Rapid Preparation and Characterization of Methacrylate-Based Monoliths for Chromatographic and Electrophoretic Separation

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Abstract

Butyl-methacrylate-based porous monoliths were rapidly prepared in the fused-silica capillary with a 10-cm stripe of polyimide removed from its exterior. The photopolymerization could be carried out in 150 s using ethylene glycol dimethacrylate as a cross-linking agent; 1-propanol, 1,4-butanediol, and water as tri-porogenic solvents; and Irgacure 1800 as a photo-initiator. The effect of different morphologies on the efficiency and retention properties was investigated using pressure-assisted CEC (p-CEC), CEC, and low pressure-assisted liquid chromatography modes (LPLC). Baseline separation of the model analytes was respectively achieved including thiourea, toluene, naphthalene, and biphenyl with the lowest theoretical height up to 8.0 µm for thiourea in the mode of *p*-CEC. Furthermore, the influence of the tri-porogenic solvents on the morphology of methacrylate-based monoliths was systematically studied with mercury intrusion porosimetry and scanning electron microscopy.

Introduction

Since the introduction of continuous polymer beds in the application of high-performance liquid chromatography (HPLC), micro-HPLC, and capillary electrochromatography (CEC), the development of monolithic separation media attracted considerable attention in the separation fields (1–2). In comparison to conventional packed columns, particular advantages of monoliths result from their unique structure, which allow the separation of both low and high molecular weight analytes. It is free from several problems associated with stationary phase packing, frit-making procedures, and usage of a high-pressure pumping system (3). Monoliths can be prepared in various methods and have an inorganic network such as silica or an organic skeleton such as polymethacrylates, polystyrenes, and polyacrylamides (4–6). For monolithic columns based on organic polymers, the polymer network is generally formed inside the capillary by a chain-polymerization reaction with the help of thermal or photo-initiation methods. Once the polymerization is complete, unreacted components such as the porogenic solvents are removed from the monolith using a syringe pump or electroosmotic flow.

Polymethacrylate-based stationary phases have been proved to be an excellent stationary phase with outstanding chemical stability in a broad pH range, which have been widely used for separation support (7–9). E.C. Peters et al. (10) have prepared this monolithic matrix for CEC within the confines of untreated fused-silica capillaries in a single step by a simple copolymerization of mixtures of butyl methacrylate (BMA), ethylene dimethacrylate (EDMA), and 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS). After that, a novel stationary phase for micro-ion chromatography was further prepared by coating quaternary amine-functionalized latex particles on the previously mentioned monoliths via simple electrostatic binding (11). R.A. Wu et al. (12) also prepared the similar monoliths for the separation of some peptides, which in situ copolymerization of lauryl methacrylate and ethylene dimethacrylate was carried in a gas chromatograph (GC) oven at 60°C for 12 h. The BMA-Co-EDMA monoliths without any charged groups have also been investigated by L.Y. Zhang et al. (13). Compared with ternary porogenic solvents (1-propanol–1,4-butanediol–water), fine control of the pore diameter and the formation of the specific surface of the monolithic polymers could be realized with the binary porogenic solvents of alcohols. Furthermore, L.J. Sondergeld et al. (14) prepared a similar monolith using butyl acrylate as the monomer; 1,3-butanedioldiacrylate as the crosslinker; 2,2'-azobisisobutyronitrile (AIBN) as the thermal initiator; and AMPS to support electroosmotic flow. Hydroxymethyl methacrylate-based monolithic columns could be designed for separation of oligonucleotides in hydrophilic-interaction capillary liquid chromatography by P. Holdsvendova et al. (15). A. Nordborg et al. extended the array of crosslinkers suitable for the preparation of polymethacrylated-based monoliths using thermal polymerization and their application for µ-HPLC separations of proteins (16). J. Urban et al. even prepared some monolithic capillary columns with porosity controlled by varying the proportions of BMA and EDMA monomers and of 1,4-butanediol and 1-propanol as the porogen solvent (17). Further studies

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were also done which investigated the main factors affecting the mesopore porosity of methacrylate-ester based monoliths (18). S. Eeltink et al. (19) prepared the methacrylate monolithic columns with different ratios of total monomers to porogens in the polymerization mixture with a thermal initiation about 20 h at 70°C. Low-density monoliths were prepared with 20% (w/w) of monomers in the mixture and high-density monoliths with 40% monomers. The low-density columns showed a higher flow permeability, but they provided less retention, and they were more difficult to prepare in a controlled, repeatable way. Later, this group grafted two ionizable monomers on this monolithic matrix, which was carried out by photopolymerization about 60 min in the porogenic solvents of decanol and cyclohexanol (20). The photochemical route to the preparation of the porous matrix has many advantages (21, 22): (i) short preparation time, (ii) control of the pore size, (iii) control over the placement and length of the porous matrix, (iv) high mechanical strength, and (v) avoidance of high temperatures that lead to cracking. Recently, the BMA-Co-EDMA monoliths have been comparatively prepared via either thermally or photochemically initiated polymerization (23). The former was carried out by immersing the capillaries in a water bath kept at 50°C for 72 h, while the photoinitiated polymerizations in the PTFE-coated UV transparent capillaries required irradiation in a Spectrolinker UV crosslinker with an exposure time of 50 min. These columns were tested in liquid chromatography–electrospray ionization-mass spectrometry mode for the separation of a model mixture of three proteins such as ribonuclease A, cytochrome *c,* and myoglobin. Generally, the prepared capillaries by thermal initiation were carried out about 50–70°C for 12–72 h. Initiation by conventional heating presented the disadvantage of long reaction time due to the slow convection of heat, while photo-polymerization necessitated use of capillaries with UV-transparent outer coatings (generally Teflon-coated capillary) in the previous studies.

This study involves the preparation and characterization of methacrylate-based monolithic columns for pressure-assisted CEC (p-CEC), CEC, and low-pressure liquid chromatography (LPLC). The porous properties of monoliths (BMA–EDMA–AMPS) could be influenced by varying the ratio of the pore-forming solvents in the polymerization mixture. The chromatographic and electrophoretic behaviors of the studied organic-based monolithic columns have comparatively been evaluated by the previous three separation modes. A key difference described here is that the preparation of BMA-Co-EDMA monoliths was carried out inside the conventional fused-silica capillary with a stripe of polyimide removed from its exterior. Due to the use of Irgacure 1800 as the photo-initiator, the polymerization time was greatly shortened to 150 s.

Experimental

Instrumentation

All CEC experiments were performed on an Agilent ^{3D}CE system (Walbronn, Germany) equipped with a diode array detector and the capability to apply up to 1.2 MPa pressure to one or both ends of the capillary. The rinse of all prepared monolithic columns was carried out using an Agilent HP1100 Series HPLC system equipped with a quaternary pump. A Spectronics XL-1500 UV cross-linker (Westbury, NY) was equipped with six 15 W blacklight tubes in which the reaction solutions were irradiated at a wavelength of 365 nm. Porosity data were obtained by using PM-33-11 Poremasters (Quantachrome Instruments, Boynton Beach, Florida) for low-pressure and high-pressure analysis, respectively. A Quanta 200 scanning electron microscope (SEM) (Philips-FEI, Eindhoven, Netherlands) was used to study the morphology of the monolith. A capillary with the monolith was sectioned into 10-mm segments without sputtering with gold prior to SEM analysis.

Materials and chemicals

Fused-silica capillaries (75 µm i.d., 375 µm o.d.) were purchased from Yongnian Ruipu Optic Fiber Plant (Yongnian, Hebei Province, China). BMA, EDMA, AMPS, γ-methacryloxypropyltrimethoxysilane (MPTMS), 1,4-butanediol, 1-propanol, acetonitrile (ACN), tris-(hydroxymethyl) aminomethane, thiourea, benzene, toluene, biphenyl, and naphthalene were purchased from Beijing Bailingwei Chemical Reagent Company (Beijing, China) and Tianjing Chemical Reagent Company (Tianjing, China). Irgacure 1800 was donated by Ciba-Geigy Company (Tianjing, China). Distilled water was obtained from a super-purification system (Danyangmen, Jiangsou, China). In all experiments, a mobile phase consisting of a mixture of ACN–phosphate buffer (2 mM, pH 8.0) (50:50, v/v) was filtered through a membrane (0.45 µm), degassed by sonication, and used at a column temperature of 25°C. In a typical chromatographic and electrophoretic experiment, aromatic compounds were dissolved in ACN and injected for peak identification.

Results and Discussion

Preparation of the Monolithic Columns

Prior to filling the reactants into the capillary, it was pretreated with the following procedure: Firstly, the capillary column with a length of 40 cm was rinsed with 1 M NaOH for 30 min and then with 0.1 M HCI for 30 min. After subsequent flushing with H_2O for 30 min, it was dried by passage of nitrogen gas. The purpose of capillary pretreatment is to increase the concentration of surface silanol groups. Because silanol groups on the capillary surface represent the principal binding sites for in situ created poly-organic-based stationary phases, higher concentration of these binding sites on the capillary surface would facilitate the formation of highly secured organic-based stationary phases through chemical bonding with the capillary inner walls. Monolithic capillary columns were fabricated in situ in 75-µm polyimide-coated capillaries whose internal walls had been modified by [3-(methacryloyloxy)propyl]-trimethoxysilane solution through a procedure described elsewhere (24). Thereby, Si-O-Si-C bonds were formed between the capillary wall and the reactive methacryloyl groups, which are available for subsequent attachment of reactant to the wall. Then the capillaries were partially filled

with the polymerization mixture, including the 40 wt% monomer solutions and 60 wt% porogenic solvents. The former consisted of BMA, EDMA as a crosslinker, AMPS for generating electroosmotic flow, Irgacure 1800 (about 1.0 wt% of monomer) for generating free radicals, and the latter was made up of 10 wt% water and 90 wt% of 1-propanol and 1,4-butanediol combined in various ratios (10–23). The composition of polymerization mixture was listed in Table I. It should be noted that the liquid moved into the capillary could be observed carefully near and under the daylight lamp when the solution was slowly forced by the specific syringe. So the moving liquid could be carefully forced and stopped to the designated position into the capillary, and the monolith length can be approximatively measured by the ruler. In the series of experiments, the ratio of the volume fractions of the monomer mixture to the porogenic solvent was kept constant (4:6, wt%) while the ratio of 1-propanol to 1,4-butanediol, in the trinary pore-forming solvent volume was varied in our experiments. The mixture was sonicated for 20 min to obtain a homogeneous mixture, and then purged with nitrogen for 10 min before filling into the capillaries. After the pretreated capillary was partially filled with the mixture to a set position, the capillary was sealed at both ends with glue and rubber stoppers. It should be noted that this photoinitiator solution was flushed through a 40-cm long capillary with a 10-cm stripe of the polyimide coating removed using a razor blade positioned at 45°C to the capillary surface (25–27). It is well-known that Teflon-coated capillaries have decreased flexibility in the preparation of monolithic columns compared to polyimide coated fused-silica. This makes them very difficult to load into commercial instrumentation, and capillary lifetime is decreased due to the fragility of the capillaries. Our attempts here could be an alternative using the polyimide-coated capillaries instead of Teflon-coated ones in UV photoinitiation. The mechanical stability of the capillary was still good despite the removal of a stripe of polyimide coating, but exercise should be done carefully in the next operations. The UV irradiation light entered the capillary only through this 10-cm stripe. No monolith was formed in the capillary where the polyimide coating ("mask") remained intact. After that, the monoliths of BMA–EDMA–AMPS within the capillary columns could be quickly formed with the help of UV photo irradiation. Prior to CEC experiments, the capillaries were flushed with mobile phase for 30 min. A preconditioning step was performed by applying a stepwise increase in voltage up to 30 kV over the column until a stable current was observed. Simultaneously with the polymerization in capillaries, the same polymerization was carried out with the same mixture in a glass vial, cut into small pieces with a razor blade, and a Soxhlet extraction was carried out with methanol for 24 h. After drying at 50°C for 4 h, mercury intrusion porosimetry and SEM experiments were performed on the monolithic materials.

Table I. Composition of the Polymerization Mixtures with Different Ratio of Monomers/Porogenic Solvents

	Monomer (%)			Porogenic solvents (%)		
MC	EDMA	BMA	AMPS	1-Propanol	1,4-Butaneoliol	water
1	40	59.4	0.6	45	45	10
2	40	59.4	0.6	50	40	10
3	40	59.4	0.6	55	35	10
4	40	59.4	0.6	60	30	10
5	40	59.4	0.6	65	25	10
6	40	59.4	0.6	70	20	10

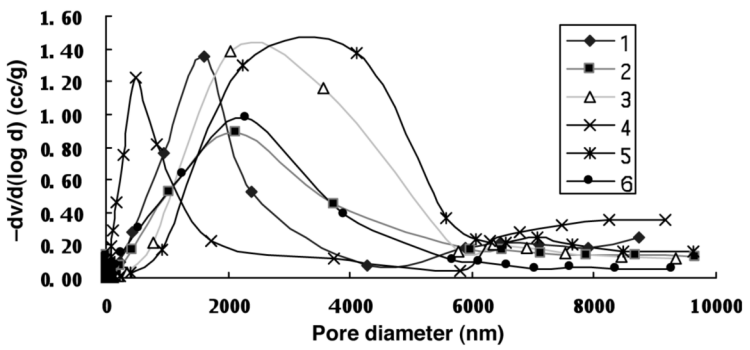

Figure 1. Typical pore-size distribution of polymethacrylate-based monoliths determined by mercury-intrusion porosimetry. See Table I for detailed description of porogenic composition.

According to the theory of nucleation and phase separation, the polymerization reaction time has an important effect on the pore and channel size and specific area of monolith (22,23). In our experiments, the polymerization within the capillary could be carried out within 150 s by using the UV photopolymerization, so increasing the irradiation time is not necessary. Photopolymerization is the process of converting a liquid monomer to a solid polymer by using UV irradiation. This photochemical process starts by the absorption of UV light by a photosensitive chemical compound (photoinitiator). Here, a high efficiency photoinitiator, Irgacure 1800, is a mixture of 25% bis(2,6-dimethoxybenzoyl)-2,4,4-trimethyl-pentylphosphineoxide and 75% 1-hydroxy-cyclohexyl-phenyl-ketone, which starts the polymerization process by producing free radicals. Free radicals are highly reactive species which break up carbon-carbon double bonds (C=C bonds) in molecules in the monomer that undergoes polymerization. By this process of destroying carbon-carbon double bonds, the molecule itself becomes highly reactive and links itself to another highly reactive molecule. By this forming of very long macromolecules, the liquid monomer changes to a solid polymer that can have totally different properties from the liquid monomer.

Characterization of the prepared monoliths

Strict control of the morphology of the monolithic stationary phase is important to obtain a generic porous monolithic material that provides a good separation efficiency and a low resistance to flow (28). The latter is of prime importance because it enables easy flushing of the column with liquids that are used in the subsequent separation in the

modes of p-CEC, CEC, and LPLC. The key variables that allow the control the pore size are the percentage of crosslinking monomer and the composition of porogenic solvent. The composition of porogenic solvent mixture is the most convenient variable to adjusting the pore size distribution because it does not require any change in the total amount of the monomers in the polymerization. Here, we only studied the influence of the composition of porogenic solvents on the morphology of the resulting material. Monoliths were prepared in glass vials and in situ in fused-silica capillaries by UV initiation. As polymerization mixtures of BMA, EDMA, and AMPS were used following a similar recipe developed by several groups (10–23). The ratio of the weight fractions of the monomers and the solvents in the polymerization mixture was kept constant, but the composition of pore-forming solvent was varied in order to adjust systematically the average size of methacrylate-based monoliths by changing the percentage of 1,4-butanediol and 1-propanol. A change of the polarity of the mixture, by changing the ratio of 1-propanol to 1,4-butanediol, resulted in the change of the average pore size (28,29). The pores in the polymer are formed during the quick polymerization. In the polymerization mixture with 1-propanol and 1,4-butanediol as the porogen solvent, the volume of BMA–EDMA–AMPS was kept 40% of total polymerization mixture in order to impart pore stabilization and rigidity to the polymer structure and to prevent pore collapse. Different morphology will be formed with the ratio of solubility parameter of the porogen to the solubility parameter of the polymer. Accordingly, the proportion of pores is necessarily controlled. Figure 1 shows pore-size distribution curves for these monolithic materials in Table I. With the change of 1-propanol–1,4-butanaediol ratio in the porogenic solvents, the pore size changed without a strong correlation. It is different from the thermally initiated monoliths, which could be precisely controlled with the pore size in the range of 250–1300 nm (28). All these photo-initiated monoliths exhibit unimodal pore-size distributions with no pores smaller than 450 nm. The pore size at the apex of the distribution curve can be controlled in a large range by adjusting the 1-propanol–1,4-butanediol ratio. A decrease in the 1-propanol content in the pore-forming solvents from 45% to 70% leads to the pore size in the range of 497–3500 nm. It should be noted that mercury intrusion porosimetry is carried out with monoliths prepared in larger glass vials and irradiated for 1 h. Because the different environment in vial and in capillary has a distinct effect on the morphology, the pore size of the bulk monolith determined by mercury porosimetry may not correspond to that of a monolith prepared in capillaries. Therefore, mercury porosimetry data are more useful to expose general trends than to measure the absolute pore size of the actual chromatographic monoliths (22).

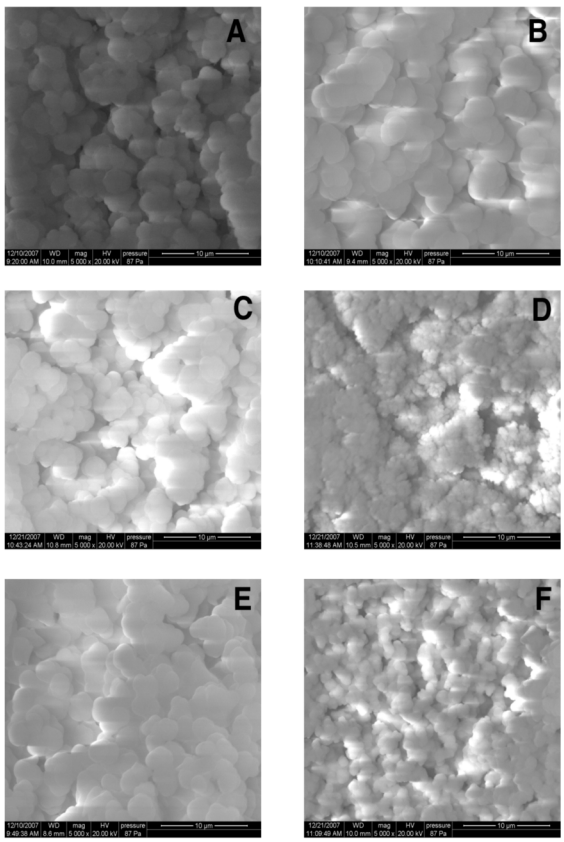

Figure 2. SEM images of the porous monolithic columns. See Table I for detailed description of porogenic composition. The scale bar corresponds to 10 µm in the inset. A, MC1; B, MC2; C, MC3; D, MC4; E, MC5; F, MC6

The forming monoliths exhibited an amazingly high mechanical strength. They could endure a high pressure over 30 Mpa, which is to be expected if covalent bonds are indeed formed between the walls and the polymer. Furthermore, characterization of these photoploymerized monoliths by SEM revealed a close association between the polymers and the composition of porogenic solvents. Figure 2 shows an interconnecting network of 1 µm or so spherical structures through which micrometer-sized macropores (as large as 4 µm) were interspersed for monoliths 1–5. Monolithic column 6 (MC6) seemed to form a smaller particle, which was less permeable and easily blocked in the next operation. Superficially, column permeability may seem irrelevant in CEC separation because electroosmotic flow as the driving force in a CEC to propel the mobile phase through the column without requiring mechanical pressure (25–27). It should be noted that columns with high permeability provide some significant advantages especially in p-CEC

Table II. Chromatographic Parameters Obtained Using the Same Operation Condition

	Retention time (min)				Resolution			Retention factor			
MC	t_1	t_2	t_3	t_4	R_{12}	R_{23}	R_{34}	k_1	k_2	k_3	N/m
1	0.94	1.32	2.05	2.72	4.95	5.21	2.79	0.40	1.18	1.89	78174
2	0.93	1.88	5.04	9.24	6.01	4.18	3.16	1.02	4.42	8.94	18317
3	1.09	1.80	3.36	4.93	7.33	7.9	4.4	0.65	2.08	3.52	65430
4	1.60	2.07	2.97	3.77	2.82	2.59	1.26	0.29	0.86	1.36	54401
5	0.91	1.18	1.71	2.19	1.88	2.13	1.14	0.30	0.88	1.41	18871

operation, LPLC operation, sample injection, or quick flushing of the capillary during column regeneration or equilibration. The suitable pore size is still the key for the preparation of monolith for capillary HPLC and CEC. The macroporous monolithic structures facilitate the mobile phase flow through the pores and thereby promote effective solute/stationary phase interaction by bringing them together. Effective solute transport mechanism operating within this monolithic structure due to mobile phase flow through the macropores together with the flat flow profile of EOF leads to high speed and separation efficiency in p-CEC (28–30).

Column Performance Studies

After columns 1–6 were prepared, they were connected to the HPLC pump for flushing. Column 6 created a high backpressure due to a high fraction of 1-propanol in the porogenic mixture, which led to unstable operation in the next chromatographic evaluation. Electrochromatographic and electrophoretic performances of other columns were comparatively evaluated under the same operation conditions in p-CEC mode using an operation voltage of 30 kV and 1.2 MPa pressured at the inlet. A reversed-phase mechanism was observed for the analyte separation using MC 1–5 (not shown). Solution partitioning between the mobile and stationary phases is the main mechanism responsible for their retention of the model compounds. The elution order of the columns is similar to that of reversed-phase chromatography; the analytes with larger molecular weight or more hydrophobic analytes were eluted later than the analytes with smaller molecular weight or more hydrophilic. The retention time, resolution, retention factor for each analyte, and the theoretic plate number for the unretained component are listed in Table II. All chromatographic parameters were obtained at the same operation condition on each monolithic stationary phase with the same length of 10 cm. It is noteworthy to see that under the same conditions, baseline separation could be obtained for the selected analytes on MC 1–3 whilst the resolution of some peak pairs for MC 4 and 5 should be improved.

As an example, a comparison of chromatograms for MC 3 is detailedly investigated by p-CEC, CEC, and LPLC. Figure 3 shows a visual illustration of three operation modes. In the mode of p-CEC, pressure only added at the sample inlet and a separation voltage was simultaneously added between the inlet and outlet. In this mode, an electroosmotic flow caused by

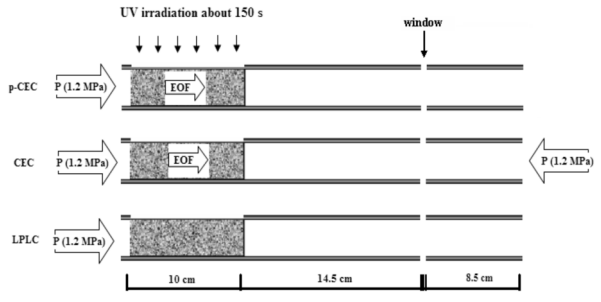

Figure 3. Schematic diagram for three separation modes.

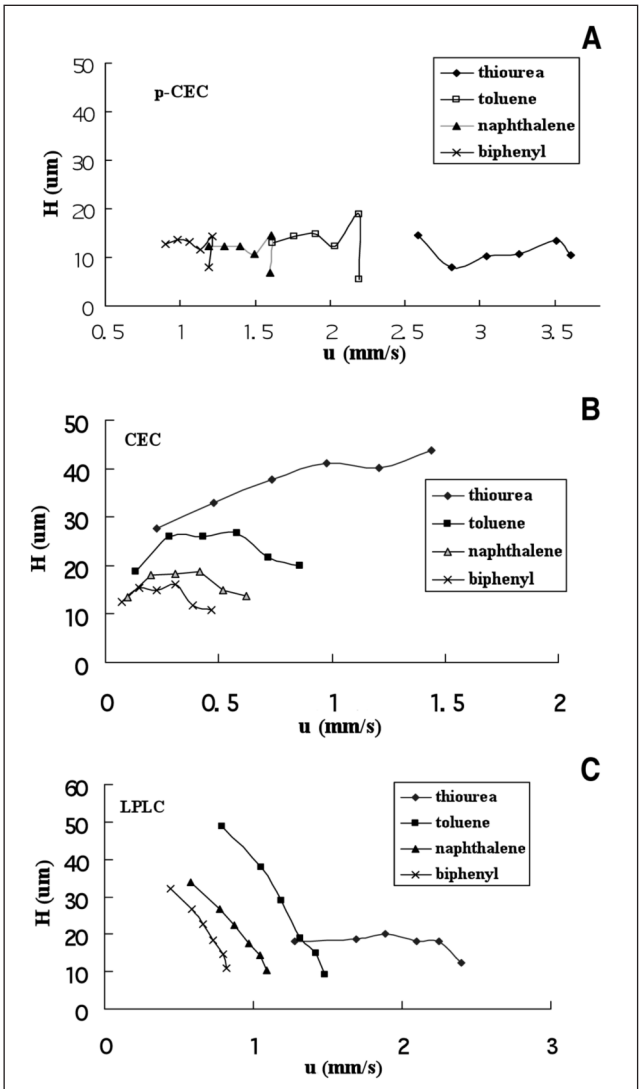

Figure 4. Van Deemter plots for MC 3 in the modes of p-CEC, CEC, and LPLC. Conditions: capillary column, 75 µm i.d., total length = 33 cm, effective length = 24.5 cm, monolith length = 10 cm; injection: 20 kV × 5 s; pH = 8.0; 214 nm; 25°C.

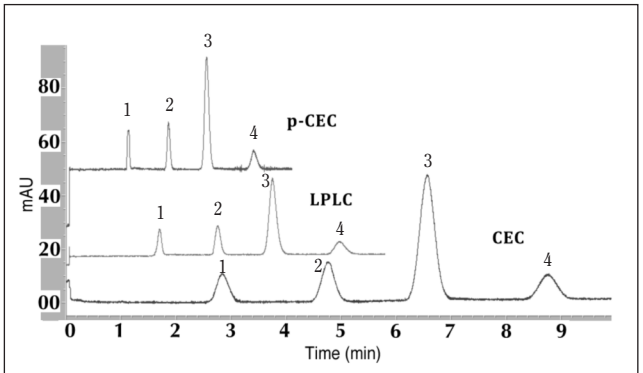

Figure 5. The chromatogram of aromatic compounds on monolithic column in the modes of p-CEC,CEC, and LPLC. p-CEC: 12 bar(inlet) + 30 kV; CEC: 12 bar (inlet and outlet) + 30 kV; LPLC: 12 bar (inlet); peak identification (in order of elution): 1, thiourea; 2, toluene; 3, naphthalene; and 4, biphenyl. Other conditions are the same as Figure 4.

the voltage is superimposed on a pressure-induced hydrodynamic flow. Low pressure and electroosmotic flow was simultaneously created for accelerating the velocity, which could improve the separation efficiency and avoid bubble formation. For the CEC mode, a pressure of 1.2 MPa was applied at both ends, and a separation voltage was simultaneously operated between the capillary inlet and outlet. For the LPLC or low-pressure driven mode, the pressure of 1.2 MPa was only applied at the inlet (the maximum limit of the Agilent ^{3D}CE system is 1.2 MPa). At this time, no high voltage was applied between the capillary inlet and outlet. The Van Deemter curves for the monolithic stationary phase (MC 3) were determined in the modes of p-CEC, CEC, and LPLC. It exhibits similar reversed-phase chromatographic retention mechanisms for the tested neutral compounds for three separation modes. The theoretical plate height values are plotted against the linear velocity of the mobile phase for the four neutral compounds in Figure 4. The plate number N per meter was calculated using the formula $N = 16 \times (t_R/\omega)^2 \times (100/L_{eff})$, where t_R is the retention time, ω is the peak width at the base and L_{eff} is the effective monolith length (10 cm in our experiments). Accordingly, the plate height H was calculated as $H = L_{eff}/N$. From the Van Deemter equation, the difference in column efficiency obtained under three eluent driven modes can be easily seen. For p-CEC mode, the linear velocities corresponded to applied field strengths between 152–909 V/cm (5–30 kV) and an applied gas pressure of 1.2 MPa at the inlet are in the range of 2.59–3.60 mm/s. For CEC mode, the linear velocities corresponded to applied voltage between 5–30 kV are in the range of 0.23–1.44 mm/s. For LPLC mode, the linear velocities corresponded to applied gas pressures at the inlet between 0.6–1.2 MPa are in the range of 1.28–2.40 mm/s. Generally, p-CEC will create the overlapping separation effect of CEC and LPLC theoretically, so it is easily understood that each analyte elute fast in the order of p-CEC, LPLC, and CEC on the prepared monolith with good permeability. A gradual decrease of the theoretical plate heights with the increase of flow rate of mobile phase in LPLC, which could be attributed to the decrease of molecular diffusion. The lowest theoretical plate height for thiourea by three separation modes could be obtained using the optimal conditions with a value of 8.0 µm, 13.0 µm, and 28.0 µm, respectively. Furthermore, the results in the modes of p-CEC and LPLC indicated that the monolith in capillary could still have a good column efficiency even at high linear velocities. But there is one exception for the unretained component in the CEC mode: the theoretical plate height of thiourea increased slightly with the increase of linear velocities of mobile phases. It was probably attributed to the mismatch of local electrosmotic flow velocities between the monolith section and the blank section, which causes a decrease in column efficiency. The typical chromatograms for three separation modes are comparatively shown in Figure 5, which baseline separation of all model compounds could be achieved with a retention time of 3.42 min, 4.97 min, and 8.75 min, respectively, for the last eluted analyte in the modes of p-CEC, CEC, and LPLC.

Reproducibility of column preparation

As an example, MC 3 was selected for the investigation of reproducibility of column preparation. The relative standard deviations of the migration time for each analyte (n = 8) were calculated between 1.18–1.91% in the mode of p-CEC. The results indicated the good run-to-run reproducibility could be easily obtained. The same polymerization mixture was used to prepare another three columns, the results of column-to-column reproducibility was satisfactory with a relative standard deviation of retention time in the range of 6.38–8.60%. It is respectively shown in Figures 6A–6B. Furthermore, the monoliths within the columns could endure the flush under the high pressure for a long time, which showed its enough mechanical strength for the usage. It should be noted that the strength and flexibility of the capillary will be heavily destroyed if more than 60% polyimide-coating of the capillary total length is scraped from its exterior. Gluing some adhesive such as epoxy on the partly scraped capillary surface will avoid the loose of polyimide coating and extend its longevity.

Conclusions

An easy and fast method for the preparation of polymethacrylate-based monolithic columns using UV photo-initiation has been attempted, which enables the suitable control of pore size by simply changing the ratio of 1-propanol to butanediol in the porogenic mixture. Furthermore, the polymerization time was apparently reduced to 150 s due to the usage of high efficient photo-initiator, and it does not require the use of expensive Teflon coated capillaries. These capillaries with short stripe are durable and perform well in our experiments. It provides an alternative not only for the in situ preparation of organic-based monoliths but also for the silica-based monoliths.

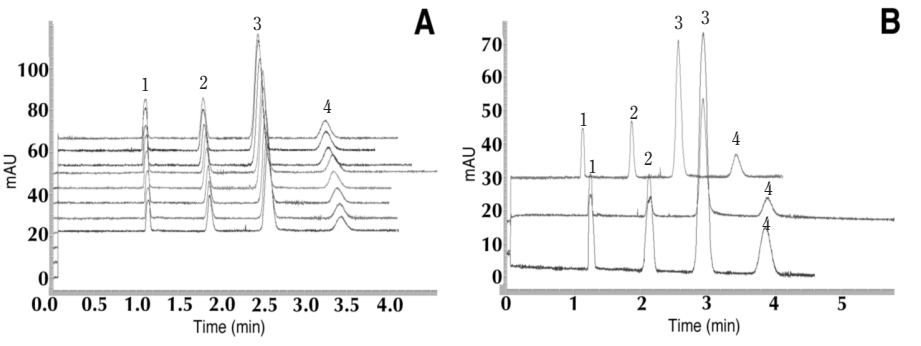

Figure 6. The chromatograms of aromatic compounds on monolithic column in the mode of p-CEC: (A) run-to-run reproducibility and (B) column-to-column reproducibility. Experimental conditions and peak order are the same as Figure 5.

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