

Renan Wu\*  
Hanfa Zou  
Mingliang Ye  
Zhengdeng Lei  
Jianyi Ni

National Chromatography R&A  
Center, Dalian Institute of  
Chemical Physics, the Chinese  
Academy of Science,  
Dalian, China

## Separation of basic, acidic and neutral compounds by capillary electrochromatography using uncharged monolithic capillary columns modified with anionic and cationic surfactants

A mode of capillary electrochromatography (CEC), based on the dynamical adsorption of surfactants on the uncharged monolithic stationary phases has been developed. The monolithic stationary phase, obtained by the *in situ* polymerization of butyl methacrylate with ethylene dimethacrylate, was dynamically modified with an ionic surfactant such as the long-chain quaternary ammonium salt of cetyltrimethylammonium bromide (CTAB) and long-chain sodium sulfate of sodium dodecyl sulfate (SDS). The ionic surfactant was adsorbed on the surface of polymeric monolith by hydrophobic interaction, and the ionic groups used to generate the electroosmotic flow (EOF). The electroosmotic mobility through these capillary columns increased with increasing the content of ionic surfactants in the mobile phase. In this way, the synthesis of the monolithic stationary phase with binary monomers can be controlled more easily than that with ternary monomers, one of which should be an ionic monomer to generate EOF. Furthermore, it is more convenient to change the direction and magnitude of EOF by changing the concentration of cationic or anionic surfactants in this system. An efficiency of monolithic capillary columns with more than 140 000 plates per meter for neutral compounds has been obtained, and the relative standard deviations observed for  $t_0$  and retention factors of neutral solutes were about 0.22% and less than 0.56% for ten consecutive runs, respectively. Effects of mobile phase composition on the EOF of the column and the retention values of the neutral solutes were investigated. Simultaneous separation of basic, neutral and acidic compounds has been achieved.

**Keywords:** Electrochromatography / Dynamically modified stationary phase / Surfactant / Uncharged monolithic column  
EL 4262

### 1 Introduction

Capillary electrochromatography (CEC) is a relatively new microseparation technique combining the high selectivity of high-performance liquid chromatography (HPLC) and the high efficiency of capillary zone electrophoresis (CZE) [1–3]. The mobile phase in CEC is driven by electroosmotic flow (EOF), and extremely high efficiencies can be obtained for CEC separations due to the plug flow profile of the mobile phase, which leads to small zone broadening. The coexistence of the stationary phase and the electric field in CEC permits the separation not only of ionic compounds but also of neutral solutes according to their different migration and/or partition properties. Ac-

ordingly, CEC has attracted more and more attention in separation sciences [4–6].

Although CEC was invented in the early 1970s [1], and its potential for packed capillary columns was demonstrated in the 1980s, the development of this promising separation method has been slowed by the following technical problems [7]: the limited stability of packed columns, the difficult fabrication of frits within a capillary, the packing of beads into a capillary with a very small diameter, and the formation of bubbles within the capillary during runs due to the existence of frits in capillary. Although no frits are used with open-tubular CEC (OT-CEC) columns, the relatively low phase ratio in OT-CEC restricts its further development and applications. Recently, the monolithic columns in CEC have attracted increasing attention due to their potential advantages, which could be easily prepared by *in situ* polymerization. Since the rod of the stationary phase is directly bonded to the inner wall of the capillary through covalent bonds, no supporting frits are necessary. In addition, the pore size of the stationary

**Correspondence:** Dr. Hanfa Zou, National Chromatography R&A Center, Dalian Institute of Chemical Physics, the Chinese Academy of Science, Dalian 116011, China  
**E-mail:** zouhfa@mail.dlptt.ln.cn  
**Fax:** +86-411-3693407

**Abbreviations:** AIBN, azobisisobutyronitrile; BMA, butyl methacrylate; EDMA, ethylene dimethacrylate;  $\gamma$ -MAPS,  $\gamma$ -methacryloxypropyltrimethoxysilane; ODS, octadecylsilane

\* On leave from Wenzhou University, Zhejiang Province, China

phase could also be adjusted during the preparation procedure to obtain optimal separation, especially in the analysis of molecules such as polymers and proteins.

Several research groups have reported works on the monolithic columns for CEC by utilizing stationary phases such as polymethacrylate [8–14] and polyacrylamide/poly(ethylene glycol) [15]. Hydrophobic ligands, such as C<sub>4</sub>, C<sub>6</sub>, C<sub>12</sub> or C<sub>18</sub> are introduced either as groups pre-existing on the monomers or by chemical modification of the polymer. Usually, in order to generate EOF as the propelling force in CEC, charge-bearing monomers such as 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) [10–13], acrylic or vinylsulfonic acid [9], and dimethyl diallylammonium chloride [8] have been introduced during polymerization. Surface-active reagents, specifically surfactants, have widely been used in various modes of liquid separation techniques, *e.g.*, to control EOF in CZE [16, 17], and to generate dynamically adsorbed stationary phases in HPLC [18]. In 1990, Pfeffer and Yeung [19] reported the use of a CTAB surfactant to control EOF and to generate a hydrophobic stationary phase in small-diameter fused-silica capillaries for OT-CLC. Later, Garner and Yeung [20] reported that a capillary coated with a hydrophobic stationary phase could be dynamically modified by CTAB to form a dynamic ion-exchanger, thereby improving the EOF and separation selectivity in OT-CEC. The separation mechanism for neutral compounds on the dynamically modified stationary phases was studied in the modes of OT-CEC [20] and packed-column CEC [21]. Seifar *et al.* [22, 23] also reported that the zeta potential (the surface charge) on octadecyl silica (ODS) packing material can be improved by the addition of anionic surfactants (SDS) into the mobile phase, which indicated that adsorption of SDS on the packing surface took place. Recently, the dynamical modification of the stationary phases has been extended to strong cation-exchange (SCX) and strong anionic-exchange (SAX) packing materials for separation of acidic, basic and neutral solutes [24] as well as enantiomers [25, 26]. Alicea-Maldonado and Colón [27] reported that the EOF on an ethylene chlorotrifluoroethylene (ECTFC) particles packed column can be generated by addition of trifluoroacetic acid (TFA) into a mobile phase in CEC, although ECTFE does not possess ionizable moieties on the surface. The generation of EOF in this type of columns was attributed to the adsorption of TFA onto the surface of the plastic material. SDS was added to the mobile phase in CEC on a C18-derivatized continuous bed containing a sulfonic acid group to improve the column EOF and peak widths [9].

In this work, CEC monolithic columns with *in situ* polymerization of neutral monomers of butyl methacrylate with ethylene dimethacrylate were prepared. By this method,

monolithic columns are more easily produced than those as reported in [28, 29] due to the absence of any charge-bearing monomer such as AMPS. Both CTAB and SDS were added to the mobile phases to form a dynamic ion-exchanger on the hydrophobic polymer surface to generate the EOF for CEC separation. The direction and the magnitude of EOF can easily be adjusted by adopting different types and concentration of surfactants (CTAB or SDS).

## 2 Materials and methods

### 2.1 Materials

Butyl methacrylate (BMA), ethylene dimethacrylate (EDMA) and  $\gamma$ -methacryloxypropyltrimethoxysilane ( $\gamma$ -MAPS) were purchased from Sigma (St. Louis, MO, USA). 1-Propanol, 1,4-butanediol and azobisisobutyronitrile (AIBN) were obtained from the Fourth Shanghai Regant Plant (Shanghai, China). Methanol (MeOH) and acetonitrile (ACN) of HPLC grade were supplied by the Yuwang Chemical Plant (Zibo, Shandong Province, China). BMA and EDMA were extracted with 5% aqueous sodium hydroxide solution and dried over anhydrous magnesium sulfate; BMA was distilled under vacuum before polymerization. All test compounds were of analytical grade. Water used in all experiments was doubly distilled and purified by a Milli-Q system (Millipore, Milford, MA, USA). The reagents of cetyltrimethyl ammonium bromide (CTAB) and SDS were purchased from the Second Beijing Reagent Factory (Beijing, China). Capillaries with 100  $\mu$ m ID and 375  $\mu$ m OD were purchased from the Yongnian Optic Fiber Plant (Hebei, China) from which a ~1 mm portion of the polyimide coating was removed for UV detection.

### 2.2 Instrumentation

Electrochromatographic experiments were carried out on a Beckman P/ACE 5510 CE system (Beckman, Palo Alto, CA, USA) equipped with a DAD UV detector. Data acquisition and processing were performed by using the Beckman ChemStation software. A Spectra-Physics HPLC pump (Spectra-Physics, San Jose, CA, USA) was used to flush the columns.

### 2.3 Samples and solutions

The sample solution was first prepared with ACN, then diluted to the appropriate concentration with the mobile phase before injection. The stock solution of phosphate buffer (100 mM) was prepared by dissolving 3.9 g NaH<sub>2</sub>PO<sub>4</sub> in 200 mL water, then adjusted to pH 7.0 by 1 M NaOH and transferred to a 250 mL flask. The stock solution of CTAB (25 mM) was prepared by dissolving 2.2779 g CTAB in 250 mL water. The stock solution of

SDS (50 mM) was prepared by dissolving 3.6048 g SDS in 250 mL water. The mobile phases were prepared by mixing 2.5 mL phosphate buffer and appropriate volumes of CTAB or SDS stock solution, ACN and water. Before running, the mobile phase was degassed in an ultrasonic bath for 10 min.

## 2.4 Preparation of the monolithic polymer capillaries

The capillary was first rinsed with 0.1 M KOH solution for 1 h and then with water to the pH value of the outlet solution at 7.0. After subsequent flushing with methanol for 10 min, it was dried by passage of nitrogen gas.  $\gamma$ -MAPS dissolved in methanol at a ratio of 1:1 v/v injected into the capillary with a syringe. It was then kept at 30°C overnight with both ends sealed with rubber. Finally, the capillary was rinsed with methanol and water, respectively, to flush out the residual reagents. Then the layer of  $\gamma$ -MAPS was introduced onto the inner wall of the capillary. AIBN (3 mg, 0.3% with respect to the monomers) was dissolved in 1.0 g of solution consisting of 50% w/w EDMA and 50% w/w BMA, respectively. The porogenic solvent contained 50% w/w 1-propanol and 50% w/w 1,4-butanediol. The monomer and porogenic solvents were mixed at a ratio of 50:50 vol.%. After ultrasonication for 15 min and sparging with nitrogen for 10 min, the small part of the mixed solution was removed using a 100  $\mu$ L syringe for capillary preparation. A 40-cm length of pretreated capillary was attached to the syringe inlet and filled with the polymerization mixture to a total length of 30 cm. The ends of the capillary were plugged with a piece of rubber tubing, and the capillary was submerged in a water bath at 60°C for 12 h. The monolithic capillary was washed with methanol and mobile phases, respectively, to flush out the residual reagents using an HPLC pump by applying the pressure at about 1000 psi; no movement of the monolithic bed was observed. The detection window was then created at the end of the continuous polymer bed. Finally, the capillary with monolithic polymer stationary phase was equilibrated with the mobile phase at low voltage for 1 h before running.

## 2.5 Electrochromatographic experiments

The capillary column was placed to the CE instrument and equilibrated by applying a voltage of 5 kV until the electric current and flow rate stabilized. Aromatic compounds were used as the model analytes, and thiourea was used as an unretained marker of the void time. The injections were made by applying a voltage of 1 kV for 1 s in CEC. The temperature was kept at 20°C and the detection wavelength was set at 214 nm. Fused-silica capillaries of 27 cm (20 cm or 6.5 cm to detector)  $\times$  100  $\mu$ m ID  $\times$  375  $\mu$ m OD were used for the CEC experiments.

## 3 Results and discussion

### 3.1 EOF in the monolithic columns

Usually, the EOF can be generated on the stationary phase in CEC due to ionized groups such as silanol, sulfonic, and amido groups. Thus, to prepare the monolithic columns in CEC, one of the monomers with ionizable groups should be adopted, with exception of the silica-based monolithic columns, where the ionizable silanol groups are involved on the surface. The addition of the ternary component to the copolymeric monomer would make it complex to control the performance of monolithic columns. In addition, once the monolithic column is prepared, the direction of the EOF is determined.

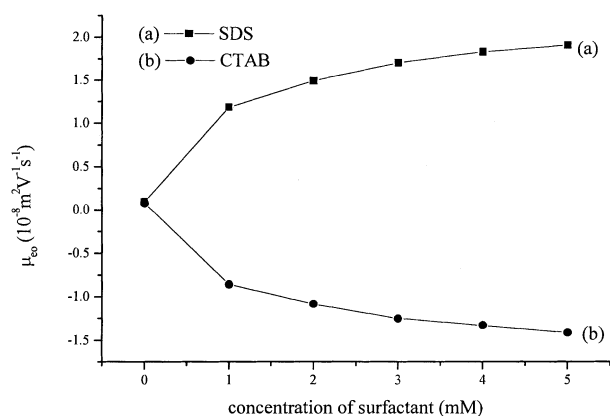
Theoretically, the magnitude of EOF depends directly on three factors *viz.* zeta potential ( $\zeta$ ) of the surface, the dielectric constant and the viscosity of the mobile phase. They are determined by other physicochemical properties such as surface charge density, solvent composition, electrolyte concentration and temperature. The sign of charge on the surface determines the direction of EOF. The magnitude of EOF ( $\mu_{eo}$ ) was calculated by the following equation:

$$\mu_{eo} = \frac{L_e L_t}{V t_0} \quad (1)$$

where  $L_t$  is the total length of column,  $L_e$  is the length of column from the inlet to the detector window,  $V$  is the applied voltage, and  $t_0$  is the eluted time of unretained compound.

Our monolithic columns were prepared by *in situ* polymerization of BMA with EDMA. No ionizable moieties exist on the monolithic bed, therefore, no EOF should be generated on this type of columns. We have measured EOF in a monolithic column with phosphate buffer (pH 7.0) containing 35% ACN as the mobile phase. The obtained EOF value is only about  $0.1 \times 10^{-8} \text{m}^2 \text{V}^{-1} \text{s}^{-1}$ . This result indicates that the EOF from the originally prepared monolithic bed is about ten times lower than that from a conventional CEC column which can not be applied to propel the mobile phase in CEC because the flow rate of the mobile phase is too small in practice.

However, EOF in those monolithic columns can be generated by the dynamical adsorption of the anionic or cationic surfactants onto the surface of the continuous bed. The effect of dynamic modification of the polymeric monolithic columns for CEC in ACN-water mobile phase was investigated. Figure 1 shows the changes of EOF on the monolithic column with CTAB and SDS concentrations in the mobile phase. The direction of EOF is from anode to cathode when SDS is added to the mobile phase; however, it



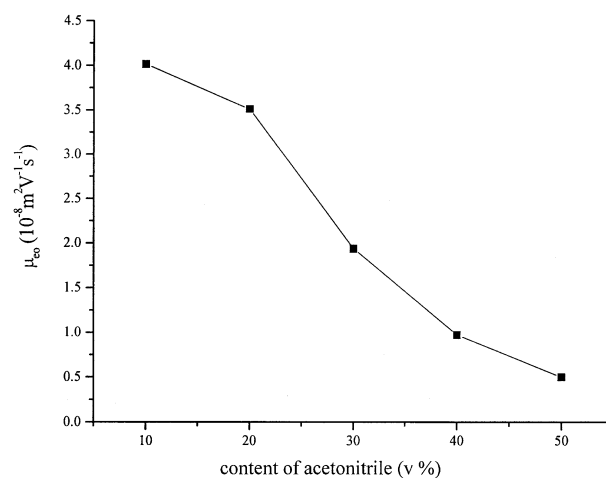
**Figure 1.** Effect of the concentration of (a) SDS and (b) CTAB on the electroosmotic mobility in uncharged monolithic stationary phase based CEC. Experimental conditions: (a) mobile phase, 5 mM phosphate buffer (pH 7.0) containing 35% ACN and various concentrations of SDS; column, capillary of 27 cm (6.5 cm effective length) with 100 μm ID and 375 μm OD; applied voltage, 5 kV; injection, 2 kV for 1 s; (b) mobile phase, 5 mM phosphate buffer (pH 7.0) containing 35% ACN and various concentrations of CTAB; other conditions as for (a).

is reversed with CTAB is added. It can be seen that the EOF on the column increases very quickly at the first stage with a surfactant concentration from 0 to 2 mM. A further increase of the surfactant concentration leads to a slight increase of the EOF values. The EOF value in the SDS system is always higher than that in the CTAB system at a fixed surfactant concentration. For example, the EOF values measured at 2 mM CTAB and SDS are about  $1.1 \times 10^{-8}$  and  $1.5 \times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ , respectively, which are sufficient to generate a relatively high flow rate of the mobile phase in CEC. This could be explained by the higher effective charge of SDS compared to that of CTAB, because in the latter case the charge may be hindered with three methyl groups.

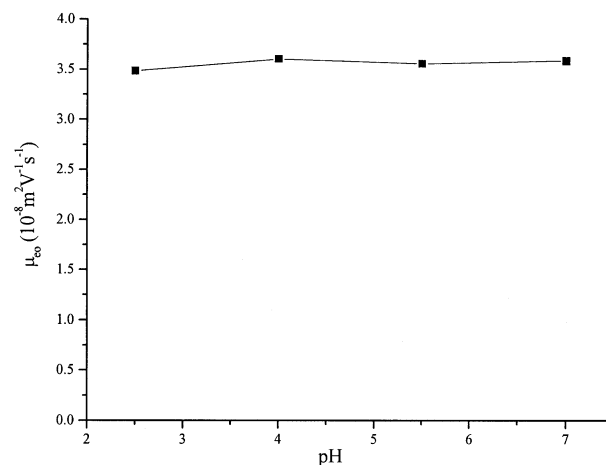
The influence of the ACN content on EOF values in CEC with addition of 2.5 mM SDS to the mobile phase was investigated, and the obtained results are shown in Fig. 2. It can be seen that the magnitude of EOF from anode to cathode in CEC decreases quickly with increasing acetonitrile content from 10 to 50%. This result can be explained by the fact that the EOF is mainly determined by the adsorbed amount of SDS on the monolithic bed through the hydrophobic interaction between the carbon chain of SDS and the hydrophobic surface; the amount of the adsorbed SDS decreases quickly with increasing acetonitrile content in the mobile phase.

Furthermore, the influence of pH on EOF was also investigated with an ACN/water mobile phase (35/75 v/v) containing 5 mM sodium phosphate and 2.5 mM SDS. As

shown in Fig. 3, the EOF changes slightly with the pH from 2.5 to 7.0. As we know, the adsorption of SDS on the monolithic bed is mainly driven by the hydrophobic interaction between them. SDS is a strong anionic surfactant, and the monolithic bed is a neutral polymer, so the change of the eluent pH value does not affect hydrophobicity of the SDS, stationary phase and even mobile phase, resulting in a minor effect on the adsorbed amount of SDS. Therefore, the effect of the eluent pH value on the EOF should be negligible. This means that the mono-



**Figure 2.** Influence of ACN concentration on the EOF on the monolithic column modified with SDS. Experimental conditions: mobile phase, 5 mM phosphate buffer (pH 7.0) containing various concentrations of ACN and 2.5 mM of SDS; other conditions as in Fig. 1, except injection with 1 kV for 1 s.



**Figure 3.** Effect of the eluent pH on the EOF on the monolithic column modified with SDS. Experimental conditions: column, 27 cm (20 cm effective length); mobile phase, 35% ACN in 5 mM phosphate buffer (pH 7.0) containing 2.5 mM SDS; applied voltage, 5 kV; electrokinetic injection, 1 kV for 1 s.

lithic columns with dynamically modified surfactants can be used in a wide pH range without sacrificing the analysis time. However, the electroosmotic mobility in silica-based RP-CEC will change seriously when the eluent pH values change, because the dissociation of silanol groups on the silica surface strongly depends on the eluent pH values [30]. In contrast to CEC based on silica packing material, this lacking effect of eluent pH values on the EOF should be one of the advantages of the surfactant-modified monolithic columns.

### 3.2 CEC performance of the monolithic column

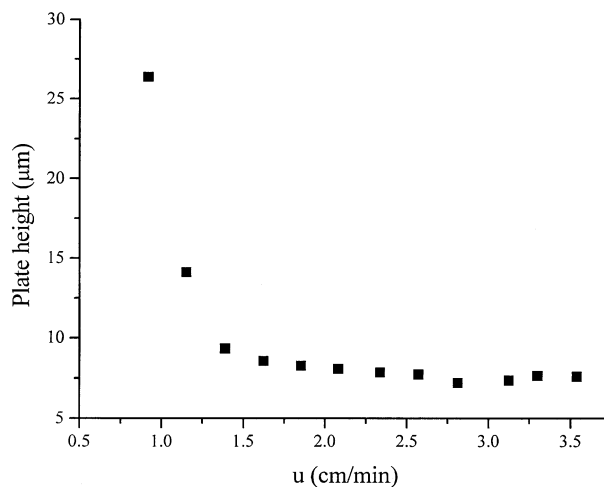
It has been observed that the current ( $I$ ) across the monolithic column (6.5 cm effective length, 27 cm total length) linearly increases with increasing applied voltage ( $V$ ) (ranging from 1 to 11 kV), the correlation coefficient being up to 0.9997. Thus, the Joule heating could be neglected in the monolithic columns dynamically modified with ionic surfactants. The reproducibility of retention times for neutral solutes on such monolithic columns was evaluated by ten consecutive injections; the relative standard deviations observed for  $t_0$  and retention factors of neutral solutes were about 0.22% and less than 0.56% for ten consecutive runs, respectively. This means that CEC with an uncharged monolithic polymer as stationary phase and modified with ionic surfactants to generate EOF can provide very good reproducibility for the retention time of solutes. The efficiencies of the monolithic column with up to 220 000 plates/m and 140 000 plates/m have been obtained for thiourea and neutral aromatic compounds, respectively. The dependence of the plate height ( $H$ ) on the velocity of the eluent ( $u$ ) through the capillary for benzyl alcohol is shown in Fig. 4. From Fig. 4 it can be seen that no significant loss of column efficiency was found as the velocity of the eluent increased from 1.5 to 3.5 cm/min. This seems to be promising for the use of such polymer monolithic columns in fast separation without loss the efficiency. In addition, our experiments show that the continuous bed was not moved or compressed even though a high eluent velocity was applied, thus demonstrating the advantages of fritless monolithic columns.

### 3.3 Retention mechanism of neutral solutes

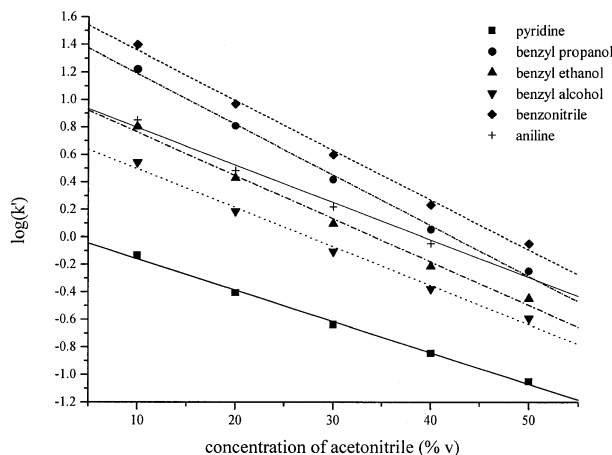
The retention of neutral solutes on RP-CEC is mainly determined by the interaction between solutes and the mobile and stationary phases, which is similar to that on RP-HPLC columns. We have measured the  $k'$  values of aromatic solutes at different concentrations of ACN by keeping the concentrations of phosphate buffer (pH 7.0) and SDS at 5 mM and 2.5 mM, respectively. The obtained results are shown in Fig. 5. It can be seen that  $\log k'$  linearly decreases with increasing concentration of ACN in

CEC. This result strongly supports the fact that the separation mechanism in CEC is based on reversed-phase partitioning, which means that the hydrophobic interaction plays a major role in the retention of tested solutes on a monolithic capillary column with dynamical modification of surfactants.

When the surfactants are over their critical micellar concentration (CMC), micelles will form in the mobile phase and act as a pseudostationary phase just like in MEKC



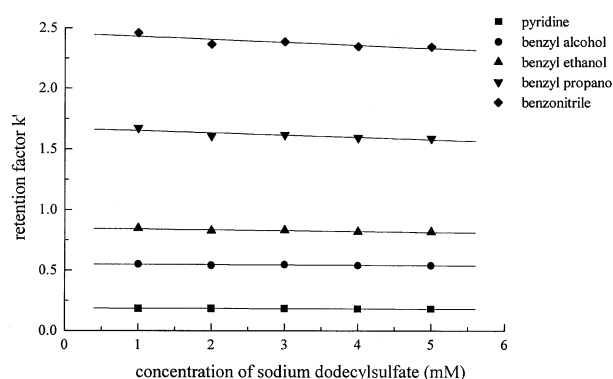
**Figure 4.** Plot of the plate height ( $H$ ) versus the linear velocity of the eluent ( $u$ ) for the solute benzyl alcohol. Experimental conditions:  $L_{\text{eff}}/L_{\text{total}}=20/27$  cm; mobile phase, 35% ACN in 5 mM phosphate buffer (pH 2.5) containing 2.5 mM SDS; applied voltage, 20 kV; injection, 1 kV for 2 s; UV detection wavelength, 214 nm.



**Figure 5.** Influence of the ACN concentration on the retention factor ( $k'$ ) of neutral compounds. Experimental conditions: column, 27 cm (effective length 6.5 cm) with 100  $\mu\text{m}$  ID, 375  $\mu\text{m}$  OD; mobile phases, 5 mM phosphate buffer (pH 7.0) containing different ACN concentrations and 2.5 mM SDS; applied voltage, 15 kV; electrokinetic injection, 1 kV for 1 s.

[32]. The separation mechanism in this system will become more complex due to the distribution of solutes into micelles. It was reported [33] that the CMC of SDS and CTAB is 8.1 and 0.92 mM in water, respectively. However, CMC of surfactants increases very quickly with increasing organic solvent concentration; CMC of CTAB measured in methanol-phosphate buffer (50/50 v/v) was about 16 mM [34]. The surfactant concentrations used in this study are less than 5 mM for the generation of EOF, which are lower than the CMCs of SDS or CTAB. The distribution of solutes in micelles as in MEKC should not contribute to the separation mechanism of CEC with monolithic columns modified by surfactants. Figure 6 shows the effect of SDS concentration in the mobile phase on the  $k'$  values of tested solutes; it can be seen that the  $k'$  values at various SDS concentrations do not change significantly showing just a slight decrease.

Since the tested solutes have no charge and no micelle is formed in the mobile phase, their separation is only due to partitioning between the mobile phase and the hydrophobic butyl groups in the copolymer. The reason for the slight decrease of  $k'$  could be due to the coverage of surfactants onto the surface of the copolymer, then the hydrophobicity of the stationary phase will decrease, and subsequently decrease the retention of solutes in the stationary phase. A similar result for the effect of CTAB on the  $k'$  values of the tested solutes has been observed. Our results may indicate that the surfactants in our system only generate the EOF, but make less contribution to the separation of neutral compounds. The influence of the eluent pH on the  $k'$  values of the neutral solutes was also investigated in our experiments, and it has been observed that the eluent pH values almost do not affect the  $k'$  values. This also means that the dynamical coating of sur-



**Figure 6.** Effect of SDS concentration on the retention factor ( $k'$ ) of neutral compounds. Experimental conditions: column, 27 cm (effective length 6.5 cm) with 100  $\mu$ m ID; mobile phase, 35% ACN in 5 mM phosphate buffer (pH 7.0) and different concentration of SDS; applied voltage, 15 kV; electrokinetic injection, 1 kV for 1 s.

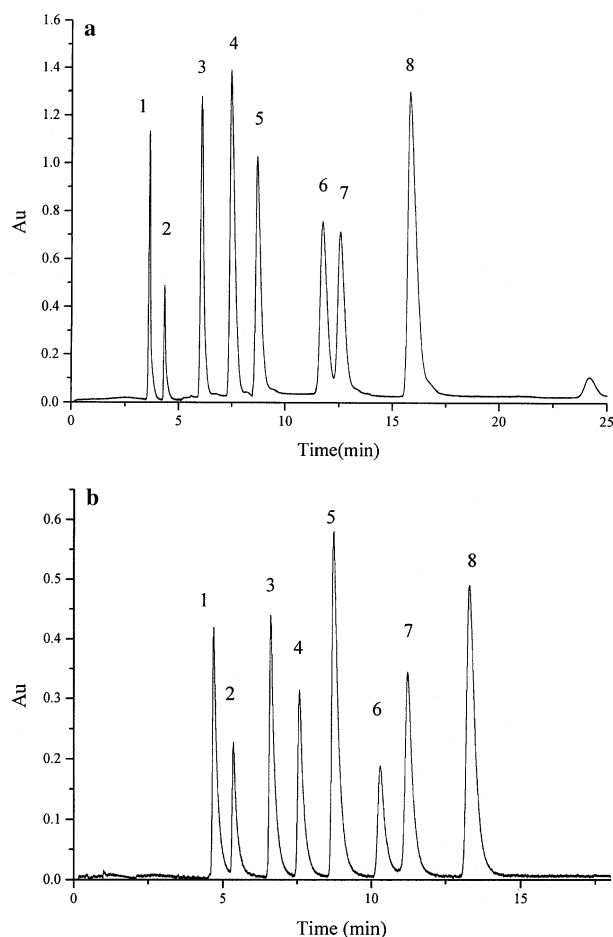
factants onto the polymer bed is well equilibrated and the amount of dynamically adsorbed surfactant will not change when the eluent pH is shifted from 2.5 to 7.0.

### 3.4 Simultaneous separation of acidic, basic and neutral solutes

Theoretically, both the ionic and neutral compounds can be separated by CEC. However, most of the reported applications of CEC focused on the analysis of neutral compounds. The separation of the acidic and basic compounds meets some difficulties in RP-CEC. It is quite complicated to apply RP-CEC to the separation of acidic solutes in their ionized forms because they tend to migrate against the EOF, causing the migration times to become relatively long or even too long to be eluted. RP-CEC with the ion-suppressed mode using a low-pH mobile phase was recommended [35]. The major difficulty of RP-CEC in separating basic compounds is peak tailing [36]. Smith and Evans [37] reported a discouragingly poor peak shape for strongly basic compounds on silica-based ODS stationary phase. Gillott *et al.* [38] demonstrated that a good peak symmetry in the separation of pharmaceutical bases could be achieved by the addition of a competing base to the mobile phase. Lurie *et al.* [39] achieved the simultaneous separation of acidic, basic and neutral compounds using RP-CEC with Hypersil-C<sub>8</sub> as the stationary phase and a low-pH buffer containing hexylamine as the mobile phase. Recently, CEC in columns packed with silica and strong cation-exchange materials, dynamically modified with CTAB, has successfully been applied to the separation of the acidic, basic and neutral compounds [24].

We performed a separation of seven compounds on the monolithic column modified with SDS and CTAB by keeping the pH of the mobile phases at 7.0; the obtained electrochromatograms are shown in Fig. 7. It can be seen that all solutes were well separated, and a good peak symmetry for nitrogen-containing solutes including pyridine and aniline has been obtained. Because all solutes eluted after the  $t_0$  marker of thiourea, the separation of these solutes must be mainly based on their partitioning mechanism.

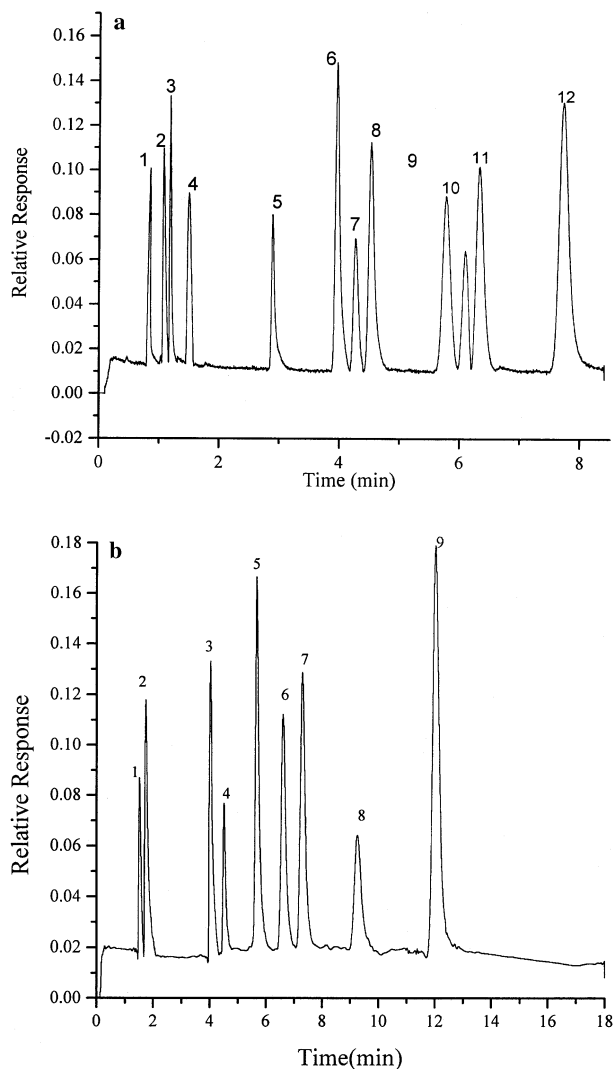
Figure 8 shows the simultaneous separation of aromatic acids and bases and the neutral compounds on CEC with a monolithic column modified by CTAB and SDS. In the latter case, a low-pH mobile phase (pH 2.5) is applied and thus pyridine, aniline and quinoline are protonated; baseline separation of 12 compounds including acidic, basic and neutral compounds is achieved. Phenylacetic acid and *o*-toluic acid are suppressed and separated based on the mechanism of RP-CEC. However, the positively charged compounds of pyridine, quinoline and the dipep-



**Figure 7.** Separation of test solutes on the monolithic column based CEC modified with (a) SDS and (b) CTAB. Experimental conditions: column, as in Fig. 1; mobile phase, 35% ACN in 5 mM phosphate buffer (pH 7.0) containing (a) 2.5 mM SDS and (b) 2.5 mM CTAB; applied voltage, 5 kV; electrokinetic injection, 1 kV for 1 s. Peaks: 1, thiourea; 2, pyridine; 3, benzyl alcohol; 4, benzyl ethanol; 5, aniline; 6, benzyl propanol; 7, benzaldehyde; 8, benzonitrile.

tides of Gly-Ala and Tyr-Trp are eluted before the void time of thiourea. This means that the separation of these compounds is mainly influenced by the electrophoresis mechanism, the contribution of partitioning and ion-exchange mechanisms to the separation being only weak. In the case of CTAB as the modified agent and the eluent of pH 7 applied, the EOF is reversed from cathode to anode. Under such a separation condition, the compounds of pyridine and quinoline are not protonated, so pyridine and quinoline and other neutral compounds were separated mainly based on the partition mechanism of RP-CEC. Whereas the phenylacetic acid and *o*-toluic acid are ionized at this condition and eluted before the void time of thiourea, their separation is mainly due to the electrophoretic migration. It can be seen from all of the above

described electrochromatograms that the peak symmetry is very good for all basic compounds at different separation conditions, which means that the irreversible adsorption of basic compounds on the capillary wall and monolithic stationary phase can be neglected.



**Figure 8.** Separation of aromatic acids and bases and neutral compounds on the monolithic column based CEC modified with (a) SDS and (b) CTAB. Experimental conditions: column, 26 cm (19 cm effective length) with 100  $\mu$ m ID and 375  $\mu$ m OD; applied voltage, 20 kV, electrokinetic injection, 3 kV for 1 s. (a) Mobile phase, 35% ACN in 5 mM phosphate buffer (pH 2.5) containing 2.5 mM SDS. Peaks: 1, pyridine; 2, quinoline; 3, Gly-Ala; 4, Tyr-Trp; 5, thiourea; 6, benzyl alcohol; 7, phenylacetic acid; 8, benzyl ethanol; 9, benzyl propanol; 10, *o*-toluic acid; 11, *p*-cresol; 12, benzonitrile. (b) Mobile phase, 35% ACN in 5 mM phosphate buffer (pH 7.0) containing 2.5 mM CTAB. Peaks: 1, *o*-toluic acid; 2, phenylacetic acid; 3, thiourea; 4, pyridine; 5, benzyl alcohol; 6, benzyl ethanol; 7, quinoline; 8, benzyl propanol; 9, benzonitrile.

## 4 Concluding remarks

The uncharged monolithic column was prepared by *in situ* polymerization of two monomers of BMA and EDMA. The EOF for CEC separation can be generated by addition of the surfactants CTAB and SDS to the mobile phase. It has been observed that the EOF on the monolithic column increases with increasing surfactant concentration and decrease of organic modifier concentration, while the pH values of the eluent had almost no effect on the EOF. The EOF value in the SDS system was always higher than that in the CTAB system at a fixed surfactant concentration, which may be explained by the higher effective charge of SDS compared to that of CTAB due to the charge of CTAB which is hindered by three methyl groups. Separation of acidic, basic and neutral solutes has been achieved with CTAB and SDS as the modifying agents, and a good peak symmetry for all tested compounds has been obtained. Ionized compounds at high or low pH-mobile phase were separated mainly based on the electrophoretic migration, but the separation of neutral compounds was always influenced by the partitioning mechanism.

*Financial support from the National Natural Science Foundation of China (No. 29635010) is gratefully acknowledged. Dr. Hanfa Zou is a recipient of the Excellent Young Scientist Award from the National Natural Science Foundation of China (No. 29725512).*

Received August 16, 2000

## 5 References

- [1] Pretorius, V., Hopkins, B. J., Schieke, J. D., *J. Chromatogr.* 1974, *99*, 23–30.
- [2] Jorgenson, J. W., Lukacs, K. D., *J. Chromatogr.* 1981, *218*, 209–216.
- [3] Knox, J. H., Grant, I. H., *Chromatographia* 1987, *24*, 135–143.
- [4] Zhang, L., Zou, H., Shi, W., Zhang, Y. K., *J. Capil. Electrophor.* 1998, *5*, 97–102.
- [5] Smith, N. W., Evans, M. B., *Chromatographia* 1994, *38*, 649–657.
- [6] Stead, D. A., Reid, R. G., Taylor, R. B., *J. Chromatogr.* 1998, *798*, 259–267.
- [7] Tsuda, T., *Anal. Chem.* 1987, *59*, 521–523.
- [8] Ericson, C., Hjertén, S., *Anal. Chem.* 1997, *71*, 1621–1627.
- [9] Liao, J., Chen, N., Ericson, C., Hjertén, S., *Anal. Chem.* 1996, *68*, 3468–3472.
- [10] Fujimoto, C., Fujise, Y., Matsuzawa, E., *Anal. Chem.* 1996, *68*, 2753–2757.
- [11] Peters, E. C., Petro, M., Svec, F., Frechet, J. M. J., *Anal. Chem.* 1998, *70*, 2288–2295.
- [12] Peters, E. C., Petro, M., Svec, F., Frechet, J. M. J., *Anal. Chem.* 1998, *70*, 2296–2302.
- [13] Schweitz, L., Andersson, L. I., Nilsson, S., *Anal. Chem.* 1997, *69*, 1179–1183.
- [14] Peters, E. C., Lewandowski, K., Petro, M., Svec, F., Frechet, J. M. J., *Anal. Commun.* 1998, *35*, 83–86.
- [15] Palm, A., Novotny, M. V., *Anal. Chem.* 1997, *69*, 4499–4507.
- [16] Tsuda, T., *J. High Resolut. Chromatogr. Chromatogr. Commun.* 1987, *10*, 622–624.
- [17] Huang, X., Luckey, J. A., Gordon, M. J., Zare, R. N., *Anal. Chem.* 1989, *61*, 766–770.
- [18] Helboe, P., Hansen, S. H., Thomsen, M., *Adv. Chromatogr.* 1989, *28*, 195–265.
- [19] Pfeffer, W. D., Yeung, E. S., *Anal. Chem.* 1990, *62*, 2178–2182.
- [20] Garner, T. W., Yeung, E. S., *J. Chromatogr.* 1993, *640*, 397–402.
- [21] Ye, M., Zou, H., Liu, Z., Ni, J., *J. Chromatogr. A* 1999, *855*, 137–145.
- [22] Seifar, R. M., Kok, W. T., Kraak, J. C., Poppe, H., *Chromatographia* 1997, *46*, 131–136.
- [23] Seifar, R. M., Kraak, J. C., Kok, W. T., Poppe, H., *J. Chromatogr. A* 1998, *808*, 71–77.
- [24] Ye, M., Zou, H., Liu, Z., Ni, J., *Anal. Chem.* 2000, *72*, 616–621.
- [25] Zou, H., Ye, M., *Electrophoresis* 2000, *21*, 4073–4095.
- [26] Ye, M., Zou, H., Liu, Z., Ni, J., Zhang, Y., *J. Chromatogr. A* 2000, *887*, 223–231.
- [27] Alicea-Maldonado, R., Colón, L. A., *Electrophoresis* 1999, *20*, 37–42.
- [28] Fujimoto, C., *Anal. Chem.* 1995, *67*, 2050–2053.
- [29] Peters, E. C., Petro, M., Svec, F., Fréchet, J. M. J., *Anal. Chem.* 1997, *69*, 3646–3649.
- [30] Smith, N., Evans, M. B., *J. Chromatogr. A* 1999, *832*, 41–54.
- [31] Zou, H., Zhang, Y., Lu, P., *High Performance Liquid Chromatography (in Chinese)*, Science Press, Beijing 1998.
- [32] Liu, Z., Zou, H., Zhang, Y., *J. High. Resol. Chromatogr.* 1998, *21*, 234–240.
- [33] Terabe, S., *Micellar Electrokinetic Chromatography*, Beckman Instruments, Palo Alto, CA, USA 1993.
- [34] Hansen, S. H., Helboe, P., Lund, U., *J. Chromatogr.* 1982, *240*, 319–327.
- [35] Cikaló, M. G., Bartle, K. D., Robson, M. M., Myers, P., Euerby, M. R., *Analyst* 1998, *123*, R87–102.
- [36] Majors, R. E., *LC.GC* 1998, *16*, 96–102.
- [37] Smith, N. W., Evans, M. B., *Chromatographia* 1995, *41*, 197–203.
- [38] Gillott, N. C., Euerby, M. R., Johnson, C. M., Barrett, D. A., Shaw, P. N., *Anal. Commun.* 1998, *35*, 217–220.
- [39] Lurie, I. S., Conner, T. S., Ford, V. L., *Anal. Chem.* 1998, *70*, 4563–4569.