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Article

Selectivity Tuning in Pressurized Capillary Electrochromatography with a Zwitterionic Monolithic Column

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A polymethacrylate-based capillary monolithic column with zwitterionic functional groups was prepared by an *in situ* copolymerization method. The column was used in pressurized capillary electrochromatography (pCEC) for the separation of a test mixture of basic, neutral and acidic analytes. Unique manipulation of selectivity in pCEC was demonstrated by fine-tuning the applied voltage. The separation mechanism for neutral compounds was primarily attributed to hydrophilic interaction, whereas the separation mechanism for charged compounds was attributed to electrophoresis. Due to the multiple retention mechanisms, the pCEC system using a zwitterionic monolithic column showed better separation of the mixture consisting of neutral and charged molecules than capillary liquid chromatography with an identical column.

Introduction

In capillary electrochromatography (CEC), formation of bubbles in the packed capillary may lead to electric current disruption and column failure. This may be overcome by combining hydraulic pressure with electroosmotic flow (EOF) as the driving force, resulting in pressurized capillary electrochromatography (pCEC). pCEC has the advantages of high efficiency, high resolution, high selectivity and fast speed, which allow it to separate complex samples. Due to its nano-scale feature, pCEC consumes 10,000 times less solvent and sample than the most frequently used high-performance liquid chromatography (HPLC); it is also economically attractive and environmentally friendly. In pCEC, the retention mechanism for neutral compounds is essentially based on chromatographic partition. However, for charged compounds, both chromatographic partition and electrophoretic mobility contribute to the separation mechanism. This dual mechanism of pCEC makes it suitable for both neutral and charged compounds and dramatically enhances the separation selectivity compared to standalone HPLC, capillary electrophoresis (CE) or CEC (1).

Using a relatively hydrophilic monolithic column is another way to partially avoid bubble formation in CEC (2, 3). The fabrication of a zwitterionic monolithic column and its utilization in hydrophilic interaction chromatography (HILIC) and ion exchange chromatography have been reported (4, 5, 6, 7, 8). The column showed excellent performance in the separation of polar compounds. In this work, a sulfoalkylbetaine-based zwitterionic monolith was used in pCEC for the separation of a test mixture consisting of neutral and charged components. The tuning of the selectivity of the separation in pCEC was clearly demonstrated by simply adjusting the applied voltage. The retention mechanism of neutral and charged compounds in pCEC with the zwitterionic column was investigated and discussed.

Materials and Methods

Materials

Pentaerythritol triacrylate (PETA) and *N*, *N*-dimethyl-N-methacryloxyethyl N-(3-sulfopropyl) ammonium betaine (SPE) were purchased from Acros (Fair Lawn, NJ). Vinylsulfonic acid (VS) sodium salt (25 wt% aqueous solution) was purchased from Aldrich (Milwaukee, WI). Azobisisobutyronitrile (AIBN) was obtained from the Forth Chemical Reagent Plant (Shanghai, China). Cyclohexanol and ethylene glycol (EG) were purchased from Tianjin Chemical Plant (Tianjin, China). HPLC-grade acetonitrile (ACN) was purchased from TEDIA (Fairfield, OH). The water used throughout the experiment was Wahaha purified water (Hangzhou China). Toluene, thiourea, benzoic acid, acetanilide, hydroquinone, phenol, ammonium formate, catechol, resorcinol, *N*, *N*-methylene-bis-(acrylamide), formic acid and ammonia solution were purchased from Sinopharm Chemical Reagent Plant (Shanghai China). *p*-Toluidine was purchased from Shanghai Jinshanting Chemical Reagent Plant (Shanghai, China). Uracil, thymine and theophylline anhydrous were purchased from Sigma (St. Louis, MO). Nicotinamide was purchased from Baitai Chemical Reagent Plant (Shanghai, China).

Instrumentation

The experiments were performed on a TriSep-2010 system (Unimicro Technologies, Pleasanton, CA), on which capillary liquid chromatography (cLC) and pCEC can be performed independently with the same capillary column (6, 9). A positive or negative voltage was applied to the outlet of the column, and the inlet of the column was grounded. A sample loop of 1 μL was used for all experiments, with a splitting ratio of 450:1. The pCEC system was equipped with a binary solvent delivery module, a variable wavelength ultraviolet-visible (UV-Vis) detector, a ± 30 kV high voltage power supply and a chromatography work station for data acquisition and analysis.

Preparation of monolithic columns

The sulfoalkylbetaine-based zwitterionic monolithic column was prepared by *in situ* copolymerization according to the reported previously procedure (10). Briefly, the monomers

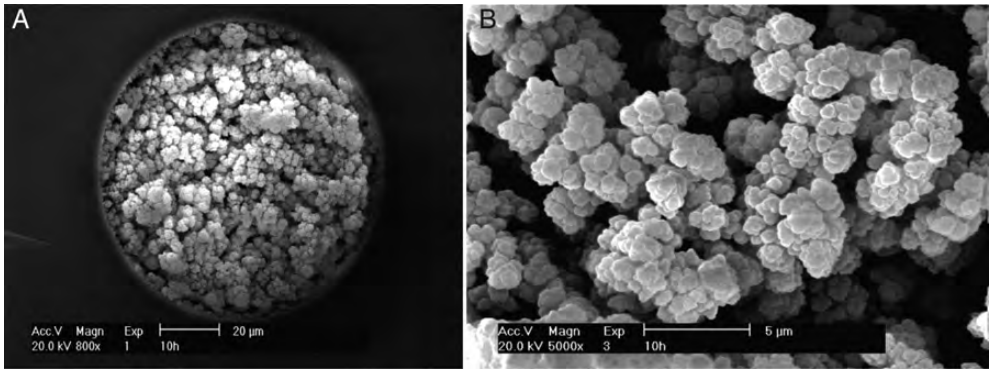

Figure 1. Scanning electron micrographs of the monolithic columns.

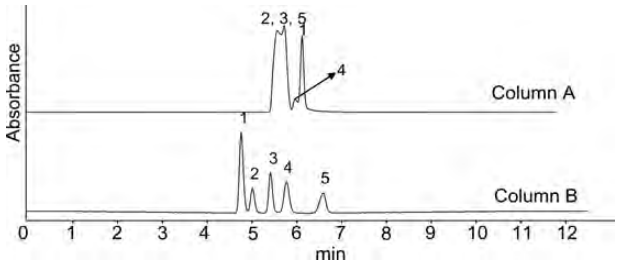

Figure 2. Chromatograms of five standard solutes on Column A (C18 column) and Column B (monolithic column). Conditions were as described in the following. Column A: 300 mm (total length 550 mm) × 100 μm i.d., packed with 3 μm C18; mobile phase, ACN–H_2O (93/7, v/v) containing 50 mmol/L ammonium formate; pump flow rate, 0.05 mL/min; backpressure, 6.2 MPa; detection, UV at 254 nm. Column B: monolithic poly(SPE-co-PETA), 250 mm (total length 500 mm) × 100 μm i.d.; mobile phase, ACN–H_2O (93/7, v/v) containing 50 mmol/L ammonium formate; pump flow rate, 0.05 mL/min; backpressure, 1.4 MPa; detection, UV at 254 nm. Samples: propyl 4-hydroxybenzoate (1), *N*, *N*′-methylenebisacrylamide (2), theophylline (3), nicotinamide (4), hydroquinone (5).

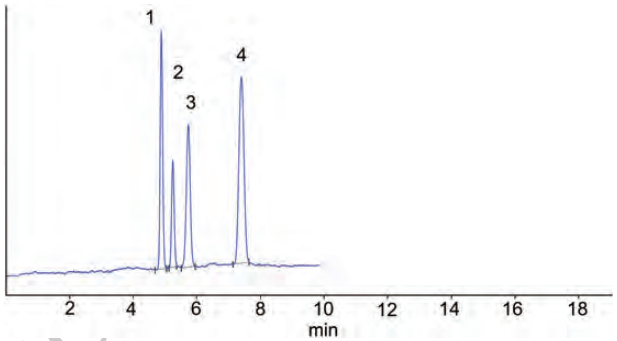

Figure 3. Chromatogram in cLC with the monolithic column. Conditions were as described in the following. Column: monolithic poly(SPE-co-PETA), 250 mm (total length 500 mm) × 100 μm i.d.; mobile phase, ACN–H_2O (93/7, v/v) containing 50 mmol/L ammonium formate; pump flow rate, 0.05 mL/min; backpressure, 1.5 MPa; detection, UV at 214 nm. Samples: toluene (1), paracresol (2), paranitrophenol (3), hydroquinone (4).

(PETA–SPE, 50/50 w/w, and VS, 0.6% w/w), the polymerization initiator (AIBN, 1 wt% with respect to the monomers), and porogens (cyclohexanol–EG, 50/50, w/w) were mixed ultrasonically for 15 min. The polymerization mixture was then bubbled with nitrogen for 10 min and introduced into the pretreated capillary. The capillary was plugged at both ends and submerged into a thermostatic bath at 60°C for 20 h. After washing with methanol for approximately 2 h to remove the porogens and unreacted monomers, a 1–2 mm detection window was created at the end of the polymer bed using a thermal wire stripper. A scanning electron micrograph, as shown in Figure 1A, reviewed a uniform PETA–SPE monolithic matrix with large through-pores within the capillary. Figure 1B shows the copolymerized monolith composed of somewhat spherical units agglomerated into larger clusters interspersed with large-pore channels, which are characteristic of monolithic structures.

Results and Discussion

Comparison of zwitterionic monolithic column and C18 packed column in cLC

To study the selectivity of the columns, a standard mixture was separated on a packed C18 column and a zwitterionic monolithic column, respectively. These polar solutes were eluted out of the C18 column near the dead time (the retention time of thiourea was 5.5 min) and could not be satisfactorily separated. In contrast, baseline separation was achieved on the monolithic column under the same experimental conditions (Figure 2).

Retention mechanism of the monolithic column

Toluene, paracresol, paranitrophenol and hydroquinone were used to investigate the retention mechanism of the monolithic column. As shown in Figure 3, baseline separation was obtained and the retention order was in accordance with the decrease of the polarity of the solute, which indicates typically hydrophilic interaction separation mechanisms.

Comparison of cLC and pCEC

Both cLC and pCEC experiments can be conducted on the same column with the same instrument, and the performances can be compared. In the cLC mode (that is, pCEC without voltage), Solutes 3 and 4 could not be well separated, as shown in Figure 4A, whereas in the pCEC mode (that is, cLC with applied voltage) the seven compounds were baseline separated, as shown in Figure 4B. The negative voltage, which was applied

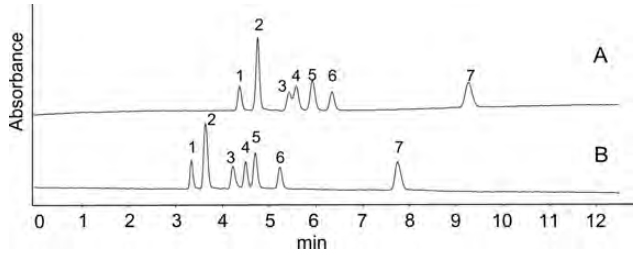

Figure 4. Separation of seven solutes: in cLC (A); in pCEC (B). Conditions were as described in the following. Column: monolithic poly(SPE-co-PETA), 250 mm (total length 500 mm) × 100 μm i.d.; mobile phase, ACN–H_2O (93/7, v/v) containing 5 mmol/L ammonium formate (pH 3.0 adjusted by formic acid); pump flow rate, 0.05 mL/min; backpressure, 1.5 MPa; detection, UV at 214 nm; applied voltage in pCEC, −10 kV (to the outlet). Samples: toluene (1), phenol (2), catechol (3), thymine (4), resorcinol (5), uacil (6), tiourea (7).

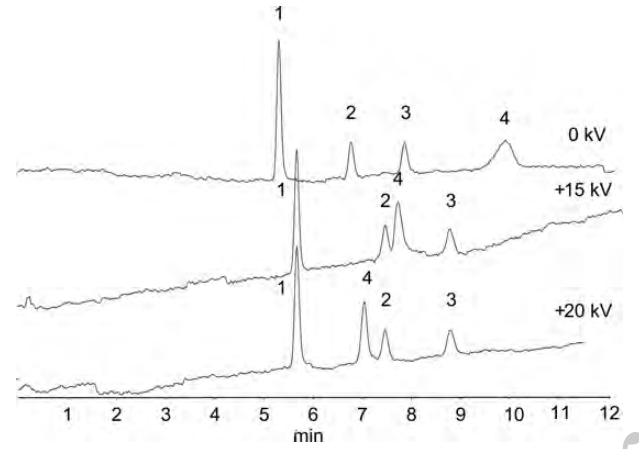

Figure 5. Chromatogram of neutral and negative charged compounds in pCEC. Conditions were as described in the following. Column: monolithic poly (SPE-co-PETA), 250 mm (total length 500 mm) × 100 μm i.d.; mobile phase, ACN–H_2O (92/8, v/v) containing 5 mmol/L ammonium formate (adjusted by ammonia solution, overall pH = 9); pump flow rate, 0.05 mL/min; backpressure, 1.5 MPa; detection, UV at 254 nm; applied voltage in pCEC, 0 kV to +20 kV (to the outlet). Samples: *p*-toluidine (1), thymine (2), uracil (3), benzoic acid (4).

to the outlet of the column, generated an EOF that was superimposed on the pressurized flow. The EOF not only improved the separation resolution, but also increased the speed.

Fine tuning of selectivity of neutral and charge compounds in pCEC

Figure 5 shows the chromatograms of *p*-toluidine, thymine, uracil and benzoic acid under various voltages. Benzoic acid with a pKa of 4.2 was negatively charged at pH 9, while the other three basic compounds, *p*-toluidine, thymine and uracil, with pKa of 8.7, 9.8 and 9.5, respectively, were neutral. The electrical field was reversed in this case and the outlet of the column was connected to the positive electrode and the inlet was still grounded. The direction of EOF was opposite to the pressurized flow. As shown in Figure 5, when the applied voltage was changed from 0 or +15 to +20 kV, the retention times of 4-toluidine, thymine and uracil were delayed slightly due to the EOF against the pressurized flow. However, there was a dramatic shortening of the retention time of the negatively charged benzoic acid because of the contribution of the electrophoretic mobility toward the outlet of the column under these experimental conditions. As shown in Figure 5, the selectivity of the mixture in pCEC can be tuned by simply adjusting the voltages/pressure ratio (11), which is a unique feature of pCEC.

Acknowledgments

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