Xiaofeng Zhu
Mary W. Kamande
Serigne Thiam
Constantina P. Kapnissi
Simon M. Mwongela
Isiah M. Warner

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

Open-tubular capillary electrochromatography/ electrospray ionization-mass spectrometry using polymeric surfactant as a stationary phase coating

We describe the use of the polymeric surfactant poly(sodium undecylenic sulfate) (poly-SUS) as a stationary phase coating in open-tubular capillary electrochromatog raphy (OT-CEC) coupled with electrospray ionization-mass spectrometry (ESI-MS) for the analysis of β-blocker and benzodiazepine analytes. The production of a polymeric surfactant coating on the capillary inner wall involves (i) adsorption of the cationic polymer poly(diallyldimethylammonium chloride) (PDADMAC) to the inner surface of capillary, and (ii) adsorption of the negatively charged poly-SUS onto the cationic polymer layer *via* strong physical interaction of the two polymer layers. As compared with micellar electrokinetic chromatography (MEKC) coupled with ESI-MS, the main advantage of this proposed method is minimization of introduction of the monomeric or polymeric surfactant into the mass spectrometer, thus avoiding the interference of the nonvolatile micelle in ESI-MS. The effects of buffer pH and applied voltage on the separation of the analytes are also discussed. Under optimum conditions, four of the five β-blockers and four benzodiazepines are separated.

Keywords: Open-tubular capillary electrochromatography / Polymeric surfactant / Stationary phase coating
DOI 10.1002/elps.200305713

1 Introduction

Interfacing capillary electrophoresis (CE) with mass spectrometry (MS) is of growing interest in separation science because CE offers rapid separation, high separation efficiency, and small sample consumption, while MS is capable of providing mass and structural elucidation information for analytes of interest. Among the CE separation modes, capillary zone electrophoresis (CZE) is the most frequently used, and its separation ability is based on the analyte's mass-to-charge ratio. In contrast to CZE, both micellar electrokinetic chromatography (MEKC) and capillary electrochromatography (CEC) have a wide application range. For either MEKC or CEC, the separation is achieved by a combination of the electrophoretic mobility difference of analytes and the partitioning interaction between the analytes and the stationary or pseudostationary phase. Therefore, both allow separation of ionic and neutral analytes. However, coupling MEKC with MS can be challenging due to the negative effect of the background surfactants on the mass signal. In electrospray ionisation-mass spectrometry (ESI-MS), nonvolatile surfactants, such as sodium dodecyl sulfate (SDS), present in the running buffer at relatively high concentrations produce low ionization efficiency. This in turn causes suppression of the analytes' mass signal and mass detector contamination [1–3].

In order to overcome the effects of surfactants in ESI-MS, Varghese and Cole [4] used the cationic surfactant cetyltrimethylammonium chloride (CTAC) at low concentration for the analysis of cationic tripeptides and other amine-containing compounds in CE/ESI-MS. However, the separation mode employed was not MEKC because the concentration of added surfactant was below its critical micelle concentration (CMC). In recent years, the use of polymeric surfactants in MEKC has received much attention [5–10]. As compared to monomeric surfactants, polymeric surfactants produce the advantage of zero CMC. The first attempt of direct coupling MEKC with ESI-MS was reported by Ozaki *et al.* [11]. In their study, the use of a high-molecular-weight surfactant (polymeric surfactant) with zero CMC made it possible to form a micelle at a very low surfactant concentration, thus reducing the interference of surfactants in ESI. Lu and coworkers [12] also developed a polymeric surfactant, poly(sodium undecylenic sulfate) (poly-SUS) for the resolution enhancement of the analytes in MEKC/ESI-MS.

Correspondence: Professor Isiah M. Warner, Department of Chemistry, Louisiana State University, Baton Rouge, LA 70803, USA
E-mail: iwarner@lsu.edu
Fax: +225-578-3971

Abbreviations: OT, open-tubular; **PDADMAC**, poly(diallyldimethylammonium chloride); **PEM**, polyelectrolyte multilayer; **poly(SUS)**, poly(sodium undecylenic sulfate); **SIC**, selected ion chromatogram

Although the use of polymeric surfactants can decrease the interference of the surfactant on mass signal, there still remains a problem. During the ESI process, the polymeric surfactant can still be introduced into the MS and the analyte-surfactant adduct mass signal may be observed [13]. In addition, the analyte and analyte-surfactant complex may have different ionization efficiencies due to the significant difference in molecular size [14]. As a result, formation of the analyte-surfactant complex may lower the ionization efficiency of the analyte, and reduce the sensitivity of MS detection by use of MEKC/ESI-MS.

Alternative methods have been developed to eliminate the introduction of pseudostationary phase into the MS. Foley and Masucci [15] used a semipermeable membrane that only allowed small molecules to permeate the membrane while retaining large molecules. Nelson *et al.* [16] interfaced partial-filling MEKC with ESI-MS. In partial-filling MEKC, the capillary was filled with a small plug of running buffer that contained micelles. The analytes first migrated into the micellar plug, and then into the mass spectrometer before the micelle plug reached the end of the capillary. In another report, Yang *et al.* [17] reversed the migration direction of the micelle such that it was opposite to the direction of the analytes. This was achieved by adjusting the pH value of the MEKC buffer. It is apparent that either partial-filling or opposite migration method is only applicable to certain kinds of analytes in MEKC/ESI-MS, and the stability of those methods still remains questionable [18].

The stationary phase in open-tubular CEC (OT-CEC) is immobilized on the inner surface of the capillary. The pseudostationary phase in MEKC though is directly added into the running buffer. In comparison with packed CEC, where the stationary phase is packed in the column, OT-CEC does not suffer from the air bubble formation problem, which exists around the frits and the packing material. Moreover, the running buffer in OT-CEC can be replenished between each run by simply flushing the capillary with fresh solution. However, one limitation of OT-CEC is the low phase ratio (the ratio of stationary phase to mobile phase). This may result in low selectivity for certain separation in comparison with packed CEC. Because of the inherent advantages of OT-CEC, it is an almost ideal separation technique that can be used for coupling with ESI-MS. Wu *et al.* [19] prepared a reversed-phase OT-CEC column coupled with MS for ultrafast analysis of a peptide mixture. After covalently binding the reversed-phase C-8 on the capillary inner wall, it is further coated with an amine on C-8 surface to increase the electroosmotic flow (EOF). However, the column preparation was tedious and time-consuming. Different kinds of coatings, such as polyelectrolytes and ionic liquids, have also been used for capillary wall modification [20–23]. However, unlike the coating for OT-CEC, those coatings on the capillary inner wall were primarily for the purpose of reducing capillary wall interactions with the analytes.

Our laboratory has recently investigated the use of the polymeric surfactant, poly(sodium *N*-undecanoyl-L-glycinate) as a stationary phase coating in OT-CEC [24]. The polymeric surfactant was successfully immobilized on the capillary inner wall by use of the polyelectrolyte multilayer (PEM) coating that was constructed *in situ* by alternating rinses of positively and negatively charged polymers. A drawback to this approach is that the multiple coating steps for ten bilayers may be time-consuming. Thereafter, we used another negatively charged polymeric surfactant (poly-SUS) and the positively charged polymer poly(diallyldimethylammonium chloride) (PDADMAC) to make a single bilayer coating on the capillary inner wall [25]. The OT-CEC column demonstrated good stability and satisfactory separation performance. In comparison with the previously used polymeric surfactant, poly-SUS can be used in a wide pH range.

In this study, we use the polymeric surfactant, poly-SUS as a stationary phase coating in OT-CEC/ESI-MS for the first time for the analysis of both β-blockers and benzodiazepines. This technique is developed by combining the favorable aspects of MEKC and OT-CEC to benefit MS detection.

2 Materials and methods

2.1 Chemicals and materials

Glacial acetic acid, methanol, and acetone of HPLC-grade were purchased from Fisher Scientific (Fair Lawn, NJ, USA) and used without further purification. Ammonium hydroxide was from Mallinckrodt Baker (Paris, KY, USA), ultrapure-grade ammonium acetate from Amresco (Solon, OH, USA). Cationic polymer PDADMAC with a molecular weight range of 200 000 and 350 000 was obtained from Aldrich Chemical (Milwaukee, WI, USA). Chlorosulfonic acid, 10-undecenyl alcohol, and the analytes alprenolol, atenolol, pindolol, propranolol, sotalol, clonazepam, flunitrazepam, lorazepam, and nitrazepam were purchased from Sigma (St. Louis, MO, USA). Poly-SUS was synthesized according to our previously reported procedure [9]. Deionized water produced by use of a USFilter system (Lowell, MA, USA) was used for the preparation of all solutions. All other chemicals were of analytical grade. The bare fused-silica capillary (50 μm ID × 360 μm OD) was purchased from Polymicro Technologies (Phoenix, AZ, USA).

2.2 Buffer and sample preparation

The running buffer solution for OT-CEC/ESI-MS containing 10 mM ammonium acetate was adjusted to pH 4.0 and 6.0 with acetic acid, and to pH 9.0 with ammonium hydroxide. The solution of the sheath liquid consisted of 0.5% acetic acid in 50:50 v/v methanol/water. Before use, each solution was sonicated for 10 min, and then filtered with 0.45 μm pore size syringe filter (Whatman, Clifton, NJ, USA). Stock standard solutions of analytes were prepared by dissolving each compound in methanol to obtain a concentration of 1 mg/mL. All solutions were stored at 4°C before use. For OT-CEC/ESI-MS experiments, the analytes from stock solution were mixed and further diluted in methanol. The concentration of each analyte in the mixture was 0.1 mg/mL. The final concentration of each analyte was achieved at 50 μg/mL by adding an equivalent volume of running buffer to the mixture.

2.3 OT-CEC column preparation

The OT-CEC column was prepared by the protocol described in a recent paper [25]. Briefly, the capillary was rinsed with 1 M NaOH for 45 min in order to enhance the dissociation of the silanol groups, followed by a 15 min rinse with deionized water. After preconditioning, the capillary was rinsed with the solution containing 0.5% w/v PDADMAC in 0.2 M NaCl for 20 min to form a cationic polymer layer. Then, the capillary was rinsed with deionized water for 5 min. Finally, 1% w/v poly-SUS solution was rinsed over the cationic layer for 20 min to immobilize the polymeric surfactant coating on the internal surface of the capillary. Any residual poly-SUS was removed by rinsing the capillary with deionized water for 5 min. The coating consisted of one bilayer.

2.4 OT-CEC/ESI-MS instrumentation

A Hewlett-Packard^{3D} CE instrument (Palo Alto, CA, USA) and an ESI-TOF-MS Mariner Biospectrometry Workstation from Applied Biosystems (Framingham, MA, USA) were employed for OT-CEC/ESI-MS experiments. All experiments were performed at room temperature. A CE-ESI sprayer from Agilent Technologies (Palo Alto, CA, USA) was used to interface the CE with the MS. As shown in Fig. 1, the sprayer is capable of providing both a coaxial sheath liquid and a nebulization gas to assist the electrospray. The capillary tip was set at an angle of 45° relative to the direction of the ESI-TOF-MS nozzle in order to obtain an optimum signal. A distance of 0.5 mm of the capillary tip was extended from the sprayer tip. The length of the poly-SUS coated capillary was 61.5 cm. The sheath liquid was delivered at a flow rate of 4 μL/min by using a Harvard Apparatus syringe pump (Holliston, MA, USA). The ESI voltage applied on the sprayer was set at 3.5 kV

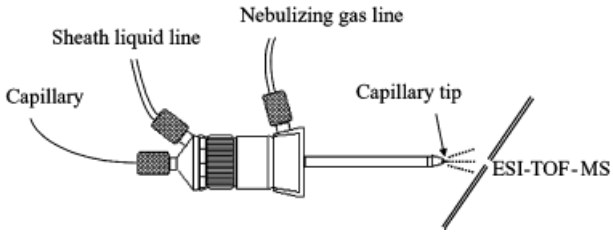


Figure 1. Schematic representation of the sheath flow interface for OT-CE/ESI-TOF-MS.

in the positive mode. Data acquisition was performed in the range of *m/z* 50–1000 at a scan rate of 3 s/spectrum. The nozzle voltage was set at 100 V, and the skimmer 1 voltage was set at 12 V. The nebulizer and curtain gases were both nitrogen, and the flow rates were optimized at 0.6 L/min and 0.7 L/min, respectively. Between runs, the capillary was rinsed with the running buffer for 2 min. Pressure was applied at 50 mbar for 5 s for sample injection.

3 Results and discussion

In OT-CEC, the stationary phase, which is ascribed to the separation by partitioning interaction with analytes, is immobilized on the capillary internal wall. In this study, the polymeric surfactant coating of poly-SUS was physically adsorbed on the capillary inner wall and used as the OT-CEC stationary phase. For a bare fused-silica capillary, as summarized in Table 1, the EOF is generated as a result of the deprotonated silanol groups on the inner surface. The EOF direction and magnitude of the capillary are related to the kind of surface charge and the surface charge density, respectively. After flushing with the strongly charged cationic polymer PDADMAC, the EOF direction is reversed with a different magnitude as compared to that of a bare fused-silica capillary. The result indicated that the cationic polymer was adsorbed on the inner surface and the overall surface charge turned out to be positive. Followed by a continuous flush with the anionic polymeric surfactant, poly-SUS, the EOF variations in terms of both direction

Table 1. Measured EOF of capillaries with different coatings

Capillary	EOF $(m^2/(S \cdot v) \times 10^{-8})$
Bare fused-silica	3.86
Coated with PDADMAC	−2.28
Coated with PDADMAC/Poly-SUS	2.88

Conditions: EOF marker, methanol; capillary, 50 μm ID × 58 cm; 20 mM borate buffer, pH 9.2; applied voltage, 20 kV; capillary temperature, 20°C. The negative value indicated the reversal of EOF direction.

and magnitude confirmed that the poly-SUS stationary phase coating for OT-CEC was successfully immobilized on the PDADMAC layer, as expected.

The pH of OT-CEC running buffer plays a crucial role on the separation of analytes because it affects not only the charge of the analyte, but the charge of the capillary inner wall as well. The influence of pH on the separation of five β-blocker analytes is shown in Fig. 2. At pH 4.0, the basic analytes carry positive charges. In addition to a partitioning interaction between the analyte and the hydrophobic core of the polymeric surfactant poly-SUS immobilized on the capillary wall, the positively charged analytes have a strong electrostatic interaction with the sulfate groups on poly-SUS molecule, thus leading to long migration times of the analytes and to broad peaks. When the pH of the buffer is increased, the charges of the analytes decrease, and therefore, the electrostatic interaction between the analytes and the polymeric surfactant is weakened. In addition, the EOF is increased with increasing buffer pH values. In this case, both the molecular interaction and the EOF resulted in good separation at pH 9.0. At this pH, the separation for the five analytes with the polymeric surfactant-coated capillary was improved as compared to the separation with a bare fused-silica capillary.

The influence of the CE applied voltage ranging from 20 to 30 kV on the separation was also investigated. As indicated in Fig. 3, the peak efficiency is improved upon increasing the voltage from 20 to 30 kV, and the migration time is decreased accordingly. Better peak resolution is

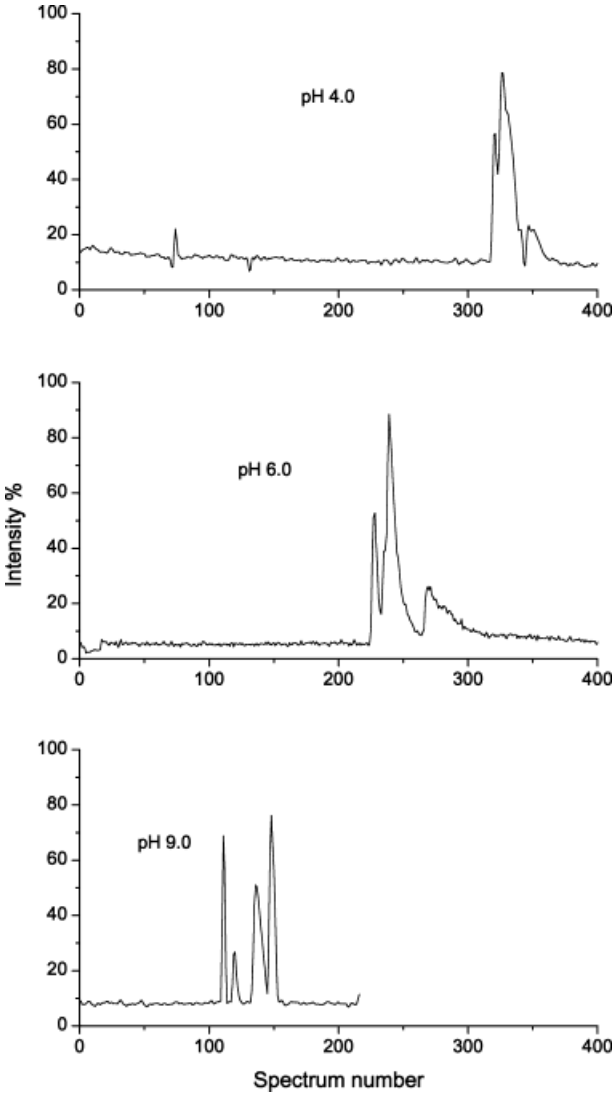


Figure 2. Effect of buffer pH on the separation of five β-blockers in OT-CEC/ESI-MS. CE separation voltage, 25 kV. For other conditions, see Section 2.

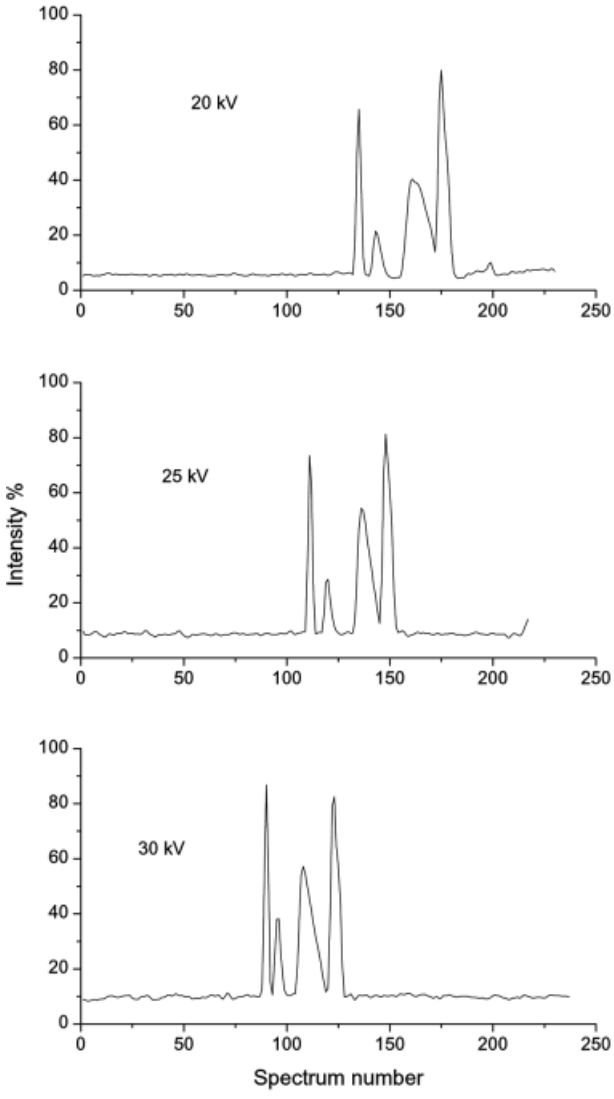


Figure 3. Effect of CE separation voltage on the separation of five β-blockers in OT-CEC/ESI-MS. Buffer pH at 9.0. For other conditions, see Section 2.

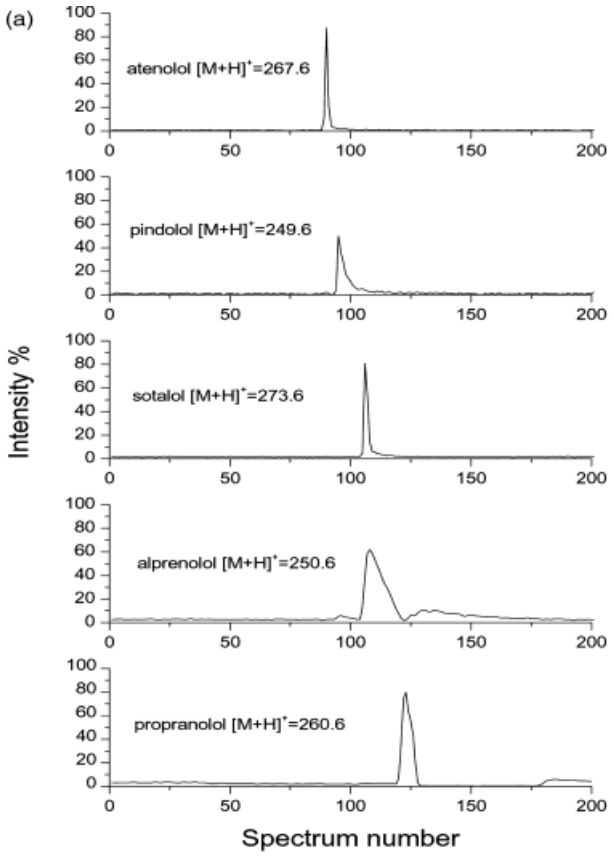
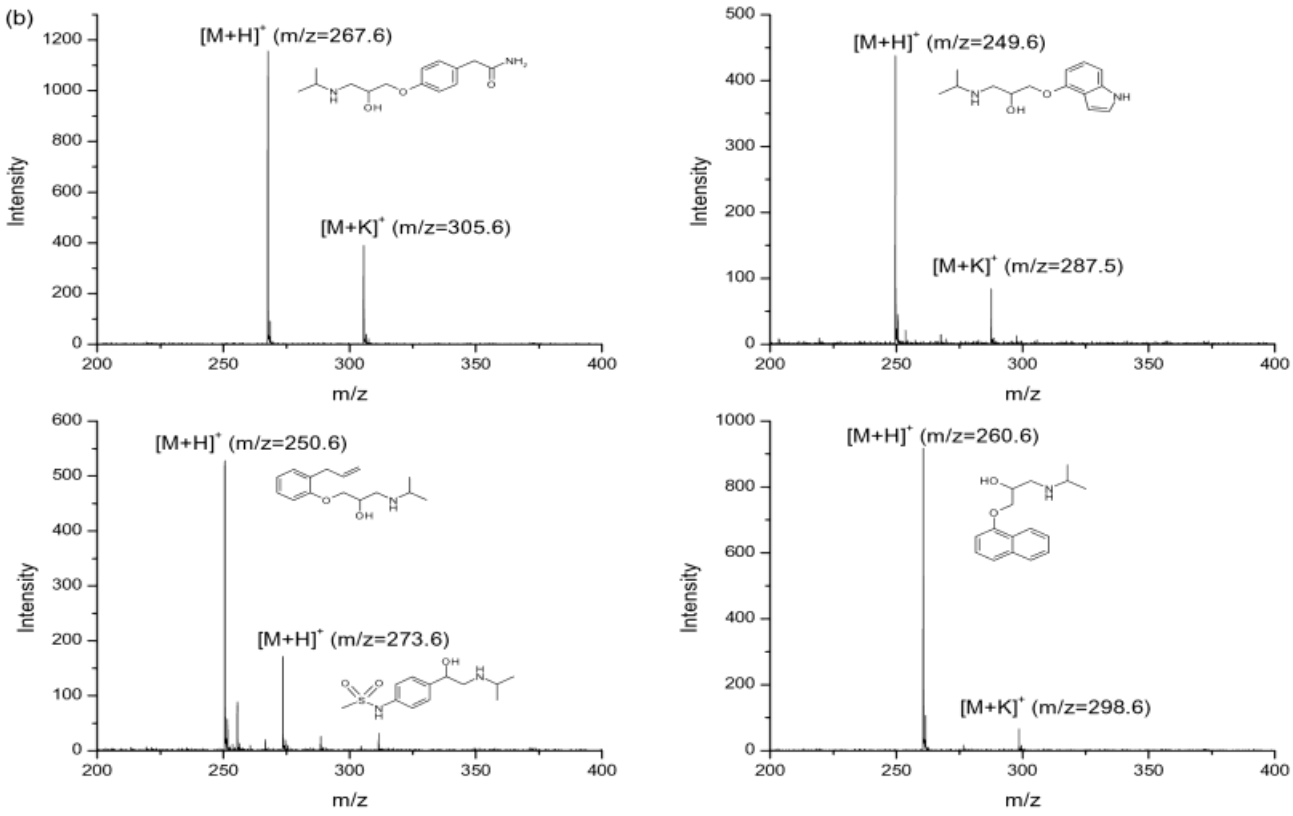


Figure 4. (a) SICs of five β-blockers obtained from OT-CEC/ESI-MS. Atenolol $[M+H]^+$ = *m/z* 267.6, pindolol $[M+H]^+$ = *m/z* 249.6, sotalol $[M+H]^+$ = *m/z* 273.6, alprenolol $[M+H]^+$ = *m/z* 250.6, propranolol $[M+H]^+$ = *m/z* 260.6. CE separation voltage, 30 kV; buffer pH at 9.0. (b) Mass spectra for the five β-blockers, the spectra were extracted individually from the SICs of the analytes as shown in (a), a number of scans were averaged, and the background was subtracted.



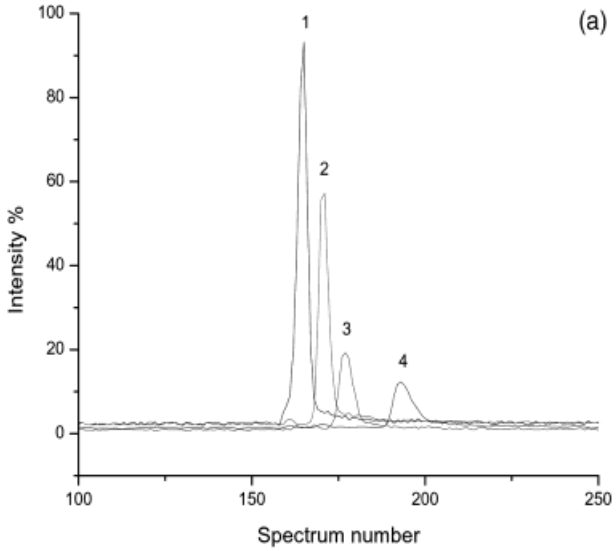
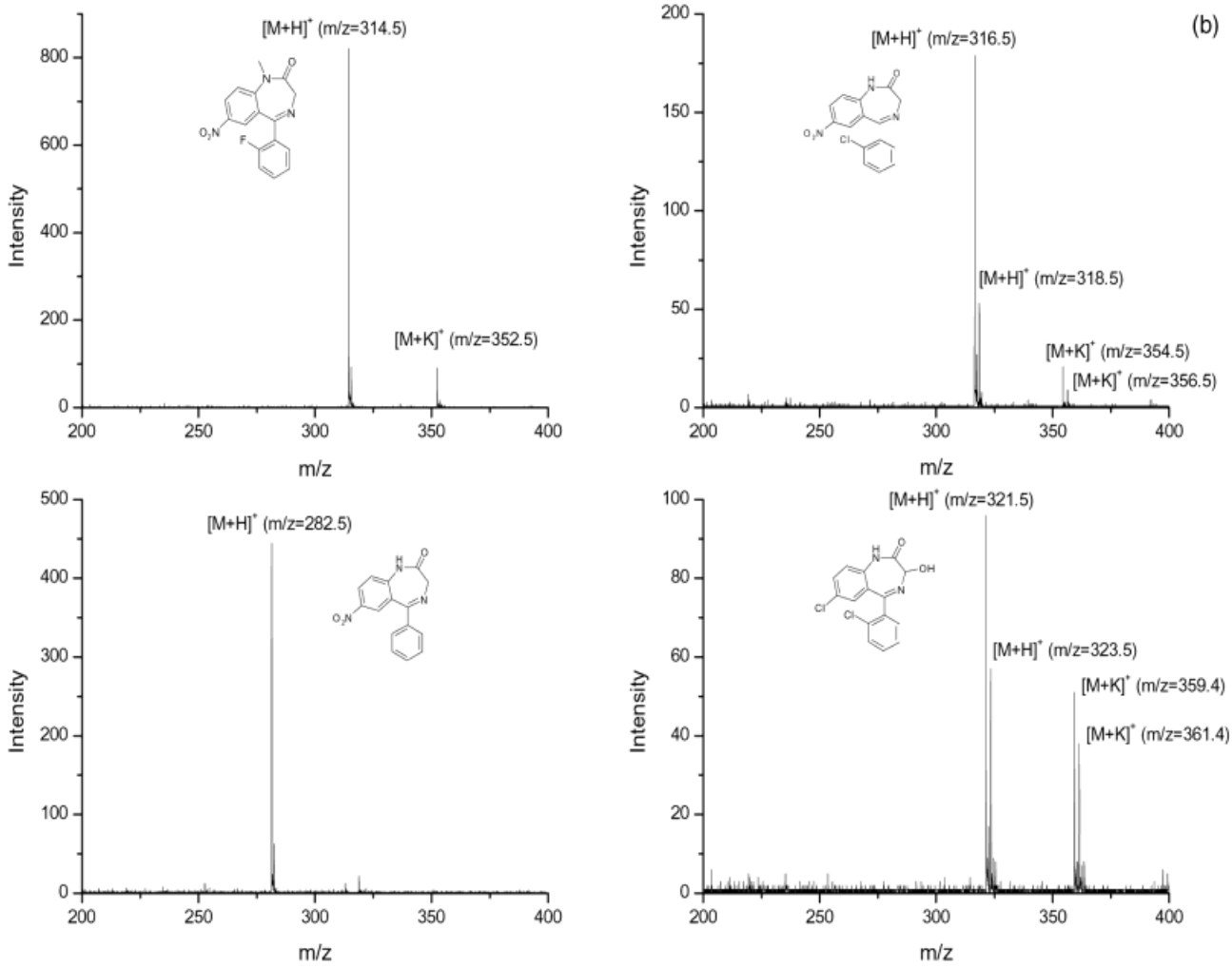


Figure 5. (a) SICs of four benzodiazepines obtained from OT-CEC/ESI-MS. Flunitrazepam (1) $[M+H]^+$ = *m/z* 314.5, nitrazepam (2) $[M+H]^+$ = *m/z* 282.5, clonazepam (3) $[M+H]^+$ = *m/z* 316.5, lorazepam (4) $[M+H]^+$ = *m/z* 321.5. CE separation voltage, 25 kV, buffer pH at 9.0. (b) Mass spectra for the four benzodiazepines, the spectra were extracted individually from the SICs of the analytes as shown in (a), a number of scans were averaged, and the background was subtracted.



achieved at a higher applied voltage. However, the effect of applied voltage on analyte separation is not as significant as varying the buffer pH.

The selected ion chromatograms (SICs) obtained from OT-CEC/ESI-MS and relative mass spectra of the analytes are shown in Figs. 4a and b, respectively. Under the optimum conditions, four of the five β-blockers are separated. The migration order of the analytes can be determined according to their mass signals without the use of standards that might be used for peak identification when a UV-vis detector is employed. The analyte sotalol coelutes with alprenolol under the selected separation conditions, while the later analyte has a broad peak with a lower peak efficiency. Among the five analytes, propranolol has a biphenyl substrate and a higher hydrophobicity. Furthermore, the analytes have similar pK_a values. Therefore, the long migration time of propranolol may result from stronger hydrophobic interactions between the analyte and the core of the poly-SUS. In the mass spectra of the analytes, analyte-potassium adducts were also found. However, the intensity of adducts differed between analytes.

Another successful application of OT-CEC/ESI-MS was the analysis of four benzodiazepines. The SIC and mass spectra of the analytes are shown in Figs. 5a and b, respectively. Baseline separation is achieved for the four analytes. However, the analytes clonazepam and lorazepam have low ionization efficiency (Fig. 5a). In addition to the analyte-potassium adduct signal, the chlorine isotope mass signals, which have two mass unit differences, are also found for the analytes clonazepam and lorazepam (Fig. 5b). The four benzodiazepine analytes failed to be separated from each other and eluted as a single peak when a bare fused-silica capillary was used (data not shown). These results indicate that the polymeric surfactant poly-SUS coating in OT-CEC is very important for baseline separation of the four benzadiazepine analytes, and also acting as a stationary phase.

4 Concluding remarks

The polymeric surfactant poly-SUS was successfully immobilized on the capillary inner wall by a PEM coating. The poly-SUS coating was used as a stationary phase in OT-CEC/ESI-MS. Therefore, it prevents the surfactant from entering the mass spectrometer, and overcomes the MS contamination and surfactant mass signal interference problems that occur in MEKC/MS. The method presented here is a hybrid of MEKC and OT-CEC, giving rise to a favorable detection state for MS.

We acknowledge the National Science Foundation (NSF) and the National Institutes of Health (NIH) for financial support of this research.

Received July 9, 2003

5 References

[1] Smith, R. D., Barinaga, C. J., Udseth, H. R., *Anal. Chem.* 1988, *60*, 1948–1952.

[2] Rundlett, K. L., Armstrong, D. W., *Anal. Chem.* 1996, *68*, 3493–3497.

[3] Lu, W., Poon, G. K., Carmichael, P. L., Cole, R. B., *Anal. Chem.* 1996, *68*, 668–674.

[4] Varghese, J., Cole, R. B., *J. Chromatogr.* 1993, *652*, 369–376.

[5] Palmer, C. P., Khaled, M. Y., McNair, H. M., *J. High Resolut. Chromatogr.* 1992, *15*, 756–762.

[6] Palmer, C. P., Terabe, S., *J. Microcol. Sep.* 1996, *8*, 115–121.

[7] Palmer, C. P., Terabe, S., *Anal. Chem.* 1997, *69*, 1852–1860.

[8] Wang, J., Warner, I. M., *Anal. Chem.* 1994, *66*, 3773–3776.

[9] Shamsi, S. A., Akbay, C., Warner, I. M., *Anal. Chem.* 1998, *70*, 3078–3084.

[10] Akbay, C., Warner, I. M., Shamsi, S. A., *Electrophoresis* 1999, *20*, 145–151.

[11] Ozaki, H., Itou, N., Terabe, S., Takada, Y., Sakairi, M., Koizumi, H., *J. Chromatogr. A* 1995, *716*, 69–79.

[12] Lu, W., Shamsi, S. A., McCarley, T. D., Warner, I. M., *Electrophoresis* 1998, *19*, 2193–2199.

[13] Fridriksson, E. K., Baird, B., McLafferty, F. W., *J. Am. Soc. Mass Spectrom.* 1999, *10*, 453–455.

[14] Kempen, E. C., Brodbelt, J. S., *Anal. Chem.* 2000, *72*, 5411–5416.

[15] Foley, J. P., Masucci, J. A., *Proceedings for the 17th International Symposium on Capillary Chromatography and Electrophoresis*, Wintergreen, VA 1995, p. 278.

[16] Nelson, W. M., Qing, T., Harrata, A. K., Lee, C. S., *J. Chromatogr. A* 1996, *749*, 219–226.

[17] Yang, L., Harrata, A. K., Lee, C. S., *Anal. Chem.* 1997, *69*, 1820–1826.

[18] Molina, M., Weidmer, S. K., Jussila, M., Silva, M., Riekkola, M. L., *J. Chromatogr. A* 2001, *927*, 191–202.

[19] Wu, J., Huang, P., Li, M. X., Qian, M. G., Lubman, D. M., *Anal. Chem.* 1997, *69*, 320–326.

[20] Graul, T. M., Schlenoff, J. B., *Anal. Chem.* 1999, *71*, 4007–4013.

[21] Katayama, H., Ishihama, Y., Asakawa, N., *Anal. Chem.* 1998, *70*, 2254–2260.

[22] Katayama, H., Ishihama, Y., Asakawa, N., *Anal. Chem.* 1998, *70*, 5272–5277.

[23] Qin, W., Li, S. F. Y., *Electrophoresis* 2002, *23*, 4110–4116.

[24] Kapnissi, C. P., Akbay, C., Schlenoff, J. B., Warner, I. M., *Anal. Chem.* 2002, *74*, 2328–2335.

[25] Kamande, M. W., Kapnissi, C. P., Zhu, X., Akbay, C., Warner, I. M., *Electrophoresis* 2003, *24*, 945–951.