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Analytical Separations Using Molecular Micelles in Open-Tubular Capillary Electrochromatography

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Open-tubular capillary electrochromatography (OT-CEC) is an alternative approach to conventional CEC. The primary advantage of OT-CEC is the elimination of problems associated with frits and silica particles in conventional CEC. This report is an investigation of the utility of using a polymeric surfactant (molecular micelle) for OT-CEC. In this approach, fused-silica capillaries coated with thin films of physically adsorbed charged polymers are developed by use of a polyelectrolyte multilayer (PEM) coating procedure. The PEM coating is constructed in situ by alternating rinses with positively and negatively charged polymers, where the negatively charged polymer is a molecular micelle. This can offer a number of advantages for separation of hydrophobic analytes. In this study, poly(diallyldimethylammonium chloride) was used as the cationic polymer and poly(sodium *N*-undecanoyl-L-glycinate) was used as the anionic polymer for PEM coating. The performance of the modified capillaries as a separation medium is evaluated by use of seven benzodiazepines as analytes. The run-to-run, day-to-day, week-to-week, and capillary-to-capillary reproducibilities of electroosmotic flow are very good with relative standard deviation values of less than 1% in all cases. In addition, the chromatographic performance of the monomeric form of the molecular micelle is compared for the separation of these analytes. The PEM-coated capillary was remarkably robust with more than 200 runs accomplished in this study. Strong stability against extreme pH values was also observed. The general utility of this approach is discussed in detail.

Capillary electrochromatography (CEC) is a hybrid electroseparation technique that couples the selectivity of high-performance liquid chromatography (HPLC) and the separation efficiency of capillary electrophoresis (CE).[1−5] Such studies have demonstrated that CEC provides high resolution, short analysis time, smaller sample and buffer consumption, and efficiencies 5−10 times higher than HPLC. The separation in CEC is based upon the electrophoretic mobility of the solutes and their partitioning between the stationary and mobile phases.

In the development of CEC, both packed and open-tubular column configurations have been reported.[1−10] The conventional form of CEC uses a fused-silica capillary with a typical internal diameter of 50−100 μm, packed with a typical HPLC stationary phase, such as an octadecyl silica (ODS) stationary phase.[2,4] However, there are several problems that need to be solved in order for packed-CEC to be a viable alternative to either CE or HPLC. One of the limitations of conventional CEC is the necessity to fabricate frits, which are required for retention of the packed particles within the column. In addition, packed capillaries have the tendency to form bubbles around the packing material or at the frit. Such problems often result in an unstable baseline, nonreproducible migration times, or even current breakdown. To circumvent this potential problem, pressurization of both ends of the column is required and the solvent must be thoroughly degassed. Another common problem of conventional CEC is that the packing procedure is more difficult than for HPLC because of the narrow inner diameter of the capillary and the small diameter of the particles. Finally, the separation of basic compounds with packed-CEC can be difficult due to dissociation of the silanol groups, which is needed to generate an adequate electroosmotic flow (EOF).

Open-tubular CEC (OT-CEC) is an alternative approach to packed-CEC.[9] None of the problems mentioned above are likely to be encountered in an open-tubular format. In this CEC format, a stable coating needs to be constructed on the inner walls of the capillary in order to provide efficient chromatographic separations and reproducible EOF.[10] The most commonly used approaches to wall coatings for modifying the capillary include the following: (1) dynamic coating performed by adding the cationic or neutral modifier to the electrolytes;[11,12] (2) adsorbed cationic modifier on the capillary wall by physical adsorption;[13−16] and (3) fixation of

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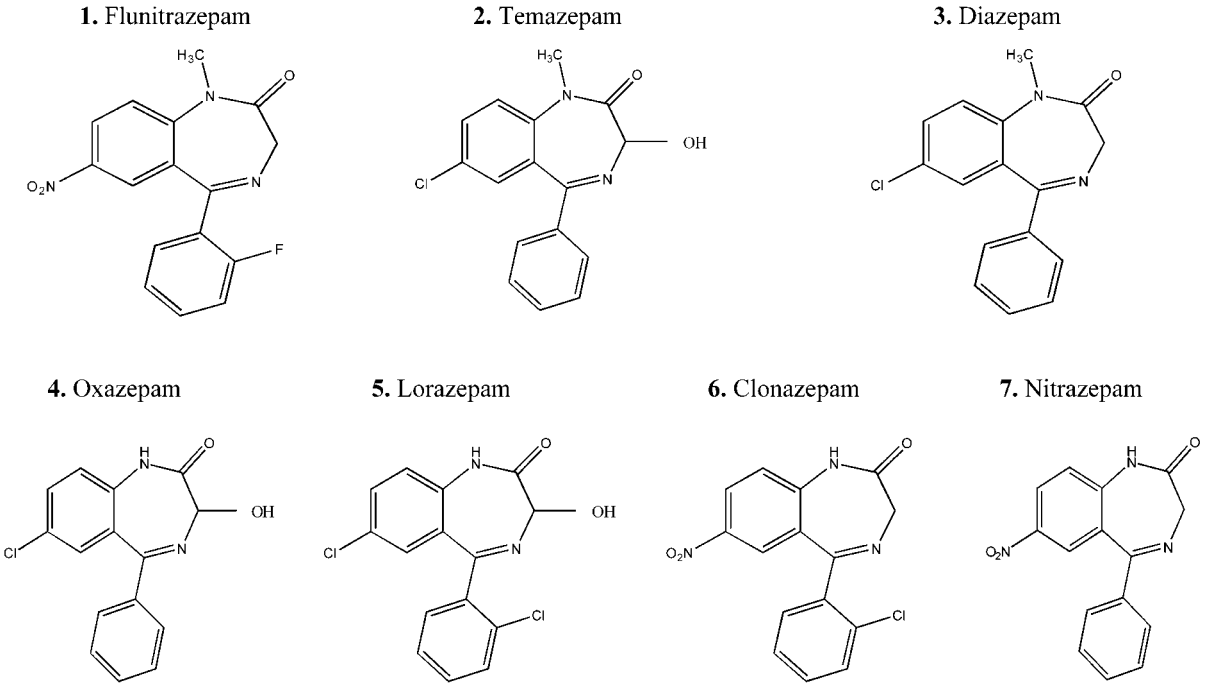

Figure 1. Structures of the seven benzodiazepine analytes.

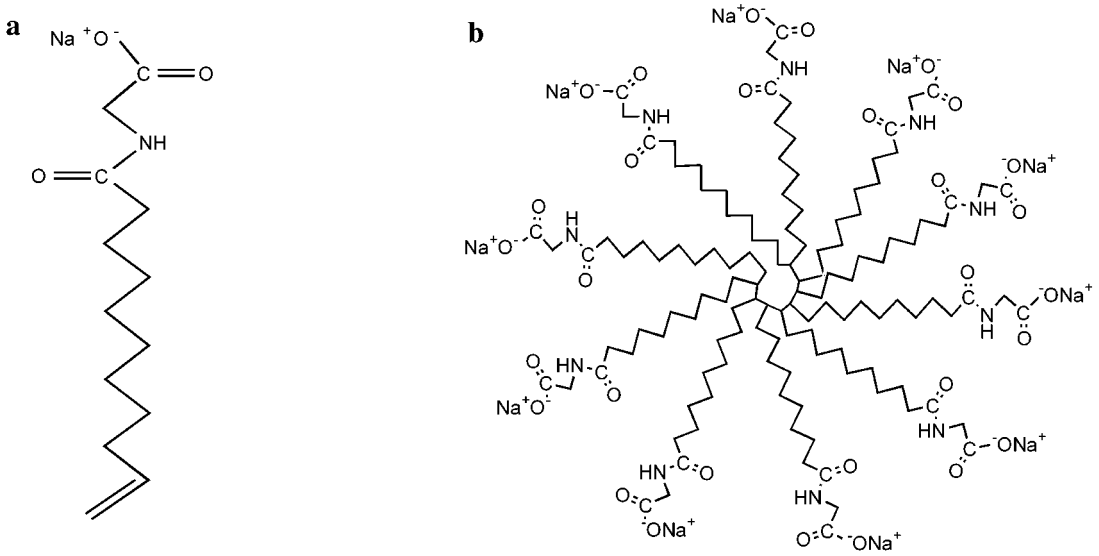

Figure 2. Structures of the (a) monomeric SUG and (b) polymeric SUG.

the hydrophilic layer by covalent bonding or cross-linking.[17−22] Harrell et al. achieved a baseline separation of seven tricyclic antidepressants by use of a novel nonionic micelle polymer, poly(*n*-undecyl α-D-glucopyranoside) (PUG) as a dynamic coating.[23] However, dynamic coating is known to cause problems when CE is coupled to mass spectrometry (CE/MS). In addition, the presence of the nonvolatile buffer constituents may deteriorate the ionization of the analytes.[24,25] Although physical adsorption has a simple and rapid coating procedure and good reproducibility, it has been shown to have a short lifetime and limited pH range.[24,26] In contrast, some of the covalent bonding or cross-linking have a long lifetime, but require a more complicated coating procedure.[24,26] Obviously, an ideal coating procedure would be one that is both simple and stable.

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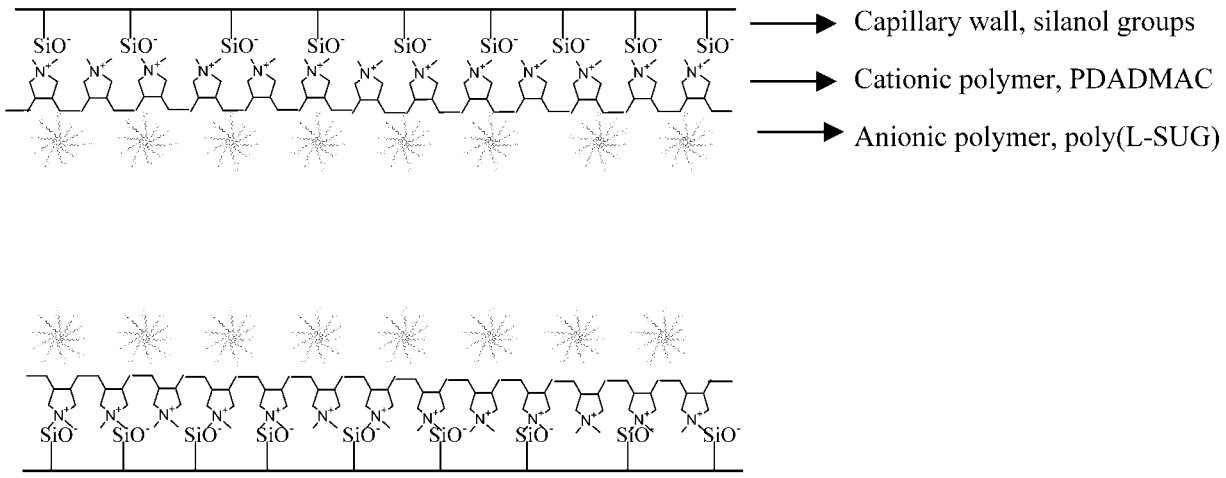

Figure 3. Scheme of the PEM-coated capillary.

In this report, we explore an alternative to covalent linking of a polymer to silica beads. In our approach, we use polymeric surfactants (molecular micelles) in a simple coating procedure, which involves layer-by-layer deposition process.[27,28] The coating is thus a polyelectrolyte multilayer (PEM), constructed in situ by alternating rinses of positively and negatively charged polymers.[29−33] Via electrostatic forces, a layer of polymer adds to the oppositely charged surface, reversing the surface charge and priming the film for the addition of the next layer. The mechanism of formation and charge balance in PEMs has been explored.[33−37] Such coatings have been found to be robust and thus highly resistant to charge and deterioration during use.[24,26,32] The advantages of our PEM coating are twofold. First, since the polymeric surfactant is coated electrostatically onto the capillary, less consumption of the reagent is required. Second, with the molecular micelle coated on the capillary, there is less detection interference with the analyte of interest, which in turn makes the system more amenable to coupling with mass spectrometry or other detectors where the polymeric surfactant reagent interferes.

We describe here our PEM coating approach for fabricating open-tubular columns for use in OT-CEC. The performance of the modified capillaries as a separation medium is evaluated by use of seven benzodiazepines as analytes. The coating was found to be remarkably stable with excellent performance for more than 200 runs.

EXPERIMENTAL SECTION

Apparatus and Conditions. Separations were performed on a Beckman P/ACE MDQ capillary electrophoresis system with UV detection (Fullerton, CA). The fused-silica capillary, 57 cm (50-cm effective length) × 50 μm i.d., was purchased from Polymicro Technologies (Phoenix, AZ) and mounted in a Beckman capillary cartridge. Unless stated otherwise, the cartridge temperature was maintained at 25 °C by use of liquid coolant. UV detection was performed at 214 nm, and the samples were injected by pressure (0.1 psi; 1 psi = 6894.76 Pa) for 1 s.

Reagents and Chemicals. Flunitrazepam, temazepam, diazepam, oxazepam, lorazepam, clonazepam, and nitrazepam were purchased from Sigma Chemical Co. (St, Louis, MO). The structures of the analytes used in this study are shown in Figure 1. Sodium phosphate (Na_2HPO_4 and NaH_2PO_4), hydrochloric acid, and sodium chloride were all obtained from Fisher Scientific (Fair Lawn, NJ). Poly(diallyldimethylammonium chloride) (PDADMAC; M_w=200 000−350 000) was obtained from Aldrich (Milwankee, WI). Other chemicals, including L-glycine, undecylenic acid, and *N*-hydroxysuccinimide, were also purchased from Sigma.

Sample and Buffer Preparation. Analytical standard benzodiazepine stock solutions were prepared in methanol−water (1:1) at concentrations of ~0.15 mg/mL each. A buffer solution of 50 mM Na_2HPO_4 was prepared by dissolving the appropriate amount of Na_2HPO_4 in 10 mL of deionized water. The solution was filtered using a polypropylene nylon filter with 0.45-μm pore size and sonicated for 15 min before use.

Synthesis of Monomeric and Polymeric Surfactant. The surfactant monomer of sodium *N*-undecylenyl-L-glycinate (mono-(L-SUG)) was synthesized from the *N*-hydroxysuccinimide ester of undecylenic acid according to a previously reported procedure.[38] A 100 mM sodium salt solution of the monomer was then polymerized by use of ^{60}Co γ radiation. After irradiation, the polymer was dialyzed by use of a 2000 molecular mass cutoff and then lyophilized to obtain the dry product. Structures of the monomeric and polymeric surfactant are illustrated in Figure 2.

Procedure for Polyelectrolyte Multilayer Coating. PEM coating was achieved by deposition of the polymer solutions using the rinse function on the Beckman CE system. Each polymer deposition solution contained 0.5% (w/v) polymer in 0.2 M aqueous NaCl solution. It was observed that the addition of NaCl to the polymer solution resulted in enhanced thickness for each polyelectrolyte layer.[32] The capillary was conditioned before coating using a 5-min rinse of water, to remove any contaminants originating from the capillary manufacturing process. The column

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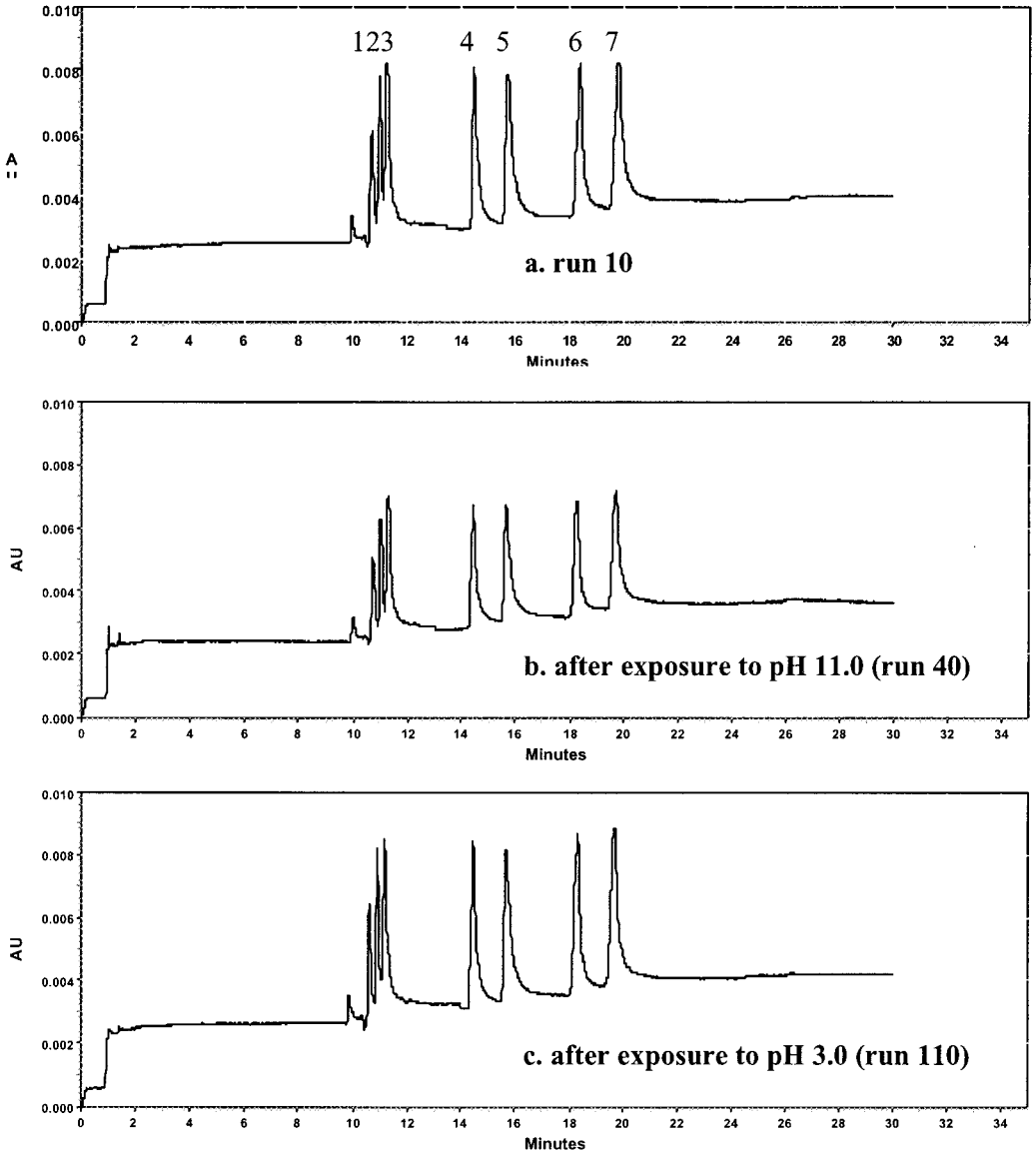

Figure 4. Stability studies of PEM coating. Conditions: 0.5% (w/v) PDADMAC and 0.5% (w/v) poly(L-SUG) with 0.2 M NaCl; pressure injection, 0.1 psi for 1 s; electrolyte, 50 mM Na_2HPO_4 (pH 9.2); applied voltage, 20 kV; temperature, 25 °C; capillary, 57 cm (50 cm effective length) × 50 μm i.d.; detection, 214 nm.

Table 1. Reproducibilities of PEM Capillary Coating[a]

	EOF average (min)	RSD (%)
run to run ($n = 50$)[b]	9.990	0.78
day to day ($n = 5$)[b]	9.982	0.81
week to week ($n = 3$)[b]	9.930	0.86
capillary to capillary ($n = 25$)[c]	9.960	0.98

[a] n, number of runs. Conditions: same as Figure 4. [b] These studies were done in the same capillary. [c] This study was done in five different capillaries. Five consecutive runs were performed in each capillary.

was then conditioned by rinsing with 1 M NaOH for 60 min. Pure deionized water was flushed through the capillary for 15 min more. The first monolayer of polymer (PDADMAC) was deposited by rinsing the solution of the cationic polymer through the capillary for 20 min followed by a 5-min water rinse. All other polymer depositions were done with 5-min rinses followed by 5-min water rinses. All processes were performed by continuous dynamic rinsing of the reagents through the capillary. A diagrammatic scheme of the PEM-coated capillary is shown in Figure 3. This diagram is not provided to give an actual structural representation of the bilayer, but only to represent the order of polymer deposition. Current studies are ongoing in our laboratory to better understand the structure of the bilayers. The multilayer coatings used for the separation of benzodiazepines and the reproducibility studies consisted of 10 layer pairs (a layer pair is a layer of cationic polymer plus a layer of anionic polymer; also termed a bilayer). The capillary was then flushed with buffer until a stable current was achieved. The columns were conditioned with buffer for 2 min between injections.

The thickness of the coating fabricated on a silicon wafer with a robotic platform was determined in one of our laboratories using a Gaertner Scientific L116B Autogain ellipsometer. The thickness of 10 bilayers on the wafer surface was ~1200 Å. Typical studies on the determination of the thickness of a poly(styrenesulfonate)

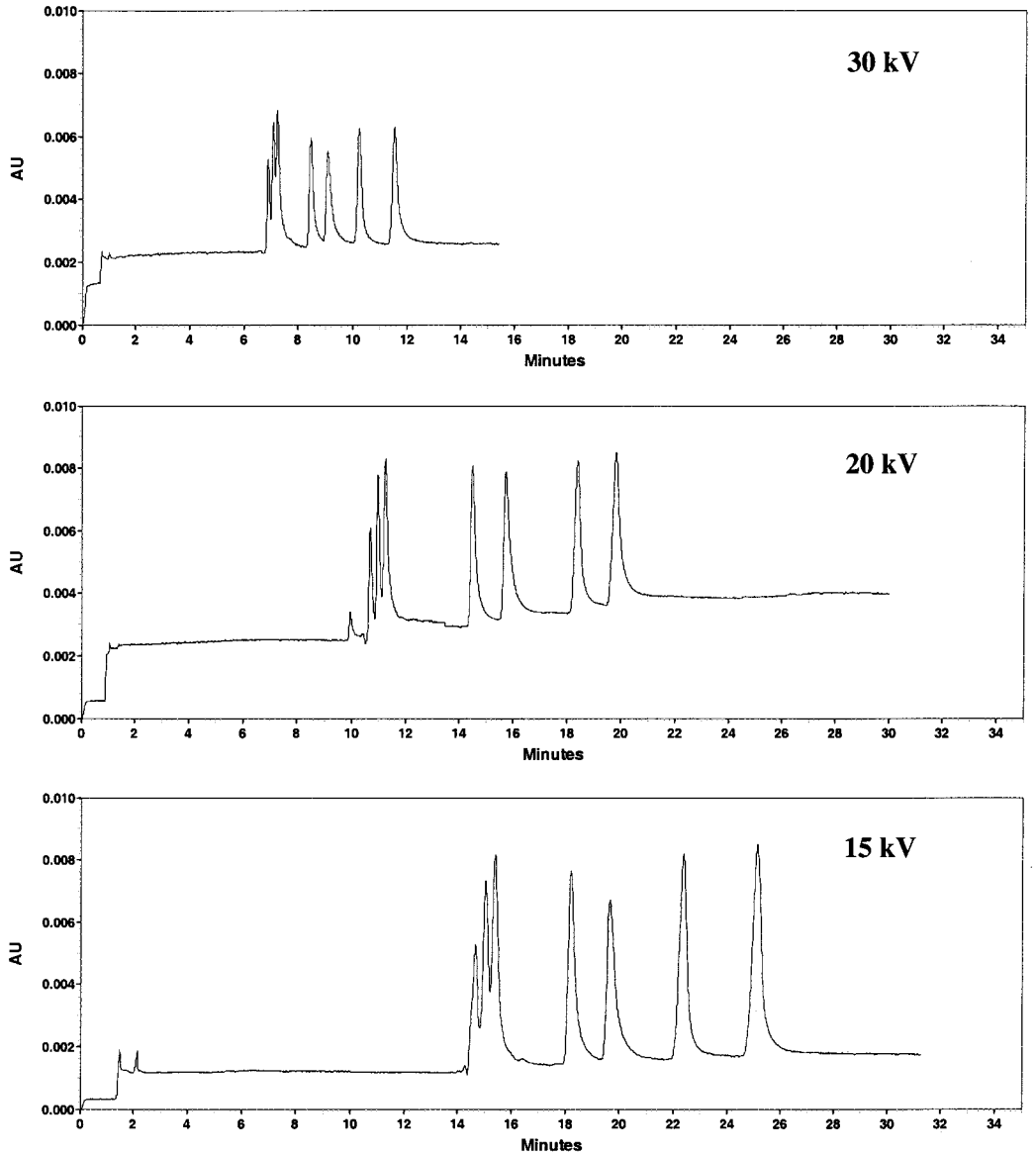

Figure 5. Effect of applied voltage on the OT-CEC separation of benzodiazepines. Conditions: same as Figure 4, except applied voltage was varied.

(PSS)/PDADMAC multiplayer have already been done by others.[35] The thickness of a 10-bilayer film deposited on silicon wafer from 0.2 M NaCl was ~500 Å.[35]

RESULTS AND DISCUSSION

Column Performance. (1) Measurement of EOF. In CEC, the transport of mobile phase through the capillary is achieved by the EOF, which is generated due to the electrical double layer that exists at the solid−liquid interface of the charged silica surface in contact with an electrolyte solution.[39] The EOF, μ_{eo}, is defined as

$$\mu_{eo} = L_d L_t / V t_o,$$

where L_d is the distance from injector to detector, L_t is the total capillary length, t_o is the migration time of the electroosmotic flow marker, and V is the applied voltage. The relative EOF can be monitored by use of the values of t_o since all other factors should be constant. In the studies reported here, the values of t_o were measured by use of methanol, which is expected to be unretained by the OT-CEC column. Electroosmotic mobility (μ_{eo}) and migration time of methanol (t_o) were also used to evaluate the stability of the PEM coating.

The retention mechanism of benzodiazepines on this PEM-coated phase is based on the differential partitioning of the analytes into the coating. Their retention is determined by hydrophobic interactions between the hydrophobic core of the polymer and the nonpolar moiety of each analyte. Therefore, the migration order of the benzodiazepines was $t_R^1 < t_R^2 < t_R^3 < t_R^4 < t_R^5 < t_R^6 < t_R^7$ (the superscripted numbers are the number designation for the analytes in Figure 1).

(2) Endurance of PEM Coating. An important aspect of this approach, which must be considered, is the lifetime of the stationary phase. Thus, we examined the stability of our coating

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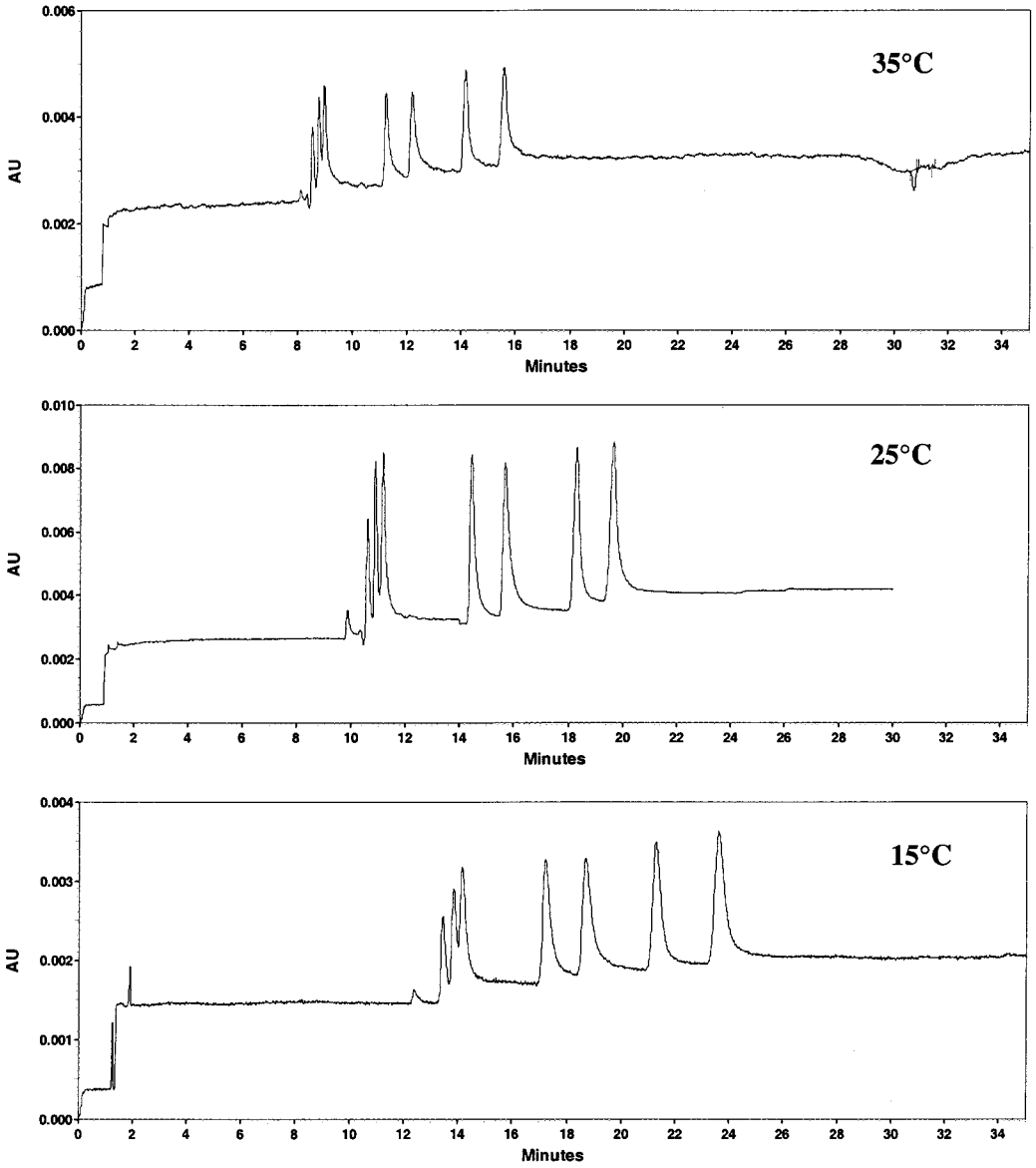

Figure 6. Effect of temperature on the OT-CEC separation of benzodiazepines. Conditions: same as Figure 4, except the temperature was varied.

by use of the following procedure. A 10-layer pair multilayer was constructed, and nearly 50 separations were performed within 5 days. Each separation was done with applied voltages of 15−30 kV at 25 °C. The electrolyte concentration was 50 mM Na_2HPO_4, and a pH range 9.2−11.0 was used. After these experiments, the capillary, filled with running buffer, was removed from the instrument, and the tips of the column were placed in deionized water vials for one week. Before the capillary was reused, it was reconditioned with the 50 mM phosphate buffer (pH 9.2). Fifty replicate runs were then performed using an applied voltage of 20 kV and a temperature of 25 °C. The capillary was again removed from the instrument and the tips were placed in water vials for an additional week, after which the column was placed back into the instrument and more than 100 runs were performed. In these studies, both the applied voltage and the temperature were varied from 15 to 30 kV and from 15 to 35 °C, respectively. Therefore, the aggregate performance of the PEM-coated capillary was evaluated for more than 200 runs.

(3) Stability of PEM Coating. Another important factor to consider when PEM-coated capillaries are used is the stability of the capillary surface, especially after exposure to solutions with extreme pH values. To evaluate this parameter, two more phosphate buffers of pH 11.0 and 3.0 were prepared. First, 30 replicate runs were performed with 50 mM phosphate buffer (pH 9.2). The 10th run (Figure 4a) yielded a μ_{eo} of 2.39×10^{-3} cm^2 V^{-1} s^{-1}. The capillary was then flushed with 50 mM phosphate buffer (pH 11.0) for 100 min and rinsed with 50 mM phosphate buffer (pH 9.2) for 30 min. One of the electropherograms obtained after the exposure to pH 11.0 (Figure 4b) gave a μ_{eo} of 2.37×10^{-3} cm^2 V^{-1} s^{-1}. After this, the same procedure was followed with the 50 mM phosphate buffer (pH 3.0). One of the runs that was performed after the last exposure (Figure 4c) yielded a μ_{eo} of 2.37×10^{-3} cm^2 V^{-1} s^{-1}. Therefore, the PEM coating was demonstrated to have extraordinary stability under extreme values of pH.

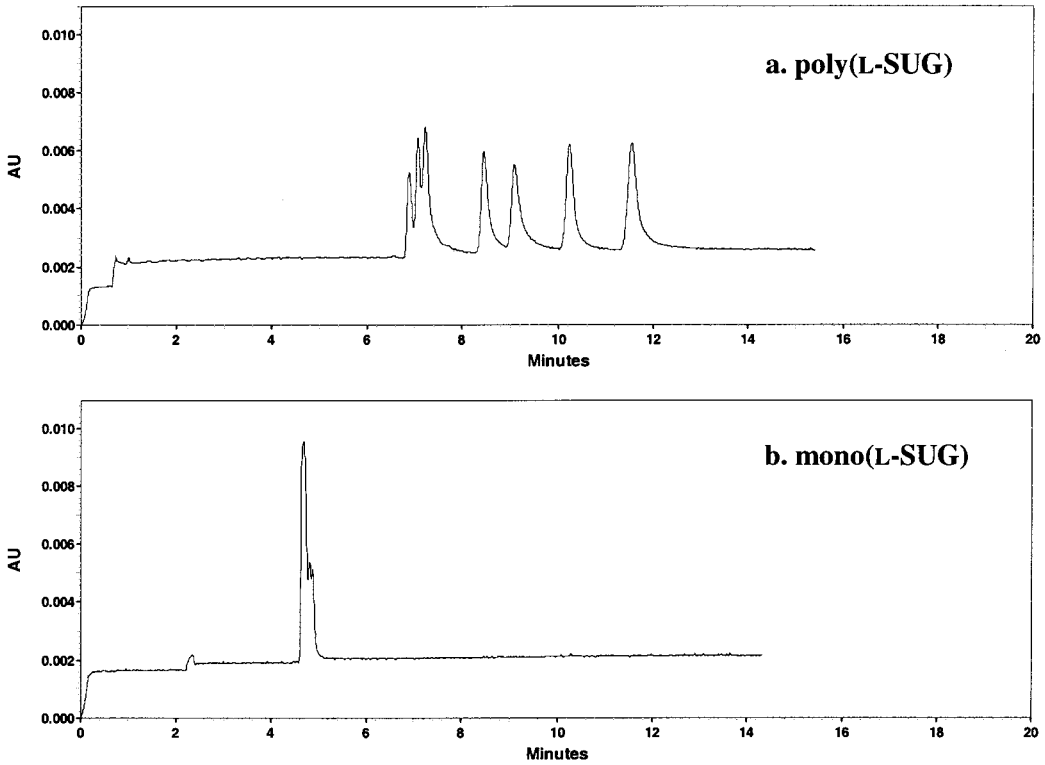

Figure 7. Comparison between monomeric and polymeric surfactants for OT-CEC separation of benzodiazepines. Conditions: same as Figure 4, except applied voltage was 30 kV: (a) 0.5% (w/v) poly(L-SUG); (b) 0.5% (w/v) mono(L-SUG).

(4) Reproducibilities. Reproducibility of the PEM coating is also an important consideration. The reproducibilities were evaluated by computing the relative standard deviations (RSDs)[40] of the EOF, which are reported in Table 1. The run-to-run RSD was obtained from 50 consecutive electrophoresis runs; the day-to-day RSD was obtained by use of 5 replicate analyses; the week-to-week RSD was obtained by use of 3 replicate analyses; the capillary-to-capillary RSD was obtained by use of 25 runs (5 consecutive runs performed in 5 different capillaries). All RSDs of the EOF were below 1%, and thus, very good reproducibilities were observed.

Application. (1) Voltage Study. The PEM-coated capillary was applied to the separation of seven benzodiazepines. The influence of the applied voltage on the efficiency, resolution, and analysis time of the benzodiazepines was evaluated using a mobile phase of 50 mM Na_2HPO_4 at 25 °C. As expected, a higher voltage decreased the retention times. At 30 kV, the analytes eluted faster with higher efficiency and lower resolution. In contrast, at 15 kV, the migration times were longer, the resolution was higher, and the efficiency was lower. However, an applied voltage of 15 kV did not have a major impact on analyte resolution, compared to the electropherogram obtained when a 20-kV voltage was applied (Figure 5).

(2) Temperature Study. The effect of temperature on the separation of benzodiazepines was also studied. The temperature for this study was varied from 35 to 15 °C. As shown in Figure 6, the retention time decreased at higher temperature, and peak efficiency decreased at lower temperature. In addition, electroosmotic mobility increased when temperature increased, likely due to a decrease in electrolyte viscosity.

(3) Comparison between Monomeric and Polymeric Surfactants. Another important consideration for this study is whether molecular micelles are needed to form an effective and stable PEM, or can the same be achieved by use of monomeric surfactants. In an effort to compare the chromatographic performance of mono(L-SUG) and poly(L-SUG) for the separation of hydrophobic analytes, benzodiazepines were used as test solutes. The separation of benzodiazepines using 50 mM Na_2HPO_4 (pH 9.2) as the electrolyte and 0.5% (w/v) poly(L-SUG) as the anionic polyelectrolyte for the construction of the PEM coating is shown in Figure 7a. Figure 7b is the electropherogram of the benzodiazepines under the same conditions as in Figure 7a. However, the anionic surfactant used for PEM coating construction in this figure is the monomeric (nonpolymerized) surfactant at the concentration of 0.5% (w/v; 19 mM). Almost no separation is noted, even though the monomeric surfactant concentration is significantly above the normal cmc of the nonpolymerized surfactant (7 mM). Thus, it is clear for this study that the molecular micelle allows better discrimination of the hydrophobic analytes than the conventional micelle. In a normal (nonpolymerized) micellar system, the dynamic equilibrium that exists between the monomers and micellar aggregates has been demonstrated to be a disadvantage for separations.[33] This dynamic equilibrium will likely reduce the stability of the PEM coating in OT-CEC. In such a case, poor analyte separation will be observed as seen here. In contrast, polymeric micelles do not have such problems because the covalent bonds formed between monomers eliminate dynamic equilibrium. Thus, the coating that is produced will be more stable as is observed in this study.

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CONCLUSIONS

A stable modified capillary has been developed by use of a simple PEM coating procedure employing a molecular micelle. Excellent reproducibility in separation is observed. In addition, the PEM-coated capillaries exhibited high stability against extreme pH values. The stability of the capillaries allowed us to perform over 200 runs with RSDs of less than 1%. This approach also shows that highly efficient and reproducible peaks can be obtained from stationary phases prepared by such a simple procedure. We conclude that this method is a promising alternative to conventional CEC and should prove very useful in both chiral and achiral separations.

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