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Capillary electrochromatographic separation of basic compounds with bare silica as stationary phase

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Abstract

The feasibility of capillary electrochromatography (CEC) was demonstrated using capillary columns packed with 3-μm silica particles and a mobile phase of organic–aqueous buffer system. The retention mechanisms of basic compounds were explored. Preliminary results indicated that the separation of the basic compounds in the CEC system involved multiple retention mechanisms including reversed-phase, cation-exchange as well as normal-phase mechanisms. In addition, electrophoretic mobility of the solutes also contributed to retention. The effects of experimental parameters such as the volume fraction of the organic modifier, pH value and ionic strength of the buffer on the retention behaviours of the solutes were investigated. A comparison of differences between CEC and CE of separations was also discussed.

Keywords: Electrochromatography; Silica; Buffer composition; Retention mechanism; Basic compounds

1. Introduction

Capillary electrochromatography (CEC) combines the advantages of micro-HPLC and CE. As a hybrid technique, it is rapidly gaining popularity owing to its separation power (high efficiency and high selectivity) and other advantages from miniaturization such as lower solvent consumption, low sample-volume requirements, increased mass sensitivity, and its compatibility to MS. Most of the reported work concerning CEC separation and analysis so far, has been focused on the use of reversed (e.g., C_{18}) stationary phases [1–10]. Recently, Smith and Evans [11] have demonstrated ion-exchange electrochromatography by separating several highly polar compounds using a strong cation-exchange column. There are three reports on chiral separations by using CEC with packed capillary columns [12–14]. Knox and Grant [4] studied the effect of particle diameter on the electroosmotic flow (EOF) and plate heights using a frontal elution method with silica-gel-packed columns. However, CEC separation utilizing bare silica as a stationary phase with a mobile phase composed of organic–aqueous buffer, has not been reported so far in our literature search. Bare-silica packings with reversed-phase elution have been used for separating basic compounds in HPLC due to better peak symmetry on silica compared with that on reversed-phase packings. The chromatographic behaviour in this system was also investigated widely in HPLC. Jane [15] first showed the possibilities and potentials of such a polar separation system for analysis of amino-group-containing drugs. Bidlingmeyer and coworkers [16,17] explained that a silica stationary phase has hydrophobic properties due to siloxane groups and the retention mechanism

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is similar to that observed in RP-HPLC. Cox and Stout [18,19] interpreted the retention of some basic compounds as a combination of ion-exchange and reversed-phase interactions. Schmid and Wolf [20] studied the characteristic selectivity of silica phase and an ion-exchange retention mechanism was confirmed. Obviously, the CEC system with silica stationary phase is very complicated owing to its dependence on electrophoresis and electroosmotic flow besides the retention mechanism in HPLC. The purpose of this work is to show the feasibility of performing CEC on bare silica using a reversed-phase elution. We also report our observations on the retention mechanisms of basic solutes in CEC with the bare-silica-packed columns. The effects of buffer ionic strength, pH value, organic solvent and applied voltage on retention and EOF were also investigated. The results should provide information for a better understanding of retention mechanisms of basic compounds in CEC with silica-gel-packed columns. The separations of basic compounds by CE and CEC were also compared.

2. Experimental

2.1. Apparatus

Experiments were performed on a P/ACE 5510 capillary electrophoresis system (Fullerton, CA, USA). The packed capillary columns were obtained from Unimicro Technologies (Pleasanton, CA, USA), which were packed electrokinetically [21] with 3-μm bare silica (Micra Scientific, Northbrook, IL, USA). The open tubular columns for CZE were obtained from Yongnia Optical Fiber Factory (Yongnian, Hebei, China). The dimension of the capillary columns was the same in both CEC and CZE, i.e., 20 cm×5 μm I.D. (27 cm total length), 20 cm from the inlet frit to the detection window. The on-line UV detector was operated at 214 nm with a detection range of 0.05 a.u.f.s, and rinse time of 0.3 s. All experiments were done at 25°C.

2.2. Reagents and chemicals

HPLC-grade acetonitrile was used for organic modifier. Water was deionized. The buffers used were trishydroxymethylaminomethane (Tris) adjusted with HCl to different pH values. The basic compounds of codeine phosphate, ephedrine hydrochloride, thebaine, berberine hydrochloride, jatrorrhizine hydrochloride and cocaine hydrochloride were reference compounds identified by thin-layer chromatography obtained from the Chinese National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Aniline was analytical grade. The structures of the model compounds are shown in Fig. 1. Samples were prepared by mixing a stock solution of 1 mg/ml with a solvent, which has a slightly lower percentage of acetonitrile than the mobile phase. The concentration of the sample mixture for model solutes was ca. 40 ppm.

2.3. Procedures

The mobile phase was prepared by mixing the buffer of desired pH with an appropriate amount of acetonitrile. Tris–HCl buffer was adjusted to the desirable pH using HCl. The mobile phase was

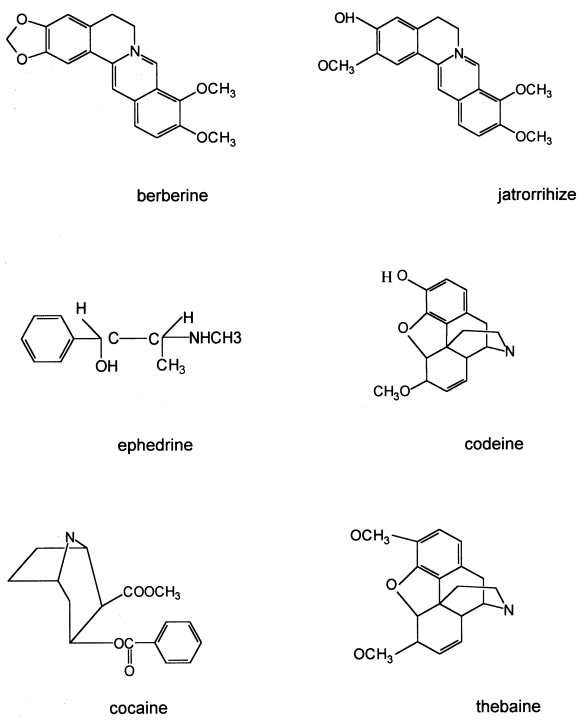

Fig. 1. The structures of basic compounds.

filtered through a 0.25-μm filter and degassed with ultrasonication for about 5 min before it was used. The packed capillary column was installed in a Beckman Model 5510 P/ACE capillary cartridge and conditioned with a mobile phase using a manual syringe pump (Unimicro Technologies) under approximately 500 p.s.i. (1 p.s.i.=6894.76 Pa). Prior to the run, the capillary was preconditioned at a relatively low voltage (5 kV) until a constant current was achieved. Electrokinetic injections run at 5 kV for 5 s.

3. Results and discussion

The samples chosen in this work were intended to represent four types of basic compounds that may interact with bare-silica surfaces. They were berberine, jatrorrhizine (quaternary ammonium cations) codeine, cocaine, thebaine (tertiary amines), ephredine (secondary amine) and aniline (first amine). The EOF was determined by solvent-disturbed peak.

3.1. Electroosmotic flow in packed capillaries

The proprieties of the EOF in packed capillaries have been regarded as the same as in open-tubular capillaries [4,5], since the surface of the silica packing particles and the inner wall of a capillary exhibit almost the same zeta potential. The equation describing the electroosmotic flow in an electrically driven system was expressed as:

$$u_{eo} = \frac{\varepsilon_r \varepsilon_0 \zeta V}{\eta L} = \mu_{eo} E \quad (1)$$

where ε_r and ε_0 are the relative and vacuum permittivity respectively, ζ and η are the zeta potential and the viscosity of the solvent, respectively. *V*, *E* and *L* are the applied voltage, electric-field strength and the total column length, respectively. μ_{eo} is the electroosmotic flow mobility.

Knox and Grant [4] pointed out that the EOF was lower in a packed bed than in an open tube, because the channels in the packed bed were not aligned axially and EOF occurred only outside the particles. Moreover, surface modification of the silica particles in RP-CEC lead to a decrease of the free silanol density on the silica surface, so EOF decreased. Dittman and Rozing [22] studied the properties of EOF on five different C_{18} stationary phases and pointed out that the EOF in a packed capillary was mainly generated from the packing particles, not from the inner surface of the capillary. Based on this assumption, EOF in a bare-silica-packed capillary column should be much stronger than that in a column packed with reversed-phase particles. The value of EOF obtained in this work was above 1.7 mm/s under the field strength of 740 V/cm, which was larger than that in the RP-CEC system [23] under similar conditions.

Fig. 2 shows the effect of acetonitrile concentration on EOF. The value of EOF increases with the increase of acetonitrile concentration. A similar result was observed in RP-CEC [22,23], but it was in contradiction with the result in CZE under similar conditions [24]. Obviously, it could not be explained only by Eq. (1), i.e., the ratio of ε/η, as stated by Dittman and Rozing [22]. We infer that both the increase of silanol density on the silica surface and the absorption of the counterion by packings, contribute to EOF. The polarity of the mobile phase decreased with the increase of acetonitrile concentration, which was more effective in drenching the hydrophobic siloxane in the stationary phase and resulted in a larger density of bare free silanol groups of stationary phase. Meanwhile, the counterion could be more easily absorbed on the silica surface compared to that in a more polar mobile phase, which resulted in the increase of charge excess on the surface of packings.

Fig. 3 shows the effect of the pH of the buffer on EOF. EOF increased with the increase in pH of the buffer. The results were consistent with what observed in RP-CEC [22,23]. It indicated that the origin of EOF was from the free silanol groups. The larger the density of the free silanol groups on the surface, the larger the EOF. Therefore, a rapid separation or a desired EOF may be obtained by mixing a different proportion of silica particles with a certain stationary phase.

3.2. Retention mechanisms

It is believed that the retention mechanisms in HPLC on a bare-silica stationary phase are complex

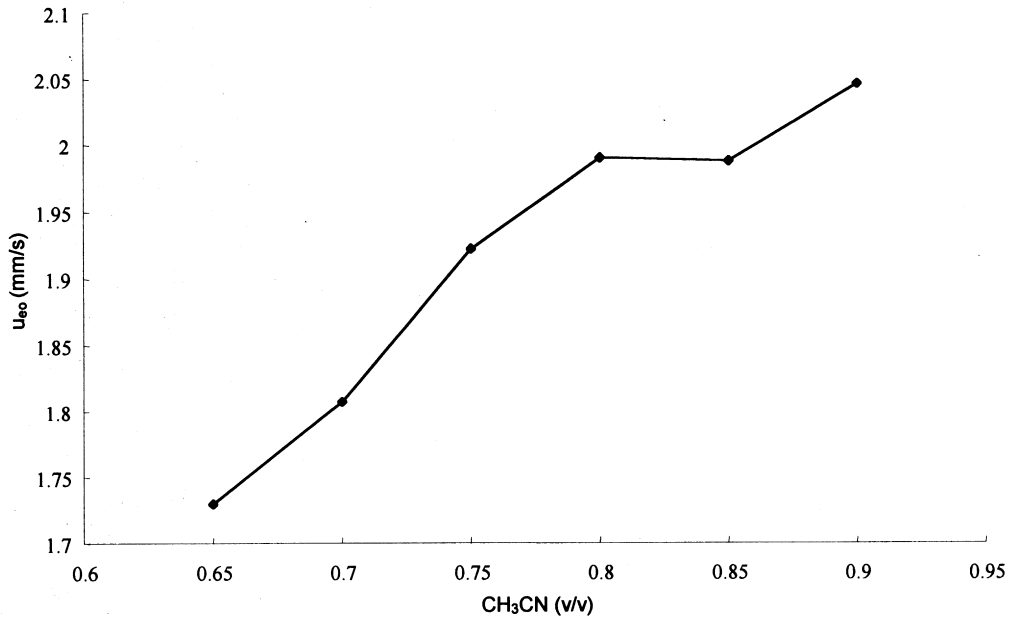

Fig. 2. Effect of organic modifier concentration on electroosmotic flow. Experimental conditions, mobile phase: 10 m*M* buffer (pH=8.29); applied voltage 20 kV; injections: 5 kV for 5 s; UV detection: λ=214 nm, 0.05 a.u.f.s.; rise time: 0.3 s; temperature: 25°C.

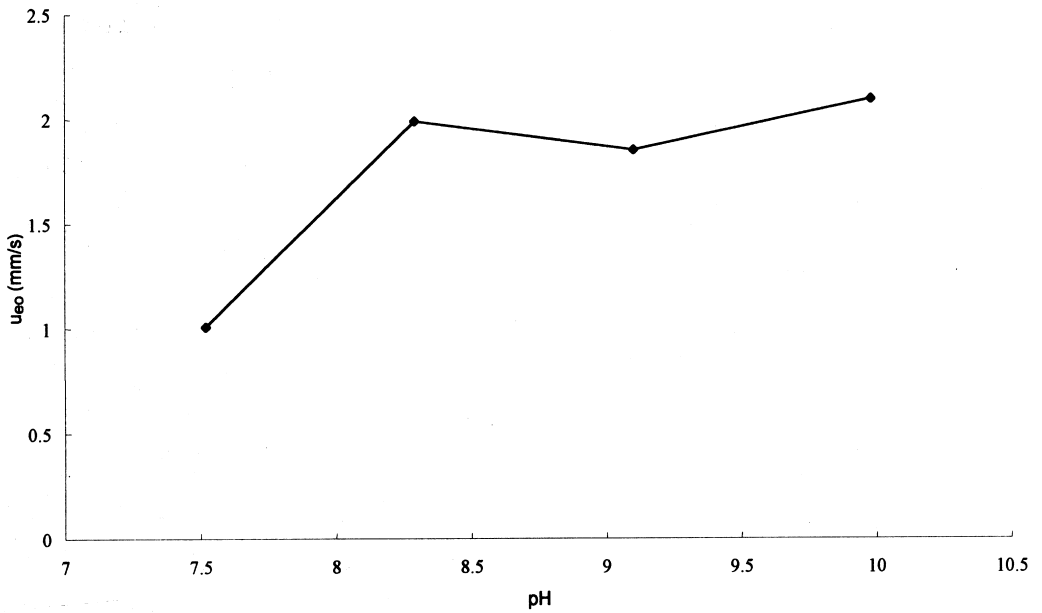

Fig. 3. Effect of pH of buffer in mobile phase on EOF. Experimental conditions, mobile phase: CH_3CN 10 m*M* buffer; other conditions as in Fig. 2.

and seem to be multifunctional, mainly involving a cation-exchange mechanism [17,18] and a reversed-phase mechanism [15,16]. In CEC, the retention becomes even more complicated owing to the additional electrophoresis effect. In order to investigate the reversed-phase retention behaviour, we varied the acetonitrile–buffer ratio while maintaining a constant pH (8.29) and ionic concentration (10 m*M*).

The effect of organic modifier is shown in Fig. 4. The capacity factor k' for the almost unretained aniline is unchanged with the change of acetonitrile concentration. For berberine, jatrorrhizine (quaternary ammonium cations) and ephidrine with pK_a 10.8 that are completely protonated, the retention should be determined by the chromatographic partition and electrophoresis. But for codeine, cocaine, and thebaine, pK_a 6~8 (tertiary amine) which are less protonated, the retention should be controlled predominantly by chromatographic partition. As the acetonitrile concentration increased from 65% to 80%, retention of these solutes decreased except ephidrine whose retention slightly increased. It shows a reversed-phase mechanism for these solutes. Ephidrine behaves differently because its interaction with the silica surface is dominated by an ion-exchange mechanism due to its more linear shape. This positively charged molecule was probably 'standing' perpendicularly to the silica surface with its amine group interacting electrostatically with the deprotonated silanol groups. The increase of the acetonitrile makes the mobile phase less polar and consequently a more hostile environment for this polar compound. Therefore, ephidrine was forced to experience more electrostatic interaction with the silica surface. Longer retention is expected. The globular compounds such as berberine and jatrorrhizine, however, cannot avoid the hydrophobic interaction with the siloxane besides the ion-exchange interaction with the silanol groups. For codeine, poor peak shapes were observed in Fig. 5, which may be attributed to the slow desorption kinetics associated with silanol–amine interactions as in reversed-phase packings.

At higher acetonitrile concentrations ranging from 80% to 90%, nearly U-shape curves for several solutes were obtained. This type of curve was

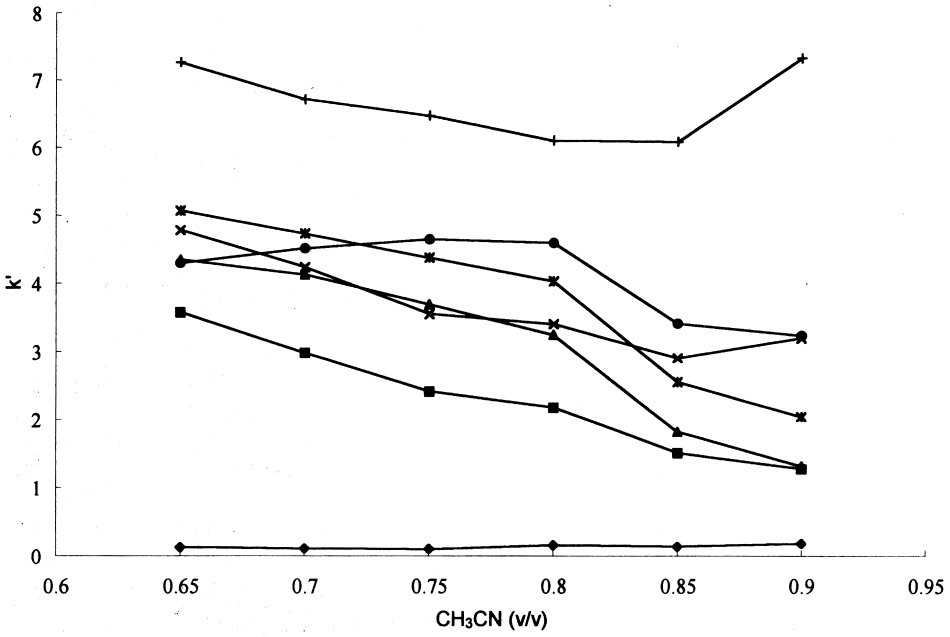

Fig. 4. Effect of organic modifier concentration on k'. Experimental conditions, mobile phase: 65–90% (v/v) acetonitrile in 10 m*M* buffer. Solutes: 1, (-✦-) aniline; 2, (-■-) cocaine hydrochloride; 3, (-▲-) berberine hydrochloride; 4, (-×-) thebaine; 5, (-*-) jatrorrhizine hydrochloride; 6, (-●-) ephridine hydrochloride; 7, (-+-) codeine phosphate; other conditions as stated in Fig. 2.

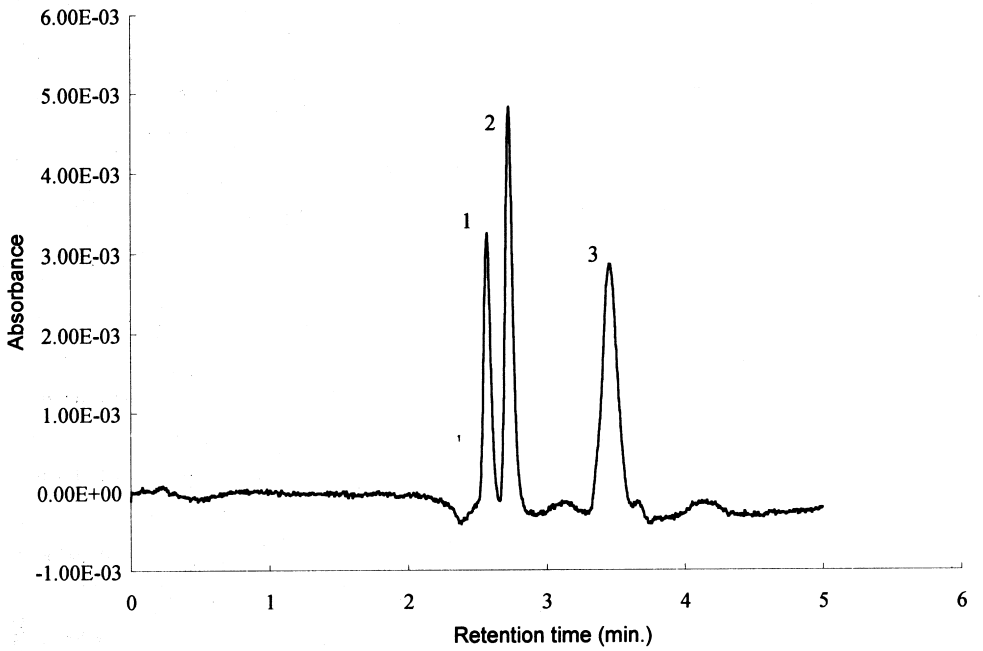

Fig. 5. Electrochromatogram of a test neutral compound on silica stationary phase. Experimental conditions, mobile phase: CH_3CN 15 m*M* buffer (95:5, v/v); applied voltage: 15 kV; other conditions as in Fig. 2. Solutes: 1, toluene; 2, aniline; 3, thiourea.

observed previously in HPLC [17,18,20]. Cox [19] explained these U-shapes as the changes in counter-ion solvation at high organic solvent concentration [19]. We infer that it is due to the change of separation model from a reversed-phase to a normal-phase. When acetonitrile concentration increased further, the stationary phase is more polarized than the mobile phase, which resulted in the change in separation model. We performed experiments in 95% acetonitrile concentration, the chromatogram was shown in Fig. 6. As expected, the elution order under this condition is toluene, aniline and thiourea, which is a typical normal-phase type of retention order.

In contrast to the hydrophobic interactions in the reversed-phase CEC, we observed predominant hydrophilic interactions in this CEC system with the silica stationary phase under the experimental conditions used. We may define this normal-phase type of separation using silica with organic–aqueous mobile phase as hydrophilic CEC in order to distinguish it from the classical normal-phase chromatography in organic mobile phase. Polar compounds (e.g., thiourea) have stronger interactions with the silica than the less polar ones (e.g., toluene) and consequently have longer retention times.

It is interesting to examine the retention of ionic compounds. For two quaternary ammonium compounds, the plots of k' vs. acetonitrile concentration are parallel, which indicates the retention behaviours are similar to each other. The value of selectivity (k'_1/k'_2) of these compounds is constant over the entire acetonitrile concentration range studied. It seems that the electrophoretic interaction also contributes to the retention for these two solutes.

3.3. Effect of ionic strength

In order to examine the effect of ionic strength, the experiments were performed at fixed organic concentration of 70% and pH 8.29. Fig. 7 shows the relationship between the k' value and the reciprocal of the concentration of Tris buffer in the mobile phase.

From Fig. 7, the k' value of aniline does not change with the variation of Tris buffer because it is neutral under the separation conditions. In the lower

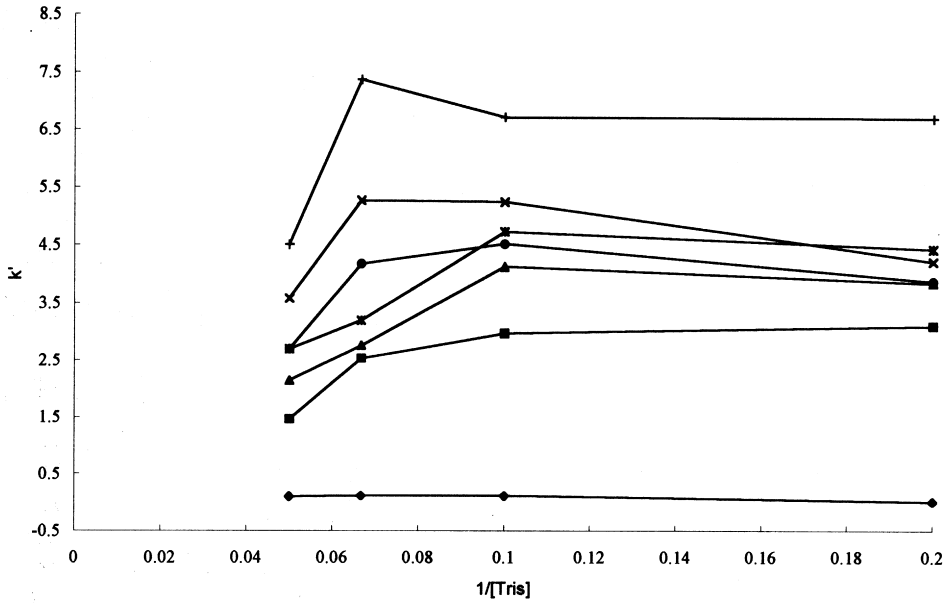

Fig. 6. Effect of ionic strength on k'. Experimental conditions, mobile phase: CH_3CN buffer (70:30). Solutes: 1, (-♦-) aniline; 2, (-■-) cocaine hydrochloride; 3, (-▲-) berberine hydrochloride, 4, (-×-) thebaine; 5, (-*-) jatrorrhizine hydrochloride; 6, (-●-) ephridine hydrochloride; 7, (-+-) codeine phosphate; other conditions as stated in Fig. 2.

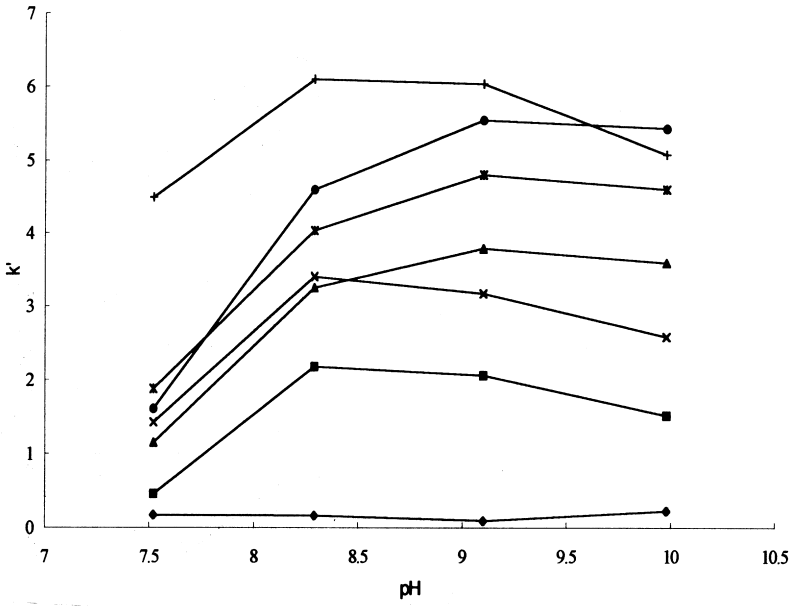

Fig. 7. Effect of mobile phase pH on k'. Experimental conditions, mobile phase: CH_3CN 10 m*M* buffer (80:20). Solutes: 1, (-♦-) aniline; 2, (-■-) cocaine hydrochloride; 3, (-▲-) berberine hydrochloride; 4, (-×-) thebaine; 5, (-*-) jatrorrhizine hydrochloride; 6, (-●-) ephridine hydrochloride; 7, (-+-) codeine phosphate; other conditions as stated in Fig. 2.

salt concentration range (from 5 to 10 m*M*), the weaker effect of ionic strength on the retention was observed. When the salt concentration increased from 10 m*M* to 20 m*M*, the k' values began to decrease and the shapes of plots for these compounds were similar. It indicated the same retention mechanism as the cation ion-exchange mechanism under the experimental conditions. However, the shapes of plots were not the same as in typical ion-exchange chromatography. This attributed to the effects of other retention mechanisms, i.e, reversed-phase and electrophoresis. In Fig. 7, the shapes of plots for berberine and jatrorrhizine were also similar and parallel to each other. It implied the contribution of the electrophoric mobility to the retention.

3.4. Effect of pH

Fig. 8 shows the retention behaviour of seven basic compounds in buffers of different pH in the mobile phase. Since aniline is neutral over the entire pH range studied, k' does not change with the increase of pH value. For three tertiary amine compounds, the shape of k' vs. pH is similar. The retention increased with pH from 7 to 8.3. When shifted to a more basic mobile phase, it decreased with pH from 8.3 to 9.98. The similar trends were observed by Schmid [19] in HPLC. The k' of three protonated compounds increased with the increase of pH, which could be explained by the cation-exchange retention [19]. The larger the charge density of the silanols on the silica surface, the larger the exchange capacity. Thus a strong retention was observed.

Again, we found that the plots of k' vs. pH of two quaternary ammonium compounds were parallel and the value of selectivity of those compounds were constant. It supports the hypothesis that the electrostatic interaction and electrophoretic mobility contribute to the retention for the protonated solutes.

3.5. Comparison of separations by CZE and CEC

It is believed that electrophoresis itself could be

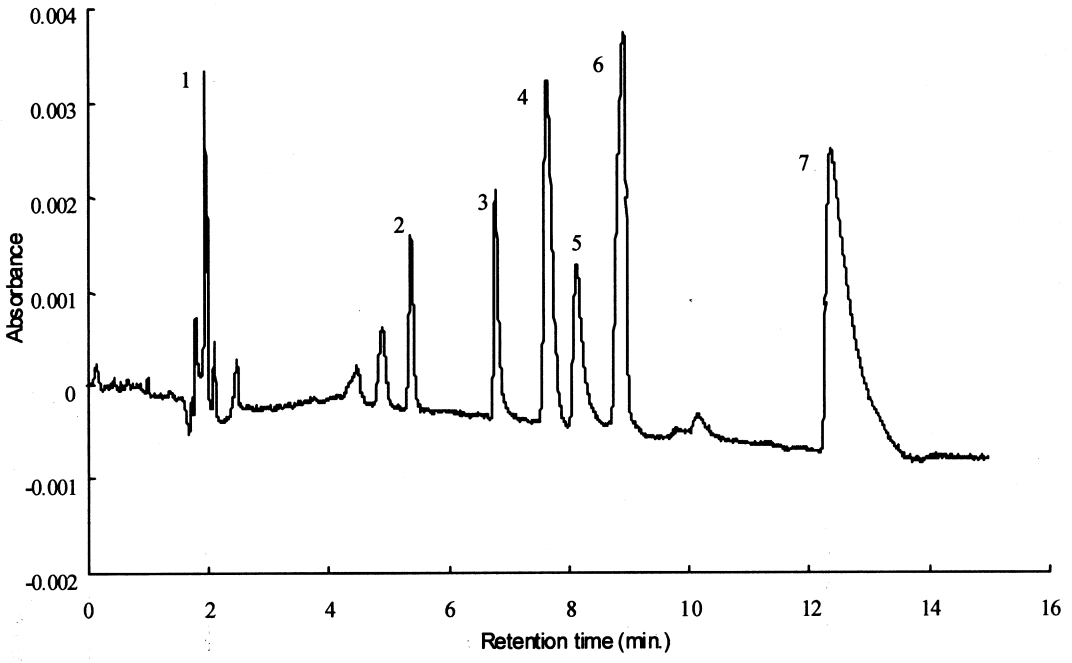

Fig. 8. Electrochromatogram of the separation of seven basic drugs on silica stationary phase. Experimental conditions, packed column: 27 cm (20 cm effective length)×75 μm I.D.; packing: 3-μm silica; mobile phase: CH_3CN–10 m*M* buffer (pH 8.29) (80:20). Solutes: 1, aniline; 2, cocaine hydrochloride; 3, berberine hydrochloride; 4, thebaine; 5, jatrorrhizine hydrochloride; 6, ephridine hydrochloride; 7, codeine phosphate.

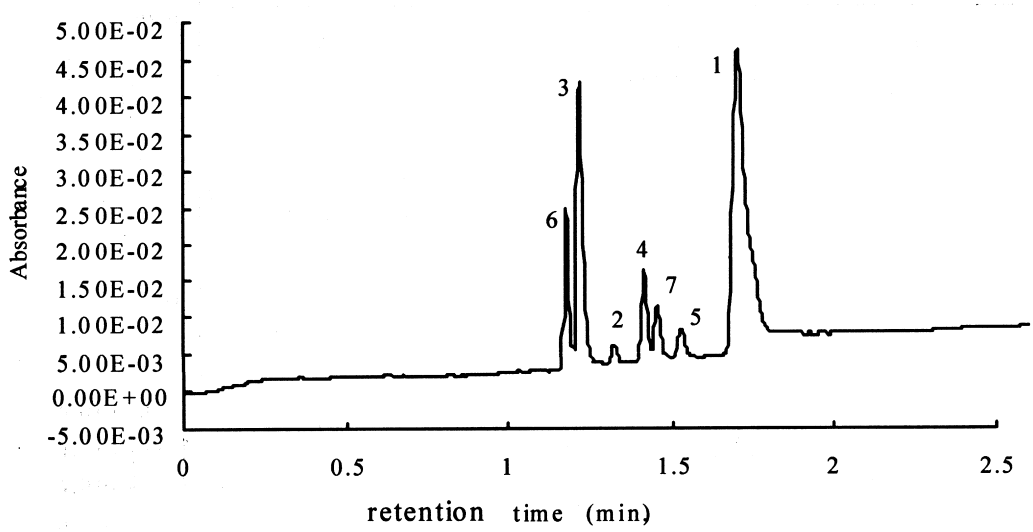

Fig. 9. Electrochromatogram of the separation of seven basic drugs by CZE. Experimental conditions, open tubular column: 27 cm (20 cm effective length)×75 μm I.D.; mobile phase: 100 m*M* buffer (pH 8.29). Solutes: 1, aniline; 2, cocaine hydrochloride; 3, berberine hydrochloride; 4, thebaine; 5, jatrorrhizine hydrochloride; 6, ephridine hydrochloride; 7, codeine phosphate.

sufficient for separations of model compounds because these selected compounds are all strong bases.

Fig. 9 shows a typical electrochromatogram of the separation of seven basic compounds in CEC. However, this could not be achieved by CZE under the same conditions due to the shorter retarded time that solutes take in open tube. A successful separation in CZE was obtained within 2 min under the 100 m*M* Tris–HCl buffer without organic modifier (Fig. 9).

While in CEC, it took 14 min to separate these compounds. A comparatively more rapid separation is due to the larger EOF in CZE than in CEC. However, resolution was poor in CZE, which could not be improved by selecting different separation conditions in our experiments. Because separation in CZE is only determined by electrophoretic mobility, while the chromatographic mechanisms (cation-exchange or reversed-phase) as well as electrophoresis in CEC may be contributed to the improved resolution.

In addition, the elution orders are also different from each other. In CEC, aniline, cocaine hydrochloride, berberine hydrochloride, thebaine, jatrorrhizine hydrochloride, ephridine hydrochloride and codeine phosphate eluted in sequence. While in CZE, the elution order is ephridine hydrochloride, berberine hydrochloride, cocaine hydrochloride, thebaine, codeine phosphate, jatrorrhizine hydrochloride and aniline. Anline, the neutral compound, eluted along with the electoosmotic flow both in CEC and CZE, the charged solutes were resolved according to the difference in electrophoretic mobilities themselves in CZE. While in CEC, the elution order is dependent on both chromatographic retention and electrophoresis.

Therefore, CEC can be chosen as an alternative for the separation of basic solutes that are often separated by CZE. Moreover, a better selectivity in CEC may be achieved potentially in the separation of the solutes with similar electrophoretic mobilities that are difficult to be resolved by CZE.

4. Conclusions

The feasibility of capillary electrochromatography (CEC) was demonstrated on capillary columns packed with 3-μm bare-silica stationary phases. The retention mechanisms of basic compounds in the CEC separation system were explored. The retention behaviours of basic compounds on bare silica seem to be multifunctional, including a reversed-phase, cation-exchange mechanism as well as normal-phase mechanisms. The separation mechanisms vary with

the composition of mobile phase. Both electrophoric mobility and electrostatic interaction contribute to the retention for charged solutes. EOF varied with organic modifier and pH of the mobile phase in the same way as those observed in RP–CEC. The selectivity is different in CZE and CEC due to their different retention mechanisms. A better selectivity in CEC may be achieved potentially in the separation of the solutes with similar electrophoretic mobilities which are difficult to resolve by CZE.

Acknowledgements

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