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Monolithic columns with mixed modes of reversed-phase and anion-exchange stationary phase for capillary electrochromatography

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Abstract

A capillary electrochromatography (CEC) monolithic column with mixed modes of reversed-phase and anion-exchange stationary phases was prepared by in situ polymerization of 2-(methacryloxy)ethyltrimethylammonium methyl sulfate (MEAMS) and ethylene dimethacrylate (EDMA) in a binary porogenic solvent consisting 1-propanol and 1,4-butanediol. The ammonium groups on the surface of the stationary phase generate an electroosmotic flow (EOF) from cathode to anode, and serve as a strong anion-exchange stationary phase at the same time. The EOF of the stationary phase can be determined by the amount of MEAMS monomer in reaction mixtures during the polymerization. The monolithic stationary phases exhibited reversed-phase chromatographic behavior toward neutral solutes. For charged solutes, hydrophobic as well as electrostatic interaction/repulsion with the monoliths was observed. Separations of aromatic compounds and basic compounds on the prepared column were performed under the mode of CEC. Peak tailing of basic compounds was avoided and the efficient separation of aromatic acids was achieved using neutral mobile phase due to the same direction of EOF and electrophorestic mobility of negatively charged solutes.

Keywords: Monolithic columns; Mixed-mode separations; Stationary phases, electrochromatography; Electrochromatography; Anilines; Alkylbenzenes; Polynuclear aromatic hydrocarbons; Caffeine; Barbitals; Benzoic acids; Terephthalic acid

1. Introduction

Monolithic stationary phases have attracted increasing attention in capillary electrochromatography (CEC) because of various advantages over the packed columns [1–4]. Up to date, the vast majority of reports on CEC concern separations in reversed-phase mode, in which stationary phases contain both hydrophobic groups and negatively charged groups that generate the cathodic electoosmotic flow (EOF) [5–8]. Recently, mixed-mode of reversed-phase and cation-exchange stationary phase for CEC was reported by several groups [9,10]. However, basic compounds will be eluted with serious peak tailing even cannot be eluted on the stationary phases with negatively charged groups because of electrostatic adsorption [11]. Therefore, the stationary phases with positively charged functionalities are one of alternative approaches to separation of basic compounds. Unfortunately, compared with the negatively charged monolithic columns, much less work has been done on the positively charged monolithic stationary phases for CEC. Generally, the positively charged monolithic CEC columns are tailored by preparation of a monolithic matrix with active groups and then functionlization with positively charged groups. Horváth and co-workers [12–14] prepared positively charged monolithic columns from poly (glycidyl methacrylate–co-ethylene dimethacrylate) and poly(chloromethylstyrene–co–divinylbenzene) monolith by further reacting with aliphatic amines. However, preparation of column with multiple steps is time-consuming and also difficult to control the amount of charged groups incorporated in the polymer. In fact, the preparation and functionlization of the matrix can typically be combined in a single step. The monolithic stationary phase is prepared by polymerization of the two functional monomers, one for generation of the chromatographic interaction sites and another for coupling of charged groups. Lämmerhofer et al. [15] prepared hydrophilic macroporous weak and strong anion-exchange stationary phases by a single

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step polymerization of 2-dimethylaminoethyl methacrylate, 2-hydroxyethyl methacrylate and ethylene dimethacrylate for CEC in normal-phase separation. A hydrophilic chiral anion-exchange monolith with chiral quinidine-based ligands, which possessed chiral recognition ability and generated anionic electroosmotic flow at the same time, was prepared in a single step by the same group [16].

In this work, monolithic columns with in situ polymerization of charged monomer of 2-(methacryloyloxy)ethyltrimethylammonium methyl sulfate (MEAMS) and ethylene dimethacrylate (EDMA) were prepared, which showed the mixed-mode of reversed-phase (RP) and strong anion-exchange (SAX) mechanisms. The positively charged monolithic capillary columns were prepared by polymerization reaction in a single step and the strength of EOF could be adjusted by the amount of MEAMS in reaction mixtures. Diverse series of neutral and ionic samples, such as aromatic hydrocarbons, anilines, basic pharmaceuticals and aromatic acids were well separated on the prepared CEC columns.

2. Experimental

2.1. Materials

MEAMS was purchased from Aldrich (Milwaukee, WI, USA). EDMA and γ-methacryloxypropyltrimethoxysilane (γ-MAPS) were obtained from Sigma (St. Louis, MO, USA). 1-Propanol, 1,4-butanediol and azobisisobutyronitrile (AIBN) were obtained from Shanghai Fourth Reagent Plant (Shanghai, China), HPLC-grade methanol and acetonitrile (ACN) were supplied by the Yuwang Chemical Plant (Zibo, Shandong Province, China). EDMA was extracted with 5% aqueous sodium hydroxide solution and dried over anhydrous magnesium sulfate. Thiourea and aromatic compounds purchased from Tianjin Chemical Plant (Tianjin, China) were of analytical grade. Pharmaceuticals were obtained from Sigma. Double distilled water purified by Milli-Q (Millipore C., Milford, MA, USA) was utilized throughout the experiments. The sample solution of aromatic hydrocarbons was prepared by dissolving them in ACN with volume ratio at 1:10, and then further diluted to the appropriate concentration ranged from 0.01 to 0.03 μg/μL with the mobile phase before injection. Anilines, pharmaceuticals and aromatic acids were directly dissolved in the mobile phase in the concentration range 0.1–2 μg/μL.

Capillaries of 100 μm inner diameter and 375 μm outer diameter were purchased from the Yongnian Optic Fiber Plant (Hebei, China).

2.2. Instruments

A Hewlett-Packed ^{3D}CE system (Hewlett-Packard, Waldbronn, Germany) was used for all CEC experiments. A Waters 510 HPLC pump (Waters, Milford, MA, USA) was utilized to flush the columns.

2.3. Calculations

The retention factor in CEC can be expressed as follows [17]:

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

where t_r is the migration time of a solute, and t_0 is the migration time of a neutral and chromatographically unretained compound. In this work thiourea was selected as the t_0 marker.

$\log P$ is octanol–water partition coefficient as a measure of molecular hydrophobicity. All the $\log P$ data of analytes were calculated accordingly [18].

The pK_a values of anilines were predicted with the soft PALLAS (CompuDrug Chemistry, Chemical Software Series, version 1.2, 1994, Hungary).

2.4. Preparation of monolithic columns

Prior to the polymerization, the capillary was pretreated with the following procedure [19]: First, the capillary column with a length of 40 cm was rinsed with 0.1 M NaOH for 1 h and then with water until the outflow reached pH 7.0. After subsequent flushing with methanol for about 10 min, it was dried by passage of nitrogen gas. γ-MAPS solution by its dilution with methanol at a volume ratio of 1:1 was injected into the capillary with a syringe. Then, the capillary was sealed with rubber at both ends and then submerged in water bath at 50 °C for overnight. Finally, the capillary was rinsed with methanol and water to flush out the residual reagent. Thereby, Si–O–Si–C bonds were formed between the capillary wall and the reactive methacryloyl groups, which were available for subsequent attachment of monolith to the wall during the polymerization reaction.

The monolithic columns were prepared from polymerization reaction of mixtures, consisting of the monomers MEAMS, EDMA, the porogens of 1-propanol and 1,4-butanediol using AIBN (0.3%, w/w, with respect to the monomers) as an initiator. The polymerization mixtures were sonicated for 20 min to obtain homogeneous solution, and then purged with nitrogen for 10 min. After the pretreated capillaries was completely filled with the mixture, they were sealed at both ends with rubber stoppers. The sealed capillaries were submerged into a water bath and allowed to react for 2 h at 50–70 °C. The resultant monolithic capillary columns were washed with methanol about 2 h using an HPLC pump to remove unreacted monomers and porogens. At the end of this period, the detection window was made by burning off 1–2 mm of both the coated polymer outside and the monoliths inside of the capillaries using flames [20]. The ashes of the organic monolith inside the capillaries were flushed out by methanol for about 30 min with the HPLC pump under the applied pressure at about 80 bar. Capillaries, without visible compression of the monolith, were cut at both ends to a total length of 32 cm

and effective length of 8.5 cm. Finally, the columns were equilibrated at 10 kV for 30 min before running.

Macroscopic materials prepared in larger amounts of corresponding mixtures in empty HPLC columns were washed with methanol by using an HPLC pump for about 6 h. After that, the polymers were flushed out from the columns, cut into small pieces, and dried under vacuum at 50 °C for 24 h. Mercury intrusion porosimetry was used to characterize the pore size and surface area of monolith.

3. Results and discussion

3.1. Monolith synthesis

Two functional monovinyl monomers, namely, MEAMS and EDMA were used for this purpose. MEAMS affords positively charged functionalities to generate anionic EOF and provide the anion-exchange interaction sites simultaneously. The preparation process of the mixed-modes monolithic columns for CEC is quite simple. However, a number of factors have to be taken into account. Among these factors, the selection of the porogenic solvents is crucial for the preparation of the monolithic CEC columns. Several porogenic solvents, i.e. cyclohexanol/dodecanol, toluene/dimethyl sulfoxide, toluene/dodecanol, 1-propanol/1,4-butanediol, which have frequently been used in the preparation of monolithic CEC columns, were tested for their compatibility. It was observed that a binary porogen of 1-propanol/1,4-butanediol is well suited for the preparation of the positively charged porous poly(MEAMS–co–EDMA) monoliths. As reported by Peters et al. [8,21], the pore structure of monolith can be adjusted by changing the composition of porogenic solvents. Effect of porogenic solvent composition on the porosity of the poly(MEAMS–co–EDMA) monolithic column was investigated by changing the ratio of 1-propanol to 1,4-butanediol (keeping monomers/porogens ratio at 4/6, v/v). Dark monoliths with low permeability were observed under microscope by using porogenic mixtures containing less than 33% 1-propanol. As the content of 1-propanol increased, the permeability of the columns became better and the drops of mobile phase can be seen at the end of the capillary columns. However, translucid gel-like beds with poor permeability were obtained using the porogenic mixtures containing more than 67% 1-propanol. Thus, a mixture with 1-propanol/1,4-butanediol at volumetric ratio of 5/5 was chosen as the binary porogenic solvent.

Reaction temperature is a convenient variable to control the process of polymerization. The polymerization reaction did not occur with mixture containing 200 μL MEAMS, 200 μL EDMA, 300 μL 1-propanol, 300 μL 1,4-butanediol and 0.15 mg AIBN even for 12 h when reaction temperature was set at 50 °C. Whereas the polymerization could be finished in 2 h at 60 °C and the monoliths with homogeneous bed were obtained. However, the permeability of monolithic columns prepared at 70 °C for 2 h is not good enough to allow the mobile phase to flow though. Thus, the reaction temperature of 60 °C and the reaction time of 2 h were selected.

Table 1 lists the porous properties of monoliths prepared with different periods of reaction time. As can be seen that the pore diameters of the poly(MAEMS–co–EDMA) are mainly in the range of 0.5–1.0 μm. With the increasing reaction time, both the surface area and pore volumes increased due to more micropores produced with longer reaction time. The scanning-electron micrographs of the end of the poly(MEAMS–co–EDMA) capillary column are shown in Fig. 1. It can be seen that the monolithic bed with macropores linked to the pretreated capillary wall.

3.2. Evaluation of column performance

A column prepared with the reaction mixture and reaction time listed in column B in Table 1 was evaluated for CEC separation performance. Thiourea as a void time marker could elute after 2.19 min under the applied inlet pressure at 2 bar, which means that the monolithic bed in capillary shows good permeability and low flow resistance. Relative standard derivations (R.S.D.s) of the retention times of thiourea and toluene in five consecutive injections using the mobile phase containing 40% acetonitrile in 10 mM phosphate buffer (pH 2.0) was below 0.18 and 0.77%, respectively. Which demonstrated the high reproducibility of retention times for the analytes on the poly(MEAMS–co–EDMA) monolithic column.

The relationships between the theoretical plate height (HEPT) and the velocities of the mobile phase for thiourea and benzyl alcohol were studied and the obtained results are shown in Fig. 2. As can be seen that even when the velocity is higher than 1.0 mm/s, no apparent loss of column

Table 1
Effect of reaction time on the pore structure of the prepared monoliths

Column	Reaction time (h)	Pore volume (cm^3/g)	Specific surface area (m^2/g)	Pore diameter distribution (%)			
				<0.15 μm	0.15−0.5 μm	0.5−1.0 μm	>1.0 μm
A	1	1.15	26.5	8	8	70	14
B	2	1.29	35.1	11	9	58	22
C	6	1.37	40.1	13	12	51	24

Polymerization conditions: MEAMS 20% (v/v), EDMA 20% (v/v), porogenic solvent (1-propanol + 1,4-butanediol) 60% (v/v) and AIBN 0.3% (wt.) with respect to the monomers; reaction temperature at 60 °C.

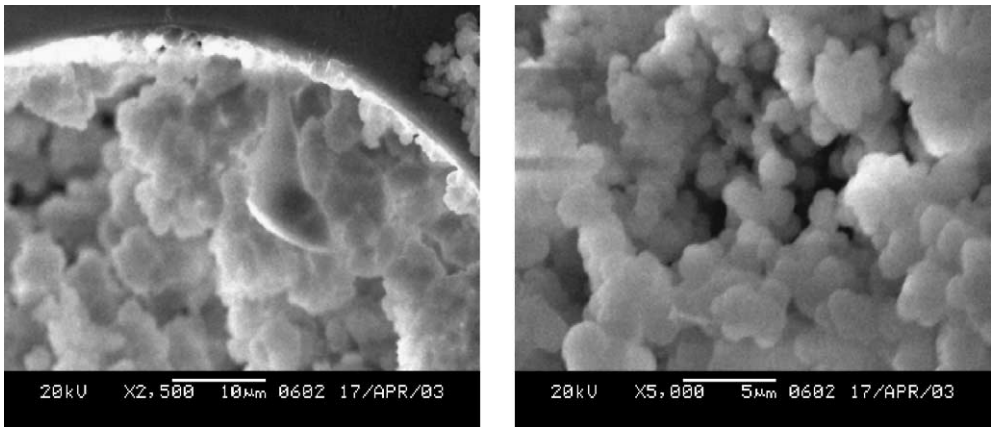

Fig. 1. Scanning-electron micrograph of the end of the poly(MEAMS–co–EDMA) monolithic stationary phase in a fused-silica capillary column with 100 μm i.d. (A) 2500×, (B) 5000×.

efficiency was observed. Similar result was also obtained by Jiang et al. [22] in the case of macrylate monolithic CEC columns with negatively charged groups. It suggests that on such columns rapid separation can be obtained with minor loss in resolution.

EOF is the driving force to transport the mobile phase through the capillary columns. An anionic EOF was observed in CEC, due to the positively charged groups on the surface of monolith and the EOF were varied from 2.12 to 0.94×10^{-8} m^2/(v s) in the pH range between 2.0 and 8.0. The decrease of the EOF with increasing pH of the mobile phase may be caused by the suppression of ionization of ammonium groups on the surface, as well as the ionization of the residual silanol groups on the bare capillary wall, which generate cationic EOF. Although CEC columns with ammonium groups on the surface of the monolithic bed generated anionic EOF over the whole pH range. Effect of the phosphate concentration in the mobile phase on the EOF was also investigated. The EOF almost stays constant value around 1.8×10^{-8} m^2/(v s) with increasing the concentration of phosphate from 5 to 40 mM in the mobile phase. The effect of acetonitrile concentration on the EOF was investigated by keeping the phosphate concentration at 10 mM and the pH at 2.0. It is observed that the EOF slightly increased from 1.83 to 2.11×10^{-8} m^2/(v s) with increasing acetonitrile concentration from 10 to 80% (v/v). This effect can be explained by a decrease of the viscosity of the mobile phase with increasing the acetonitrile content, thereby leading to an increase of the EOF.

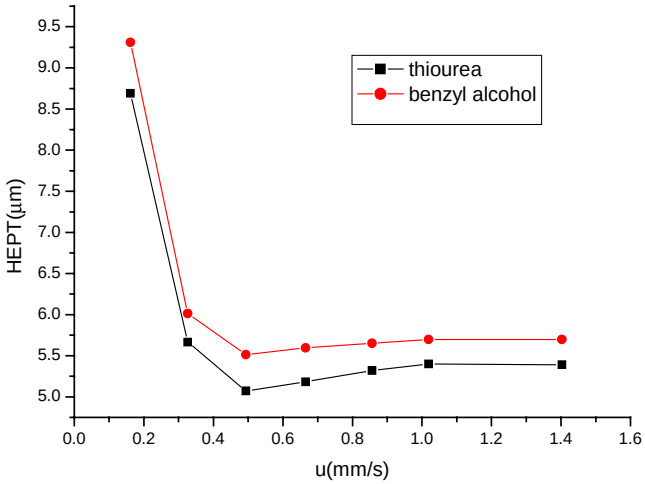

Fig. 2. Plot of theoretic plate height (HEPT) vs. the linear velocity of thiourea and benzyl alcohol. Experimental conditions: columns prepared with polymerization mixtures containing 200 μL MEAMS, 200 μL EDMA, 300 μL 1-propanol, 300 μL 1,4-butanediol and 0.15 mg AIBN at 60 °C for 2 h with effective length of 8.5 cm (total length 32 cm) × 100 μm i.d. × 375 μm o.d.; mobile phase, 10 mM phosphate buffer containing 40% (v/v) acetonitrile, pH 2.0; applied voltages, from 5 to 25 kV; injection, 5 kV × 5 s.

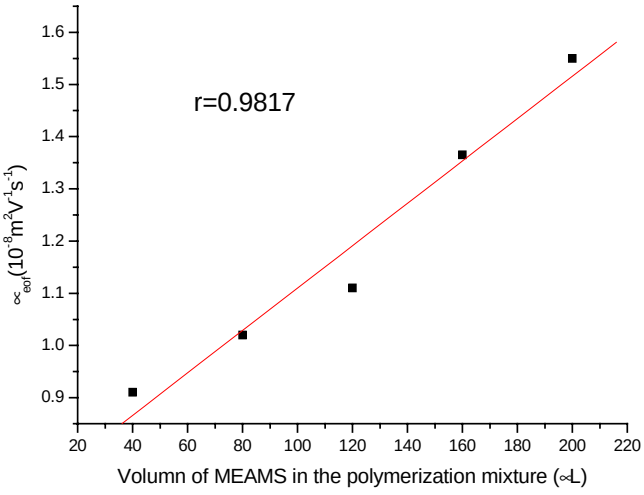

Fig. 3. Relationship between the electroosmotic mobility on the monolithic columns and the content of ionic monomer in polymerization mixture. Experimental conditions: columns prepared with the polymerization mixtures containing 400 μL (MEAMS + EDMA) with various content of MEAMS, 300 μL 1-propanol, 300 μL 1,4-butanediol and 0.15 mg AIBN at 60 °C for 2 h; mobile phase, 10 mM phosphate buffer (pH 2.0) containing 40% (v/v) acetonitrile; applied voltage, 10 kV. Other conditions as in Fig. 2.

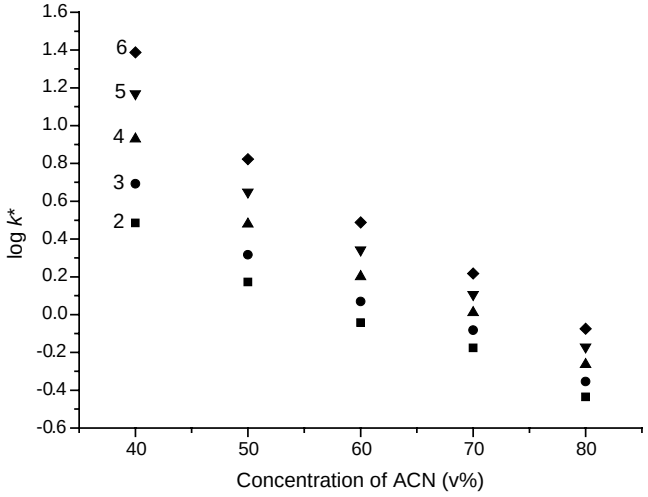

Fig. 4. Effect of acetonitrile concentration on the retention of alkylbenzenes. Experimental conditions: mobile phase, 10 mM phosphate buffer (pH 2.0) containing various content of acetonitrile; applied voltage, 10 kV. Solutes: (1) thiourea; (2) benzene; (3) toluene; (4) ethylbenzene; (5) propylbenzene; (6) butylbenzene. Other conditions as in Fig. 2.

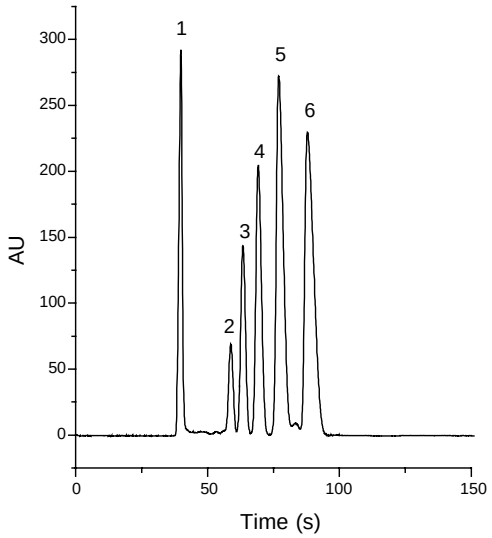

Fig. 5. Fast separation of alkylbenzenes on poly(MEAMS–co–EDMA) column. Experimental conditions: mobile phase, 10 mM phosphate buffer (pH 2.0) containing 70% (v/v) acetonitrile; applied voltage, 30 kV. Other conditions as in Fig. 4.

Monolithic columns with different amount of charged groups were prepared by changing amount of MEAMS monomer during polymerization reaction and the relationship between the EOF of the prepared monolithic columns and the content of ionic monomer is shown in Fig. 3. The EOF of monolithic stationary phases increased almost linearly with the increasing content of MEAMS in the polymerization mixture. So, the EOF of the poly(MEAMS–co–EDMA) monoliths is mainly determined by the amount of ionic monomer in mixtures during polymerization.

3.3. Separation of different types of solutes on monolithic columns

The prepared monolithic columns were applied for the separation of neutral compounds using acidic mobile phases. Alkylbenzenes were eluted in the order of thiourea < benzene < toluene < ethylbenzene < propylbenzene < butylbenzene according to their hydrophobicity on the poly(MEAMS−co−EDMA) monolithic columns. The effect of acetonitrile concentration in the mobile phase on the retention factors of alkyl benzenes is shown in Fig. 4. It can be seen that $\log k^*$ almost linearly decreased with the increasing concentration of acetonitrile in the mobile phase ($r > 0.9864$), and it can be deduced that the separation of alkylbenenes on the poly(MEAMS–co–EDMA) is mainly based on the reversed-phase mechanism. The retention factors of alkylbenzenes on the monolithic columns prepared with different amount of MEAMS monomers are listed in Table 2. The alkylbenzenes are less retarded on the mono-

Table 2
Retention factors of alkylbenzenes on poly(MEAMS–co–EDMA) monolithic columns with different amount of MEAMS in reaction mixture[a]

MEAMS (%)[b]	$k^*_{benzene}$	$k^*_{toluene}$	$k^*_{ethylbenzene}$	$k^*_{propylbenzene}$	$k^*_{butylbenzene}$
20	2.948	3.737	4.617	6.600	8.485
40	1.234	1.524	1.897	2.608	3.319
50	1.184	1.384	1.646	2.177	2.631

Experimental conditions: mobile phase, 10 mM phosphate buffer containing 60% (v/v) acetonitrile, pH 2.0; applied voltage, 20 kV.

[a] Polymerization conditions: reaction mixtures containing (MEAMS + EDMA) 400 μL, 1-propanol 300 μL, 1,4-butanediol 300 μL, AIBN 0.15 mg; reaction temperature 60 °C; polymerization reaction time 2 h.

[b] Volume percentage of MEAMS in the monomer mixture.

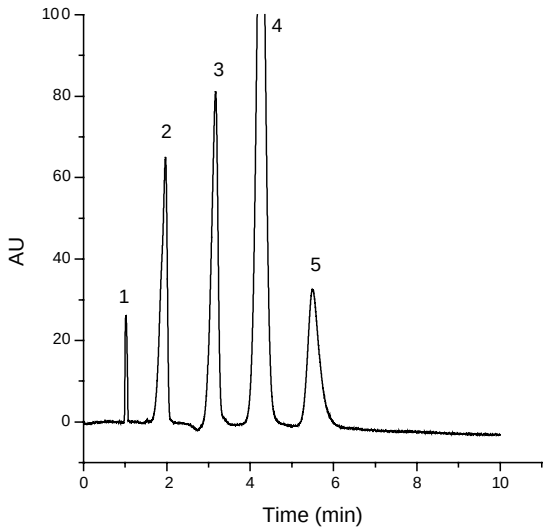

Fig. 6. Separation of polycyclic aromatic hydrocarbons on the monolithic column. Experimental conditions: mobile phase, 10 mM phosphate buffer (pH 2.0) containing 60% (v/v) acetonitrile; applied voltage, 20 kV. Solutes: (1) thiourea; (2) benzene; (3) naphthalene; (4) acenaphthene; (5) anthracene. Other conditions as in Fig. 4.

lithic columns prepared with higher amount of positively charged monomers due to weak hydrophobicity of monolithic rod. The effect of applied voltage on the retention factors of alkylbenzenes was investigated and it was observed that retention factors of alkylbenzenes kept almost constant with the applied voltages varied from 5 to 30 kV, therefore the separation can be performed with high applied voltage without loss of separation efficiency. Fig. 5 shows the fast separation of five alkylbenzenes within 100 s by applying voltage at 30 kV. Typical separation of a mixture of polycyclic aromatic hydrocarbons (PAHs) on the column is shown in Fig. 6. It clearly indicated that the elution order of PAHs is according to their hydrophobicity.

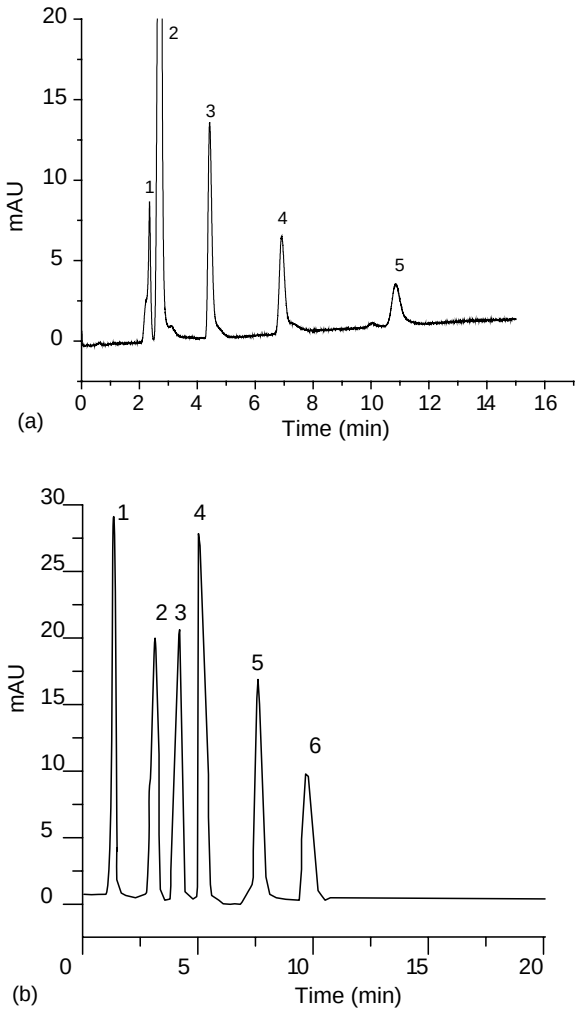

Fig. 7. Electrochromatograms of anilines on the poly(MEAMS–co–EDMA) monolithic column. Experimental conditions: mobile phases, (a) 10 mM phoshate buffer (pH 4.0) containing 40% acetonitrile; (b) 10 mM phosphate buffer (pH 6.0) containing 60% (v/v) acetonitrile; applied voltage, 20 kV. Solutes in (a): (1) 1,2-phenylenediamine (pK_b 9.12); (2) 1,3-phenylenediamine (pK_b 9.12); (3) 2,5-dimethylpyridine (pK_b 7.63); (4) 2-nitroaniline (pK_b 9.42); (5) 2,6-dinitroaniline (pK_b 9.42). Solutes in (b): (1) 2,4-diaminotoluene (pK_b 9.02); (2) 4-aminobiphenyl (pK_b 9.39); (3) 3,3′-dimethoxybenzidine (pK_b 8.41); (4) 3,5-dinitroaniline (pK_b 9.42); (5) 2,6-dinitroaniline (pK_b 9.12); (6) 1,2-diphenylhydrazine. Other experimental conditions as in Fig. 2.

Separation of basic compounds somewhat is still a troublesome task in HPLC and CEC due to the undesirable interaction between basic analytes and residual silanols on the surface of silica-based stationary phase. Monolithic polymer stationary phase is an alternative choice for the separation of basic compounds. Lämmerhofer et al. [15] reported the separation of basic compounds in normal phase CEC by using a strong anionic exchange monolithic column in the non-aqueous organic mobile phase. In this work, anilines were separated on the poly(MEAMS–co–EDMA) monolith by using the ‘counterdirectional mode’, an approach suggested by Hjertén and co-worker [6]. In this CEC system, basic compounds migrated electrophoretically in a direction opposite to that of the EOF and the electrostatic adsorption between the basic solutes and the stationary phase was avoided. Fig. 7 shows the typical electrochromatograms of anilines on the monolithic column with mobile phases at pH 4.0 and 6.0. No obvious peak tailing of the basic anilines was observed owing to avoidance of the electrostatic

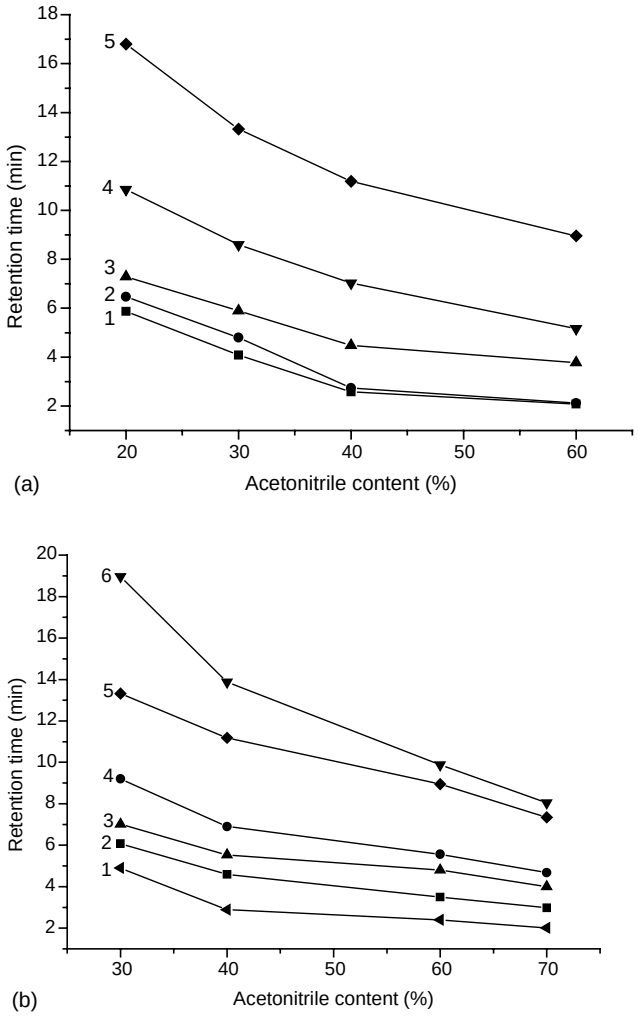

Fig. 8. Effect of acetonitrile concentration on the retention times of anilines. Experimental conditions: mobile phase, 10 mM phosphate buffer (pH 4.0) containing various concentration of acetonitrile; applied voltage, 20 kV. Other conditions as in Fig. 7.

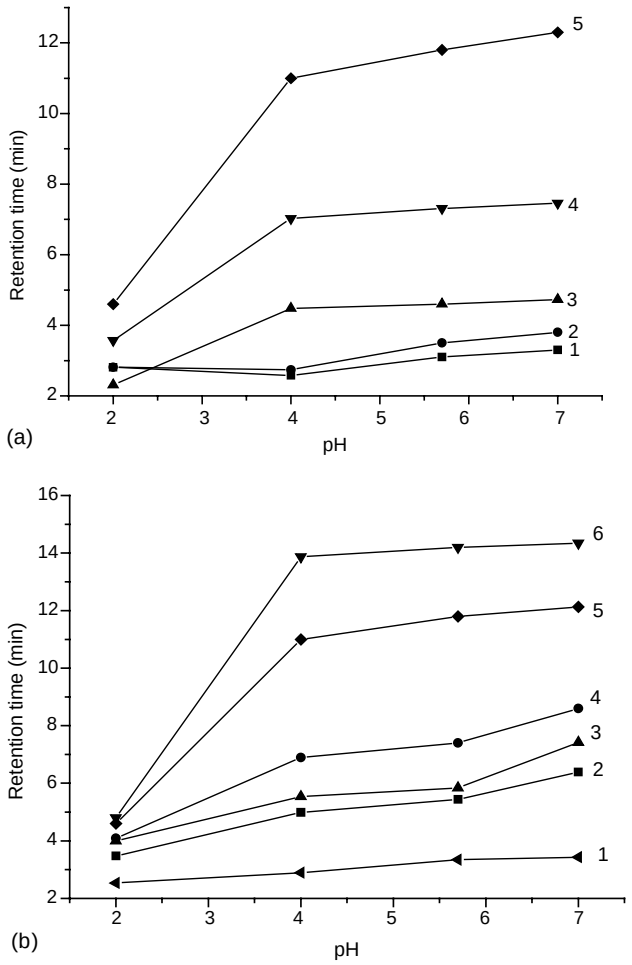

Fig. 9. Effect of pH on the retention times of anilines. Experimental conditions: (a) mobile phase, 10 mM phosphate buffer containing 40% (v/v) acetonitrile with various pH; (b) mobile phase, 10 mM phosphate buffer containing 60% acetonitrile with various pH. Other conditions as in Fig. 7.

adsorption between the basic analytes and charged groups on the surface of the stationary phase. The effect of the acetonitrile concentration in the mobile phase on the retention times of anilines is shown in Fig. 8. The retention times of aniline decreased with the increasing acetonitrile concentration in the mobile phase and it can be deduced that the hydrophobic interaction was responsible for the retention of anilines on the poly(MEAMS–co–EDMA) column even anilines were positively charged under acidic conditions. Fig. 9 illustrates the dependence of retention times of anilines on the pH values of the mobile phase. As can be seen that the retention of the anilines is remarkably dependent on the pH values of the mobile phase. Most of anilines had weak retention on the poly(MEAMS–co–EDMA) column at low pH value because the anilines were positively charged at low pH value and therefore, hydrophobic interaction between anilines and the stationary phase was not strong though electrophoresis accelerates the positively charged anilines towards the cathode. As the pH value of mobile phase increased, the retention times of anilines increased because anilines deprotonated gradually with the increasing pH values and therefore hydrophobic interaction and suppression of electrophoretic mobility will make a positive contribution to the retention of the anilines. However, the retention times of both 1,2-phenylenediamine and 1,3-phenylenediamine anilines decreased when the pH value of mobile phase increased from 2.0 to 4.0 and then increased with the increasing of pH values. This is because both 1,2-phenylenediamine and 1,3-phenylenediamine have more positive charges and tend to migrate against the EOF, and thereby the migration times at pH 2.0 are relatively long. With the increasing of pH value of mobile phase, the two analytes gradually deprotonated and the influence of electrophoretic mechanism lost importance and the hydrophobic interaction gradually played an important role on their retention. Separation of basic pharmaceutical also carried out on the poly(MEAMS–co–EDMA) monolithic column and the obtained electrochromatogram is shown in Fig. 10. Linear relationship ($r = 0.9926$) was observed between the $\log P$ and $\log k^*$, which indicated that the main interaction between the pharmaceuticals and the stationary phase was hydrophobic interaction.

Separation of acidic compounds was often carried out in ion-suppressed mode of CEC on the silica-based stationary phases. However, it will take long time to accomplish the separation because of low EOF generated by silanols under acidic conditions [23,24]. Separation of acidic profens was achieved on a polar anionic exchange monolithic column by using non-aqueous mobile phase with long separation time [15]. Fig. 11 shows the typical electrochromatograms of acidic analytes on poly(MEAMS–co–EDMA) monolithic

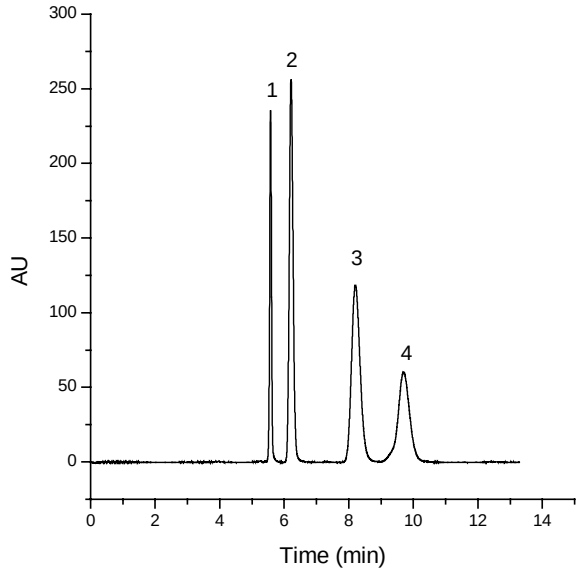

Fig. 10. Electrochromatogram of basic pharmaceuticals on the poly(MEAMS−co−EDMA) monolithic CEC column. Experimental conditions: mobile phase, 50 mM phosphate buffer (pH 2.0) containing 20% (v/v) acetonitrile; applied voltage, 20 kV. Solutes: (1) caffeine; (2) barbital; (3) phenobarbital; (4) amobarbital. Other conditions as in Fig. 2.

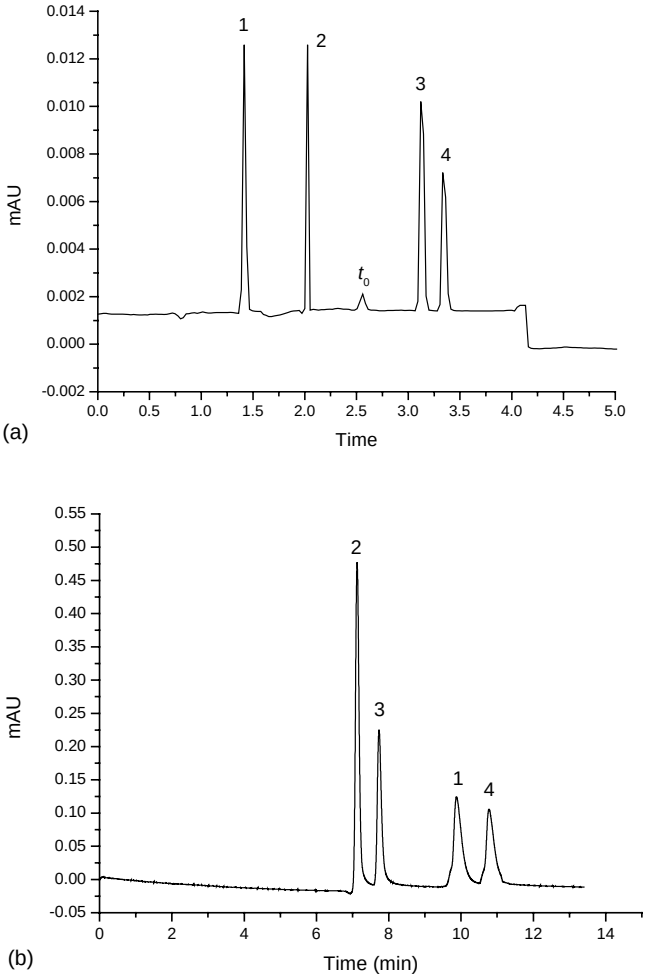

Fig. 11. Electrochromatograms of aromatic acids on poly(MEAMS–co–EDMA) monolithic CEC column. Experimental conditions, mobile phase: 20% (v/v) acetonitrile in 20 mM phosphate buffer at (a) pH 7.0 and (b) pH 2.0; applied voltage, 20 kV. Solutes: (1) terephthalic acid; (2) 3,5-dinitrobenzoic acid; (3) *p*-nitrobenzoic acid; (4) benzoic acid.

column with mobile phase at pH 7.0 and 2.0. As can be seen that the aromatic acids were eluted in different order using mobile phase with different pH values. Moreover, more efficient separation of acidic analytes was obtained using neutral mobile phase because of the same direction of EOF and electrophoretic mobility of negatively charged acids.

4. Conclusion

Monolithic columns with quaternary amino groups affording mixed-modes of reversed phase and anion-exchange stationary phase for capillary electrochromatography within the confined capillary columns were obtained by the copolymerization of MEAMS and EDMA in the presence of porogens. The monolithic CEC columns generated anionic EOF in the whole pH range, and the EOF mobility almost linearly increased by increasing the amount of ionic monomer MEAMS in the polymerization mixture. Neutral compounds, such as alkylbenzenes and PAHs, retained on the stationary phases based on the reversed-phase mechanism. Basic compounds such as aniline and basic pharmaceutical were well separated on the poly(MEAMS–co–EDMA) monolithic columns in the 'counterdirectional mode' to avoid the electrostatic adsorption between basic analytes and the charged groups on the surface of the stationary phases. Efficient separations of aromatic acids were achieved with neutral mobile phase on the column because of the same direction of the electrophoretic mobility of acids and the anionic EOF.

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