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Journal of Chromatography A, 1049 (2004) 43–49
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Optimization of binary porogen solvent composition for preparation of butyl methacrylate monoliths in capillary liquid chromatography

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Received 21 April 2004; received in revised form 3 August 2004; accepted 3 August 2004

Dedicated to Professor Jaroslav Janák on the occasion of his 80th birthday.

Abstract

Butyl methacrylate monolithic columns in 320 μm i.d. fused silica capillaries for reversed-phase capillary liquid chromatography were prepared by radical polymerization initiated thermally with azobisisobutyronitrile (AIBN). Polymerization mixture contained butyl methacrylate (BMA) as the function monomer and ethylene dimethacrylate (EDMA) as the crosslinking agent with 1,4-butanediol and 1-propanol as a binary porogen solvent. Ratio of 1,4-butanediol to 1-propanol in the porogen solvent was optimized regarding the monolithic column efficiency and performance. Total porosity, column permeability, separation impedance, Walters hydrophobicity index, retention factors, peak asymmetry factors, height equivalents to a theoretical plate and peak resolutions were used for characterization of the prepared monolithic columns. The polymerization mixture consisting of 17.8% of BMA, 21.8% of EDMA, 18.0% of 1,4-butanediol, 42.0% of 1-propanol and 0.4% AIBN generated monolithic columns of the best performance having a sufficient permeability and the lowest separation impedance. It was also demonstrated that monolithic columns of this composition exhibited good preparation reproducibility and an excellent pressure resistance when applied in capillary liquid chromatography.

Keywords: Porogen solvent; Monoliths; Butyl methacrylate; Pressure resistance; Reproducibility

1. Introduction

Simplicity of chromatographic column preparation without loss of their separation performance becomes one of the important goals in the area of separation techniques. Chromatographic columns based on monoliths may be a potential solution of this target. The first attempts of making this type of "single-piece" separation media can already be found in 1960s and 1970s [1–3], but practical use of these columns failed because of their low permeability and stability in some organic solvents. In 1989, Hjertén et al. [4] developed compressed soft polyacrylamide gels called "continuous beds" and applied these monolithic media successfully in chromatographic separations for the first time. Švec and Fréchet [5] in 1992 introduced a new type of stationary phases based on rigid macroporous organic polymer monoliths, which have become, thanks to their excellent properties, one of the widely developing stationary phases for liquid chromatography and electrochromatography [6–11].

Abbreviations: AIBN, α,α′-azobisisobutyronitril; $b/a_{10\%}$, asymmetry factor at 10% of peak height; BMA, butyl methacrylate; CLC, capillary liquid chromatography; *E*, separation impedance; EDMA, ethylene dimethacrylate; *H*, height equivalent to a theoretical plate; *k*, retention factor; *K*, column permeability; *N*,*N*-DMA, *N*,*N*-dimethylaniline; PEEK, polyether ether ketone; PTFE, poly(tetrafluoroethylene); t_R, retention time; $R_{i,j}$, peak resolution

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doi:10.1016/j.chroma.2004.08.009

Wide range of polymers, copolymers and derivational agents might be used for preparation of monolithic stationary phases. Monoliths based on polyacrylamide [12–14], including chiral stationary phases [15,16], polystyrene [17,18], poly(butyl methacrylate) [7,8,10,19–21], polymethacrylate [22–25] and poly(glycidyl methacrylate) [6,26,27] were prepared as stationary phases. The possibility to synthesize monoliths with various chemical properties and functionalities enables their application to a broad range of analytes like peptides and proteins [6,17,24,28–31], oligonucleotides [32], oligosacharides [13], DNA fragments [33] and neutral, alkaline and acidic compounds [34].

The butyl methacrylate monoliths have already been prepared in 320 μm i.d. fused silica capillaries to be used in capillary liquid chromatography (CLC) [21,35–37]. The ternary porogen solvent, commonly used, was replaced with a binary one to simplify the preparation procedure for electrochromatographic purposes [25,38]. The same trend seems to be useful for synthesis of monolithic stationary phases for CLC. Therefore, the aim of this study is optimization of the binary porogen solvent composition for preparation of butyl methacrylate monolithic CLC columns. Influence of the ratio of 1,4-butanediol to 1-propanol in the binary porogen solvent on the chemical properties, permeability, separation impedance and performance of monolithic columns is investigated in detail. In addition, a pressure resistance of these monoliths is evaluated to show benefits of the methacrylate-based stationary phases prepared in this way.

2. Experimental

2.1. Chemicals

Methanol (99.8%), 1,4-butanediol (99%), 1-propanol (99%), 3-(trimethoxysilyl)propyl methacrylate (99%), α,α′-azoisobutyronitril (AIBN) (98%) and acetic acid (99%) were purchased from Fluka (Buchs, Switzerland). Ethylene dimethacrylate (EDMA) (98%), butyl methacrylate (BMA) (99%) and 4-ethylaniline (98%) were provided by Merck (Darmstadt, Germany). Uracil (99%) used as unretained compound, phenol (99%), aniline (98%), *N*,*N*-dimethylaniline (*N*,*N*-DMA) (98%), toluene (99%), naphthalene (99%) and anthracene (99%) were supplied by Sigma (St. Louis, USA). Ethylbenzene (99%) and acetonitrile (99.9%) were purchased from Aldrich (Steinheim, Germany), sodium hydroxide (p.a.) and benzene (p.a.) from Lachema (Brno, Czech Republic). The water used in this work was purified with a Milli-Q water purification system (Millipore, USA). The mobile phase used in all experiments was composed of acetonitrile–water 65:35 (v/v).

2.2. Apparatus

Monolithic columns were prepared in polyimide-coated fused silica capillaries of 320 μm i.d. and 450 μm o.d. provided by Supelco (Bellefonte, USA). A UL 400 Memmert oven (Schwabach, Germany) was used for thermostatting the capillaries during silanization and polymerization. An ISCO 100 DM syringe pump (Lincoln, USA), a Valco International 60 nL injection valve (Schenkon, Switzerland), and a Linear UVIS 205 absorbance detector equipped with an on-column flow cell (San Jose, USA) were used for the CLC experiments. The column inlet was installed in the injection valve using PEEK sleeve and finger-tight fitting. The column outlet was connected by a piece of shielding PTFE tubing to a 100 μm i.d. fused silica capillary with a detection window burnt in a position of 80 mm from the separation column outlet. This capillary was placed in the absorbance detector. Detection was performed at two parallel wavelengths of 214 and 254 nm. Chromatograms were recorded and evaluated through the CSW 1.7 computer software provided by DataApex (Prague, Czech Republic). Flow rates from 0.5 to 4.0 μL/min and ambient temperature were applied to measure the chromatographic data.

2.3. Preparation of butyl methacrylate monolithic columns

The preparation procedure of butyl methacrylate monolithic columns in 100 and 150 μm i.d. fused silica capillaries was developed by Peters et al. [10]. This procedure was adopted in several points for preparation of butyl methacrylate monoliths for CLC in 320 μm i.d. fused silica capillaries [21,35,36]. Firstly, the capillaries of 25 or 22 cm length were flushed through a hydrodynamic gravitation with 1 M NaOH for 6 h and then with deionized H_2O for another hour. Secondly, the capillaries were filled with silanization solution containing 40 μL of 3-(trimethoxysilyl)propyl methacrylate in 10 mL of 6 M CH_3COOH, both ends of the capillary were immersed into small amounts of this solution and thermostatted at 60 °C for 20 h. In the next step, the capillaries were flushed with deionized H_2O for 30 min and dried by a flow of N_2 for 5 min. Finally, the capillaries were filled with polymerization mixture, their both ends were dipped in small vials containing the polymerization mixture and then thermostatted at 60 °C for 20 h. After polymerization, the capillary ends were cut off to the final column length of 20 cm (columns A1–X1) or 15 cm (columns B2–B9). The prepared columns connected to the injection valve were carefully flushed with the mobile phase consisting of CH_3CN/H_2O (65:35, v/v) and subsequently tested by Walters test [39] for reversed stationary phases.

The polymerization mixture contained 40% (w/w) of monomer mixture and 60% (w/w) of porogen solvent. The monomer mixture consisted of 44.5% (w/w) BMA, 54.5 % (w/w) EDMA and 1.0% (w/w) AIBN as already optimized in [21]. Composition of the porogen solvent was optimized in this study and all the compositions investigated are listed in Table 1.

3. Results and discussion

3.1. Optimization of the porogen solvent composition

The porogen solvents for preparation of butyl methacrylate monoliths used commonly in the literature [7,8,10,13] are ternary mixtures containing 1-propanol, 1,4-butanediol and water as they were developed for capillary electrochromatography monoliths which have to carry ionizable functionalities generating electroosmotic flow. For CLC purposes, the water can be removed from the porogen solvent to get a binary mixture and hence simplify the polymerization mixture. Along with the binary porogen solvent containing 1-propanol and 1,4-butanediol, some other binary porogen solvents for preparation of butyl methacrylate monoliths were investigated before [40] and in this work. Based on our experiments, methanol and 1,4-butanediol as the porogen solvents gave monoliths exhibiting a low separation efficiency and poor separation selectivity. On the other hand, polymerization mixtures with methanol and 1-propanol generated monolithic columns having a high flow resistance. Acetonitrile was not proved to be a promising component of the porogen solvent because of a low solubility of monomers in acetonitrile. Based on these preliminary experiments, 1-propanol and 1,4-butanediol were selected as components of the binary porogen solvent for preparation of butyl methacrylate monolithic columns in this work.

3.2. Influence of the porogen solvent composition on column permeability and separation impedance

Eight monolithic columns (A1–H1) were prepared keeping the ratio of the monomer mixture to the porogen solvent and the composition of the monomer mixture constant, while varying the composition of the porogen solvent according to Table 1. The percentage of 1,4-butanediol in the binary porogen solvent ranged within an interval from 25 to 80% (w/w) and, consequently, the percentage of 1,4-butanediol and 1-propanol in the polymerization mixture varied from 15 to 48% and from 45 to 12% (w/w), respectively. A set of 10 compounds differing in their hydrophobicity, acidity and alkalinity (i.e., uracil, phenol, aniline, 4-ethylaniline, *N*,*N*-dimethylaniline, benzene, toluene, ethylbenzene, naphthalene and anthracene) were used to test all the prepared columns. The testing was done under the same experimental conditions to get reliable data for comparison of the monoliths.

1-Propanol and 1,4-butanediol considerably differ in their viscosities (2.3×10^{-3} and 89.1×10^{-3} Pa s) and dipole moments (5.2×10^{-30} and 13.6×10^{-30} C m), while their densities (0.8 and 1.0 g/mL) and relative permittivities (20.3 and 31.1) are very close. When a low percentage of 1,4-butanediol in the porogen solvent is used, columns with a high number of theoretical plates showing high back pressures are obtained as it is evident from Fig. 1, panels A and B. It is obvious that the corresponding dependencies from the panels A and B matched each other conforming the same trend of the separation efficiency and the column resistance. Columns A1 and B1 prepared from porogen solvents having the lowest concentrations of 1,4-butanediol exhibited also the steepest back pressure dependencies on the linear velocity of eluent as demonstrated for column B1 in Fig. 1C. With high concentration of 1,4-butanediol in the porogen solvent, monolithic columns F1–H1 having more plate numbers and higher back pressures were also obtained compared to the medium region of 1,4-butanediol content (columns D1 and E1) but this difference was much less significant than for the low content of 1,4-butanediol. The columns D1 and E1 also exhibited the most flat dependencies of the back pressure on the linear velocity of eluent.

All the columns prepared were characterized in Table 1 through total porosities calculated from retention times of the unretained compound (i.e., uracil), Walters hydrophobicity indices [39], column permeabilities K (Eq. (1)) and separation impedances E (Eq. (2)) [41]:

$$K = \frac{u\eta L}{\Delta p} \quad (1)$$

$$E = \frac{H^2}{K} \quad (2)$$

where u is the linear velocity of eluent, η the dynamic viscosity of eluent, L the column length, Δp the pressure drop

Table 1
Porogen solvent compositions, total porosities, Walters hydrophobicity indices, column permeabilities K and separation impedances E of columns A1–H1 based on retention data of ethylbenzene in the eluent acetonitrile–water (65:35, v/v) at a flow-rate of 3 μL/min using UV detection at 214 nm

Column label	Porogen solvent composition (w/w)		Total porosity	Walters hydrophobicity index	Permeability K [10^{-14} m^2]	Separation impedance E [10^3]
	1,4-Butanediol	1-Propanol				
A1	25	75	0.69 ± 0.01	4.07 ± 0.07	0.3	370
B1	30	70	0.69 ± 0.01	3.96 ± 0.03	0.9	230
C1	35	65	0.61 ± 0.02	4.18 ± 0.08	5.0	1240
D1	40	60	0.64 ± 0.02	3.63 ± 0.10	11.6	8230
E1	50	50	0.65 ± 0.01	3.67 ± 0.15	9.6	2720
F1	60	40	0.61 ± 0.01	4.32 ± 0.22	6.3	780
G1	70	30	0.59 ± 0.02	3.74 ± 0.07	4.4	4020
H1	80	20	0.74 ± 0.01	3.88 ± 0.03	2.8	620

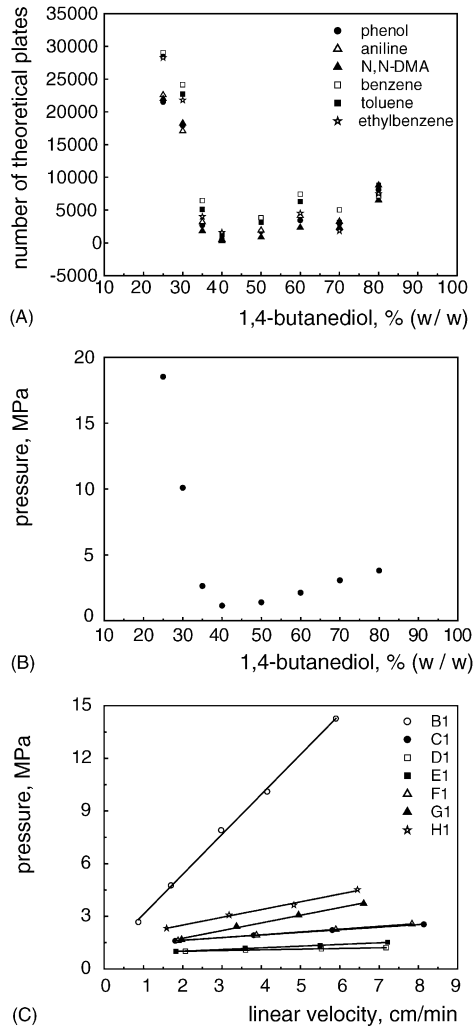

Fig. 1. Number of theoretical plates per column meter (panel A) for phenol, aniline, *N*,*N*-dimethylaniline (*N*,*N*-DMA), benzene, toluene and ethylbenzene and back pressure (panel B) of monolithic columns A1–H1 as a function of 1,4-butanediol percentage in the porogen solvent at an eluent flow-rate of 3 μL/min. Linear dependencies (panel C) of the back pressure of columns B1–H1 on the linear velocity of eluent (acetonitrile–water, 65:35, v/v).

and *H* the height equivalent to a theoretical plate. The value of dynamic viscosity $\eta = 0.65 \times 10^{-3}$ Pa s of 65% (v/v) acetonitrile needed for calculations was adopted from [42]. The columns showed very similar total porosities except of columns A1, B1 and H1 at both ends of the 1,4-butanediol concentration range with a significantly higher porosity. Walters hydrophobicity indices of all the prepared monoliths were very close regarding an error of this parameter and gave values of the same magnitude as published previously [21,37]. Based on this observation, hydrophobicity of the monolith surface seems not to be influenced by the ratio of the porogen solvent components. Separation impedances summarized in Table 1 were evaluated from the retention data of ethylbenzene, however, similar values were obtained for the other test compounds. The highest permeability and the largest separation impedance were observed for column D1 with 24% of 1,4-butanediol in the polymerization mixture which represents the worst composition for monolith preparation. Column permeabilities of the columns C1 and E1–H1 are similar to the values published for commercially available columns packed with 5 μm particles, but approximately 10-fold lower than permeabilities of silica-based monoliths [43]. The most promising column B1 with 18% of 1,4-butanediol in the polymerization mixture exhibited the lowest separation impedance combined with a satisfactory column permeability. The separation impedance of the column B1 is 10-times above and the permeability one-tenth of the values reported for capillary columns packed with 3 μm C18 particles [44]. The increased separation impedance of methacrylate monoliths was caused by their decreased column permeability. The column B1 prepared using the binary porogen solvent was evaluated as comparable or even better than similar monolithic columns reported in the literature [21,35–37]. These results also confirm the observation that porogen solvents with low percentages of 1,4-butanediol generate monoliths with small through-pores and large mesopores [11,36]. The monoliths with large mesopores exhibit a high specific surface needed for a successful separation of small organic molecules.

To compare the monolithic column B1 prepared using the binary porogen solvent with a monolithic column polymerized from the ternary porogen solvent containing water, a monolithic column X1 of the same composition as previously reported [21] (i.e. 17.8% BMA, 21.8% EDMA, 36.0% 1-propanol, 18.0% 1,4-butanediol, 6.0% water and 0.4% AIBN) was synthesized. Both columns were tested under the same experimental conditions with 10 polar and non-polar compounds. Retention factors, asymmetry factors and height equivalents to a theoretical plate were evaluated as listed in Table 2. The retention factors are almost identical and the asymmetry factors are very close for both columns. The *H* values are lower for six compounds on the column B1 and for four substances on the column X1. A very positive result was obtained for the most hydrophobic compounds naphthalene and anthracene. The *H* value is dramatically lower

Table 2
Retention factors *k*, asymmetry factors at 10% of peak height $b/a_{10\%}$ and height equivalents to a theoretical plate *H* of 10 test compounds on the column B1 and column X1 in the eluent acetonitrile–water (65:35, v/v) at a flow-rate of 3 μL/min using UV detection at 214 nm

Compound	Column B1			Column X1		
	k	$b/a_{10\%}$	*H* [μm]	*k*	$b/a_{10\%}$	*H* [μm]
Uracil	0.00	1.51	58	0.00	1.60	56
Phenol	0.41	1.35	48	0.43	1.30	59
Aniline	0.50	1.66	46	0.51	1.42	59
4-Ethylaniline	0.67	1.51	50	0.69	1.20	92
N,*N*-DMA	1.27	1.30	45	1.30	1.29	75
Benzene	1.23	1.65	39	1.24	1.56	37
Toluene	1.53	1.57	39	1.54	1.22	37
Ethylbenzene	1.93	1.53	40	1.95	1.22	39
Naphthalene	2.48	1.74	54	2.48	1.34	81
Anthracene	4.94	1.85	69	4.91	1.51	137

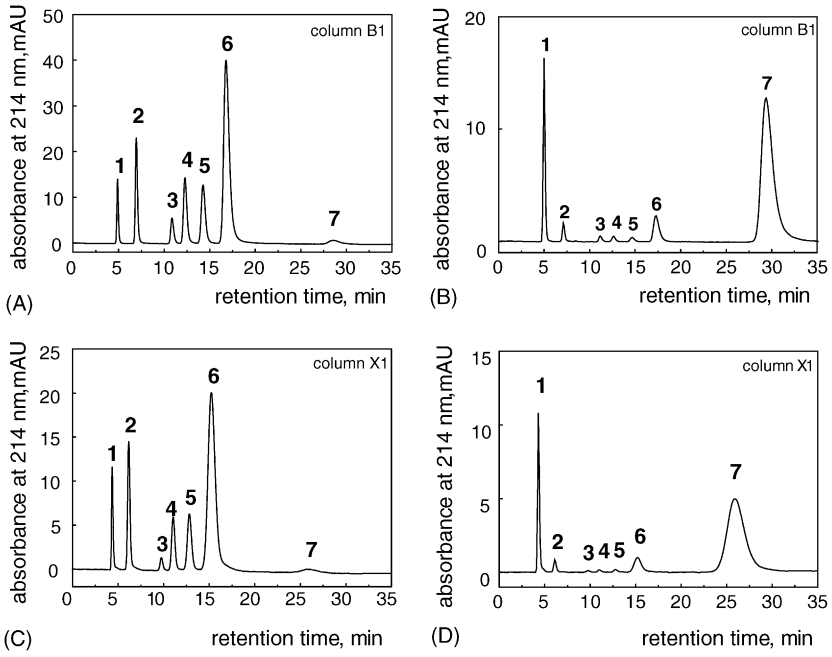

Fig. 2. Separation of a test mixture containing: uracil (1), phenol (2), benzene (3), toluene (4), ethylbenzene (5), naphthalene (6) and anthracene (7) on the column B1 (panels A and B) and the column X1 (panels C and D). Effective column lengths, 20 cm; eluent, acetonitrile–water (65:35, v/v); flow-rate, 3 μL/min; injection, 60 nL; detection, 214 nm (panels A and C), 254 nm (panels B and D).

on the column B1 compared to the column X1, especially for anthracene. Chromatograms of separation of seven test compounds on both columns under the same experimental conditions detected at 214 and 254 nm are depicted in Fig. 2. It is evident that both columns are very similar in several parameters, however, the column B1 is easier to prepare and shows markedly better separation efficiencies for naphthalene and anthracene with slightly higher asymmetry factors. Again, the efficiency of the column B1 is similar or even better when compared with values already published for columns of the same type and size [21,35–37].

3.3. Pressure resistance of butyl methacrylate monolithic columns

Pressure resistance is a very important factor of separation columns since the stationary phase can easily be damaged or even destroyed with a high pressure applied to the beginning of the column. To investigate the pressure resistance of butyl methacrylate monolithic columns, a column B2 was prepared from the same polymerization mixture as the column B1. The column B2 was tested with seven analytes in five different chromatographic parameters as retention time, retention factor, asymmetry factor, height equivalent and resolution. Values of these parameters measured at a flow rate of 3 μL/min are listed in Table 3. In the next step, the column B2 was pressurized to 15 MPa for 1 h and after depressurization to 7.7 MPa corresponding to the flow rate of 3 μL/min, the column was tested again with the same set of analytes on the previously measured parameters, values of which are summarized in Table 3. The set flow rate of 3 μL/min was verified with a flow-rate meter connected to the column outlet to be 2.95 ± 0.04 μL/min. Subsequently, the same pressure tests of 20 and 30 MPa were applied to the column B2. Differences between the values determined before pressure tests and after the last pressure test expressed in percentage in Table 3 show that application of a high pressure causes changes in t_R and k lower than 2%, $b/a_{10\%}$ and $R_{i,j}$ below 10% and H not exceeding 20%. These results clearly demonstrate that butyl methacrylate columns of 320 μm i.d. can be used with pressures up to 30 MPa since the changes of these capillary columns in chromatographic parameters are negligible when the monolith is tightly bound to the capillary wall with covalent bonds.

3.4. Repeatability and reproducibility of monolithic columns

There are only few data on the reproducibility of preparation of methacrylate monolithic columns in the literature [6,37]. In this work, the repeatability and reproducibility of preparation of butyl methacrylate monoliths were studied with eight monolithic columns B2–B9 synthesized from a polymerization mixture of the same composition as that for column B1. The columns B2–B5 were prepared from one polymerization mixture and the columns B6–B9 from another polymerization mixture but both of the same composition. The repeatability and reproducibility were expressed as relative standard deviations of the same chromatographic parameters measured with the same set of analytes as those used for tests of the pressure resistance (see Table 4). The run-to-run repeatability was evaluated with the column B2

Table 3
Retention times t_R, retention factors k, asymmetry factors $b/a_{10\%}$, height equivalents to a theoretical plate H and peak resolutions $R_{i,j}$ of seven test compounds on the column B2 before and after a pressure test with increased pressures of 15, 20 and 30 MPa

Parameter	Compound	Before pressure test	After (15 MPa)	After (20 MPa)	After (30 MPa)	Difference (%)
t_R (min)	Uracil	2.88	2.80	3.00	2.91	1.0
	Phenol	4.06	3.94	4.22	4.11	1.2
	Benzene	6.22	6.04	6.49	6.33	1.8
	Toluene	7.12	6.90	7.43	7.22	1.4
	Ethylbenzene	8.25	8.00	8.62	8.37	1.5
	Naphthalene	9.78	9.49	10.23	9.91	1.3
	Anthracene	16.47	16.03	17.25	16.79	1.9
k	Uracil	0.00	0.00	0.00	0.00	–
	Phenol	0.41	0.41	0.41	0.41	0.0
	Benzene	1.16	1.16	1.17	1.18	1.7
	Toluene	1.47	1.47	1.48	1.48	0.7
	Ethylbenzene	1.87	1.86	1.88	1.88	0.5
	Naphthalene	2.40	2.39	2.42	2.41	0.4
	Anthracene	4.72	4.73	4.76	4.77	1.1
$b/a_{10\%}$	Uracil	1.37	1.50	1.40	1.43	4.4
	Phenol	1.37	1.34	1.43	1.39	1.5
	Benzene	2.33	2.21	2.39	2.10	9.9
	Toluene	1.55	1.45	1.52	1.47	5.2
	Ethylbenzene	1.54	1.50	1.51	1.44	6.5
	Naphthalene	1.52	1.45	1.54	1.51	0.7
	Anthracene	1.65	1.80	1.76	1.78	7.9
H (μm)	Uracil	42	44	42	48	14.3
	Phenol	49	49	45	41	16.3
	Benzene	46	43	45	41	10.9
	Toluene	38	37	36	36	5.3
	Ethylbenzene	37	37	37	35	5.4
	Naphthalene	51	50	51	49	3.9
	Anthracene	66	71	71	76	15.2
$R_{i,j}$	Uracil	–	–	–	–	–
	Phenol	4.77	4.70	4.89	4.77	0.0
	Benzene	5.85	5.97	6.01	6.25	6.8
	Toluene	2.00	2.01	2.03	2.02	1.0
	Ethylbenzene	2.29	2.30	2.33	2.38	3.9
	Naphthalene	2.43	2.46	2.45	2.48	2.1
	Anthracene	6.26	6.18	6.16	6.07	3.0

Effective column length, 15 cm; eluent, acetonitrile–water (65:35, v/v); flow-rate, 3 μL/min; detection, 214 nm. Difference characterizes a change of the investigated parameter in percentage between the values before the pressure test and after the highest pressure test.

from 10 parallel measurements. The column-to-column repeatability was calculated within two independent sets of four columns (i.e. B2–B5 and B6–B9). The run-to-run repeatability was lower than 5.5% for all the parameters tested and the column-to-column repeatability was below 16% within the second column set and below 23% within the first set. The reproducibility of eight butyl methacrylate monolithic columns (i.e. B2–B9) prepared from two different polymerization mixtures of the same composition was better than 3.5% regarding the retention times and retention factors, around 10% in the asymmetry factors and resolution and 21% considering the height equivalents. The repeatability and reproducibility

Table 4
Repeatability and reproducibility of preparation of butyl methacrylate monolithic columns expressed as relative standard deviations (R.S.D., %) of retention time t_R, retention factor k, asymmetry factor $b/a_{10\%}$, height equivalent to a theoretical plate H and peak resolution $R_{i,j}$

Parameter	Repeatability			Reproducibility
	Run-to-run	Column-to-column		Mixture-to-mixture
	Column B2 (n = 10)	Columns B2–B5 (n = 4)	Columns B6–B9 (n = 4)	Columns B2–B9 (n = 8)
t_R (min)	2.5	2.2	3.6	3.3
k	1.8	0.9	3.7	3.3
$b/a_{10\%}$	4.1	9.3	7.3	9.2
H (μm)	5.3	22.2	15.7	20.3
$R_{i,j}$	1.6	10.6	10.7	10.6

observed in this study are similar to the reproducibilities of commercial packed capillary columns available on the market.

4. Conclusions

The porogen solvent for polymerization of butyl methacrylate monoliths was optimized. It was shown that the porogen solvent can be simplified to a binary mixture consisting of 1,4-butanediol and 1-propanol. The polymerization mixture containing 18% of 1,4-butanediol and 42% of 1-propanol (i.e. ratio 30:70 (w/w), in the porogen solvent) generates the monolithic column with the lowest separation impedance and a satisfactory column permeability suitable for an efficient separation of small organic molecules. These monolithic capillary columns show approximately two-times higher plate heights than commonly noticed at conventional HPLC packed columns. They exhibit a good repeatability and reproducibility of preparation with R.S.D. values below 10% in majority of the investigated chromatographic parameters. An excellent pressure resistance of the monolith up to 30 MPa is an important benefit of monolithic columns prepared in the way optimized in this paper.

Acknowledgement

Financial support by Grant no. 203/03/0161 of the Grant Agency of the Czech Republic is gratefully acknowledged. Salaries of the scientists were supported by Research Projects no. J13/98:113100002 and J13/98:113100001 of the Charles University.

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