Journal of Chromatographic Science 2014;52:201–210
doi:10.1093/chromsci/bmt011 Advance Access publication February 18, 2013

Article

Preparation and Evaluation of Benzyl Methacrylate Monoliths for Capillary Chromatography

Ahmad Aqel[1]*, Zeid A. ALOthman[1,2], Kareem Yusuf[2], A. Yacine Badjah-Hadj-Ahmed[2] and Abdulrahman A. Alwarthan[2]

[1]King Abdullah Institute for Nanotechnology, College of Science, King Saud University, P.O. Box 2454 Riyadh 11451 Saudi Arabia, Kingdom of Saudi Arabia, and [2]Advanced Materials Research Chair, Department of Chemistry, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Kingdom of Saudi Arabia

*Author to whom correspondence should be addressed. Email: ahmad3qel@yahoo.com, aifseisi@ksu.edu.sa

Received 24 December 2012; revised 23 January 2013

This paper describes the comprehensive fabrication of monolithic materials for use as stationary phases in capillary liquid chromatography. Several columns were synthesized in the confines of 320 μm i.d. fused-silica capillaries by single-step *in situ* copolymerization of benzyl methacrylate and ethylene dimethacrylate (EDMA). The polymerization procedure was optimized by varying the reaction time within the range of 0.5–20 h, and by changing the composition contents of the polymeric mixture. The EDMA content showed a predominant influence on the characteristics of the columns and hence, on their chromatographic properties. The optimum value of the thermal initiator corresponded to 5 mg/mL.

Changes of the porous, hydrodynamic properties and morphology of the prepared columns were thoroughly investigated and characterized. Different solvents were used as the mobile phase to demonstrate that the resulting monoliths exhibited good permeability and mechanical stability, whereas swelling and shrinking behaviors were observed and discussed. The efficiency and performance toward different sets of analytes were obtained; mixtures of aromatic hydrocarbons and phenolic compounds were successfully separated and evaluated, and adding tetrahydrofuran to the mobile phase showed improvement in both resolution and peak shapes. The characteristics of the columns were also checked in terms of repeatability and reproducibility.

Introduction

Capillary liquid chromatography has been one of the most important developments in separation and analysis technology in the few last years. Capillary liquid chromatography offers several advantages over conventional normal scales. The advantages include increased chromatographic resolution, higher efficiency, lower consumption of samples and solvents, the ability to analyze and isolate rare compounds of interest, greater mass sensitivity and ease of online connection to a mass spectrometer (1–3). Traditionally, capillary liquid chromatography uses fused silica capillaries prepared with a variety of stationary phases; this technique seems to be very promising in separating a wide variety of analytes in different application fields (4–10). However, its successful development is closely related to the technical challenges associated with column manufacturing.

Monolithic columns have rapidly become highly popular and attracted increasing interest as separation media in all chromatographic methods. The unique structure of the monoliths in addition to their ease of preparation offer improved chromatographic performance and favorable properties for high efficiency (11–14). Hjertén *et al.* (15) first introduced the use of monoliths with capillary liquid chromatography in 1989, and since that time, monolithic stationary phases have been extensively studied for use in capillary liquid chromatography (16–20). The chemical and physical properties of the monolithic polymer depend, in addition to the preparation conditions such as the reaction time and temperature, on the type and concentrations of the monomer, crosslinker, porogenic solvent and initiator. Several monoliths, including methacrylate polymers (21–30), have been widely prepared and studied in the literature. Several advantages are associated with using methacrylate based polymers as monolithic stationary phases, such as high stability in a wide range of mobile phase pH (2–12), fast and simple preparation and easy functionalization. Methacrylate monolithic columns have also various selectivities toward monomers with wide ranging polarities (31, 32).

Poly(benzyl methacrylate-co-bisphenol A dimethacrylate and ethylendimethacrylate) monoliths have been prepared by ultraviolet (UV)-initiated copolymerization in the presence of decanol and cyclohexanol as porogenic solvents and used for capillary electrochromatography (CEC) (33). In this study, more than 30 monolithic columns (including preliminary and reproducibility experiments) were thermally prepared by one-step *in situ* copolymerization of benzyl methacrylate (BMA) and ethylene dimethacrylate (EDMA) in the presence of a suitable porogen mixture (1-propanol, 1,4-butanediol and water) and 2,2'-azobisisobutyronitrile (AIBN). The concentrations of each component, including AIBN initiator, were optimized and compared; the upper and lower limits of percentage compositions were set based on several preliminary experiments; the columns were synthesized at 200 mm length and 320 μm i.d. fused silica tubing. The characterization and physical properties of the prepared monolithic columns were thoroughly investigated. The columns were chromatographically evaluated and applied to the separation of different mixtures, including aromatic hydrocarbons and phenols, by using a modified high-performance liquid chromatography (HPLC) system. Repeatability and reproducibility studies of the prepared columns were also performed.

Experimental

Chemicals and columns

Formic acid, benzene, naphthalene, 4-aminophenol, *m*-nitrophenol and other chemicals used in the experiments were of analytical grade and purchased from BDH (Lutterworth, UK). 2-Naphthol and anthracene were provided by Merck

(Schuchardt, Germany). Polystyrene standard with molecular mass of 140,000 was purchased from Aldrich (Steinheim, Germany). Standard solutions were prepared as follows: aromatic compounds were dissolved in HPLC-grade hexane to provide 30 μg/mL of benzene and 60 μg/mL of naphthalene and anthracene solutions. All phenol compounds were dissolved in purified water at levels of 160 μg/mL for 4-aminophenol and 60 μg/mL for *m*-nitrophenol and 2-naphthol.

HPLC-grade acetonitrile, methanol, tetrahydrofuran and hexane were purchased from BDH. The purified water used throughout all experiments was prepared on a Milli-Q system (Advantage with Elix; Millipore, Molsheim, France) and then filtered with 0.2 μm nylon membrane filter from Whatman (Maidstone, UK). Before use, the mixed mobile phases were filtered using a vacuum glass filtration system through the same nylon membrane filters and degassed ultrasonically for 30 min.

Fused silica tubing (0.32 mm i.d.) was purchased from Restek (Bellefonte, PA). The chemicals used for monolithic materials preparation in this work were purchased from Aldrich as follows: 98% 3-(trimethoxysilyl)propyl methacrylate (TMSM), 98% EDMA used as crosslinker, 98% BMA as monomer and AIBN as thermal initiator. Toluene, hydrochloric acid, sodium hydroxide, 1-propanol and 1,4-butandiol were acquired from BDH. All chemicals were used without further purification.

Preparation of capillary monolithic columns

To clean and activate the inner capillary surface, the fused-silica tubing (0.32 mm i.d.) was rinsed first with 1.0M NaOH solution for 10 min and left for 30 min with the same solution, then rinsed with water and dried in air for 10 min, twice for each step. The tubing was then flushed with 1.0M HCl for 20 min and dried with air for 5 min. After that, the capillary was rinsed with toluene for 10 min, flushed with 20% TMSM solution in toluene for 10 min and left with the same solution for 4 h, then rinsed with toluene for 10 min and dried with air for 5 min. The activated capillary was then cut into four pieces (200 mm each in length) with a razor blade. In this study, a series of monoliths was prepared with different compositions of the polymerization mixtures (percentage, v/v), which are described in Table I.

Table I
Composition of the Polymerization Mixtures (Percentage v/v) Used for the Preparation of Capillary Monolithic Columns

Column	BMA	EDMA	1-Propanol	1,4-Butandiol	Water	Porogen content	AIBN (mg/mL)
C1	10	10	48	24	8	80	10
C2	15	15	42	21	7	70	10
C3	20	20	36	18	6	60	10
C4	25	25	30	15	5	50	10
C5	10	15	45	22.5	7.5	75	10
C6	20	15	39	19.5	6.5	65	10
C7	25	15	36	18	6	60	10
C8	20	10	42	21	7	70	10
C9	10	20	42	21	7	70	10
C10	15	10	45	22.5	7.5	75	10
C11	15	20	39	19.5	6.5	65	10
C12	15	25	36	18	6	60	10
C13	30	20	30	15	5	50	10
C14	20	15	42	23	0	65	10
C15	20	15	36	16	13	65	10
C16	20	15	39	19.5	6.5	65	20
C17	20	15	39	19.5	6.5	65	15
C18	20	15	39	19.5	6.5	65	5
C19	20	15	39	19.5	6.5	65	3
C20	20	15	39	19.5	6.5	65	2

The monomer mixture and the porogen solvents were mixed into a homogenous solution, then sonicated and purged with helium gas for 5 min. Each capillary column was filled with the corresponding reactant solution and both ends were plugged with a piece of rubber. The polymerization was performed at 70°C for varying polymerization times (ranging from 0.5 to 20 h) to obtain rigid macroporous polymer monoliths. After the polymerization, the seals were removed; the resulting columns were connected to an HPLC pump and thoroughly washed with acetonitrile to remove the unreacted materials and porogenic solvents. The schematic procedure showing the preparation of the reaction of monolithic columns is demonstrated in Figure 1.

Porosity and bed permeability

The total column porosity (ε_T) is an important parameter for column evaluation. In the literature, various methods are available to measure ε_T, such as the flow method (34), the

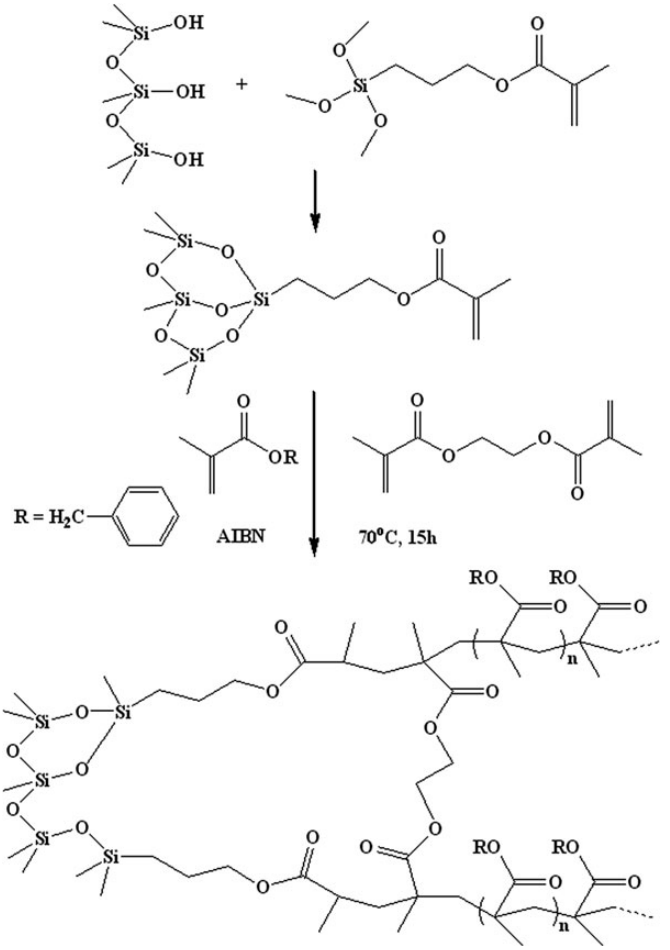

Figure 1. Schematic representation of the preparation procedure of monolithic columns.

conductivity method (35) and the gravimetric method (36). In this study, the flow method was used to evaluate ε_T. This method is based on the determination of retention volume of an unretained marker (uracil was used in this work) and the geometrical volume of the empty column (because it can be considered to be a long cylindrical tube), after correction for extra-column volume contributions, depending on the tubes used for connection. Because of the presence of two types of pores in monolithic continuous separation media, both through pores (corresponding to the external porosity, ε_e) and mesopores (internal porosity, ε_i) should be characterized. The retention data of a polystyrene standard with 140,000 molecular mass using pure tetrahydrofuran as the mobile phase was used for this purpose (37).

The permeability (K^o) of a porous medium is a measure of its capacity to transmit a fluid driven by an imposed pressure drop across the column. Darcy's law links the solvent viscosity, pressure drop and ε_T to K^o (38). Also, the Hagen–Poiseuille equation (39) provides the pressure drop in a fluid flowing through a long cylindrical pipe; this physical law has been used to calculate the average diameter of monolithic channels (macropores). The average velocity of the mobile phase over the channel section (v) is obtained from integration of the momentum transport equation that is derived on the basis of Navier–Stokes equation.

Characterization of the monolithic columns

After chromatographic experiments were finished, the monolithic rods in the tubes were washed and cut into small pieces, then dried. The dried columns and monolith materials were subjected to optical microscopy, scanning electron microscopy (SEM), Fourier transform infrared (FT-IR) spectroscopy and thermogravimetric analysis (TGA) characterization. The optical microscope images were obtained using a Micromaster Fisher Scientific optical microscope (G2009-A 702-042; Shanghai, China), typically with 100-fold magnification. The pore properties and microscopic morphology of the polymers were characterized by a Jeol (JSM-6380LA) analytical scanning electron microscope (Tokyo, Japan) at 5 kV after the column samples were splattered with platinum.

The FT-IR spectra were recorded on a Thermo Nicolet 6700 FT-IR spectrophotometer (Madison, WI). The resultant porous monolith was removed from the vial, then crushed. The powder was immersed in 1 mL (50:50, v/v) acetonitrile–water and shaken for 10 min to remove any soluble compounds; these processes were repeated twice. After vacuum drying, the monolithic powder was thoroughly mixed with KBr in an approximate ratio of 1:20 and pressed into a pellet. The FT-IR spectrum was then recorded at a resolution of 4 cm^{-1} over the full mid-IR range (400–4,000 cm^{-1}). TGA was conducted for the monolithic material, prepared in the same way, with a Mettler Toledo TGA/DSC 1 Stare System (Schwerzenbach, Switzerland). The sample was heated from 25 to 400°C with a heating rate of 10°C/min.

HPLC modification and conditions

All analyses were performed with a Shimadzu HPLC system (Kyoto, Japan), including a pump (LC-6A), a Rheodyne 7125 manual injector, a UV detector (SPD-6A) and a C-R6A integrator. The detector was set at different wavelengths according to the analyzed compounds. Acetonitrile–water solutions with or without acid additives at different ratios were used as the mobile phase. All solutions were filtered through 0.2 μm nylon membrane filter from Whatman prior to use. All experiments were conducted at room temperature.

The HPLC system was successfully modified for the use of micro-columns. The detector was equipped with a 2 cm path length with 320 μm i.d. and 1.6 μL volume homemade cell. A simple system was constructed for splitting both mobile phase flow and injection volume, controlled by a custom-built adjustable flow splitter based upon a T-derivation piece connected between the injector and the column. In this configuration, both column and split flows could be adjusted by changing the inner diameter or length of the splitting PEEK tubing (Varian, Palo Alto, CA). In the present work, the selected splitting ratio was 1:200 and the actual injection volume was fixed at 5 nL.

Results

Preparation and optimization of the monolithic columns

A series of monolithic columns was prepared with different monomer, crosslinker, porogen and initiator percentage compositions, which are summarized in Table I. According to preliminary studies, the BMA monomer (percentage v/v) in the polymerization mixture was set in the range of 10–30%, whereas the EDMA crosslinker (percentage v/v) was set in the range of 10–25%. On the other hand, the AIBN initiator content was varied between 2 and 20 mg/mL. The content of each component of the ternary porogenic mixture, 1-propanol, 1,4-butanediol and water, was in the range of 30–48, 15–24 and 5–13%, respectively.

Figure 2 shows the influence of polymerization time on the permeability of Column C6 using acetonitrile as eluent at a 5 μL/min flow rate in the range of 0.5–20 h. Fifteen hours were set for the next experiments, which corresponds to a complete polymerization reaction.

Characterization of the monolithic columns

Hydrodynamic properties and porosity

Among the four solvents used, the highest measured back-pressure using the same flow rate corresponded to tetrahydrofuran. This

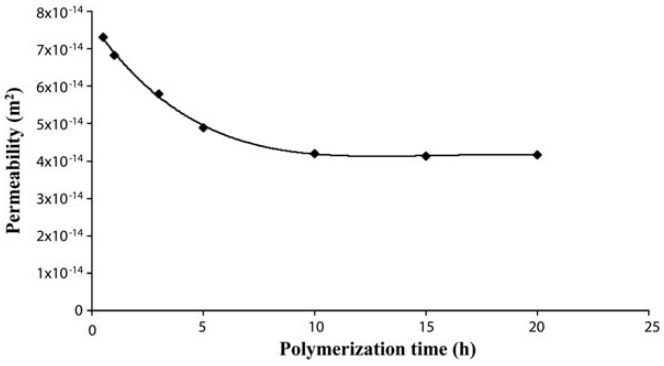

Figure 2. Dependence of hydrodynamic permeability on polymerization time for Monolithic Column C6.

result confirms that tetrahydrofuran causes a distinctive swelling of the polymer rod in contact with this organic solvent, inducing a decrease of the permeability and a higher back-pressure than expected.

Numerical values for the permeability of the monolithic capillary columns were determined using acetonitrile, methanol, water and tetrahydrofuran, which were passed through the columns at a volumetric flow rate of 5 μL/min (corresponding to a 1.04 mm/s linear flow velocity) and a temperature of 25°C (room temperature). The pressure drops were measured and the permeabilities were calculated and summarized in Table II. The permeabilities with methanol and water are higher than with acetonitrile and tetrahydrofuran in all cases.

Uracil was injected as a non-retained marker with acetonitrile–water as the mobile phase (50/50, v/v) to determine the total porosity of all prepared monolithic columns, the values of which are reported in Table II. The total porosity for all columns ranged from 57% (C4) to 80% (C10), except for C1, which corresponded to 87% (the porosities of all other columns were between the values for Columns C1 and C4).

1-Propanol, 1,4-butanediol and water were used as ternary porogenic solvents; a higher fraction of 1,4-butanediol or water induces smaller pores in the monolithic bed, which means lower total porosity and permeability, whereas 1-propanol has an opposite effect. In addition, studying the effect of the content of AIBN initiator showed that the lowest total porosity and permeability values were achieved when the AIBN content was 5 mg/mL (C18); this concentration corresponds to the optimum value of the thermal initiator.

For more accurate characterization of the performance of the capillary columns, the pore-size distribution in monoliths was characterized by the determination of the external, ε_e, and internal, ε_i, porosities. The results suggest that the external porosities of the prepared monolithic columns lie in the range of 0.55–0.59, whereas the internal porosities are in the range of 0.15–0.19.

Table II
Porosities and Permeabilities of all Prepared Columns Using Acetonitrile, Methanol, Water and Tetrahydrofuran as Mobile Phases

Column	Porosity (ε_T)	Permeabilities (K^o) (m^2)			
		Acetonitrile	Methanol	Water	Tetrahydrofuran
C1	0.87	1.03×10^{-13}	1.19×10^{-13}	1.84×10^{-13}	8.91×10^{-14}
C2	0.78	5.19×10^{-14}	6.42×10^{-14}	9.95×10^{-14}	4.50×10^{-14}
C3	0.66	2.98×10^{-14}	3.54×10^{-14}	5.50×10^{-14}	2.58×10^{-14}
C4	0.57	2.39×10^{-14}	3.08×10^{-14}	4.82×10^{-14}	2.09×10^{-14}
C5	0.79	5.53×10^{-14}	6.73×10^{-14}	1.05×10^{-13}	4.76×10^{-14}
C6	0.72	4.11×10^{-14}	4.82×10^{-14}	7.57×10^{-14}	3.81×10^{-14}
C7	0.71	3.74×10^{-14}	4.72×10^{-14}	7.34×10^{-14}	3.29×10^{-14}
C8	0.76	4.65×10^{-14}	5.80×10^{-14}	9.01×10^{-14}	4.18×10^{-14}
C9	0.74	4.27×10^{-14}	5.30×10^{-14}	8.24×10^{-14}	3.85×10^{-14}
C10	0.80	5.65×10^{-14}	7.11×10^{-14}	1.10×10^{-13}	5.15×10^{-14}
C11	0.70	3.58×10^{-14}	4.55×10^{-14}	7.00×10^{-14}	3.16×10^{-14}
C12	0.69	3.45×10^{-14}	4.44×10^{-14}	6.89×10^{-14}	3.08×10^{-14}
C13	0.65	3.01×10^{-14}	3.95×10^{-14}	5.97×10^{-14}	2.70×10^{-14}
C14	0.74	4.23×10^{-14}	5.23×10^{-14}	8.09×10^{-14}	3.73×10^{-14}
C15	0.67	3.16×10^{-14}	4.21×10^{-14}	6.38×10^{-14}	2.90×10^{-14}
C16	0.79	5.47×10^{-14}	6.68×10^{-14}	1.05×10^{-13}	4.88×10^{-14}
C17	0.77	4.90×10^{-14}	6.15×10^{-14}	9.39×10^{-14}	4.33×10^{-14}
C18	0.68	3.32×10^{-14}	4.31×10^{-14}	6.55×10^{-14}	2.96×10^{-14}
C19	0.71	3.73×10^{-14}	4.79×10^{-14}	7.36×10^{-14}	3.31×10^{-14}
C20	0.75	4.57×10^{-14}	5.51×10^{-14}	8.56×10^{-14}	3.91×10^{-14}

Morphology of monoliths

Several experiments, including optical and SEM microscopy, FT-IR spectroscopy and TGA, were conducted to evaluate the morphology and surface property of the monoliths.

Some optical micrographs and SEM images of these monolithic columns are shown in Figure 3. As shown in the figure, the formed monoliths were well attached to the surface of the capillaries. A cross section of intact and homogeneous column beds is also shown. The microglobules that appear in the figures have an approximate diameter in the range of 1–2 μm.

The primary observed frequencies on the FT-IR spectrum (Figure 4A) can be attributed as follows: 748 cm^{-1}, aromatic C–H bending; 1,147 cm^{-1}, ester C–O stretching; 1,257 cm^{-1}, aromatic C–H bending; 1,388 cm^{-1}, CH_3 symmetrical bending; 1,457 cm^{-1}, aromatic C = C stretching; 1,730 cm^{-1}, ester C = O stretching; 2,952 cm^{-1}, CH_3 asymmetric stretching; 3,017 cm^{-1}, aromatic C–H stretching.

Typical TGA data shown in Figure 4B indicate that the porous poly(benzylmethacrylate) monolith does not undergo any significant thermal degradation below 230°C, showing a relatively high degree of thermal stability.

Separation and efficiency of monolithic columns

In the HPLC procedures, the effect of the flow rate and composition of the mobile phase on the retention time of the analyte (t_R, min), the width at half peak ($w_{0.5}$, min), the resolution between two neighboring peaks (R_s) and the number of theoretical plates (N) were studied in all cases. The sample injection volume was 5 nL.

Separation of aromatic compounds

The prepared columns were successfully applied to achieve the reproducible separation of three aromatic hydrocarbons (benzene, naphthalene and anthracene) with good separation efficiency using different experimental conditions. As an example, Figure 5A shows the separation of the three components on Column C6 at a 6 μL/min flow rate in 24 min and a 260 nm detection wavelength using a binary acetonitrile–water (50:50, v/v) mobile phase. By varying the flow rate of the mobile phase, the best performance was obtained for naphthalene at 4 μL/min, which corresponded to a column efficiency of 8,200 plates/m. By using the same chromatographic conditions, the other prepared columns gave 5,520, 8,600 and 10,180 plates/m for C1, C3 and C4, respectively.

Adding tetrahydrofuran for the composition of the mobile phase indicates that both peak shape and resolution of the three aromatics were improved. A comparison of the two chromatograms in Figures 5A and 5B obviously shows a noticeable influence when 10% of tetrahydrofuran was added to the mobile phase. The separation was achieved in approximately 8 min less, whereas the plate number slightly increased by approximately 100 units.

Fast separation of phenol compounds

Some of the prepared columns were also applied for the separation of three phenolic compounds (aminophenol, nitrophenol and 2-naphthol). A chromatogram of the phenol separation is shown in Figure 5C. This example shows that the three compounds were completely separated in less than 5 min, with an

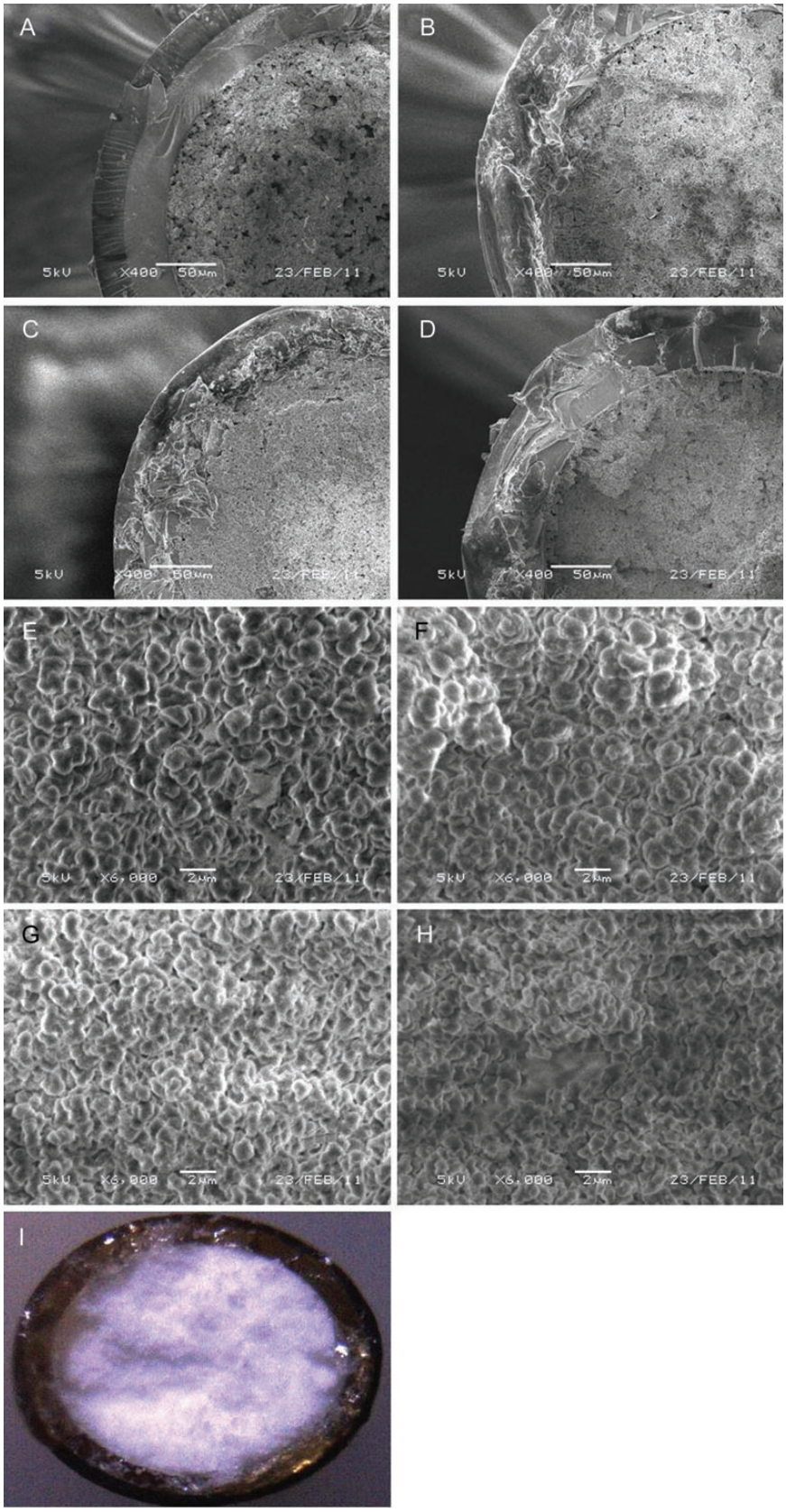

Figure 3. Cross-section SEM images: Column C1 (A); Column C3 (B); Column C4 (C); Column C6 (D). Bulk region SEM images: Column C1 (E); Column C3 (F); Column C4 (G); Column C6 (H). Column C6 photograph (I).

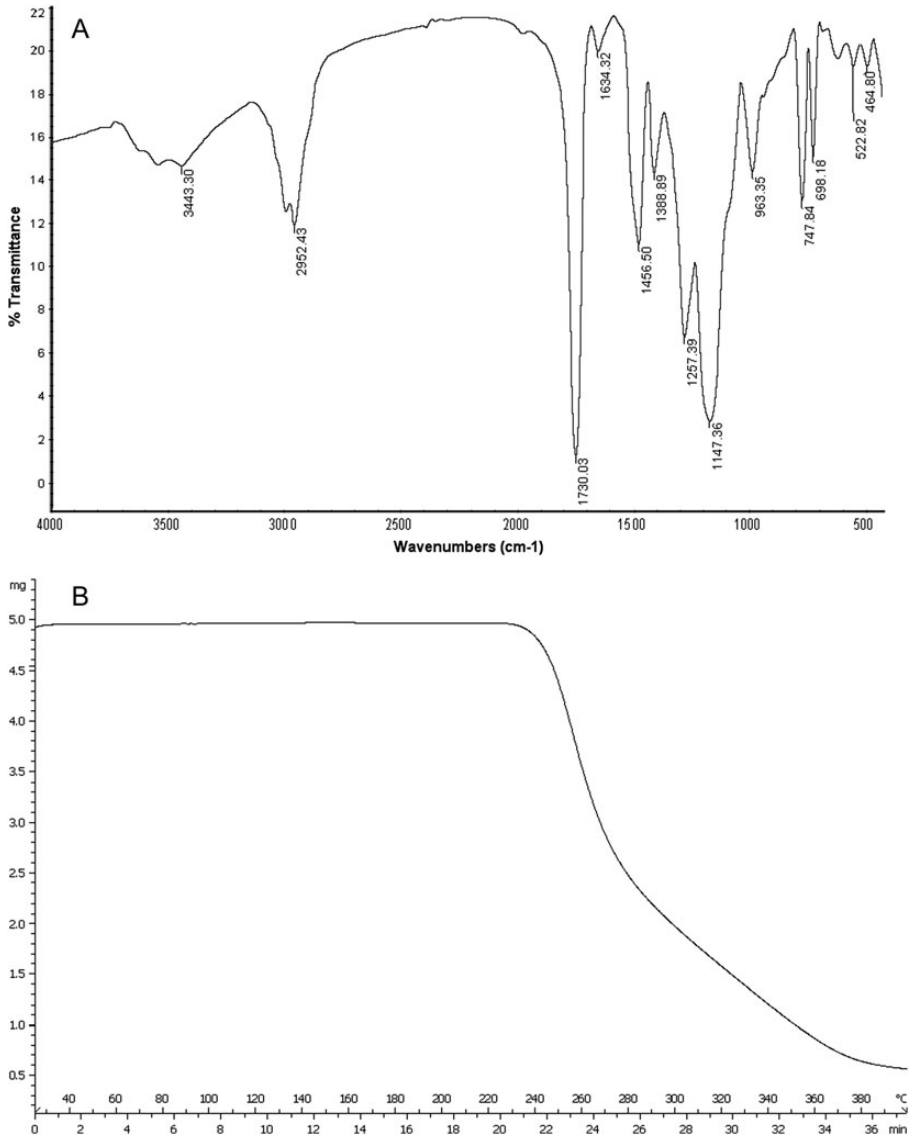

Figure 4. Infrared spectra of the C6 monolithic stationary phase (A); thermal behavior of the C6 monolithic polymer (B); 5 mg of the sample was heated from 25 to 400°C with a heating rate of 10°C/min.

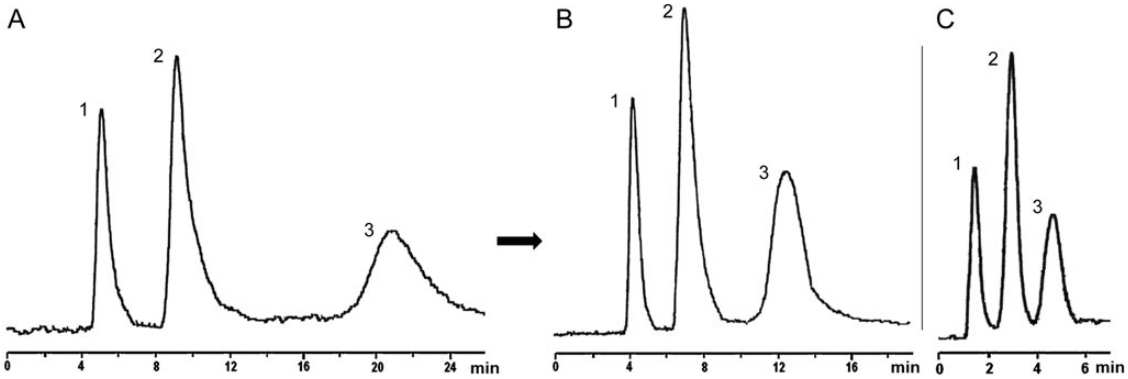

Figure 5. Chromatograms on Column C6: separation of aromatic hydrocarbons with a binary acetonitrile–water (50:50, v/v) mobile phase, where 1 is benzene, 2 is naphthalene and 3 is anthracene (A); separation of aromatic hydrocarbons with a ternary acetonitrile–water–tetrahydrofuran (45:45:10, v/v) mobile phase, where 1 is benzene, 2 is naphthalene and 3 is anthracene (B); separation of phenols using acetonitrile–water (40:60, v/v) with 1% formic acid mobile phase, where 1 is aminophenol, 2 is nitrophenol and 3 is 2-naphthol (C).

Table III
Repeatability and Reproducibility of Preparation of Monolithic Columns Expressed as RSD on t_M, t_R, H, R_s, ε_T and K^o

Repeatability (RSD, %)

Parameters values	Parameter	Run-to-run ($n = 5$)	Day-to-day ($n = 5$)
t_M (min)	3.50	0.31	1.94
Benzene			
t_R (min)	5.66	0.33	2.94
H (mm)	0.17	0.65	5.87
Naphthalene			
t_R (min)	10.07	0.22	3.27
H (mm)	0.12	0.44	6.54
R_s (Ben-Nap)	5.2	0.84	3.66
Anthracene			
t_R (min)	21.99	1.19	2.99
H (mm)	0.16	2.38	5.96
R_s (Nap-Ant)	5.8	2.03	2.38

Reproducibility (RSD, %)

Parameters	Column-to-column ($n = 3$)	Batch-to-batch ($n = 3$)
ε_T	3.1	5.8
K^o	6.4	10.2

acceptable resolution ($R_s > 1.5$) at a flow rate of 10 μL/min and detection wavelength of 254 nm using acetonitrile–water (40:60, v/v) with 1% formic acid mobile phase. By using other columns with lower porosities and permeabilities, such as C3 and C4, the same analytes were separated in more than 12 min with higher resolutions.

The plate height, H, was calculated for each constituent at different flow rates. For all aromatic hydrocarbons and phenolic compounds, the height equivalent to a theoretical plate remains within the range of 0.1–0.3 mm on range of the the investigated flow rate, which was 4–10 μL/min.

Repeatability and reproducibility

Repeatability and reproducibility studies of the prepared columns were also performed. Run-to-run and day-to-day repeatability were investigated in terms of t_R, H and R_s, whereas column-to-column and batch-to-batch reproducibility were evaluated by a comparison of ε_T and K^o of the columns prepared according to the same procedure as C6. Using acetonitrile–water (50:50, v/v) as the mobile phase at a flow rate of 6 μL/min, the repeatability levels based on run-to-run and day-to-day injections were less than 2.4% ($n = 5$) and 6.6% ($n = 5$), whereas the reproducibility values based on column-to-column preparation from the same batch of polymerization solution and batch-to-batch preparation of monoliths were 6.4% ($n = 3$) and 10.2% ($n = 3$) relative standard deviation (RSD), respectively. The results are listed in Table III.

Discussion

The composition of the polymerization mixture has a significant effect on the chromatographic performance of the yielded monolith and should be carefully optimized.

The polymerization procedure was optimized by varying several factors and the morphology and hydrodynamic properties of all prepared columns were studied and compared. The effect of polymerization time was also investigated and set according to experimental results. A trend to lower permeabilities is observed when increasing polymerization duration while rapidly approaching values close to those found at a complete polymerization reaction. Fifteen hours has been set for the next experiments; this polymerization time corresponds to a maximum conversion of monomeric precursors.

To set the upper and lower limits of percentage compositions, the composition percentages prepared in Table I were set based on preliminary experiments, in which some of the factors can only be varied over a restricted area. These experiments show that when the total contents of the monomer and crosslinker are below 20%, the porosity and permeability were excessive, and hence, the monolithic capillary column showed poor chromatographic performance. When the total contents of the monomer and crosslinker were above 50%, the backpressure exceeded 5,000 psi due to the low porosity. When the total contents of the monomer and crosslinker were more than 60%, the polymeric mixture was inhomogeneous.

The influence of the content of AIBN initiator was also investigated; when higher than 20 mg/mL, the generated monolith was found to have high porosity and permeability, whereas AIBN contents lower than 2 mg/mL led to an incomplete polymerization. The porogenic mixture was a ternary solution composed of 1-propanol, 1,4-butanediol and water; the range of its composition was set as a compromise to provide a clear and homogenous monolith matrix and to provide good column resolutions for the separation of aromatic and phenolic mixtures. However, higher contents of 1,4-butanediol and water provided an inhomogeneous polymerization mixture, whereas lower contents produced high porosity and permeability, and hence, poor resolution.

Varying the ratio of each component of the polymerization mixture generates monolithic columns with different physical properties and chromatographic behavior. All column preparations were controlled by varying six factors (percentage v/v): concentration of monomer (BMA), concentration of crosslinker (EDMA), concentration of each solvent in porogenic mixture (1-propanol, 1,4-butanediol and water) and concentration of AIBN initiator.

Polymer monolithic packing materials are sensitive to solvent changes. Good polymer solvents such as tetrahydrofuran often

lead to swelling, whereas other polymer non-solvents like methanol or water may result in an irreversible shrinking of the packing bed. The results confirm that tetrahydrofuran causes swelling of the polymer rod, which induces a decrease in the permeability of the columns. However, this observation is not relevant for HPLC because tetrahydrofuran is not commonly used as the mobile phase, unlike acetonitrile, methanol or water.

The permeability values confirm that some swelling of the stationary phase occurs with acetonitrile, but to a much lesser degree than with tetrahydrofuran, indicating that these two solvents induce a restriction of the accessible pore volume (40, 41).

Total porosity results confirm that, as expected and as mentioned by previous works (26, 42), increasing the porogen ratio in the polymerization mixture corresponding to the BMA monomer and the EDMA crosslinker induced larger pores, whereas the total porosity and permeability increased. Furthermore, it is noticeable that the content of EDMA crosslinker plays a more predominant role than the content of BMA monomer regarding porosity, permeability, specific area and column performance. This effect is clearly observed when comparing Columns C5/C10, C6/C11, C7/C12 and C8/C9.

The composition of the porogenic mixture and content of AIBN initiator also play an important role in characteristics of the column. In contrast to the effect of 1-propanol, increasing the percentage of 1,4-butanediol or water solvents induces smaller pores, which means lower total porosity and permeability; this influence is clearly observed when comparing Columns C6, C14 and C15. On the other hand, a lower AIBN content induces a decrease in the polymerization reaction ratio, whereas if its content is higher than 5 mg/mL, the number of active polymerization sites increases and the average polymer chain length decreases, affecting the crosslinking ratio and the performance of the column.

The external porosity, corresponding to the volume of the through-pores in the monolithic column, was determined directly from the elution volume of polystyrene standard (140,000 molecular weight in this study) using tetrahydrofuran as solvent. The internal porosity, corresponding to the mesopore volume of the monolithic column, was calculated as the difference between the total porosity, ε_T, and the external porosity. The measured values agree with values found in literature (31, 43, 44).

The morphology of the monolithic bed is one of the key factors affecting the separation capability of the capillary column. To obtain high efficiency and stability, homogeneity and rigidity of the polymer bed are needed (45). Therefore, it is important to investigate and control the parameters governing morphology during the synthesis of the monoliths. The morphology and surface property of the monoliths were evaluated to characterize the stationary phases.

SEM pictures demonstrate that the procedure for synthesis renders permeable monoliths with a uniform structure and porosity, completely filling the capillary tubings and bonding to the surface of the inner capillary walls through the TMSM linker. Optical microscopy examinations (at a magnification of 100 times) revealed that the continuous beds were homogeneous and also close to the wall, indicating that the synthesis was properly made.

For further characterization, the monoliths were examined by FT-IR spectroscopy to identify the organic functional groups of the polymeric phase. The FT-IR spectrum shows the presence of the primary groups corresponding to the ester functional group of the methacrylate, in addition to the alkyl substituents. On the other hand, the absence of C = C and =C–H stretching bands at 1,650 and 3,090 cm^{-1}, respectively, confirms the completion of the polymerization reaction. These results demonstrated that the proposed polymerization conditions were suitable to produce a homogenous and continuous monolith resulting from the reaction of EDMA and BMA.

TGA data showed a relatively high degree of thermal stability; this excellent thermal stability allows these monolithic columns to operate routinely at temperatures up to 200°C, and even up to 220°C for short periods of time, without observing any deterioration of their properties. When the temperature is raised above 230°C, the curve rapidly dropped and stabilized over 370°C, indicating a complete degradation of the monolith. The thermal stability of the porous monolith is an important characteristic when used either in CEC or gas chromatography, because of the voltages and temperatures involved in these techniques.

The column efficiency is the key factor for column evaluation; several factors like morphology, specific surface area, accessibility of the surface and chemical structure have to be considered to determine the performance of the column. The efficiencies of the columns used in this study were calculated at room temperature and the Van Deemter curves were plotted for selected columns.

The BMA monomer has benzyl groups, which are supposed to provide π–π interactions between the stationary phase and aromatic analytes such as polycyclic aromatic hydrocarbons and phenol compounds. Some of the prepared capillary columns were successfully applied for the separation of a mixture of aromatic hydrocarbons using different experimental conditions. Next, the effect of adding tetrahydrofuran for the composition of mobile phase on the separation of aromatic hydrocarbons was examined. The results indicated that both peak shape and resolution of the three aromatics were improved by adding tetrahydrofuran to the mobile phase.

Column C6, which has relatively higher porosity and permeability than the other prepared, columns was also used for the fast separation of three phenolic compounds (aminophenol, nitrophenol and 2-naphthol). The plate height, *H*, was calculated for each phenol constituent at different flow rates. For the three phenolic compounds, the height equivalent to a theoretical plate remains almost constant on the investigated flow rate range. This fact indicates that the column performance is slightly affected by increasing the mobile phase velocity, which has been previously established for capillary columns in both liquid and gas chromatography (46–48) for faster analysis without reduction of their separation ability.

To evaluate the qualitative and quantitative performances of the prepared columns, the repeatability and reproducibility of columns preparation were assessed through the RSD of selected parameters for the three aromatics used as model analytes. The results prove that the prepared capillary monolithic columns offer several advantages in terms of run-to-run and day-to-day repeatability and column-to-column and batch-to-batch reproducibility.

Conclusion

More than 30 monolithic capillary columns were reproducibly prepared by single-step *in situ* free radical polymerization of BMA in fused silica tubing using different compositions. The procedure proved to be rapid, simple and efficient; it needed only small quantities of solvents and reagents. The prepared monoliths were characterized by optical microscopy, SEM, FT-IR and TGA. Their porosity and permeability were also determined and compared to the morphology parameters obtained from the micrographs. The capillary columns were successfully applied to achieve reproducible separation of different kinds of compounds, such as aromatic hydrocarbons and phenols, with good separation efficiency. The chromatographic performances were satisfactory and confirmed that capillary columns offer several advantages over packed conventional columns in liquid chromatography.

Acknowledgments

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding the work through the Research Group No RGP-VPP-043.

References

1. Gu, C., He, J., Jia, J., Fang, N., Simmons, R., Shamsi, S.A.; Surfactant-bound monolithic columns for separation of proteins in capillary high performance liquid chromatography; *Journal Chromatography A*, (2010); 1217: 530–539.
2. Song, Y., Liu, Y.M.; Quantitation of cardioexcitatory Asn-D-Trp-Phe-NH2 diastereomers in Aplysia's central nervous system by nanoscale liquid chromatography-tandem mass spectrometry; *Journal of Mass Spectrometry*, (2008); 43: 1285–1290.
3. Tastet, L., Schaumloffel, D., Bouyssiere, B., Lobinski, R.; Identification of selenium-containing proteins in selenium-rich yeast aqueous extract by 2D gel electrophoresis, nanoHPLC-ICP MS and nanoHPLC-ESI MS/MS; *Talanta*, (2008); 75: 1140–1145.
4. Bailón-Pérez, M.I., García-Campaña, A.M, Del Olmo-Iruela, M., Gámiz-Gracia, L., Cruces-Blanco, C.; Trace determination of 10 β-lactam antibiotics in environmental and food samples by capillary liquid chromatography; *Journal Chromatography A*, (2009); 1216: 8355–8361.
5. Petrovic, M., Farré, M., De Alda, M.L., Perez, S., Postigo, C., Köck, M., *et al.*; Recent trends in the liquid chromatography-mass spectrometry analysis of organic contaminants in environmental samples; *Journal of Chromatography A*, (2010); 1217: 4004–4017.
6. ALOthman, A., Aqel, A., Alharbi, M., Yacine Badjah-Hadj-Ahmed, A., Alwarthan, A.; Fast chromatographic determination of caffeine in food using a capillary hexyl methacrylate monolithic column; *Food Chemistry*, (2012); 132: 2217–2223.
7. Qian, K., Tang, T., Shi, T., Wang, F., Li, J., Cao, Y.; Residue determination of glyphosate in environmental water samples with high-performance liquid chromatography and UV detection after derivatization with 4-chloro-3,5-dinitrobenzotrifluoride; *Analytica Chimica Acta*, (2009); 635: 222–236.
8. Issaq, H.J., Chan, K.C., Blonder, J., Ye, X., Veenstra, T.D., Separation detection and quantitation of peptides by liquid chromatography and capillary electrochromatography; *Journal of Chromatography A*, (2009); 1216: 1825–1837.
9. D'Orazio, G., Cifuentes, A., Fanali, S.; Chiral nano-liquid chromatography-mass spectrometry applied to amino acids analysis for orange juice profiling; *Food Chemistry*, (2008); 108: 1114–1121.
10. Dakna, M., He, Z., Yu, W.C., Mischak, H., Kolch, W., Technical bioinformatical and statistical aspects of liquid chromatography-mass spectrometry (LC-MS) and capillary electrophoresis-mass spectrometry (CE-MS) based clinical proteomics: A critical assessment; *Journal of Chromatography B*, (2009); 877: 1250–1258.
11. Unger, K.K, Skudas, R., Schulte, M.M.; Particle packed columns and monolithic columns in high-performance liquid chromatography—Comparison and critical appraisal; *Journal of Chromatography A*, (2008); 1184: 393–415.
12. Wu, R., Hu, L., Wang, F., Ye, M., Zou, H.; Recent development of monolithic stationary phases with emphasis on microscale chromatographic separation; *Journal of Chromatography A*, (2008); 1184: 369–392.
13. Buszewski, B., Szumski, M.; Study of bed homogenity of methacrylate-based monolithic columns for micro-HPLC and CEC; *Chromatographia*, (2004); 60: S261–S267.
14. Yang, C., Ikegami, T., Hara, T., Tanaka, N.; Improved endcapping method of monolithic silica columns; *Journal of Chromatography A*, (2006); 1130: 175–181.
15. Hjertén, S., Liao, J.L., Zhang, R. High performance liquid chromatography on continuous polymer beds; *Journal of Chromatography*, (1989); 473: 273–275.
16. Svec, F., Fréchet, J.M.J.; Continuous rods of macroporous polymer as high-performance liquid chromatography separation media; *Analytical Chemistry*, (1992); 54: 820–822.
17. Minakuchi, H., Nakanishi, K., Soga, N., Ishizuka, N., Tanaka, N.; Octadecylsilylated porous silica rods as separation media for reversed-phase liquid chromatography; *Analytical Chemistry*, (1996); 68: 3498–3501.
18. Coufal, P., Čihák, M., Suchánková, J., Tesařová, E., Bosáková, Z., Štulík, K.; Methacrylate monolithic columns of 320 mm I.D. for capillary liquid chromatography; *Journal of Chromatography A*, (2002); 946: 99–106.
19. Holdšvendová, P., Coufal, P., Suchánková, J., Tesaøová, E., Bosáková, Z.; Methacrylate monolithic columns for capillary liquid chromatography polymerized using ammonium peroxodisulfate as initiator; *Journal of Separation Science*, (2003); 26: 1623–1628.
20. Grafnetter, J., Coufal, P., Tesařová, E., Suchánková, J., Bosáková, Z., Ševčík, J.; Optimization of binary porogen solvent composition for preparation of butyl methacrylate monoliths in capillary liquid chromatography; *Journal of Chromatography A*, (2004); 1049: 43–49.
21. Dong, X.L., Wu, R., Dong, J., Wu, M.H., Zhu, Y., Zou, H.F.; Polyacrylamide-based monolithic capillary column with coating of cellulose tris(3,5-dimethylphenyl-carbamate) for enantiomer separation in capillary electrochromatography; *Electrophoresis*, (2008); 29: 919–927.
22. Zhang, Y.P., Ye, X.W., Tian, M.K., Qu, L.B., Choi, S.H., Gopalan, A.I., *et al.*; Novel method to prepare polystyrene-based monolithic columns for chromatographic and electrophoretic separations by microwave irradiation; *Journal of Chromatography A*, (2008); 1188: 43–49.
23. Vlakh, E.G., Tennikova, T.B.; Applications of polymethacrylate-based monoliths in high-performance liquid chromatography; *Journal of Chromatography A*, (2009); 1216: 2637–2650.
24. Mangelings, D., Tanret, I., Meert, V., Eeltink, S., Schoenmakers, P.J., Kok, W.T., *et al.*; Evaluation of polymeric methacrylate-based monoliths in capillary electrochromatography for their potential to separate pharmaceutical compounds; *Journal of Chromatographic Science*, (2007); 45: 578–586.
25. Ueki, Y., Umemura, T., Iwashita, Y., Odake, T., Haraguchi, H., Tsunoda, K.; Preparation of low flow-resistant methacrylate-based monolithic stationary phases of different hydrophobicity and the application to rapid reversed-phase liquid chromatographic separation of alkylbenzenes at high flow rate and elevated temperature; *Journal of Chromatography A*, (2006); 1106: 106–111.
26. Jiang, Z., Smith, N.W., Ferguson, P.D., Taylor, M.R., Preparation and characterization of long alkyl chain methacrylate-based monolithic column for capillary chromatography; *Journal of Biochemical and Biophysical Methods*, (2007); 70: 39–45.

27. ALOthman, A., Aqel, A., Abdelmoneim, H., Yacine Badjah-Hadj-Ahmed, A., Alwarthan, A.; Preparation and evaluation of long chain alkyl methacrylate monoliths for capillary chromatography; *Chromatographia*, (2011); 74: 1–8.
28. Fan, L.Q., Zhang, Y.P., Chen, N., Gong, W.J., Qu, L.B., Lee, K.P.; Rapid preparation and characterization of methacrylate-based monoliths for chromatographic and electrophoretic separation; *Journal of Chromatographic Science*, (2010); 48: 399–405.
29. Li, Y., Zhang, J., Xiang, R., Yang, Y., Horváth, C.; Preparation and characterization of alkylated polymethacrylate monolithic columns for micro-HPLC of proteins; *Journal of Separation Science*, (2004); 27: 1467–1474.
30. Svec, F.; Porous polymer monoliths: Amazingly wide variety of techniques enabling their preparation; *Journal of Chromatography A*, (2010); 1217: 902–924.
31. Moravcova, D., Jandera, P., Urban, J., Planeta, J.; Characterization of polymer monolithic stationary phases for capillary HPLC; *Journal of Separation Science*, (2003); 26: 1005–1016.
32. Shu, X., Chen, L., Yang, B., Guan, Y.; Preparation and characterization of long methacrylate monolithic column for capillary liquid chromatography; *Journal of Chromatography A*, (2004); 1052: 205–209.
33. Junjie, O., Gibson, G.T.T., Oleschuk, R.D.; Fast preparation of photopolymerized poly(benzyl methacrylate-co-bisphenol A dimethacrylate) monoliths for capillary electrochromatography; *Journal of Chromatography A*, (2010); 1217: 3628–3634.
34. Horváth, C., Lin, H.J.; Movement and band spreading of unsorbed solutes in liquid chromatography; *Journal of Chromatography*, (1976); 126: 401–420.
35. Bear, J. (ed). Dynamics of fluids in porous media. Dover Publications, New York, NY, (1988), 113.
36. Unger, K.K. (ed). Porous silica, its properties and use as support in columns liquid chromatography. Elsevier, Amsterdam, (1979), 171.
37. Al-Bokari, M., Cherrak, D., Guiochon, C.; Determination of the porosities of monolithic columns by inverse size-exclusion chromatography; *Journal of Chromatography A*, (2002); 975: 275–284.
38. Meyers, V.M. (ed). Practical high-performance liquid chromatography. Wiley, Hoboken, NJ, (2005).
39. Zabka, M., Minceva, M., Rodrigues, A.E.; Experimental characterization and modelling of analytical monolithic column; *Journal of Biochemical and Biophysical Methods*, (2007); 70: 95–105.
40. Oberacher, H., Premstaller, A., Huber, C.G.; Characterization of some physical and chromatographic properties of monolithic poly-(styrene-co-divinylbenzene) columns; *Journal of Chromatography A*, (2004); 1030: 201–208.
41. Lubbad, S.H., Buchmeiser, M.R.; Fast separation of low molecular weight analytes on structurally optimized polymeric capillary monoliths; *Journal of Chromatography A*, (2010); 1217: 3223–3230.
42. Jiang, Z., Reilly, J., Everatt, B., Smith, N.W.; Novel zwitterionic polyphosphorylcholine monolithic column for hydrophilic interaction chromatography; *Journal of Chromatography A*, (2009); 1216: 2439–2448.
43. Cabooter, D., Lynen, F., Sandra, P., Desmet, G.; Total pore blocking as an alternative method for the on-column determination of the external porosity of packed and monolithic reversed-phase columns; *Journal of Chromatography A*, (2007); 1157: 131–141.
44. Ishizuka, N., Minakuchi, H., Nakanishi, K., Soga, N., Tanaka, N.; Designing monolithic double-pore silica for high-speed liquid-chromatography; *Journal of Chromatography A*, (1998); 797: 133–137.
45. Cong, L., Huang, B., Chen, Q., Lu, B., Zhang, J., Ren, Y.; Determination of trace amount of microcystins in water samples using liquid chromatography coupled with triple quadrupole mass spectrometry; *Analytica Chimica Acta*, (2006); 569: 157–168.
46. Zhang, Y.P., Deng, P.H., Chen, N., Liu, P., Yang, Z.J.; Preparation and evaluation of tandem stationary phases for μ-HPLC and capillary electrochromatography; *Microchimica Acta*, (2010); 170: 1–7.
47. Fekete, S., Fekete, J., Ganzler, K.; Characterization of new types of stationary phases for fast liquid chromatographic applications; *Journal of Pharmaceutical and Biomedical Analysis*, (2009); 50: 703–709.
48. Guiochon, G.; Monolithic columns in high-performance liquid chromatography; *Journal of Chromatography A*, (2007); 1168: 101–168.