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Using the cationic surfactants *N*-cetyl-*N*-methylpyrrolidinium bromide and 1-cetyl-3-methylimidazolium bromide for sweeping–micellar electrokinetic chromatography

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ABSTRACT

This paper describes a sweeping–micellar electrokinetic chromatography (sweeping–MEKC) technique for the determination of seven benzodiazepines, using, as sweeping carriers, the ionic liquid-type cationic surfactants 1-cetyl-3-methylimidazolium bromide (C_{16}MIMBr) and *N*-cetyl-*N*-methylpyrrolidinium bromide (C_{16}MPYB). These surfactants resemble the commonly employed cationic surfactant cetyltrimethylammonium bromide (CTAB), but they provide different separation efficiencies. We optimized the separation and sweeping conditions, including the pH, the concentrations of organic modifier and surfactant, and the sample injection volume. Adding C_{16}MIMBr or C_{16}MPYB to the background electrolyte enhanced the separation efficiency and detection sensitivity during the sweeping–MEKC analyses of the benzodiazepines. C_{16}MIMBr enhanced the sensitivity for each benzodiazepine 31–59-fold; C_{16}MPYB, 86–165-fold. In the presence of C_{16}MPYB, the limits of detection for the seven analytes ranged from 4.68 to 9.75 ng/mL. We adopted the sweeping–MEKC conditions optimized for C_{16}MPYB to satisfactorily analyze a human urine sample spiked with the seven benzodiazepines. To minimize the matrix effects, we subjected this urine sample to off-line solid phase extraction (SPE) prior to analysis. The recoveries of the analytes after SPE were satisfactory (ca. 77.0–88.3%). Our experimental results reveal that the cationic surfactant C_{16}MPYB exhibits superior sweeping power relative to those of C_{16}MIMBr and CTAB and that it can be applied in sweeping–MEKC analyses for the on-line concentrating and analyzing of benzodiazepines present in real samples at nanogram-per-milliliter concentrations.

1. Introduction

Ionic liquids (ILs) are a class of nonmolecular ionic solvents with melting points fixed at or below 100 °C. They are called room temperature ILs (RTILs) if the melting points of ILs are below room temperature (ca. 25 °C) [1]. ILs usually contain bulky nitrogen- or phosphorous-centered organic cations and inorganic or organic counteranions [2]. ILs can be regarded as designer solvents because the choice of anion and cation affects their properties [3]. Although ILs exhibit many of the properties of conventional organic solvents, including excellent solvation over a wide range of viscosities and temperature [4], their negligible vapor pressure and nonvolatility make them more useful in green chemistry applications [1].

A common feature of ILs involves a charged hydrophilic "head" group and one or more hydrophobic "tails"; such ILs resemble conventional surfactants [5] and, indeed, micelles form in solution when the ILs concentration exceeds the critical micelle concentration (CMC) [6–10]. Bowers et al. reported that ILs based on 1-alkyl-3-methylimidazolium salts behave just like short-chain cationic surfactants in aqueous solution; they used surface tension, conductivity, and small-angle neutron scattering (SANS) measurements to determine the aggregation behavior of 1-butyl-3-methylimidazolium tetrafluoroborate (C_4MIMBF$_4$), 1-octyl-3-methylimidazolium chloride (C_8MIMCl), and 1-octyl-3-methylimidazolium iodide (C_8MIMI) [6]. Baker et al. studied the micellization of a homologous series of *N*-alkyl-*N*-methylpyrrolidinium bromides (C_nMPYB; n = 10–18) and suggested that these salts could constitute a new class of surfactants [7]. After Dong et al. studied micelle formation from the long-chain ILs C_{14}MIMBr to C_{16}MIMBr, they concluded that the CMCs of these imidazolium-based systems are considerably lower than those of typical cationic alkyltrimethylammonium (C_nTAB)-based surfactants, i.e., they form micelles more readily [10].

Micellar electrokinetic chromatography (MEKC) is a powerful extension of capillary electrophoresis (CE) that allows the separation of mixtures of uncharged and/or charged compounds [11–13]. ILs have received much attention for their use in separation science, and have been applied in recent years as additives

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to improve MEKC-based separations (i.e., for improved resolution and peak efficiency) because of their high conductivity, hydrophobicity, and solvating property [14–16]. Several groups have studied the separation mechanism that occurs in MEKC when ILs are added into the background electrolyte (BGE) containing surfactants. Borissova et al. introduced the surfactant 1-tetradecyl-3-methylimidazolium chloride into the BGE as a pseudostationary phase to successfully separate neutral analytes, such as methylresorcinol isomers and other benzene derivatives [15]. From a study of pyrrolidinium-based surfactants in MEKC, Schnee et al. found that the presence of C_nMPYB micellar pseudophase provided highly efficient separations because the interactions of these less-hydrophobic surfactants with polar compounds are stronger than those of cetyltrimethylammonium bromide (CTAB), a commonly employed surfactant [16]. According to these findings, imidazolium- and pyrrolidinium-based surfactants appear to have great potential for application in MEKC and other analytical methods.

The rapidity, efficiency, and selectivity of CE make it a suitable method for separating benzodiazepines. Although several groups have combined MEKC with sodium dodecyl sulfate (SDS) micelles to determine benzodiazepines [17–19], the short optical path length for UV detection usually results in poor sensitivity. Quirino and Terabe developed sweeping–MEKC as an on-line preconcentration technique that improves the sensitivity of MEKC [20,21]. In sweeping–MEKC, a surfactant is added to the BGE at a concentration above its CMC. The sensitivity enhancement depends upon the analyte's retention factor, the ratio of the number of solute species in the micellar phase to that in the aqueous phase [22]. The most widely used surfactants in sweeping–MEKC are anionic (e.g., SDS) [23], cationic (e.g., CTAB) [24], nonionic [25], and polymeric [26] surfactants.

In this paper, we report the use of IL-type cationic surfactants as sweeping carriers for the pre-concentration of benzodiazepines during the sweeping–MEKC process. We investigated 1-cetyl-3-methylimidazolium bromide (C_{16}MIMBr) and *N*-cetyl-*N*-methylpyrrolidinium bromide (C_{16}MPYB) as sweeping cationic surfactants because (i) imidazolium- and pyrrolidinium-based surfactants both possess favorable micellar properties [7,10] and (ii) of the known IL-type surfactants, they exhibit micelle characteristics most similar to those of the surfactant CTAB (e.g., an ammonium head group and a 16-carbon linear hydrocarbon tail), while displaying different separation selectivities when applied to MEKC. We compared C_{16}MIMBr and C_{16}MPYB with CTAB, for their abilities to pre-concentrate benzodiazepines, predicting that these IL-type surfactants would display sweeping powers that would enhance the sensitivity of on-line concentration techniques. To test the sweeping efficiencies of C_{16}MIMBr and C_{16}MPYB, we employed them to determine the optimal separation and sweeping conditions for the analyses of seven benzodiazepines using sweeping–MEKC. We then applied the optimized method to analyze SPE-treated benzodiazepine-spiked human urine samples.

2. Experimental

2.1. Chemicals

All reagents and chemicals used were of analytical grade. Diazepam (MW = 284.7 g/mol), clorazepate (MW = 408.9 g/mol), chlordiazepoxide (MW = 336.2 g/mol), bromazepam (MW = 316.2 g/mol), nitrazepam (MW = 281.3 g/mol), alprazeolam (MW = 308.8 g/mol), and flunitrazepam (MW = 313.3 g/mol) standards were obtained from Sigma–Aldrich (St. Louis, MO, USA); their chemical structures are presented in Fig. 1. Borax ($Na_2B_4O_7{\cdot}10H_2O$), hydrochloric acid (HCl), ammonium hydroxide (NH_4OH), 2-propanol (IPA), and *d*-chloroform ($CDCl_3$) were also purchased from Sigma–Aldrich. 1-Methylimidazole (99%), 1-bromohexadecane ($C_{16}H_{33}Br$, 99%), and 1-methylpyrrolidine (98%) were obtained from Acros Organics (Geel, Belgium). Disodium hydrogenphosphate (Na_2HPO_4), cetyltrimethylammonium bromide (CTAB), and sodium hydroxide (NaOH) were acquired from Fluka (Buchs, Switzerland). Methanol was purchased from Echo Chemical (Miaoli, Taiwan). Citric acid was obtained from Merck (Darmstadt, Germany). Ethyl acetate was purchased from Grand Chemical (Bangkok, Thailand). Dichloromethane was obtained from Tedia (Fairfield, Ohio, USA). Deionized water was purified through a Millipore Milli-Q water system (Milford, MA, USA).

2.2. Apparatus

A Beckman P/ACE MDQ CE system (Fullerton, CA, USA) was used to effect the separations. A diode array detector was employed for detection. Separations were performed in a 50-cm (40 cm effective length) × 50-μm I.D. fused silica capillary tube (Polymicro Technologies, Phoenix, AZ, USA). The capillary tube was assembled in the cartridge format. 32 Karat software, running on a personal computer, controlled the P/ACE instrument and data analysis. Prior to actual procedure, the separation capillary was pre-conditioned sequentially with MeOH (10 min), 1 M HCl (10 min), deionized water (2 min), 1 M NaOH (10 min), and then deionized water again (2 min). In between runs of the sweeping–MEKC and MEKC modes, the capillary was flushed with the separation BGE (3 min).

2.3. Synthesis of C_{16}MIMBr and C_{16}MPYB

C_{16}MIMBr was prepared according to Vanyúr's procedure [27]. 1-Methylimidazole (0.15 mol) was mixed with 1-bromohexadecane (0.15 mol) in toluene for 24 h at 80 °C. The resulting salt was recrystallized from EtOAc three times to yield a white crystalline solid, which was dried in a vacuum container for 2 days prior to use. C_{16}MPYB was synthesized using the procedure reported by Baker et al. [7]. A slight molar in excess of 1-bromohexadecane (100 g, 0.33 mol) was stirred in dropwise to 1-methylpyrrolidine (32 mL, 0.31 mol) at 0 °C. After 1 h, the mixture was slowly warmed to room temperature and stirred for an additional 12 h. The resulting white solid was filtered off using a cooled Büchner funnel and washed with cold EtOAc. Residual solvent was removed using rotary evaporation. The product was recrystallized three times more from acetone; the resulting white crystalline power was dried overnight in a vacuum oven at 40 °C. The synthesized C_{16}MIMBr and C_{16}MPYB were analyzed qualitatively using ^{1}H NMR spectroscopy; their structures are displayed in Fig. 1.

2.4. Preparing standards and urine samples

Stock standard solutions (1 mg/mL) of the seven benzodiazepines were prepared in MeOH and refrigerated at 4 °C. Prior to analysis in the sweeping–MEKC mode, the stock solution was diluted with MeOH to 100 μg/mL. The working standard was then diluted with the 15 mM borate buffer (pH 9.0, 6 mL 0.05 M $Na_2B_4O_7{\cdot}10H_2O$ and 1.1 mL 0.1 M HCl diluted to 20 mL with H_2O) to provide a conductivity similar to that of the BGE [15 mM borate buffer (pH 9.0) containing 30% MeOH and 20 mM surfactant] at the desired concentration. For the MEKC mode, the sample was diluted with MeOH to 50 μg/mL. No filtration was performed for the samples. Calibration curves were obtained after preparing solutions of the standards individually at 0.125, 0.25, 0.5, 1.0, 2.0, and 3.0 μg/mL for C_{16}MIMBr and at 0.03, 0.1, 0.5, 1.0, 2.0, and 3.0 μg/mL for C_{16}MPYB. Experiments were performed five times at each concentration. Samples of human urine were collected and frozen at

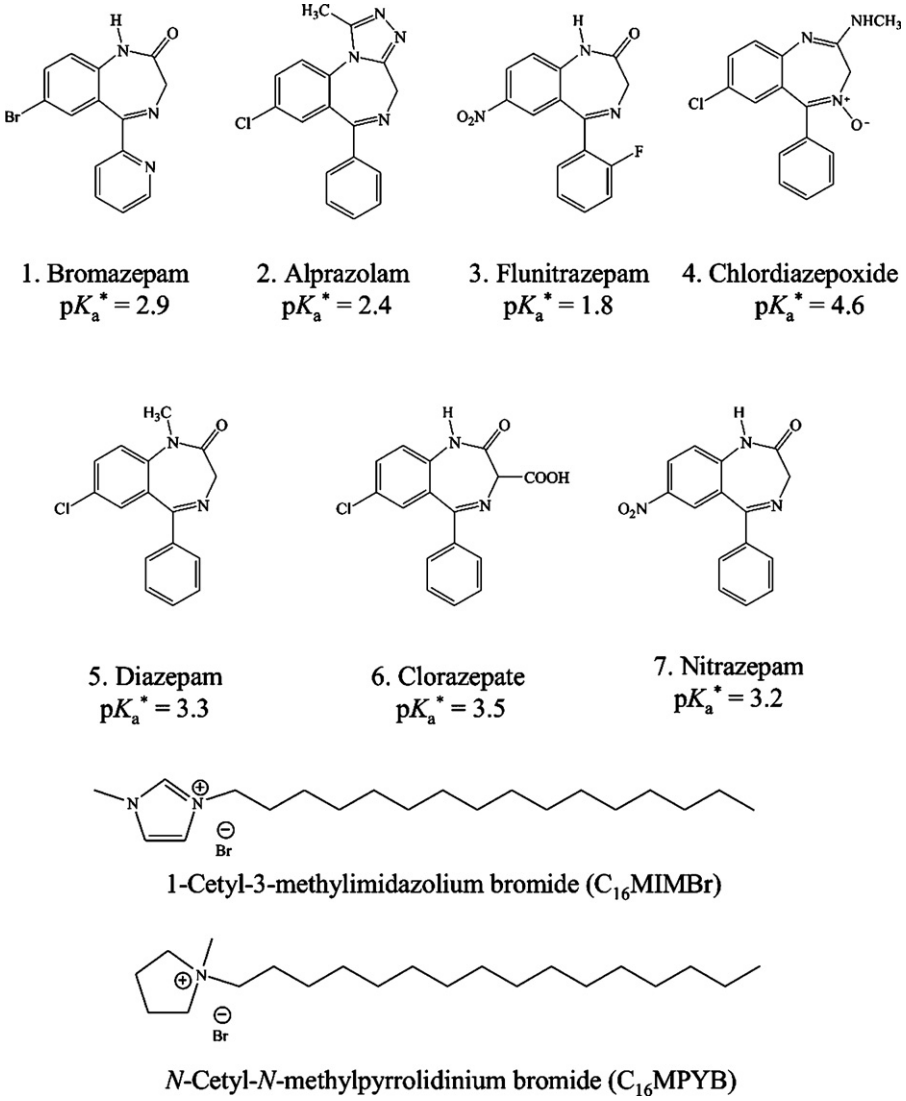

Fig. 1. Molecular structures of the seven benzodiazepines and cationic surfactants C_{16}MIMBr and C_{16}MPYB. *Values of pK_a are those of the conjugate acids of the benzodiazepines.

−20 °C; when required for an assay, they were thawed and warmed to room temperature.

2.5. Method procedures

The bare fused silica capillary was conditioned initially using a micellar BGE. In the MEKC procedure, samples were pressure-injected at 3.45 kPa for 3 s. The detection wavelength was set at 230 nm. The separation proceeded with the micellar BGE under a negative applied potential (−25 kV). In the sweeping–MEKC procedure, the capillary was initially filled with micellar BGE and then the samples were pressure-injected at 3.45 kPa for 120–420 s. Finally, a negative voltage (−25 kV) was applied to perform the sweeping process.

2.6. Solid phase extraction

Oasis® MCX column-type cartridges were obtained from Waters (Milford, MA, USA). The cartridges (3 mL/60 mg) were first conditioned with MeOH (2 mL) and H_2O (2 mL). The loading sample was a drug-free urine sample (3 mL) spiked with seven 40 μL benzodiazepines (5 μg/mL) and 60 μL citric acid/disodium hydrogenphosphate buffer (1 M, 3.2 mL 1 M citric acid and 3.4 mL 2 M Na_2HPO_4 diluted to 10 mL with H_2O); the pH was ca. 5.0. The concentration of benzodiazepines in the loading sample was 64.5 ng/mL. The column was washed sequentially with 0.1 M HCl (2 mL) and MeOH (2 mL). The elution solution was a mixture of dichloromethane, isopropyl alcohol, and sat. NH_4OH (78:20:2, v/v); the mixture was dried in a stream of nitrogen gas [24]. For assaying, the residue was dissolved in a mixture of MeOH (10 μL) and 15 mM borate buffer (pH 9.0, 190 μL).

3. Results and discussion

3.1. Sweeping with IL-type cationic surfactants

We employed C_{16}MIMBr and C_{16}MPYB as cationic surfactants for sweeping–MEKC. The sweeping process began by filling the capillary with a micelles loaded BGE. A large volume of the analytes, which were prepared in a BGE lacking a cationic surfactant, was injected hydrodynamically into the capillary. When a BGE containing a cationic surfactant fills a capillary column, the capillary walls become dynamically coated with the cation. The electroosmotic flow (EOF) within the capillary is then reversed when a sufficient surfactant concentration is reached. C_{16}MIMBr and C_{16}MPYB form

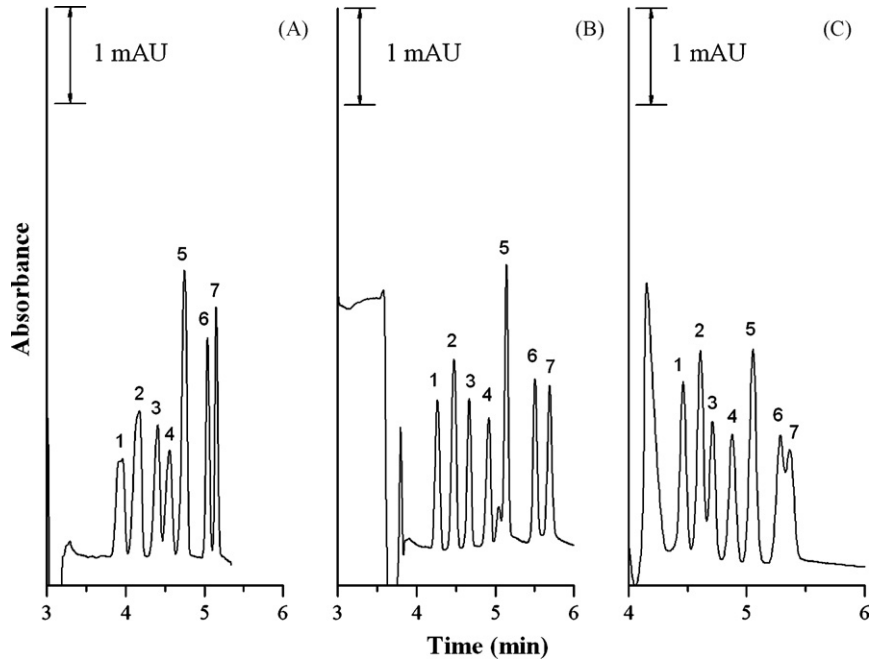

Fig. 2. Effect of (A) 20%, (B) 30%, and (C) 40% MeOH in the BGE on the sweeping–MEKC analyses. Sweeping–MEKC conditions: BGE, 15 mM borate (pH 9.0) containing 20 mM C_{16}MPYB; sample injection at 3.45 kPa for 300 s; sample concentration, 1 μg/mL in BGE (without C_{16}MPYB); separation voltage, −25 kV; detection at 230 nm; effective capillary length, 40 cm. Peak identification: (1) bromazepam; (2) alprazeolam; (3) flunitrazepam; (4) chlordiazepoxide; (5) diazepam; (6) clorazepate; (7) nitrazepam.

micelles at concentrations greater than 0.61 and 0.83 mM, respectively [16,27]. In other words, when the concentrations of these surfactants were above their CMCs, we applied a reversed polarity voltage to perform MEKC sweeping and separation. When the micelles migrated through the sample zone in a direction opposite to that of the EOF, they effectively swept the analytes into a narrow zone. Finally, the separation was continued and the analytes were carried by the micelles and the EOF to the detector. The success of the sweeping process relies on strong interactions between the analytes and the cationic surfactant micelles, and the potent interaction enhances the sweeping–MEKC analysis.

3.2. Optimizing sweeping conditions using C_{16}MIMBr and C_{16}MPYB

To obtain good separation and enhancement efficiencies with both C_{16}MIMBr and C_{16}MPYB, we studied the effects of several parameters of the sweeping–MEKC method, including the pH, the concentrations of the organic modifier and surfactant, and the injection time of the sample. Our attempts to optimize each of these conditions are described in detail below.

3.3. Effect of pH and organic modifier

In initial sweeping experiments, we inspected the influence of the BGE at pH 8.0, 9.0, and 10.0, where the analytes possess no net charge thus facilitating favorable interactions with the micelles. For both C_{16}MIMBr and C_{16}MPYB, the analyses of the seven benzodiazepines occurred with highest resolution at pH 9.0. Indeed, the analytes were not completely separated at pH 8.0 and 10.0. At pH 8.0–10.0, the changes in pH resulted in minor variations in the EOF, which affected the migration times and peak resolutions. For subsequent experiments, we chose a BGE with a pH of 9.0.

The effect of the organic modifier was the most important factor influencing the separation efficiency of present MEKC technique. In the absence of any organic modifier in the BGE, the seven benzodiazepines migrated at the same velocity and could not be identified. Fig. 2 displays the effect of MeOH in the BGE on the sweeping–MEKC separation. When we added 20% MeOH to the BGE, the resolution was not ideal; increasing the BGE's MeOH content to 30% improved the resolution, and lead to complete separation of the analytes. The resolution deteriorated when the BGE contained 40% MeOH. We suspect that the presence of the organic modifier increased the solubility of the hydrophobic solutes in the aqueous phase, thereby affecting the retention factors of the analytes and the resolution of their separation. The optimal separation conditions for the BGEs containing C_{16}MIMBr and C_{16}MPYB were both achieved when 30% MeOH was added.

3.4. Effect of cationic surfactant

To evaluate their sweeping enhancements, we added C_{16}MIMBr and C_{16}MPYB individually to the BGEs at concentrations in the range of 10–40 mM. The analytes were not completely separated at a 10 mM concentration of either cationic surfactant. The best peak enhancement occurred, with no loss of separation efficiency, when the cationic surfactant concentration in the BGE was 20 mM. At 30 mM or greater, the peaks broadened, the resolution decreased, and the signal enhancement did not improve significantly. Sweeping occurred when the positively charged C_{16}MIMBr and C_{16}MPYB micelles interacted with the neutral analytes; the EOF migrated toward the outlet and the cationic micelles traveled toward the inlet in the reversed polarity mode. Therefore, an increase in the number of micelles would increase the migration time of the analyte. After comparing the separation efficiencies and peak enhancement factors, we selected cationic surfactant concentrations of 20 mM for both C_{16}MIMBr and C_{16}MPYB.

Increasing the injection time of a sample should theoretically increase the peak signal of the analytes. Note, however, that the introduced volume is limited by the capillary length because a sufficient length of the capillary must be left free of sample solution for the separation to proceed after sweeping [28]. In this study, we varied the sample injection time from 120 to 420 s at 3.45 kPa. The peak heights of the seven benzodiazepines all increased until the injection time reached 300 s; beyond that point, the peak height

did not increase further either because of peak broadening or because the baseline separation was destroyed. Thus, the optimal injection time was determined to be 300 s at 3.45 kPa; it provided the highest separation resolution and maximum peak enhancement.

3.5. Sensitivity enhancements of C_{16}MIMBr and C_{16}MPYB

From the studies above, the optimal conditions for performing sweeping–MEKC involved using a 15 mM borate buffer (pH 9.0) containing 30% MeOH and 20 mM C_{16}MIMBr or C_{16}MPYB, with hydrodynamic injection of the sample at 3.45 kPa for 300 s. Figs. 3 and 4 display the electropherograms obtained when using C_{16}MIMBr and C_{16}MPYB, respectively, as cationic surfactants in both the conventional MEKC and sweeping–MEKC methods for the analysis of the seven benzodiazepines. The analyte peak intensity was indeed enhanced using the sweeping–MEKC method with either C_{16}MIMBr or C_{16}MPYB as surfactant. Table 1 reveals that the sensitivity enhancements for the sweeping–MEKC method, relative to conventional MEKC, were improved by factors of 31–59 for C_{16}MIMBr and factors of 86–165 for C_{16}MPYB. We suspect that C_{16}MPYB interacts better with the benzodiazepines, thereby achieving its superior sweeping efficiency relative to that of C_{16}MIMBr. We also used CTAB as a reference surfactant to compare the sweeping efficiency with C_{16}MIMBr and C_{16}MPYB. Table 1 lists the optimized sweeping conditions for CTAB; we found that the sensitivity enhancements for the benzodiazepines when sweeping with CTAB were improved by factors of 75–104. Hence, C_{16}MPYB also performed better than CTAB when applied

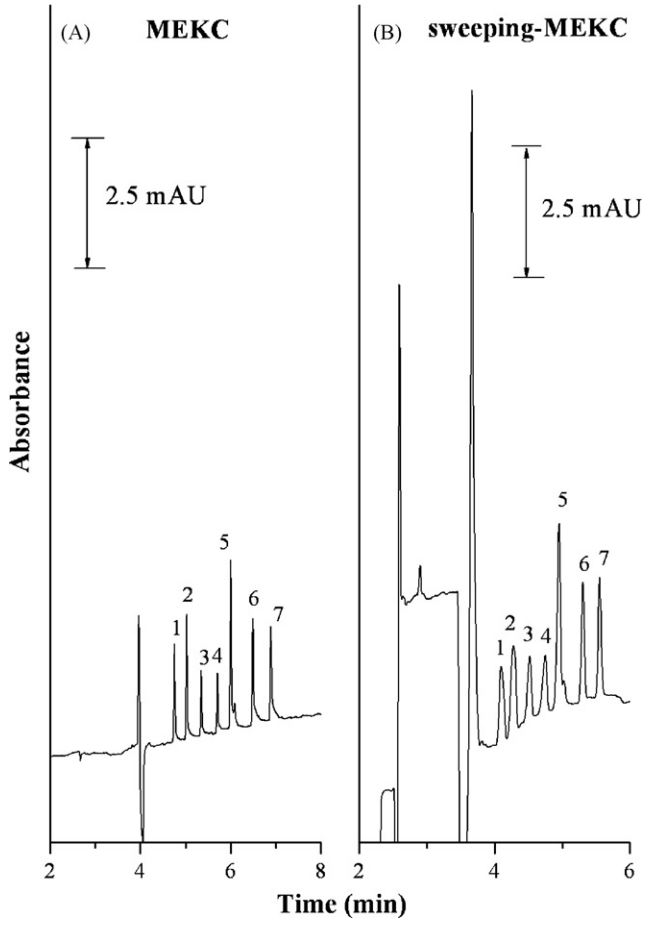

Fig. 3. Analyses of the seven benzodiazepines using (A) MEKC and (B) sweeping–MEKC with C_{16}MIMBr as the cationic surfactant. MEKC conditions: BGE, 15 mM borate (pH 9.0) containing 30% MeOH and 20 mM C_{16}MIMBr; sample injection at 2.07 kPa for 3 s; sample concentration, 50 μg/mL; injection length, 0.9 mm. Sweeping–MEKC conditions: BGE, 15 mM borate (pH 9.0) containing 30% MeOH and 20 mM C_{16}MIMBr; sample injection at 3.45 kPa for 300 s; sample concentration, 1 μg/mL in BGE (without C_{16}MIMBr); injection length, 150 mm. Other conditions were the same as those used to obtain Fig. 2.

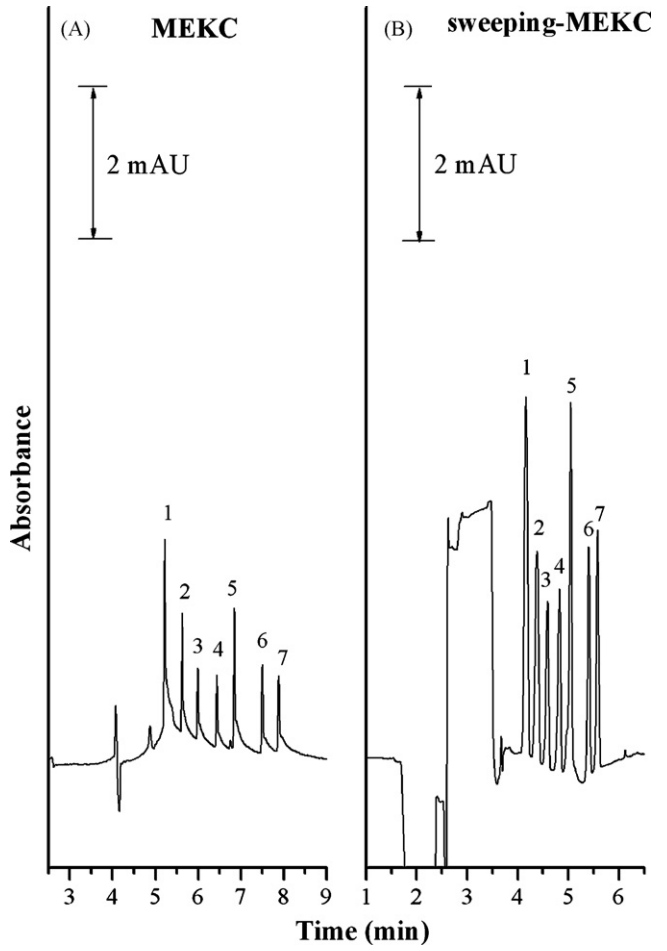

Fig. 4. Analyses of the seven benzodiazepines using (A) MEKC and (B) sweeping–MEKC with C_{16}MPYB as the cationic surfactant. MEKC conditions: BGE, 15 mM borate (pH 9.0) containing 30% MeOH and 20 mM C_{16}MPYB; sample injection at 2.07 kPa for 3 s; sample concentration, 50 μg/mL; injection length, 0.9 mm. Sweeping–MEKC conditions: BGE, 15 mM borate (pH 9.0) containing 30% MeOH and 20 mM C_{16}MPYB; sample injection at 3.45 kPa for 300 s; sample concentration, 1 μg/mL in BGE (without C_{16}MPYB); injection length, 150 mm. Other conditions were the same as those used to obtain Fig. 2.

Table 1
Sensitivity enhancements for C_{16}MIMBr, C_{16}MPYB, and CTAB.

Compounds	SE_{height}[a]		
	C_{16}MIMBr	C_{16}MPYB	CTAB[b]
Bromazepam	35	92	78
Alprazeolam	31	86	75
Flunitrazepam	38	115	86
Chlordiazepoxide	40	122	89
Diazepam	52	127	89
Clorazepate	51	127	92
Nitrazepam	59	165	104

[a] SE_{height}: Peak height from sweeping–MEKC/peak height from MEKC × dilution factor.

[b] MEKC conditions: BGE, 25 mM borate (pH 9.6) containing 30% MeOH and 50 mM CTAB; sample injection at 2.07 kPa for 3 s; sample concentration, 50 μg/mL; injection length, 0.9 mm. Sweeping–MEKC conditions: BGE is the same as MEKC; sample injection at 3.45 kPa for 270 s; sample concentration, 1 μg/mL in BGE (without CTAB); injection length, 135 mm.

Table 2
Calibration curves, coefficients of determination (r^2), limits of detection (LODs), and values of RSD for the analyses of benzodiazepines using sweeping–MEKC with C_{16}MIMBr and C_{16}MPYB.

	Bromazepam	Alprazeolam	Flunitrazepam	Chlordiazepoxide	Diazepam	Clorazepate	Nitrazepam
C_{16}MIMBr							
Calibration curve[a]	$y = 6.14x + 102$	$y = 7.14x + 83.1$	$y = 3.44x + 218$	$y = 3.64x + 91.3$	$y = 10.0x + 240$	$y = 5.58x - 97.2$	$y = 6.16x + 136$
Coefficient of determination	0.9950	0.9994	0.9936	0.9971	0.9929	0.9970	0.9982
LOD (S/N = 3; ng/mL)	33.35	25.89	38.25	31.29	9.39	13.52	11.49
LOQ (S/N = 10; ng/mL)	111.2	86.30	127.5	104.3	31.3	45.10	38.30
RSD (%; $n = 5$)							
(a) Migration time (min)	1.59	1.60	1.73	1.81	1.83	1.94	2.00
(b) Peak area	2.52	2.49	5.24	1.40	3.93	4.58	1.88
(c) Peak height	3.61	3.11	7.04	2.95	3.51	7.49	3.82
C_{16}MPYB							
Calibration curve[b]	$y = 18.0x + 700$	$y = 10.7x + 13.9$	$y = 6.48x - 120$	$y = 6.93x - 30.8$	$y = 12.2x - 94.5$	$y = 6.69x + 112$	$y = 7.94x + 285$
Coefficient of determination	0.9959	0.9994	0.9984	0.9985	0.9991	0.9973	0.9958
LOD (S/N = 3; ng/mL)	4.68	8.17	9.75	8.40	5.31	8.34	7.22
LOQ (S/N = 10; ng/mL)	15.6	27.2	32.5	28.0	17.7	27.8	24.1
RSD (%; $n = 5$)							
(a) Migration time (min)	0.33	0.29	0.31	0.33	0.37	0.39	0.46
(b) Peak area	1.72	3.19	1.98	5.79	6.89	8.26	2.61
(c) Peak height	1.78	2.94	1.65	3.84	3.91	4.28	2.74

[a] Calibration line (0.125–3 μg/mL): peak area (arbitrary units) = slope × concentration (ng/mL) + y-intercept.
[b] Calibration line (0.03–3 μg/mL): peak area (arbitrary units) = slope × concentration (ng/mL) + y-intercept.

in sweeping–MEKC. This result is similar to that of a previous study in which the interactions of the C_{16}MPYB surfactant with polar compounds were found to be stronger than those of CTAB [16,29]. Therefore, C_{16}MPYB outperforms both C_{16}MIMBr and CTAB in the on-line pre-concentration and analysis of benzodiazepines.

3.6. Calibration curve and detection limits

Under the optimal conditions, we performed a quantitative analysis (Table 2) to determine the range of linearity, the limits of detection (LODs), and the relative standard deviations (RSDs) of the migration times and peak heights when employing C_{16}MIMBr and C_{16}MPYB as surfactants. For the sweeping–MEKC method using C_{16}MIMBr, the calibration curve was linear from 0.125 to 3.0 μg/mL; for C_{16}MPYB, the linear range was from 0.03 to 3.0 μg/mL; over these ranges, the coefficients of determination (r^2) were all greater than 0.9929. The LODs [signal-to-noise (S/N) ratio = 3] of the seven analytes ranged from 9.39 to 38.25 ng/mL when using C_{16}MIMBr and from 4.68 to 9.75 ng/mL when using C_{16}MPYB. Our experimental results clearly indicate that sweeping–MEKC using these cationic surfactants provides superior sensitivity and linearity for the determination of benzodiazepine analytes; the use of C_{16}MPYB as the cationic surfactant enhanced the sensitivity and reproducibility relative to those obtained using C_{16}MIMBr.

3.7. Analyzing spiked urine samples using sweeping–MEKC and C_{16}MPYB

Because of its superior sensitivity enhancement, we employed C_{16}MPYB as the cationic surfactant for the sweeping–MEKC analysis of the seven benzodiazepines spiked into a urine sample. We employed SPE prior to performing sweeping–MEKC to eliminate interference from the urine sample and to concentrate the analytes. The resulting electropherogram in Fig. 5 reveals that SPE treatment allowed us to detect the presence of these analytes in urine samples without interference from any unknown compounds. The analytes were identified in terms of their migration times and absorbance spectra. Under the optimized SPE conditions, the recoveries were 77.0% for bromazepam, 88.3% for alprazeolam, 83.4% for flunitrazepam, 83.6% for chlordiazepoxide, 86.7% for diazepam, 81.7% for clorazepate, and 76.6% for nitrazepam; the reproducibilities (RSD; $n = 5$) of the extractions were 6.8, 4.5, 6.3, 5.2, 4.0, 7.2 and 8.0%, respectively.

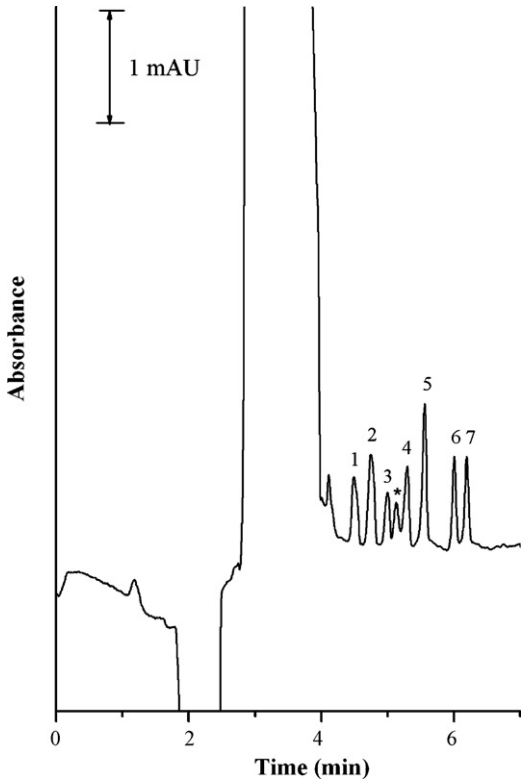

Fig. 5. Sweeping–MEKC electropherogram, recorded using C_{16}MPYB in the BGE, of a urine sample spiked with the seven benzodiazepines. Analysis was performed according to the optimized conditions of the sweeping–MEKC method. *Unknown peak. Other conditions were the same as those used to obtain Fig. 4(B).

4. Conclusions

The IL-type surfactants C_{16}MIMBr and C_{16}MPYB are suitable sweeping–MEKC additives for the on-line sample preconcentration and determination of seven benzodiazepines. Under

the optimized separation parameters, the enrichment factors for the seven compounds when using sweeping–MEKC, relative to MEKC, fell within the ranges 31–59 when employing the C_{16}MIMBr surfactant and 86–165 when employing C_{16}MPYB; the enhanced sweeping efficiency of C_{16}MPYB presumably arises from its stronger interactions with these benzodiazepines. The LODs for the seven benzodiazepines when using C_{16}MPYB as the sweeping carrier ranged from 4.68 to 9.75 ng/mL. The reproducibility of this developed sweeping–MEKC method was adequate. We also applied this sweeping–MEKC method—with C_{16}MPYB as surfactant—to the successful analysis of the seven benzodiazepines in spiked human urine samples after SPE. Therefore, it appears that performing sweeping–MEKC with IL-type surfactants in the sweeping buffer is potentially a new approach toward the on-line concentration and analysis of benzodiazepines present at low nanogram-per-milliliter concentrations in real samples.

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