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Monolithic silica columns for high-efficiency separations by high-performance liquid chromatography

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Abstract

Generation of a large number of theoretical plates was attempted by capillary HPLC. Monolithic silica columns having small skeletons (ca. 2 μm) and large through-pores (ca. 8 μm) were prepared by a sol–gel method in a fused-silica capillary (50 μm I.D.), and derivatized to C_{18} phase by on-column reaction. High external porosity (>80%) and large through-pores resulted in high permeability ($K=1.2\times10^{-12}$ m^2). The monolithic silica column in the capillary produced a plate height of about 12 μm in 80% acetonitrile at a linear velocity of 1 mm/s. Separation impedance, E value, was found to be as low as 200, that was about an order of magnitude lower than reported values for conventional columns packed with 5 μm particles. Reproducibility of preparation within ±15% was obtained for column efficiency and for pressure drop. It was possible to generate 100,000 plates by using a 130-cm column at very low pressure (<7 kg/cm^2). A considerable decrease in column efficiency was observed at high linear velocity, and for solutes with large retention factors due to the slow mobile-phase mass transfer in the large through-pores. The monolithic silica columns, however, showed performance beyond the limit of conventional particle-packed columns in HPLC under favorable conditions.

Keywords: Monolithic silica columns; Stationary phases, LC; Columns

1. Introduction

Higher column efficiency and reduction of analysis time in HPLC have been achieved by using smaller particles that cause smaller eddy diffusion and faster mass transfer [1–3]. The limitation associated with this approach was realized with conventional HPLC instrumentation having a pressure limit of ca. 350–400 kg/cm^2 [3]. Many researchers have been trying to overcome the problem of high pressure drop associated with the use of small particles by employing ultrahigh-pressure liquid chromatography (UHPLC) [4,5], capillary electrochromatography (CEC) [6], or by open tube liquid chromatography [7]. Although CEC has been extensively studied and is known to provide high column efficiency in short time, up to 200,000 plates with 120 s t_0 (column dead time) [8], CEC has not been widely used in routine applications due to practical difficulties including frit failure, or bubble formation.

The problems associated with particle-packed columns for CEC were alleviated by using a monolithic column that was either prepared from monomers or from a particle-packed capillary column. The

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single-piece structure can be formed starting from a packed bed of silica particles in a capillary by a sol–gel process or by sintering [9–12]. The monolithic silica columns provided 40,000–70,000 theoretical plates per 20–25 cm with t_0 of about 200 s that were fewer than that of a column packed with particles without such treatment. Monolithic columns based on organic polymers in a capillary have also been reported [13–17]. Polymer monolithic columns generally produced low efficiency for small molecules compared with columns packed with silica particles due to the presence of micropores in a polymer-gel structure, although column efficiency of up to 150,000 plates/m for small molecules has been reported in CEC with very long separation time [13]. It is not feasible to operate these columns under pressure-driven conditions, because of low permeability and/or inadequate rigidity.

The preparation of monolithic silica columns has also been reported by using a sol–gel process starting from tetramethoxysilane [18–22]. It is possible to create such a monolithic column that has small-sized skeletons and large through-pores with the through-pore size–skeleton size ratios much greater than 0.25–0.4 found for conventional columns packed with particles [23]. The monolithic silica columns simultaneously showed lower pressure drop and higher efficiency than a particle-packed column. The advantages of such a monolithic column were proven in high-speed separation of both small and large molecules [18,24–26]. The preparation of monolithic silica columns in a mold, however, limits the length of a column to be less than 15 cm or so, otherwise a straight monolithic column cannot be prepared causing problems in the subsequent column fabrication process that includes cladding with an engineering plastic. Therefore the monolithic silica columns (4–6 mm I.D.) for conventional HPLC prepared in a mold can provide high separation speed, while the maximum number of theoretical plates per column is rather limited. The generation of a large number of theoretical plates requires a series of such columns connected.

We also prepared a continuous silica gel structure in a 100-μm fused-silica capillary and evaluated in HPLC and CEC [21]. Under pressure-driven conditions, the monolithic silica column with 2 μm skeletons and large through-pores of up to 10 μm showed column efficiency of 6–8000 plates/25 cm at very low pressure drop. Although the CEC operation with a 25-cm column was not advantageous due to the slow electroosmotic flow generating only 30,000 theoretical plates, the results indicated the potentially high-performance of such a monolith in a capillary in a pressure-driven mode based on the extremely high permeability. Here we report an improved preparation method of monolithic silica columns in a long 50-μm diameter capillary as well as their evaluation in HPLC that showed high performance and fair reproducibility.

2. Experimental

The preparation of monolithic silica gel for capillary HPLC was as follows: tetramethoxysilane (4 ml) was added to a solution of poly(ethylene glycol) (0.88 g, $M_w = 10{,}000$, Aldrich) and urea (0.90 g) in 0.01 *M* acetic acid (10 ml) and stirred at 0 °C for 45 min. The resultant homogeneous solution was charged into a fused-silica capillary tube (Polymicro), which had been treated with 1 *M* NaOH solution at 40 °C for 3 h in advance, and allowed to react at 40 °C. The gelation occurred within 2 h and the gel was subsequently aged in the capillary overnight at the same temperature. The monolithic silica column formed was treated at higher temperatures with ammonia generated by the hydrolysis of urea [27], for 3 h at 120 °C to complete the formation of mesopores, followed by a wash with water and methanol. After drying, heat-treatment was carried out at 330 °C for 25 h, resulting in the decomposition of organic moieties in the capillary. This process was accompanied by no serious deformation or fracture of gel structure. Usually two 100–200-cm capillary columns were prepared from the same reaction mixture. After preparation, each end (10–15 cm) of the capillary having large voids was cut off, and 2–4 33.5-cm-long columns were obtained from two 100-cm capillaries. A detection window (2 mm) was made by removing polyimide coating at a specified distance from the capillary inlet to allow on-column detection through the monolithic bed with some loss of sensitivity. The surface modification of the monolithic silica was carried out on-column by continuously feeding the solution of

octadecyldimethyl-*N*,*N*-diethylaminosilane (2 ml) in 8 ml of toluene under the pressure of 0.05 kg/cm^2 at 60 °C for 3 h.

The morphology of the monolithic silica was examined by scanning electron microscopy (SEM, S-510 Hitachi) using a fractured surface. A split-injection HPLC system used in this study consists of the conventional equipment, a pump (LC-10A, Shimadzu), a UV detector (CE 971, JASCO) that allowed on-column detection operated at 210 nm or 254 nm, a data processor (C-R6A, Shimadzu), and an injection valve (model 7125, Rheodyne) fitted with a T-joint which serves as a splitter, one end connected to a capillary column and the other end to a flow restrictor that is a stainless steel column (4.6 mm I.D., 10–15 cm long) packed with ODS-silica particles (5–15 μm particle size). In order to avoid an excessive pressure pulse during sample injection, a by-pass was created by connecting the 0.25-mm I.D. tubings from the pump to the injector and from the splitting T-joint to the restrictor with 0.1-mm I.D. tubing and two other T-joints. Split ratio was controlled at about 1/1000 or smaller with 0.2 ml/min or greater solvent delivery from the conventional pump in order to maintain the high efficiency and reproducibility with a consistent flow at sub-μl/min in capillary. Chromatography measurement was carried out at an ambient temperature (20–25 °C). Commercial columns packed with 5-μm ODS-silica particles, Mightysil RP18 and Inertsil ODS3 were obtained from Kanto Chemical and GL Science, respectively.

3. Results and discussion

Fig. 1 shows the SEM photographs of the fractured surfaces of the monolithic silica columns in a capillary. Large voids due to the shrinkage of silica were observed along the wall of a large diameter capillary (250 μm), but not in smaller capillaries. The shrinkage of the whole network structure could be avoided by attaching the silica skeletons to the tube wall of a small-diameter capillary that has the greater wall area–volume ratio. The attachment of the silica skeletons to the wall, however, made large through-pores. The uniform-sized silica skeletons of about 2 μm and the through-pores of up to 8 μm were distributed evenly over the cross section of the 50-μm I.D. capillary. The silica morphologies possess appearance of aggregates of silica spheres, or a corpuscular system [28], different from the network structures of smooth cylindrical skeletons, or a spongy system, found with large-sized skeletons of monolithic silica columns prepared in a mold reported previously [18].

Because a micro-HPLC system that can operate a 50-μm column is not readily available, we had to compose a split-injection system as described in the Experimental section. Another way of operating the small sized column will be provided by a CE instrument, either in CEC mode or in HPLC mode. Because the permeability is so high, pressure below 10 kg/cm^2 used in CEC is sufficient to cause a significant flow for pressure-driven mode. A larger-sized capillary type monolithic silica column will have advantages including the availability of commercial instruments and the compatibility with an injection system without splitting, in addition to the smaller contribution of the extra-column effects.

Continuous silica structure was prepared by hydrolysis and polycondensation of tetramethoxysilane which involves the concurrent occurrence of phase separation based on spinodal decomposition and sol–gel transition to form bicontinuous domain structures [29]. Since the resultant gel morphology reflects the domain structure frozen by the gel formation on coarsening, it mainly depends on parameters such as starting composition and reaction temperature that affect the onset of phase separation relative to the occurrence of sol–gel transition. The present silica skeleton structures indicate that the phase separation relative to the gelation proceeded further as compared with the case of silica monoliths prepared in a mold having smooth cylindrical surfaces. Nakanishi et al. reported the preparation method of mesopores that can be tailored by aging under basic conditions by utilizing the hydrolysis of urea [27]. The addition of urea in the starting preparation mixture has made the preparation process simpler, since the mesopores are formed just by heating the whole reaction mixture in the capillary without introducing aqueous ammonium hydroxide solution.

The silica skeletons are more densely distributed when prepared in urea solution compared to the

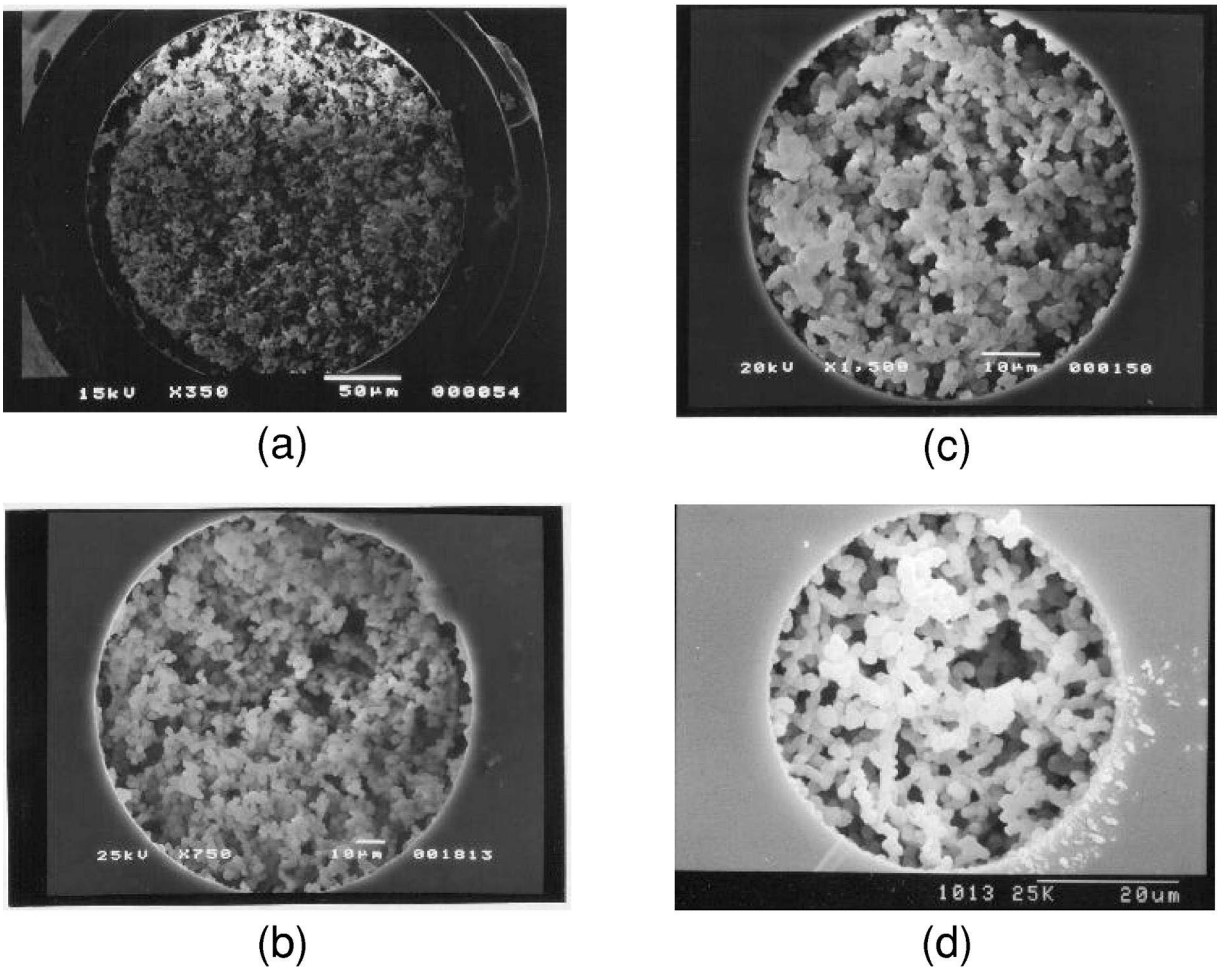

Fig. 1. Scanning electron micrographs of monolithic silica prepared in fused-silica capillaries prepared in 250 μm (a), 100 μm (b), 75 μm (c), and 50 μm (MS(50)) capillary (d).

previous monoliths prepared in the absence of urea [22]. The size of through-pores was much larger than skeleton size resulting in the through-pore size–skeleton size ratio of up to about 4. Through-pore size–skeleton size ratios were reported to be 0.25–0.4 with a column packed with particles [23], and 1.2–1.5 for monolithic silica columns prepared in a mold with shrinkage of the skeletons [18]. The MS(50) columns possessed ca. 92% total porosity and 83% external porosity, as determined by SEC, the porosities being slightly lower than those of monolithic silica columns in a capillary prepared in the absence of urea [21]. The internal porosity of silica skeletons was similar to that of monolithic silica columns prepared in a mold.

The permeability of monolithic silica columns was much higher than that of packed columns. Fig. 2 shows the plots of pressure drop against the linear velocity of mobile phase. The permeability, K, calculated according to Eq. (1) [2] (η, viscosity of the mobile phase; L, column length; ΔP, back pressure; u, linear velocity of mobile phase) was typically $K = 1.2 \times 10^{-12}$ m^2, that is about 25–30 times higher than a column packed with 5-μm particles, as shown in Table 1, and about 3–15 times greater than that of a monolithic silica column

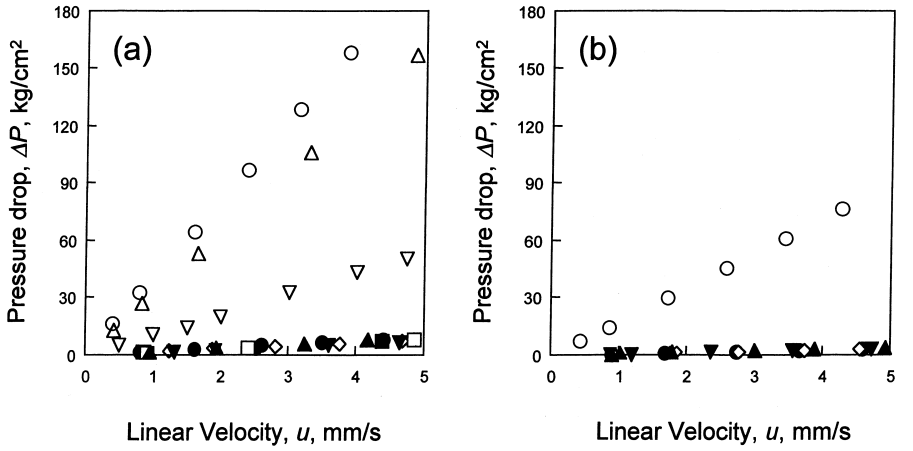

Fig. 2. Plots of column back pressure against linear velocity of mobile phase. Mobile phase: (a) 80% methanol, (b) 80% acetonitrile. The pressures were normalized to the column length of 15 cm. Columns: 5 μm silica-C_{18} particles, Inertsil ODS3 (△), Mightysil RP18 (○). Monolithic silica column prepared in a mold, SR-(B)-C_{18} (▽) [20]. Monolithic silica column in capillary, MS(50)-A (●), MS(50)-B (▲), MS(50)-C (▼), MS(50)-D (■), MS(50)-E (□), MS(50)-F (◇). MS(50)D–F, 50 μm I.D.× 33.5 cm, effective length 25 cm.

prepared in a mold [19], reflecting the high porosity and the large through-pore size–skeleton size ratios. The monolithic silica column was stable against 200 kg/cm^2 inlet pressure, as shown later.

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

Fig. 3a–c shows the chromatograms obtained with three monolithic silica columns (MS(50)-A, 25 cm; MS(50)-B, 45 cm; MS(50)-C, 130 cm) for alkylbenzenes in 80% acetonitrile at a linear velocity of ca. 1 mm/s. MS(50)-A and MS(50)-C were prepared from the same batch of starting mixture in different capillaries, while MS(50)-B was prepared independently. Excellent peak symmetry was observed. At low linear velocity of 0.9 mm/s, MS(50)-A produced 16,000 theoretical plates in 80% acetonitrile that are similar to the efficiency of a common 15-cm column packed with 5-μm particles, but at much lower pressure drop of 0.7 kg/cm^2. Fig. 3c shows that the 130-cm column (MS(50)-C) produced 106,000 theoretical plates for hexylbenzene at 0.9 mm/s linear velocity with pressure drop of 3.7 kg/cm^2. It is noted, however, that these high efficiencies are only realized for solutes with very low k values. The k values obtained with the present monolithic silica columns in capillary were much smaller than those with the monolithic silica of conventional-size or with the particle-packed columns as previously reported [21]. Due to the small retention factor (k), the effective plate number ($N_{\text{eff}} = N\{k/(1+k)\}^2$) was about 19,000 for hexylbenzene.

Table 1
Permeability of columns

Column material	Permeability, K ($\times 10^{-14}$ m^2)
Inertsil ODS3[a]	5.0
Mightysil RP18[a]	3.9
Monolithic silica[b]	17
MS(50)-A	120
MS(50)-B	100
MS(50)-C	130

[a] A column packed with 5-μm silica-C_{18} particles.
[b] Monolithic silica column prepared in a mold, SR-(B)-C_{18} in Ref. [20] (through-pore size: 2.2 μm, skeleton size: 1.8 μm).

Fig. 4 shows the van Deemter plots obtained with hexylbenzene as a solute in 80% methanol and 80% acetonitrile. The MS(50) columns showed lower column efficiency than a monolithic silica column prepared in a mold. Plate height minimum was not found above linear velocity of 0.8 mm/s. The MS(50) columns, however, showed much better performance than the monolithic silica columns prepared in the absence of urea in a capillary of 100 μm diameter [21]. The reproducibility of preparation in terms of plate height was within ±15% in most

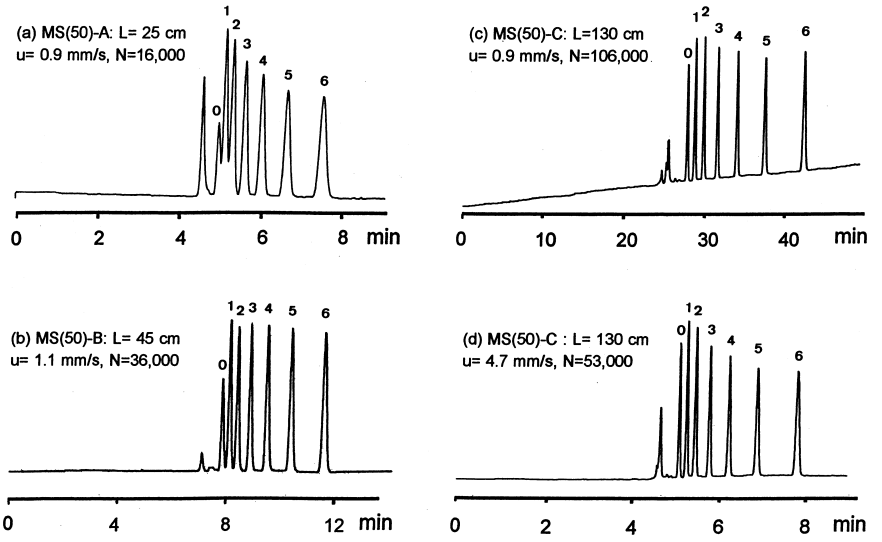

Fig. 3. Chromatograms obtained for alkylbenzenes ($C_6H_5(CH_2)_nH$, $n=0$–6). Column: (a) MS(50)-A: column size: 50 μm I.D.×33.5 cm (effective length 25 cm), (b) MS(50)-B: 50 μm I.D.×53.5 cm (effective length 45 cm), (c, d) MS(50)-C: 50 μm I.D.×138.5 cm (effective length 130 cm). Mobile phase: 80% acetonitrile. Linear velocity: (a) 0.9 mm/s, (b) 1.1 mm/s, (c) 0.9 mm/s, (d) 4.7 mm/s.

cases, while the pressure drop was reproducible within ±15% for several preparations that are much better than the preparations in 100 μm capillary. The longer columns showed lower plate height than the shorter ones presumably due to the smaller contribution of extra-column effects. The plots for MS(50)-E (25 cm) in Figs. 2 and 4 were obtained after delivering 80% methanol to MS(50)-D column at 200 kg/cm^2 for 1 h.

The column efficiency of the MS(50) per unit length was lower than that of a column packed with 5-μm particles by a factor of about two in a low velocity region. The pressure drop of MS(50), however, was about 1/25–1/30 that of such a

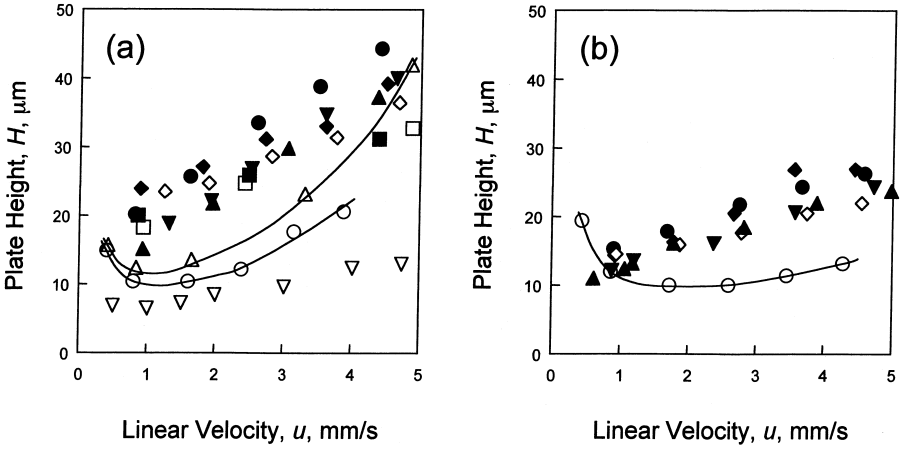

Fig. 4. The van Deemter plots obtained for C_{18} monolithic silica columns and silica-C_{18} packed column with hexylbenzene as a solute. Mobile phase: (a) 80% methanol, (b) 80% acetonitrile. Symbols as in Fig. 2 for the columns.

conventional column. Thus it was possible to generate more than 100,000 theoretical plates at very low pressure, as shown in Fig. 3c. At a higher linear velocity (4.7 mm/s), MS(50)-C produced 50,000–60,000 theoretical plates at 28 kg/cm^2 with t_0 of 4.5 min (Fig. 3d). The results suggest that it will be possible to generate 100,000 plates at ca. 60 kg/cm^2 with t_0 of 9 min by using a 250-cm column. Such a column would generate nearly 200,000 theoretical plates at a lower linear velocity. In order to achieve high efficiency at high linear velocities, it is necessary to further optimize the silica structure in capillary to have smaller domain sizes (a combined size of the through-pore size and the skeleton size) as discussed below, although it will be accompanied by the reduction in permeability.

The total performance of an MS(50) column is much higher than that of a particle-packed column, as indicated by the smaller separation impedance, E value (Eq. (2), N, number of theoretical plates; H, a height equivalent of a theoretical plate), as shown in Fig. 5. The minimum separation impedance, E value, of 100–200 was obtained, while a column packed with 5-μm particles gave minimum E values of 2500–3000. The results can also be compared favorably with the performance of a capillary column packed with 1-μm ODS-silica particles generating 125,000 plates under 2300 kg/cm^2 with 2 min t_0 in UHPLC [5]. Long monolithic silica columns possess potential utility for generating large numbers of theoretical plates with current instrumentation, although they need considerably longer separation time than a column packed with small silica particles to be used in CEC or UHPLC.

$$E = \frac{t_0 \Delta P}{N^2 \eta} = (\Delta P/N)(t_0/N)(1/\eta) = H^2/K \quad (2)$$

$$H = 1/[(1/A_e) + (1/A_m u)] + B/u + Cu \quad (3)$$

$$H = 1/[(1/C_e d_p) + (D_m/C_m d_p^2 u)] + C_d D_m/u + C_{sm} d_p^2 u/D_m \quad (4)$$

$$H = \frac{2D_m}{u} + \frac{1 + 6k + 11k^2}{96(1+k)^2} \cdot \frac{d_c^2}{D_m} u + \frac{2k}{3(1+k)^2} \cdot \frac{d_f^2}{D_s} u \quad (5)$$

Fig. 6 shows the van Deemter plots obtained for MS(50)-C for several alkylbenzenes as solutes. Eqs. (3) and (4) are commonly employed for the evaluation of column performance (d_p, particle size; D_m, diffusion coefficient of a solute in the mobile phase; A_x, B, C_x, coefficient for the contribution of each term). The slopes of the plots for MS(50) in Figs. 4 and 6 are steep as columns consisting of 2 μm silica skeletons. However, the slopes of the van Deemter plots obtained for monolithic silica columns prepared

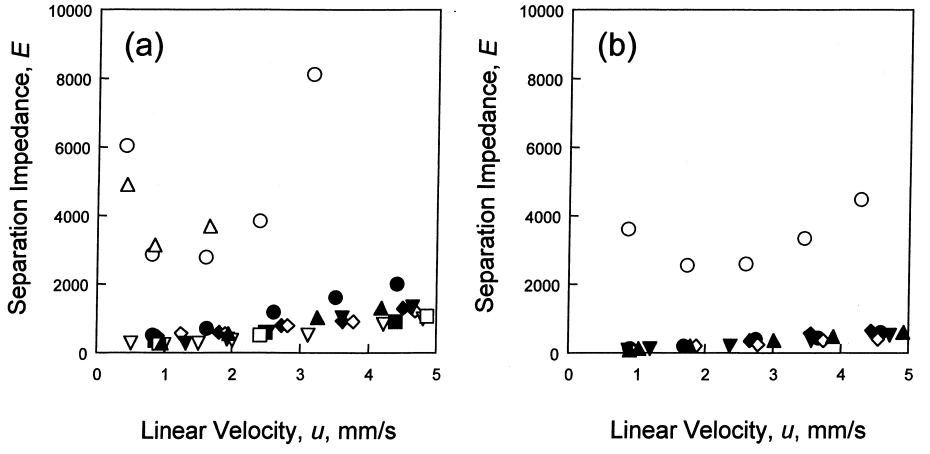

Fig. 5. Plots of separation impedance against linear velocity of mobile phase calculated for hexylbenzene as a solute. Mobile phase: (a) 80% methanol, (b) 80% acetonitrile. Symbols as in Fig. 2 for the columns.

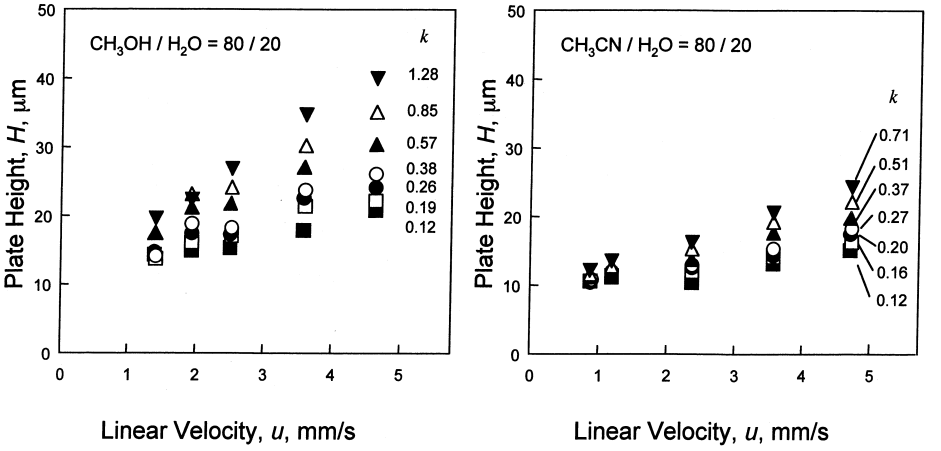

Fig. 6. The van Deemter plots for alkylbenzenes as solutes. Mobile phase: 80% methanol. Column: MS(50)-C. Samples: alkylbenzenes ($C_6H_5(CH_2)_nH$, $n=0$–6). $n=0$ (■), 1 (□), 2 (●), 3 (○), 4 (▲), 5 (△), 6 (▼).

in a mold having similar skeleton size and smaller through-pores (1–2.3 μm) were small in a similar linear velocity range in HPLC, and comparable with those of columns packed with 2–3-μm particles [19]. The use in CEC provided much lower plate height than in HPLC at linear velocity above 1 mm/s for a monolithic silica column of 2 μm skeleton size and 8–10 μm through-pores in the previous study [21].

The results shown in Fig. 6 also reveal the dependency of plate height on a retention factor (k value of up to 1.3 in Fig. 6a) especially at high linear velocity. The effect is greater in 80% methanol than in 80% acetonitrile. The dependency of plate heights on k value based on the mobile-phase mass transfer term is well known in open tube liquid chromatography, as described by the Golay equation (Eq. (5), D_s, diffusion coefficient in the stationary phase; d_c, inner diameter of the capillary; d_f, thickness of the stationary layer) [7], where small d_c would predict small effect of k on plate height. However, in the case of a column packed with particles or monolithic silica columns that possess much smaller size of through-pores (interstitial voids), the dependency of plate height on k value was not clearly observed [18,25].

These results suggest that the contributions of the stationary-phase mass transfer term and the mobile-phase mass transfer term to plate height are minor for a monolithic silica column with small domain size (ca. 2 μm size through-pores and skeletons), but the mobile-phase mass transfer term can make a major contribution in the pressure-driven operation of a monolithic silica column that has much larger through-pores. In other words, the present results shown in Figs. 4 and 6 suggest that the major cause of the steep slope of van Deemter plots, or the poor efficiency at high speed separation, is related to an A-term component ($A_m u$ in Eq. (3)) that has dependency on the linear velocity. The plate heights obtained in 80% acetonitrile are lower than in 80% methanol, presumably due to the larger diffusion coefficients of solutes in the mobile phase having lower viscosity. Knox recently pointed out that the A-term (Eq. (3)) makes a major contribution to band broadening on a particle-packed column in HPLC, while the C-term contribution is minor [30].

The contributions of multiple streampath (eddy diffusion) that is independent of linear velocity, and slow mobile phase mass transfer that is proportional to linear velocity, should be considered in combination for the characterization of a column packed with particles, as in Eqs. (3) and (4) [1,30]. The present results can be consistent with the following interpretations.

(i) The contribution of eddy diffusion is potentially very large, because of the presence of both very large through-pores and very small ones (Fig. 1). There is little chance for a streampath to meet

constriction in straight, large through-pores. Flow of mobile phase is not fast enough to reach the high-velocity region where the effect of eddy diffusion is dominating the band broadening.

(ii) If the through-pores in monolithic silica columns are straight and uniform in size [18–20], they could result in small contribution of eddy diffusion to plate height. In this case, the situation can be similar to that in open tube capillary chromatography where plate height is expressed by Eq. (5). But this is unlikely. The monolith structure is far from a straight open tube, as Fig. 1d shows the presence of both tiny space and large openings in the network structure.

Under the first assumption (i), the coefficient representing the band broadening due to eddy diffusion (A_e in Eq. (3)) should be very large for the monolithic silica columns having large through-pores. Then in a low linear velocity region, band broadening is dominated by the contribution of slow mobile-phase mass transfer ($A_m u$ term in Eq. (3)), as experimentally observed. The plots in Fig. 6 can be approximated by Eq. (6) or Eq. (3a) assuming minor contributions of B term (molecular diffusion in the mobile phase) and C term (stationary phase mass transfer) in Eqs. (3) and (4) in the linear-velocity range studied [30]. Monolithic silica columns prepared in a mold having similar skeleton size and smaller through-pores (1–2.3 μm) showed much smaller H values than MS(50) columns in a similar linear velocity range in HPLC [19]:

$$H = A' u^n \tag{6}$$

$$H = 1/(1/A'_e + D_m/A'_m u) \tag{3a}$$

Knox approximated the contribution of A_e and A_m terms in Eq. (3) by Eq. (6), and suggested that an exponent of 0.3–0.4 was likely for a particle-packed column, where the A term made the major contribution along with a C-term contribution at less than 5% of total plate height at much greater linear velocity [30]. The contributions of slow mass transfer in the mobile phase and eddy diffusion are coupled in Eqs. (3) and (4), but described collectively in Eq. (6). For MS(50) columns, Eq. (6) gave the best fit to the experimental results with an exponent of 0.50–0.60 when examined at 0.05 intervals, as shown in Fig. 7.

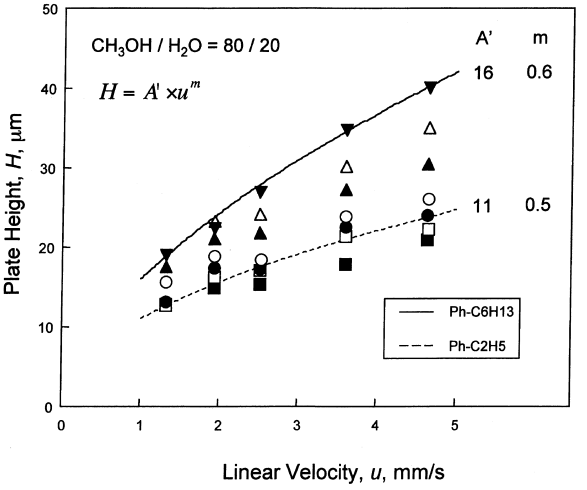

Fig. 7. The simulation with Eq. (6) for the van Deemter plots in 80% methanol for ethylbenzene and hexylbenzene as solutes. Column: MS(50)-C. Symbols as in Fig. 6 for samples. The curves were obtained with $n = 0.50$ (ethylbenzene) and 0.60 (hexylbenzene) in Eq. (6), as shown in the figure. A' value (Eq. (6)) was adjusted to give the best fit to the experimental points.

The larger exponent in Eq. (6) found for MS(50) than for a particle-packed column should indicate the greater contribution of A_m term in Eq. (3).

Major contribution of mobile-phase mass transfer term to plate height with a monolithic silica column having large through-pores can also be realized by the large A'_e value of 50–80 in Eq. (3a) that resulted in the curves in Fig. 8, showing fair fit for ethylbenzene and hexylbenzene, respectively. (The D_m values were calculated according to the Wilkie–Chang equation [31].) The large A'_e values agree with the assumption that eddy diffusion has little effect in determining the band broadening with the present MS(50) columns. The large A'_e value should reflect the irregularity of structure, or the presence of both large and small through-pores in a monolithic silica column, as well as the inefficient exchange of streampaths due to the characteristic structure of through-pores that are wide and relatively straight compared to interstitial voids of a particle-packed column.

Because the performance of the present monolithic silica in capillary seems to be dominated by the A term in the velocity range studied, particularly large A_m caused by the presence of the large through-pores

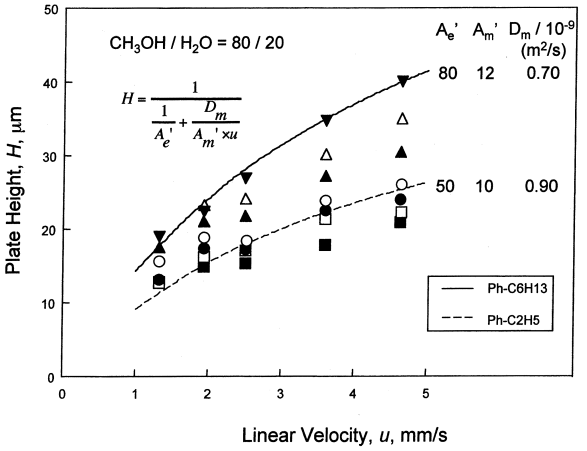

Fig. 8. The simulation with Eq. (3a) for the van Deemter plots in 80% methanol for ethylbenzene and hexylbenzene as solutes. Column: MS(50)-C. Symbols as in Fig. 6 for samples. The curves were obtained with $A_e' = 50$ (ethylbenzene) and 80 (hexylbenzene) in Eq. (3a), as shown in the figure. The D_m values were calculated by using the Wilkie–Chang equation [31]. A_m' value was adjusted to give the best fit to the experimental points.

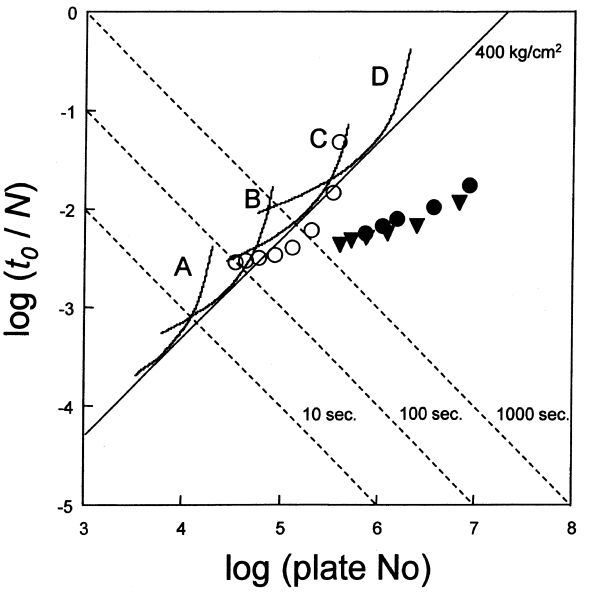

Fig. 9. Plots of plate time, (t_0/N), vs. plate number with particle-packed column and monolithic columns (adopted for 80% acetonitrile mobile phase from Ref. [3]). The curves for a column packed with particles were obtained by assuming the parameters, maximum pressure: 400 kg/cm^2, $\eta = 0.00047$ Pa/s, $\phi = 1000$, $D_m = 2.1 \times 10^{-9}$ m^2/s, and Knox equation $h = \nu^{1/3} + 1.5/\nu + 0.05\nu$. Particle diameter: (A) 1 μm, (B) 2 μm, (C) 5 μm, (D) 10 μm. The points are based on the experimental results in 80% acetonitrile on a monolithic column, (●) MS(50)-A, (▼) MS(50)-C, and a particle-packed column, Mightysil RP18 (○), extrapolated to the limiting conditions of 400 kg/cm^2. The dashed lines indicate the required t_0 values in seconds.

together with large A_e, much better performance can be expected in CEC, as observed [21], and for monolithic silica columns having smaller through-pore size:

$$t_0/N = h^2 \phi N \eta / \Delta P \quad (7)$$

Fig. 9 shows the plots of plate time (t_0/N) which is a measure of separation speed in HPLC against the number of theoretical plates in a logarithmic scale [3]. The curves in dotted lines are for particle-packed columns based on the calculated values for particle size of 1, 2, 5, and 10 μm, according to Eq. (7) [3]. A curve for a particular particle diameter was obtained on the basis of the column performance at various linear velocities of the mobile phase according to the equation, $h = A\nu^{1/3} + B/\nu + C\nu$, with the parameters $A = 1$, $B = 1.5$, and $C = 0.05$, where h and ν represent reduced plate height and reduced velocity, respectively. The maximum plate number attainable with a column packed with particles of each size could be calculated, assuming 400 kg/cm^2 as the pressure limit [3]. The plots for the 5-μm C_{18} particle-packed column, Mightysil RP18, and the C_{18} monolithic silica in capillary, MS(50)-A and MS(50)-C, are based on the experimental results, extrapolated to the limiting pressure of 400 kg/cm^2. The commercial column, Mightysil RP18, provided high efficiency close to the predicted performance for 5-μm particles.

The envelope for the curves obtained for various d_p values represents the limit of performance of a particle-packed column in HPLC obtainable under a practical pressure limit of a conventional pump, leading to the Knox and Saleem limit [3]. Under optimized HPLC conditions, a conventional particle-packed column would produce 100,000 theoretical plates with $t_0 = 1000$ s at pressure drop of 400 kg/cm^2 by using 4-μm particles. In the course of the column development in HPLC, the use of small particles actually shortened separation times with the reduction in the number of theoretical plates obtainable per unit time under certain pressure drop. The

use of small particles in a range, 1–2 μm, can generate large plate numbers only when the problem of high pressure drop is solved by such means as CEC or UHPLC.

The plots experimentally obtained for MS(50)-A and MS(50)-C are actually located close to the limiting performance of a particle-packed column under the pressure limit of 4000 kg/cm^2, and clearly indicate the advantage of the monolithic structure for producing high column efficiency. The results suggest that it is possible to generate large numbers of theoretical plates by using a long monolithic silica column in a capillary. The plots in Fig. 9 suggest that a column of MS(50)-C type can potentially produce ~100,000 with 400 s t_0 at 100 kg/m^2. A particle-packed column would need 400 kg/m^2 to achieve such efficiency. The present study showed that one way to generate a large number of theoretical plates in HPLC is to use a long capillary-type monolithic silica column possessing high permeability. The use of such a column in HPLC, however, will need longer separation time to provide similar efficiency than CEC or UHPLC using columns packed with small particles. Because the major source of band broadening is the slow mobile-phase mass transfer, monolithic silica columns with smaller domain size than the present columns will generate much higher column efficiency. The way of increasing column efficiency per unit time is very similar to the reduction in particle size for a column packed with particles, an approach taken for the last 30 years. Monolithic silica columns having smaller domain size are needed to meet current need for high speed separations. The use of monolithic silica columns in CEC mode is another way of increasing column efficiency and reducing the separation time, although the present monolithic silica columns showed limited performance due to the slow electroosmotic flow.

4. Conclusion

Monolithic silica columns prepared in a fused-silica capillary in the presence of urea from tetramethoxysilane were evaluated in HPLC. The preparation in a capillary has made the fabrication process simpler and the preparation of a long column feasible. The monolithic silica columns showed extremely high permeability based on the large through-pores. In spite of the adverse effect of the large through-pores on column efficiency, or the significant band broadening caused by the slow mobile phase mass transfer in the presence of a large eddy diffusion term, the total performance of such monolithic silica columns in terms of separation impedance was shown to be much higher than that of particle-packed columns. It was possible to generate 100,000 theoretical plates under favorable conditions. The present results suggest that one way to achieve high efficiency in liquid phase separations is micro-HPLC using a monolithic silica column, although CEC or UHPLC can still provide higher efficiency and shorter separation time by using columns packed with small particles.

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