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Capillary electrochromatography with a silica column with a dynamically modified cationic surfactant

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Abstract

A novel mode of capillary electrochromatography (CEC), called dynamically modified silica-capillary electrochromatography, is described in this paper. The column packed with bare silica was dynamically modified with long chain quaternary ammonium salt, cetyltrimethylammonium bromide (CTAB), which was added into the mobile phase. CTAB ions were adsorbed onto the surface of bare silica, and the resulted hydrophobic layer on the silica gel was used as the stationary phase. Using the dynamically modified silica column, neutral solutes were separated by CEC. The highest number of theoretical plates obtained was about 71 500/m and the relative standard deviations for t_0 and capacity factor of toluene were 4.7% and 4.9% for 20 consecutive runs, respectively. The separation mechanism of neutral solutes and the influence of mobile phase composition on the separation was investigated. The separation of nitrogen-containing solutes was carried out with this mode and the peak tailing of basic solute was effectively eliminated because the adsorption of basic solute on silica was blocked by the preferred adsorption of CTAB. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Electrochromatography; Dynamically modified silica capillary electrochromatography; Stationary phases, LC; Anilines; Benzenes; Peptides

1. Introduction

Capillary electrochromatography (CEC), which combines the high efficiency of capillary zone electrophoresis (CZE) and the high selectivity of high-performance liquid chromatography (HPLC), has received more and more attention recently. Since CEC is a hybrid of HPLC and CZE, the separation mechanism is more complex than that of HPLC and CZE. Neutral compounds are separated in CEC due to the differences in partitioning between the mobile and stationary phases, while ionic compounds are separated due to the combination of partitioning and electrophoresis [1].

The focus of CEC nowadays is on the separation of neutral compounds such as aromatic compounds and neutral drugs, but little attention is paid to charged compounds especially basic compounds [2]. The major difficulty of reversed-phase CEC to separate basic compounds is peak tailing [3]. Smith and Evans [4] reported discouragingly poor peak shape of strong basic compounds on the silica-based ODS stationary phase. Gillott et al. [5] reported that the good peak symmetry in the separation of pharma-

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ceutical bases can be achieved by the addition of competing base into the mobile phase. Lurie et al. [6] also reported simultaneous separation of acidic, basic and neutral organic compounds by CEC with a mobile phase containing hexylamine. But the addition of competing base to the mobile phase will increase the ionic strength of mobile phase and Joule heating. Therefore a pressure of 8 bar [5] or 10 bar [6] was applied to both sides of capillary to avoid bubble formation by utilization of those mobile phases, which requires a more complex instrument and cannot be performed on most of the standard capillary electrophoresis instrument.

Both CEC and micellar electrokinetic capillary chromatography (MECC) can be used for separation of charged and uncharged compounds, but the choice of surfactants in MECC is limited, while the choice of stationary phase in CEC is extremely wide because many kinds of stationary phases in HPLC are available and can be packed into CEC columns. Up to now, a range of packings have been used in CEC. Among these, silica based ODS is most commonly used since the early 90s. Several researchers [5,7–10] have reported a number of separations on these packings. Cation-exchange phase has also been used in CEC, Smith and Evans [4] have reported the highest column efficiency (up to $8\cdot10^6$ plates/m) in CEC for the separation of basic tricyclic antidepressants by using these packings. A series of chiral packings [11–13] including β-cyclodextrin (β-CD) bonded silica and protein bonded silica have also been used in the separation of enantiomers.

Chromatography on dynamically modified silica in HPLC was first reported by Ghaemi and Wall [14], in which, HPLC column was packed with bare silica and a certain amount of long chain quaternary ammonium salts, such as cetyltrimethylammonium bromide (CTAB), was added into the mobile phase [15]. CTAB ions have considerable affinity to ionized silanol groups. Therefore CTAB ions will adsorb onto the silica surface in such a way that the C_{16} carbon chain is pointing away from the surface. The CTAB ions adsorbed on the silica surface form an apolar layer similar to that of a chemically bonded ODS-silica material. The separation mechanism for neutral compounds is based on the reversed-phase partition according to the literature [14,15]. In this work, the idea of dynamically modified silica column was used in CEC and a novel mode of CEC established, which is called dynamically modified silica-CEC (DMS-CEC). DMS-CEC can be performed on a commercial capillary electrophoresis instrument which cannot apply pressure on both sides of the capillary. It was also found that the peak tailing of basic compounds can be effectively eliminated in this mode because the surfactant has more affinity to silanol than common basic compounds.

2. Experimental

2.1. Instrumentation and materials

All the CEC experiments were performed on a P/ACE system 5010 (Beckman, Fullerton, CA, USA). The CZE experiments were carried out on a P/ACE system MDQ (Beckman). A Spectra-Physics pump (Spectra-Physics, San Jose, CA, USA) was used to pack capillary columns. Fused-silica capillary (100 μm I.D.×365 μm O.D.) was obtained from Yongnian Optic Fiber Plant (Hebei, China). Five μm Spherisorb-silica gel and 5 μm Spherisorb-ODS I were purchased from Waters (Milford, MA, USA).

2.2. Samples and solution

Methanol was of chromatographic grade, ethylbenzene was of chemical grade, and the other reagents used were of analytical-reagent grade. The ultra-pure water used for preparing solutions was produced by the Milli-Q water system (Millipore, Bedford, MA, USA). The sample solution was first prepared with methanol, then diluted to appropriate concentration with the mobile phase before injection. The stock solution of phosphate buffer (100 m*M*) was prepared by dissolving 3.90 g NaH_2PO_4 in 200 ml ultra-pure water, then adjusting to pH 7.5 by 1 *M* NaOH and transferring to a 250-ml flask. The stock solution of CTAB (10 m*M*) was prepared by dissolving 0.911 g CTAB in 250 ml ultra-pure water. The mobile phases were prepared by mixing 2.5 ml phosphate buffer and appropriate volume of CTAB stock solution, methanol and ultra-pure water. Before running, the mobile phase was degassed in an ultrasonic bath for 10 min.

2.3. Separation conditions

All the separations were performed at the following conditions except otherwise stated: experiments for DMS-CEC, reversed-phase CEC and CZE were performed at various voltages from 15 kV to 25 kV. The injections were made by applying a voltage of 10 kV for 1 s in CEC and by pressurizing the inlet and outlet vials to 8.17 kPa for 5 s in CZE. The temperature was kept at 25°C and the detection wavelength was set at 214 nm. Fused-silica capillaries of 27 cm (20 cm to detector)×100 μm I.D. were used for the CEC experiments and those of 50.2 cm (40 cm to detector)×100 μm I.D. were used for the CZE experiments.

2.4. Column preparation

CEC columns with Spherisorb-silica gel and Spherisorb-ODS I were packed by slurry packing method as reported in the literature [7].

3. Results and discussion

3.1. Electroosmotic flow (EOF)

It was found previously [16] that in CEC, bubble formation seldom occurred on the bare silica packed column even when the mobile phase contained 60% methanol and 10 m*M* phosphate buffer, and Joule heating effects could be negligible even when the current was more than 20 μA. In this work, CEC on bare silica column with the addition of surfactant into the mobile phase can also be performed without applying pressure. The current observed was about 10 μA at an applied voltage of 20 kV with a mobile phase containing 50% methanol, 2 m*M* CTAB and 5 m*M* phosphate buffer (pH 7.5). In the DMS-CEC mode, bubbles occurred occasionally. The formation of bubble resulted in decreasing current, increasing retention time and reducing efficiency. But the bubbles can be easily be pushed out by purging the column for 30 min with water and then for 30 min with the mobile phase by using a syringe, of which the needle is connected to the outlet of the CEC column with a PTFE tube. Changing mobile phase can simply be performed by changing the vials in the capillary electrophoresis instrument. But the equilibration times on the dynamically modified silica column is relatively long. It takes about 50 min to equilibrate the column during mobile phase changing.

The effects of the surfactant concentration and the methanol content on the EOF in DMS-CEC were investigated and compared with that in CZE with the same mobile phase. Formamide was selected as the t_0 marker in both systems. The magnitude of EOF (μ_{eo}) was calculated by the following equation:

$$\mu_{eo} = (L_d L_t)/(V t_0) \quad (1)$$

where L_t is the total length of capillary, L_d is the length of capillary from the inlet to the detector window and V is the applied voltage. Theoretically, the magnitude of EOF depends directly on three factors viz. the zeta potential of the surface, the dielectric constant and the viscosity of the mobile phase. These are determined by other physico–chemical properties such as surface charge density, solvent composition, electrolyte concentration and temperature [1]. The direction of EOF is determined by the sign of charge on the surface. The surface of an unmodified capillary and of most silica-based HPLC packings is negatively charged, therefore, the direction of EOF is from anode to cathode. However, this direction can be reversed by the addition of cationic surfactants like CTAB into the running buffer in CZE [18]. The influence of cationic surfactants on EOF can be divided into two stages with increasing surfactant concentration. Firstly, the cationic surfactant will be adsorbed onto the capillary surface due to the electrostatic attraction between the positively charged head group of the cationic surfactant and the negatively charged SiO^- groups. Thus the non-polar chains of the surfactant will form a hydrophobic layer. In this stage, with increasing of the surfactant concentration, the surface charge density of capillary will decrease due to the neutralization of the negative charge by the surfactant ions, thereby the EOF rate will decrease. If the negative charge has been completely neutralized, EOF will be completely suppressed. In the first stage, the direction of EOF is still from anode to cathode because the surface charge is still negative. Secondly, upon further increase of the surfactant concentration a

bilayer is formed by the hydrophobic interaction between the non-polar chains of surfactant. The cationic sites of the second layer molecules are now faced towards the buffer solution, resulting in a positive surface charge, and the reversal of EOF direction occurs. So it can be determined from the direction of EOF, whether only mono layer or double layers of cationic surfactant is formed on the silica surface.

Since the structure of the bare silica surface is similar to that of the unmodified capillaries, the law of EOF in CEC columns packed with bare silica must be similar to that of the CZE with unmodified capillary. Fig. 1 shows the influence of the CTAB concentration in CZE and CEC with bare silica as the stationary phase. It can be seen from Fig. 1 that the magnitude of EOF from anode to cathode in CZE seriously decreases with increasing CTAB concentration, and the EOF is reversed when the CTAB concentration exceeds about 0.5 m*M*, then the magnitude of EOF from cathode to anode increases with further increasing the CTAB concentration. In DMS-CEC, the effect of the CTAB concentration on EOF was quite different from that in CZE. Firstly, the EOF direction does not change throughout the range of the CTAB concentration investigated, which is always from anode to cathode. Secondly, the CTAB concentration has less influence on the magnitude of EOF in DMS-CEC than that in CZE. This result can be explained by the fact that a CEC column packed with silica particle has a higher surface area and thus a higher number of negative groups to be neutralized by CTAB than a CZE capillary. The effect of the CTAB concentration on the magnitude of EOF was also investigated with CTAB higher than 4 m*M*, but it was difficult to perform the experiments due to unstable baselines and currents probably caused by bubble formation. The fact that the magnitude of EOF in DMS-CEC slightly decreased with the increase of the CTAB concentration means that more CTAB ions have been adsorbed onto the bare silica surface, and the surface of silica become more hydrophobic with increasing the CTAB concentration.

The influence of the methanol content on EOF in DMS-CEC and CZE was studied as shown in Fig. 2. It can be seen that the magnitude of EOF from cathode to anode in CZE decreased quickly with increasing the methanol content from 50% to 60%. Upon further increase of the methanol concentration to 70%, the EOF direction was reversed and the magnitude of EOF from anode to cathode increased. The reason for this might be that the amount of the adsorbed CTAB ions decreases quickly with increasing the methanol content. However, the magnitude of

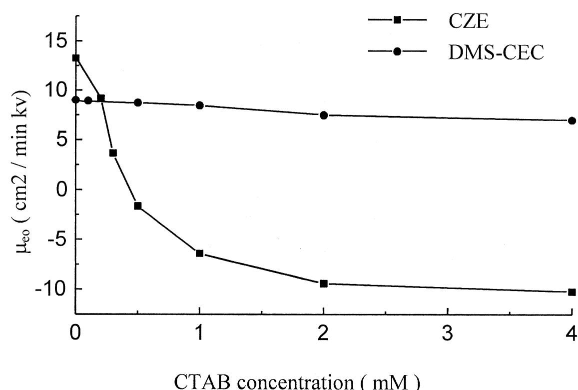

Fig. 1. Influence of the CTAB concentration on electroosmotic mobility (μ_{eo}) in DMS-CEC and CZE. Experimental conditions: mobile phase, 5 m*M* phosphate buffer (pH 7.5) containing 50% methanol and various concentrations of CTAB. (a) DMS-CEC: applied voltage, 25 kV; electrokinetic injection, 1 s×10 kV; other conditions are the same as in Fig. 3. (b) CZE: column, capillary of 50.2 cm (effective length 40 cm)×100 μm I.D.×375 μm O.D.; applied voltage, 20 kV; hydrodynamic injection, 8.17 kPa×5 s.

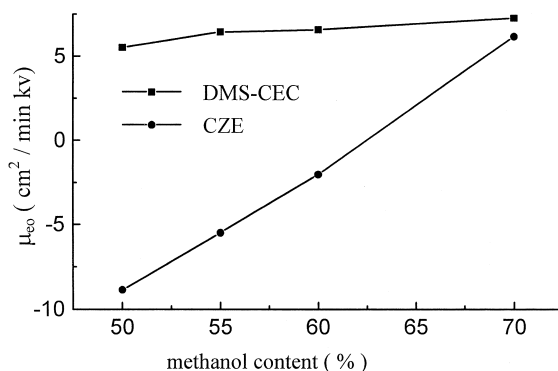

Fig. 2. Influence of the methanol content on electroosmotic mobility (μ_{eo}) in DMS-CEC and CZE. Experimental conditions: mobile phase, 5 m*M* phosphate buffer (pH 7.5) containing 2 m*M* CTAB and various concentrations of methanol. (a) DMS-CEC, applied voltage: 20 kV; electrokinetic injection: 1 s×10 kV, other conditions as in Fig. 3. (b) CZE, experimental conditions as in Fig. 1b.

EOF from anode to cathode in DMS-CEC always increases slightly with increasing the methanol content, but there is much less effect of methanol concentration on the magnitude of EOF in DMS-CEC than that in CZE. This result indicated that there is much less effect of the methanol concentration on the amount of absorbed CTAB ions in DMS-CEC because only a mono layer of CTAB is formed in DMS-CEC.

3.2. Chromatographic behavior

The DMS-CEC system was established by the adsorption of CTAB ions on bare silica particle, and the adsorbed CTAB forms a hydrophobic layer which acts as stationary phase. Neutral solutes can be separated due to the difference of their partitioning between the mobile phase and the hydrophobic layer. The efficiency of column was evaluated with formamide and five alkyl benzene homologous as test solutes. A typical chromatogram is shown in Fig. 3. The highest number of theoretical plates obtained was about 71 500/m, which was not very good for the CEC column packed with 5 μm particles. Relatively low column efficiency may be explained by a fact that the dynamic adsorption of CTAB on silica will contribute to the resistance to mass transfer of solute from the mobile to stationary phases. The reproducibility was evaluated with test solutes of formamide and toluene. The relative standard deviation (RSD) for t_0 marked by formamide was 4.7%, and the RSD for capacity factor of toluene was 4.9% for 20 consecutive runs. But those in CEC with Spherisorb-ODS I packing were 3.6% and 2.7%, respectively, according to our previous experiments [17], which means that the reproducibility of retention values in DMS-CEC was not as good as that in the reversed-phase CEC with silica-based ODS as stationary phase.

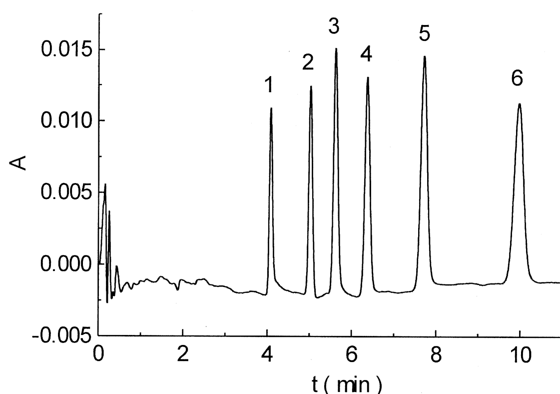

Fig. 3. Chromatogram for separation of aromatic compounds by DMS-CEC. Experimental conditions: column, 27 cm (packed length 20 cm)×100 μm I.D.×375 μm O.D. packed with 5 μm Spherisorb-silica gel; mobile phase, 5 m*M* phosphate buffer (pH 7.5) containing 60% methanol and 2 m*M* CTAB; applied voltage, 20 kV; electrokinetic injection: 5 s×10 kV; UV detection wavelength, 214 nm. Peaks: 1=formamide, 2=benzene, 3=toluene, 4=ethylbenzene, 5=*n*-propylbenzene, 6=*n*-butylbenzene.

The separation mechanism of neutral solutes in this system was studied through the relationship of capacity factors versus the concentration of CTAB and methanol and the carbon number of solutes. t_0 was measured by formamide and k' was calculated by the following equation:

$$k' = (t_R - t_0)/t_0 \quad (2)$$

where t_R is the retention time of the solutes. It is well known that there is a linear relationship between the logarithm of the capacity factor (log k') of homologous solutes and their carbon numbers (N_c) in reversed-phase HPLC. Fig. 4 shows the log k' vs. N_c plots for test solutes in DMS-CEC with the mixed solutions of methanol and phosphate buffer containing 2 m*M* and 4 m*M* CTAB as the mobile phases. As can be seen from Fig. 4, log k' and N_c obey excellent linear relationships (r>0.999), and

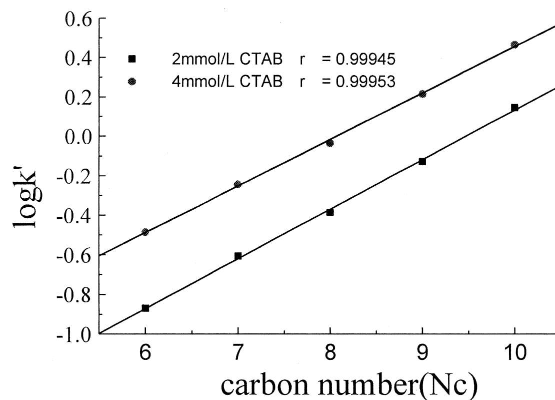

Fig. 4. Linear relationship of log k' and carbon number of solutes (N_c) in DMS-CEC. Experimental conditions and solutes as in Figs. 1a and 3, respectively.

the positive value of the slope means that the retention value in DMS-CEC increases with the hydrophobicity of the solute. This result strongly supports that the separation mechanism in DMS-CEC is based on the reversed-phase partitioning.

When the CTAB concentration is over its critical micellar concentration (CMC) micelles will form in the mobile phase. The micelles will act as a pseudo-stationary phase just like in MECC [19] and micellar liquid chromatography (MLC) [20]. The separation mechanism in this case will become more complex due to the distribution of solutes into micelles. However, CTAB concentration used in this study is less than 4 m*M* which is far lower than the CMC of CTAB (about 16 m*M* in 50% methanol solution [21]), the distribution of solute in micelle in a way of MECC does not contribute to separation mechanism of DMS-CEC. In order to investigate whether the electrophoresis mechanism contributes to the separation of neutral solutes in DMS-CEC, five alkyl benzenes as the test solutes have been separated in CZE and DMS-CEC with the same mobile phase of 5 m*M* phosphate buffer containing 50% methanol and 2 m*M* CTAB, and the chromatograms obtained are shown in Fig. 5a and c. Fig. 5b shows the separation of five alkylbenzenes in CEC with a silica-packed column and the same mobile phase as in DMS-CEC with absence of CTAB. It can be seen from Fig. 5a and c that all the six solutes (the first one is the t_0 marker) were baseline separated in DMS-CEC, but only *n*-propylbenzene and *n*-butylbenzene were partially separated in CZE. Since neutral solutes have no charge and no micelle was formed in the mobile phase in CZE, the separation of *n*-propylbenzene and *n*-butylbenzene was obtained only due to their partitioning between the mobile phase and the adsorbed CTAB double layer as reported in our previous papers [22]. It was reported that siloxane bridge of bare silica has some hydrophobicity and can be used as a hydrophobic stationary phase in HPLC [23]. Partial separation of five alkylbenzenes in CEC with the silica-packed column was achieved as shown in Fig. 5b through hydrophobic interaction of neutral solutes with the silica surface. This result means that the hydrophobicity of silica surface by the dynamic adsorption of CTAB in DMS-CEC is high enough to separate neutral solutes as that in the reversed-phase CEC.

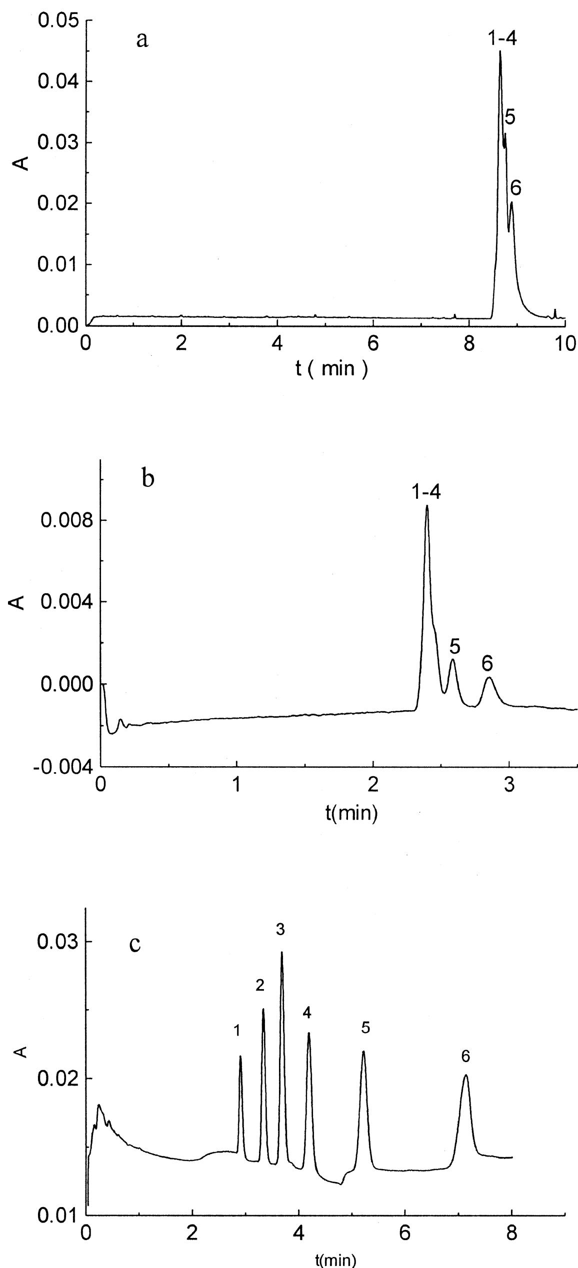

Fig. 5. Chromatogram for separation of the test solutes in CZE, CEC with silica-packed column and DMS-CEC. Experimental conditions: (a) CZE with mobile phase of 5 m*M* phosphate buffer (pH 7.5) containing 50% methanol and 2 m*M* CTAB; applied voltage, 25 kV; other conditions as in Fig. 1b. (b) CEC with the silica packed column and mobile phase of 5 m*M* phosphate buffer (pH 7.5) containing 50% methanol; other conditions as in Fig. 3. (c) DMS-CEC with mobile phase of 5 m*M* phosphate buffer (pH 7.5) containing 50% methanol and 2 m*M* CTAB, other conditions as in Fig. 3.

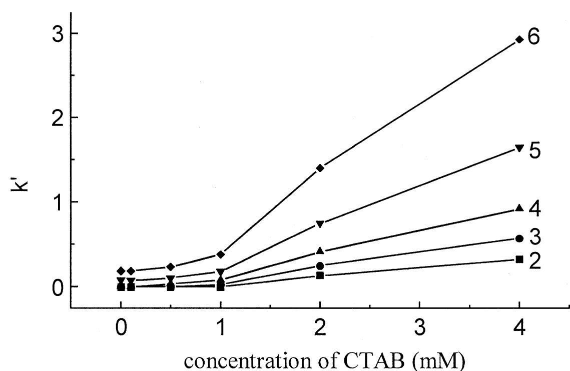

Fig. 6. Influence of the CTAB concentration on capacity factor (k') in DMS-CEC. Experimental conditions and solutes as in Figs. 1a and 3, respectively.

The influence of CTAB concentration and methanol content on the capacity factors of alkylbenzenes was also investigated in this study. The results are shown in Figs. 6 and 7. As can be seen in Fig. 6, the capacity factors increased with increasing CTAB concentration. The reason is that more CTAB ions were adsorbed onto the silica surface which became more hydrophobic with increasing the CTAB concentration as discussed above. As can be seen in Fig. 7, the capacity factors decreased with increasing the methanol content, which may be caused by the facts that the elution strength of mobile phase increased as in reversed-phase CEC and the amount of adsorbed CTAB ions on the silica surface decreased with increasing the methanol content in the mobile phase.

The analysis of basic solutes by reversed-phase CEC on chemically-modified ODS silica gel is hindered by the occurrence of badly tailing peak. For example, poor peak shape of strong basic compounds was observed on the silica based ODS stationary phase [3]. A way to resolve the problem is the addition of competing base to the mobile phase [5,6], but special instrumentation with pressurization of both the capillary ends is required in order to avoid

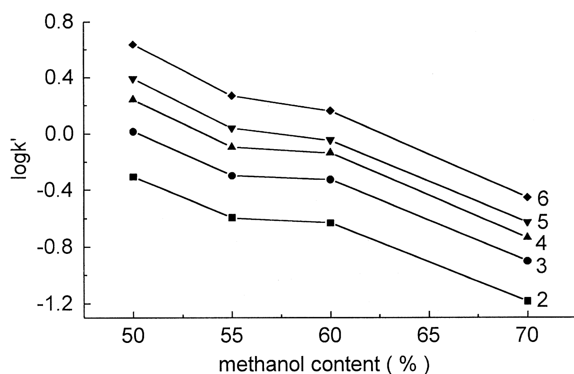

Fig. 7. Influence of the methanol content on capacity factor (k') in DMS-CEC. Experimental conditions and solutes as in Figs. 2 and 3, respectively.

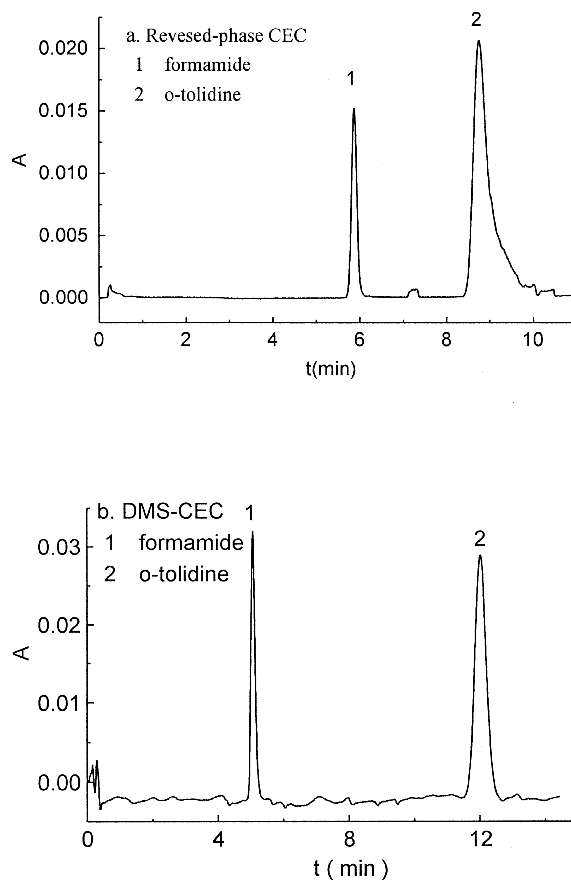

Fig. 8. Chromatogram of basic solutes in reversed-phase CEC and DMS-CEC. Experimental conditions: (a) reversed-phase CEC: column, 27 cm (packed length 20 cm)×100 μm I.D. capillary packed with 5 μm Spherisorb-ODS I; mobile phase, 1 m*M* phosphate buffer (pH 7.5) containing 70% methanol; applied voltage, 15 kV; electrokinetic injection, 1 s×10 kV; detection wavelength, 214 nm. (b) DMS-CEC: column, 27 cm (packed length 20 cm)×100 μm I.D. capillary packed with 5 μm Spherisorb-silica gel; mobile phase, 5 m*M* phosphate buffer (pH 7.5) containing 55% methanol and 2 m*M* CTAB; applied voltage, 20 kV; electrokinetic injection, 1 s×10 kV; detection wavelength, 214 nm.

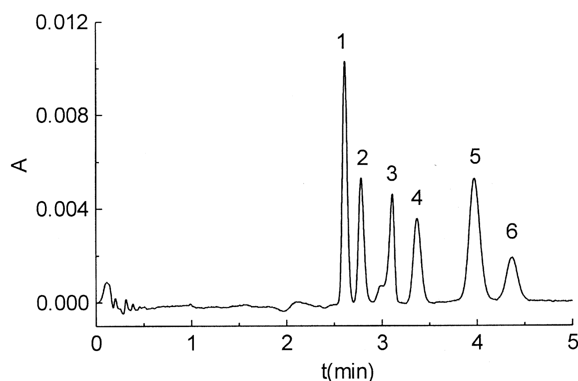

Fig. 9. Chromatogram of aniline compounds by DMS-CEC. Experimental conditions: column, 27 cm (packed length 20 cm)×100 μm I.D. capillary packed with 5 μm Spherisorb-silica gel; mobile phase, 5 m*M* phosphate buffer (pH 7.5) containing 40% methanol and 0.9 m*M* CTAB; separation voltage, 30 kV; electrokinetic injection, 1 s×10 kV; detection, 214 nm. Peaks: 1=aniline, 2=*m*-nitroaniline, 3=*o*-nitroaniline, 4=3,5-dinitroaniline, 5=*o*-tolidine, 6=2,6-dichloro-4-nitroaniline.

bubble formation. However, the peak tailing of basic compounds can be effectively eliminated with the DMS-CEC mode because the surfactant used has stronger affinity to silanol group than the common basic compound does. Fig. 8a and b show the chromatograms for separation of formamide (neutral solute, peak 1) and *o*-tolidine (basic solute, peak 2) for reversed-phase CEC and DMS-CEC. As can be seen from Fig. 8, the neutral solute has good peak symmetry in both systems, while the basic solute *o*-tolidine has poor peak shape in reversed-phase CEC but good peak shape in DMS-CEC. Figs. 9 and 10 show the chromatograms for the separation of six compounds containing nitrogen and small basic peptides, respectively. It can be seen that all of the basic compounds have good peak symmetry.

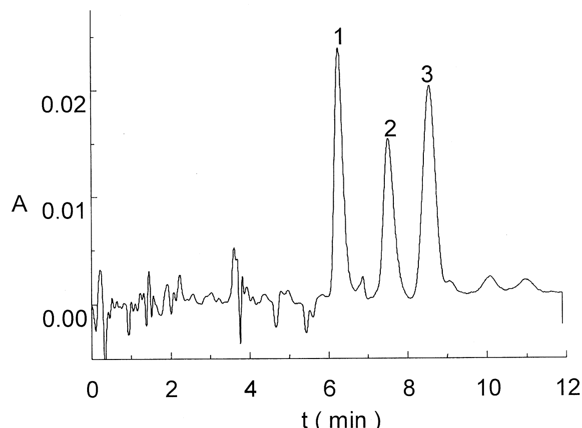

Fig. 10. Chromatogram for separation of three basic peptides by DMS-CEC. Experimental conditions: 31 cm (packed length 10 cm)×100 μm I.D. capillary packed with 5 μm Spherisorb-silica gel; mobile phase, 10 m*M* phosphate buffer (pH 7.65) containing 60% methanol and 2 m*M* CTAB; separation voltage, 15 kV; electrokinetic injection, 10 s×5 kV; detection, 200 nm. Peaks: 1=Arg–Gly, 2=Arg–Trp, 3=Lys–Tyr–Ser.

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