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Short communication

Preparation and characterization of long methacrylate monolithic column for capillary liquid chromatography

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Abstract

Long methacrylate monolithic columns (100 cm × 320 μm i.d.) were prepared from silanized fused-silica capillaries of 320 μm i.d. by in situ copolymerization of butyl methacrylate (BMA) with ethylene dimethacrylate (EDMA) in the presence of a suitable porogen. The separation performance and selectivity of the column were evaluated and compared with a 25 cm × 320 μm i.d. column prepared in the same way by capillary high-performance liquid chromatography (μ-HPLC). The results showed that the 1 m long monolithic column can generate 33×10^3 plate number and exhibited good permeability, higher sample loadability, and separation capability.
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Keywords: Monolithic column; Butyl methacrylate phase; Stationary phases, LC

1. Introduction

Packed capillary high-performance liquid chromatography (μ-HPLC) has been used extensively in the field of biomedical and environmental sciences [1,2]. It provides high separation efficiency and facile sample adaptability as that of conventional HPLC, while reducing the consumption of mobile phase, stationary phase, sample, operation cost, and secondary pollution. The head pressure of the packed capillary HPLC column is proportional to the length of the column. For example, the head pressure of a 30 cm long column packed with 5 μm particles is about 4–8 MPa, and is tripled for a 100 cm long column, which is 12–20 MPa. Although the long column has higher separation efficiency, the preparation of the column is rather difficult because of the high packing pressure required. The monolithic column developed in recent years possesses the advantages of good hydrodynamic characteristic, low flow resistance as well as easy preparation [3–7], which made the monolithic material an ideal support for long columns.

The methacrylate monolithic column has varied selectivity and good stability between pH 2 and 12 [8–10]. It is often synthesized using R-methacrylate (R is a functional group, for example, butyl, glycidyl, etc.), crosslinker, porogen solvent and polymerization initiator. Jiang et al. [8] described the preparation of 100 μm i.d. monolithic column based on polymer media for capillary electrochromatography. Holdšvendová et al. [9] and Moravcová et al. [10] reported the preparation of polymer monolithic columns (320 μm i.d. × 20–24 cm) for capillary liquid chromatography. Both the sample capacity and allowable detection cell volume of the 320 μm i.d. columns are five to nine times higher than that of 100–150 μm i.d. columns. This is beneficial for practical use of capillary liquid chromatography.

In this study, we described a procedure for the preparation of methacrylate monolithic μ-HPLC column of 100 cm (eff) × 320 μm i.d., which is four times longer than that previously reported. The columns were prepared using butyl methacrylate (BMA) as functional monomer, ethylene dimethacrylate (EDMA) as crosslinker, a ternary solvent consisting of 1-propanol, 1,4-butanediol and water as porogen solvent, and azoisobutyronitrile as polymerization initiator.

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2. Experimental

2.1. Materials

Butyl methacrylate (BMA, 99%), ethylene dimethacrylate (EDMA, 98%), 1-propanol (99%), (γ-methacryloyloxypropyl) trimethoxysilane (γ-MAPS, 98%) were supplied by Acros (USA). Uracil (ultra pure grade) was from Amresco (USA). Acetonitrile (HPLC grade purity) was from Tedia (USA). 1,4-Butanediol (AR) and azoisobutyronitrile (AIBN, AR) were provided by Tianjin Fuchen Chemical Reagents Factory (Tianjin, China). Acetone (AR), benzene (AR) and toluene (AR) were from Shenyang Lianbang Chemical Reagents Factory (Shenyang, China). Ethylbenzene (CR) was from Beijing Chemical Plant (Beijing, China). Sodium hydroxide (AR) was from Liaoning Medical Reagents Plant (Liaoning, China). Butyl methacrylate, 1-propanol and 1,4-butanediol were used after vacuum distillation. Azoisobutyronitrile was used after secondary crystallization. The water was bi-distilled water starting from purified water of Wahaha (Wahaha, Hangzhou, China).

2.2. Instruments

Fused-silica capillary of 320 μm i.d. was from Yongnian Optical Fibre Factory (Hebei, China). A thermostat (Shanghai Experimental Instrument Factory, China) was used during polymerization. A Jasco CE-975 on column UV–vis detector, a Jasco PU-1580 Intelligent HPLC pump (JASCO, Japan) and a Valco injection valve with a 500 nL internal loop (VICI, Switzerland) were employed in the chromatographic measurements. Detection wavelength was 254 nm. Mobile phase was acetonitrile–water (60:40, v/v). Chromatographic data was collected and manipulated by using a KeFen Chromstation (Dalian Scien-Tech Instrument Inc. Ltd., Dalian, China). The column morphology was studied by a JSM-6360LV scanning electron microscope (SEM) (JEOL Inc., Tokyo, Japan).

2.3. Capillary inner wall modification

Since the columns are going to be used at HPLC mode, the monolith must be firmly bounded to the capillary wall to prevent displacement of the monolithic stationary phase by the mobile phase at high pressure.

The fused-silica capillary tubing was first washed with 0.1 M sodium hydroxide solution for 2 h, and then flushed with purified water until the pH value of the outlet solution reached 7. Thereafter, the capillary was washed with 50 column-volumes of acetone. The capillary was dried at room temperature under purge of nitrogen for 5 h. The capillary was filled with silanization solution containing 30% (v/v) γ-MAPS in acetone, sealed with rubber and stayed overnight. After silanization, the capillary was flushed with 50 column-volumes of acetone and dried by the purge of nitrogen gas.

A comparison of the stability of monolith columns prepared by using modified (C6 and C7) and unmodified (C4 and C5) (see Table 1) capillaries were made under the same chromatographic conditions. It showed that the polymer monolith in C4 and C5 were flushed out of the capillaries by the mobile phase during elution, while the monolith was held inside the tubing in C6 and C7, proving that the polymer monolith in C6 and C7 were covalently bounded to the inner wall of the capillaries. The modified capillaries were used for the preparation of methacrylate monolithic columns in the following experiments.

2.4. Monolithic column preparation

The monomers (BMA and EDMA), the polymerization initiator (AIBN, 1 wt.% with respect to the monomer amount) and porogen (1-propanol, 1,4-butanediol and water)

Table 1
Compositions of the polymerization mixtures used for preparation of methacrylate monolithic columns and their total porosities

Column	Porogen mixture (wt.%)			Polymerization mixture (vol.%)		Total porosity[a]
	1-Propanol	1,4-Butanediol	Water	Monomer mixture	Porogen solvent	
C1	50	40	10	30	70	0.79
C2	50	40	10	35	65	0.76
C3	50	40	10	40	60	0.66
C4[c]	55	35	10	50	50	NM[b]
C5[c]	45	45	10	25	75	NM
C6	55	35	10	50	50	NM
C7	45	45	10	25	75	0.80
C8	45	45	10	15	85	NM
C9	45	45	10	20	80	0.83
C10	50	40	10	50	50	NM

C1–C10: total length, 110 cm; effective length, 100 cm. Monomer mixture (wt.%): BMA, 59.5%; EDMA, 39.5%; AIBN, 1%.

[a] The total porosity was calculated from the dead retention time: $\varepsilon_t = 4Ft_0/d^2\pi L$ (F: volumetric flow-rate; t_0: the retention time of an unretained marker (uracil); d: the column inner diameter; L: column length).

[b] NM, not measured.

[c] The inner wall of C4 and C5 were unmodified, others were modified.

were mixed ultrasonically into a homogenous solution. The monomer mixture and the porogen solvent were mixed at various ratios to prepare monoliths with various porosities, as detailed in Table 1. Subsequently, the reactant solution was purged with nitrogen gas for 3 min to remove oxygen before it was aspirated into the capillary to a certain length (at the distance of 10 cm from the outlet end). Both ends of the capillary were plugged with a piece of rubber and the capillary was submerged in a water bath at 60 °C for 20 h. The capillary column was then attached to the HPLC pump. Mobile phase was pumped through the column to remove the porogen solvents and other unreacted soluble compounds.

A 2–3 mm segment of the outer coating of the column, at a distance of 8 cm from the outlet end, was removed using a razor blade for detection window.

3. Results and discussion

3.1. Optimization of polymerization mixture composition

We choose the ratio of BMA/EDMA = 60/40 (w/w) in the monomer mixture in our experiments based on the literatures [7,8,11]. The content of water in the porogen solvent were kept constant at 10 wt.%. The influence of the contents of 1-propanol and the porogen solvent in the polymerization mixture on the porosity and performance of the methacrylate monolithic columns were investigated in our study. Different contents of 1-propanol and porogen were used in the preparation of columns as detailed in Table 1.

The fact that the pressure drop across the column is proportional to the column length brings much more strict requirement for column permeation characteristic in long column preparation. Therefore, the porosity control of the monolithic columns is especially important in our study. The monolith porosity increases as the content of the porogen solvent increases. When the content of the porogen solvent in the polymerization mixture reached 85%, the polymer monolith in C8 (see Table 1) were flushed out of the capillary by the mobile phase due to the excessively large pore size formed in the polymerization and accordingly the loosening polymeric bed formed. In contrast, when the content of the porogen solvent reduced to 50% (see C6, C10 in Table 1), the pressure drop across the columns reached 18 MPa at a mobile phase flow-rate of less than 5 μL/min due to the highly dense polymeric bed formed. Further experiments at the porogen solvent content of 60, 65, 70, and 80%, respectively, showed that all the columns exhibited a poor separation performance at mobile phase flow-rates of 1–8 μL/min range. A chromatogram of the test mixture obtained with column C9 (porogen solvent content is 80%) was depicted in Fig. 1. The column performance was drastically improved when the porogen solvent content was 75% (column C7) as demonstrated in Fig. 2, where all the test compounds were eluted at the similar operating conditions as in Fig. 1.

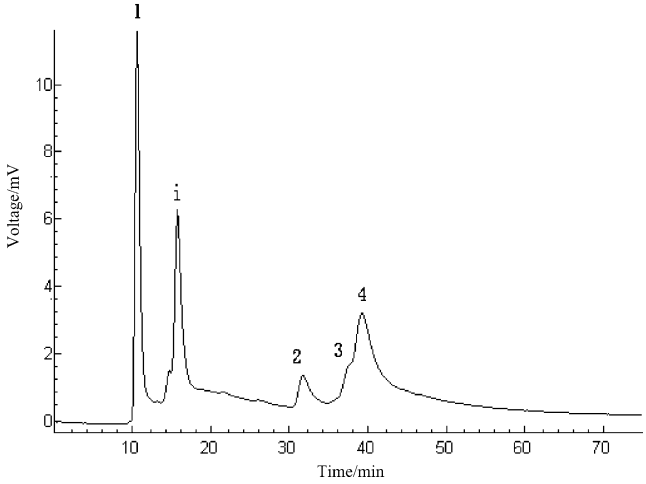

Fig. 1. Chromatogram on column C9. Peak identities: (1) uracil, (2) benzene, (3) toluene, (4) ethylbenzene, (i) impurity. Effective column length: 100 cm; mobile phase flow-rate: 6 μL/min; back pressure: 20 MPa.

We found that the porosity was also sensitive to the amount of 1-propanol. As the content of 1-propanol in porogen solvent increased, the monolith porosity decreased. Experiments showed that when the content of 1-propanol in porogen solvent reached 55%, the column exhibited a very high resistance (very low permeability) and showed a very poor mechanical stability. As the content of 1-propanol in porogen solvent reduced to 45%, a satisfactory result was obtained.

Considering all the above aspects, we choose the composition of C7 (see Table 1) as the optimum composition for the preparation of 100 cm long columns. The following experiments were carried out on C7 type columns.

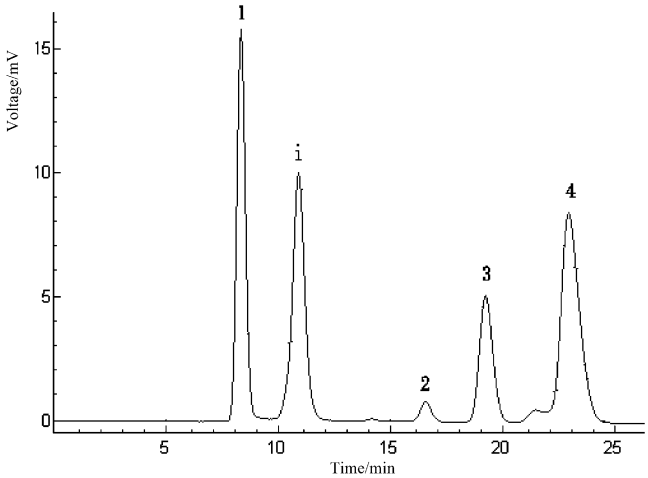

Fig. 2. Chromatogram on column C7. Peak identities: (1) uracil, (2) benzene, (3) toluene, (4) ethylbenzene, (i) impurity. Effective column length: 100 cm; mobile phase flow-rate: 8 μL/min; back pressure: 16 MPa.

3.2. Column characterization

The performance of the column C7 for the test compounds of various polarities, including uracil, benzene, toluene and ethylbenzene, were evaluated at flow-rates of 1–8 μL/min. During injection, the valve was remained in INJECT position for 3 s, and then turned back to LOAD position. The injection volume is then 3 s × (1–8) μL/min, which is 50–400 nL.

3.2.1. Column permeability

In general, the permeability of monolithic columns was higher than that of packed columns. The permeability [12], K, calculated according to Eq. (1), was $K = 7.2 \times 10^{-14}\ m^2$, that is about three times higher than that of a column (15 cm × 320 μm i.d.) packed with 5 μm C_{18} particles. The results show that the column head pressure was directly proportional to the flow-rate of mobile phase and exhibited a good linearity in the range of 1–8 μL/min (data not shown). This should be attributed to the stable structural characteristics of the synthesized monolithic material. The SEM image of the monolithic column is shown in Fig. 3, which indicates that the largest channels in the polymer reached several μm, resulting in higher permeability but lower mass transfer process. On the other hand, the good linearity between column head pressure and the mobile phase flow-rate clearly demonstrates that the cross-linked monoliths were mechanically stable to withstand the pressure of the liquid passing through the column up to 17 MPa.

$$K = \frac{u\eta L}{\Delta P} \tag{1}$$

where η is the viscosity of the mobile phase; L the column length; ΔP the back pressure; and u the linear velocity of mobile phase.

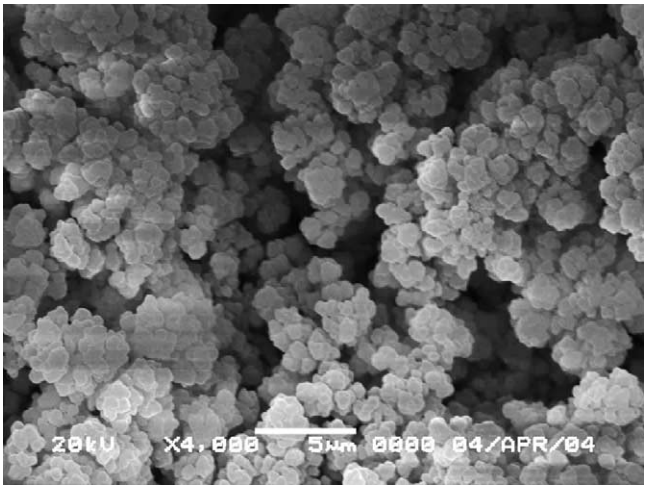

Fig. 3. Scanning electron micrograph of 100 cm long monolithic column.

Table 2
Maximum injection volumes for packed and monolithic microcolumns with different length

Column i.d. (μm)	Column length (cm)	V_{inj} (nl)	
		Packed column (d_p = 5 μm)	Home made monolithic column
75	25	5.7	–
320	25	105	136
	100	–	544

A 5% loss in column efficiency was allowed ($\theta^2 = 0.05$). The total porosity of packed column was taken as 0.70 and plate height ($3d_p$) was assumed. The total porosity of monolithic column was taken as 0.80. Ideal injection profiles ($K^2 = 12$), optimum mobile phase flow-rate was assumed. The retention factor k equalled 0.

3.2.2. Sample loadability

In μ-HPLC, the maximum allowable injection volume V_{inj} for a non-retained compound can be estimated by:

$$V_{inj}^2 = K^2\sigma_{inj}^2 = \theta^2(K\pi r^2\varepsilon_t)^2 HL \tag{2}$$

where V_{inj} is the maximum allowable injection volume; θ^2 the loss in column efficiency; ε_t the total porosity; r the column radius; H the plate height; and L the column length; ideal injection profiles ($K^2 = 12$).

From Eq. (2), the maximum allowable injection volume can be calculated when a 5% loss in column efficiency ($\theta^2 = 0.05$) is permitted. The results for packed and monolithic microcolumns with different length are given in Table 2. From the results, we can seen that the 100 cm × 320 μm i.d. monolithic column possess a much higher sample loadability (more than 500 nL) which is compatible with a larger-volume detection cell and results in a higher detection sensitivity.

3.2.3. Column performance

The performance of the prepared monolithic column was evaluated by using uracil, benzene, toluene and ethylbenzene as test compounds. Fig. 2 demonstrates the chromatogram of test compounds on column C7. The peak asymmetry factors for uracil, benzene, toluene and ethylbenzene peaks were 1.08, 0.98, 1.27, and 1.57, respectively.

The R.S.D. of retention time of uracil, benzene, toluene and ethylbenzene for four repeated injections was 1.16, 0.63, 0.60 and 0.58%, respectively, at a flow-rate of 6 μL/min. These data proved that the monolithic column processed both the mechanical and chemical stability. The column-to-column reproducibility of five columns prepared in the same way were about ±15% (25,000–33,000 plate/column) on column efficiencies, and 5.4, 3.7, 4.8 and 4.9% R.S.D. on retention time, respectively, for the four test compounds.

The van Deemter curves of all test samples in a flow-rate range of 1–8 μL/min are plotted in Fig. 4. Considering the linear plots for all the test compounds, the resistance to mass transfer between the stationary phase and mobile phase seemed to be the dominating factor for band broad-

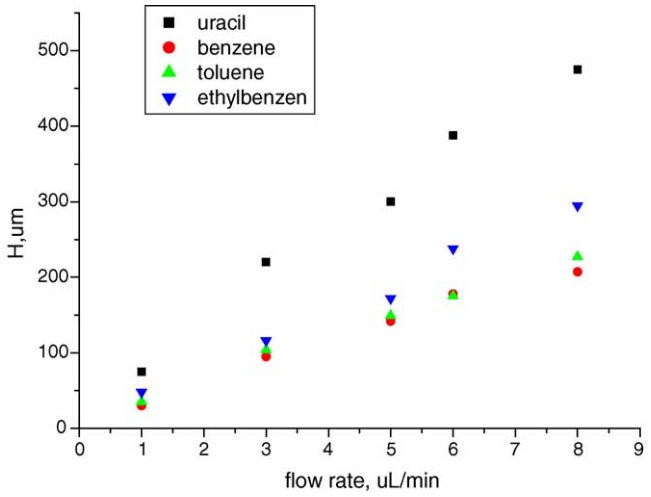

Fig. 4. Dependence of plate height (H) on the flow-rate of the mobile phase. Column: 100 cm × 320 μm i.d. methacrylate monolithic column.

Table 3
Comparison of total plate number of 100 cm and 25 cm monolithic columns

Flow-rate (μL/min)	N (sample: benzene)	
	100 cm	25 cm
1	33.3×10^3	6.41×10^3
3	10.5×10^3	3.01×10^3
5	7.04×10^3	2.23×10^3
8	4.83×10^3	1.70×10^3

ening mechanism. The lowest plate height values of the compounds, obtained from Fig. 4, were 30 μm (benzene) to 75 μm (uracil), depending on the properties of compounds, which is similar to those obtained in the literature [11]. Compared with a 25 cm × 320 μm i.d. column prepared in the same way, the 1 m long monolithic column exhibited the same column efficiency per unit length and generated 33,000 plates/column, as shown in Table 3.

A reversed-phase chromatographic retention mechanism of the butyl methacrylate monolith was evident from two facts: the retention order of the test compounds, and that the capacity factors are independent of the flow-rate.

4. Conclusion

BMA monolithic μ-HPLC columns of 100 cm (eff) × 320 μm i.d. prepared in this study could separate both polar and apolar small molecules with plate height of tens of μm. It was proved that the long monolithic columns exhibited good permeability, higher sample loadability, and can generate much higher total plate number and separation capability than short column, which is especially important for the analysis of complex samples. Compared with slurry method, the preparation of long monolithic column was easier, and there is no limit for the length of the column.

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