β-blocker to (−)-Alp were obtained and plotted versus the concentration. The calibration curves for all β-blockers were linear with correlation coefficients between 0.955 and 0.996 for UV and 0.962 and 0.997 for MS detection. Last, the concentration limit of detection (CLOD) for all β-blockers were found to range from 2 to 13 μM with UV and from 0.9 to 7.5 μM with MS detection at a S/N ratio of 3. Based on the higher S/N ratios in MS detection as compared to UV detection, much lower CLOD values were expected. One reason for this inconsistency could be the fact that, at lower concentration of β-blockers, the ratio of [surfactant]/[β-blocker] increases in the ESI chamber. This is believed to reduce the ionization ability of the solutes due to the presence of a relatively high concentration of oppositely charged surfactant molecules. This drawback can be overcome by using lower surfactant concentration.

CONCLUSIONS

For the first time, tandem CMEKC−UV and CMEKC−ESI-MS was studied for simultaneous enantioseparation and detection of eight structurally similar β-blockers using a new and more versatile synthetic chiral surfactant (i.e., poly-L-SUCL) with a volatile buffer system. Three optimization approaches, i.e., DI-MS, CZE-MS, and CMEKC−MS, were applied to optimize sheath liquid parameters, spray chamber parameters, and CMEKC separation parameters. Although both DI-MS and CZE-MS approaches provided similar trends in signal intensity, CZE-MS was found to mimic CMEKC−MS better due to the fact that the latter approach provides baseline noise, an important figure of merit required in obtaining true detection limits. No effect of sheath liquid and spray chamber parameters (except nebulizing pressure) was found on CMEKC separation time and chiral Rs. However, these parameters were found to affect signal intensity and sensitivity of the system. On the other hand, nebulizing pressure was found to be critical on signal intensity, retention time of highly hydrophobic β-blockers, and chiral Rs. To obtain a reasonable tradeoff between Rs and S/N, the last optimization approach, i.e., CMEKC−MS, was applied using optimized sheath liquid and spray chamber parameters to find the optimum BGE concentration, pH, and polymeric surfactant concentration.

Comparison of monomeric and polymeric L-SUCL under optimum experimental conditions revealed that, overall, poly-L-SUCL showed better performance than monomeric L-SUCL. This is because unpolymerized micelles of L-SUCL surfactant tends to

break down once introduced to the ESI chamber, thus resulting in unstable electrospray, which in turn increases background noise of the mass spectrometer.[30] It should also be noted that polymeric surfactant provided separation of all β-blockers even at the lowest surfactant concentration studied (i.e., 5 mM poly-L-SUCL). However, at the same concentration (i.e., 5 mM) of monomeric L-SUCL, chiral resolution of only four (i.e., Ate, Met, Car, and Pind) out of eight β-blockers was possible. The CMEKC with tandem UV and MS detections was also compared. On the average, CMEKC−ESI-MS provides ∼16 times better S/N ratio than CMEKC−UV. Furthermore, due to the shorter distance between inlet and UV window, the β-blockers eluted faster in UV with relatively smaller RSD values for migration times and peak areas as compared to MS detection. However, higher peak areas with high Rs and N as well as better CLOD were always obtained in MS than in UV.

In general, it is unlikely that any patient would be prescribed more than one β-blocker. However, compared with other techniques, the simultaneous determination of β-blockers using a highly selective chiral molecular micelle has several advantages: (i) a single and universal analytical method with the same pH, buffer concentration, and chiral selector concentration eliminates the need for developing individual protocols for analysis of each β-blocker; (ii) all chiral β-blockers and their chiral/achiral metabolites can be simultaneously separated provided a very selective chiral reagent is employed; (iii) physicochemical properties such as micelle−water partition coefficient can be correlated with log P and pK_a's can be determined in high-throughput fashion for combinatorial mixtures of chiral drugs. Thus, it is likely that the use of highly selective molecular micelles for CMEKC compatible to MS detection will attract great interest in the years to come.

ACKNOWLEDGMENT

Financial support for this project was provided by the National Institute of Health (Grant GM 62314-02).

Received for review July 26, 2004. Accepted December 2, 2004.

AC0401422