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Methacrylate monolithic columns of 320 μm I.D. for capillary liquid chromatography

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Abstract

Monolithic capillary columns (320 μm I.D.) were prepared for capillary liquid chromatography (CLC) by radical polymerization of butylmethacrylate (BMA) and ethylenedimethacrylate (EDMA) in the presence of a porogen solvent containing propan-1-ol, butane-1,4-diol and water. The influence of the contents of the porogen solvent and EDMA in the polymerization mixture on the monolith porosity and column efficiency was investigated. The composition of the polymerization mixture was optimized to attain a minimum HETP of the order of tens of μm for test compounds with various polarities. The separation performance and selectivity of the most efficient monolithic column prepared was characterized by van Deemter curves, peak asymmetry factors and Walters hydrophobicity and silanol indices. It was demonstrated that the 320-μm I.D. monolithic column exhibited CLC separation performance similar to that observed for 100- and 150-μm I.D. monolithic columns reported in the literature; moreover, the 320-μm I.D. column was easier to operate in CLC and exhibited a higher sample loadability.

Keywords: Monolithic columns; Stationary phases, LC; Methacrylate

1. Introduction

Miniaturization of columns in high-performance liquid chromatography (HPLC) has been one of the main trends in this separation technique within the last 10 years [1,2]. Separation columns of the common inner diameters (i.e. 3.2–4.6 mm) used in conventional HPLC have been substituted with 1.5–3.2 mm I.D. columns in microbore HPLC, 0.5–1.5 mm I.D. columns in microcolumn LC, 150–500 μm I.D. columns in capillary liquid chromatography (CLC) and 10–150 μm I.D. columns in nanoscale LC [3]. Miniaturization brings some benefits to the separation process, e.g. decreased amounts of samples necessary for injection, small flow-rates of eluents leading to their reduced consumption and a lowered dilution of analytes in the separation column resulting in an increased sensitivity of detection [4,5].

Continuous polymer beds (monoliths) were introduced by Hjertén et al. in 1989 [6] and subsequently investigated by other research groups [7–9]. Monolithic columns have recently been prepared and

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applied to capillary liquid chromatography and capillary electrochromatography (CEC) [10–12]. The monolithic columns are prepared by polymerization of a monomer mixture with a porogen solvent, forming a porous polymer bed which fills into the separation column and acts as a stationary phase. Monolithic capillary columns are prepared much more easily than packed capillary columns and need no frits as the monolith can be permanently fixed in the separation capillary by covalent bonding to the capillary inner surface [10,13].

Various polymers and copolymers have been used for the preparation of monolithic columns. Monolithic columns based on acrylamide and methacrylamide [14,15] are synthesized by polymerization of acrylamide, methacrylamide or their derivatives, with methylenebisacrylamide or piperazine diacrylamide as cross-linking agents. Polystyrene-based monolithic columns [16] are prepared by copolymerization of styrene or its derivatives with divinylbenzene as the crosslinker. Methacrylate ester-based monolithic columns [13,17,18] are obtained by polymerization of butyl methacrylate or other methacrylate esters, ethylene glycol dimethacrylate acting as the crosslinking agent. In CLC, the monolith should covalently be bound to the inner capillary surface to avoid any displacement of the monolithic bed by pumping the mobile phase through the separation column. This is very often attained by silanizing the capillary wall with 3-(methoxysilyl)propyl methacrylate which covalently binds methacrylate ester-based monoliths [19].

Monolithic stationary phases for separation of proteins and polynucleotides [20] and for enantioselective separations [21] have also recently been prepared and investigated.

The literature describes a preparation procedure for methacrylate ester-based monolithic columns of 100- and 150-μm I.D. and their successful application in CEC [13,17,18,22–24] and CLC [22–24]. However, there are only a few reports dealing with columns of larger diameters and their use in CLC [10,25,26]. Therefore, the aim of this work was to prepare a butyl methacrylate monolithic column of 320 μm I.D., to optimize the preparation procedure with regard to the column efficiency, to test the separation performance and selectivity of the column in CLC and to find out how its performance compares with that of columns with smaller diameters. Columns of 320-μm I.D. can be expected to be easier to operate in CLC compared to 100- and 150-μm I.D. ones, considering simple column installation into the injection valve, simple connection to the capillary with the detector window, simple establishment of the optimal mobile phase flow-rate, a decreased hydrodynamic resistance and an increased sample loadability resulting in a higher detection sensitivity.

2. Experimental

2.1. Chemicals

Acetic acid (99%), 3-(trimethoxysilyl)propyl methacrylate (99%), 1-propanol (99%) and 1,4-butanediol (99%) were supplied by Fluka (Buchs, Switzerland). Butylmethacrylate (BMA) (99%), ethyleneglycol dimethacrylate (EDMA) (98%), α,α′-azoisobutyronitrile (AIBN) (98%), *N,N*-diethyltoluamide (purity of USP) and acetonitrile (gradient grade) were provided by Merck (Darmstadt, Germany). Uracil (99%) used as the dead retention time marker, phenol (99%), aniline (99%), benzene (99%), toluene (99.5%), ethylbenzene (99%), anthracene (99%) and sodium hydroxide (99%) were purchased from Sigma (St. Louis, MO, USA). The water used for preparation of all the solutions was purified with a Milli-Q water purification system (Millipore, USA).

2.2. Equipment and materials

Capillary monolithic columns were prepared in a polyimide-clad fused-silica capillary of 320 μm I.D. and 450 μm O.D. provided by Supelco (Bellefonte, PA, USA). An oven UL 400 Memmert (Schwabach, Germany) was used for thermostating the capillaries during silanization and polymerization. An ISCO syringe pump model 100 DM (Lincoln, NE, USA), a Valco International injection valve with a 60-nl internal loop (Schenkon, Switzerland) and a Linear UVIS-205 absorbance detector (San Jose, CA, USA) were employed in the chromatographic measurements. The monolithic column inlet was installed in the injection valve using a 50-mm PEEK sleeve (500

μm I.D.) and a PEEK fingertight fitting. The monolithic column outlet was directly connected, using a piece of PTFE tubing, to a 100-μm I.D. fused-silica capillary with the detection window positioned 90 mm from the connection of these two capillaries. The 100-μm capillary with the detection window was placed in the absorbance detector operated at wavelengths of 214 and 254 nm. Chromatograms were recorded and evaluated using the CSW v. 1.7 computer software supplied by DataApex (Prague, Czech Republic). The monolithic columns prepared were tested under mobile phase flow-rates from 1 to 4 μl/min applying eluent pressures of 1–10 MPa depending on the monolith porosity.

3. Results and discussion

3.1. Preparation of methacrylate monolithic columns

The principles of the preparation procedure for methacrylate ester-based monolithic columns of 100- and 150-μm I.D. used in CEC and published by Peters et al. [13] were adopted. However, the procedure was modified as follows: as no electroosmotic flow is involved in CLC, 2-acrylamido-2-methylpropan-1-sulfonic acid (AMPS) was omitted from the polymerization mixture. On the other hand, the monolith must be firmly bound to the capillary wall in CLC and thus the capillary was silanized prior to the polymerization reaction.

A 22-cm piece of 320-μm I.D. fused-silica capillary was flushed with 1 *M* sodium hydroxide at a flow-rate of 5 μl/min for 6 h to activate the capillary inner surface and convert its siloxane groups into silanol ones. The capillary was then flushed with deionized water at a flow-rate of 5 μl/min for 1 h. To create double bonds on the surface, several published procedures based on (γ-methacryloyloxypropyl)trimethoxysilane (γ-MAPS) were tested at room temperature with various reaction time periods [19,27,28]. None of these procedures prevented displacement of the monolith by the flowing mobile phase, but a moderate modification of the silanization procedure solved the problem. The capillary was filled with the silanization solution containing 40 μl of γ-MAPS in 10 ml of 6 *M* acetic acid, sealed with septa and then thermostatted at 60 °C for 20 h. After silanization, the capillary was flushed with deionized water at a flow-rate of 5 μl/min for 30 min and dried by the passage of nitrogen for 5 min.

In the next step, the capillary was filled with the polymerization mixture (Table 1), sealed with septa or its outlets immersed in polypropylene microtubes containing the polymerization mixture and then thermostatted at 60 °C for 20 h. Keeping the capillary ends in the polymerization mixture was found advantageous because a moderate shrinking of the monolith could occur during the polymerization and thus the open space would be filled up. The columns prepared in this way exhibited a better performance than those polymerized with septa at the ends and thus the latter approach was used throughout.

The polymerization mixture (Table 1) consisted of a monomer mixture (containing functional monomer BMA, crosslinking agent EDMA and 1% (w/w) of AIBN) and a porogen solvent (mixed from 60% (w/w) of propan-1-ol, 30% (w/w) of butane-1,4-diol and 10% (w/w) of deionized water; this ratio was kept constant). The radical polymerization produced monoliths with the chemical structure outlined in

Table 1
Compositions of the monomer and polymerization mixtures (%, w/w) used for preparation of methacrylate monolithic columns and their total porosities

Column	Monomer mixture			Polymerization mixture		Total porosity[a]
	BMA	EDMA	AIBN	Monomer mixture	Porogen solvent	
C1	59.5	39.5	1	40	60	0.72
C2	59.5	39.5	1	50	50	0.69
C3	49.5	49.5	1	40	60	0.65
C4	44.5	54.5	1	40	60	0.63

[a] The total porosity was calculated from the dead retention time.

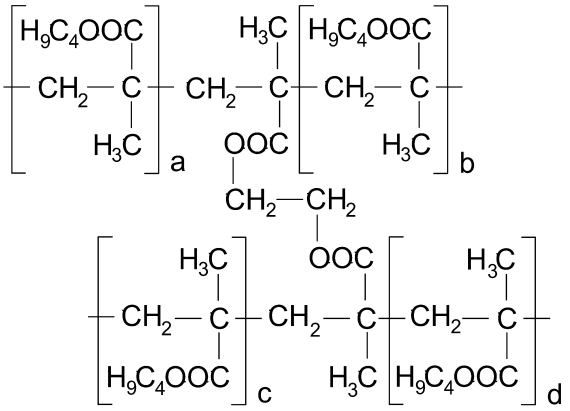

Fig. 1. Chemical structure of the butylmethacrylate monolith.

Fig. 1. The monomer mixture and the porogen solvent were mixed at various ratios to prepare monoliths with various porosities (Table 1). All the polymerization mixtures were sonicated for 10 min and bubbled with nitrogen for another 5 min to remove gases before they were aspirated into the capillary.

After the polymerization, the capillary ends 10–15 mm long were cut off and the monolithic column, about 20 cm long, was connected to the injection valve and flushed with the mobile phase.

3.2. Optimization of the polymerization mixture composition

The influence of the contents of AMPS, propane-1-ol and the porogen solvent in the polymerization mixture on the porosity and performance of methacrylate monolithic columns of 100- and 150-μm I.D. was investigated in CEC and CLC [13,17,23,24]. All these works used the same ratio of BMA to EDMA (i.e. 60:40%, w/w) in the monomer mixture. Based on this fact, the monomer mixture applied by us to the preparation of column C1 (see Table 1) contained the same ratio of BMA and EDMA, however no AMPS was added to the polymerization mixture. The column C1 exhibited a poor separation performance using a mobile phase acetonitrile–water (65:35, v/v) at flow-rates of 1–4 μl/min for all the test compounds, i.e. uracil, phenol, aniline, benzene, toluene, ethylbenzene and anthracene. The best performance of column C1 under the above conditions was attained for uracil at a flow-rate of 4 μl/min with a HETP of 490 μm.

To decrease the monolith porosity and improve the column performance, the content of the porogen solvent in the polymerization mixture was reduced from 60 to 50% for column C2 (Table 1) using the same BMA:EDMA ratio. The porosity decreased, as demonstrated by the total porosity values in Table 1 and by a higher pressure required to attain the same eluent flow-rates compared to column C1 (Fig. 2), but the column performance did not improve. These experiments showed that the preparation procedure published for methacrylate monolithic columns of 100- and 150-μm I.D. exhibiting good performance cannot simply be adopted for the preparation of columns of 320-μm I.D.

It was expected that not only the content of the porogen solvent in the polymerization mixture but also the percentage of EDMA in the monomer mixture could influence the monolith porosity and, consequently, the column efficiency. Therefore, the content of EDMA was increased from 39.5 to 49.5% in the monomer mixture with column C3, keeping the percentage of the porogen solvent at 60% (Table 1). This column exhibited a very good performance for uracil but poor efficiencies for the other test compounds as demonstrated on a chromatogram of uracil, aniline and toluene in Fig. 3. The HETP

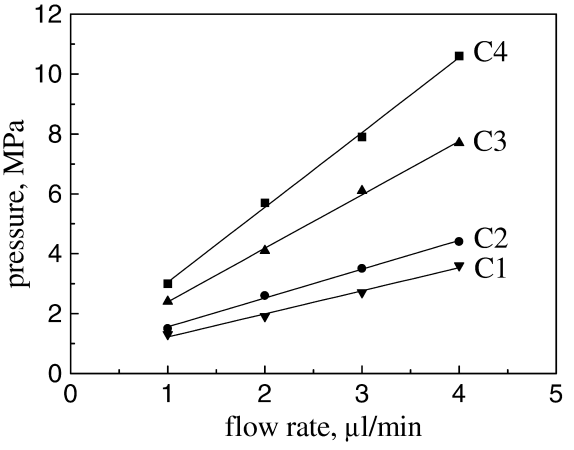

Fig. 2. Dependence of the back pressure on the eluent flow-rate for columns C1–C4.

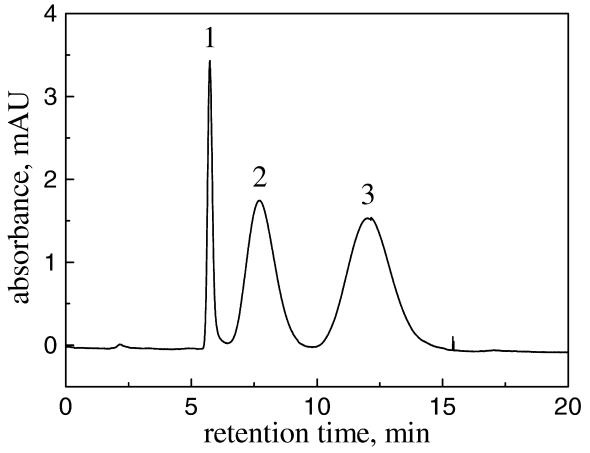

Fig. 3. Separation of uracil (1), aniline (2) and toluene (3) on the column C3. Effective column length, 21 cm; mobile phase, acetonitrile–water (65:35, v/v); flow-rate, 2 μl/min; injection, 60 nl; detection, 214 nm.

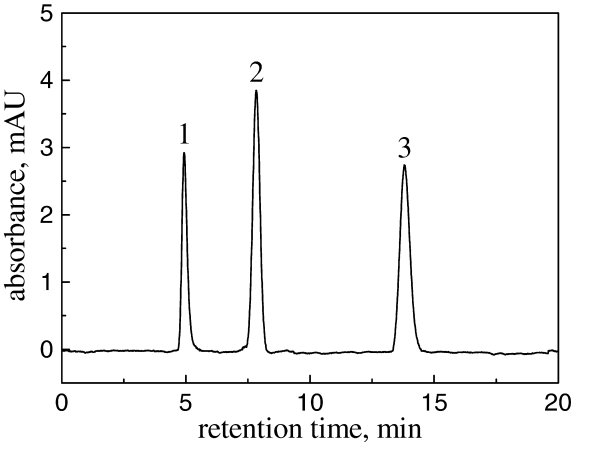

Fig. 4. Separation of uracil (1), aniline (2) and toluene (3) on the column C4. Effective column length, 19 cm; mobile phase, acetonitrile–water (65:35, v/v); flow-rate, 2 μl/min; injection, 60 nl; detection, 214 nm.

values of 50, 980 and 990 μm were obtained for uracil, aniline and toluene, with the highest peak asymmetry factor of $b/a_{10\%} = 1.4$ for aniline.

The promising result obtained with the column C3 initiated the preparation of column C4 with even a higher content of EDMA (i.e. 54.5%, w/w) in the monomer mixture (Table 1). The column C4 yielded sharp peaks of all the test compounds with HETP values of 80 μm for uracil, 60 μm and $b/a_{10\%} = 1.0$ for aniline and 40 μm for toluene. A chromatogram of the test mixture obtained with this column is depicted in Fig. 4. Therefore, column C4 was used in further experiments.

3.3. Separation performance and selectivity of the optimized column

The separation efficiency of the column C4 for the test compounds of various polarities, uracil, phenol, aniline, benzene, toluene, ethylbenzene and anthracene, was determined with the acetonitrile–water (65:35, v/v) eluent at flow-rates from 1 to 4 μl/min, corresponding to mobile phase linear velocities of 1.9–7.6 cm/min. The chromatograms obtained at mobile phase flow-rates of 1 and 4 μl/min are presented in Figs. 5 and 6, respectively. It is apparent that a better separation performance with a higher resolution of the recorded peaks is achieved at the flow-rate of 1 μl/min. However, the analysis time is four times as long as that at the flow-rate of 4 μl/min. Van Deemter dependences for all the test substances within a flow-rate interval from 1 to 4 μl/min are plotted in Fig. 7. The resistance to mass transfer in the stationary and mobile phase seems to be the main band broadening mechanism as follows

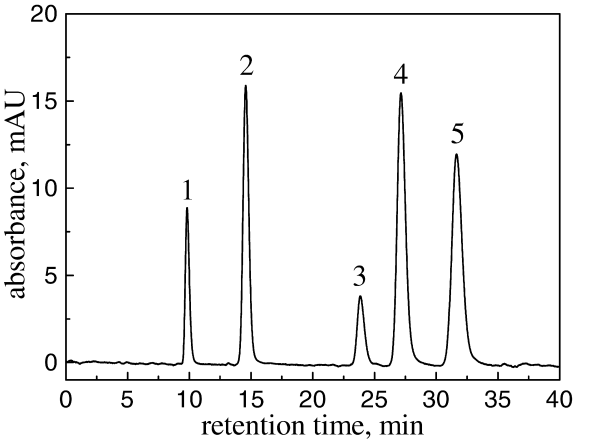

Fig. 5. Separation of uracil (1), phenol (2), benzene (3), toluene (4) and ethylbenzene (5) on the column C4. Effective column length, 19 cm; mobile phase, acetonitrile–water (65:35, v/v); flow-rate, 1 μl/min; injection, 60 nl; detection, 214 nm.

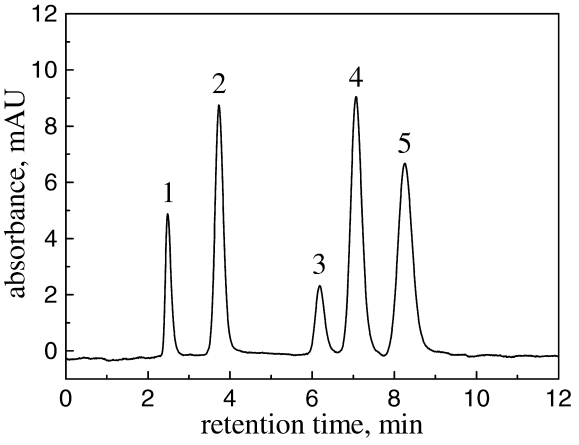

Fig. 6. Separation of uracil (1), phenol (2), benzene (3), toluene (4) and ethylbenzene (5) on the column C4. Effective column length, 19 cm; mobile phase, acetonitrile–water (65:35, v/v); flow-rate, 4 μl/min; injection, 60 nl; detection, 214 nm.

from the linear character of the van Deemter plots for all the test compounds. The lowest HETP values were obtained for benzene (27 and 57 μm at 1 and 4 μl/min, respectively) and the highest ones for anthracene (100 and 320 μm at 1 and 4 μl/min, respectively).

A reversed-phase character of the butylmethacrylate monolith is evident from the retention order of the test compounds. The retention factors for the test compounds are summarized in Table 2. The symmetry of the peaks are satisfactory, with the peak asymmetry factors of 1.0–1.3 for polar compounds (phenol and aniline), 1.2–1.4 for hydrophobic substances (benzene, toluene, ethylbenzene and anthracene) and 1.4–1.8 for uracil.

The retention factors of anthracene and benzene determined in acetonitrile–water (65:35, v/v) eluent were used to calculate the hydrophobicity index (HI) according to Walters [29] of the BMA monolith HI = 3.9±0.1. Based on this value, the hydrophobicity of the monolithic column is comparable to that of conventional C_{18} reversed stationary phases. The retention factors of *N,N*-diethyl-*m*-toluamide and anthracene measured in pure acetonitrile as the eluent were used in the calculation of a formal Walters silanol index [29] of the monolith SI = 0.5±0.1. This relatively low value assumes a negligible affinity of the monolith to strongly basic

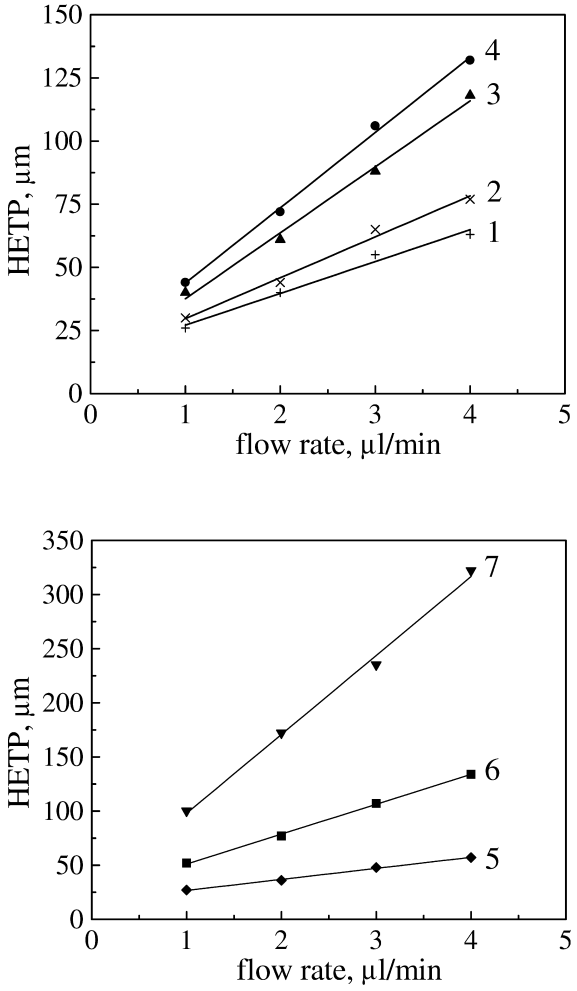

Fig. 7. Dependence of the height equivalent to a theoretical plate on the flow-rate of the mobile phase for toluene (1), ethylbenzene (2), aniline (3), phenol (4), benzene (5), uracil (6) and anthracene (7) on the monolithic column C4 with acetonitrile–water (65:35, v/v) eluent.

analytes and this assumption is also supported by the symmetrical peak observed for aniline.

The 100- and 150-μm I.D. methacrylate monolithic columns were mostly used in CEC [13,17,18,22–24] with HETP from units to hundreds of μm and also in CLC [22–24] with HETP of tens of μm. The optimized 320-μm I.D. methacrylate monolithic column prepared in this work exhibited performance that was similar to those obtained in the literature with 100- and 150-μm I.D. columns in the

Table 2
Retention factors of the test compounds on the column C4 with acetonitrile–water (65:35, v/v) eluent at different flow-rates

Compound	Flow-rate (μl/min)			
	1	2	3	4
Uracil	0.00	0.00	0.00	0.00
Phenol	0.49	0.49	0.49	0.50
Aniline	0.59	0.59	0.59	0.59
Benzene	1.44	1.46	1.46	1.49
Toluene	1.81	1.80	1.80	1.84
Ethylbenzene	2.28	2.27	2.28	2.32
Anthracene	5.81	5.77	5.78	5.85

CLC separation mode [22–24], higher than those published for 8-mm I.D. columns used in HPLC [30] and poorer than that of 8-mm I.D. columns [7] and newly introduced monolithic silica columns [31,32]. It seems that there is no dramatic decrease in the performance when thin capillaries are replaced by thicker ones as already demonstrated in the literature [10]. However, the 320-μm I.D. columns are easier to prepare and operate in the CLC mode and possess a higher sample loadability (60–100 nl compared to 1 nl for 100- and 150-μm I.D. columns [24]) which is compatible with a larger-volume detection cell (100- or 220-μm I.D. capillary with the detection window) and results in a higher detection sensitivity.

4. Conclusion

This work demonstrated that BMA monolithic columns of 320 μm I.D., yielding satisfactory separation performance for hydrophobic and polar compounds with HETP of tens of μm, can be prepared. It was proved that not only the porogen solvent percentage, but also the content of EDMA in the polymerization mixture, affect the monomer porosity and the resultant column efficiency. The optimized 320 μm I.D. column exhibited HETP values comparable to those published for 100- and 150-μm I.D. columns, however, the 320-μm I.D. columns are easier to operate in CLC and have a higher sample loadability. Van Deemter curves measured on the monolithic column indicated the resistance to mass transfer as the major band broadening mechanism. The Walters hydrophobicity and silanol indices demonstrated that the hydrophobicity of the butyl methacrylate monolith was similar to that of conventional C_{18} stationary phases and that the monolith had a low affinity to strongly basic analytes.

5. Symbols and abbreviations

AIBN	α,α′-azoisobutyronitrile
AMPS	2-acrylamido-2-methylpropan-1-sulfonic acid
BMA	butylmethacrylate
$b/a_{10\%}$	peak asymmetry factor at 10% of peak height
CEC	capillary electrochromatography
CLC	capillary liquid chromatography
EDMA	ethylene dimethacrylate
HETP	height equivalent to a theoretical plate
HI	Walters hydrophobicity index
HPLC	high-performance liquid chromatography
I.D.	inner diameter
LC	liquid chromatography
γ-MAPS	(γ-methacryloyloxypropyl)trimethoxysilan,3-(trimethoxysilyl)propyl methacrylate
O.D.	outer diameter
PEEK	polyetheretherketone
SI	Walters silanol index
USP	United States Pharmacopoeia

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