Separation of Basic Drugs Using Pressurized Capillary Electrochromatography

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A novel pressurized capillary electrochromatography (PCEC) was developed to separate basic drugs on strong cation exchange (SCX) column. The separation result by using PCEC was better than that by using micro-HPLC. The effects of electrical field and pressure on plate height and resolution were investigated. Influences of organic modifier, ionic strength and pH value of buffer on retention behavior were evaluated, and the separation mechanism was also discussed.

Keywords pressurized capillary electrochromatography, strong cation exchange, mixed mode, basic drug

Introduction

Capillary electrochromatography (CEC) has attracted relative attention and become a powerful and useful separation tool, which affords much high column efficiency as compared with high performance liquid chromatography (HPLC) due to its pluglike flow profile.[1-3] At present this technique is not yet mature enough and some practical difficulties, such as bubble formation and column dry-out during experiment, still hold back the progress of CEC.[4,5]

To suppress bubble formation and obtain good repeatability of experiment, Tsuda[6,7] introduced a pressurized flow by an HPLC pump, thus the mobile phase is driven by combination of electroosmotic flow (EOF) and supplementary pressure. This separation mode is named pressurized CEC (PCEC). The application of the pressure changes the flow type of mobile phase in CEC, and some reports have shown that the separation efficiency in PCEC is intermediate between those in pure CEC and HPLC.[7,8]

PCEC can provide a special advantage for the separation of charged species, because the electrophoretic migration rate and the mobile phase flow rate can be operated independently.[8,9] The retention factor, in theory, can be tuned well by adjusting pressure and electrical field, and thereby selectivity in the separation is improved. Another advantage of PCEC is that gradient elution in CEC can be set up easily,[10-12] thus it is very powerful for the separation of complicated samples, while it is very difficult that PCEC as a novel separation mode is performed on commercial CE instrument or "home-built" instrument. Development of PCEC provides attractive opportunities for special CEC separation system.

In our laboratory, a novel special CEC instrument, Trisep™ 2000 GV CEC system, has been developed, in which the mobile phase is driven by EOF and pressurized flow. Three separation modes, PCEC, CE and micro-HPLC, can be operated on this versatile instrument. A high pressure can be applied on the column, since samples can be inspected to take whether the normal or reversal applied voltage mode. A continuous gradient elution can be set up easily and accurately quantitative injection though a rotary-type injector can be carried on the instrument.[13,14]

CEC is attractive for basic pharmaceutical separation because of its high efficiency and speed. Basic drugs can be separated by reversed phase CEC, while it would require a high pH in the mobile in order to become neutral. In reversed phase system, the peak shape is badly tailing and the stationary phase is detrimental at too high pH. To improve the peak shape, some amines can be added in the mobile.[15,16] Another solution to solve peak tailing is using ionic exchange column in CEC.[17,18] Smith and Evan[17] suggested using strong cation exchange (SCX) stationary phase to separate basic drugs in CEC, because the SCX stationary phase can not only improve separation results, but also provide strong and stable EOF.[19] Some other separation modes in CEC for basic drugs were also reported. Wei *et al.*[20] reported that the basic compounds could be separated with silica column in CEC, and the separation mechanism was very complicated. In these studies, little was reported on the separation according to mixed mode of ionic exchange and hydrophilic interaction in PCEC.

In this paper, the separation of six basic drugs was achieved by PCEC and micro-HPLC with mixed mode of hydrophilic and ionic interaction. The separation results and column efficiency obtained in PCEC and micro-HPLC were

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compared. The effect of normal and reversal applied voltage mode on separation was studied. The influences of organic concentration, ionic strength and pH of buffer on retention behavior of basic drugs in PCEC were also evaluated.

Experimental

Apparatus

PCEC was carried out with a Trisep™ 2000 GV CEC system (Unimicro Trchnologies Inc., Pleasanton, CA, USA) which comprised a solvent gradient delivery module (two PU-1580 intelligent HPLC pumps purchased from JASCO, Japan), a high voltage power supply, a variable wavelength UV-vis detector, a micro fluid manipulation module (including a 20 nL four port injector) and a data acquisition module.

Materials

SCX capillary column (250 mm × 100 (150) μm i.d.) packed with 5 μm particles was supplied from Unimicro Technologies Inc. (Pleasanon, CA, USA). Potassium dihydrogen phosphate, phosphoric acid, potassium hydroxide, sodium chloride and toluene were all of analytical grade (Tianjin Chemical Company, Tianjin, China). Acetonitrile was chromatographic grade (Xinke Chemical Inc., Hebei, China). Double deionized water was used. Samples of indapamide, methadone, celiprolol, atenolol, timolol and isoprenaline were obtained from Tianjin Institute of Drug Control (Tianjin, China).

Procedures

Drugs were first dissolved in water to obtain a solution containing 1 mg/mL each drug, then the resulting solutions were further diluted with mobile phase to give the drug an approximate concentration of 0.1 mg/mL. All these solutions were filtered with 0.22 μm micro filter. Mobile phase solutions were first prepared by adjusting the potassium dihydrogen phosphate buffer to the desired pH value and then mixing with the appropriate amount of acetonitrile and sodium chloride buffer. Mobile phase solution was degassed in an ultrasonic bath for 10 min before it was used. A negative or positive voltage was added on the column outlet and the column inlet was grounded. Pressure was applied to the column inlet during the separation. Total flow rate of the two pumps was 30 μL/min. The wavelength of the UV-vis detector was set at 214 nm. The injector has an internal loop of 20 nL.

Results and discussion

Basic drug separation by CEC *and micro*-HPLC

In this paper, separation of six basic drugs by PCEC was compared with that by micro-HPLC, as shown in Fig. 1. In this PCEC separation system, the directions of pressure flow, EOF and electrophoretic mobility of solute were the same, since the solute flowed out faster than by micro-HPLC in the same experiment condition. A good resolution can be obtained in shorter time by PCEC than by micro-HPLC.

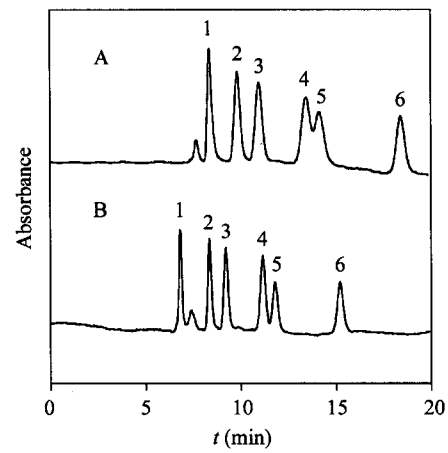

Fig. 1 Electrochromatograms showing the comparison of micro-HPLC (A) and PCEC (B) for the separation of six basic drugs. Column: EP-100-25-5-SCX; mobile phase: 70% (*V*/*V*) acetonitrile in 5 mmol·L^{-1} KH_2PO_4 (pH 4.5); flow rate: 0.03 mL/min; voltage: (A) 0 kV, (B) 5 kV, pressure: 3.44 × 10^6 Pa; injection: 20 nL; detection wavelength: 214 nm; peaks: (1) indapamide, (2) methadone, (3) celiprolol, (4) atenolol, (5) timolol and (6) isoprenaline.

The effect of pressure on reduced plate height in PCEC and micro-HPLC was studied by changing back pressure regulators. It can be seen from Fig. 2 that the plate height was lower in PCEC than in micro-HPLC. The voltage causes a low plate height and a high efficiency in PCEC. It can be explained in theory that the contribution of electrophoretic mobility of solute from electrical field makes the values of eddy diffusion, axial molecular diffusion and resistance to mass transfer in PCEC smaller than in HPLC.[7] It is also shown in Fig. 2 that the plate height in PCEC decreased with the increase of pressure from 1.72 × 10^6 Pa to 3.44 × 10^6 Pa, while increased with the increase of pressure from 3.44 × 10^6 Pa to 1.72 × 10^7 Pa. It implied the relationship between plate height and linear velocity of flow on column, as similar with those reported.[12]

Effect of voltage on separation

To further study the influence of voltage on separation, three basic drugs and a neutral compound were separated by PCEC using normal and reversal applied voltage modes. The results are shown in Fig. 3. It can be seen from Fig. 3 that the elution time and the elution orders of solutes were both altered by tuning the applied voltage, because the positively charged atenolol eluted more rapidly than neutral thiourea with increase of electrical field. The retention behavior of thiourea in Fig. 3 demonstrates that the interaction of solute and SCX stationary phase is hydrophilic interaction rather than hydrophobic interaction. The influence of electric field on column was also investigated as shown in Fig. 4.

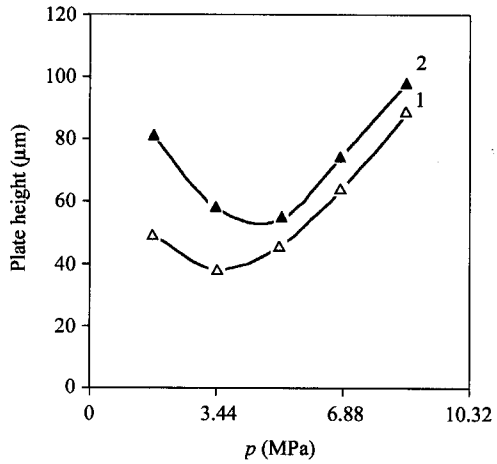

Fig. 2 Effect of pressure on plate height. Column: EP-100-25-5-SCX; mobile phase: 70% ($V:V$) acetonitrile in 5 mmol·L^{-1} KH_2PO_4 buffer (pH 4.5); flow rate: 0.03 mL/min; voltage: (1) 5 kV, (2) 0 kV; detection wavelength: 214 nm; sample: isoprenaline.

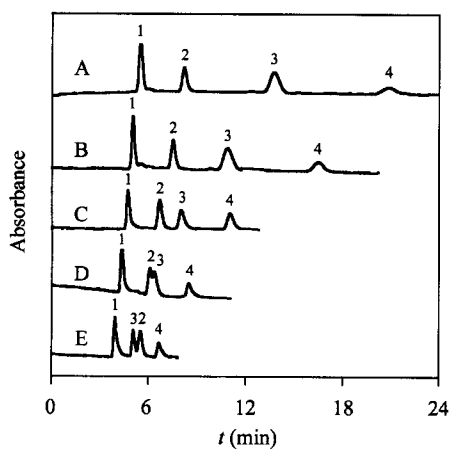

Fig. 3 Effect of voltage on retention. Column: EP-150-25-5-SCX; mobile phase: 70% ($V:V$) acetonitrile in 5 mmol·L^{-1} KH_2PO_4 buffer (pH 4.5); flow rate: 0.03 mL/min; pressure: 3.44×10^6 Pa; voltage: (A) −5 kV, (B) 0 kV, (C) 5 kV, (D) 10 kV, (E) 15 kV; detection wavelength: 214 nm; peaks: (1) indapamide, (2) thiourea, (3) atenolol, (4) isoprenaline.

Effect of organic modifier, ionic strength and pH *value on separation by* PCEC

To investigate further the retention mechanism of basic drugs on this stationary phase in PCEC, the mobile phases containing different acetonitrile concentration, ionic strength and pH were tried on SCX column in PCEC. The capacity factor of PCEC k^* was defined as the following equation.[8,9]

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

where t_r and t_0 are the retention time for the retained solute and unretained neutral solute (toluene), respectively.

Fig. 5 shows the relationship between k^* and concentration of acetonitrile. The k^* of atenolol increased rapidly

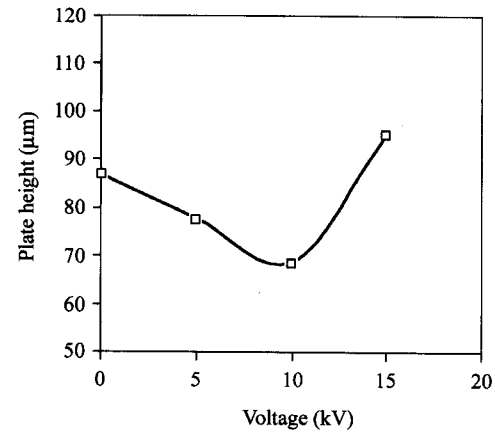

Fig. 4 Effect of voltage on plate height. Column: EP-100-25-5-SCX; mobile phase: 70% ($V:V$) acetonitrile in 5 mmol·L^{-1} KH_2PO_4 buffer (pH 4.5); flow rate: 0.03 mL/min; pressure: 5.16×10^6 Pa; voltage: 5 kV; detection wavelength: 214 nm; sample: celiprolol.

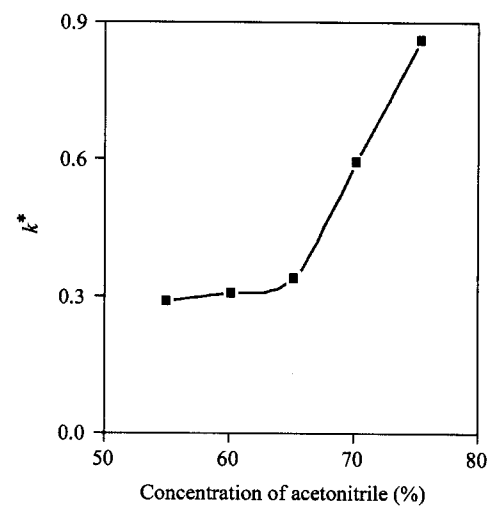

Fig. 5 Effect of concentration of acetontrile on retention. Column: EP-100-25-5-SCX; mobile phase: varied concentration of acetonitrile in 5 mmol·L^{-1} KH_2PO_4 buffer (pH 3.5); flow rate: 0.03 mL/min; pressure: 3.44×10^6 Pa; voltage: 5 kV; detection wavelength: 214 nm; sample: atenolol.

with the increase of acetontrile concentration in PCEC. It illustrates that hydrophilic interaction between solutes and the SCX stationary phase was very strong in the experimental condition. This separation mechanism is similar to the peptide separation in SCX-HPLC.[21,22] It also implied that retention behavior of solute was based strongly on the chromatographic mechanism. Effect of ionic strength was studied over the range 0—100 mmol·L^{-1} NaCl in the mobile phase consisting of 70% acetonitrile and 5 mmol·L^{-1} KH_2PO_4 (pH 4.5) in PCEC. k^* of atenolol decreased obviously with increase of ionic strength as shown in Fig. 6. It illustrates that ion-exchange interaction still influenced strongly the retention behavior of drug in the experiment.

Effect of pH on retention in PCEC was studied by using phosphate buffer mobile phase under different pH from 2.5 to 6.5, and it was found that the migration time of drugs

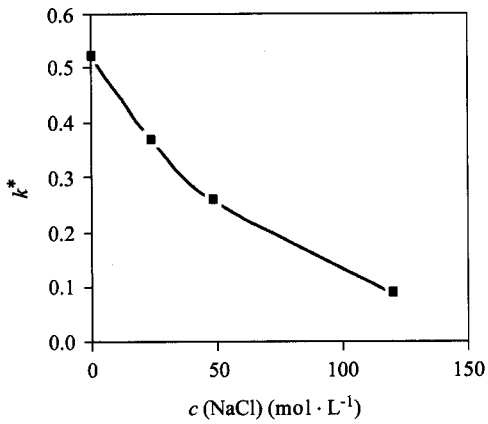

Fig. 6 Effect of ionic strength on PCEC separation. Column: EP-100-25-5-SCX; mobile phase: varied concentration of NaCl in 5 mmol·L^{-1} KH_2PO_4 buffer (pH 4.5) consisting of 70% acetonitrile; flow rate: 0.03 mL/min; Pressure: 3.44×10^6 Pa; voltage: 5 kV; detection wavelength: 214 nm; sample: atenolol.

increased with the increase of pH value from 2.5 to 3.5; while decreased from 3.5 to 6.5. As above discussed, the interaction between the solutes and stationary phase is the important factor for separation of solutes. Ionizations of drugs decreased with the increase of pH from 3.5 to 6.5, so the abilities of ion-exchange between solutes and stationary phase decreased and the migration time of solutes decreased. While ionizations of sulphonic groups on stationary phase was practically suppressed at low pH and increased with the increase of pH value from 2.5 to 3.5, and it led to that the ion exchange interaction between drugs and stationary phase also increased with the increase of pH, so the migration time of solutes increased.

Conclusion

Baseline separation of six basic drugs was obtained by PCEC and micro-HPLC with SCX stationary phase. It is better and faster by using PCEC than that by using micro-HPLC. The separation mechanism was investigated by studying the effects of organic modifier, ionic strength and pH value on separation. The separation results show that the separation mechanism is a complex mixed separation mode and the migration time of solutes is based on hydrophilic and ionic interaction in chromatographic aspect, total electromigration of solute in different ionic state in electrophoretic aspect and ionizations of solutes in the mobile phase. Voltage can tune the elution orders of solutes and cause a low reduced plate height.

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