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Separation of acidic compounds by strong anion-exchange capillary electrochromatography

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Abstract

Separation of the acidic compounds in the ion-exchange capillary electrochromatography (IE-CEC) with strong anion-exchange packing as the stationary phase was studied. It was observed that the electroosmotic flow (EOF) in strong anion-exchange CEC moderately changed with increase of the eluent ionic strength and decrease of the eluent pH, but the acetonitrile concentration in the eluent had almost no effect on the EOF. The EOF in strong anion-exchange CEC with eluent of low pH value was much larger than that in RP-CEC with Spherisorb-ODS as the stationary phase. The retention of acidic compounds on the strong anion-exchange packing was relatively weak due to only partial ionization of them, and both chromatographic and electrophoretic processes contributed to separation. It was observed that the retention values of acidic compounds decreased with the increase of phosphate buffer and acetonitrile concentration in the eluent as well as the decrease of the applied voltage, and even the acidic compounds could elute before the void time. These factors also made an important contribution to the separation selectivity for tested acidic compounds, which could be separated rapidly with high column efficiency of more than 220 000 plates/m under the optimized separation conditions.

Keywords: Electrochromatography; Ion-exchange electrochromatography; Mobile phase composition; Benzoic acids

1. Introduction

The major difference between capillary electrochromatography (CEC) and high-performance liquid chromatography (HPLC) is the difference in driving force. In CEC the electroosmotic flow (EOF) is used to transport mobile phase other than the pressurized flow as used in HPLC. The use of EOF as a pump for chromatographic separations offers great promise for the analysis of neutral and charged solutes. Higher efficiency can be obtained in CEC due to the plug-like flow profile than that in HPLC, and the length of capillary column can be longer and the size of packing particles can be smaller in CEC because no pressure drop exists in CEC columns. Additional selectivity can be obtained for charged solutes because of the involvement of electrophoretic process in CEC. Therefore the technique of CEC has been rapidly developed recently since the first paper was reported by Pretorious et al. [1] in 1974.

Although both neutral and charged solutes can be separated by CEC, the analysis of acidic compounds in the reversed-phase CEC (RP-CEC) is relatively difficult. EOF results from the negatively charged packing surface in RP-CEC due to the ionization of silanol groups, and its direction is from the anode to cathode. But the ionized acid with negative charge will attempt to electrophoretically migrate towards

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the anode. Therefore the anionic acids will eventually elute out from detection window only when the EOF is greater than the electrophoretic mobility of anionic acids. Choudhary and Horváth [2] and Moffatt et al. [3] have reported the analysis of acidic compounds with high pH eluent by RP-CEC. However, if the electrophoretic mobility of the anionic acids is higher than that of the EOF, the solute cannot even be loaded into the CEC column. Huber et al. [4] have found that some acidic phenylthiohydantoin (PTH)-amino acids were subject to counterdirectional electrophoretic migration and did not enter the column on ODS column at pH 7.55. In order to solve this problem, it was recommended [5] to operate CEC in ion-suppressed mode where low pH electrolytes are used as the eluent. Euerby et al. [6] have reported that it is essential to run CEC in ion-suppressed mode in order to separate both acidic and neutral analytes. Lurie et al. [7] have achieved the simultaneous separation of acidic, basic and neutral compounds in the reversed-phase CEC with Hypersil-C_8 as the stationary phase and low pH buffer containing hexylamine as the mobile phase. However, the rate of EOF is poor with low pH buffer as the eluent because the amount of ionized silanol groups on stationary phase is small, which results in low electroosmotic flow and long analysis time. In order to generate acceptably fast EOF rates at low pH value of the eluent, specific stationary phase for CEC [8] has been developed in which the silica particles are coated with a mixture of sulfonic acid and alkyl chain moieties. Altria et al. [5] have separated eight acidic drugs in less than 8 min with a mobile phase of pH 1.5 on a 7-cm packed column with such a specific stationary phase. A typical way to analyze the small anionic solutes in CE is that the direction of EOF is reversed by the addition of some amine base, therefore anionic solutes and EOF will both migrate to the anode and separation can be achieved rapidly. A similar method was also reported in CEC [9] for the separation of acidic enantiomers by using β-cyclodextrin as a chiral selector and triethylammonium as an additive of the mobile phase to reverse the direction of EOF. It was reported [10] recently that five aromatic acids have been successfully separated at low pH of the eluent on the strong cation-exchange (SCX) column, dynamically modified by cetyltrimethylammonium bromide.

Several papers on ion-exchange CEC (IE-CEC) using SCX packings have been reported in literature [11–14] for separation of cationic compounds, while research on CEC using strong anion-exchange (SAX) packings is seldom reported. Strong anion-exchange CEC has been used for the separation of inorganic anions including iodide, iodate and perrhenate by Li et al. [15], as well as sulfate, sulfite and thiosulfate by Kitagawa et al. [16]. Anion-exchange liquid chromatography was successfully used to separate acidic compounds [17,18]. The surface of anion-exchange packings is positively charged, if these materials are packed into CEC column, the direction of EOF will be from cathode to anode. Acidic compounds also migrate at the same direction as EOF in anion-exchange CEC. Therefore, anion-exchange CEC is preferred to analyze anionic compounds. In this paper, the separation of acidic compounds by CEC on SAX packings is presented. The influences of the buffer concentration, organic modifier content, and applied voltage on separation have been investigated.

2. Experimental

2.1. Instrumentation and material

All the CEC experiments were performed on a P/ACE system MDQ (Beckman, Fullerton, CA, USA), a Spectra-Physics pump (Spectra-Physics, San Jose, CA, USA) was used to pack capillary columns. The fused-silica capillary (50 μm I.D.×365 μm O.D.) was obtained from Yongnian Optic Fiber Plant (Hebei, China). 5-μm Spherisorb-SAX and 5-μm Spherisorb-ODS I were purchased from Waters (Milford, MA, USA).

2.2. Chemicals and buffers

Acetonitrile was of chromatographic grade, benzyl alcohol, benzoic acid, α-naphthylacetic acid, *m*-bromobenzoic acid were of chemical grade, and the other reagents used were of the analytical reagent grade. Ultra-pure water used for preparing solutions was produced by a Milli-Q water system (Millipore, Bedford, MA, USA). Stock solutions of 100 m*M* phosphate buffer, at pH 2.2, pH 3.0 and pH 7.01,

were prepared by mixing appropriate concentrations of sodium dihydrogenphosphate solution with phosphoric acid or disodium hydrogenphosphate solution. The mobile phase was prepared by mixing appropriate volumes of acetonitrile, stock buffer solution and water. Before running, mobile phase was degassed in an ultrasonic bath for 30 min. The pH value reported in this paper is that of the stock buffer solution.

2.3. Column preparation and separation conditions

CEC columns were packed in this laboratory by slurry packing technique as reported in literature [10,19]. All columns were 31 cm long with packed length of 10 cm. Before CEC experiment, the column was first flushed with mobile phase for 30 min by a syringe. Then the column was conditioned on the instrument with the mobile phase for at least 1 h. The applied voltage was first ramped from 0 to 10 kV for 10 min and then operated 10 kV. The temperature was kept at 25°C and the detection wavelength was set at 214 nm. The separation voltage was at 10 kV if not otherwise stated.

3. Results and discussion

Separation of the acidic solutes in their ionized form in RP-CEC is relatively difficult because they tend to migrate against EOF, and thereby the migration times are relatively long or cannot even elute out from the detection window. Six acids, including benzoic acid (pK_a 4.20), *o*-toluic acid (pK_a 3.91), *p*-nitrobenzoic acid (pK_a 3.43), *p*-bromobenzoic acid (pK_a 4.00), 3,5-dinitrobenzoic acid (pK_a 2.82) and *o*-bromobenzoic acid (pK_a 2.85), were selected as the test solutes. In order to investigate if they can elute out from detection window in RP-CEC, mobile phases containing 60% acetonitrile in 2 m*M* phosphate buffers, with pH values of 7.01, 3.0 and 2.2, respectively, were used. It was found that none of the six acids could be eluted out at an eluent pH of 7.01. This means that the electrophoretic mobility of all tested acids from cathode to anode was greater than or very close to that of EOF, therefore they could not be loaded into the column packed with Spherisorb-ODS. When the eluent pH decreased to 3.0, only three acids including, benzoic acid, *o*-toluic acid and *p*-bromobenzoic acid, with relatively high pK_a values eluted from the column. Huber et al. [4] also reported that some acidic PTH-amino acids did not enter the column packed with 3.5-μm Zorbax ODS at pH 7.55. However, all acids, except 3,5-dinitrobenzoic acid, eluted out from the column in RP-CEC mode with an eluent pH of 2.2, and the chromatogram is shown in Fig. 1a. It can be seen that *p*-nitrobenzoic acid and *o*-bromobenzoic acid coeluted out from the column, and the column efficiency for them was relatively poor. The poor chromatographic performance may be due to the interaction of the fully dissociated carboxylic acid with unbounded silanol groups on the surface of ODS packing materials [20]. In order to lower the silanophilic interactions, increasing the phosphate concentration or decreasing the eluent pH value may be necessary. Altria et al. [5] have reported a mobile phase with a pH value of 1.5 used for the separation of eight acidic drugs in the ion-suppressed mode. The mobile phase at such a low pH value must be harmful to the silica-based stationary phase. The disadvantage of this method is obvious that the void time of column marked by thiourea increased about 3-fold from 1.97 to 5.63 min, when the eluent pH value decreased from 7.01 to 2.2. This resulted in relatively long analysis time. The poor column efficiency was also observed with low pH value of the eluent. For example, the column efficiency for benzene decreased from 132 000 to 58 000 plates/m when the eluent pH decreased from 7.01 to 2.2. The column efficiency for the early-eluted benzoic acid and *o*-toluic acid as shown in Fig. 1a was even less than 50 000 plates/m.

The surface of the SAX packing is positively charged, which results in the direction of EOF from cathode to anode on the SAX packed column. Therefore, anionic acid will migrate with the direction of EOF. Six acids were also separated in the strong anion-exchange CEC with Spherisorb-SAX as the stationary phase and 50% acetonitrile in 20 m*M* phosphate buffer (pH 2.2) as the mobile phase, and the chromatogram is shown in Fig. 1b. It can be seen from Fig. 1b that the six acids were well separated within 3.7 min, which was even far less than the void time of 5.63 min in RP-CEC with the Spherisorb-ODS packed column. Void time with the eluent of pH

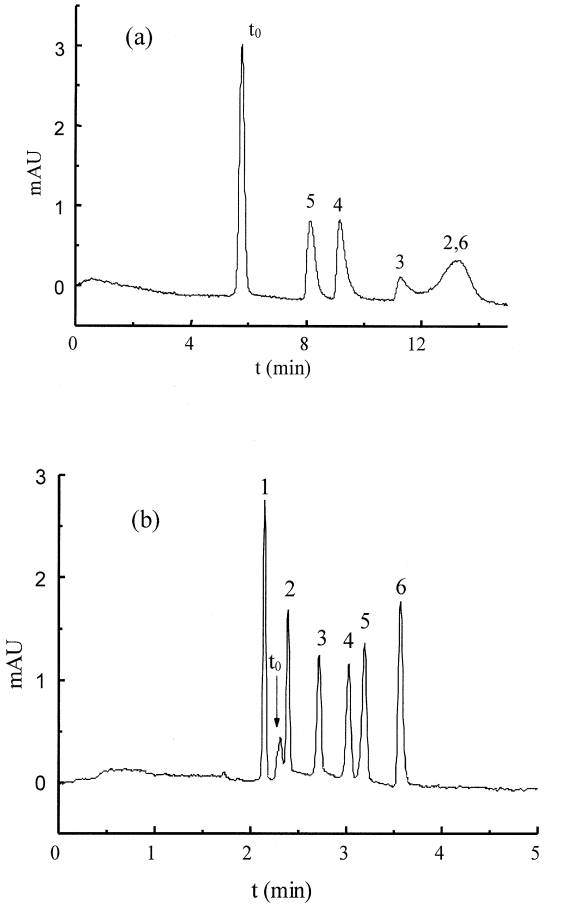

Fig. 1. Chromatograms for separation of the test acids in RP-CEC and strong anion-exchange CEC. Experimental conditions: column, 31 cm (packed length 10 cm)×50 μm I.D. with 375 μm O.D.; applied voltage, 10 kV; UV detection wavelength, 214 nm. (a) Column packed with 5-μm Spherisorb-ODS I; mobile phase, 60% acetonitrile in 2 m*M* phosphate buffer (pH 2.2); electrokinetic injection, 5 kV, 20 s; t_0 was marked by thiourea. (b) Column packed with 5-μm Spherisorb-SAX; mobile phase, 50% acetonitrile in 20 m*M* phosphate buffer (pH 2.2); electrokinetic injection, 5 kV, 2 s; t_0 was marked by solvent peak. Peaks: (1) 3,5-dinitrobenzoic acid (pK_a 2.82); (2) *p*-nitrobenzoic acid (pK_a 3.43); (3) *p*-bromobenzoic acid (pK_a 4.00); (4) *o*-toluic acid (pK_a 3.91); (5) benzoic acid (pK_a 4.2); (6) *o*-bromobenzoic acid (pK_a 2.85).

2.2 in IE-CEC with SAX packed column was about 2.30 min, which was less than that in RP-CEC with Spherisorb-ODS packed column with the eluent of pH 7.01. The relatively large void time at the higher ionic strength of the mobile phase used in IE-CEC may result from low ζ-potential and thereby slow EOF.

The separation mechanism in IE-CEC with the SAX stationary phase is the combination of electrophoresis and strong anion-exchange, while that in RP-CEC with non-polar stationary phase is based on the electrophoresis and reversed-phase partition. As shown in Fig. 1, the different separation mechanisms resulted in the different selectivity for separation of acidic compounds in two systems. All solutes in HPLC should elute after void time because their retention on the stationary phase, while the charged solutes in CEC may elute before void time if the mobility of solutes and that of EOF have the same direction, and their retention on stationary phase is not too strong. It can be seen from Fig. 1b that 3,5-dinitrobenzoic acid eluted before the t_0 marker.

The separation of charged solutes in capillary zone electrophoresis (CZE) is only based on the difference of mass-to-charge ratios, therefore it is difficult to separate the solutes with the same mass-to-charge ratios. However the separation of such kinds of charged solutes can be achieved by CEC, due to the contribution of the chromatographic mechanism. Zhang et al. [21] have reported that dinucleotides and tRNAs, which have similar mass-to-charge ratios, can be well resolved in CEC. Separation of three positional isomers of bromobenzoic acid at baseline was also achieved in our system, as shown in Fig. 2. They cannot be well separated by CZE if they are full dissociated because they have the same mass-to-charge ratios. The acidic isomers with the lower pK_a values should have a higher overall negative charge and a greater electrophoretic mobility, thereby eluting more rapidly if the electrophoresis has the same direction as electroosmotic mobilities in IE-CEC with the SAX stationary phase. Therefore, the positional isomers of bromobenzoic acids in our system should be in the order of *o*-bromobenzoic acid (pK_a 2.85), *m*-bromobenzoic acid (pK_a 3.81) and *p*-bromobenzoic acid (pK_a 4.00). But actually, from Fig. 2, the elution order was reversed as expected. The reason is that electrostatic interaction between the anionic solutes and stationary phase took place in this system. The solute with higher charge was more strongly retained by SAX packing. Therefore the selectivity for separation of anionic solutes in strong anion-exchange CEC was different

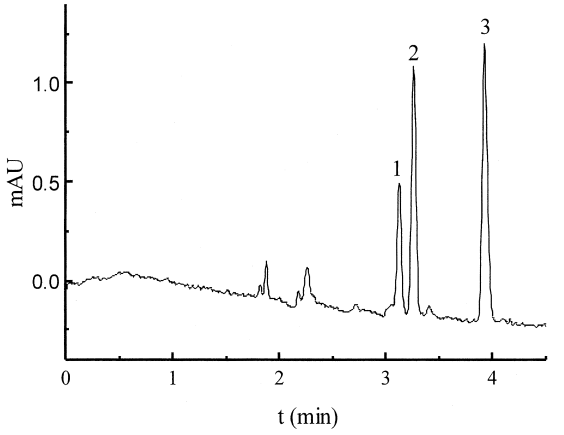

Fig. 2. Chromatogram for separation of positional isomers of bromobenzoic acid in strong anion-exchange CEC. Experimental conditions: mobile phase, 50% acetonitrile in 10 m*M* phosphate buffer (pH 2.2). Other experimental conditions as in Fig. 1b. Peaks: (1) *p*-bromobenzoic acid (pK_a 4.00); (2) *m*-bromobenzoic acid (pK_a 3.81); (3) *o*-bromobenzoic acid (pK_a 2.85).

from that in CZE; such a result was also found in the separation of small peptides by CZE and strong cation-exchange CEC in our previous paper [14]. The direction of the electrophoretic mobility of anion is different from that of the electroosmotic mobility in CZE. In order to shorten the analysis time, it is suggested to add some amine base to running buffer to reverse the direction of EOF for separation of anionic solutes in CE. However, the acidic compounds could be separated quickly in IE-CEC with SAX packings without addition of the amine base into the mobile phase as shown in Figs. 1b and 2, because the packing surface was positively charged.

The chromatographic retention factor k' is always used for describing the migration process in HPLC, and can also be used for neutral solutes in CEC because their retention is based on a purely chromatographic mechanism. However, it is not suitable for charged solutes in CEC because of the coupled electrophoretic migration. Therefore it is necessary to define an equivalent parameter to describe the migration process in CEC [22]. This parameter should include both electrophoretic and chromatographic process because they are the characteristic components in CEC. Rathore and Horváth [23] have defined the electrochromatographic retention factor as the ratio of the separative and non-separative virtual migration length for describing the migration process. The relationship between this parameter with the retention factor caused by chromatography alone, the electrophoretic mobility of the solute and the electroosmotic mobility, was obtained from a unified theory using virtual migration distances [22,23]. As we know, the retention factor in HPLC can be easily calculated from the migration time of the solute and the void time, which can be directly obtained from a chromatogram. Similarly, the k^* in CEC was defined with the following equation by some authors [24,25]:

$$k^* = (t_r - t_0)/t_0 \quad (1)$$

where t_r is the migration time of a solute, and t_0 is the migration time of a neutral and chromatographically unretained solute. In this experiment, solvent peak was selected as t_0 marker in IE-CEC. The electrochromatographic retention factor k^* has been used to describe the migration process of charged solutes both in RP-CEC [24] and IE-CEC [12]. Theoretically, k^* can also be expressed as follows [24,25]:

$$k^* = \frac{k' - \mu_{ep}/\mu_{eo}}{1 + \mu_{ep}/\mu_{eo}} \quad (2)$$

where k' is the retention factor caused only by chromatography alone in CEC, μ_{eo} and μ_{ep} are the electroosmotic mobility and electrophoretic mobility of solute, respectively. It can be seen from Eq. (2) that the k^* value reflects the concurrence of the chromatographic and electrophoretic processes. For neutral solutes, $\mu_{ep} = 0$ and k^* becomes k', reflecting a purely chromatographic process. However, for a charged unretained solutes, $k' =$ zero, reflecting a purely electrophoretic process. If the solutes are charged and retained in the stationary phase, the two processes will all be involved. The value of k' in HPLC is always positive because the retention time of solutes is always greater than void time. Because the sign is associated with the electrophoretic and electroosmotic mobilities, the value of k^* in CEC can be either positive or negative. For example, if the signs of μ_{ep} and μ_{eo} are the same and the value of k' is not too great, the sign of the k^* can be negative. In other words, if the direction of the electrophoretic migration of charged solute is the same as that of

electroosmotic flow and the retention of the solute in the stationary phase is not too strong, the migration time of the solute can be smaller than the void time.

The repeatability and efficiency of the SAX packed column were evaluated in strong anion-exchange CEC with 50% acetonitrile in 10 m*M* phosphate buffer (pH 2.2) as the mobile phase, and *p*-nitrobenzoic acid, *p*-bromobenzoic acid, benzoic acid and *o*-bromobenzoic acid were selected as test solutes. The relative standard deviation (RSD) for t_0 was 0.6% and the RSDs for the k^* values of four acids were less than 2.5% for 10 consecutive runs. Average column efficiencies of four acids by 10 consecutive runs were varied from 220 000 to 310 000 plates/m with RSD less than 7.7%, which was relatively high. But it was found that the column efficiency for neutral solutes of benzyl alcohol and benzene was only about 140 000 plates/m in the same system under the same experimental conditions. It was observed [12] that the preparation of sample by lower ionic strength of solution than that of mobile phase in strong cation-exchange CEC could improve the column efficiency. Both acids and neutral solutes were prepared in the same mobile phase in this work, but the column efficiency for acids was about two times higher than that for neutral solutes. Some kind of sample stacking might be responsible for that during the separation of acidic compounds. The unusually high column efficiency for the charged solutes was also found both in strong cation-exchange CEC with 5 μm Spherisorb-SCX as the stationary phase [12] and in RP-CEC with 3-μm Hypersil-ODS as the stationary phase [3].

The influence of the eluent ionic strength on the separation was studied by changing phosphate buffer concentration from 5 to 30 m*M* in the mobile phase, but keeping the eluent pH at 2.2. It was observed that the mobility of EOF decreased about 50% when phosphate buffer concentration increased from 5 to 30 m*M*. Generally, elution strength of mobile phase in ion-exchange chromatography depends on the ionic strength of the mobile phase, and the retention of solutes will decrease with increase of ionic strength. Such a tendency was also found in strong anion-exchange CEC in this work, the electrochromatographic retention (k^*) decreased with the increase of phosphate buffer concentration, as shown in Fig. 3. The linear relationship between log k^* of peptide and the logarithm of the buffer concentration (log[*c*]), which is a well-known relationship in ion-exchange liquid chromatography, was observed in IE-CEC with SCX stationary phase [12]. This result indicates that the peptides were strongly retained on SCX packings and the influence of electrophoretic mechanism on the separation was moderate. However, the retention of acidic solutes on the SAX packed column in this work was not very strong, some acids eluted even before void time, and the k^* values of some acidic solutes were negative. Because the logarithm of negative data is meaningless, straight lines were fitted only for the later eluted three acids, and the correlation coefficient was below 0.95. This result suggested that both the chromatographic and electrophoretic processes simultaneously contributed to separation of acidic solutes in this study.

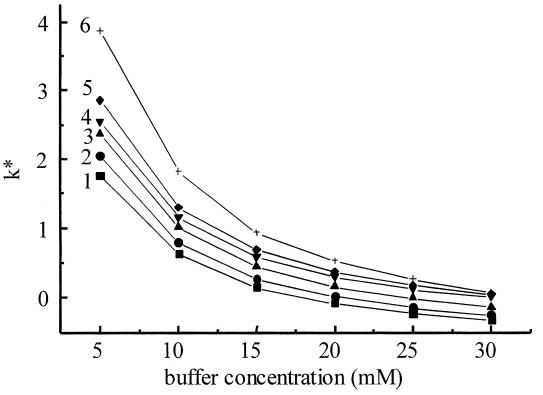

Fig. 3. Effect of the eluent ionic strength on the k^* values in strong anion-exchange CEC. Experimental conditions: mobile phase, 50% acetonitrile in phosphate buffers with varied concentration from 5 to 30 m*M* (pH 2.2). Other conditions were as in Fig. 1b. Solutes tested were as in Fig. 1b.

The effect of acetonitrile concentration on separation was also studied. It was observed that the influence of acetonitrile content on EOF in strong anion-exchange CEC with Spherisorb-SAX as the stationary phase was almost negligible, and the RSD of EOF measured at acetonitrile concentrations of 20, 30, 40 and 50% in 10 m*M* phosphate buffer (pH 2.2) was within 2.4%. The k^* values of acidic compounds at various acetonitrile concentration were also measured, and results are shown in Fig. 4. It can

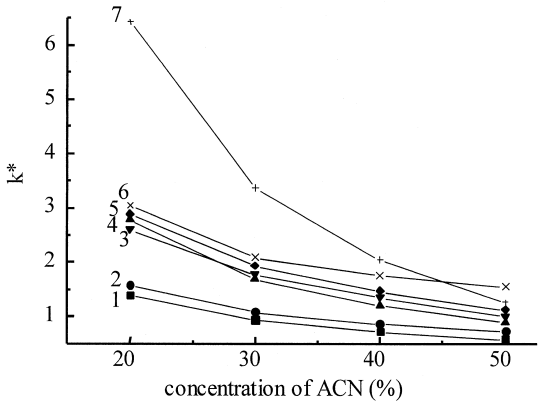

Fig. 4. Effect of acetonitrile (ACN) concentration on the k^* value in strong anion-exchange CEC. Experimental conditions: mobile phase, acetonitrile concentration from 20 to 50% in 10 m*M* phosphate buffer (pH 2.2). Other conditions were as in Fig. 1b. Solutes: (1) 3,5-dinitrobenzoic acid (pK_a 2.82); (2) *p*-nitrobenzoic acid (pK_a 3.43); (3) *p*-bromobenzoic acid (pK_a 4.00); (4) *o*-toluic acid (pK_a 3.91); (5) benzoic acid (pK_a 4.2); (6) *o*-bromobenzoic acid (pK_a 2.85); (7) α-naphthylacetic acid.

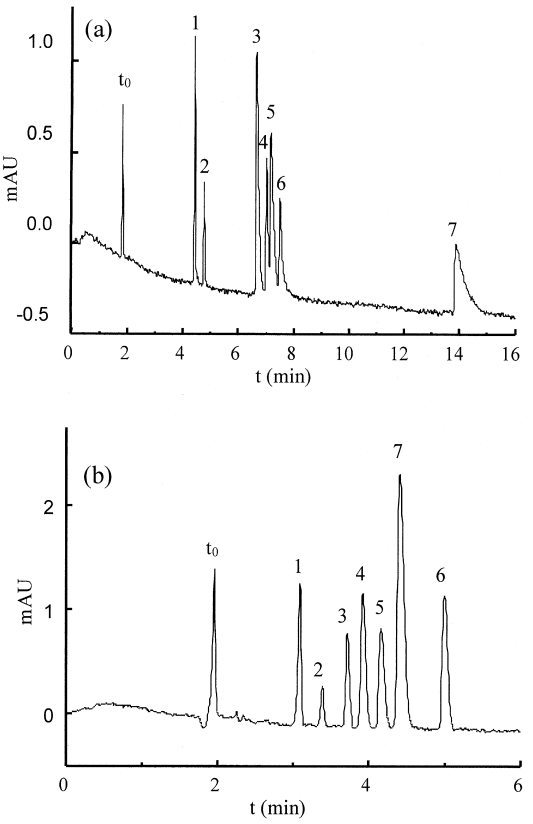

Fig. 5. Chromatograms for separation of acidic compounds at different acetonitrile contents in strong anion-exchange CEC. Experimental conditions: (a) 20% and (b) 50% acetonitrile in 10 m*M* phosphate buffer (pH 2.2). Other conditions were as in Fig. 1b.

be seen that the k^* values decrease rapidly with increase of acetonitrile content. The k^* value of the more hydrophobic solutes, such as α-naphthylacetic acid, decreases more obviously. One of the possible reasons is that the reversed-phase partition mechanism might also contribute to separation at low organic content. Fig. 5 shows the typical chromatograms for separation of acidic compounds by strong anion-exchange CEC with 20 and 50% acetonitrile in 10 m*M* phosphate buffer (pH 2.2) as the eluents, respectively. It can be seen that the peak shape of acidic compounds was obviously improved, and the separation selectivity also changed with the increase of acetonitrile content, as shown in Fig. 5. For example, the asymmetry value for α-naphthylacetic acid decreased from 3.47 to 1.5 when acetonitrile content increased from 20 to 50%.

In CEC, the mobile phase is driven by EOF generated by applying electric field. The influence of electric field on EOF and column efficiency has been extensively studied [26–29], but the influence of electric field on retention was seldom reported. Zhang et al. [19] observed that the retention factors of 27 neutral solutes were not significantly different among RP-CEC, micro-column HPLC and pressurized RP-CEC (P-CEC). But Vissers and Claessens [30] reported that the retention factors for neutral solutes in RP-CEC were about 1–1.4 times higher than those in micro-column HPLC. The separation selectivity for ionic compounds in the pressurized CEC could also be tuned by changing the applied voltage or pressure [23,31]. Kitagawa and Tsuda [32] reported that electric field could induce a variation of the distribution coefficient in CEC packed with anion-exchange resin, and resulted in the alternation of retention factors of ionic solutes. We have investigated the effect of electric field on the retention of acidic compounds in strong anion-exchange CEC, and it was observed that the k^* values of acidic compounds increased with the

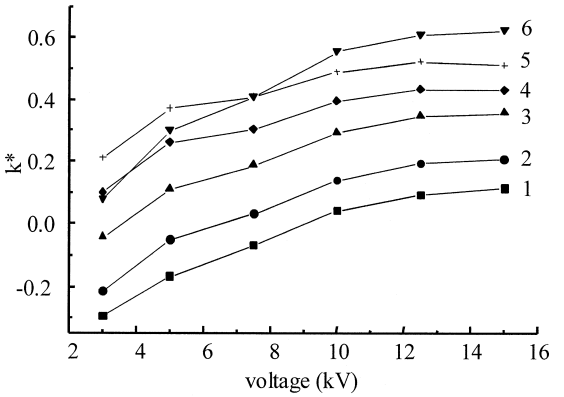

Fig. 6. Effect of the applied voltage on the k^* values in strong anion-exchange CEC. Experimental conditions: mobile phase, 50% acetonitrile in 10 m*M* phosphate buffer (pH 2.2); applied voltages varied from 3 to 15 kV. Other conditions were as in Fig. 1b. Solutes: (1) 3,5-dinitrobenzoic acid (pK_a 2.82); (2) *p*-nitrobenzoic acid (pK_a 3.43); (3) *p*-bromobenzoic acid (pK_a 4.00); (4) *o*-toluic acid (pK_a 3.91); (5) phenylacetic acid (pK_a 4.28); (6) *o*-chlorobenzoic acid (pK_a 2.92).

increase of applied voltage, as shown in Fig. 6. Fig. 7 shows the typical chromatograms for separation of six acidic compounds with applied voltages at 5 and 15 kV, respectively. As can be seen from Fig. 7, the retention of acidic compounds at 15 kV by strong anion-exchange CEC was relatively strong and all solutes eluted after t_0, while that at 5 kV was relatively weak, and two acidic compounds eluted from detection window before t_0. The elution order of acidic compounds at different applied voltages is also changeable, which means that the separation selectivity for acidic compounds could also be tuned by the applied voltage in strong anion-exchange CEC. It is observed that there is an excellent linear relationship between the linear velocity of EOF and the applied voltage ($r>0.999$), which means that the influence of Joule heating on retention can be neglected. One of the possible reasons for the change of the retention values and separation selectivity might be that the nature of the stationary phase was induced to be changed with application of an electric field on the column [32], which resulted in changing the distribution constant of acidic compounds between the stationary phase and mobile phase. This subject should be studied more thoroughly in future.

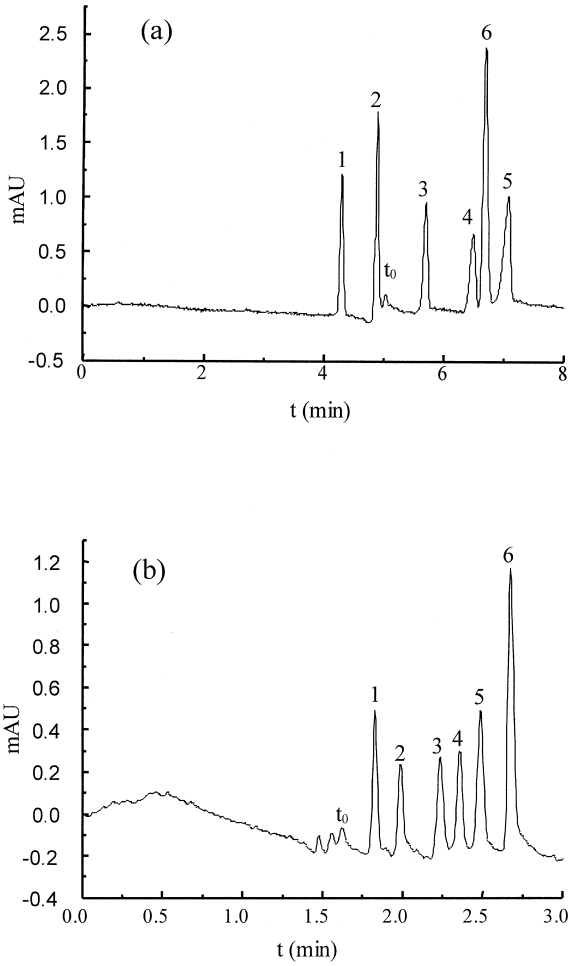

Fig. 7. Typical chromatograms for separation of acidic compounds at different applied voltages in strong anion-exchange CEC. Experimental conditions: applied voltages at (a) 5 and (b) 15 kV. Other conditions and solutes were as in Fig. 6.

4. Conclusions

It has been shown that IE-CEC with SAX packings is a very good separation technique for the analysis of acidic compounds. The separation selectivity of IE-CEC is different from that of RP-CEC and CZE because of the different mechanisms involved, and it can be adjusted by changing the concentration of the organic modifier and applied voltage. The retention of the solutes from the ion-exchange contribution can be controlled by the

eluent ionic strength. It has also been shown that the separation of acidic compounds can be performed in a short time with high efficiency in IE-CEC.

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