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Octyl-type monolithic columns of 530 μm i.d. for capillary liquid chromatography

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Abstract

A novel monolithic capillary column (530 μm i.d.) was prepared for capillary liquid chromatography (CLC) by in situ copolymerization of octyl methacrylate (MAOE) and ethylene dimethacrylate (EDMA) in the presence of a porogen solvent containing 1-propanol, 1,4-butanediol, and water with azobisisobutyronitrile as the initiator. The influences of the contents of the porogen solvent, EDMA and the various concentration ratios of 1-propanol to 1,4-butanediol in the polymerization mixture on the morphology, porosity, globule size, stability and column efficiency were investigated. The morphology and pore size distribution of monolithic capillary columns were characterized by SEM and mercury intrusion porosimetry, respectively. Chromatographic evaluations of the columns were performed under CLC mode. The results showed that good permeability and stability can be obtained under optimal experimental conditions. The separation results of some acid, neutral and basic analytes demonstrated the hydrophobicity and low affinity to basic analytes of the new column. Three metal ions, i.e. Mg(II), Zn(II) and Cd(II) were also separated under ion-pair mode on the new monolithic capillary column and the results were acceptable.

Keywords: Monolithic columns; Stationary phases; Capillary liquid chromatography; Methacrylate

1. Introduction

Capillary liquid chromatography (CLC) has been one of the main trends in the separation technique in the past decade because of its low sample and solvent consumption, high sensitivity of detection and ease of coupling with special detectors (MS, AES, NMR) [1,2]. However, some serious technical problems have slowed down the development of CLC. These problems include the packing of micron-sized particles into a narrow-bore tube, the limited stability of packed columns, and the difficult preparation of solid and stable frits at both ends of the column. At the same time it is extremely difficult to avoid side effects arising from the frits [3,4], i.e. bubbles formation within the capillary during runs.

In order to avoid the problems described above for making packed columns, a good strategy is to prepare monolithic media which was introduced by Hjerten et al. [5] and subsequently investigated by other research groups [6–9]. 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. Presently, there are two main types of monolithic materials, silica sol–gels [7,10–12] and porous organic polymers [13–17]. In principle, the polymeric approach exhibits more potential advantages and a more promising future compared to the silica-based counterparts. This is due to the simpler preparation process, easier pore size control, and more facile adaptability to adjust column selectivity. Up to now, three types of polymers, i.e. polyacrylamides [18–20], poly(methacrylate esters) [21–28] and polystyrenes [14,29–31], have been used for the preparation of organic monolithic columns.

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Although monolithic columns have recently been prepared and applied to capillary liquid chromatography and capillary electrochromatography (CEC), most of the work reported to date has been focused on the development of small size capillary monolithic column and was applied in CEC [7,8,10–23]. There are only a few papers dealing with columns of larger diameters and their use in CLC [24–27,29,30,32]. Therefore, this paper reports the use of octyl methacrylate as monomer to prepare octyl-type capillary monolithic column (OTCM) of 530 μm i.d. Compared with that of columns with smaller diameters, i.e. 100, 150, 200 and 320 μm i.d. [23–27,29–35], larger columns can be expected to be easier to operate in CLC mode, i.e. simple column installation to the injection valve, simple connection with the detector window, lower column head pressure and increased sample loadability. The effects of contents of monomer and porogen solvent on the characters of OTCM were investigated in detail. The chromatographic evaluations of the columns were also performed under CLC mode.

2. Experimental

2.1. Chemicals

Octyl methacrylate (99%), ethylene dimethacrylate (EDMA) (97%) and 3-(trimethoxysilyl)-propyl methacrylate (γ-MAPS) (95%) were supplied by Tokyo kasei kogyo Co. (TCI, Japan); azobisisobutyronitrile (AIBN) (97%, recrystallized before use), 1-propanol (97%) and 1,4-butanediol (98%) (distilled before use) were purchased from Shanghai Chemical Co. (China). Fused-silica tubing (530 μm i.d.) was got from Ruifeng Instrumental Co. (Hebei, China); cadmium(II), zinc(II), and magnesium(II) stock solutions were prepared by dissolving specpure Cd, Zn, and MgO (Shanghai, China) with HNO_3 and diluted to 1.0 μg/mL, respectively. HPLC-grade acetonitrile was purchased from Tedia Company (Fairfield, USA). Water used throughout this study was purified using a Milli-Q water purification system (Millipore, USA). Other reagents used in this study were of at least analytical grade, and the mobile phases as well as the samples were degassed before use.

2.2. Equipments and materials

A micro HPLC system was consisted of a pump (LC10ADvp, Shimadzu), a system controller (SCL-10Avp, Shimadzu), a SPD-M10Avp UV detector (2.5 μL flow cell), a fluorescence detector with 2.0 μL flow cell (RF-10AXL, Shimadzu) and Shimadzu CLASS-VP 5.0 chromatography workstation. Sample injection was performed using a Model 7520 manual micro-scale sample injector with a 0.2 μL loop (Rheodyne, Cotati, California, USA). All experiments were performed at room temperature and dead time was detected by thiourea.

The pore size distribution of the polymer was measured on mercury intrusion porosimeter (Porous Materials Inc., Ithaca, NY). The samples were made in a 2.0 mL vial by the same polymerization process as used for the corresponding columns. Before measurement, the bulk polymer was cut into small pieces, Soxhlet-extracted with methanol for 12 h, vacuum-dried for 6 h at 70 °C. The column morphology was studied by a Model XL30 SEM instrument (Philip, Netherlands).

2.3. Column pretreatment

The inner wall of the fused-silica capillary was treated with 0.5 mol/L NaOH for 30 min, flushed with 0.10 mol/L HC1 for 1 h, rinsed with deionized water for 1 h and then with acetone 10 min. Thereafter, the capillary was dried with a stream of nitrogen. There are several published procedures for creating double bonds on the inner surface used γ-MAPS as coupler [23–26], we also adopted the procedures reported but added some pyridine in the reactant as catalyst to favor the reaction of γ-MAPS with silanol [36], which would enhance the degree of vinylization and improve the stability of capillary monolithic columns.

2.4. In situ polymerization

AIBN was used as polymerization initiator (1 wt% of the total monomer amount) in the all polymerization reaction. Different monomers, porogen concentrations and volume fractions were used for different columns, as detailed in Table 1. The monomer mixtures and porogen (1-propanol, 1,4-butanediol and 10 wt% water) were mixed ultrasonically into a homogenous solution. Subsequently, the reactant solution was purged with nitrogen for 3 min before a small part of the reactant mixture was introduced into a silanized capillary by a 10 mL syringe. After both ends of the capillary were sealed with septa, it was kept at 60 °C for 12 h. After the polymerization, the capillary ends (10–15 mm long) were cut off, and the remaining monolithic column (about 20 cm long) was connected to the injection valve and flushed with acetonitrile to remove the residue monomers, porogen, uncross-linked polymers and initiator, then conditioned with the mobile phase.

3. Results and discussion

3.1. Effect of the contents of porogen solvent

The monolith porosity (ε_T) decreased as the content of the porogen solvent decreased. When porogen fraction decreased from 65% (column a) to 50% (column d), the ε_T decreased from 0.803 to 0.651 (Table 1), which demonstrated the permeability descended with decreasing the usage of porogen, so a higher pressure must be required to attain the same eluent flow-rates (data not shown). When the contents of porogen in polymerization mixture decreased to 45% (column e), the permeability was so bad that cannot be flushed by high pres-

Table 1
Characteristics of different columns

Column	Monomer mixture[a]		Polymerization mixture			Porosity (ε_T)[b]	Mean pore size (nm)[c]	C (%)[d]	H in CLC (μm)[e]
	MAOE (%, w/w)	EDMA (%, w/w)	Monomer mixture (%, w/w)	Porogen solvent (%, w/w)	Concentration of 1-propanol in porogen (%, w/w)				
a	59.5	39.5	35	65	60	0.803	84.2	1.1	473
b	59.5	39.5	40	60	60	0.722	68.7	0.6	436
c	59.5	39.5	45	55	60	0.708	56.9	0	430
d	59.5	39.5	50	50	60	0.651	40.1	0	272
e	59.5	39.5	55	45	60	NM[f]	NM	NM	NM
f	54.5	44.5	40	60	60	0.711	NM	0.4	603
g	49.5	49.5	40	60	56	0.765	70.1	1.2	870
h	49.5	49.5	40	60	58	0.712	62.8	0.8	410
i	49.5	49.5	40	60	60	0.703	56.1	0.2	172
j	49.5	49.5	40	60	64	NM	37.1	NM	NM
k	44.5	54.5	40	60	60	0.688	NM	0	748

[a] Weight fraction in monomer mixture.
[b] $\varepsilon_T = (Ft_0 - v_{ex})/v_{col}$, F: flow rate (μL/min); t_0: dead time (min); v_{ex}: extra-column volume (μL); v_{col} capillary volume (μL).
[c] Got from mercury intrusion porosimeter.
[d] Compressibility, $C\% = (L_0 - L_1)/L_0 \times 100\%$; L_0: initial column length; L_1: column length after high pressure was applied.
[e] Plate height (H) under CLC conditions. Column efficiency was calculated by half peak width method.
[f] NM, not measured.

sure pump. The microglobule size of the polymer a–e was estimated according to the SEM pictures. With the decrease of the porogen content from 65% to 50%, the microglobules became smaller (from about 1515 nm for column a down to 606 nm for column e) and the globule of polymer became denser so the ε_T decreased. The pore size distribution profiles and data of mean pore size of the polymers a–d were shown in Fig. l. and Table 1. It can be seen from the figure and table that higher porogen contents a larger pore size can be got. For example, the column a has a higher porogen fraction 65%, the size range was around 1800 nm and the mean pore size was 84.2 nm. While the column d with lower porogen 50%, the size range and the mean pore size decreased to 57.4 and 40.1 nm, respectively. This change trend of pore size with porogen content is consistent with earlier reported [23]. At the same time, when porogen volume fraction decreased from 65% to 50%, the column efficiency increased (showed in Table 1). Although the columns c and d and provided higher efficiency than column b, their permeability was too bad for further study. Therefore, the porogen volume fraction 60% was used in further experiments. At the same time, with the contents of porogen solvent in the polymerization mixture decreased, the polymer became harder so the compressibility decreased which means the column has higher stability (Table 1).

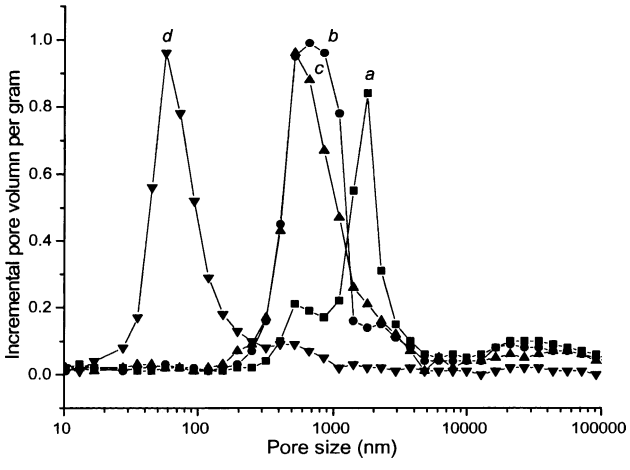

Fig. l. Pore size distribution profiles of the columns a–d.

3.2. Effect of the contents of EDMA

The contents of EDMA in monomer mixture have some effects on the characters of OTCM but not as drastic as the effect of the contents of porogen. As the EDMA fraction increased the ε_T and microglobule size decreased (Table 1). Fig. 2 depicted the dependence of plate height on the flow rate on column f, i and k. It can be seen from the figure and

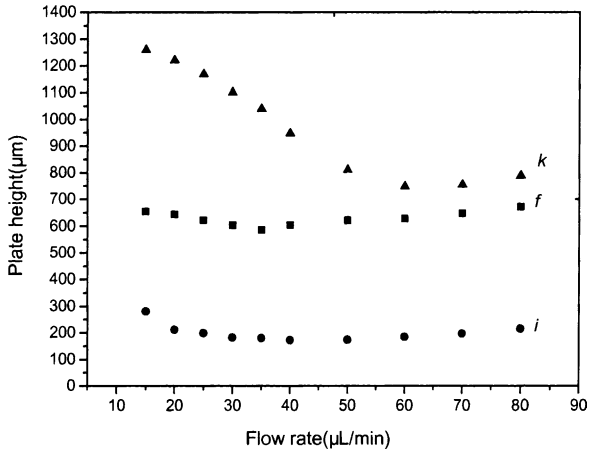

Fig. 2. Plots of plate height as a function of flow rate in CLC mode for columns f, i and k.

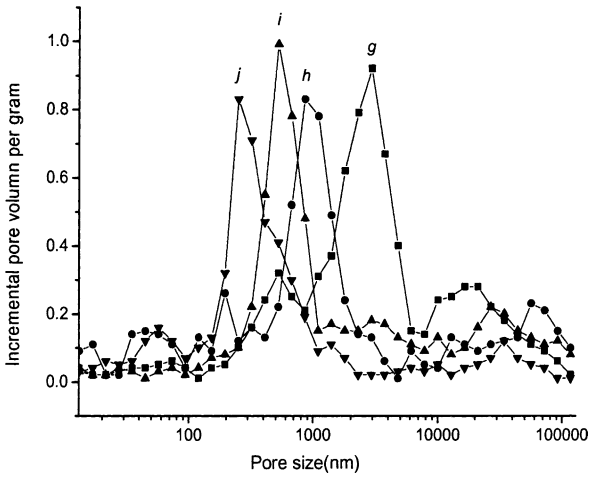

Fig. 3. Pore size distribution profiles of the columns g–j.

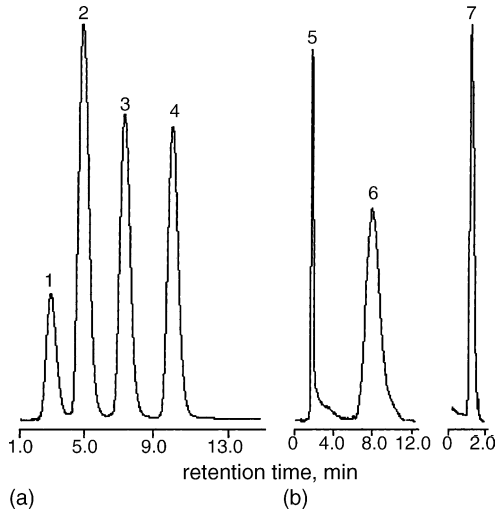

Fig. 5. Separation of some compounds on column i (a) and column b (b). Conditions: (a) on column i; effective column length, 19.95 cm; mobile phase, acetonitrile–water (20/80, v/v); flow rate, 40 μL/min; detection, 230 nm; (b) on column b; effective column length, 20.1 cm; mobile phase, 5 mmol/L 8-hydroxyquinoline-5-sulphonicacid, 3 mmol/L hexadecyltrimethyl ammoniumbromide, 10 mmol/L NaAc-HAc (pH 4.4)-acetonitrile (70/30, v/v); flow rate, 40 μL/min; detection, λ ex = 388 nm; λ em = 518 nm. Peaks: 1. bipyridine; 2. phenol; 3. *o*-tolunidine; 4. *p*-nitrophenol; 5. Mg(II); 6. Zn(II); 7. Cd(II).

Table 1 that there were not regular changes in the column efficiency with the contents of EDMA in monomer mixture. The column i with EDMA fraction 49.5% showed the higher efficiency than the columns b, f and k. It also can be seen from Fig. 2 that there is not obvious change in column efficiency when flow rate from 30 to 80 μL/min, which demonstrates the advantage of monolithic column[9,13]. The result showed that the preparation procedure published for butyl methacrylate monolithic columns of 100, 150 and 320 μm i.d. exhibiting good performance cannot simply be adopted for the preparation of 530 μm i.d. used octyl methacrylate as monomer.

3.3. Effect of the contents of 1-propanol in porogen

The constitutes of porogen solvent have important effect on the ε_T, pore size distribution and other characters of OTCM. As the contents of 1-propanol fraction in the porogen increased the value of ε_T decreased (Table 1). When the fraction of 1-propanol increased to 64%, the permeability was so bad that it can not be flushed through with mobile phase even under 15.0 MPa pressure. The pore size distribution profile was shown in Fig. 3. It can be seen from that when the fraction of 1-propanol was 56%, the size range was around 2300 nm and the mean pore size was 70.1 nm (Table 1). With highest content of 1-propanol 64%, the size range and mean pore size decreased to 251 and 37.1 nm, respectively. The globule size became smaller with the increase of 1-propanol (the SEM shown in Fig. 4). It is strange that the permeability of columns g, h and i was not obvious difference, the three columns all have a good permeability, but the pressure drop across column j reached 20 MPa at a mobile phase flow rate of 5 μL/min. The plate heights of columns g–i were showed in Table 1. The data clearly showed that the column efficiency was very sensitive to the amount of 1-propanol. This is consistent with early reported results [21–23,27]. It also was worthy to notice that the dosage of 1-propanol increased, the polymer became harder and the compressibility decreased which means the column was more stable (Table 1).

3.4. Chromatographic evaluation

The retention factors of aniline, phenol and benzaldehyde against the content of acetonitrile in mobile phase were inves-

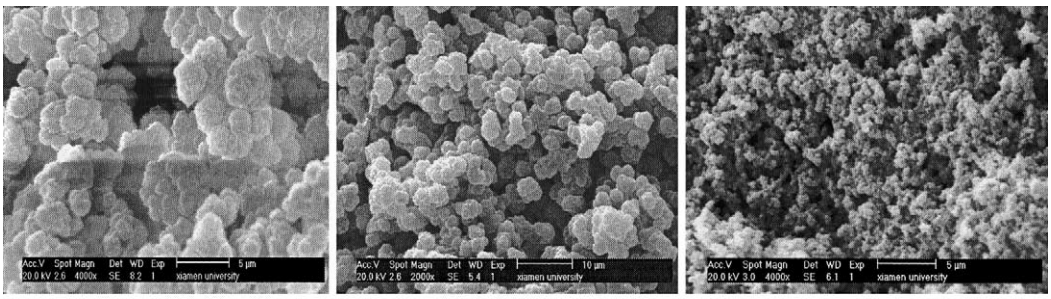

Fig. 4. Scanning electron microscope (SEM) pictures of columns g, i and j.

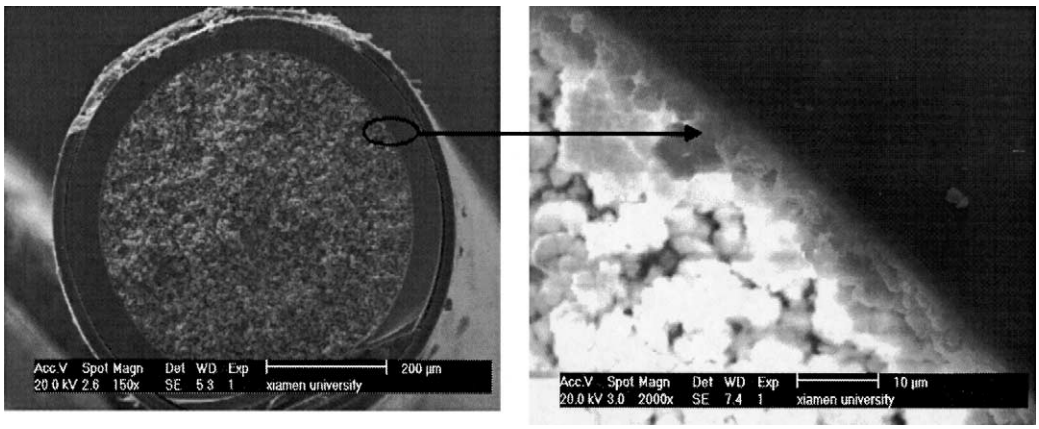

Fig. 6. SEM photos of a polymer rod column k with an inner wall modified fused-silica capillary.

tigated. Experiment showed that the log *k* decreased linearly with the fraction of acetonitrile increasing, which demonstrates that the column was operated by a reversed-phase mechanism [37]. Fig. 5a showed the separations of four small moleculars on column i under acetonitrile/water as binary mobile phase. The peaks in the chromatograms all have good peak symmetry, for example, the asymmetry factor As was 1.26 for bipyridine and 1.23 for *o*-toluidine. Three metal ions, i.e. Mg(II), Zn(II) and Cd(II), were also separated on column b under ion-pair mode (shown in Fig. 5b) and the results were acceptable, which provided a feasible method to separate metal ions using monolithic columns.

The chromatographic stability of the new columns was studied using the pyridine, 3-nitrophenol and benzene as test analytes. After flushing the column i about 8000 column volumes, the R.S.D.% ($n = 30$) of retention factor *k* of the three compounds were 1.60, 0.79 and 0.18, respectively and the R.S.D.% ($n = 30$) of Rs of adjacent two peaks were 0.68 and 2.92, respectively. The mechanical stability of the columns was also studied. After using the columns under high pressure (up to 14.0 MPa) for about 8 h, there may be some long empty part at inlet of the column was observed. It was more obvious for columns prepared using high porogen content and high 1-propanol fraction (Table 1). Although there was some shrinkage for the columns after flushing a period of time under high pressure, the chromatographic and mechanical stability are good enough for daily routine practice as a whole. The good stability is the result of the pretreatment of fused silica capillary with an acrylic double bond on the inner wall. As seen in Fig. 6, the polymer monolith in this column is covalently bounded to the inner wall of the capillary and there is no cleft between the polymer rod and the inner wall. Therefore, modified capillaries may be a better alternative than unmodified fused-silica capillaries in order to make more stabile monolithic columns.

3.5. Conclusion

A novel capillary monolithic column was prepared with using octyl methacrylate as monomer in 530 μm i.d. fused-silica capillary, which has not reported before. It was proved that not only the porogen solvent percentage, but also the content of EDMA and the fraction of 1-propanol in the porogen affect the porosity, permeability and other characters of the new column. Good permeability and stability can be obtained under optimal experimental conditions. The separation results of some acid, neutral and basic analytes demonstrated the reversed phase retention mechanism and low affinity to basic analytes of the octyl methacrylate monolith. The further study about the chromatographic characters and the application, especially in elemental speciation, of the new capillary monolithic column are undergoing.

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