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Preparation and evaluation of a neutral methacrylate-based monolithic column for hydrophilic interaction stationary phase by pressurized capillary electrochromatography

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ABSTRACT

A polar and neutral polymethacrylate-based monolithic column was evaluated as a hydrophilic interaction capillary electrochromatography (HI-CEC) stationary phase with small polar–neutral or charged solutes. The polar sites on the surface of the monolithic solid phase responsible for hydrophilic interactions were provided from the hydroxy and ester groups on the surface of the monolithic stationary phase. These polar functionalities also attract ions from the mobile phase and impart the monolithic solid phase with a given zeta potential to generate electro-osmotic flow (EOF). The monolith was prepared by *in situ* copolymerization of a neutral monomer 2-hydroxyethyl methacrylate (HEMA) and a polar cross-linker with hydroxy group, pentaerythritol triacrylate (PETA), in the presence of a binary porogenic solvent consisting cyclohexanol and dodecanol. A typical HI-CEC mechanism was observed on the neutral polar stationary phase for both neutral and charged analytes. The composition of the polymerization mixture was systematically altered and optimized by altering the amount of HEMA in the polymerization solution as well as the composition of the porogenic solvent. The monoliths were tested in the pCEC mode. The resulting monoliths had different characteristics of hydrophilicity, column permeability, and efficiency. The effects of pH, salt concentration, and organic solvent content on the EOF velocity and the separation of nucleic acids and nucleosides on the optimized monolithic column were investigated. The optimized monolithic column resulted in good separation and with greater than 140,000 theoretical plates/m for pCEC.

1. Introduction

Recently, because of their potential advantages over packed columns and open tubular (OT) columns, capillary columns with monolithic stationary phases have gained increasing attention in capillary electrochromatography (CEC). These advantages include easy control of permeability and surface charge, the fact that they are fritless, and have greater phase ratios than OT columns [1–3]. In CEC, the mobile phase was driven by electroosmotic flow (EOF), and it is believed that EOF is necessary for separation of analytes. EOF is promoted by incorporation of ionizable functional groups, such as acrylic acid, sulfonic acid, or ammonium monomers for porous polymeric monolithic columns and the ionization of residual silanol groups for silica sol–gel monoliths or other inorganic continuous beds. In fact, the neutral monoliths, which are devoid of ionizable functional groups on the surface of stationary phases, would also generate a significant EOF. The inherent property of this type of monolith yielded a stationary phase essentially free of strong electrostatic attraction toward charged solutes but with the ability of generating a significant EOF for separation of analytes [4]. Previously, Okanda and El Rassi [5] reported a neutral, nonpolar monolith for CEC produced by co-polymerising pentaerythritol diacrylate mono-stearate (PEDAS). Despite the fact that the monolith carried no electric charge, it in fact had an EOF due to the adsorption of ions from the mobile phase electrolyte [4,5]. No peak tailing and greater efficiencies for separation of the analytes were observed in the relatively strong hydrophobic monoliths and the separation mechanism were mainly based on hydrophobic interaction between the solutes and hydrophobic stationary phase in a reverse-phase (RP) mode.

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Table 1
Composition of the polymerization solutions used in the preparation of the different monolithic columns.

Column designation[a]	Monomers (wt%)		Dodecanol[b] (wt%)	Permeability, K ($\times 10^{-14}$ m^2)
	HEMA	PETA		
A	90	10	50.0	1.08
B	90	10	59.9	0.82
C	90	10	70.0	0.74
D	90	10	80.0	0.55
E	95	5	70.0	0.69
F	80	20	70.0	0.99
G	70	30	70.0	1.19

[a] Monomer-to-solvent ratio in the polymerization solutions was 40:60.
[b] Percentage of dodecanol in the porogenic solvent.

The separation of more polar compounds is still difficult in RP mode because highly or totally aqueous mobile phases combined with a non-wetted nonpolar stationary phase are often required in order to achieve sufficient retention, which can lead to a number of issues such as bubble formation in CEC and interruption of the separation process and even the stationary phase collapse [6]. An alternative to RP sorbents is the use of polar stationary phases, which exhibit a different retention mechanism. Recently, hydrophilic interaction CEC (HI-CEC) based on polar stationary phases has attracted increasing attention [3,7]. HI-CEC is expected to be a more effective approach than RP-CEC for the separation of polar and charged analytes. Normally, HI-CEC is run using polar stationary phases in a greater-organic and lesser-aqueous mobile phase to achieve retention of very polar compounds that could not be retained using RP methods [3,8]. However, few studies have investigated the application of polymer-based monolithic columns as the polar stationary phases in HI-CEC mode [8–14]. The lack of information on the use of polymer-based monoliths in HI-CEC is likely due to several factors. First, different from the hydrophilicity of silica gel stationary phase, the polymer skeleton bone of polymer-based monolith is hydrophobic. Second, the limited solubility of very polar monomers in most commonly used solvent mixtures [15]. Recently, our group prepared polar monoliths with mixed modes of HI and electrostatic interaction for the separation of polar and charged species [8,9]. To promote the development of HI-CEC, further effort was needed to research the separation mechanism of polar analytes on polymer-based monolithic column with HI-CEC mode and develop polar stationary phases with novel properties for efficient separation of polar compounds.

The study on which we report here involved the preparation and characterization of a novel polar and neutral monolithic column with a relatively strong EOF for the HI-CEC of polar and charged species. The monolithic column was prepared by *in situ* copolymerization of a neutral monomer HEMA and a polar cross-linker with hydroxy group, pentaerythritol triacrylate (PETA), in the presence of a binary porogenic solvent consisting cyclohexanol and dodecanol. This polar stationary phase combines the advantages of the neutral monoliths in that: (i) it could generate EOF despite the fact that the monolith carried no electric charge and (ii) the neutral monolith devoid of fixed charges on the surface was one of alternative solutions to separate the polar and basic compounds. The resultant monolithic column was evaluated as a HI-CEC stationary phase for pressurized CEC (pCEC). pCEC is a powerful separation system in which a mobile phase is driven by both a pressurized flow and an EOF, and has been proved to be reliable and repeatable in the analysis of various compounds [16,17]. The ratio of PETA to HEMA and the composition of the pore-forming solvent was systematically altered and optimized to obtain satisfactory hydrophilicity and column efficiency. The properties of the resultant monolithic column were investigated by using it to separate several neutral and charged analytes in HI mode.

2. Experimental

2.1. Reagents and materials

HEMA, PETA, EDMA, and 3-trimethoxysilyl propyl methacrylate (γ-MAPS) were purchased from Acros (NJ, USA). Azobisisobutyronitrile (AIBN) was obtained from the Forth Chemical Reagent Plant (Shanghai, China). Cyclohexanol and dodecanol were purchased from Tianjin Chemical Plant (Tianjin, China). HPLC-grade methanol and acetonitrile (ACN) were purchased from Chemical Reagent Corporation (Shanghai, China). The water used throughout all experiments was purified with a Millipore Milli-Q purification system (Milford, MA, USA). Uracil, uridine, adenine, adenosine, cytosine, cytidine, guanine, and guanosine were purchased from Sigma (St. Louis, MO, USA). Phosphoric acid, phenol, hydroquinone, resorcinol, catechol, pyrogallol, phloroglucinol, toluene, thiourea, formamide, acetamide, *N*,*N*-dimethylformamide (DMF), *N*,*N*′-dimethylenebisacrylamide and trimethylamine (TEA) (Chemical Reagent Plant, Shanghai, China) were of analytical grade. All test compounds were of analytical grade. The fused-silica capillaries (100-μm I.D.; 375-μm O.D.) were purchased from the Yongnian Optic Fiber Plant (Hebei, China).

2.2. Instrumentation

pCEC was conducted on a TrisepTM 2100GV CEC system (Unimicro Technologies, Pleasanton, CA, USA) which was comprised of a solvent gradient delivery module, a high-voltage power supply (+30 and −30 kV), a variable wavelength UV–vis detector, a microfluid manipulation module (including a six-port injector) and a data acquisition module. The detailed structure of the TrisepTM 2100GV CEC system has been presented previously [18]. Negative voltage was applied to the outlet of the column, and the inlet of the column was connected to the split valve and grounded. A supplementary pressure (6.9 MPa) was applied to the column inlet and the flow rate of the pump was 0.050 mL/min during the separation. In these studies, the isocratic elution system was used. An HPLC pump was used to flush monolithic columns. Scanning electron microscopy (SEM) of the monolith was carried out on a XL30 E scanning electron microscope (Philips, Netherlands).

2.3. Single-step preparation of polymeric monolithic columns

Prior to use, the inner wall of a capillary was treated with γ-MAPS, according to the procedure reported previously [19]. The monomers (HEMA and PETA), the polymerization initiator (AIBN, 1 wt% with respect to the monomer), and porogens composed of cyclohexanol and dodecanol were mixed ultrasonically for 15 min to yield a homogeneous solution. The ratio of PETA to HEMA and the composition of the pore-forming solvent were optimized to prepare the monolith (Table 1). After sonication and bubbling with nitrogen for 10 min, the polymerization mixture was introduced into

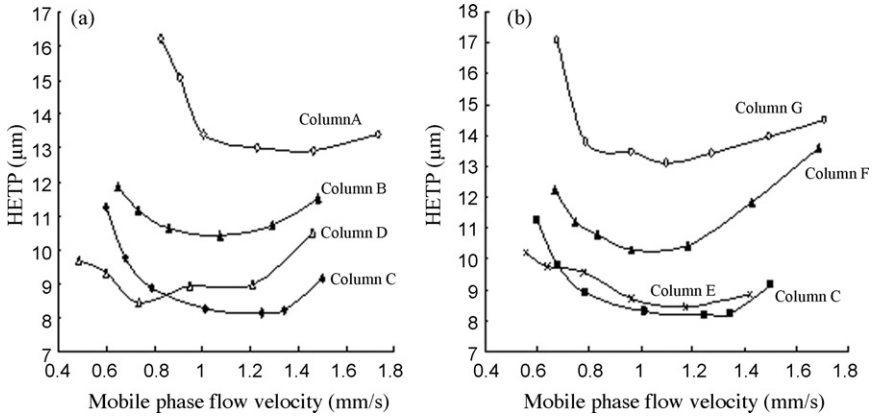

Fig. 1. Van Deemter plots showing average plate height as a function of apparent mobile-phase flow velocity for various monolithic columns prepared from polymerization solution at different (a) wt% of dodecanol and (b) wt% of HEMA. The plate height is the average taken for DMF, formamide and thiourea. Capillary column, 30 cm effective length, 50 cm total length × 100 μm I.D.; experimental conditions: mobile phase, 5 mmol/L TEAP buffer pH 7.5 in ACN/H_2O (90/10, v/v); running voltage from −3 to −25 kV; pump flow rate: 0.05 mL/min; supplement pressure: 6.9 MPa; detection wavelength: 214 nm.

the pretreated capillary. The capillary was plugged at both ends with GC septa and submerged into a thermostatic bath at 60 °C for 20 h. The resultant monolithic capillary column was washed with methanol for about 2 h using an HPLC pump to remove porogens and unreacted monomers. A 1–2-mm detection window was created immediately after the end of the polymer bed using a thermal wire stripper. Finally, the column was cut to a total length of 50 cm with an effective length of 30 cm. A 2-cm length of the capillary containing the monolith inside was cut for SEM analysis.

2.4. Electrochromatography procedures

The stock solution of TEA phosphate buffer (TEAP, 0.5 mol/L in phosphate), was prepared by adding TEA to a concentrated solution of phosphoric acid until the desired pH was obtained, followed by an appropriate dilution. Mobile phases were prepared by mixing appropriate volumes of ACN, stock buffer solution and water. Electrolyte solutions were filtered through 0.22 μm membrane. Mobile phase solutions were degassed in an ultrasonic bath for 20 min before use. Toluene was used as the unretained neutral compound [20]. Before pCEC experiments, the monolithic column was conditioned on the instrument with the mobile phase for 1 h, applied voltage was firstly ramped from 0 to 20 kV and then operated at 20 kV. The column was equilibrated for about 30 min after the mobile phase was changed and the temperature of the column was kept at room temperature (25 °C).

3. Results and discussion

3.1. Column characterization

3.1.1. Optimization of polymerization mixture composition for pCEC

Different from silica gel stationary phases, the hydrophilicity of the polymer skeleton bone of methacrylate-based monolith is hydrophobic, so this type of monolith is mainly designed for RP-

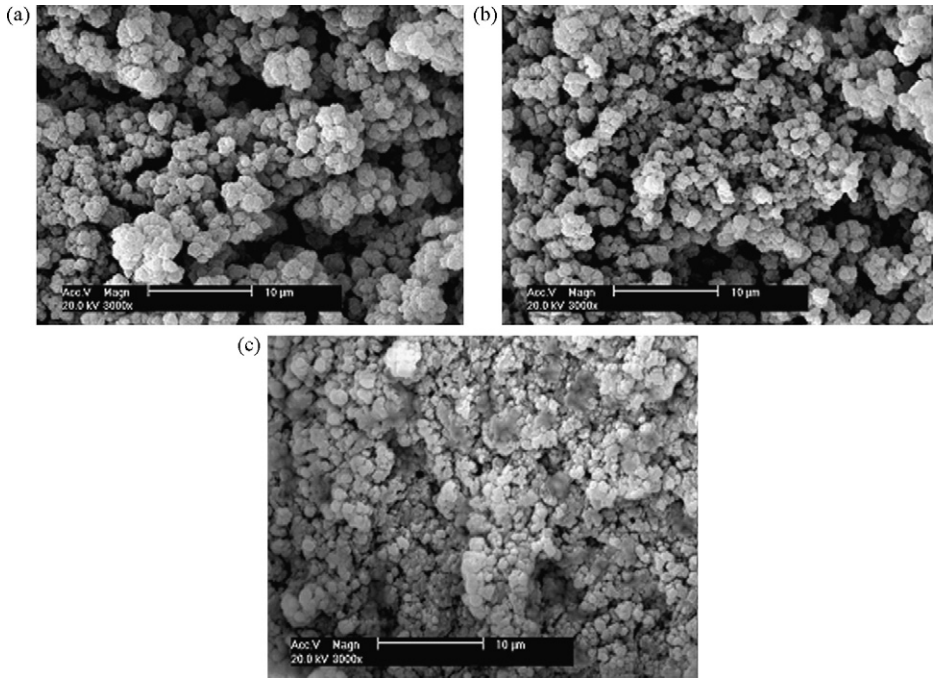

Fig. 2. Scanning electron microphotographs of monolithic columns prepared with different dodecanol weight fraction: (a) column B; (b) column C; (c) column D.

CEC [21,22]. In order to prepare a hydrophilic methacrylate-based monolith, the incorporation of polar monomers into the monoliths is necessary. The functional monovinyl and trivinyl monomers, HEMA and PETA, were used for this purpose. The preparation process of the monolithic columns was quite simple. However, a number of factors have to be considered and optimized. It is known that the properties of monolithic phases can be altered by minor changes to the composition of the polymerization mixture [23]. The plate height at various wt% dodecanol and HEMA measured as a function of mobile phase linear velocity by varying the applied voltage, combined with the permeability, K value [24] ($K = u\eta L/\Delta P$, where η is the viscosity of the mobile phase, L is the column length, ΔP is the back pressure and u is the linear velocity of mobile phase), and SEM analysis, were used to investigate the properties of the monoliths (Table 1 and Figs. 1 and 2).

To investigate the influence of the porogen composition on the preparation of poly(HEMA-co-PETA) monolith, the ratio of HEMA/PETA (w/w) was kept constant at 90/10, while the proportion of dodecanol was varied from 50.0% (column A) to 80.0% (column D). Plate height was determined on several monolithic columns containing various amount of the dodecanol (columns A–D) in monolithic stationary phases (Fig. 1a). The plate shown in Fig. 1 was the average taken for DMF, formamide and thiourea. A mixture of DMF, formamide, and thiourea was injected repetitively five times to calculate the average plate height. The RSD value of the average plate height was 3.0%. Plots for polymerization solutions keeping the weight content of the dodecanol at 70.0% but different amount of HEMA in monomer mixture (columns C, E–G) are also given (Fig. 1b). Increasing the wt% of dodecanol resulted in a lower plate height except at values greater than 80.0% (column D) (Fig. 1a). Increasing the wt% of dodecanol decreased the column permeability (Table 1). This result indicated that the average pore size became smaller with increasing wt% of dodecanol, which is in good agreement with earlier findings [25]. Examination by SEM also showed that the pore size became smaller with the increase of dodecanol content (Fig. 2). When the percentage of dodecanol was increased from 50% to 80%, the permeability decreased from 1.08×10^{-14} m^2 to 0.55×10^{-14} m^2 (Table 1). At the same time, when the percentage of dodecanol was increased from 50% to 80%, a lower plate height was measured except at 80.0% or greater (column D) (Fig. 1a). Therefore, considering both peak efficiency and column permeability, a dodecanol proportion of 70.0% was considered to be optimal and thus used in further experiments.

It was also found that the column characteristics changed when the ratio of HEMA to PETA (w/w) in the monomer mixture changed. Column porosity was sensitive to the ratio of HEMA/PETA (w/w) in the monomer mixture. Increasing the wt% of HEMA resulted in greater plate height except when the HEMA weight fraction was 95% or greater (column E) (Fig. 1b). At constant wt% dodecanol (70.0%) increasing the wt% of HEMA resulted in less column permeability (Table 1), which is likely due to the lesser average pore size associated with greater wt% of HEMA. The pore size seen in SEM was less at the greater wt% of HEMA (figure not shown). HEMA concentration in the monomer mixture affected retention factors (k^*) of DMF, formamide and thiourea with retention factors increasing as the HEMA content increased from 70% to 95% (Fig. 3). This result indicated that increasing with the amount of HEMA included in the polymerization mixture, the hydrophilicity of the monolith also increased. Therefore, in order to compromise between hydrophilicity and peak efficiency, the monolithic column C was selected for all further experiments.

3.1.2. EOF

In pCEC, the mobile phase was driven by EOF as well as pressurized flow. The linear velocity of analytes in the mobile phase (u_m) was the sum of the electrophoretic mobility (u_{ep}), pressurized flow (u_p), and EOF (u_{eo}) and it could be expressed (Eqs. (1) and (2)) [26].

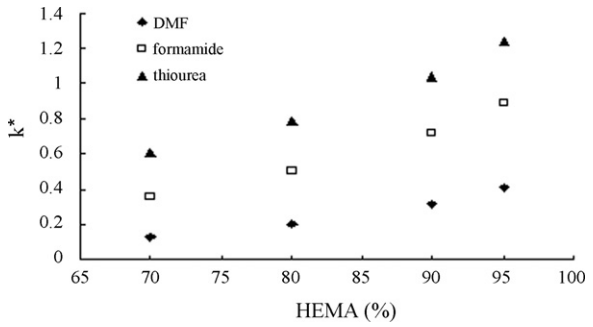

Fig. 3. Plots of retention factors (k^*) versus HEMA (wt%) in the monomer mixture. Experimental conditions: applied voltage: −20 kV; other conditions as in Fig. 1.

$$u_m = u_{ep} + u_p + u_{eo} \quad (1)$$

$$u_{eo} = u_m - u_{ep} \quad (2)$$

For neutral species, $u_{ep} = 0$. The electroosmotic mobility was measured with toluene as the unretained marker. The elution time of toluene with the application of the pressure only was measured to be t_{01}, and the elution time of toluene with the application of both the pressure and voltage was measured to be t_{02}. The EOF was calculated by use of the following equation:

$$u_{eo} = \frac{L}{t_{01}} - \frac{L}{t_{02}} \quad (3)$$

where L is the column effective length. The effect of the buffer pH on the EOF was investigated in this study by changing the pH from 3.5 to 9.5 with an applied voltage of 20 kV and a mobile phase of 5 mmol/L TEAP in ACN/H_2O (90/10, v/v). The EOF was found to increase slightly from 0.78 to 0.84 mm/s by increasing the pH of TEAP buffer from 3.5 to 9.5. The observed EOF can be attributed to that the polar functionalities on the surface of the stationary phase attract ions from the mobile phase and thus impart the monolith with a given zeta potential to generate the EOF. Similar behaviors were also observed with a PEDAS monolith [4,5]. The effect of ACN content on the EOF, u_p and u_m was also investigated by keeping the TEAP buffer concentration at 5 mmol/L and the pH at 8.5 (Fig. 4). The increase in EOF observed at greater content of ACN was thought to be caused by changes in the viscosity and the zeta potential [27]. As an increase of the organic content of the mobile phase, the electroosmotic mobility in proportion to the ratio of the dielectric constant to the viscosity was increased. That led to an increase of the EOF. An increase in u_p was also observed at greater content of ACN caused by the change of viscosity. By superimposing a supplementary u_p on an EOF in the electrochromatographic separation, pCEC could shorten the elution time and avoid bubble formation [28,29], which is the main problem encountered in the pure CEC systems.

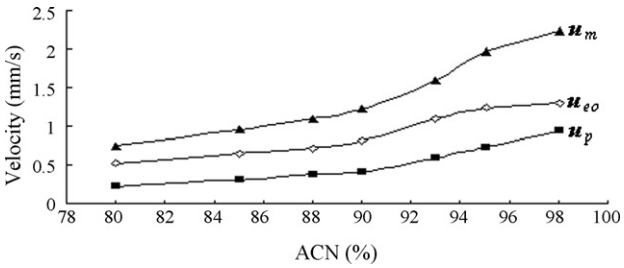

Fig. 4. Effect of ACN content on the EOF, u_p and u_m. Capillary column, column C; experimental conditions: mobile phase, 5 mmol/L TEAP buffer pH 8.5 in ACN/H_2O (90/10, v/v); applied voltage: −20 kV; other conditions as in Fig. 1.

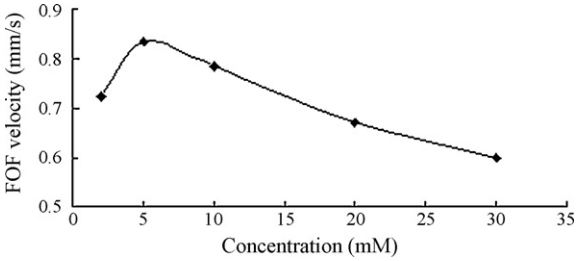

Fig. 5. Plots of the apparent EOF velocity versus the concentration of the TEAP buffer. Capillary column, column C; experimental conditions: mobile phase, TEAP buffer pH 7.5; other conditions as in Fig. 4.

The influence of TEAP buffer concentration in the mobile phase on the EOF was also investigated in the range of 2–30 mmol/L by keeping the ACN content at 90% (v/v) and the pH at 7.5. EOF velocity increased initially as the concentration increased from 2 to 5 mmol/L, and then decreased slightly when it further increased to 30 mmol/L (Fig. 5). This increase can be attributed to a hydrophilic partitioning process. A greater salt concentration would drive more solvated ions into the water-rich liquid layer and part of them might be immobilize on the monolithic stationary phase, and thus resulted in the increase of the zeta potential [15,30]. As the TEAP buffer concentration was increased to 30 mmol/L, the decreasing EOF can be attributed to that the water-enriched layer on the surface of more polar stationary phases could behave similarly to the classic electrical double-layer whose thickness decreased with increasing salt concentration in buffer and consequently the zeta potential was decreased.

While the preparation of neutral monoliths having strong EOF is a rather delicate task and can only be achieved with particular monoliths, the surfaces of which have the tendency to adsorb electrolyte ions from the mobile phase. These surface-adsorbed ions impart the zeta potential, thus supporting the EOF necessary for mass transport across the monolithic column [4,5]. In order to prepare polar and neutral monolith, the HEMA was introduced as a neutral polar monomer and PETA was used a polar cross-linker. To further illuminate the properties of the poly(HEMA-co-PETA) monolith, the EDMA was added to substitute PETA as the cross-linker in the monomer mixture while keeping the percentage of dodecanol in the porogenic solvent the same and the ratio of monomers to porogen constant. With the same amount of HEMA included in the monomer mixture, the poly(HEMA-co-PETA) and poly(HEMA-co-EDMA) monoliths exhibited similar hydrophilicity indicated by the retention values of the thiourea on these stationary phases. The EOF velocity on the poly(HEMA-co-PETA) monolith was nearly constant as the wt% cross-linker in the monomer mixture increases, while EOF velocity on the poly(HEMA-co-EDMA) monolith decreased as the amount of cross-linker increased (Table 2). Therefore, the character of the PETA cross-linker was an important development in the design of polar and neutral monolith with relatively strong EOF.

Table 2
Effects of percent cross-linker (wt%) in the monomer mixture on the apparent EOF velocity and retention factor.

Cross-linker	Monomers (wt%)		$k'_{thiourea}$	u_{eo} (mm/s)
	HEMA	Cross-linker		
PETA	90	10	1.04	0.82
	80	20	0.79	0.84
	70	30	0.61	0.81
EDMA	90	10	1.05	0.74
	80	20	0.74	0.63
	70	30	0.59	0.41

Capillary column, 30 cm effective length, 50 cm total length × 100 μm I.D.; experimental conditions: mobile phase, 5 mmol/L TEAP buffer pH 7.5 in ACN/H_2O (90/10, v/v); applied voltage: −20 kV; pump flow rate: 0.05 mL/min; supplement pressure: 6.9 MPa; detection wavelength: 214 nm.

3.1.3. Monolithic column reproducibility and stability

The reproducibility of column production, which was prepared from the identical polymerization mixture, was assessed through the percent relative standard deviation (RSD) using DMF, formamide, and thiourea as model solutes. Toluene was used as the unretained marker to measure the EOF. As it can be seen in Table 3, the observed RSD from column-to-column ($n = 9$) for column C were <2.2%, <3.1% and <5.1% for the EOF velocity, retention factors and separation efficiency in pCEC. Run-to-run ($n = 5$) and day-to-day ($n = 3$) repeatability were satisfactory with RSD values less than 1.8% for EOF, <2.2% for retention factors and <4.0% for column efficiency in the pCEC mode. These reproducibility data are in close agreement to those reported in the literature [31,32].

3.2. Characterization of the poly(HEMA-co-PETA) with polar solutes

3.2.1. Separation of phenols

The polar poly(HEMA-co-PETA) surface can provide a hydrophilic partitioning process. A series of phenols including phenol, hydroquinone, resorcinol, catechol, pyrogallol and phloroglucinol were separated on the neutral polar monolith (Fig. 6). As expected for a hydrophilic monolith, retention increase with an increase in the number of hydroxyl groups within the molecule. The elution order of the phenols using the hydrophilic stationary phase was as follows: less polar phenol with one hydroxyl group first, and then followed by more polar three positional isomers of hydroquinone, resorcinol and catechol with two phenolic hydroxyl groups, and finally the most polar positional isomers of pyrogallol and phloroglucinol with three phenolic hydroxyl groups.

3.2.2. Separation of amides

A mixture of toluene, DMF, formamide, and thiourea with different polarity were separated on the neutral polar monolith. This elution order (Fig. 7), which indicates the typical hydrophilic interaction separation mechanisms, is well in according to the polarity of the solute. A mixture of lesser molecular-mass amides including DMF, *N*,*N*′-dimethylenebisacrylamide, formamide and acetamide

Table 3
Reproducibility of EOF, retention factor, and efficiency on column C.

	EOF (%RSD)	Retention factors (%RSD)			Capillary efficiency (%RSD)		
		DMF	Formamide	Thiourea	DMF	Formamide	Thiourea
Column-to-column[a] ($n = 9$)	2.2	2.8	3.1	3.1	4.9	5.0	5.1
Run-to-run[b] ($n = 5$)	1.5	1.8	1.7	1.8	2.9	2.9	3.2
Day-to-day[b] ($n = 3$)	1.8	2.0	2.1	2.2	3.6	3.8	4.0

[a] The meaning of column-to-column ($n = 9$) was that the monolithic columns were prepared from three batches and every batch contained three columns.
[b] The %RSD of run-to-run and day-to-day were calculated by the same column.

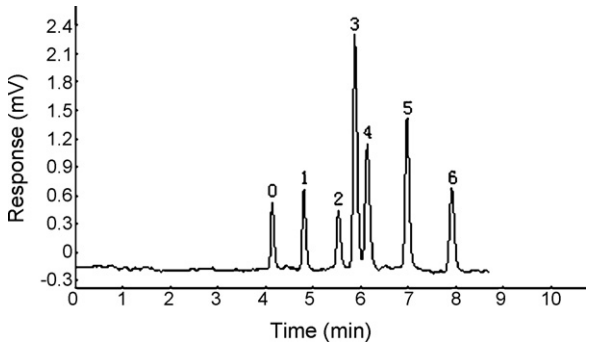

Fig. 6. Electrochromatogram of phenols. Capillary column, column C; experimental conditions: mobile phase, 5 mmol/L TEAP buffer; other conditions as in Fig. 5. Solutes: (0) toluene, (1) phenol, (2) catechol, (3) resorcinol, (4) hydroquinone, (5) pyrogallol, (6) phloroglucinol.

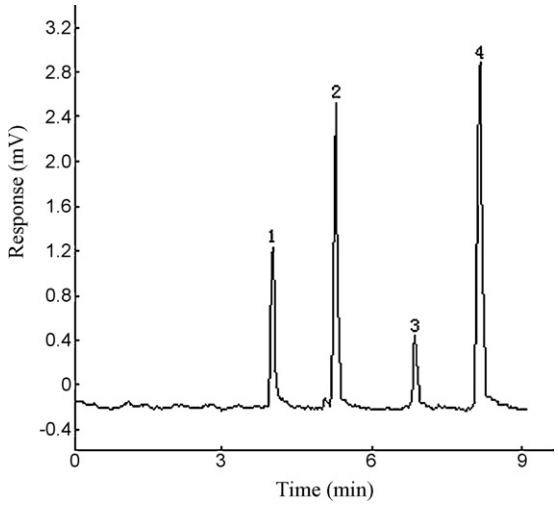

Fig. 7. Electrochromatogram of some model compounds. Capillary column, column C; experimental conditions as in Fig. 6. Solutes: (1) toluene, (2) DMF, (3) formamide, (4) thiourea.

was used to investigate closely related neutral compounds that are difficult to retain and separate by reversed-phase chromatography (Fig. 8).

3.2.3. Charged compounds

For this neutral poly(HEMA-co-PETA) monolith, no strong electrostatic interaction was involved in the separation of charged

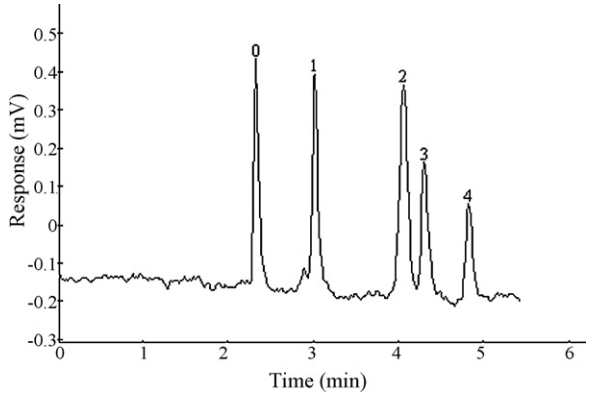

Fig. 8. Electrochromatogram of amides. Capillary column, column C; expermental conditions: mobile phase, TEAP buffer in ACN/H_2O (98/2, v/v); other conditions as in Fig. 6. Solutes: (0) toluene, (1) DMF, (2) *N,N′*-dimethylenebisacrylamide, (3) formamide, (4) acetamide.

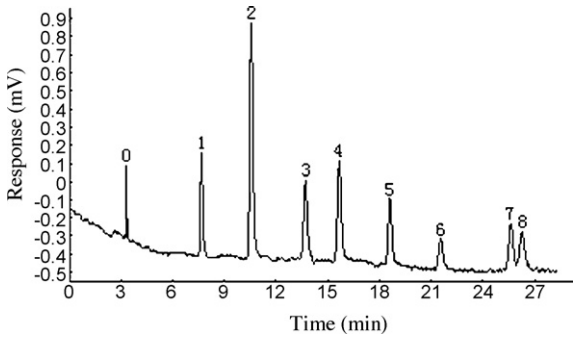

Fig. 9. Electrochromatogram of nucleic acid bases and nucleosides. Capillary column, column C; experimental conditions: mobile phase, TEAP buffer in ACN/H_2O (93/7, v/v); the detection wavelength was 214 nm until 5 min, and then was changed to 254 nm; other conditions as in Fig. 6. Solutes: (0) toluene, (1) uracil (p*Ka* = 9.20), (2) uridine (p*Ka* = 8.81), (3) adenosine (p*Ka* = 3.25), (4) adenine (p*Ka* = 2.95), (5) cytosine (p*Ka* = 4.60), (6) cytidine (p*Ka* = 4.47), (7) guanine (p*Ka* = 3.33), (8) guanosine (p*Ka* = 2.90) [33,34].

species, and thus the separation of polar charged analytes was mainly based on HI-CEC mechanism. In order to investigate the separation characteristics of this polar monolith in HI-CEC mode, more polar compounds, such as nucleic acid bases and nucleosides separated on the poly(HEMA-co-PETA) monolith, were used for further evaluation. Due to the multiplicity of their polar groups, the nucleic acid bases and nucleosides are important probes for evaluating the polarity of the stationary phases. For the strong polarity of the nucleic acid bases and nucleosides, it is especially difficult to separate them in the typical RP mode. Furthermore, the absence of fixed charges on the surface of the neutral poly(HEMA-co-PETA) monolith would allow the efficient separations of basic nucleic acid bases and nucleoside without peak tailing. Good separations on the poly(HEMA-co-PETA) monolith were obtained with optimized electrochromatographic conditions (Fig. 9).

The content of organic modifier in the mobile phase has great influence on the resolution and selectivity of polar compounds, and hydrophilic interactions are promoted by increasing the organic modifier content. There is linear dependence of the log k^* of four nucleic acid bases and four nucleosides on the ACN concentration in the mobile phase from 88% to 95% (Fig. 10). This result indicated that the separation of these solutes was governed by the hydrophilic interaction between the analytes and the monolithic stationary phase. HI mode separations commonly employ water and ACN as the mobile phase, but require a greater organic content in order to ensure significant hydrophilic interaction [15]. The

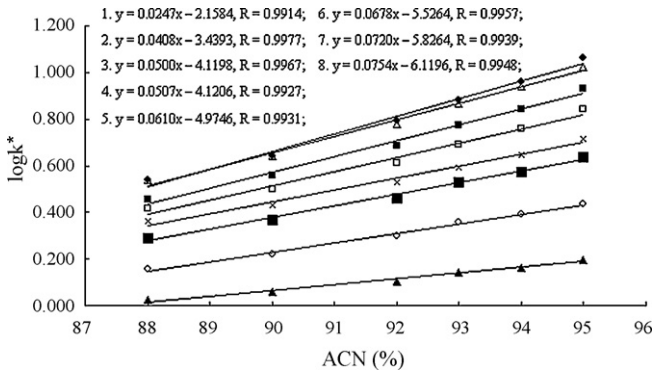

Fig. 10. Plots of log k^* for nucleic acid bases and nucleosides versus percent ACN (v/v) in the mobile phase. Capillary column, column C; experimental conditions: mobile phase: TEAP buffer in ACN/H_2O (v/v); other conditions as in Fig. 6. Lines: (1) uracil, (2) uridine, (3) adenosine, (4) adenine, (5) cytosine, (6) cytidine, (7) guanine, (8) guanosine.

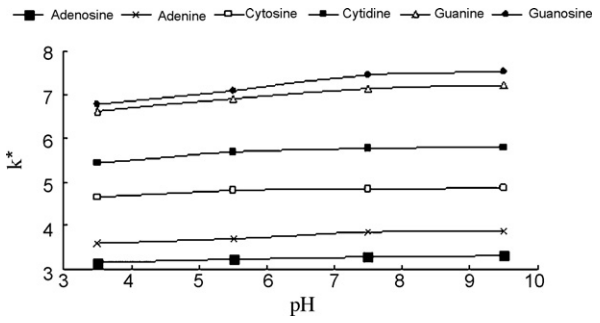

Fig. 11. Effect of pH on the retention factor (k^*). Capillary column, column C; experimental conditions: mobile phase: TEAP buffer in ACN/H_2O (93/7, v/v); other conditions as in Fig. 6.

hydrophilic interaction increases with increasing ACN content [30].

Mobile-phase pH was used to investigate the separation of the nucleic acid bases and nucleosides. In our experiments the pH of buffer solution was measured before mixing with the organic solvent. The effect of pH on HI-CEC separation on the poly(HEMA-co-PETA) monolith was investigated by varying the pH of TEAP buffer to 3.5, 5.5, 7.5 and 9.5. The values of k^* for uracil and uridine remained almost unchanged since there are no significant changes in positive charge, so no electrophoretic mobilities in the pH range studied were contributed to their retentions. The k^* of the other nucleic acid bases and nucleosides kept almost constant with the pH decreasing from 9.5 to 7.5 and then decreased slightly when the pH further decreased to 3.5 (Fig. 11). When the mobile phase pH decreased from 7.5 to 3.5, the six analytes were protonated partly and therefore positively charged and more hydrophilic, thus leading to stronger hydrophilic interaction and thus stronger retention, which would make a positive contribution to the retention. On the other hand, when the six nucleic acid bases and nucleosides became protonated and took more positive charges, their electrophoresis effect resulted in decreased retention of the solutes. The behavior of the analytes with decreasing pH value of the mobile phase was probably related to the balance of opposing effects. These results indicated that the retention mechanism of nucleic acid bases and nucleosides was based on the hydrophilic interaction with the stationary phase combined with the electrophoresis.

4. Conclusion

A neutral poly(HEMA-co-PETA) monolithic column, which was prepared by thermal copolymerizing HEMA monomer and PETA cross-linker, has been successfully used as a polar stationary phase in HI mode. The polar sites on the surface of the monolithic solid phase that are responsible for hydrophilic interactions were provided from the hydroxy and ester groups. The observed EOF on the neutral monolith can be attributed to that the polar functionalities on the surface of the stationary phase attract ions from the mobile phase and thus impart the monolith with a given zeta potential to generate the EOF. It was found that the character of the PETA cross-linker was an important development in the design of polar and neutral monolith with relatively strong EOF. The effects of pH and ACN content on the separation of nucleic acid bases and nucleoside showed that typical HI-CEC mechanism was contributed to the separation. The absence of fixed charges on the surface of the neutral poly(HEMA-co-PETA) monolith allowed the efficient separations of polar charged compounds, such as basic nucleic acid bases and nucleoside without peak tailing.

Acknowledgements

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