The performance of hybrid monolithic silica capillary columns prepared by changing feed ratios of tetramethoxysilane and methyltrimethoxysilane

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A R T I C L E   I N F O

A B S T R A C T

The effect of a feed ratio of methyltrimethoxysilane (MTMS) to tetramethoxysilane (TMOS) was studied to improve the performance of a hybrid monolithic silica capillary column with 100-μm i.d. in HPLC in a range MTMS/TMOS (v/v) = 10/90–25/75. The domain size was also varied by adjusting the amount of PEG to control permeability ($K = 2.8 \times 10^{-14}$–6.9×10^{-14} m^2). Evaluation of the performance for those capillary columns following octadecylsilylation proved an increase in retention factor (k) and a decrease in steric selectivity α(triphenylene/ortho-terphenyl) with the increase in MTMS content in the feed. The effect of the feed ratio was also observed in porosity and hydrophobic property of the C18 stationary phase from the results of size exclusion chromatography (SEC) and reversed phase characterization. The monolithic silica capillary columns prepared under new preparation conditions were able to produce a plate height of 4.6–6.0 μm for hexylbenzene in a mobile phase acetonitrile/water = 80/20 at a linear velocity of 2 mm/s. Consequently, it was possible to prepare hybrid monolithic silica capillary columns with higher performance than those reported previously while maintaining the retention factors in a similar range by reducing the MTMS/TMOS ratio and increasing the total silane concentration in feed.

1. Introduction

Fast and high-efficiency separations are desired in many applications of HPLC, for example, pharmaceutical, food, proteome as well as metabolome analysis, etc. To meet such demand, ultrahigh-pressure liquid chromatography (UHPLC) using particle sizes below 2 μm has been studied [1–3]. Recently, many HPLC companies started to focus on the high-pressure, high-speed columns packed with sub-2 μm particles. Furthermore, fused-core silica particles which possess a solid core and a porous shell have been reported by Kirkland and co-workers [4–6]. It has been reported by Guiochon and co-workers that the column can provide superior performance to a column packed with fully porous particles of similar size [7].

On the other hand, monolithic silica columns have also been studied as another candidate for a high-speed or high-efficiency column, because it is known that they can realize the higher permeability and higher column efficiency than a particulate column simultaneously in HPLC [8–10]. Monolithic silica columns can be prepared as capillary columns [11,12]. A monolithic silica capillary column, especially a long one, is easy to prepare, compared to a particulate column which needs frits to keep particles and high pressure to pack small particles in a long capillary column.

In the case of a monolithic silica column, it is important to control the shrinkage inside a fused-silica capillary tube to attach silica skeletons to the inner walls of the capillary. Up to now, some results have been reported to control the shrinkage with methyltrimethoxysilane (MTMS) [13–15] and it was shown to be possible to prepare a column of i.d. 530 μm at maximum [16].

These approaches to a high-efficiency column are mainly related to reducing the resistance against mass transfer of a solute based on a small skeleton size or particle size, or even a thin porous shell. If we discuss column efficiency on the basis of van Deemter equation (Eq. (1), H: a plate height, u_0: linear velocity of the mobile phase, A, B, C: coefficients), the challenges are how to control the A-term (eddy diffusion) and the C-term (mass transfer in a mobile phase and in a stationary phase) that are directly related to the through-pore size and the skeleton size (particle size) as well as the structural homogeneity, because the B-term corresponding to the longitudinal diffusion hardly affects a plate height under practical HPLC conditions [17–20].

$$H = A + \frac{B}{u_0} + Cu_0 \tag{1}$$

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The comparison of columns packed with small particles, superficially porous particles, and monolithic columns have been reported [21–26]. Desmet and co-workers compared the column performance between particulate columns and monolithic silica columns, utilizing kinetic plots [23,24]. They showed that the column efficiency of a monolithic silica capillary column was inferior to that of a particulate column packed with particles below 3 μm in a region below 80,000 theoretical plates at 40 MPa pressure limit, or at relatively high linear velocity. This is because it had not been possible to prepare a monolithic silica column with a small domain size (a combined size of a through-pore and a skeleton) that could provide such high column efficiency per unit time as a column packed with small particles. Such a monolithic column must possess high structural homogeneity.

Another problem of a monolithic silica capillary column is the smaller retention factors and the smaller sample loading capacity than those observed with a particulate column. The smaller retention factors could lead to the poorer resolution on a monolithic silica column in comparison with a particulate column. The latter problem was clearly observed for a large-volume injections or the injection of strong solvent [27]. The problem is related to high porosity observed with a monolithic silica capillary column (90–95%) which leads to small phase ratios resulting in small retention factors and small sample loading capacity [28,29]. In other words, the column porosity should be reduced to provide large retention factors and a high sample loading capacity. To improve the efficiency at high speed and the sample loading capacity, we prepared monolithic silica columns with increased silane concentrations in the feed solution by 40–60% for a monolith prepared from TMOS in our preceding study [30]. The columns provided larger retention factors accordingly and greater numbers of theoretical plates than previous columns. The performance was similar to that of a column packed with 2–2.5 μm particles. These results agreed with the suggestion given by Desmet and co-workers, recommending the reduction in the column porosity to generate higher column efficiencies at high speed [22,24]. However, the retention factors provided by a monolithic silica capillary column prepared from TMOS were still much smaller than those obtainable with a particulate column in reverse-phase liquid chromatography (RPLC).

Generally, hybrid monolithic silica columns prepared from a mixture of TMOS and MTMS can have higher phase ratios, leading to greater retention factors than those prepared from TMOS in RPLC after octadecylsilylation [12], although slightly lower column efficiencies were observed with the hybrid silica columns, so far similar to a column packed with 3.5–4 μm particles. In order to examine whether it is possible to achieve the high column efficiencies per unit time shown by the TMOS monolithic silica columns having increased phase ratios [30] while maintaining the larger retention factors of hybrid monolithic silica columns, we examined preparation of hybrid columns by changing the MTMS/TMOS ratio and the total silane concentrations in the feed solution in this study. We carried out the characterization of the products by size exclusion chromatography (SEC) using polystyrene standard samples in THF mobile phase and evaluated the chromatographic performance in RPLC.

2. Experimental

2.1. Materials

Tetramethoxysilane (TMOS), methyltrimethoxysilane (MTMS), and octadecyldimethylchlorosilane (ODS-Cl) were obtained from Shinnetsu Chemicals (Tokyo, Japan), poly(ethylene glycol) (PEG; M_n = 10,000) from Sigma–Aldrich (Steinheim, Germany), urea and acetic acid from Wako Pure Chemicals (Osaka, Japan), and diethy-

lamine (DEA) from Nacalai Tesque (Kyoto, Japan). Fused-silica capillaries of 100-μm i.d. and 375-μm o.d. were purchased from Polymicro Technologies (Phoenix, AZ, USA).

2.2. Preparation of monolithic silica columns

The preparation conditions of the monolithic silica columns were similar to those reported earlier [12,29]. Typical conditions are as follows. A fused-silica capillary tubing (2–3 m in length) was treated with a 1N aqueous sodium hydroxide solution at 40 °C for 3 h, washed with water and acetone, and then dried. Monolithic silica capillary columns were prepared from a mixture of MTMS and TMOS (V_{MTMS}/V_{TMOS} = 25/75) to form a hybrid structure. A MTMS/TMOS mixture was prepared by mixing 75 ml of TMOS and 25 ml of MTMS. The mixture (18 ml) was added to a solution of PEG (1.90 g) and urea (4.05 g) in 0.01 M acetic acid (40 ml) at 0 °C and stirred for 30 min. The homogeneous solution was then stirred for 10 min at 40 °C, filtered with a 0.45 μm PTFE filter, charged into a fused-silica capillary tube, and allowed to react at 40 °C. The resultant gel was subsequently aged in the capillary overnight at the same temperature. Then, the temperature was raised slowly (over 10–20 h for long capillary columns), and the monolithic silica columns were treated for 4 h at 120 °C to form mesopores with the ammonia generated by the hydrolysis of urea, then cooled and washed with methanol. After drying, heat treatment was carried out at 330 °C for 25 h, resulting in the decomposition of the organic moieties in the capillary.

Surface modification of the monolithic silica was carried out on-column by continuously feeding a solution of octadecyldimethyl-N,N-diethylaminosilane (ODS-DEA prepared from ODS-Cl and DEA, 2 ml) in 8 ml of toluene driven by a nitrogen pressure of 5 bar at 60 °C for 3 h. As shown in Table 1, we changed the ratio between MTMS and TMOS in feed solutions. The PEG concentration was also varied along with the silane concentration. The morphology of the monolithic silica was examined by a high resolution scanning electron microscope (HSEM; Leo Gemini 982, Leo (Zeiss), Oberkochen, Germany) using a fractured surface. The through-pore size and skeleton size were measured manually from the photographs by averaging the sizes of more than 150 through-pores or skeletons clearly observed.

A series of columns designated as MS(100)-Hy(10)-I, MS(100)-Hy(15)-II, and MS(100)-Hy(25)-IV were prepared with the volume ratios, MTMS/TMOS = 10/90, 15/85, and 25/75 in a feed, respectively, and used for the pore size characterization by SEC with and without surface modification, and for evaluation in RPLC. The abbreviation, MS, stands for monolithic silica followed by the capillary diameter in parentheses, and Hy for the support material, hybrid, followed by the MTMS content (%) in the silane mixture in the preparation feed. MS(100)-T-IV, MS(100)-Hy(10)-I, -II, and -III, MS(100)-Hy(15)-I, -II, and -III, MS(100)-Hy(25)-IV shown in Table 1 were utilized to compare the column performance and selectivity. MS(100)-T-IV and MS(100)-Hy(25)-IV were prepared according to the preparation conditions in previous report [12,30].

2.3. HPLC instrument

Two sets of HPLC instruments were used for the characterization and evaluation of the monolithic silica capillary columns. One set consisted of a MP 681 pump (GL Sciences, Tokyo, Japan), C4-00R-0.01 10-nl injector (Valco, TX, USA), and CE1575 detector (JASCO, Tokyo, Japan) for SEC, and the other set was X-LC 3085PU Pump (JASCO) with split injection/flow using a Rheodyne 7725 injector (Rheodyne, Cotati, CA, USA) and CE-2075 UV detector (JASCO) for the other chromatographic measurement. The chromatographic measurements using split injection/flow mode were performed as previously described [12]. The chromatographic data were pro-

Table 1
Feed composition for the preparation of monolithic silica capillary columns.

Column	TMOS (ml)	MTMS/TMOS mixture (ml)	PEG (g)	Urea (g)	AcOH (ml)	Temperature (°C)[a]
MS(100)-T-IV[b]	56		11.9	9.00	100	25
MS(100)-Hy(10)-I[c]		22	2.40	4.05	40	35
MS(100)-Hy(10)-II		22	2.44	4.05	40	35
MS(100)-Hy(10)-III		22	2.52	4.05	40	35
MS(100)-Hy(15)-I		22	1.80	4.05	40	35
MS(100)-Hy(15)-II		22	1.84	4.05	40	35
MS(100)-Hy(15)-III		22	1.88	4.05	40	35
MS(100)-Hy(25)-IV[d]		18	1.90	4.05	40	40

[a] Temperature for gel formation.

[b] The TMOS column was prepared according to the condition in Ref. [30].

[c] Following number after Hy indicates the extent of volume ratio between TMOS and hybrid. For example, Hy(10) means the feed solution prepared by mixing 90 ml of TMOS and 10 ml of MTMS.

[d] The hybrid column was prepared according to previous condition in Ref. [12].

cessed with EZChrom Elite software (GL Sciences). SEC was carried out in tetrahydrofuran (THF) in order to characterize the pore properties of the monolithic silica columns using polystyrene standards (Chemco, Osaka, Japan).

In the SEC measurement, we used a flowmeter attached to the end of the column, and always measured the elution times of an excluded peak and the peak corresponding to total permeation from one chromatographic run. Furthermore, we measured the total column porosity and the external porosity for a monolithic silica capillary column six times resulting in the relative standard deviation (RSD (%)) of less than 0.1% for the volume of mesopores. Therefore, the estimated values for the amount of stationary phase for the capillary columns can be discussed with some confidence.

3. Result and discussion

3.1. SEM observation and column permeability

SEM photographs for capillary columns with 100-μm i.d. prepared by two kinds of feed solutions are shown in Figs. 1 and 2. In these photographs, MS(100)-Hy(10) series were prepared from the feed solution with MTMS/TMOS = 10/90, and MS(100)-Hy(15) series the volume ratio MTMS/TMOS = 15/85. The concentration of

PEG was increased in the order from -I to -III in each series. Fig. 1 shows that it was possible to prepare monolithic silica structures filling the capillary with 100 μm diameters with the range of ratios of MTMS to TMOS in the feed solution. Then monolithic silica structures with a different domain size were prepared by changing the concentration of PEG as shown in Fig. 2, where a smaller domain size is observed with monoliths prepared with the greatest amount of PEG (MS(100)-Hy(15)-III and MS(100)-Hy(10)-III) compared to corresponding monoliths -I or -II.

Table 2 lists a domain size calculated from SEM photographs and the permeability in methanol/water = 80/20 mobile phase at 30 °C for capillary columns after octadecylsilylation. The permeability (K) based on *Darcy's law* was calculated by using Eq. (2) [17,26,31];

$$K = \frac{\varepsilon u_0 \eta L}{\Delta P} \qquad (2)$$

where u_0 is linear velocity and η viscosity of a mobile phase, L column length, and ε total column porosity, respectively. With respect to the monolithic silica capillary columns studied here, the parameters in Eq. (2) can be regarded as constant at constant linear velocity except column pressure drop ΔP and length L, because the column porosity after octadecylsilylation is supposed to be constant at roughly 90% with the present feed composition in this study (see Table 3). Column permeability reflects through-pore size and exter-
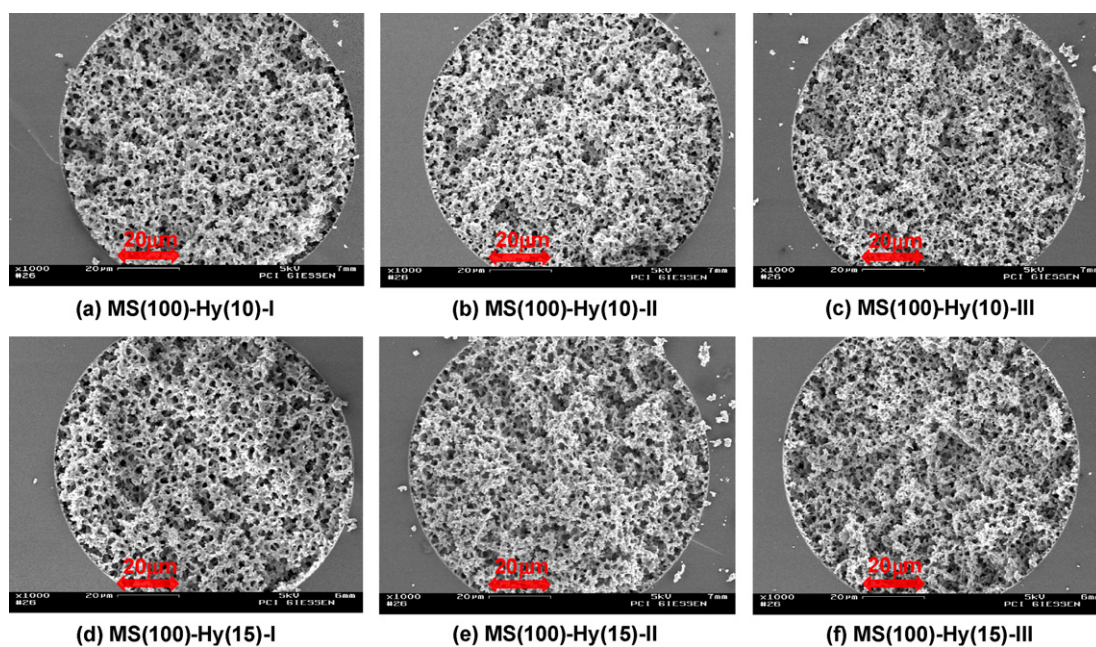

Fig. 1. Scanning electron micrographs of monolithic silica columns prepared from TMOS and MTMS in a 100-μm fused-silica capillary. Scale bars correspond to 50 μm. (a)–(c) MS(100)-Hy(10)-I–III; (d)–(f) MS(100)-Hy(15)-I–III.
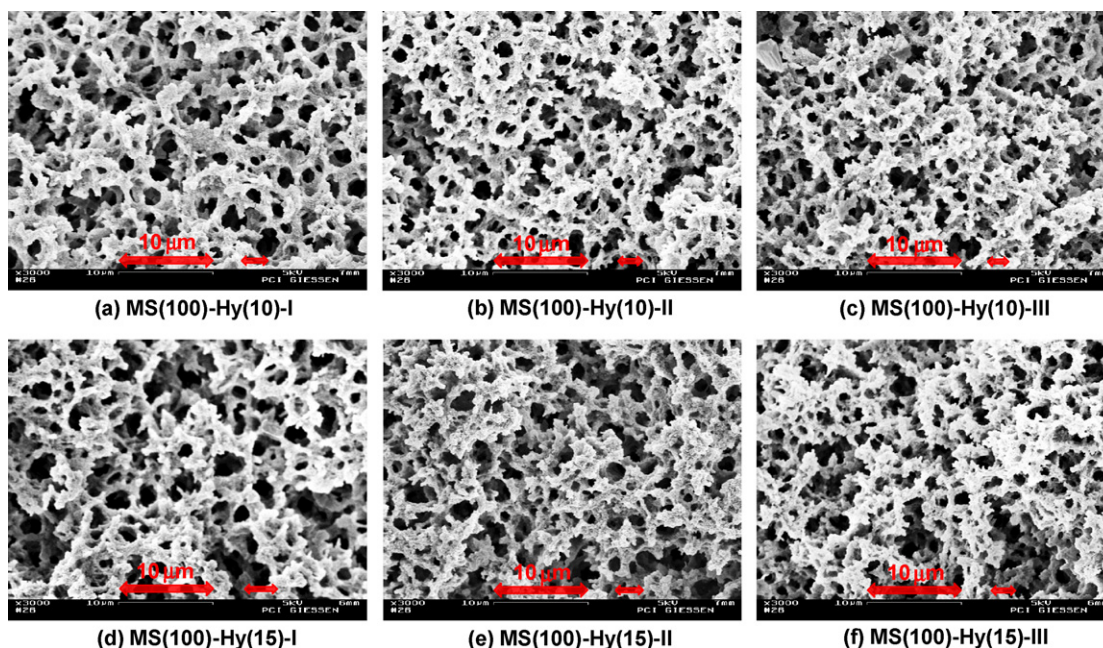
Fig. 2. Scanning electron micrographs of monolithic silica columns prepared from TMOS and MTMS in a 100-µm fused-silica capillary. Scale bars correspond to 10 µm. (a)–(c) MS(100)-Hy(10)-I–III; (d)–(f) MS(100)-Hy(15)-I–III. The smaller scale bar shown in each figure corresponds to the domain size of the monolithic silica.

Table 2
Domain size, surface/volume ratio and permeability (K) for monolithic silica capillary columns[a].

Column	Skeleton size, D_s (µm)	Through-pore size, D_p (µm)	Domain size (µm)	Surface/volume of the[b] skeletons (M m^{-1})	Permeability[c], K ($\times 10^{-14}$ m^2)
MS(100)-T-IV	1.1	1.5	2.6	2.7	3.5
MS(100)-Hy(10)-I	1.2	1.6	2.8	2.5	5.5
MS(100)-Hy(10)-II	1.1	1.5	2.6	2.7	3.4
MS(100)-Hy(10)-III	1.0	1.4	2.4	3.0	2.8
MS(100)-Hy(15)-I	1.4	2.0	3.4	2.1	6.9
MS(100)-Hy(15)-II	1.3	1.7	3.0	2.3	5.6
MS(100)-Hy(15)-III	1.1	1.5	2.6	2.7	3.1
MS(100)-Hy(25)-IV	1.3	1.8	3.1	2.3	6.2

[a] The measurement for a skeleton size and a through-pore size was carried out manually.
[b] The value was calculated as s/v = 3/D_s according to the suggestion from Ref. [33].
[c] Permeability was measured in methanol/water = 80/20 at 30 °C. According to definition in Ref. [26,31], permeability (K) was calculated as the total porosity of monolithic silica capillary columns is 90%.

nal porosity, or a domain size (a combined size of a through-pore and a skeleton) at a constant through-pore size/skeleton size ratio.

The RSDs (%) for the through-pores and skeletons of monolithic silica capillary columns were calculated to be 27–30% for both skeleton size and through-pore size, based on the SEM photographs. In this study, the average values in Table 2 were obtained from the measurement at more than 150 locations for through-pores and for skeletons to confirm the relationship between the domain size and the permeability, because the error of those measurements from SEM photographs could be large [32]. Guiochon and co-workers reported that the RSD (%) value of a particle size was 13% for 3 µm particles and 5% for 2.7 µm fused-core silica particles [7]. A narrow particle size distribution (PSD) is important in order to attain column efficiency and permeability expected from an average particle size [31].

In another approach, Unger and co-workers recently reported a correlation between the column permeability and surface area to volume ratio of the skeletons for a monolithic silica column [33].

Table 3
Relationship between column porosity and retention factor for monolithic silica capillary columns[a].

Column	Total porosity (V_m)	Through-pore (V_0)	Mesopore	Bond phase (V_s)	Phase ratio (V_s/V_m)	k(hexylbenzene)	α(CH$_2$)[b]
MS(100)-Hy(10)-I (silica)	0.937	0.676	0.261				
MS(100)-Hy(10)-I (ODS)	0.906	0.674	0.232	0.029	0.032	2.60	1.48
MS(100)-Hy(15)-II (silica)	0.934	0.670	0.264				
MS(100)-Hy(15)-II (ODS)	0.903	0.667	0.236	0.028	0.031	2.87	1.48
MS(100)-Hy(25)-IV (silica)	0.917	0.665	0.252				
MS(100)-Hy(25)-IV (ODS)	0.895	0.666	0.229	0.023	0.026	2.91	1.48
MS(100)-T1.4-A[c] (silica)	0.938	0.689	0.249				
MS(100)-T1.4-A[c] (ODS)	0.898	0.679	0.219	0.030	0.033	2.16	1.50

[a] Mobile phase, THF. Temperature, 30 °C.
[b] Measured in methanol/water = 80/20 at 30 °C. The ratio of the retention factors was calculated as α(CH$_2$) = k(hexylbenzene)/k(amylbenzene).
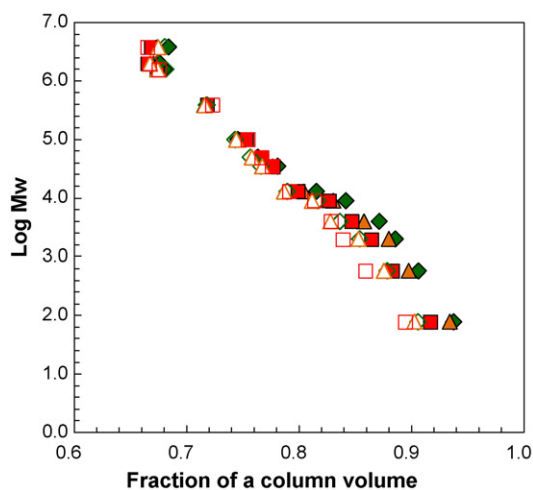[c] This data is from Ref. [30].

Fig. 3. Selective permeation of polystyrene standards in THF with columns changing the mixed ratios. Diamonds: MS(100)-Hy(10)-I, triangles: MS(100)-Hy(15)-II, and squares: MS(100)-Hy(25)-IV. Solid symbols stand for silica columns, and open symbols for ODS-modified monolithic silica columns.

The results from the skeleton size measurement are also shown in Table 2. High column permeability seems to reflect small surface area to volume ratios or large through-pore size (or domain size), as reported [33]. A reliable way to evaluate monolithic structures should be established, because it is important for a researcher to confirm homogeneity of monolithic silica structures inside a col-

umn. Laser scanning confocal microscopy (LSCM) may be one of useful ways for the evaluation [34,35].

For practical convenience in this study, it is assumed that a value of permeability under constant HPLC conditions reflects an average through-pore size of a monolithic silica column to be used for discussing the performance, that is to say a domain size or a through-pore size dictates permeability as a particle size does for a particulate column [36].

3.2. Characterization for monolithic silica capillary columns by SEC and RPLC

As shown in Fig. 3, the relationship between elution volume and the molecular weight of a polystyrene standard sample was obtained for the monolithic silica capillary columns by SEC in THF, for MS(100)-Hy(10)-I, MS(100)-Hy(15)-II, and MS(100)-Hy(25)-IV with and without octadecylsylilation. Although the total porosity found was similar at ca. 93% between MS(100)-Hy(10)-I and MS(100)-Hy(15)-II, that of MS(100)-Hy(25)-IV was found to be ~91% despite the lower silane concentrations in the feed. The results suggest that the shrinkage of silica skeletons was reduced by increasing MTMS concentrations [16]. The results obtained utilizing these relationships are summarised in Table 3, including the results of TMOS column from our previous study [30].

The difference in mesopore volume before and after ODS modification can be regarded as the volume occupied by the ODS moieties inside a column. The phase ratio was calculated by dividing the volume of the stationary phase by the total pore volume obtained from the elution volume of benzene.
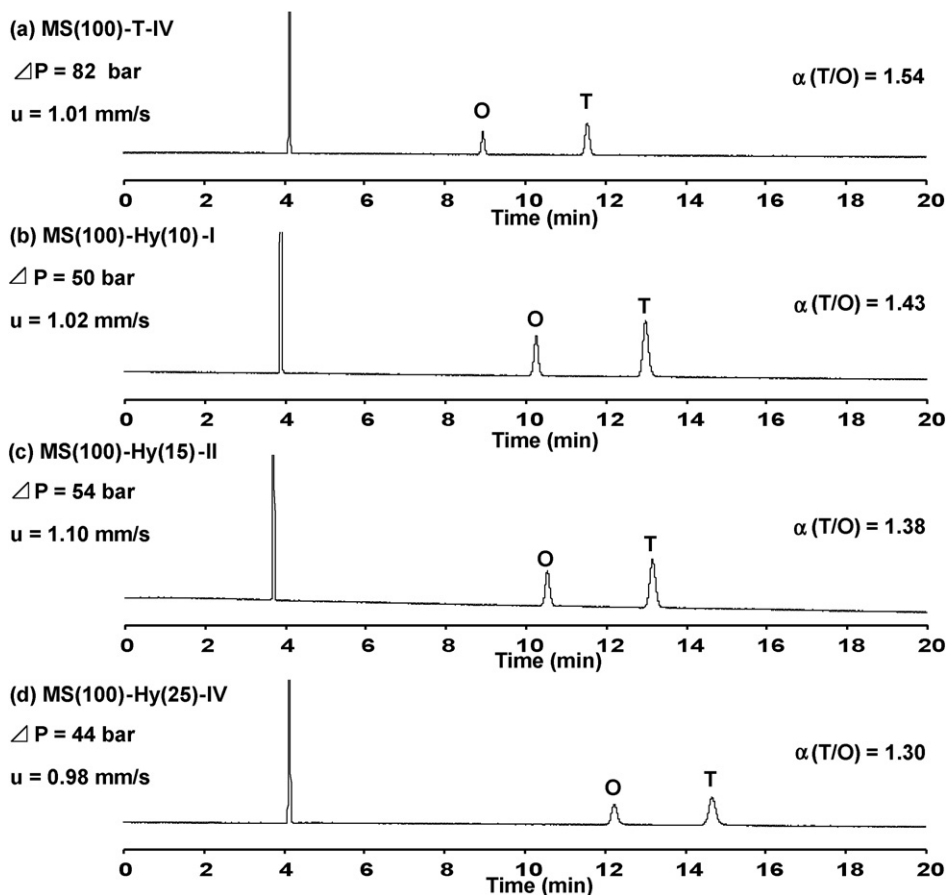

Fig. 4. Chromatograms obtained for o-terphenyl (O) and triphenylene (T). Column: (a) MS(100)-T-IV 30.0 cm (effective length 25.0 cm), (b) MS(100)-Hy(10)-I 28.9 cm (effective length 23.9 cm), (c) MS(100)-Hy(15)-II 29.5 cm (effective length 24.5 cm), and (d) MS(100)-Hy(25)-IV 29.4 cm (effective length 24.4 cm). Column diameter: 100 μm. Mobile phase: methanol/water = 80/20. Temperature: 30 °C. Detection: 254 nm. The pressure drop, linear velocity, and steric selectivity α(T/O) are indicated.

Table 4
Retention factor (k), $\alpha(CH_2)$, and $\alpha(T/O)$ for monolithic silica capillary columns[a].

Column	k(hexylbenzene)	$\alpha(CH_2)$	$\alpha(T/O)$	Temperature (°C)
MS(100)-T-IV	2.06	1.49	1.54	29.8
MS(100)-Hy(10)-I	2.60	1.48	1.43	29.9
MS(100)-Hy(10)-II	2.53	1.48	1.40	29.9
MS(100)-Hy(10)-III	2.65	1.48	1.41	29.9
MS(100)-Hy(15)-I	2.91	1.49	1.40	29.8
MS(100)-Hy(15)-II	2.87	1.48	1.38	29.9
MS(100)-Hy(15)-III	2.84	1.48	1.39	29.9
MS(100)-Hy(25)-IV	2.91	1.48	1.30	30.0

[a] Measurement was carried out in methanol/water = 80/20 at 30 °C. The ratio of the retention factors was calculated as $\alpha(CH_2) = k$(hexylbenzene)$/k$(amylbenzene) and $\alpha(T/O) = k$(triphenylene)$/k$(ortho-terphenyl).

In Table 3, it can be recognized that the volume occupied by ODS chains or the amount of stationary phase depends on silane concentration in the feed solution. For example, MS(100)-Hy(25)-IV was prepared with a lower silane concentration than the other columns. The amount of bonded stationary phase was less than that of the other ones, presumably because silanol groups on silica surface which can react with a reagent for ODS modification could be less due to the smaller amount of silica. Also, we can notice that the amount of bonded phase moieties does not strongly depend on the MTMS concentrations in the feed solution when we compare the three other columns, not including MS(100)-Hy(25)-IV.

The retention factor (k) of hexylbenzene was found to be ca. 2.6 for MS(100)-Hy(10)-I, ca. 2.9 for MS(100)-Hy(15)-II, and ca. 2.9 for MS(100)-Hy(25)-IV in methanol/water = 80/20 at 30 °C. It has been reported that the retention factor was ca. 2.2 for ODS-modified TMOS column which was designated as MS(100)-T1.4-A in previous report [30]. The retention factor (k) of hexylbenzene in RPLC tended to be larger with the increase in MTMS concentration in a feed solution. It is obvious when we compare MS(100)-Hy(25)-IV possessing smaller amount of ODS chain due to the low silane concentration in the feed with MS(100)-Hy(15)-II. Similar tendency can be observed through the comparison of MS-Hy(10)-I or MS(100)-Hy(15)-II with the TMOS column in Table 3. In summary, the results obtained from SEC and RPLC indicate that the methyl groups on the silica surface introduced by MTMS contribute significantly to solute retention, although the hydrophobic property of the stationary phase indicated by an $\alpha(CH_2)$ value may not be high after ODS modification.

It is known that ODS columns show steric selectivity for planar compounds such as polynuclear aromatic hydrocarbons (PAHs) compared to a bulky aromatic compound of similar hydrophobic property [37,38], and that the selectivity is dependent on a presence of methyl group on the silica surface for monolithic silica columns between TMOS column and hybrid column [39]. In this study, o-terphenyl (O) and triphenylene (T) were employed as solutes to obtain steric selectivity, $\alpha(T/O)$ [40], in order to recognize the dependency of the selectivity on the difference in the surface concentration of methyl groups caused by the change of MTMS concentrations for preparation.

Fig. 4, the chromatograms for o-terphenyl (O) and triphenylene (T) in methanol/water = 80/20 at 30 °C, shows that the steric selectivity $\alpha(T/O)$ decreases with the increase in the methyl group content in monolithic silica based on the starting MTMS concentrations in the preparation feed. Generally, the steric selectivity $\alpha(T/O)$ is dependent on surface coverage of ODS groups on silica surface and the length of alkyl chains in the stationary phase [38,40]. It has been reported that the selectivity tends to be larger with longer alkyl chains, and becomes smaller with endcapping by
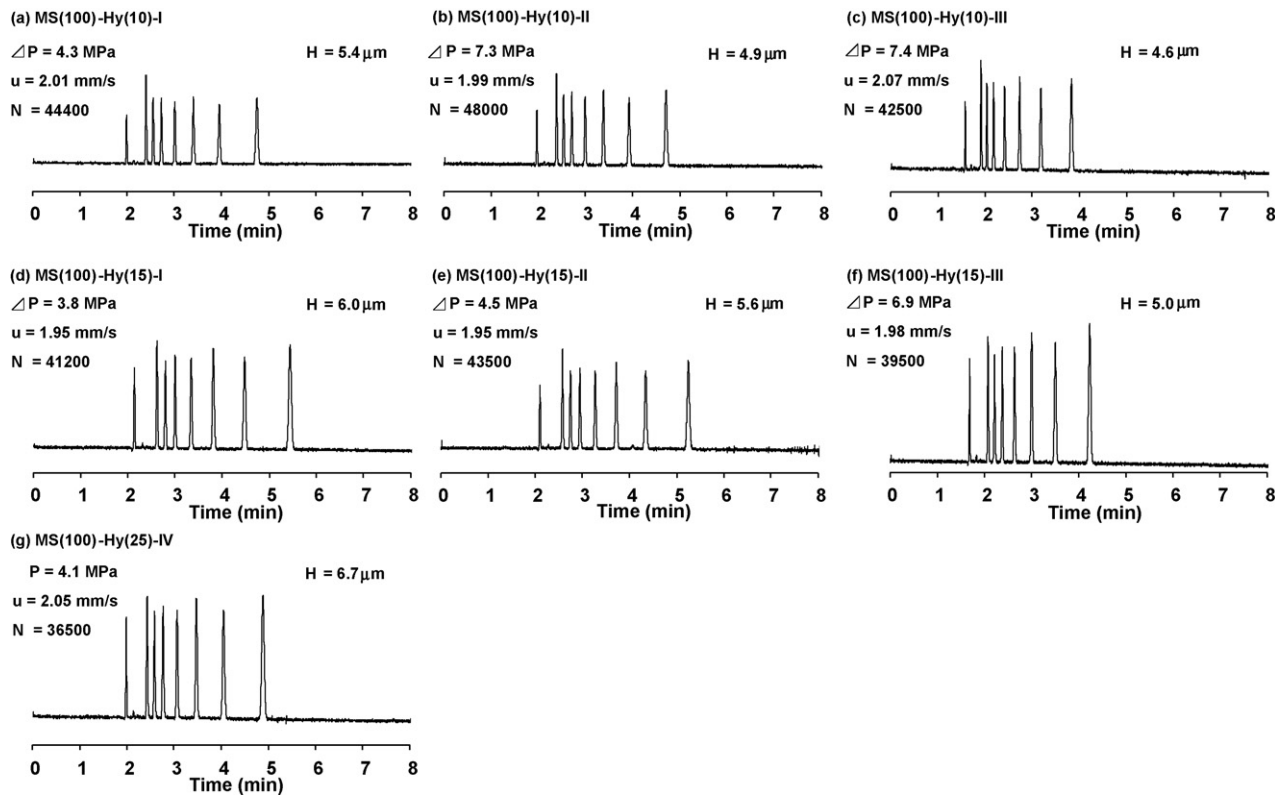

Fig. 5. Chromatograms obtained for alkyl benzenes ($C_6H_5(CH_2)_nH$, $n = 0$–6). Column: (a) MS(100)-Hy(10)-I 28.9 cm (effective length 23.9 cm), (b) MS(100)-Hy(10)-II 28.5 cm (effective length 23.5 cm), (c) MS(100)-Hy(10)-III 24.6 cm (effective length 19.6 cm), (d) MS(100)-Hy(15)-I 29.9 cm (effective length 24.9 cm), (e) MS(100)-Hy(15)-II 29.5 cm (effective length 24.5 cm), (f) MS(100)-Hy(15)-III 25.0 cm (effective length 20.0 cm), and (g) MS(100)-Hy(25)-IV 29.4 cm (effective length 24.4 cm). Column diameter: 100 μm. Mobile phase: acetonitrile/water = 80/20. Temperature: 30 °C. Detection: 210 nm. The pressure drop, linear velocity, the number of theoretical plates and plate height for hexylbenzene are indicated.
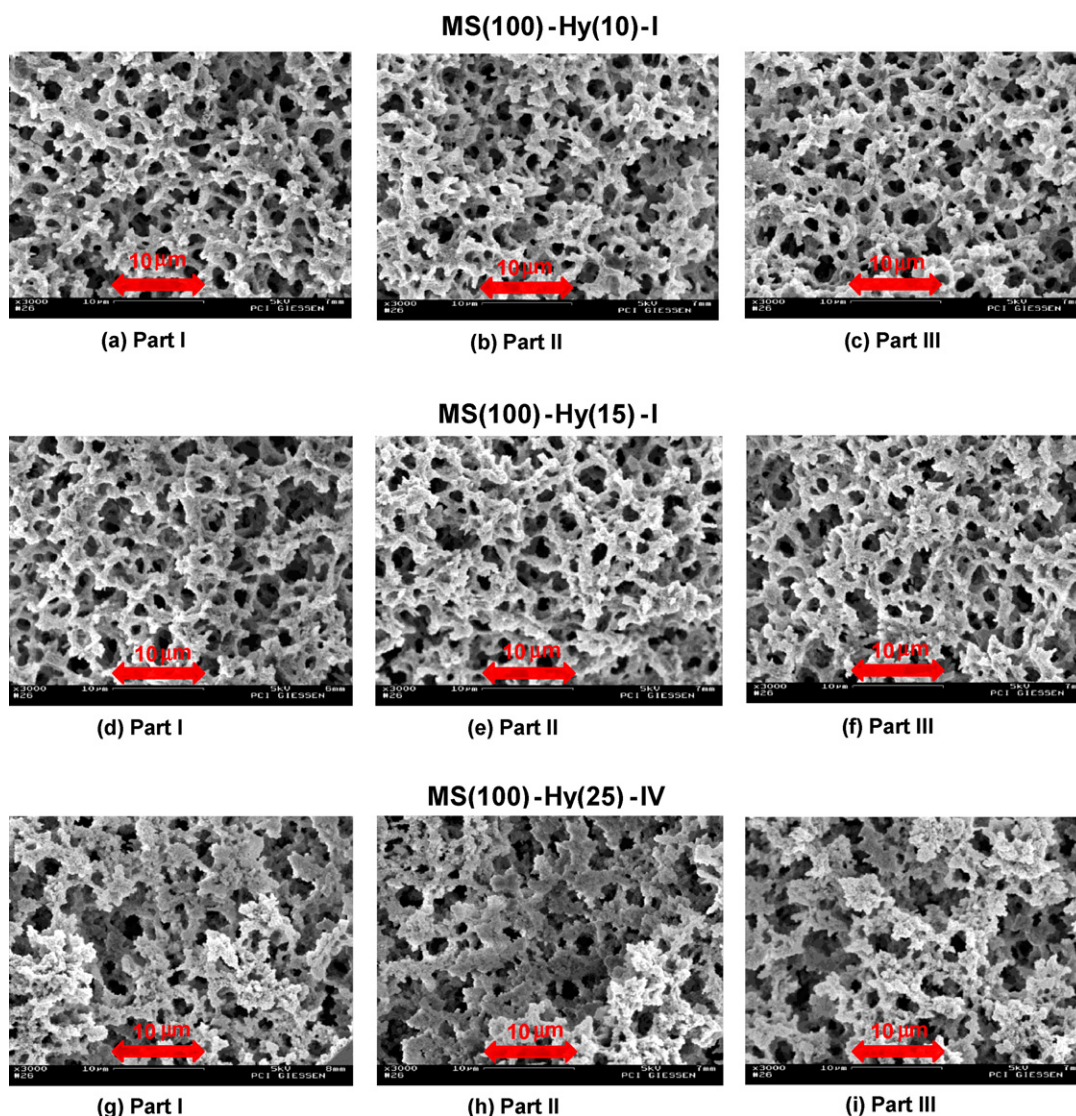
MS(100)-Hy(10)-I

(a) Part I

(b) Part II

(c) Part III

MS(100)-Hy(15)-I

(d) Part I

(e) Part II

(f) Part III

MS(100)-Hy(25)-IV

(g) Part I

(h) Part II

(i) Part III

Fig. 6. Scanning electron micrographs obtained from a different part of the column for MS(100)-Hy(10)-I, MS(100)-Hy(15)-I, and MS(100)-Hy(25)-IV. Scale bars correspond to 10 μm. (a)–(c) Part I–III from MS(100)-Hy(10)-I, (d)–(f) from MS(100)-Hy(15)-I, and (g)–(i) from MS(100)-Hy(25)-IV.

trimethylchlorosilane (TMS-Cl) [40]. Recently, a small separation factor based on the presence of methyl groups was also reported for a monolithic silica capillary column prepared only from MTMS [41]. Methyl groups in silicon oxide structures prepared from MTMS are known to be stable below 400 °C [42,43]. In this study, heat treatment was carried out for the monolithic silica columns at 330 °C, and trimethylsylilation or endcapping was not carried out for any monolithic silica column used. Therefore, in the case of the hybrid columns, it can be assumed that the selectivity based on the ODS groups is reduced by the presence of the methyl groups derived from MTMS. In other words, methyl groups contribute to the retention of hydrophobic species, but not discriminate planar and nonplanar compounds just like trimethylsilyl groups.

Furthermore, an $\alpha(CH_2)$ value for an ODS column tends to increase after endcapping due to the conversion of silanol groups to TMS groups [40]. The amount of ODS groups is nearly the same between MS(100)-T1.4-A, MS(100)-Hy(10)-I and MS(100)-Hy(15)-II from the results in Table 3. The results shown in Table 4 suggest that the surface coverage (distribution) of ODS groups for the hybrid columns is lower than those for the TMOS column because $\alpha(CH_2)$ values for the hybrid columns with methyl groups tend to be slightly smaller than that of the TMOS column. Table 4 shows the

retention factor (k) for hexylbenzene, $\alpha(CH_2)$, and $\alpha(T/O)$ for all monolithic silica capillary columns used in this study. The results show the dependency of retention characteristics on the MTMS concentration in the feed rather than the amount of ODS groups bonded.

3.3. Column efficiency for monolithic silica capillary columns

Fig. 5 shows the column performance of ODS-modified MS(100)-Hy(10) and MS(100)-Hy(15) series columns in acetonitrile/water = 80/20 at 30 °C, confirming the effect of the domain size of a monolithic silica column. The performance of MS(100)-Hy(25)-IV is also shown to indicate the efficiency of a previous hybrid column. It is noticeable that the column efficiency was improved with a smaller domain which was obtained with increasing PEG concentrations in the preparation feed, as expected. MS(100)-Hy(10)-III having the smallest domain size generated a plate height of 4.6 μm for hexylbenzene with a retention factor of ca. 1.4 at $u = 2$ mm/s, and MS(100)-Hy(15)-III ca. 5.0 μm with the retention factor of ca. 1.5 at similar linear velocity. It is also interesting to note that both MS(100)-Hy(10) and MS(100)-Hy(15) series could provide higher column efficiency than MS(100)-Hy(25)-IV which
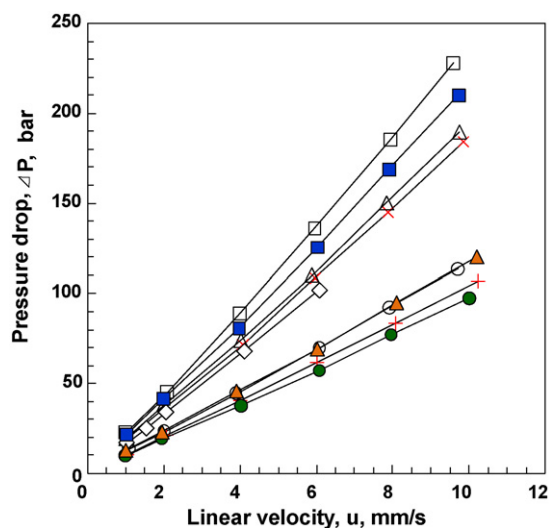
Fig. 7. Plots of column back pressure against linear velocity of mobile phase. Mobile phase: acetonitrile/water = 80/20. Temperature: 30 °C. The pressure were normalized to a column length 15 cm. Columns: Mightysil-RP18 packed with 5 μm C18 particles (◇), MS(100)-T-IV (×), MS(100)-Hy(10)-I (○), MS(100)-Hy(10)-II (△), MS(100)-Hy(10)-III (□), MS(100)-Hy(15)-I (●), MS(100)-Hy(15)-II (▲), MS(100)-Hy(15)-III (■), MS(100)-Hy(25)-IV (+).

Fig. 8. Van Deemter plots obtained for ODS-modified monolithic silica columns and a silica-C18 packed column with hexylbenzene as a solute. Mobile phase: acetonitrile/water = 80/20. Temperature: 30 °C. The symbols are the same as in Fig. 7 for the columns.

provided the plate height of 6.7 μm. Therefore, we are able to prepare the hybrid monolithic silica columns with higher column efficiency than previous hybrid columns, and achieved greater solute retention in comparison with the monolithic silica-C18 column prepared from TMOS which gave a plate height of 4.8 μm at u = 2 mm/s with the retention factor of ca. 1.1 for hexylbenzene [30].

The SEM photographs for MS(100)-Hy(10)-I, MS(100)-Hy(15)-I, and MS(100)-Hy(25)-IV are shown in Fig. 6, to show the structural homogeneity of each hybrid column. The increased structural homogeneity for MS(100)-Hy(10)-I and MS(100)-Hy(15)-I is noticeable in comparison with MS(100)-Hy(25)-IV. Such results can also be obtained for every series in Fig. 2. It is tempting to assume that the increased column efficiency of MS(100)-Hy(10) and MS(100)-Hy(15) series compared to MS(100)-Hy(25)-IV is related to the increase in the homogeneity of the hybrid monolithic structure, as reported for a second-generation monolithic silica column prepared from TMOS previously [30]. Actually the preparation conditions for MS(100)-Hy(10) and MS(100)-Hy(15) series columns included a higher concentration of silanes in the starting feed and a lower temperature of gelation than those for MS(100)-Hy(25)-IV. The conditions were similar to those employed for the preparation of the TMOS-derived monolithic silica column, MS(100)-T-IV in this study.

The relationship between a linear velocity and a column pressure for a monolithic silica capillary column and a particulate column in acetonitrile/water = 80/20 at 30 °C is shown in Fig. 7. An increase in column pressure drop corresponds to a decrease in domain size or permeability as shown in Table 2. The permeability (pressure drop) values seen with MS(100)-T-IV or MS(100)-Hy(10)-II were comparable to that of a column packed with 5 μm particles. Generally, changing the amount of PEG contributes to the change of a domain size or the change of a silica skeleton size and a through-pore size simultaneously [9,32]. As discussed below, permeability can be regarded as the parameter which indicates an average domain size. The results in Fig. 5 indicate that MS(100)-Hy(10) series generate a slight higher column efficiency for hexylbenzene with similar permeability than MS(100)-Hy(15) series, when comparison is made between MS(100)-Hy(10)-I and MS(100)-Hy(15)-II or MS(100)-Hy(10)-II and MS(100)-Hy(15)-III.
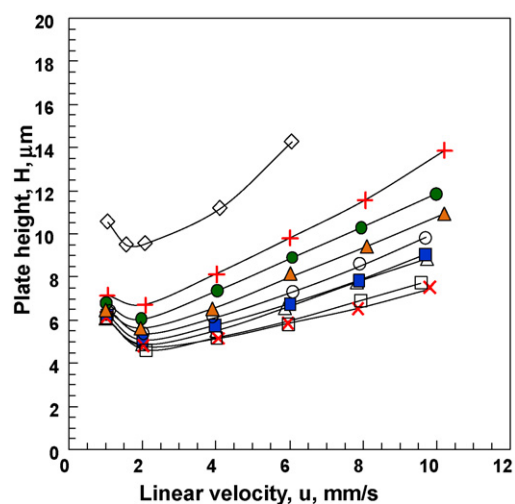
Fig. 8 shows the plot of a plate height (H) against linear velocity for hexylbenzene in acetonitrile/water = 80/20. Smaller minimum plate height and the shift of the optimum linear velocity toward a higher u value are observed with the decrease in a domain size. It has been reported that the domain size of a monolithic silica column corresponds to the size of particles that have been expected to generate similar column efficiency [8]. MS(100)-Hy(10)-III and MS(100)-Hy(15)-III provided plate height values expected for a column packed with 2.5 μm silica particles or smaller. However, the performance of the hybrid columns is still lower than that of MS(100)-T-IV at higher linear velocity. Plate height values are 4.8, 4.6, and 5.0 μm at ca. 2 mm/s, but 6.6, 6.9, and 7.8 μm at ca. 8 mm/s for MS(100)-T-IV, MS(100)-Hy(10)-III, and MS(100)-Hy(15)-III, respectively. Despite the similar or lower permeability compared to that of MS(100)-T-IV, the efficiency of hybrid columns decreases at higher linear velocity. Similar result can be also observed for comparison of MS(100)-Hy(10)-I and MS(100)-Hy(15)-II.

It is known that the presence of small pores contributes to slow mass transfer due to the hindrance of diffusion of a solute within pores [17,31,44]. Actually, the greater amount of smaller pores may exist in a hybrid material than those prepared from TMOS, because it could be harder to make mesopores by alkali treatment with the increase in MTMS concentration in a feed solution [14]. However, for a conventional monolithic silica column, the effect of hindrance for a small molecule like hexylbenzene within pores is negligible, as recently reported by Guiochon and co-workers [20]. Furthermore, it has been reported by Unger and co-workers that mesopores for a monolithic silica capillary column is roughly similar to or a little bigger than those for a conventional monolithic silica column by inverse size exclusion chromatography (ISEC) [45]. Therefore, the effect of mesopore size was assumed to be negligible with respect to the column efficiency for hexybenzene.

It is possible that structural homogeneity decreases with an increase in MTMS concentration in a feed solution, because of the difference in hydrolysis and condensation rates between TMOS and MTMS [46,47].

When the column efficiency of MS(100)-Hy(15)-I was compared to that of MS(100)-Hy(25)-IV prepared by the previous preparation method [12], the former can generate higher efficiency than the latter, although the former has a larger domain size, showing higher permeability. In addition, both columns gave a similar
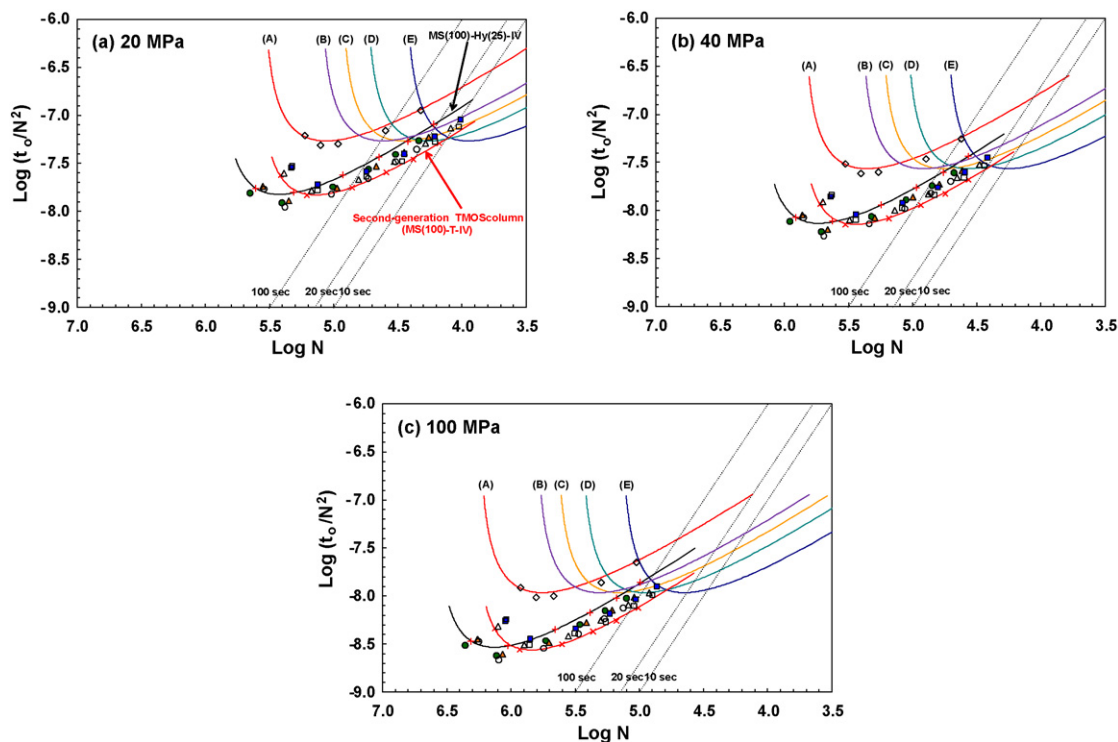
Fig. 9. Plots of $\log(t_0/N^2)$ against $\log(N)$ for the columns evaluated. The curves were obtained by assuming the following parameters, $\eta = 0.00046$ Pa s, $\varphi = 700$, $D_m = 2.22 \times 10^{-9}$ m^2/s, and Knox equation, $h = 0.65\nu^{1/3} + 2/\nu + 0.08\nu$. Maximum pressure: (a) 20 MPa, (b) 40 MPa, (c) 100 MPa, particle diameter: (A) 5 μm, (B) 3 μm, (C) 2.5 μm, (D) 2 μm (E) 1.4 μm. Symbols as in Fig. 7 for the columns.

retention factor for hexylbenzene in acetonitrile/water = 80/20. The results indicate that the monolithic silica structure of MS(100)-Hy(15)-I is more homogeneous than that of MS(100)-Hy(25)-IV. Tables 1 and 3 show that the large retention factor on MS(100)-Hy(25)-IV is obtained by increasing MTMS concentrations in the feed mixture. In addition, by increasing the total silane concentrations in preparation, the increased retention on MS(100)-Hy(15)-I and -II was achieved. Therefore, it is suggested that the preparation method for MS(100)-Hy(10) series or MS(100)-Hy(15) series with an increased silane concentration achieved the higher homogeneity of the monolithic silica structures in comparison with the hybrid materials in the past [12], as observed in Fig. 6.

3.4. Comparison of performance by using kinetic plots

Fig. 9 shows the plot of the log (t_0/N^2) values against $\log(N)$, so-called kinetic plots, proposed by Desmet and co-workers [23], where t_0 is the column dead time and N the number of theoretical plates. The plots are very useful when comparing the performance of various types of columns in term of attainable N and t_0 at a specified pressure. The performance of particulate columns packed with various particle sizes was estimated by using Knox equation, $h = A\nu^{1/3} + B/\nu + C\nu$ [31], where h is a reduced plate height, ν reduced velocity, and A, B, and C for coefficients for the contribution of each term. In this study, we utilized the value for $A = 0.65$, $B = 2$, $C = 0.08$ based on the result obtained from Mightysil-RP18 packed with 5 μm particles, which demonstrates a higher column efficiency than that employed by Poppe at $A = 1.0$, $B = 1.5$, and $C = 0.05$ [21]. The diffusion coefficient of hexylbenzene in acetonitrile/water = 80/20 was calculated by Wilke–Chang equation [17]. According to these parameters, the plots for particulate columns were made at a pressure of 20 MPa, because most HPLC separations have been carried out at a pressure of 20 MPa or lower. In

Fig. 9, the plot for MS(100)-Hy(25)-IV made for a previous preparation merges that of the particulate columns at $t_0 = $ ca. 40 s, or $N = $ ca. 27,000 and that of MS(100)-Hy(15)-I with the largest domain size in this study does it at $t_0 = $ ca. 26 s or $N = $ ca. 22,000. Fig. 9b and c shows the comparison in the kinetic plots at 40 and 100 MPa pressure drop. With the increase in the pressure, the cross over point shifts to a higher number of theoretical plates.

As in the case of Fig. 8, the higher performance than a column packed with 2.0 μm particles was observed for MS(100)-Hy(10)-III with the smallest domain size in the range of $N = 16,000$ or more at around $t_0 = \sim 15$ s or longer, and for MS(100)-Hy(15)-III in the range of $N = 19,000$ or more at $t_0 = \sim 20$ s or longer. These results mean that monolithic silica column would provide higher performance than particulate columns if more than 20,000 theoretical plates were desired at 20 MPa. However, it can be recognized that the column performance for the monolithic silica columns tends to be lower with the increase in MTMS concentrations in a preparation feed. MS(100)-T-IV can generate $N = 14,000$ at $t_0 = 10$ s as reported previously [30], which showed similar performance as a column packed with 2–2.5 μm particles with a comparable column pressure drop to a column packed with 5 μm particles. MS(100)-Hy(10)-III can generate $N = 12,000$ and MS(100)-Hy(15)-III, $N = 10,000$ with $t_0 = 10$ s, although its domain size or the permeability is similar to or smaller than that of MS(100)-T-IV. The results suggest that it is necessary to achieve better structural homogeneity with the small domain size for hybrid columns than those for TMOS-derived columns. Therefore, in the range $N < 20,000$ at a pressure of 20 MPa, a column packed with sub-2 μm particles or a second-generation TMOS monolithic silica column is superior to the hybrid monolithic silica series. The preparation method of the monolithic silica columns still needs improvement and a further study is desired for the development of monolithic silica columns with a smaller domain size and greater retentivity.

4. Conclusion

In this study, it was possible to prepare hybrid monolithic silica columns with an increased total silane concentration and the lower MTMS concentration compared to previous hybrid columns. It was also observed that column permeability of a monolithic silica column reflects a domain size or a surface/volume ratio, as shown in the recent report [33]. The relationship between the column porosity shown by the SEC measurement and the retention factor for hexylbenzene in 80% MeOH for these columns indicated that the solute retention strongly depends on the content of methyl groups on silica surface. It was possible to increase the retentivity by increasing MTMS concentrations in the preparation feed. The steric selectivity α(T/O) for a planar compound was also clearly related to the MTMS concentration in a feed. The column efficiency of hybrid monolithic silica columns prepared with the lower MTMS content and with the increased silane concentration was superior to that of previous hybrid columns. The SEM observations suggest that the increased structural homogeneity for a present hybrid column contributes to the higher column efficiency than that of a previous hybrid column. The kinetic plots indicated that the column efficiency of these hybrid columns was comparable with that of a particulate column packed with 2.0–2.5 μm particles, generating more than 20,000 theoretical plates with $t_0 = 25$ s at a pressure of 20 MPa. However, it should be recognized that the column efficiency of the monolithic silica columns tends to be lower with increased MTMS concentrations. The performance of present hybrid monolithic silica columns was actually a little lower than that of the second-generation TMOS monolithic silica column, although much higher than that of previous hybrid columns. It is essential to achieve better structural homogeneity of monolithic silica with a small domain size of below 2.5 μm for hybrid columns if column efficiency comparable with a particulate column packed with sub-2 μm particles is desired.

Acknowledgements

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