ELSEVIER

Journal of Chromatography A, 961 (2002) 53–63

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Monolithic silica columns with various skeleton sizes and through-pore sizes for capillary liquid chromatography

Masanori Motokawa[a], Hiroshi Kobayashi[a], Norio Ishizuka[b], Hiroyoshi Minakuchi[b], Kazuki Nakanishi[c], Hiroshi Jinnai[a], Ken Hosoya[a], Tohru Ikegami[a], Nobuo Tanaka[a,*]

[a]*Department of Polymer Science and Engineering, Kyoto Institute of Technology, Gosho-kaido-cho, Matsugasaki, Sakyo-ku, Kyoto 606-8585, Japan*

[b]*Kyoto Monotech, 376-5-206 Tsukiyama-cho, Kuze, Minami-ku, Kyoto 601-8203, Japan*

[c]*Department of Material Chemistry, Graduate School of Engineering, Kyoto University, Yoshida, Sakyo-ku, Kyoto 606-8501, Japan*

Abstract

Reduction of through-pore size and skeleton size of a monolithic silica column was attempted to provide high separation efficiency in a short time. Monolithic silica columns were prepared to have various sizes of skeletons (~1–2 μm) and through-pores (~2–8 μm) in a fused-silica capillary (50–200 μm I.D.). The columns were evaluated in HPLC after derivatization to C_{18} phase. It was possible to prepare monolithic silica structures in capillaries of up to 200 μm I.D. from a mixture of tetramethoxysilane and methyltrimethoxysilane. As expected, a monolithic silica column with smaller domain size showed higher column efficiency and higher pressure drop. High external porosity (>80%) and large through-pores resulted in high permeability ($K=8\cdot10^{-14}$–$1.3\cdot10^{-12}$ m^2) that was 2–30 times higher than that of a column packed with 5-μm silica particles. The monolithic silica columns prepared in capillaries produced a plate height of about 8–12 μm with an 80% aqueous acetonitrile mobile phase at a linear velocity of 1 mm/s. Separation impedance, *E*, was found to be as low as 100 under optimum conditions, a value about an order of magnitude lower than reported for conventional columns packed with 5-μm particles. Although a column with smaller domain size generally resulted in higher separation impedance and the lower total performance, the monolithic silica columns showed performance beyond the limit of conventional particle-packed columns under pressure-driven conditions.

Keywords: Monolithic columns; Stationary phases, LC; Alkylbenzenes

1. Introduction

Higher column efficiency and reduction of analysis time in HPLC has been achieved by using smaller particles [1–3]. Conventional HPLC instrumentation having a pressure limit of ~350–400 kg/cm^2 limits what can be realized by this approach. In order to overcome the problem of high-pressure drop associated with the use of small particles, ultrahigh-pressure liquid chromatography (UHPLC) [4,5], capillary electrochromatography (CEC) [6,7], and open tubular liquid chromatography [8] have been studied. These high-performance methods, however, have not been widely used in routine applications due to practical difficulties.

We have shown that it was possible to prepare a monolithic silica column with small-sized skeletons (1–3 μm) and large through-pores (1.5–5 μm) with large through-pore size/skeleton size ratios (~1.2–1.5) and that such a column could give performance

*Corresponding author. Tel.: +81-75-724-7809; fax: +81-75-724-7710.

E-mail address: nobuo@ipc.kit.ac.jp (N. Tanaka).

0021-9673/02/$ – see front matter
PII: S0021-9673(02)00133-4

beyond the limit of a column packed with particles in pressure-driven liquid chromatography [9]. The monolithic silica columns prepared by using a sol–gel process starting from tetramethoxysilane [9–14] showed much lower separation impedance than a particle-packed column. The advantages of such a monolithic column were proven in high-speed separation of both small and large molecules [10,12–14]. Monolithic silica columns have been prepared in 50–100-μm fused-silica capillaries for use in HPLC and CEC [13,15–17]. Under pressure-driven conditions, a monolithic silica column with 2 μm skeletons and large through-pores of up to 10 μm in a 50-μm capillary showed efficiency of 16 000 plates/25 cm column length and 100 000 plates/130 cm column length at very low pressure drop. A similar column in a 100-μm capillary though showed lower performance. While this high efficiency was obtained with a considerably longer separation time than with CEC or UHPLC, the results indicated, based on the extremely high permeability, the potentially high-performance of such a monolith in a capillary in the pressure-driven mode. It is necessary to prepare monolithic silica columns with smaller skeleton size and through-pore size to make the separation faster, and also in a capillary of larger diameter for easy operation. Here we report a study on monolithic silica columns having various skeleton sizes and through-pore sizes in 100–200 μm capillaries, and the evaluation of their performance under pressure driven-conditions.

2. Experimental

The preparation conditions of monolithic silica columns were similar to those reported earlier [16,18,19]. Typical conditions are as follows: tetramethoxysilane (TMOS, 4 ml) was added to a solution of poly(ethylene glycol) (PEG, 0.88 g, M_r = 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, AZ, USA), which had been treated in advance with 1 *M* NaOH solution at 40 °C for 3 h, and allowed to react at 40 °C. Gelation occurred within 2 h and the gel was subsequently aged in the capillary overnight at the same temperature. Then, temperature was raised, and the monolithic silica column was treated for 3 h at 120 °C to complete the formation of the mesopores with ammonia generated by the hydrolysis of urea [19], followed by water and methanol washes. After drying, heat-treatment was carried out at 330 °C for 25 h, resulting in the decomposition of organic moieties in the capillary. 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 two to four 33.5-cm long columns were obtained from two 100-cm capillaries. A detection window (2 mm) was made by removing the polyimide coating at a specified distance from the capillary inlet to allow on-column detection through the silica monolith. 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 a pressure of 50 mbar at 60 °C for 3 h.

Morphology of the monolithic silica was examined by scanning electron microscopy (SEM, S-510 Hitachi, Tokyo, Japan) using a fractured surface. Three-dimensional structures of the monolithic silica were observed by laser scanning confocal microscopy (LSCM) [20] (Carl Zeiss, LSM510, Gottingen, Germany). The surface of the monolithic silica was labeled with a fluorescent compound, fluorescein 5-isothiocyanate (FITC isomer I), using the procedure detailed elsewhere [21]. The monolithic silica in the dry state is turbid and does not allow transmission of the laser light of the LSCM. A transparent, almost invisible, monolithic silica was necessary for the LSCM observation, which was attained by immersing the silica specimen in toluene–chloroform (ca. 17:83, w/w). An argon ion laser (wavelength: 488 nm) was used to excite the FITC moieties that were chemically attached to the silica skeleton. A band pass filter (515–565 nm) was installed in front of the detector (photomultiplier) so that only fluorescent light (approximately 519 nm) was detected using an oil-immersed 40×/NA = 1.3 (Plan-Neofluar, Carl Zeiss) objective, where NA represents numerical aperture. The silica monolith was scanned in the focal plane, measuring fluorescent intensity in a two-dimensional optically sliced image comprised of N^2 pixel^2 (resolution is 0.22 μm/pixel), where N is the number of pixels along the edge of the two-dimensional image; $N = 512$ was used for most of the

LSCM observations. In most cases, seventy optically sliced images were taken along the optical axis of the microscope (the *z* axis), perpendicular to the focal plane, with an increment of 0.4 μm.

A split-injection HPLC system used in this study consists of a pump (LC-10A, Shimadzu, Kyoto, Japan), a UV detector (CE 971, Jasco, Tokyo, Japan) operated at 210 or 254 nm, a data processor (C-R6A, Shimadzu), and an injection valve (model 7125, Rheodyne, Cotati, CA, USA) fitted with a T-union which serves as a splitter, with one end connected to a capillary column and the other end to a flow restrictor that is a stainless steel column (10–15 cm×4.6 mm I.D.) packed with ODS–silica particles (5–15 μm particle size). In order to avoid an excessive pressure pulse during sample injection, a bypass was created by connecting 0.25 mm I.D. tubing 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-unions. The split ratio was controlled at about 1/1000 to 1/100 for the 50–200 μm capillary columns in order to maintain the high efficiency and reproducibility of the pump above 0.2 ml/min solvent delivery. Chromatographic measurements were carried out at an ambient temperature (20–25 °C). A commercial column packed with 5-μm ODS–silica particles, Mightysil RP18, was obtained from Kanto (Tokyo, Japan).

3. Results and discussion

The major objective of this study was to develop monolithic silica columns having a domain size (a combined size of skeleton and through-pore, or a unit size of network structure after phase separation in the preparation mixture) of ~10 μm or smaller, and to prepare silica monoliths in capillaries larger than 50 μm I.D. In the preparation of MS(50)-A, B, C, and D from TMOS, the amount of PEG and the reaction temperature were varied to reduce the domain size. Column preparation using larger internal diameter capillaries proved difficult due to shrinkage of silica skeletons. By increasing the PEG content of the reaction mixture, silica monolith of smaller domain size was formed as shown in Tables 1 and 2. Similar results were obtained in the preparation of silica monoliths from a mixture of TMOS and methyltrimethoxysilane (MTMS). It was also possible to prepare a monolith in a 100–200 μm capillary using the latter approach. The relation between preparation conditions and resultant gel morphologies was described recently [19].

Fig. 1 shows the SEM photographs of the fractured surfaces of monolithic silica columns, MS(50)-A, -B, -C and -D, prepared from tetramethoxysilane in a capillary. Attachment of the silica skeletons to the tube wall seems to be better with monoliths having smaller domain sizes. The reduction of skeleton size and through-pore size with the increase in PEG content in the preparation mixture is clearly observed.

Fig. 2 shows the SEM photographs of monolithic silica columns prepared from TMOS and MTMS. Fig. 2a and b shows the hybrid-type monolithic silica columns in 50-μm capillary, while Fig. 2c and d shows the silica structure prepared in 100- and 200-μm capillaries, respectively. Monolithic silica columns were successfully prepared in the larger-sized capillaries that will make their operation easier.

Table 1
Feed composition for the preparation of monolithic silica columns

Column	PEG (g)	TMOS (ml)	TMOS + MTMS (ml)	Urea (g)	AcOH (ml)	Temperature[a] (°C)
MS(50)-A	8.8	40		9.0	100	40
MS(50)-B	12.4	40		9.0	100	30
MS(50)-C	12.6	40		9.0	100	30
MS(50)-D	12.8	40		9.0	100	30
MS-H(50)-I	1.00		9	2.03	20	40
MS-H(50)-II	1.05		9	2.03	20	40
MS-H(100)-II	1.05		9	2.03	20	40
MS-H(200)-II	1.05		9	2.03	20	40

[a] Temperature for gel formation.

Table 2
Pore properties and permeability of columns

Column	Skeleton size (μm)	Through-pore size (μm)	Permeability, K ($\sim 10^{-14}$ m^2)
MS(50)-A	2.0	8.0	130
MS(50)-B	1.4	2.8	25
MS(50)-C	1.1	2.2	15
MS(50)-D	1.0	2.0	8
MS-H(50)-I	2.0	4.5	56
MS-H(50)-II	1.5	2.0	19
Mightysil	5.0	(1.3–2.0)[a]	4

[a] Estimate at 25–40% of particle size.

Fig. 3 shows the images obtained by LSCM for MS(50)-A and MS(50)-C. Fig. 3a panel 1 is the side view of MS(50)-A at ~100 μm from the column end. Clusters of the silica skeletons that were observed by detecting fluorescence of FITC chemically attached to the silica surface were clearly seen in the figure. Fig. 3a panels 2–5 are views of the same part of the column but from different angles. Fig. 3a panel 5 shows the diagonal (almost cross-sectional) view of the monolith from the upper-right of the portion shown in Fig. 3a panel 1. Several clusters of silica skeleton can be observed across the column in radial direction. The domain size of MS(50)-A was estimated to be about 10 μm. When these images are successively shown as animation on a computer, one can see the three-dimensional internal structures

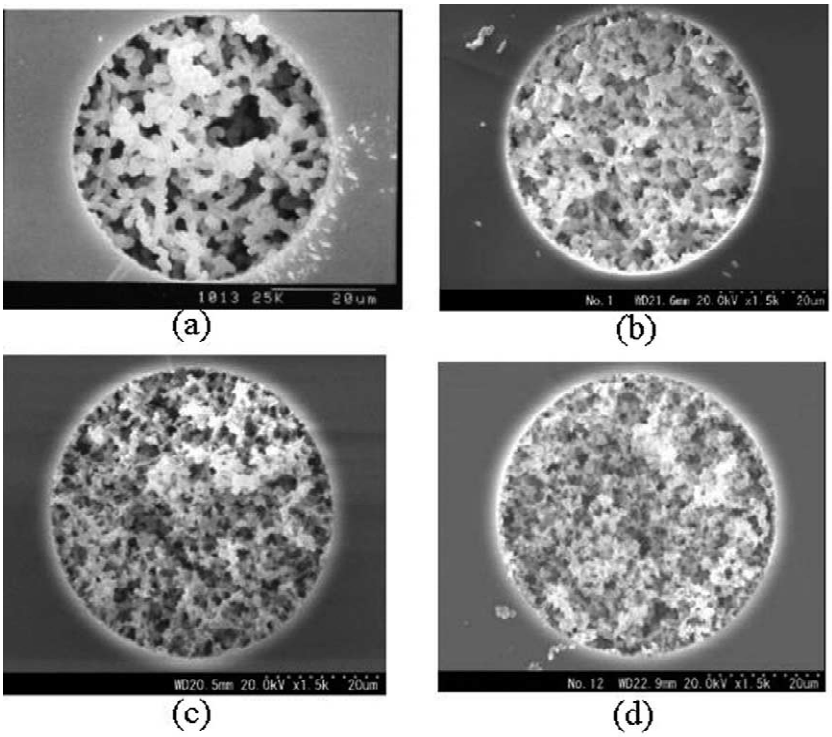

Fig. 1. Scanning electron micrographs of monolithic silica prepared from tetramethoxysilane in a 50-μm fused-silica capillary. (a) MS(50)-A; (b) MS(50)-B; (c) MS(50)-C; (d) MS(50)-D.

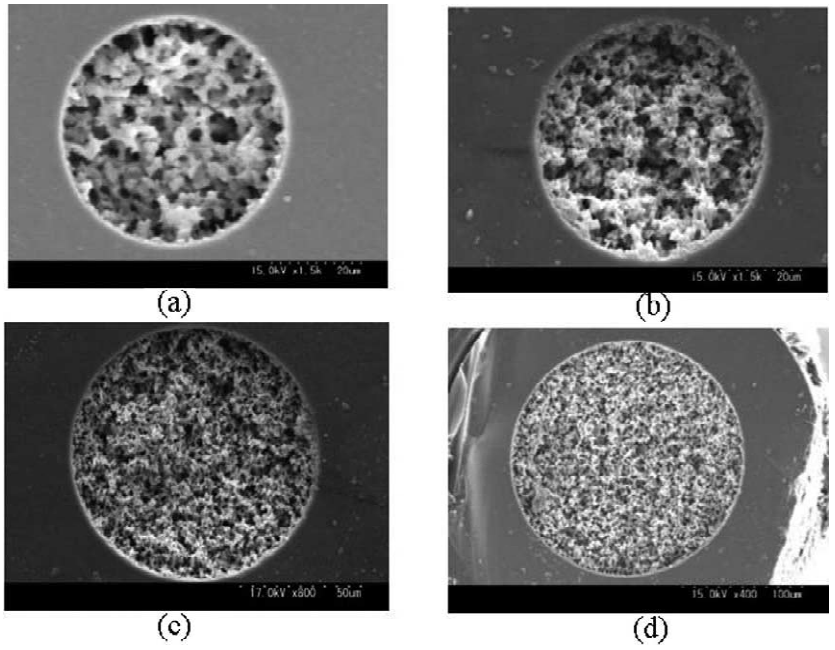

Fig. 2. Scanning electron micrographs of monolithic silica prepared from methyltrimethoxysilane and tetramethoxysilane; (a) MS-H(50)-I and (b) MS-H(50)-II in a 50-μm fused-silica capillary, (c) MS-H(100)-II in a 100-μm fused-silica capillary and (d) MS-H(200)-II in a 200-μm fused silica capillary.

inside the silica monolith. Shown in Fig. 3b are three-dimensional pictures of MS(50)-C similarly to those for MS(50)-A. The domain size of the network structure seems to be close to, or a little bit below, the resolution of LSCM. However, more clusters of skeletons than in Fig. 3a panel 1 can be readily observed, indicating that the domain size of MS(50)-C is much smaller than that of MS(50)-A. The network structures of monolithic silica columns will be studied in detail in the future using LSCM.

Fig. 4 shows the relation between through-pore size and skeleton size. The monolithic silica columns prepared from TMOS seem to give network structures with larger through-pore size/skeleton size ratios. The through-pore size/skeleton size ratio of 2–4 is much greater than 0.25–0.4 found in a column packed with particles [22], shown by the bars in Fig. 4. The results, however, indicate that the domain size was not well controlled over the range studied. A small change in preparation conditions resulted in a significant change in the gel structure. This is also related to the reproducibility of pore properties.

The permeabilities of monolithic silica columns with large domain sizes were much higher than those of monolithic silica columns of smaller domain sizes, as expected, and than that of a particle-packed column. Fig. 5 shows the plots of pressure drop against the linear velocity of mobile phase. The permeability, K value, ($K = u\eta L/\Delta P$, where η is the viscosity of the mobile phase, L is the column length, ΔP is the back pressure and u is the linear velocity of mobile phase) was $8–130 \cdot 10^{-14}$ m^2, that is, about 2–30 times higher than a column packed with 5-μm particles, as shown in Table 2, and reflects the high porosity and the large through-pore size/skeleton size ratio.

Fig. 6a–d shows the chromatograms obtained with the four monolithic silica columns [MS(50)-A, -B, -C and -D; 45 cm] prepared from TMOS for alkylbenzenes in an 80% aqueous acetonitrile mobile phase at a linear velocity of ~1 mm/s. Excellent peak symmetry was observed. At low linear velocities, the 45-cm columns produced 36 000–57 000 theoretical plates in 80% aqueous acetonitrile that are similar to or greater than the efficiency of a 25-cm

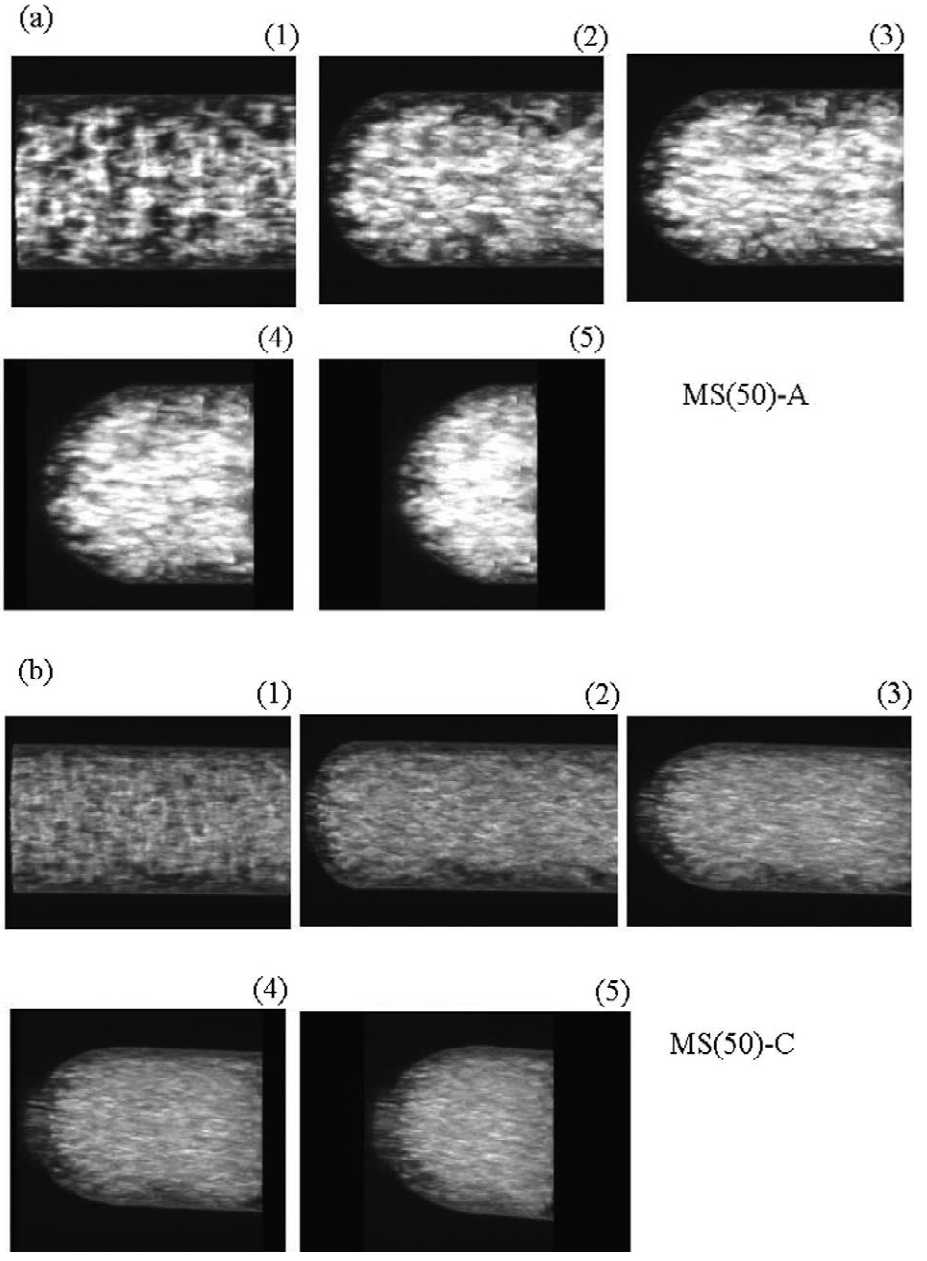

Fig. 3. Laser scanning confocal micrographs of monolithic silica prepared in a 50-μm fused-silica capillary. Two kinds of monolithic silica samples were subjected to the LSCM observation: (a) MS(50)-A, (b) MS(50)-C. Five images viewed from different angles are shown in panels 1–5 for each monolith. Image (1) for both samples shows a side view.

column packed with 3–5 μm silica–C_{18} particles, at a much lower pressure drop. Fig. 6 shows that the monolithic silica columns after chemical bonding provided similar retention of these hydrocarbons. Retention factors, $k = 0.78 \pm 0.02$, were obtained on MS(50)-B, -C, and -D, while a smaller k value (0.64) was obtained for MS(50)-A.

Fig. 7 shows chromatograms obtained with hy-

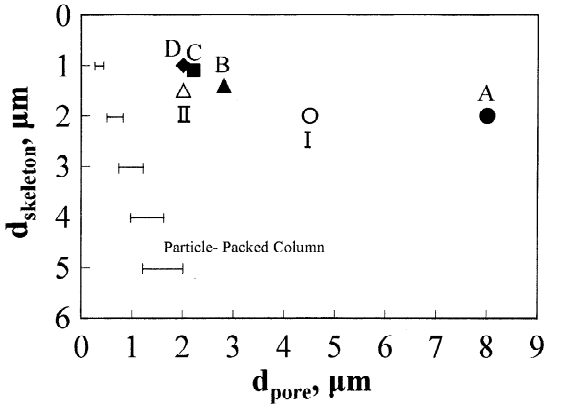

Fig. 4. Plots of the through-pore size against the skeleton size of the monolithic silica in capillary. MS(50)-A (●), MS(50)-B (▲), MS(50)-C (■), MS(50)-D (♦), MS-H(50)-I (○), MS-H(50)-II (△). Bars are shown to indicate the size of interstitial voids at 25–40% of particle size in a column packed with particles.

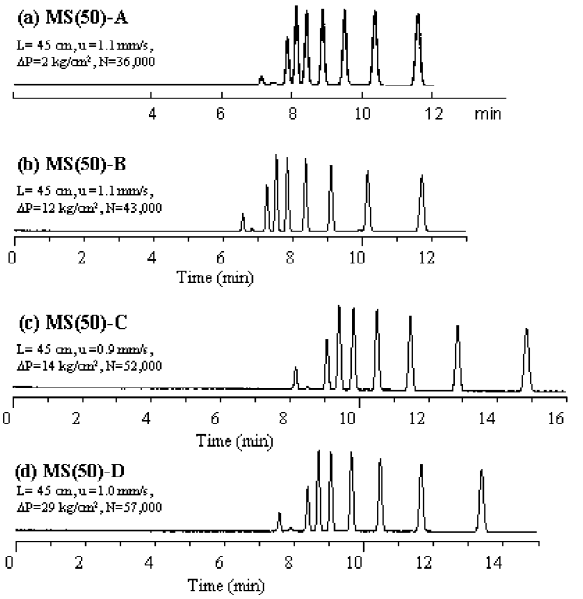

Fig. 6. Chromatograms obtained for alkylbenzenes ($C_6H_5(CH_2)_nH$, $n=0$–6). Column: (a) MS(50)-A, (b) MS(50)-B, (c) MS(50)-C, (d) MS(50)-D. Column size: 53.5 cm (effective length 45 cm)×50 μm I.D. Mobile phase: 80% aqueous acetonitrile. Linear velocity: (a) 1.1 mm/s, (b) 1.1 mm/s, (c) 0.9 mm/s, (d) 1.0 mm/s.

brid-type columns (MS-H) prepared from a mixture of TMOS and MTMS in capillaries. MS-H columns were successfully prepared in 100–200 μm capillaries, to produce 21 000–27 000 theoretical plates/25 cm column. MS-H(50)-I and MS-H(50)-II having different through-pore size and skeleton size showed very similar chromatograms, while MS-H(200)-II prepared from the same feed as MS-H(50)-II also gave similar chromatograms. The retention factors of alkylbenzenes on MS-H columns are very similar to each other and slightly larger than those on MS(50) columns prepared from TMOS. This is presumably due to the more hydrophobic nature of the MS-H

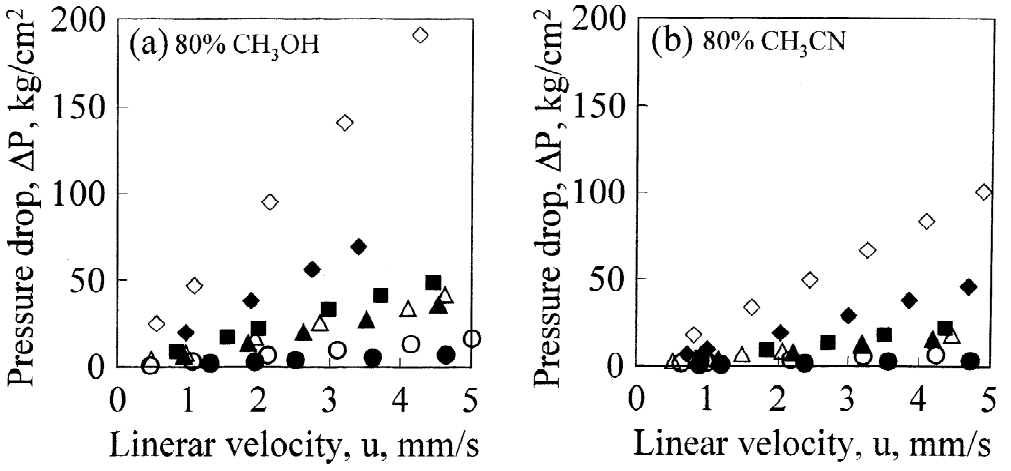

Fig. 5. Plots of column back pressure against linear velocity of mobile phase. Mobile phase: (a) 80% methanol, (b) 80% aqueous acetonitrile. The pressures were normalized to a column length of 15 cm. Columns: 5 μm silica–C_{18} particles, Mightysil RP18 (◇). Monolithic silica column in capillary, MS(50)-A (●), MS(50)-B (▲), MS(50)-C (■), MS(50)-D (♦), MS-H(50)-I (○), MS-H(50)-II (△).

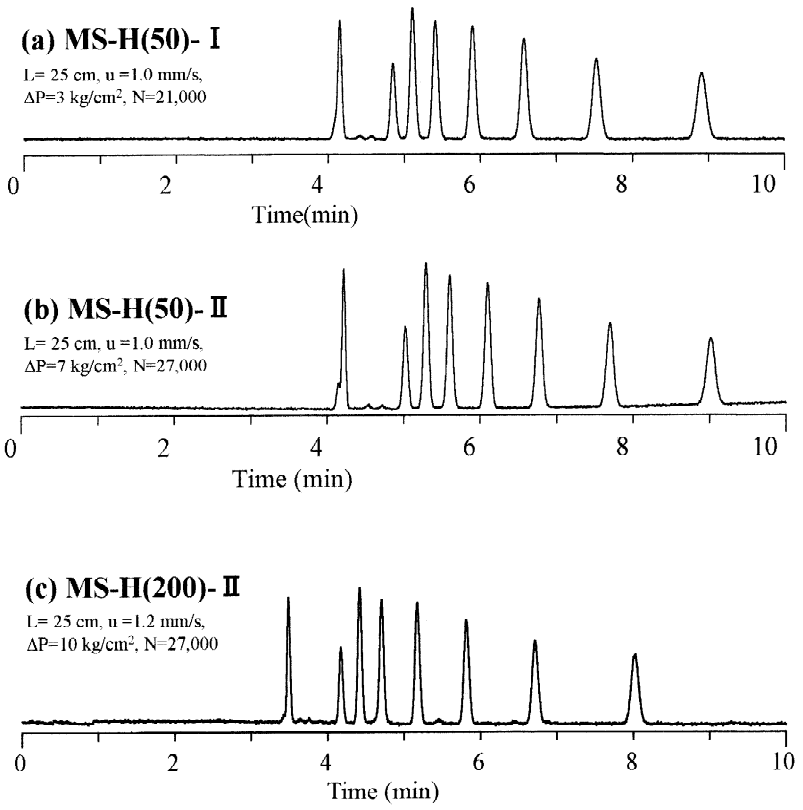

Fig. 7. Chromatograms obtained for alkylbenzenes ($C_6H_5(CH_2)_nH$, $n=0$–6). Column: (a) MS-H(50)-I: Column size: 33.5 cm (effective length 25 cm)×50 μm I.D. (b) MS-H(50)-II: 50 μm I.D.×33.5 cm (effective length 25 cm), (c) MS-H(200)-II: 33.5 cm (effective length 25 cm)×200 μm I.D. Mobile phase: 80% aqueous acetonitrile. Linear velocity: (a) 1.0 mm/s, (b) 1.0 mm/s, (c) 1.2 mm/s.

columns that possess abundant methyl groups as well as C_{18} alkyl chains after chemical bonding. The separation factor for hexylbenzene and amylbenzene, α_{CH_2}, was 1.51 on MS-H columns, while 1.50 was obtained for MS(50) columns. The column performance of the MS-H columns was slightly poorer than that of MS(50) columns of small through-pore size and skeleton size. Because it is easier to work with a 200-μm capillary column than with a 50-μm capillary column, the optimization of preparation conditions and the control of domain size of MS-H columns in a wider range is of much importance for practical applications.

Fig. 8 shows the van Deemter plots obtained with hexylbenzene as a solute in mobile phases that contain 80% methanol and 80% acetonitrile. The MS(50) columns of smaller domain size showed a plate height minimum, while those with larger domain sizes did not show the minimum at above 0.5 mm/s linear velocity. The adverse effect of the large through-pores, i.e. the significant band broadening that is caused by slow mobile phase mass transfer in the presence of a large eddy diffusion term observed with monolithic silica having large through-pores was minimized by the reduction in domain size. The plate height values obtained with these monolithic silica columns were actually much larger than what one might expect based on the size of the silica skeletons (1–2 μm) that should result in small C-term in Eq. (1) (H, height equivalent of a theoretical plate, A_x, B, C: coefficient for the contribution of each term). The slopes of the plots for monolithic silica columns are not much smaller than for a column packed with 5-μm particles (Fig. 8). The results can be explained by considering the contributions of the large through-pores (2–8 μm). Through-

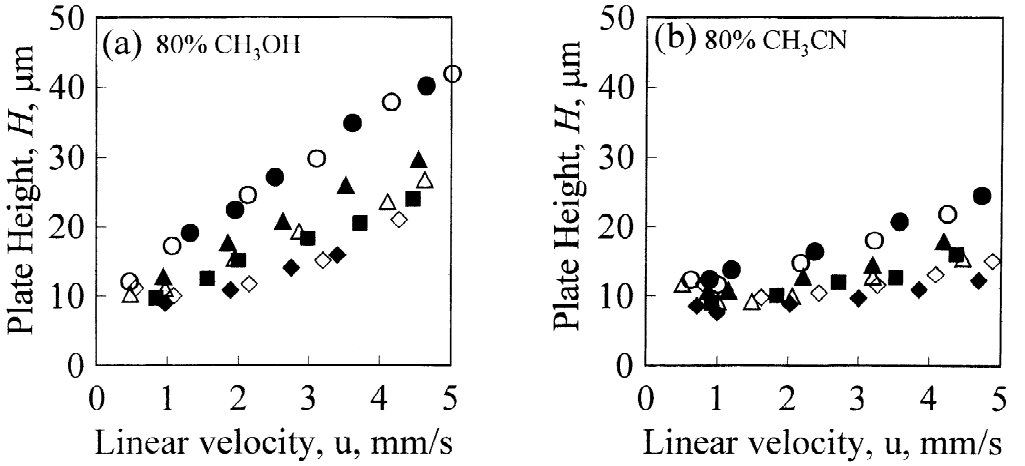

Fig. 8. Van Deemter plots obtained for C_{18} monolithic silica columns and a silica–C_{18} packed column with hexylbenzene as a solute. Mobile phase: (a) 80% aqueous methanol, (b) 80% aqueous acetonitrile. Symbols as in Fig. 5 for the columns.

pores of 2 μm would be found in a column packed with 5-μm or larger-sized packing materials. The large and relatively straight through-pores (which lead to high porosity) cause significant band broadening due to the slow mobile-phase mass transfer (large A_m) and inefficient exchange between streamlines which means large eddy diffusion (large A_e) [23]:

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

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

While the plate heights observed with MS(50)-D column (showing the highest efficiency among the monolithic columns) was comparable with that of a column packed with 5-μm particles, the total performance of the monolithic columns is much better than that of the particle-packed column. The separation impedance, E value [Eq. (2), N, number of theoretical plates, t_0, time required for an unretained solute to travel through the column) at minimum is close to 100 for MS(50)-A, and is about 300 for MS-H(50)-I. These values are much smaller than the minimum E value, ~3000, obtained with the column packed with 5-μm particles, as shown in Fig. 9. Generally, monolithic silica columns with larger domain sizes provided smaller values, or better total performance. Despite the lower column efficiency observed with the large domain size monolithic silica column (Figs. 6 and 7), generation of large numbers of theoretical plates will be easier with these columns because their permeability is high. It should be possible with the monolithic columns to generate 50 000 theoretical plates with t_0 values of less than 4 min at a pressure drop less than 100 kg/cm^2 by employing a longer column and a higher flow-rate than indicated in Figs. 6 or 7. Monolithic silica columns with smaller domain sizes will be useful for fast separations just like columns packed with small particles.

The results can be compared favorably with those reported with monolithic columns prepared from a particle-packed capillary in HPLC. High permeability was reported for monolithic columns prepared from a column packed with silica particles [24] or those from organic monomers [25], although the use of high-efficiency organic polymer monoliths is often accompanied by high pressure drop [26]. In most cases [27–30] columns prepared from particle-packed capillary were used for CEC.

The main advantages of a monolithic silica column having a network structure are the high permeability contributed by the large through-pores and the small C term contributed by the small skeleton sizes both of which are favorable factors for high-speed separations. Thus, at a similar pressure drop one can use a longer monolithic column to produce a

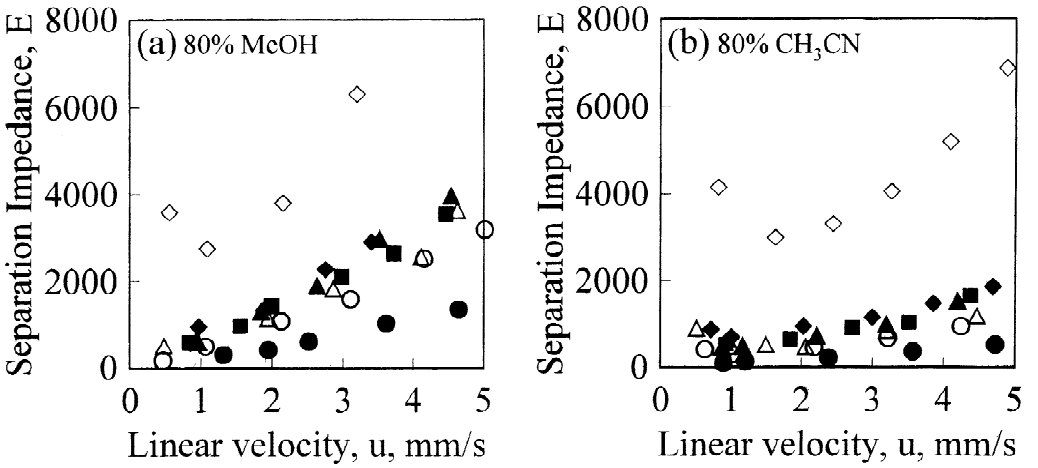

Fig. 9. Plots of separation impedance against linear velocity of mobile phase calculated for hexylbenzene as a solute. Mobile phase: (a) 80% aqueous methanol, (b) 80% aqueous acetonitrile. Symbols as in Fig. 5 for the columns.

greater number of theoretical plates than with a column packed with particles. Further work is required to control the domain size (through-pore size and skeleton size) of monolithic silica columns in a wider range to find optimum structures for various separation needs.

4. Conclusion

Monolithic silica columns prepared in a fused-silica capillary in the presence of urea from TMOS or from a mixture of TMOS and MTMS were evaluated in HPLC. Reduction in through-pore size and skeleton size resulted in the increase of column efficiency at the expense of permeability. The monolithic silica columns, however, showed higher permeability due to the larger through-pores and higher porosity than a column packed with 5-μm particles. Hybrid-type silica monoliths were successfully prepared from a mixture of TMOS and MTMS in 50–200 μm capillaries, and showed similar column efficiencies as those in 50-μm capillary. The monolithic silica column with the smallest domain size provided a plate height of <10 μm under a pressure drop lower than a column packed with 5-μm particles. LSCM seems to be a promising tool to study the three-dimensional silica network structure.

Acknowledgements

This work was supported in part by Merck Japan, and by a Grant-in-Aid for Scientific Research funded by the Ministry of Education, Sports, Culture, Science and Technology.

References

[1] J.C. Giddings, Unified Separation Science, Wiley, New York, 1991.
[2] P.A. Bristow, J.H. Knox, Chromatographia 10 (1977) 279.
[3] H. Poppe, J. Chromatogr. A 778 (1997) 3.
[4] J.E. MacNair, K.C. Lewis, J.W. Jorgenson, Anal. Chem. 69 (1997) 983.
[5] J.E. MacNair, K.D. Patel, J.W. Jorgenson, Anal. Chem. 71 (1997) 700.
[6] M.M. Dittmann, G.P. Rozing, J. Chromatogr. A 744 (1996) 63.
[7] R. Dadoo, R.N. Zare, C. Yan, D.S. Anex, Anal. Chem. 70 (1998) 4787.
[8] P.P.H. Tock, C. Boshoven, H. Poppe, J.C. Kraak, J. Chromatogr. 477 (1989) 95.
[9] H. Minakuchi, K. Nakanishi, N. Soga, N. Ishizuka, N. Tanaka, Anal. Chem. 68 (1996) 3498.
[10] H. Minakuchi, K. Nakanishi, N. Soga, N. Ishizuka, N. Tanaka, J. Chromatogr. A 762 (1997) 135.
[11] H. Minakuchi, K. Nakanishi, N. Soga, N. Ishizuka, N. Tanaka, J. Chromatogr. A 797 (1998) 121.
[12] H. Minakuchi, N. Ishizuka, K. Nakanishi, N. Soga, N. Tanaka, J. Chromatogr. A 828 (1998) 83.

[13] N. Tanaka, H. Nagayama, H. Kobayashi, T. Ikegumi, K. Hosoya, N. Ishizuka, H. Minakuchi, K. Nakanishi, K. Cabrera, D. Lubda, J. High Resolut. Chromatogr. 23 (2000) 111.
[14] K. Cabrera, G. Wieland, D. Lubda, K. Nakanishi, N. Soga, H. Minakuchi, K.K. Unger, Trends Anal. Chem. 17 (1998) 50.
[15] C. Fujimoto, J. High Resolut. Chromatogr. 23 (2000) 89.
[16] N. Ishizuka, H. Minakuchi, K. Nakanishi, N. Soga, H. Nagayama, K. Hosoya, N. Tanaka, Anal. Chem. 72 (2000) 1275.
[17] J.D. Hayes, A. Malik, Anal. Chem. 72 (2000) 4090.
[18] K. Nakanishi, H. Shikata, N. Ishizuka, N. Koheiya, N. Soga, J. High Resolut. Chromatogr. 23 (2000) 106.
[19] N. Tanaka, H. Kobayashi, K. Nakanishi, H. Minakuchi, N. Ishizuka, Anal. Chem. 73 (2001) 420A.
[20] T. Wilson, in: T. Wilson (Ed.), Confocal Microscopy, Academic Press, London, 1990, p. 1.
[21] H. Jinnai, K. Nakanishi, Y. Nishikawa, J. Yamanaka, T. Hashimoto, Langmuir 17 (2001) 619.
[22] K.K. Unger, in: Porous Silica, Elsevier, Amsterdam, 1979, Chapter 5.
[23] J.H. Knox, J. Chromatogr. A 831 (1999) 3.
[24] I. Gusev, X. Huang, C. Horvath, J. Chromatogr. A 855 (1999) 273.
[25] C.K. Ratnayake, C.S. Oh, M.P. Henry, J. Chromatogr. A 887 (2000) 277.
[26] A. Maruska, C. Ericson, A. Vegvari, S. Hjerten, J. Chromatogr. A 837 (1999) 25.
[27] C.K. Ratnayake, C.S. Oh, M.P. Henry, J. High Resolut. Chromatogr. 23 (2000) 81.
[28] R. Asiaie, X. Huang, D. Farnan, C. Horvath, J. Chromatogr. A 806 (1998) 251.
[29] Q. Tang, M.L. Lee, J. High Resolut. Chromatogr. 23 (2000) 73.
[30] T. Adam, K.K. Unger, M.M. Dittmann, G.P. Rozing, J. Chromatogr. A 887 (2000) 327.