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Mechanistic Studies of Partial-Filling Micellar Electrokinetic Chromatography

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The need for coupling micellar electrokinetic chromatography (MEKC) with electrospray mass spectrometry initiates the development of partial-filling MEKC. In comparison with conventional MEKC, only a small portion of the capillary is filled with a micellar solution for performing the separation in partial-filling MEKC. Analytes first migrate into the micellar plug, where the separation occurs, and then into the leading electrophoresis buffer, which is free of surfactants. A theoretical model is proposed for predicting the separation behavior of triazine herbicides in partial-filling MEKC. The comparisons between conventional and partial-filling MEKC in terms of separation efficiency and resolution of triazine herbicides are presented and discussed. The optimization techniques, possible applications, and advantages of partial-filling MEKC are similarly addressed.

Micellar electrokinetic chromatography (MEKC) was introduced by Terabe[1,2] for the separation of neutral compounds. MEKC involves the solubilization of surfactant monomers in solution at a concentration above the critical micelle concentration. At these concentrations, surfactant monomers form roughly spherical aggregates, or micelles. Neutral solutes differentially partition between the aqueous phase and the hydrophobic interior of the micelles. The partitioning difference among neutral solutes contributes to the separation in MEKC.

The potential interference of surfactants used in MEKC with on-line electrospray mass spectrometry detection has been presented by several research groups.[3−5] In the presence of a relatively high concentration of nonvolatile surfactant, the electrospray efficiency and the mass sensitivity of analytes decrease significantly relative to those observed in the absence of surfactant. The need for coupling MEKC with electrospray mass spectrometry has led to several new approaches including the use of high molecular weight surfactants,[4] semipermeable membrane interface,[5] and partial-filling MEKC.[4] The advantage of high molecular weight surfactants is their low critical micelle concentration. Additionally, the use of high molecular weight surfactants avoid the production of high levels of low-mass background ions in the mass spectrum and the potential interference with the mass detection of small analytes. The new interface introduced by Foley and Masucci[5] utilizes a semipermeable membrane that selectively permeates small analyte molecules to the mass spectrometer while retaining the relatively larger surfactants.

In comparison with conventional MEKC, partial-filling MEKC involves filling a small portion of the capillary with a micellar solution to achieve a separation. The capillary in partial-filling MEKC mode is filled with electrophoresis buffer, followed by an introduction of micellar solution, and finally sample injection. Analytes will first migrate into the micellar plug where the separation occurs and then into the electrophoresis buffer, which is free of surfactants. This paper describes the mechanistic studies of partial-filling MEKC by a comparison with conventional MEKC using triazine herbicides as a model system. The effects of micellar concentration, plug length, and overall capillary length on the separation resolution and efficiency of partial-filling MEKC are investigated both experimentally and theoretically. Optimization of partial-filling MEKC and advantages of partial-filling MEKC in the manipulation of retention window are also presented and discussed.

EXPERIMENTAL SECTION

Electrokinetic separations were performed using fused-silica tubing from Polymicro Technologies (Phoenix, AZ). The capillary dimensions were 50 μm i.d. × 360 μm o.d. with a total length of 45 cm, 30 cm to the detector. Detection was carried out by a Linear UVIS 200 detector from Linear Instruments (Reno, NV) modified for on-column detection. Detection wavelength was set at 226 nm. A Spellman CZE 1000R high-voltage power supply (Plainview, NY) delivered −11.25 kV to the detector end of the capillary for electrokinetic injection and for electrophoretic separation. All experiments were performed at room temperature, 24 °C. Data collection was performed by HP 35900D analog to digital interface board with the HP G1250C General Purpose Chemstation Software (Hewlett-Packard, Fullerton, CA).

Sodium dodecyl sulfate (SDS) of protein research grade was purchased from Boehringer Mannheim Gmbh (Mannheim, Germany) and used as received. Phosphate buffer was prepared by titrating 10 mM solutions of monobasic with dibasic sodium phosphate (Fisher Scientific, Pittsburgh, PA) to pH 7. HPLC grade methanol (Fisher Scientific) and quinine hydrochloride (Aldrich, Milwaukee, WI) were used as the electroosmotic flow marker and the micellar marker, respectively.

Ametryne, atrazine, prometryne, propazine, and simazine were purchased from ChemService (West Chester, PA). Triazine herbicides with an individual concentration of 10^{-5} M were prepared in phosphate buffer containing 5% (v/v) methanol. The standard herbicide solution did not contain any SDS surfactant. All solutions were prepared using water purified by a NANOpure II system (Dubuque, IA) and further filtered with a 0.2-μm Supor-200 membrane filter from Gelman Sciences (Ann Arbor, MI).

(1) Terabe, S.; Otsuka, K.; Ichikawa, K.; Tsuchiya, A.; Ando, T. *Anal. Chem.* **1984,** *56,* 113.
(2) Terabe, S.; Otsuka, K.; Ando, T. *Anal. Chem.* **1985,** *57,* 834.
(3) Varghese, J.; Cole, R. B. *J. Chromatogr. A* **1993,** *652,* 369.
(4) Terabe, S.; Ozaki, H.; Takada, Y.; Sakairi, M.; Koizuni, H. *Proceedings for the Seventh International Symposium on High Performance Capillary Electrophoresis,* 1995; p 53.
(5) Foley, J. P.; Masucci, J. A. *Proceedings for the Seventeenth International Symposium on Capillary Chromatography and Electrophoresis,* 1995; p 278.

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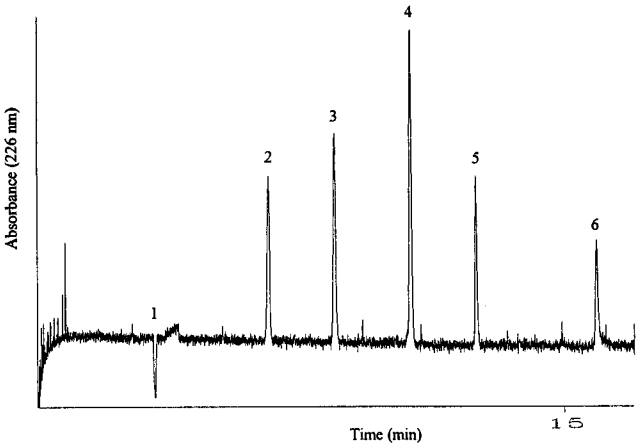
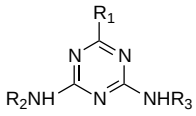


Figure 1. Conventional MEKC separation of 10^{-5} M each of (2) simazine, (3) atrazine, (4) propazine and ametryne, and (5) prometryne. Methanol (1) and quinine hydrochloride (6) were used as the electroosmotic flow marker and the micellar marker, respectively. Buffer, 10 mM phosphate/20 mM SDS, pH 7; capillary, 45-cm total length, 50-μm i.d., and 360-μm o.d., length to detector 30 cm; voltage, −11.25 kV and 3 s for injection, −11.25 kV for electrophoresis; UV detection at 226 nm.

Table 1. Triazine Herbicides with Corresponding Structures and pK_a Values[a]



triazines	R1	R2	R3	pK_a
simazine	Cl	ethyl	ethyl	1.65−1.80
atrazine	Cl	isopropyl	ethyl	1.68−1.85
propazine	Cl	isopropyl	isopropyl	1.50−1.85
ametryne	*S*-methyl	ethyl	isopropyl	4.00−4.10
prometryne	*S*-methyl	isopropyl	isopropyl	4.05−4.10

[a] Taken from refs 6−9.

RESULTS AND DISCUSSION

For conventional MEKC separation of triazine herbicides, a fused-silica capillary was filled with electrophoresis buffer containing 20 mM SDS and 10 mM phosphate buffer at pH 7. The standard herbicide solution with the addition of 10^{-5} M quinine hydrochloride was electrokinetically injected for 3 s at the running voltage. The running voltage of −11.25 kV was applied at the cathodic end (the UV detector end) for obtaining the separation of triazine herbicides shown in Figure 1. Triazine herbicides with their corresponding structures and pK_a values[6−9] were listed in Table 1. The elution order and the capacity factor of triazine herbicides were summarized in Table 2 with the more hydrophobic herbicides eluting latter in the micelle phase. Propazine coeluted with ametryne.

To compare with conventional MEKC, a fused-silica capillary was initially filled with 10 mM phosphate buffer at pH 7 containing no SDS. A 20 mM SDS solution (in 10 mM phosphate buffer at pH 7) and a standard herbicide solution were sequentially injected at the anodic end of capillary by using a −11.25 kV for 20 and 3 s, respectively. The separation of triazine herbicides in partial-filling MEKC is shown in Figure 2. To identify the elution of micellar plug, quinine hydrochloride was added to a 20 mM SDS solution with a final concentration of 3.2×10^{-4} M. To mimic the injection conditions in Figure 2, a SDS solution containing quinine hydrochloride and a phosphate buffer free of SDS and triazine herbicides were injected sequentially by using a −11.25 kV for 20 and 3 s, respectively. As shown in Figure 3, a SDS plug with a peak width of ∼20 s was eluted at 7.38 min. In comparison with Figure 2, all triazine herbicides other than prometryne were eluted ahead of the SDS micelle plug. Prometryne was eluted within the SDS micelle plug.

Several dramatic differences between conventional and partial-filling MEKC were observed by comparing the electropherograms shown in Figures 1−3. The decrease in the migration times of triazine herbicides was observed in partial-filling MEKC. The separation efficiencies of triazine herbicides in conventional and partial-filling MEKC were summarized and compared in Table 3. The separation efficiencies of triazine herbicides other than prometryne were lower in partial-filling than in conventional MEKC. However, prometryne coeluted within the SDS micelle plug and exhibited a greater separation efficiency in partial-filling than in conventional MEKC. Propazine and ametryne were baseline separated and resolved in partial-filling MEKC, but coeluted in conventional MEKC. In contrast, the separation resolutions of the other triazine herbicides were reduced in partial-filling MEKC. Furthermore, the migration time of SDS micelle, t_{MC}, was reduced from 15.95 min in conventional MEKC to 7.38 min in partial-filling MEKC.

Table 2. Elution Order and Capacity Factor for Triazine Herbicides

analyte peak	elution order	capacity factor
methanol	1	
simazine	2	1.66
atrazine	3	3.27
propazine	4	6.62
ametryne	4	6.62
prometryne	5	12.79
quinine hydrochloride	6	

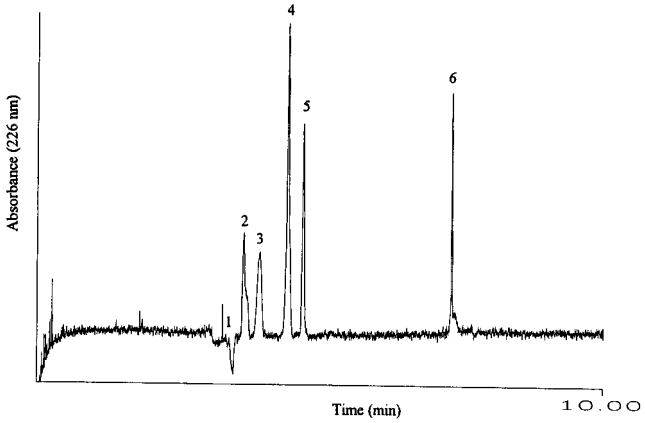


Figure 2. Partial-filling MEKC separation of 10^{-5} M each of (2) simazine, (3) atrazine, (4) propazine, (5) ametryne, and (6) prometryne. Methanol (1) was used as the electroosmotic flow marker. Electrophoresis buffer, 10 mM phosphate, pH 7; capillary, 45-cm total length, 50-μm i.d., and 360-μm o.d., length to detector 30 cm; voltage, −11.25 kV and 20 s for SDS injection (20 mM SDS in 10 mM phosphate buffer at pH 7), −11.25 kV and 3 s for triazine injection, −11.25 kV for electrophoresis; UV detection at 226 nm.

(6) Vermeulen, N. M. J.; Apostolides, Z.; Potgieter, D. J.; Nel, P. C.; Smit, S. H. *J. Chromatogr.* **1982,** *240*, 247.
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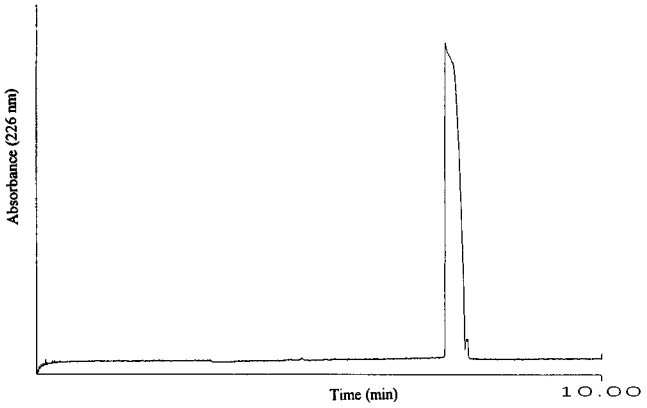

Figure 3. Partial-filling MEKC separation of 3.2×10^{-4} M quinine hydrochloride in 20 mM SDS, 10 mM phosphate buffer, pH 7. Electrophoresis buffer, 10 mM phosphate, pH 7; voltage, −11.25 kV and 20 s for quinine hydrochloride injection, −11.25 kV and 3 s for electrophoresis buffer injection, −11.25 kV for electrophoresis. Other conditions are the same as in Figure 2.

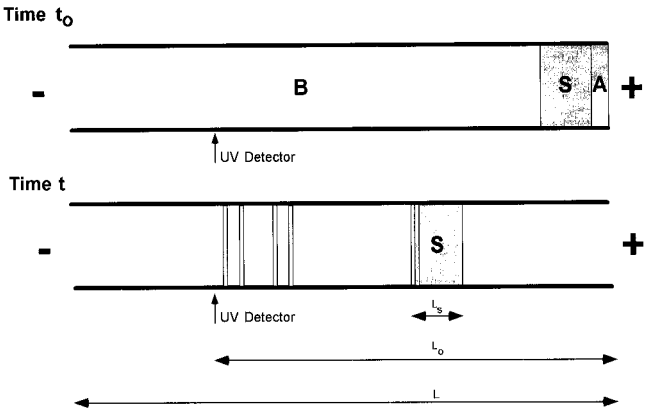

Figure 4. Schematic diagram of partial-filling MEKC: B, background phosphate buffer; S, SDS micelle plug; A, sample analytes; L_S, SDS micelle plug length; L_O, distance between the injection point and the UV detector; L, total capillary length.

Table 3. Separation Efficiency of Triazine Herbicides in Conventional and Partial-Filling MEKC

	no. of theoretical plates	
triazine herbicides	conventional MEKC	partial-filling MEKC
simazine	64 000	21 000
atrazine	85 000	15 000
propazine	114 000	58 000
ametryne	114 000	76 000
prometryne	240 000	562 000

Theoretical Model of Partial-Filling MEKC. A theoretical model for partial-filling MEKC separation of triazine herbicides was proposed and is illustrated in Figure 4. Due to the presence of 20 mM SDS, the SDS micelle plug exhibited a higher solution conductivity and a correspondingly lower electric field strength than those of the surrounding phosphate buffer. In partial-filling MEKC, a constant electric current, I, was applied across the entire capillary as

$$I = V_S/(L_S R_S) = V_B/(L_B R_B) \quad (1)$$

where V, L, and R were the applied electric potential, capillary length, and specific resistance in each region with the subscripts of S and B for the surfactant plug and the phosphate buffer, respectively. The total capillary length, L, was 45 cm and the total applied electric potential, V, was 11.25 kV. The total capillary length and the total applied electric potential were given as

$$L = L_S + L_B \quad (2)$$

and

$$V = V_S + V_B \quad (3)$$

Table 4. Distribution of Applied Electric Potentials and Electric Field Strengths in Partial-Filling and Conventional MEKC

capillary region	length in each region (cm)	electric potential (V)	electric field strength (V/cm)
partial-filling MEKC			
micelle plug	1.32	174	132
phosphate buffer	43.68	11 076	254
conventional MEKC	45	11 250	250

The micellar electrophoretic mobility, μ_{MC}, which was measured from the quinine hydrochloride peak in conventional MEKC (Figure 1) was -4.75×10^{-4} cm^2/(V s). In comparison with phosphoric acid, the ionic mobilities of phosphoric acid were reported as -3.4×10^{-4}, -5.8×10^{-4}, and -7.2×10^{-4} cm^2/(V s) with the corresponding pK_a values of 2.1, 7.2, and 12.3.[10] The effective ion mobility of our background phosphate buffer at pH 7 was therefore estimated around -4.6×10^{-4} cm^2/(V s) and was close to that of SDS micelles. Thus, the SDS micellar plug of partial-filling MEKC separation could be treated, in first-order approximation, as a steady migrating zone. A SDS plug which was injected by a −11.25 kV for 20 s was shown in Figure 3 with a peak width of ~20 s. No significant band broadening of SDS micellar plug was observed. The SDS plug length, L_S, in partial-filling MEKC was estimated as

$$L_S = (L_O t_{inj}/t_{MC}) \quad (4)$$

where L_O and t_{inj} were the distance between the injection point and the UV detector and the electrokinetic injection time, respectively. The calculated value for L_S was 1.32 cm. According to the current measurements, the solution resistances of phosphate buffer and 20 mM SDS solution in a 50 μm i.d. × 360 μm o.d. capillary, R_B and R_S, were calculated as 3.55×10^7 and 1.85×10^7 Ω/cm, respectively. By using eqs 1−4, the electric potentials and the electric field strengths in the micelle plug and in the phosphate buffer were calculated and summarized in Table 4. In comparison with a field strength of 254 V/cm at the phosphate buffer region, a much lower electric field strength of 132 V/cm was estimated in the micelle plug.

Band Broadening of Analyte Solutes in Partial-Filling MEKC. Since the electric field strength in the micelle plug was significantly different from the electric field strength in the phosphate buffer, additional band broadening occurred in the

(10) Pospichal, J.; Gebauer, P.; Bocek, P. *Chem. Rev.* **1989,** *89*, 419.

analyte band. More specifically, the analyte band in the micelle plug migrated slower due to its partitioning with the micelle and the presence of a lower electric field. Thus, all analyte molecules that diffuse past the micelle front on the left (see Figure 4) encountered a higher electric field, and therefore, migration speed driven by electroosmosis increased. In comparison with conventional MEKC, the presence of an additional band-broadening phenomenon at the interface between the micelle plug and the leading phosphate buffer contributed to generally lower separation efficiencies of the triazine herbicides in partial-filling MEKC (see Table 3). As shown in Figures 2 and 3, prometryne, however, coeluted within the SDS micelle plug at 7.33 min. By reducing its migration time from 12.50 min in conventional MEKC to 7.33 min in partial-filling MEKC, the increase in the separation efficiency of prometryne was thus observed in partial-filling MEKC.

In partial-filling MEKC, the electroosmotic velocities were different between the micelle plug and the phosphate buffer region due to the difference in their electric field strength. However, the bulk velocity of fluid at each cross section in the capillary had to be the same for a noncompressible fluid. The difference between the electroosmotic velocities and the bulk velocity generated hydrostatic pressures and additional laminar flows in both the micelle plug and the phosphate buffer region.[11] In general, laminar flows display a characteristic parabolic flow pattern which gives rise to additional band broadening of analytes in partial-filling MEKC.

Migration Times of Analyte Solutes in Partial-Filling MEKC. According to Chien's theory,[11] the bulk velocity, ν_b, in partial-filling MEKC is shown to be

$$\nu_b = (L_S \nu_{eo,S} + L_B \nu_{eo,B})/L \quad (5)$$

where $\nu_{eo,S}$ and $\nu_{eo,B}$ were the electroosmotic velocities in the surfactant plug and the phosphate buffer, respectively. By assuming the same electroosmotic mobility across the entire capillary, the bulk velocity in partial-filling MEKC would therefore be the same as the electroosmotic velocity in conventional MEKC. This was confirmed by comparing the migration times of methanol peaks shown in Figures 2 and 3.

The bulk velocity, ν_b, in partial-filling MEKC was measured as 30 cm/3.33 min = 9.01 cm/min. The micellar electrophoretic mobility, μ_{MC}, was -4.75×10^{-4} cm^2/(V s). The migration time of triazine herbicides in partial-filling MEKC, t_R, was given as

$$t_R = t_S + t_B \quad (6)$$

where t_S and t_B were the migration times of analyte band moving through the SDS micelle plug and the phosphate buffer, respectively. The migration velocity of any analyte molecule in the SDS micelle plug, ν_s, was estimated as

$$\nu_s = \nu_b + [\mu_{MC} E_S k'/(1 + k')] \quad (7)$$

where k' was the capacity factor measured in conventional MEKC and summarized in Table 2. The difference in the migration velocity between the analyte band and the SDS micelle plug, ν', was obtained as

(11) Chien, R. L.; Helmer, J. C. *Anal. Chem.* **1991,** *63,* 1354.

Table 5. Migration Time and Apparent Capacity Factor of Triazine Herbicides in Partial-Filling MEKC

analyte peak	migration time (min)		capacity factor
	predicted	measured	
simazine	3.57	3.66	0.349
atrazine	3.80	3.94	1.02
propazine	4.29	4.42	3.44
ametryne	4.29	4.69	3.44
prometryne	5.20	7.20	11.59

$$\nu' = \nu_s - (\nu_b + \mu_{MC} E_S) = -\mu_{MC} E_S/(1 + k') \quad (8)$$

Thus, t_S as the migration time of analyte band moving through the SDS micelle plug was calculated as

$$t_S = L_S/\nu' \quad (9)$$

The remaining migration distance and the migration time in the phosphate buffer before the analyte band reached the UV detector window were given as

$$L' = L_O - t_S \nu_s \quad (10)$$

and

$$t_B = L'/\nu_b \quad (11)$$

By using eqs 6−11, the migration times of triazine herbicides in partial-filling MEKC were predicted and compared with the measured migration times in Table 5. Clearly, the predicted migration times of triazine herbicides other than prometryne were in good agreement with the observed migration times in partial-filling MEKC. The difference between the predicted and the measured migration times of prometryne was mainly contributed by the estimation of SDS micelle plug length, L_S.

Furthermore, the capacity factor in partial-filling MEKC, $k'_{PF-MEKC}$, was given as

$$k'_{PF-MEKC} = k'(L_O - L')/L_O \quad (12)$$

where k' was the capacity factor in conventional MEKC. On the basis of eq 10, L' was the remaining migration distance in the phosphate buffer before the analyte reached the UV detector window. Before the analyte migrated into the phosphate buffer, the analyte remained in the SDS micelle plug and traveled within the micelle plug for the distance of $L_O - L'$. The capacity factors in partial-filling MEKC were calculated and are listed in Table 5. In comparison with conventional MEKC, the reduction of the capacity factors for propazine and ametryne contributed to the baseline separation resolution obtained in partial-filling MEKC.

Effect of Micelle Plug Length on Partial-Filling MEKC Separation. To demonstrate the effect of micelle plug length on the separation of triazine herbicides in partial-filling MEKC, the electrokinetic injection of a 20 mM SDS solution was reduced from 20 to 10 s with the application of a −11.25 kV. As shown in Figure 5, prometryne with a migration time of 4.27 min was ahead of the SDS micelle plug with a migration time of 6.88 min (not shown). A significantly lower separation efficiency of prometryne

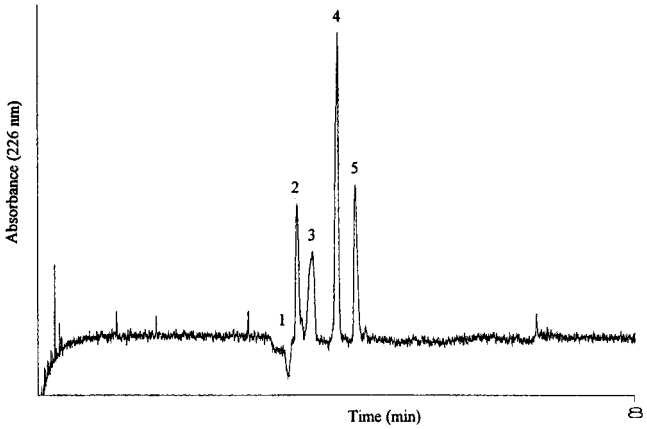

Figure 5. Partial-filling MEKC separation of 10^{-5} M each of (2) simazine, (3) atrazine, (4) propazine and ametryne, and (5) prometryne. Methanol (1) was used as the electroosmotic flow marker. Voltage, −11.25 kV and 10 s for SDS injection (20 mM SDS in 10 mM phosphate buffer at pH 7), −11.25 kV and 3 s for triazine injection, −11.25 kV for electrophoresis. Other conditions were the same as in Figure 2.

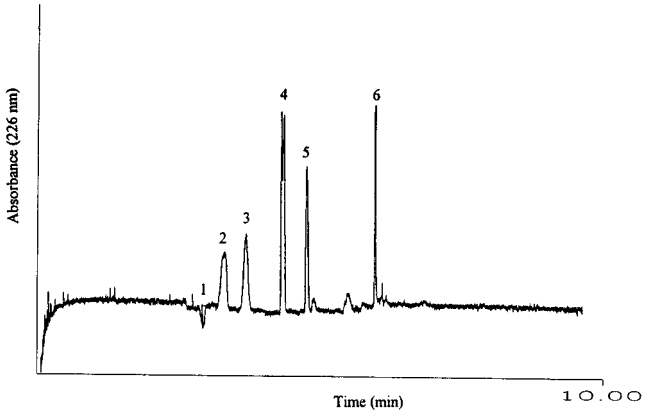

Figure 6. Partial-filling MEKC separation of 10^{-5} M each of (2) simazine, (3) atrazine, (4) propazine, (5) ametryne, and (6) prometryne. Methanol (1) was used as the electroosmotic flow marker. Voltage, −11.25 kV and 15 s for SDS injection (30 mM SDS in 10 mM phosphate buffer at pH 7), −11.25 kV and 3 s for triazine injection, −11.25 kV for electrophoresis. Other conditions were the same as in Figure 2.

was thus observed in Figure 5 due to the experience of an additional band-broadening phenomenon at the interface between the micelle plug and the leading electrophoresis buffer. In the presence of a shorter SDS micelle plug, all triazine herbicides eluted earlier and separated within 5 min of the analysis time. However, a 10-s SDS micelle plug was too short to achieve any measurable separation resolution between propazine and ametryne.

Effect of SDS Concentration on Partial-Filling MEKC Separation. A 30 mM SDS solution (in 10 mM phosphate buffer at pH 7) and a standard herbicide solution were sequentially injected at the anodic end of the capillary by using −11.25 kV for 15 and 3 s, respectively. By comparing the electropherograms shown in Figures 5 and 6, the separation resolutions of simazine/atrazine and propazine/ametryne pairs were enhanced by raising the SDS concentration from 20 to 30 mM in the micelle plug. The selection of a 15-s micelle injection in partial-filling MEKC also ensured the elution of prometryne ahead of the SDS micelle plug, which was eluted with a migration time of 7.87 min (not shown).

According to the theoretical model of partial-filling MEKC proposed in this study, the effects of micellar concentration, plug length, and overall capillary length on the separation resolution and the migration time of analytes can be predicted. All these predictions only require the solution conductivity and the capacity factor obtained from conventional MEKC experiments. The use of partial-filling MEKC not only provides a potential solution for interfacing MEKC separation with on-line electrospray mass spectrometry detection but also contributes to an additional manipulation of separation resolution in MEKC.

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