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Separation of peptides by strong cation-exchange capillary electrochromatography

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Abstract

Separation of small peptides on ion-exchange capillary electrochromatography (IE-CEC) with strong cation-exchange packing (SCX) as stationary phase was investigated. It was observed that the number of theoretical plates for small peptides varied from 240 000 to 460 000/m, and the relative standard deviation for t_0 and the migration time of peptides were less than 0.57% and 0.27%, respectively for ten consecutive runs. Unusually high column efficiency has been explained by the capillary electrophoretic stacking and chromatofocusing phenomena during the injection and separation of positively charged peptides. The sample buffer concentration had a marked effect on the column efficiency and peak area of the retained peptides. The influences of the buffer concentration and pH value as well as the applied voltage on the separation were investigated. It has been shown that the electrostatic interaction between the positively charged peptides and the SCX stationary phase played a very important role in IE-CEC, which provided the different separation selectivity from those in the capillary electrophoresis and reversed-phase liquid chromatography. A fast separation of ten peptides in less than 3.5 min on IE-CEC by adoption of the highly applied voltage was demonstrated.

Keywords: Capillary electrochromatography; Strong cation exchange; Separation mechanism; Peptides

1. Introduction

Capillary electrochromatography (CEC) is a hybrid technique which combines the high efficiency of capillary electrophoresis (CE) and the high selectivity of high-performance liquid chromatography (HPLC). In CEC, the capillary column is packed with HPLC stationary phase and the mobile phase is driven by the electroosmotic flow (EOF) rather than by a pressurized flow as in HPLC. Adoption of EOF in CEC results in two important advantages for CEC over conventional HPLC. First, the nearly flat profile of EOF reduces the band broadening caused by transchannel diffusion and eddy diffusion, therefore higher column efficiency can be obtained in CEC than that in HPLC. Second, the EOF is independent of particle size and does not generate back pressure, therefore smaller particles and longer columns can be used in CEC than those used in HPLC, and the plate numbers can be further improved. Since the first report on CEC in 1974 [1], several research groups [2–5] have demonstrated the high separation efficiency of CEC.

The initially described CEC separations were accomplished by using ODS as stationary phase and with neutral aromatic hydrocarbons and pharmaceuticals as the separation targets [3–8]. At present, the list of compounds separated by CEC has expanded gradually to include textile dyes, food coloring, nucleosides, peptide and protein digests [9–12]. The

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stationary phases used in CEC also have expanded to C_8 derivatized silica, ion-exchange packings, chiral materials and various polymer phases [5,7,13–18]. Recently, a specially designed stationary phase for CEC has been developed [19] by chemically bonding the strong sulfonic acid and octadecyl groups onto silica. It was found that this stationary phase has strong EOF at wide range of pH values. More recently, silica and strong cation-exchange material packed columns with dynamically modified by cetyltrimethylammonium bromide as the reversed-phase stationary phase in CEC were developed [20,21].

To date, only a few CEC separations have been performed on the ion-exchange packings. Smith and Evans [5] as well as Choudhary and Horváth [22] have reported the separation of basic tricyclic antidepressants and three peptides by IE-CEC with SCX stationary phase. Li et al. [23] reported that CEC on a strong anion-exchanger can be used as an analytical tool for probing the Hanford nuclear site environment. Recently, Cikalo et al. [24] have studied the behavior of cation-exchange materials in capillary electrochromatography. In this paper, the separation of peptides by CEC on strong cation-exchange (SCX) packings is presented and the influence of buffer concentration, pH, and applied voltage on the separation is studied.

2. Experimental

2.1. Instrumentation and materials

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. Fused-silica capillary (50 μm I.D.×365 μm O.D.) was obtained from Yongnian Optic Fiber Plant (Hebei, China). Spherisorb-SCX (5 μm) was purchased from the Waters Phase Separation (Milford, MA, USA).

2.2. Samples and solutions

The peptides used in this study were purchased from Serva (Heidelberg, Germany). Acetonitrile was of chromatographic grade, benzyl alcohol was of chemical grade, and the other reagents used were of the analytical reagent grade. Ultra-pure water used for preparation of solutions was produced by a Milli-Q water system (Millipore, Bedford, MA, USA). Stock solution of phosphate buffer (100 m*M*) was prepared by dissolving 3.40 g KH_2PO_4 in 200 ml ultra-pure water, then adjusting to appropriate pH by KOH or H_3PO_4 solution and then transferring to a 250-ml flask. Mobile phases were prepared by mixing appropriate volume of phosphate buffer, acetonitrile and ultra-pure water. Before running, the mobile phase was degassed in an ultrasonic bath for 30 min. If not otherwise stated, the sample solution was first prepared in water, then diluted to appropriate concentration by the mobile phases containing 60% acetonitrile in 10 m*M* KH_2PO_4 (pH 3.0) before injection.

2.3. Column preparation

CEC columns with Spherisorb-SCX were packed by slurry packing technique as reported in the literature [21,25]. All columns were 31 cm long with a packed length of 10 cm. Before experiment, the column was first flushed with mobile phase for 30 min by a syringe, whose needle was connected to the outlet of the CEC column by a PTFE tube. Then the column was conditioned on the instrument with the mobile phase for another 30 min. The applied voltage first ramped from 0 to 15 kV in 10 min and then held at 15 kV for 20 min. In order to avoid bubble formation, 6.9 bar pressure was also applied to both ends of the capillary.

2.4. Separation conditions

The P/ACE MDQ system can apply 6.9 bar pressure to both ends of the capillary. It takes about 0.3 min to reach 6.9 bar while it only takes 0.17 min for the voltage to reach the separation voltage. It is possible for bubbles to form when the voltage has reached to the separation voltage while the pressure is still low. Therefore, it is necessary to set the ramp time of the voltage longer than that of the pressure in order to prevent bubble formation. In this experiment, the ramp time for the voltage was 0.5 min and the separation voltage was 15 kV for all CEC separations unless otherwise stated. The injections

were made by applying a voltage of 5 kV for 10 s unless otherwise stated. The temperature was kept at 20°C and the detection wavelength was set at 200 nm. Fused-silica capillaries with dimension of 31 cm (10 cm to detection)×50 μm I.D. were used for CEC experiments.

The column used for CE was 50 cm long with an effective length of 40 cm. The column was conditioned with 0.1 *M* HCl solution for more than 16 h prior to first use. The capillary was rinsed with 0.1 *M* HCl for 2 min, water for 1 min and mobile phase for 3 min successively before each run. The operation voltage was 25 kV with ramp time of 0.17 min. The temperature was kept at 20°C and the detection wavelength was set at 200 nm as in CEC. The injection was made by applying a pressure of 0.035 bar for 5 s.

3. Results and discussion

3.1. Selectivity and efficiency

Both CE and IEC can be used to separate peptides [26,27]. The separation mechanisms in CE and IEC are mainly based on the differences in electrophoretic mobility of the solutes and electrostatic interaction of solutes with stationary phase, respectively. IE-CEC is a relatively new separation technique based on the combination of ion-exchange chromatographic and electrophoretic separation mechanisms. Therefore the selectivity of CE and IE-CEC should be different due to the different mechanisms involved. Five peptides, Gly–Thr, Gly–Ala–Gly, Glu–Glu, Gly–Gly–Asn–Ala and Glu–Glu–Glu were selected as test solutes, and the neutral compound, benzyl alcohol, was selected to approximately mark the EOF. The typical chromatograms obtained in IE-CEC and CE are shown in Fig. 1 with the same mobile phase containing 60% acetonitrile in 30 m*M* KH_2PO_4 buffer (pH 3.0) applied. Marked differences in selectivity are apparent between the two systems. Because all the peptides were positively charged at pH 3, the electrophoretic migration direction of peptides was the same as the direction of the EOF, which resulted in faster migration than EOF and all the peptides eluted before the t_0 marker in CE. But all peptides eluted after the t_0 marker in CEC, which meant the strongly electrostatic interactions between the peptides and SCX packings took place. The elution orders of the peptides was also different in the two systems. For example, Gly–Thr eluted before Gly–Ala–Gly in CEC, but the elution order was reversed in CE (Fig. 1).

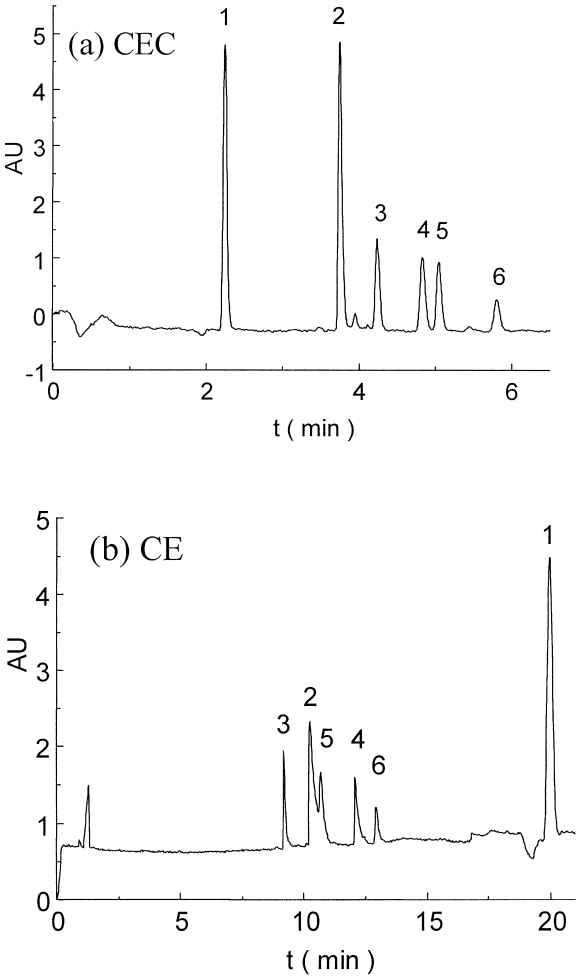

Fig. 1. Separation of small peptides in IE-CEC and CE experimental conditions: mobile phase, 60% acetonitrile in 30 m*M* KH_2PO_4 buffer (pH 3.0). (a) IE-CEC: column, 75 μm I.D.×375 μm O.D. packed with 5 μm Spherisorb-SCX, packed/total length=10/31 cm; applied voltage, 15 kV; electrokinetic injection, 5 kV×10 s; UV detection, 200 nm. (b) CE: column, capillary with 50 μm I.D.×375 μm O.D.; capillary length, total/effective length of 50.2/40 cm; applied voltage, 25 kV; hydrodynamic injection, 0.035 bar×5 s; UV detection, 200 nm. Peaks: 1=benzyl alcohol; 2=Gly–Thr; 3=Gly–Ala–Gly; 4=Glu–Glu; 5=Gly–Gly–Asn–Ala; 6=Glu–Glu–Glu.

It is obvious that the running buffer in Fig. 1b was not optimized for the CE separation of peptides, two of five peptides could not be baseline separated due to peak tailing, which may be caused by the adsorption of positively charged peptide on the ionized silanol groups of the capillary wall at relatively low rate. This problem can be solved by further lowering the pH of the running buffer to suppress silanol groups on capillary wall, but this results in longer migration times. However, the peak shape of peptides in CEC was very good (Fig. 1a). The reason may be that the surface area of the packing was much greater than that of the capillary wall, and the peptides adsorbed to the sulfonic groups at a relatively high rate, with little adsorption of the peptides to the capillary wall and therefore little peak tailing. It has been reported that excessive tailing has been a characteristic feature in the separation of basic compounds on some commercially available silica-based SCX materials [28]. But the peak asymmetry for separation of peptides at all conditions was satisfied in this study.

Peptides can also be separated in the reversed-phase liquid chromatography (RP-HPLC) [29] mainly based on the interaction between the non-polar subunit of peptides and the alkyl chains of the stationary phase. The separation selectivity in RP-HPLC is also different from that in IE-CEC. Fig. 2a and b shows the chromatograms for the separation of two pairs of dipeptide isomers in IE-CEC. The elution orders observed were reversed compared with RP-HPLC [29]. The above result meant that IE-CEC could be a complementary technique for the reversed-phase CEC and CE in the separation of peptides.

The major advantages of CEC is its high column efficiency due to the flat profile of EOF. Peptides including Gly–Gly, Gly–Gly–Gly, Gly–Gly–Gly–Gly, Gly–Gly–Gly–Gly–Gly and Gly–Gly–Gly–Gly–Gly–Gly were selected to investigate the repeatability of the migration time and the efficiency of IE-CEC. Table 1 gives the results of ten consecutive runs. The relative standard deviations (RSDs) for migration time of peptides were less than 0.27% and the RSD of the void time was about 0.56%; this means that the repeatability of IE-CEC was very good. The column efficiencies for five peptides varied from 247 000 to 464 000 plates/m. The peak

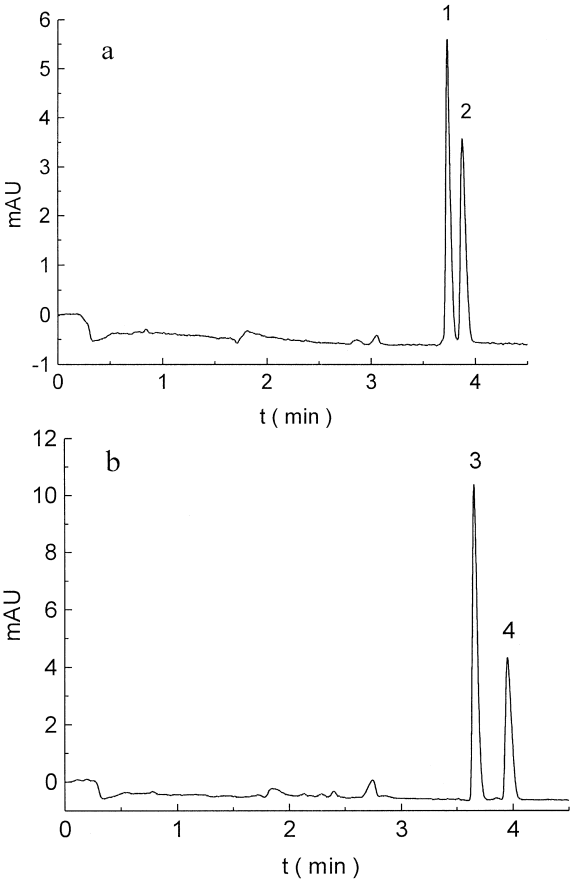

Fig. 2. Chromatogram for separation of peptide isomers in IE-CEC. Experimental conditions as in Fig. 1a. Peaks: 1=Gly–Leu; 2=Leu–Gly; 3=Gly–Phe; 4=Phe–Gly.

of the solvent appeared before the t_0 marker, which means benzyl alcohol had little retention on stationary phase. Smith et al. [5] and Li et al. [23] also reported the unusually high efficiency of IE-CEC. Sample stacking might be responsible for the high efficiency, but there was no report which studied this effect in detail. Here the effect of ionic strength of sample buffer on the efficiency was investigated using Gly–Gly, Ala–Ala–Ala and Glu–Glu as the test solutes. Samples were prepared in the solutions containing 60% acetonitrile in 0, 10, 20, 30 and 40 m*M* KH_2PO_4 buffer (pH 3.0). The concentration of Gly–Gly, Ala–Ala–Ala, Glu–Glu in above solutions were kept at 35.0, 19.5, 25.5 μg/ml, respectively. The mobile phase containing 60% acetonitrile in 40 m*M* KH_2PO_4 buffer (pH 3.0) was used in this

Table 1
Repeatability of the migration time (t_m) and column efficiency (N) for ten consecutive runs; experimental conditions as in Fig. 1

Solutes	t_m (min)	RSD (%)	N (plate number/m)	RSD (%)
Benzyl alcohol	1.83	0.55	97 000	12.10
Gly–Gly	3.64	0.15	247 000	3.26
Gly–Gly–Gly	4.61	0.26	321 000	4.59
Gly–Gly–Gly–Gly	5.33	0.27	359 000	5.27
Gly–Gly–Gly–Gly–Gly	6.11	0.13	449 000	8.39
Gly–Gly–Gly–Gly–Gly–Gly	6.72	0.19	464 000	3.86

experiment. The results of the influence of sample buffer concentration on column efficiency are shown in Fig. 3. It can be seen from Fig. 3 that the column efficiency increased with the decreasing of sample buffer concentration from 40 to 10 m*M*. This effect might be attributed to the focusing processes of sampling including the on-line sample stacking as in CE (CE stacking) and the chromatofocusing as in HPLC. The chromatofocusing has already been used [30,31], but the CE stacking has not been used intentionally to improve the detection in CEC to our knowledge. If the ionic strength of sample solution was lower than that of the mobile phase, the electric resistance and the field strength of the sample zone increased, which in turn caused the positively charged peptides to migrate fast and stack as a sharp band at the boundary between the sample zone and the mobile phase. The positively charged peptides stacked at the cathode side of sample zone (at the head of sample zone) at this condition. The elution strength of mobile phase with low ionic strength was weak in IEC, which led the analytes in sample solution with low ionic strength to be concentrated at the end of sample zone but at the front of column. One of these two stacking processes might mainly be responsible for the high column efficiency. It was obvious that CE stacking was effective only for the solutes in solution. Most of the solutes in the sample zone were retained on the SCX packing because of the sample solution with weak elution strength. Therefore, the quantity of solutes in solution was relatively small and thereby the influence of CE stacking on efficiency was relatively moderate. Chromatofocusing that might mainly be responsible for the high efficiency as shown in Table 1, which was supported by a fact that the later eluted peptides with greater k' values had the higher column efficiency because of the more effective chromatofocusing.

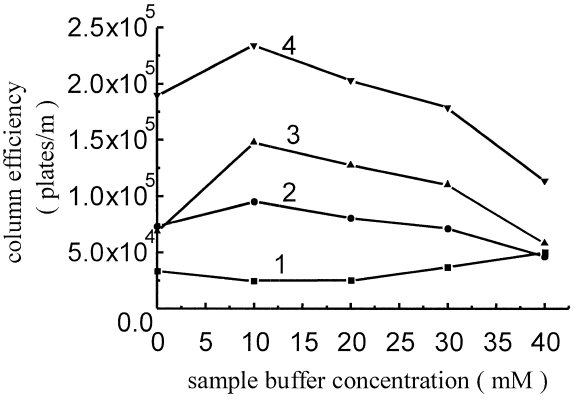

Fig. 3. Dependence of column efficiency on sample buffer concentration. Experimental conditions: mobile phase, 60% acetonitrile in 40 m*M* KH_2PO_4 buffer (pH 3.0). Samples were prepared in solutions containing 60% acetonitrile in 0, 10, 20, 30, 40 m*M* KH_2PO_4 buffer (pH 3.0); electrokinetic injection, 5 kV×20 s. Other conditions as in Fig. 1a. Solutes: 1=benzyl alcohol; 2=Gly–Gly; 3=Ala–Ala–Ala; 4=Glu–Glu.

The column efficiency increased with decreasing of the ionic strength of sample solution, therefore if the sample was prepared by the solution without buffer, it was expected that the highest column efficiency should be obtained. But it was found experimentally that the stacking was not very effective in practice as shown in Fig. 3, which might be caused by the similar reason as reported in CE [32]. Since electroosmosis occurs much more rapidly in the diluted sample solution than the running mobile phase, the mismatch in the flow-rate causes a laminar flow inside the capillary that reduces the effectiveness of the stacking processes. An interesting phenomenon as shown in Fig. 3 was that the column efficiency of neutral solutes increased with increasing of buffer concentration in sample solutions from

10 to 40 m*M* KH_2PO_4 buffers, the reason for which is unclear.

It was reported that the peak area of charged solutes decreased with an increase in the ionic strength of the sample buffer in CE [33], when the electrokinetic injection with highly ionic strength of the sample solution was applied. It may be that the excess of ions in the sample solution led to less of the analytes being introduced. A similar result was also found in CEC. Fig. 4 shows the relationship between the peak area and the sample buffer concentration. The peak area of three peptides increased slightly when the sample buffer concentration decreased from 40 to 10 m*M* KH_2PO_4, but increased by 6–10 fold from the sample solution with 10 m*M* buffer to that without buffer. The increase of peak area meant the amount of charged peptides electrokinetically injected increased with decreasing of the ionic strength of sample solutions.

3.2. Effect of mobile phase ionic strength, pH and voltage on the separation

The characteristic capacity factor (k'), used in HPLC, is no longer valid for describing the migration process of ionic compound in CEC due to the coupled electrophoretic migration. So it is necessary to define the electrochromatographic capacity factor (k^*) [12,34]

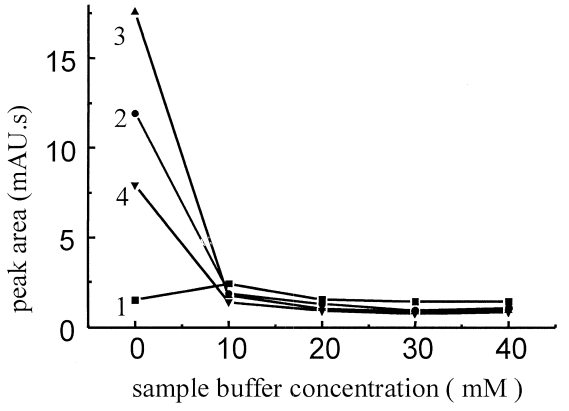

Fig. 4. Effect of sample buffer concentration on peak area. Experimental conditions as in Fig. 3.

$$k^* = \frac{(t_r - t_0)}{t_0} \quad (1)$$

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, benzyl alcohol was selected as t_0 marker, and k^* can also be expressed theoretically as follows [12,34]

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

where k' is the actual capacity factor caused by chromatography alone in CEC, μ_{eo} and μ_{ep} are the electroosmotic and electrophoretic mobilities, respectively.

The effect of the eluent ionic strength on the separation was studied using mobile phase containing 60% acetonitrile in 10–40 m*M* KH_2PO_4 buffer (pH 3.0). The ionic strength of the mobile phase influenced the magnitude of the EOF as shown in Table 2. The value of μ_{eo} decreased with increasing buffer concentration. Secondly, the ionic strength could adjust the elution strength of mobile phase in IEC, and the elution strength of the eluent increased with the ionic strength of eluent. It was observed that

Table 2
Dependence of the electroosmotic mobility (μ_{eo}) on the buffer concentration and pH of the eluents

	μ_{eo} (cm^2/kV min)
Buffer concentration (m*M*)	
40	8.72
35	8.80
30	9.26
25	9.41
20	9.35
10	10.40
Eluent pH value[a]	
2.3	9.11
3.0	10.91
4.0	11.56
5.0	11.99
6.0	11.98

[a] CEC columns used with the same dimensions but different batches.

the retention of peptides increased with decreasing of ionic strength in IE-CEC in this experiment. The logarithm of the capacity factor (log k') of ionic solutes linearly decreases with the logarithm of the counter ions concentration (log[c]) in IEC [35,36]. Since it was very difficult to measure the k' value of charged solutes in IE-CEC, k' was substituted by k^*, which was averaged by the data of three runs with RSDs $<2.0\%$. Fig. 5 shows the log k^* vs. log[c] plots for test solutes in IE-CEC. Log k' and log[c] followed the excellent linear relationships ($r>0.989$), which meant that the separation was mainly based on the ion-exchange mechanism under these conditions, and the influence of electrophoretic mechanism on the separation is negligible. This phenomenon can be explained by Eq. (2) that if μ_{ep} is relatively small and μ_{eo} is relatively large, the influence of μ_{ep} on k' can be neglected. The magnitude of μ_{eo} in this system was very large because of the adoption of SCX packings, which had high density of negative charge on the surface. Therefore the value of μ_{ep}/μ_{eo} was much smaller than 1 in this system. The k' value could be adjusted by the ionic strength of the mobile phase in IE-CEC leading to much greater than the μ_{ep}/μ_{eo} value, then the influence of electrophoresis (μ_{ep}) on separation could be neglected according to Eq. (2). But if k' is relatively small, such an influence can remain. As shown in Fig. 5 that the stronger the retention of the peptides, the better the linear relationship. In other words, if the separation was performed at high pH of the eluent when the peptides were negatively charged or under strong ionic strength of the eluent, the peptides nearly not retained on the stationary phase, the electrophoretic mechanism took the main role in the separation of peptides. Therefore, the separation of ionic solutes can be improved by lowing the ionic strength of mobile phase in IE-CEC.

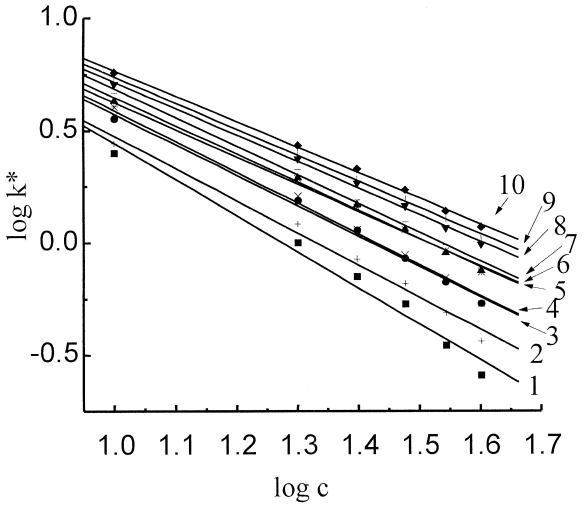

Fig. 5. Linear relationship of log k^* and log[c]. Solutes: 1=Gly–Gly, log $k^*=2.05-1.60$ log[c], $r=0.9886$; 2=Gly–Thr, log $k^*=1.90-1.43$ log[c], $r=0.9929$; 3=Gly–Gly–Gly, log $k^*=1.93-1.35$ log[c], $r=0.9974$; 4=Gly–Ala–Gly, log $k^*=1.96-1.37$ log[c], $r=0.9963$; 5=Glu–Glu, log $k^*=1.83-1.21$ log[c], $r=0.9969$; 6=Gly–Gly–Gly–Gly, log $k^*=1.90-1.25$ log[c], $r=0.9979$; 7=Gly–Gly–Asn–Ala, log $k^*=1.96-1.27$ log[c], $r=0.9972$; 8=Gly–Gly–Gly–Gly–Gly, log $k^*=1.89-1.18$ log[c], $r=0.9986$; 9=Glu–Glu–Glu, log $k^*=1.90-1.16$ log[c], $r=0.9982$; 10=Gly–Gly–Gly–Gly–Gly–Gly, log $k^*=1.90-1.13$ log[c], $r=0.9989$.

The effect of the eluent pH on the separation was studied with a mobile phase containing 60% acetonitrile in 30 m*M* KH_2PO_4 buffer. The pH of the buffer was varied from 2.3 to 6.0. The dependence of EOF on the eluent pH in IE-CEC is shown in Table 2. It was observed that the μ_{eo} value increased by 16% when the pH increased from 2.3 to 3.0, but the increase of μ_{eo} was moderate when the eluent pH was raised further. This indicated that the strong sulfonic acid groups were almost completely ionized at pH>3, and partial ionization of sulfonic groups was suppressed at pH<3. The slight increase of μ_{eo} from pH 3.0 to 6.0 might be due to the increase in the ionization of silanol groups on the capillary wall and packing surface. Therefore, the effect of the pH on EOF was not very strong because the strong sulfonic acid groups were ionized at wide range of the eluent pH values.

As one of important factors, the pH of the mobile phase could be used to adjust the separation selectivity of ionizable solutes in HPLC. But the usage of this method in reversed-phase CEC was limited because the velocity of EOF strongly depended on the eluent pH [37]. As we know that the magnitude of EOF depended on the charge density of the packing surface, the amount of ionized silanol groups on ODS packing material were suppressed at acidic eluent and the magnitude of EOF was relatively small, which resulted in the long analysis time. But in IE-CEC with SCX packing, the wide range of

pH could be adopted with keeping high EOF velocity like as in HPLC as shown in Table 2. So it was still reasonable to adjust the separation selectivity by changing the eluent pH in IE-CEC.

The effect of pH on k^* was studied in the range 2.3–6.0, and the results are shown in Fig. 6. For all peptides, k^* increased with increasing the pH from 2.3 to 3.0, which may be attributed by following factors: (1) the electroosimotic mobility (μ_{eo}) increased by 16% when pH increased from 2.3 to 3.0, which resulted in increase of k^* as indicated by Eq. (2); (2) sulfonic groups partially suppressed at low pH were ionized with increasing of pH from 2.3 to 3.0, which made the retention of peptides on the SCX packing stronger. Further increase of the eluent pH decreased the k^* values for most of solutes as shown in Fig. 6. Only positively charged solutes are retained on SCX packings. So the retention of peptides on SCX decreased quickly with increasing of the pH in IE-CEC because the amount of positively charged peptides decreased, but the influence was relatively small compared with that in IEC; the value of k^* for Gly–Gly even increased with increasing pH. Those phenomena can be explained by Eq. (2) that the influences of k' and μ_{ep} on k^* were counteracted with increasing pH. The retention of peptides decreased on SCX packings when pH increased, therefore k' decreased. However, the vector μ_{ep} decreased with increasing of pH, which resulted in increasing of k^* according to Eq. (2). It can be seen from Fig. 6 that the selectivity in separation of peptides changed with the pH of the mobile phase. Fig. 7 gives the typical chromatograms for separation of 6 peptides at pH 3.0 and pH 6.0, and the elution orders of three pairs of peptides were reversed.

In IEC the mobile phase is often of relatively high ionic strength, but high voltage must be applied in IE-CEC. In order to attain high efficiency in IE-CEC,

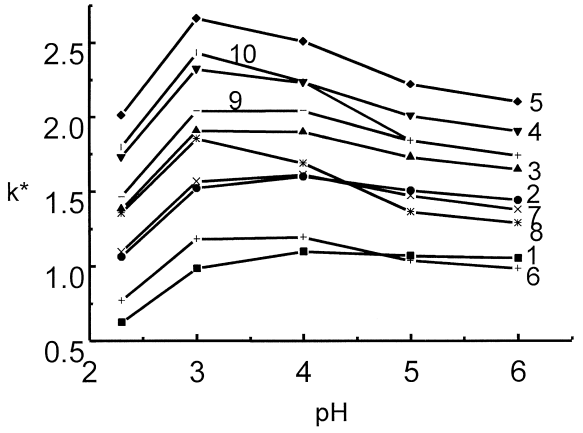

Fig. 6. Influence of the pH on k^* values. Solutes: 1=Gly–Gly; 2=Gly–Gly–Gly; 3=Gly–Gly–Gly–Gly; 4=Gly–Gly–Gly–Gly–Gly; 5=Gly–Gly–Gly–Gly–Gly–Gly; 6=Gly–Thr; 7=Gly–Ala–Gly; 8=Glu–Glu; 9=Gly–Gly–Asn–Ala; 10=Glu–Glu–Glu.

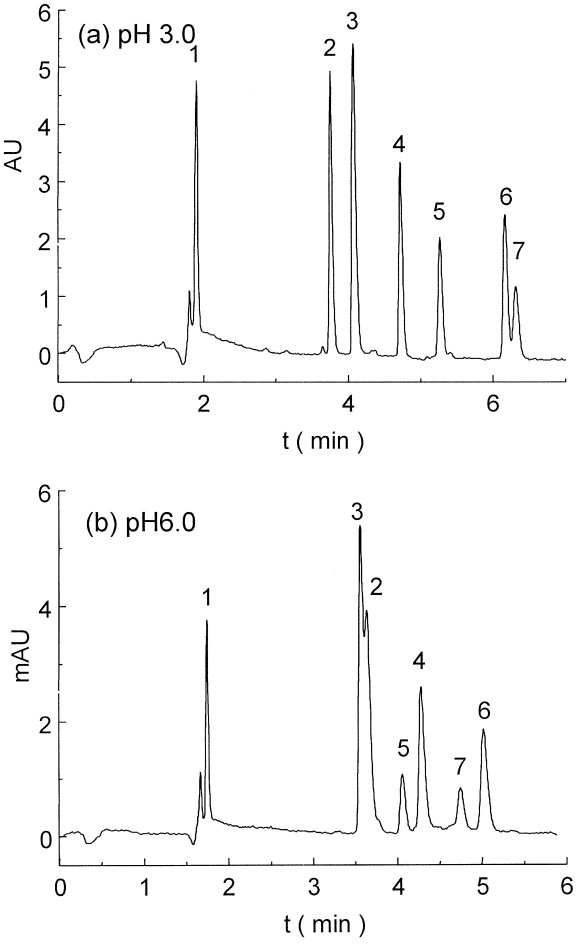

Fig. 7. Chromatograms for separation of peptides at different eluent pH values Experimental conditions: mobile phase, 60% acetonitrile in 30 mM KH_2PO_4 buffer with (a) pH 3.0 and (b) pH 6.0. Other conditions as in Fig. 1a. Peaks: 1=benzyl alcohol; 2=Gly–Gly; 3=Gly–Thr; 4=Gly–Gly–Gly; 5=Glu–Glu; 6=Gly–Gly–Gly–Gly–Gly; 7=Glu–Glu–Glu.

it was essential to ensure that an effective heat dissipation was accomplished in this system. Therefore 50 μm I.D. capillary was used in this experiment. According to the Ohm's law, the relationship of voltage (V) to current (I) should be linear if the Joule heating can be ignored, and the relationships experimentally obtained was shown as follows:

$$I = 0.081 + 0.776\,V, \quad r = 0.998$$

A good linear relationship between the current and voltage with a regression coefficient >0.998 meant that the Joule heating was not serious when the mobile phase containing 30 mM KH_2PO_4 buffer was used. The velocity of EOF could be adjusted by the changing the applied voltage in IE-CEC, and the relationship of the velocity of EOF (v_{eof}) and the applied voltage (V) obtained was shown as follows

$$v_{eof} = -0.172 + 0.336\,V, \quad r = 0.999$$

The velocity of EOF increased linearly with increasing of the applied voltage. Therefore, fast-speed separation in IE-CEC could be accomplished by applying high voltage with relatively high efficiency. Dadoo et al. [38] have reported that five polycyclic hydrocarbons were separated in <5 s in RP-CEC with a short column and a high applied voltage. We have successfully separated ten peptides in <3.5 min by applying voltage of 25 kV, and the chromatogram is shown in Fig. 8.

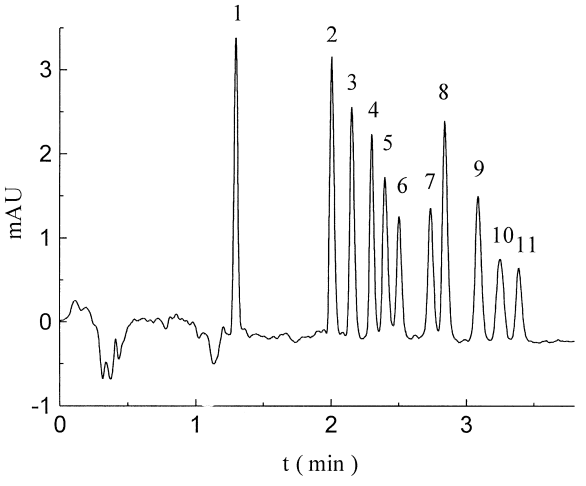

Fig. 8. Chromatogram for separation of ten peptides. Experimental conditions: applied voltage, 25 kV. Other conditions as in Fig. 1a. Peaks: 1=benzyl alcohol; 2=Gly–Gly; 3=Gly–Thr; 4=Ala–Ala–Ala; 5=Gly–Gly–Gly; 6=Ala–Ala–Ala–Ala; 7=Gly–Gly–Gly–Gly; 8=Gly–Gly–Asn–Ala; 9=Gly–Gly–Gly–Gly–Gly; 10=Glu–Glu–Glu; 11=Gly–Gly–Gly–Gly–Gly–Gly.

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References

[1] V. Pretorious, B.J. Hopkins, J.D. Schieke, J. Chromatogr. 99 (1974) 23.
[2] J.W. Jorgenson, K.D. Lukacs, J. Chromatogr. 218 (1981) 209.
[3] J.H. Knox, I.H. Grant, Chromatographia 32 (1991) 317.
[4] F. Lelièvre, C. Yan, R.N. Zare, P. Gareil, J. Chromatogr. A 723 (1996) 145.
[5] N.W. Smith, M.B. Evans, Chromatographia 41 (1995) 197.
[6] H. Yamamoto, J. Baumann, F. Erni, J. Chromatogr. 593 (1992) 313.
[7] M.R. Euerby, D. Gilligan, C.M. Johnson, S.C.P. Rouline, P. Meyers, K.D. Bartle, J. Microcol. Sep. 9 (1997) 373.
[8] P.D.A. Angus, E. Victorino, K.M. Payne, C.W. Demarest, T. Catalano, J.F. Stobaugh, Electrophoresis 19 (1998) 2073.
[9] M. Hugener, A.P. Tinke, W.M.A. Niessen, U.R. Tjaden, V. Greef, J. Chromatogr. A 647 (1993) 375.
[10] G.A. Lord, D.B. Gordon, L.W. Tetler, P.M. Carr, J. Chromatogr. A 700 (1995) 27.
[11] K. Schmeer, B. Behnke, E. Bayer, Anal. Chem. 67 (1995) 3656.
[12] J.T. Wu, P. Huang, M.X. Li, D.M. Lubman, Anal. Chem. 69 (1997) 2908.
[13] E.C. Peters, P. Miroslav, F. Svec, M.J. Fréchet, Anal. Chem. 69 (1997) 3646.
[14] Z.J. Tan, T. Remcho, Anal. Chem. 69 (1997) 581.
[15] S. Li, D.K. Lloyd, Anal. Chem. 65 (1993) 3684.
[16] F. Leviévré, C. Yan, R.N. Zare, P. Gareil, J. Chromatogr. A 723 (1996) 145.
[17] C. Wolf, P.L. Spence, W.H. Pirkle, E.M. Derrico, D.M. Cavender, G.P. Rozing, J. Chromatogr. A 782 (1997) 175.
[18] M.M. Dittmann, G.P. Rozing, J. Microcol. Sep. 9 (1997) 399.
[19] M. Zhang, Z.E. Rassi, Electrophoresis 19 (1998) 2068.

[20] M. Ye, H. Zou, Z. Liu, J. Ni, Y. Zhang, J. Chromatogr. A 855 (1999) 137.
[21] M. Ye, H. Zou, Z. Liu, J. Ni, Anal. Chem., in press.
[22] G. Choudhary, C. Horváth, J. Chromatogr. A 781 (1997) 161.
[23] D. Li, H.H. Knobel, V.T. Remcho, J. Chromatogr. A 695 (1997) 169.
[24] M.G. Cikalo, K.D. Bartle, P. Myers, Anal Chem. 71 (1999) 1826.
[25] Y. Zhang, W. Shi, L. Zhang, H. Zou, J. Chromatogr. A 802 (1998) 59.
[26] N. Chen, L. Wang, Y. Zhang, Chromatographia 37 (1993) 429.
[27] J.R. Mcdermmott, A.M. Kidd, J. Chromatogr. 296 (1984) 231.
[28] J.H. Miyama, M.S. Alasandro, LC·GC 16 (1998) 36.
[29] H. Zou, Y. Zhang, P. Lu, Chromatogrphia 31 (1991) 27.
[30] D.A. Stead, R.G. Reid, R.B. Taylor, J. Chromatogr. A 798 (1998) 259.
[31] U. Pyell, H. Rebscher, J. Banholczer, J. Chromatogr. A 779 (1997) 155.
[32] M.J. Sepaniak, R.O. Cole, Anal. Chem. 59 (1987) 472.
[33] D. Demorest, R. Dubrow, J. Chromatogr. 559 (1991) 43.
[34] M. Ye, H. Zou, Z. Liu, J. Zhu, J. Ni, Y. Zhang, Sci. China B 42 (1999) 639.
[35] R.D. Rocklin, C.A. Pohl, J.A. Schibler, J. Chromatogr. 411 (1987) 107.
[36] R.R. Drager, F.E. Regnier, J. Chromatogr. 359 (1986) 147.
[37] R.M. Seifar, J.C. Kraak, W.Th. Kok, H. Poppe, J. Chromatogr. A 808 (1998) 71.
[38] R. Dadoo, R.N. Zare, C. Yan, D.S. Anex, Anal Chem. 70 (1998) 4787.