Semi-micro-monolithic columns using macroporous silica rods with improved performance

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ABSTRACT

Monolithic silica columns in semi-micro-format have been synthesized using poly(acrylic acid) as a phase-separation inducer via a sol–gel route. The absence of a thick skin layer accompanied by deformation of the micrometer-sized gelling skeletons on the outermost part of the macroporous silica rod contributed to improve the efficiency of monolithic silica columns as thick as 2.4 mm in diameter. The kinetic plot analysis revealed that monolithic silica columns with macropore diameter of 1 μm and skeleton thickness of 1 μm with decreased macroporosity behave similarly to columns packed with 3 μm particles with slightly lower back pressure.

1. Introduction

Monolithic silica columns have been accepted as well-performing analytical columns with lower backpressure than that of particle-packed columns. However, there still exists a gap between real and "ideal" performances, although theories predict significant advantages of monolithic columns over particle-packed columns [1]. In this context, the effect of structural homogeneity on the micrometer scale on the column performance has recently been discussed extensively. In general, with a decrease in column diameter, the structural homogeneity over the cross-section becomes a dominant factor which influences the column performance, well known as a wall-effect in particle-packed columns [2]. This structural inhomogeneity is mainly due to the deformed macroporous structure in the vicinity of outermost part of the column [3], as well as to the incoherent or disturbed interfaces between the silica rod and the clad material.

A water-soluble organic polymer is often used to induce the phase separation in the sol–gel processes to fabricate macroporous silica rods [4]. On the event of phase separation, the added water-soluble polymer is distributed either to solvent-rich phase or silica-rich phase depending on the mutual interaction with the silica-polymer. In many cases, monolithic silica columns are produced using poly(ethylene glycol) (PEG) as a phase-separation inducer. In this conventional PEG-containing system, PEG is distributed to the silica-rich phase, so that the surface of the gelling phase becomes hydrophobic as a result of the specific adsorption of PEG chains onto surface silanol groups of silica oligomers [5]. In usual preparations using a hydrophobic mold, silica rods prepared in PEG-containing system tend to form a dense layer, generally called "skin layer", spreading on the mold wall (i.e. outermost part of the gelled silica rods). Accompanied by this skin layer formation, the micrometer-sized gel framework tends to deform before gelation due to the surface energy requirement. In addition, during the formation process of the skin layer, some deformation of the micrometer-sized gelling skeletons, e.g. the formation of elongated pillars, may take place, which produces structural inhomogeneity just beneath the skin layer (outermost part of the column). Both the presence of the skin layer and the structural inhomogeneity have harmful effects on the performance of monolithic columns.

On the other hand, it is reported that poly(acrylic acid) (HPAA) is distributed to the solvent phase on phase separation [6–9]. It was expected that the silica rods prepared in the HPAA-containing system behave differently from the PEG-containing system in the formation of the skin layer, as well as the tendency to give deformed structure in the vicinity of the mold wall. Moreover, in the HPAA-containing system, it is much easier to fabricate monolithic silica rods with reduced macroporosity than the case of PEG-containing systems. The reduced macroporosity is expected to improve the column performance in terms of plate numbers with some sacrifice in pressure drop [10–12].

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In present study, we have prepared monolithic macroporous silica rods with increased structural homogeneity over the cross-section using HPAA as a phase-separation inducer. The cladding method using partly molten glass tube was also employed to fabricate columns with larger plate numbers per unit column length. The results are discussed in comparison with those of commercially available Chromolith Performance column.

2. Experimental

2.1. Preparation of monolithic macroporous silica

Poly(acrylic acid) (HPAA, molecular weight 100 000, Sigma–Aldrich, USA) was dissolved in 2 M aqueous nitric acid and formamide (FA, both from Kishida Reagent Chemicals, Osaka, Japan). Tetraethoxysilane (TEOS, Shin-Etsu Chemical Co., Tokyo, Japan) was added to the above solution under vigorous stirring in an ice-cooled condition. The molar ratio of starting composition was TEOS:H_2O:FA:HPAA = 5:31:4:0.01.

After 30 min of stirring, the resultant homogeneous solution was poured into a polypropylene tube (3 mm I.D., 250 mm length) and allowed to gel at 40 °C in a closed condition. After aging at the same temperature for 10 h, the resultant gel was immersed in water for 3 h in order to remove residual acids, and solvent-exchanged with 1.5 M aqueous urea for 3 h. The following hydrothermal treatment took 4 h at 110 °C in 1.5 M aqueous urea. After additional solvent exchange with water, the obtained gels were dried and heat treated at 600 °C for 5 h in order to remove residual organics.

2.2. Structural characterization

A scanning electron microscope (SEM, JSM-6060S, JEOL, Tokyo, Japan) was employed for the observation of morphology of the gels. A mercury intrusion apparatus (PoreMaster 60GT, Quantachrome Instruments, USA) was used to determine the size distribution of pores larger than 5 nm in diameter.

2.3. Chromatographic measurements

Macroporous monolithic silica gels in semi-micro-size diameter (ca. 2.4 mm) were fabricated into HPLC columns using silicate glass as a cladding material; that is, the monolithic silica gels were put in the silicate glass tubes and the glass wall was adhered by heating while evacuating the inside of the tube. Surface modification of the monolithic silica was carried out on-column by circulating a solution of octadecyldimethyl-*N,N*-diethylaminosilane (ODS-DEA prepared from ODS-Cl and DEA) in toluene at 80 °C. The carbon content of resultant ODS-modified monolithic silica columns was 15.7%. This value is slightly lower than those of commercially available Chromolith PR-18e columns.

The evaluation of chromatographic performance was carried out using MP610 pump, MU701 detector (both from GL Sciences, Tokyo, Japan) and Rheodyne 8125 (Rheodyne, CA, USA) injector. The chromatographic data were processed with EZChrom Elite software (GL Sciences).

3. Results and discussion

3.1. Structural characterization

Fig. 1 shows SEM photographs of morphology of monolithic silica rods. Fig. 1(a) focuses on the central part of a rod prepared using HPAA as a phase-separation inducer. Fig. 1b and c focus on the outermost parts of monolithic silica rods, which are formed in the vicinity of mold walls. By using HPAA as a phase-separation inducer (b), no thick skin layers on the outermost part were observed in any of the monolithic silica rods prepared. On the other hand, by using PEG as a phase-separation inducer (c), relatively thick skin layers (often exceed 1 μm in thickness) and deformation of the micrometer-sized gelling skeletons, e.g. the formation of pillars, was observed. The thin, submicrometer-sized outermost layer and the absence of deformation of the micrometer-sized gelling skeletons of the monolithic silica ensure that the structural inhomogeneity, as well as the resultant inhomogeneous flow of mobile phase, should be minimized in the outermost region of the columns.

Fig. 2 shows the pore size distribution curves of the gels fabricated into HPLC columns determined by the mercury intrusion. The macroporous gels with ca. 1.0 μm macropore, 10 nm mesopore, a surface area of 370 m^2/g and a total porosity of 80% were reproducibly obtained. However, on the column basis, there observed a substantial difference in pressure drop by ca. 20% in spite of only a slight difference in the measured pore diameters between HPAA-silica column 1 (Fig. 2a, 1.14 μm) and HPAA-silica column 2 (Fig. 2b, 1.20 μm). This result indicates that the overall homogeneity across the column length is not fully regulated even in the present improved gel synthesis.

3.2. Performance of monolithic silica column with reduced porosity and smaller domain size

Fig. 3 shows the chromatogram of the monolithic silica column using macroporous gel in the elution of alkylbenzenes in 70% acetonitrile. The monolithic HPAA-silica column exhibited as large as 140 000 theoretical plates/m at the linear velocity of 4.19 mm/s. Even at the experimentally available maximum linear velocity of 8.28 mm/s, 120 000 theoretical plates/m was obtained.

Figs. 4 and 5 respectively show the van Deemter plots and the separation impedance plots, for reversed phase separation of amylbenzene with ODS-modified HPAA columns and commercially obtained Chromolith Performance RP-18e (3.0 mm I.D., 100 mm length). The HPAA columns showed lower plate heights, *H*, with
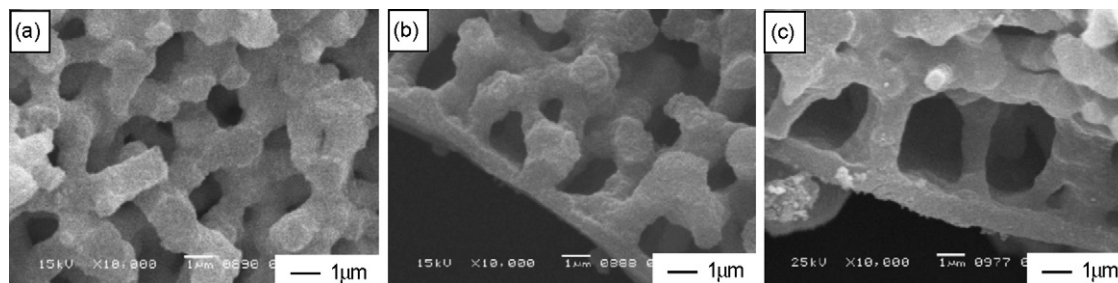

Fig. 1. SEM photographs of morphology of monolithic macroporous silica rods. (a) Central part of a rod prepared using HPAA as a phase-separation inducer; (b) outermost part of the same rod showing a thin skin layer with undeformed silica skeletons; (c) outermost part of a rod prepared using PEG as a phase-separation inducer showing a relatively thick skin layer accompanied by the deformation of adjoining silica skeletons.
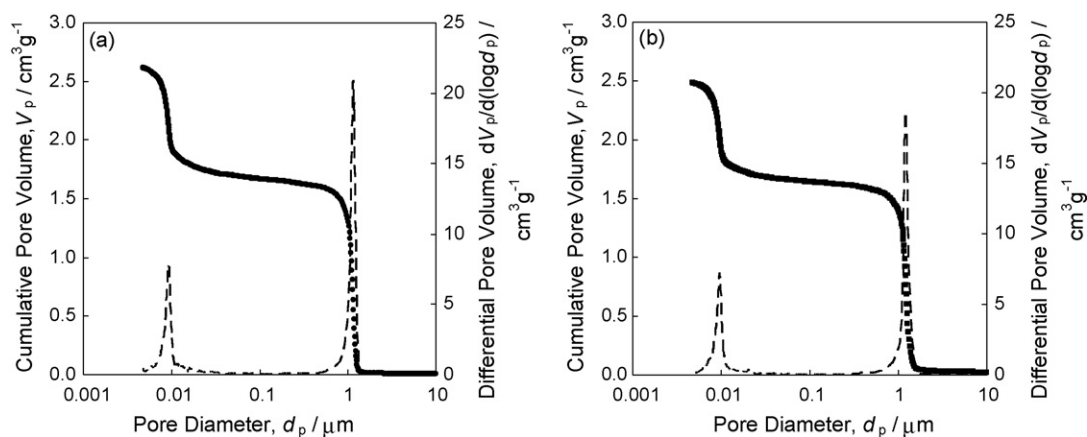
Fig. 2. Pore size distribution curves of macroporous gel using HPAA as a phase-separation inducer. (a) HPAA-silica column 1 and (b) HPAA-silica column 2.
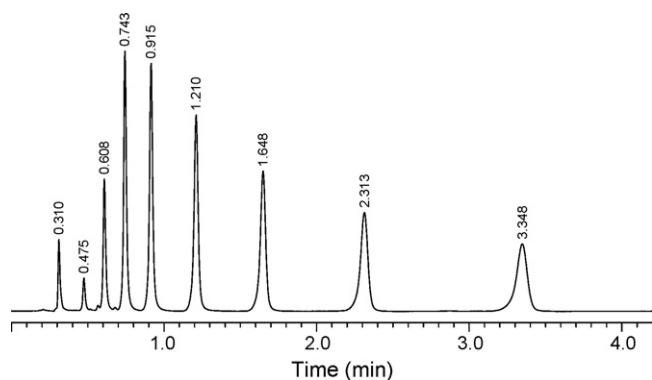

Fig. 3. Chromatogram of HPAA-silica column 1 obtained in elution of uracil and alkylbenzenes ($C_6H_5(CH_2)_nH$, $n=0$–6) in 70% acetonitrile. Column size is 90 mm × 2.4 mm I.D.

their minimum being located at higher linear velocity, u, and less steep H–u slopes than the case of Chromolith Performance RP-18e. Considering that the thickness of gel skeletons is slightly thinner in HPAA columns, these results are reasonable in that HPAA columns with reduced porosity behave similarly to the columns packed with smaller particles. The minimum separation impedances of HPAA columns are roughly 30% larger than that of commercial Chromolith Performance RP-18e. This result again coincides with the lower back
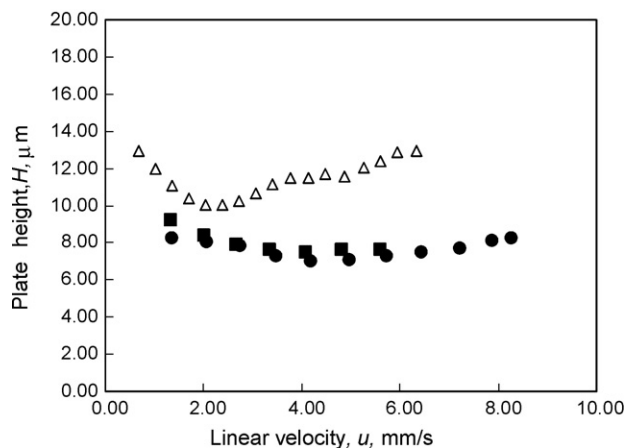
pressure due to the higher macroporosity of the latter. At u values larger than 4 mm/s, however, the separation impedance values become comparable for all the columns, again due to the flat H–u dependence of HPAA columns. Comparison between HPAA columns 1 and 2 indicates that the separation impedance, E, of column 2 is slightly lower than that of column 1, reflecting the measured difference in back pressure. As mentioned above, their still remains room for the structural optimization to generate lower plate height and lower back pressure.

Since the present HPAA-silica columns behave differently from commercial Chromolith columns, their analytical performance has been compared with that of calculated columns packed with small particles using the kinetic plot method. Fig. 6 shows the kinetic plots, $\log(t_0/N^2) - \log N$, for RP separation of amylbenzene of the columns with maximum lengths calculated with respective permeabilities at a fixed back pressure of 40 MPa. The curves for particle-packed columns were calculated using Knox equation, $h = 0.65\nu^{1/3} + 2/\nu + 0.08\nu$, where h and ν are reduced plate height and reduced velocity. The assumed values of viscosity, permeability, and diffusion coefficients are shown in the caption [11]. The plotted values for HPAA-silica columns are located almost vertically below those of 3 μm particle-packed column. Thus, the monolithic silica columns with reduced porosity and smaller domain size of ca. 2 μm (prepared with HPAA) are revealed to exhibit kinetic properties similar to those of 3 μm particle-packed column rather than commercial Chromolith column with high porosity. This result also indicates that the HPAA-columns are potentially
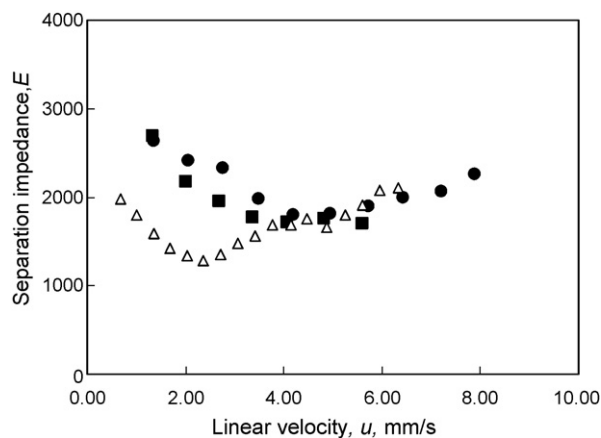

Fig. 4. van Deemter plots for reversed phase separation of amylbenzene with the monolithic silica columns prepared in HPAA system and Chromolith Performance RP-18e. Filled circle: HPAA-silica column 1; filled square: HPAA-silica column 2; open triangle: Chromolith Performance RP-18e.

Fig. 5. Separation impedance plots for reversed-phase separation of amylbenzene with the monolithic silica column and Chromolith Performance RP-18e. Filled circle: HPAA-silica column 1; filled square: HPAA-silica column 2; open triangle: Chromolith Performance RP-18e.
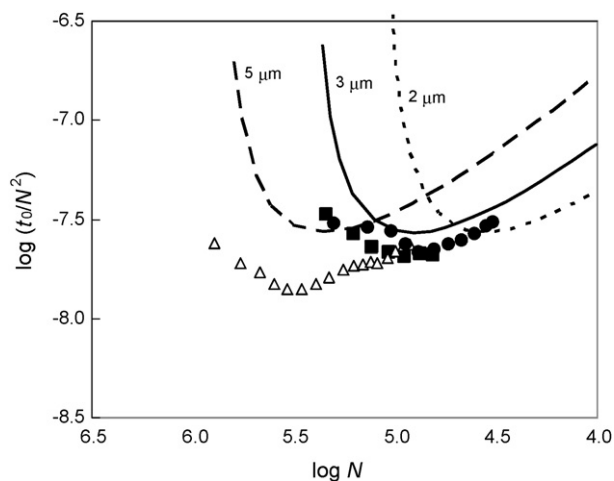
Fig. 6. Kinetic plots for reversed phase separation of amylbenzene with the monolithic silica columns and Chromolith Performance RP-18e, and calculated curves for the particle-packed column with particle diameter, $d_p = 2$, 3 and 5 µm. The curves were obtained by the following parameters: dynamic viscosity, $\eta = 0.00046$ Pa s, flow resistance, $\phi = 700$, molecular diffusion coefficient in mobile phase, $D_m = 2.22 \times 10^{-9}$ m^2/s, and Knox equation, $h = 0.65\nu^{1/3} + 2/\nu + 0.08\nu$, where h is reduced plate height ($h = H/d_p$), and ν is reduced velocity [11]. Filled circle: HPAA-silica column 1; filled square: HPAA-silica column 2; open triangle: Chromolith Performance RP-18e.

able to perform separations at shorter times with the plate number equivalent to 3 µm particle-packed column. The above plots under the constraint of 40 MPa applied pressure suggest that near their respective minimum plate heights (optimum linear velocities); a 3 µm particle-packed column with 20 cm length will generate $N = 30\,000$ with $t_0 = 36$ s, while HPAA-silica columns with 40 cm length generate $N = 60\,000$ with $t_0 = 80$ s. Due to their flat H–u dependence, the possible length and predicted performance of the latter columns at higher linear velocities approach that of the particle-packed column at around $u = 10$ mm/s with shortened t_0 of ca. 20 s. Considering the mechanical robustness of the monolithic columns, HPAA-silica columns with reduced porosity and finer domains will be suitable for the UPLC-type fast separations.

4. Conclusion

Semi-micro-monolithic columns using macroporous silica rods have been prepared using poly(acrylic acid), HPAA, as a phase-separation inducer. Macroporous silica rods with improved homogeneity over the cross-section have been obtained.

The obtained monolithic macroporous silica rods were fabricated into HPLC columns using silicate glass clad and evaluated in reversed-phase mode HPLC using alkylbenzenes as solutes. The column prepared using HPAA-containing silica rod performed as large as 140 000 plates/m ($H \sim 7$ µm) and exhibited very gentle H–u slope at higher linear velocity.

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