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Monolithic silica rod columns for high-efficiency reversed-phase liquid chromatography

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ABSTRACT

Chromatographic properties of a new type of monolithic silica rod columns were examined. Silica rod columns employed for the study were prepared from tetramethoxysilane, modified with octadecylsilyl moieties, and encased in a stainless-steel protective column with two polymer layers between the silica and the stainless-steel tubing. A 25 cm column provided up to 45,000 theoretical plates for aromatic hydrocarbons, or a minimum plate height of about 5.5 μm, at optimum linear velocity of ca. 2.3 mm/s and back pressure of 7.5 MPa in an acetonitrile–water (80/20, v/v) mobile phase at 40 °C. The permeability of the column was similar to that of a column packed with 5 μm particles, with K_F about 2.4×10^{-14} m^2 (based on the superficial linear velocity of the mobile phase), while the plate height value equivalent to that of a column packed with 2.5 μm particles. Generation of 80,000–120,000 theoretical plates was feasible with back pressure below 30 MPa by employing two or three 25 cm columns connected in series. The use of the long columns enabled facile generation of large numbers of theoretical plates in comparison with conventional monolithic silica columns or particulate columns. Kinetic plot analysis indicates that the monolithic columns operated at 30 MPa can provide faster separations than a column packed with totally porous 3-μm particles operated at 40 MPa in a range where the number of theoretical plates (N) is greater than 50,000.

1. Introduction

The development of columns for reversed-phase HPLC that can provide higher overall performance (column efficiency, permeability, and separation time combined) compared to columns packed with small totally porous particles has been attracting attention. While columns packed with sub-2 μm totally porous particles provide high efficiency at high speed, they require high pressure to achieve fast separations, and the chromatographic system needs to be optimized for efficient operation of small-sized columns [1–3]. Columns packed with 2.6–2.7 μm superficially porous particles showed similar column efficiency as columns packed with sub-2 μm particles with significantly higher permeability [1,4]. The properties of such columns have been reported in detail [4]. Several types of superficially porous particles are commercially available, and the development of smaller core–shell particles has been continued [5].

Monolithic silica columns can also provide higher efficiency per unit pressure drop compared to conventional columns packed with totally porous particles. This is based on the presence of small-sized skeletons and large through-pores, or the large (through-pore size/skeleton size) ratios [6,7]. The high permeability allowed the operation of a long column system to yield large numbers of theoretical plates under moderate back pressure [8,9]. The chromatographic properties of rod-type monolithic silica columns were discussed in relation to the silica structure in early reports [10,11] as well as for commercial materials in detail [6,12,13].

A monolithic silica rod column prepared from tetramethoxysilane (TMOS), Chromolith, was first commercialized in 2000, followed by similar products, Onyx, distributed later by another company. The silica rod columns with through-pore size of about 2 μm, mesopore size of 13 nm [14,15] clad with an engineering plastic, polyether–ether–ketone (PEEK), provided column efficiency equivalent to a column packed with 3.5–4 μm particles, or

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10,000–12,000 theoretical plates with a 10 cm column. Chromolith columns having high porosity, 85% total porosity and 70% external porosity, showed permeability equivalent to that of a column packed with 8–10 μm particles [6,15–17].

In the case of capillary monolithic silica columns, improved performance was achieved by increasing the skeleton volume fraction in a column compared to earlier preparations, which resulted in the greater homogeneity of silica structures [18,19]. Those prepared from TMOS or hybrid columns prepared from a mixture of TMOS and methyltrimethoxysilane in the presence of polyethylene glycol were able to show a plate height of 5–5.5 μm, roughly equivalent to a column packed with 2.5 μm particles, with back pressure equivalent to a column packed with 5 μm particles.

Improvement of a preparation method aiming at higher column efficiency per unit time is also of much interest for rod-type columns [20–22]. In addition, it was thought to be desirable to provide longer monolithic silica rod columns than those currently available to generate greater numbers of theoretical plates with conventional HPLC equipment. This is because conventional-sized HPLC columns (2–4.6 mmID) are most popular, and used with pumps having ca. 40 MPa pressure limit in a wide range of applications today. The availability of specialty columns from more than one supplier will be desirable for column selection and continuous use in any applications. The length of commercially available monolithic silica columns for analytical scale HPLC has been limited to 10 cm or less. Although it has been shown to be possible to connect the rod-type monolithic silica columns to form a long column system to achieve high-efficiency separations, a considerable decrease in column performance was observed for the connected columns [8]. These considerations prompted the development of another method of preparation of monolithic silica columns, different from that of Chromolith [21]. We report here the properties of monolithic silica rod columns of up to 25 cm in length that can provide higher column efficiency per unit time and column length than currently available rod-type monolithic silica columns, and a brief comparison of the performance with a column packed with superficially porous particles of 2.6 or 2.7 μm diameters.

2. Experimental

2.1. Instrument

A scanning electron microscope (S-3000N, Hitachi, Tokyo, Japan) was used to study the morphology of silica rods. Characterization by nitrogen adsorption was performed by using ASAP2010 equipment (Micromeritics Instrument, Norcross, GA, U.S.A.). Mercury intrusion apparatus (PoreMaster 60GT, Quantachrome Instruments, Boynton Beach, FL, U.S.A.) was used to characterize macropores. Chromatographic measurement was carried out by using an LC800 HPLC system (GL Sciences, Tokyo, Japan) equipped with a UV detector (MU701, GL Sciences) operated at 254 nm, unless noted otherwise. A fluorescence detector (GL-7453A, GL Sciences) was used for the detection of pyridylamino derivatives of glucose oligomers with excitation at 310 nm and emission at 380 nm. The optical unit of the UV detector and an injector of the auto-sampler unit were contained in an air-circulating column oven in order to minimize the extra-column dead volume of the chromatographic system and to attain thermal equilibrium of the mobile phase before entering a column. The signal from the UV optical cell unit was transmitted to the electronic unit via optical fibers. PU714 pump (GL Sciences) was used to supplement the solvent delivery of the LC800 instrument.

2.2. Columns

Monolithic silica rod columns, MonoClad C18-HS, 3 mmID and 5, 10, 15, and 25 cm long (GL Sciences), were selected from commercial columns (available only in Japan at present) for the study. When connecting two or three columns to form a long column system, stainless-steel tubing of 0.1 mmID/1.6 mmOD and 10 cm long was used with PEEK compression screws. Ascentis Express C18 column (3 mmID, 15 cm long, particle size 2.7 μm, Supelco, St. Louis, MO, U.S.A.), Kinetex C18 column (3 mmID, 15 cm long, particle size 2.6 μm, Phenomenex, Torrance, CA, U.S.A.), and Inertsil ODS-3 column (3 mmID, 15 cm long, particle size 3 μm, GL Sciences) were used for performance comparison.

2.3. Chemicals

Polynuclear aromatic hydrocarbons (PAHs), 1: naphthalene, 2: fluorene, 3: anthracene (Janssen Chimica, New Brunswick, NJ, U.S.A.), 4: fluoranthene, 5: o-terphenyl, 6: triphenylene, 7: 1,2-benzanthracene (Sigma–Aldrich, St. Louis, MO, U.S.A.), 8: benz(b)fluoranthene, 9: benz(a)pyrene, and 10: perylene, were obtained from Tokyo Chemical Industry (Tokyo, Japan), unless noted otherwise. Antidepressant drugs including imipramine hydrochloride, amitriptyline hydrochloride, mianserine hydrochloride, clomipramine hydrochloride, and nortriptyline hydrochloride obtained from Sigma–Aldrich and dextromethorphan hydrobromide from LKT Laboratories (St. Paul, MN, U.S.A.) were used in combination with phenol (Wako Pure Chemical Indutries, Osaka, Japan) for testing silanol activities of monolithic silica columns. Uracil was obtained from Wako Pure Chemical Indutries.

PAHs and a mixture of pyridylamino derivatives of glucose oligomers (PA-Glucose Oligomer (DP = 3–15), Takara Bio Co., Otsu, Japan) (DP: degree of polymerization) were used for comparing the monolithic silica rod columns and the columns packed with superficially porous particles. Phosphate buffer was prepared from sodium dihydrogen phosphate and sodium hydrogen phosphate (Kishida Chemical, Osaka, Japan). HPLC grade acetonitrile and methanol were obtained from Kishida Chemical. Water was purified by using Milli-Q equipment (Millipore, Billerica, MA, U.S.A.). Mobile phase was prepared based on the volume of each solvent by using volumetric flasks.

2.4. Chromatographic measurement

Column temperature was controlled at 40 °C within 0.1 °C with an air-circulating oven equipped with heating and cooling functions. Solution of PAHs in acetonitrile at 0.03–0.07 mg/mL each was used for testing chromatographic performance with 1 μL injection in most cases. The chromatographic measurement was carried out in duplicate, and the data collected and processed by EZChrom Elite software (Agilent Technologies, Santa Clara, CA, U.S.A.). The number of theoretical plates of a column (N) was calculated based on the peak width at half height for the peaks having high symmetry factor, between 0.95 and 1.10. The band broadening caused by the instrument without a column ($\sigma^2_{\mathrm{Extra}}$) was measured by replacing a column with a zero-dead-volume union, and the second central moment was calculated for the peak of naphthalene. Data acquisition rate of 50 Hz was employed for column evaluation, and the maximum rate of 200 Hz was employed for assessing the extra-column band broadening at the fastest detector response, 10 ms. The contribution of the extra-column band broadening to total band broadening was estimated by calculating the second central moment of the eluted band of naphthalene, having a retention factor, $k = 0.46$.

Peak capacity values under gradient conditions were obtained by calculating the sum of resolution (R_s) for each neighbouring peak pair for the elution range between DP = 3 and DP = 15 of PA-Glucose Oligomer (DP = 3–15). The R_s values were calculated by dividing the

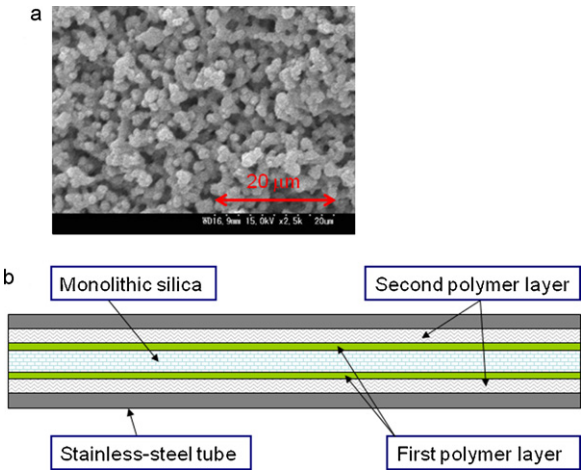

Fig. 1. (a) SEM photograph of a monolithic silica. Scale bar is 20 μm. (b) Schematic illustration of a MonoClad column.

difference in retention times for two neighbouring peaks by the average peak width (4σ of a Gaussian peak) of the two peaks.

3. Results and discussion

3.1. Silica rod and column structure

The SEM photograph of a monolithic silica and the schematic illustration of a MonoClad column are shown in Fig. 1a and b, respectively. The through-pore size is 1.2 μm as measured by mercury intrusion, smaller than that of a Chromolith column (through-pore size, 2 μm), while mesopores (18 nm determined by nitrogen adsorption) are larger (13 nm for Chromolith), and the specific surface area (200 m^2/g determined by nitrogen adsorption) smaller (300 m^2/g for Chromolith). Total porosity and external porosity are ca. 75% and 55%, respectively, for a MonoClad column, that are smaller than those of a Chromolith column, by about 10% and 15%, respectively. The carbon content, 14.6% (after endcapping), corresponds to a near maximum surface coverage.

The monolithic silica rod (3 mm diameter) was covered with a chemically inert first polymer layer, then embedded with a second polymeric material in a stainless-steel tube (8 mmOD, 2 mm thickness). The first polymer layer is compatible with organic solvents commonly employed for reversed-phase HPLC, including alcohols, acetonitrile, dichloromethane, and THF. The structure allowed the fabrication of 25 cm long columns, much longer than the first-generation monolithic silica rod columns available, allowing full use of high permeability. The suggested pressure limit is 30 MPa, higher than 20 MPa for a Chromolith column. With a continuous operation at pressure above 20 MPa, a decrease in column efficiency of up to 3–4% was noticeable, as also found for Chromolith columns for a 10–20 MPa range [8]. The effect was reproducible, and presumably caused by the compressibility of the polymer layers at high pressure. The efficiency of the column was recovered when used or stored at lower pressure afterward.

3.2. Extra-column band broadening

The operation of small sized columns packed with small particles either fully porous or superficially porous, needs HPLC or ultrahigh pressure equipment having small extra-column volume to minimize band broadening outside the column. Very high performance, reduced plate height of less than 2, was achieved with a small-sized column (2.1 mmID) packed with superficially porous particles with such instrumentation [1]. The second central moment, or the variance for the extra-column band broadening (σ^2_{Extra}) with the present instrument, LC800, was found to be 3–5 μL^2 for flow rate of 0.2–0.8 mL/min with the dead volume of the instrument about 6 μL calculated as the first moment. The contribution of the extra-column band broadening was found to be about 20% for a naphthalene peak (k = 0.46) on a 3 mmID, 5 cm column at a flow rate of 0.8 mL/min. In the case of the evaluation of a 25 cm column, the contribution was much less, approximately 4.6%, 1.5%, and 0.8% for the peaks of naphthalene (retention factor k = 0.46), triphenylene (k = 1.3), and benz(a)pyrene (k = 2.3), respectively, at 0.8 mL/min or at 2.3 mm/s linear velocity, and the effect was neglected in the preparation of van Deemter plots or kinetic plots. The kinetic plot was prepared using the results obtained for benz(a)pyrene.

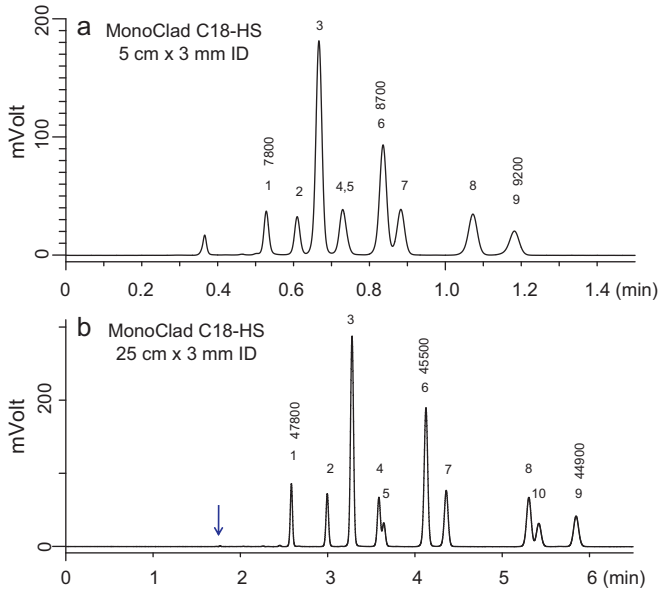

Fig. 2. Chromatograms of PAHs on MonoClad C18-HS. Mobile phase: acetonitrile/water = 80/20 (v/v), 0.8 mL/min. Temperature: 40 °C. Solutes: PAH mixture, 1. naphthalene, 2. fluorene, 3. anthracene, 4. fluoranthene, 5. o-terphenyl, 6. triphenylene, 7. 1,2-benzanthracene, 8. benz(b)fluoranthene, 9. benz(a)pyrene, and 10 perylene. The numbers of theoretical plates are indicated for peaks 1, 6, and 9. (a) Column length: 5 cm. Solute: PAH 1–9. (b) Column length: 25 cm. Solute: PAH 1–10. An arrow indicates t_0.

3.3. Column efficiency, peak symmetry, and permeability

A 5 cm column provided theoretical plates ranging from 7800 to 9200 for the PAHs at the optimum linear velocity of 2.3 mm/s (Fig. 2a). Following the correction for the extra-column band broadening, the column was found to provide height equivalent to a theoretical plate, ca. H = 5.5 μm, for the first peak of the chromatogram of the PAHs, as observed for the last peak. With the increase in flow rate, the column efficiency decreased, but the column still provided 7000 theoretical plates for the last peak at linear velocity of 5 mm/s. Peak symmetry coefficients (US Pharmacopeia) at 5% peak height were found to be between 0.89 and 1.04, although a slight increase in peak fronting was observed at a higher flow rate. The results indicate the necessity of improvement of the column fabrication method. Cladding of a silica rod with polymers seems to be the crucial step for column preparation, although radial inhomogeneity of silica rods could contribute to peak deformation [23].

Longer monolithic silica rod columns showed better peak shape and more consistent column efficiency. A chromatogram obtained with a 25 cm column is shown in Fig. 2b. The column showed peak symmetry factors of 0.96–1.02 at 0.8 (2.3 mm/s)–2.8 mL/min

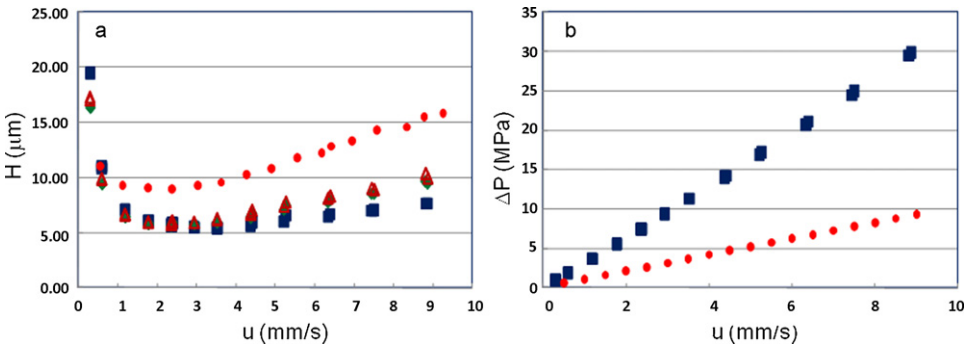

Fig. 3. Performance of a monolithic silica rod column. Column: MonoClad C18-HS, 3 mmID, 25 cm long. Mobile phase: acetonitrile/water = 80/20 (v/v). Temperature: 40 °C. (a) The plot of height equivalent to a theoretical plate against linear velocity of the mobile phase. Flow rate: 0.1–3.0 mL/min. Solute: naphthalene (■), triphenylene (△), benzpyrene (♦). (b) The plot of back pressure against linear velocity of the mobile phase. The plots for Chromolith column (●) are obtained for anthracene in 60% acetonitrile (courtesy of Dr. Cabrera, Merck).

(8 mm/s), although the peak of an unretained marker (uracil) showed considerable fronting. The column provided about 45,000 theoretical plates for the peaks of PAHs at an optimum flow rate, 0.8 mL/min, with decreasing tendency for the solutes showing the greater retention and at a higher flow rate. (Peaks 4 and 5 overlap in these chromatograms.) Two other 25 cm columns provided similar results, or about 40,000–45,000 theoretical plates for the PAHs at the optimum flow rate and peak symmetry coefficients of 0.96–1.04, and were used for studying the performance of columns connected in series. Minimum height equivalent to a theoretical plate was found to be 5.5–5.7 μm for naphthalene, triphenylene, and benz(a)pyrene.

The van Deemter plots shown in Fig. 3a indicate that the column efficiency is considerably better than that of the conventional silica rod columns (the van Deemter plot for Chromolith obtained for anthracene in 60% acetonitrile, by courtesy of Dr. K. Cabrera, Merck) [8,14,17]. High peak symmetry and considerably higher numbers of theoretical plates are the primary features of the present monolithic silica rod columns. The slope of the van Deemter plots in the high linear velocity region is smaller than that for Chromolith, but not as small as for columns packed with particles around 2 μm or smaller, in spite of the presence of small-sized skeletons in the silica rods. This is presumably caused by the contribution of the large through-pores (ca. 1.2 μm) that could cause greater eddy diffusion and slower mass transfer effect in the mobile phase than those in a particulate column with ca. 2 μm particle size. The permeability calculated on the basis of the superficial linear velocity of the mobile phase, V_F, was similar to that of a column packed with 5 μm particles, $K_F = 2.4 \times 10^{-14}$ m^2, smaller than that of Chromolith by a factor of about 4. In other words, the present monolithic silica rod columns demonstrated the column efficiency corresponding to 2.5 μm particles and back pressure equivalent to a column packed with ca. 5 μm particles (Fig. 3b) at optimum flow rate. This is another feature of the present silica rod columns.

In a methanol–water (80/20, v/v) mobile phase at 30 °C, a greater minimum plate height of ca. 6.2–7.0 μm was observed for the PAHs at lower linear velocity of 1.1 mm/s than in acetonitrile–water (80/20, v/v). Retention factors for alkylbenzenes were in a similar range with those observed on Chromolith columns [8]. A smaller porosity and the smaller specific surface area of the silica rod counteracted, resulting in a similar retention factor range of solutes on the two monolithic silica rod columns, MonoClad-C18 and on Chromolith-RP.

3.4. Operation of a long column

When two or three 25 cm columns were connected in series, large numbers of theoretical plates can be generated. Such examples are shown in Fig. 4. The 50 and 75 cm column systems afforded 80,000 theoretical plates and 120,000 theoretical plates, respectively, for the PAHs in a pressure range, 15–25 MPa, The partially overlapping peaks of fluoranthene and o-terphenyl (peak nos. 4 and 5), and benzfluoranthene and perylene (peak nos. 8 and 10) on each single column (Fig. 2) were separated with greater numbers of theoretical plates.

The results compare favorably with those reported with columns packed with 1.7 μm totally porous particles or 2.7 μm superficially porous particles at higher pressure and with shorter t_0 [24,25], although the latter was examined for different types of solutes. While the separation on the connected monolithic silica columns needs longer time for generating similar numbers of theoretical plates based on the use of a longer column system, similar column efficiency was obtained at much lower pressure, 15–25 MPa, which can be delivered with conventional HPLC equipment.

3.5. Silanol effects on monolithic silica columns

Fig. 5 shows the chromatograms of ionized amines eluted from a 10 cm MonoClad column at pH 7 with phosphate buffer. Amitripty-

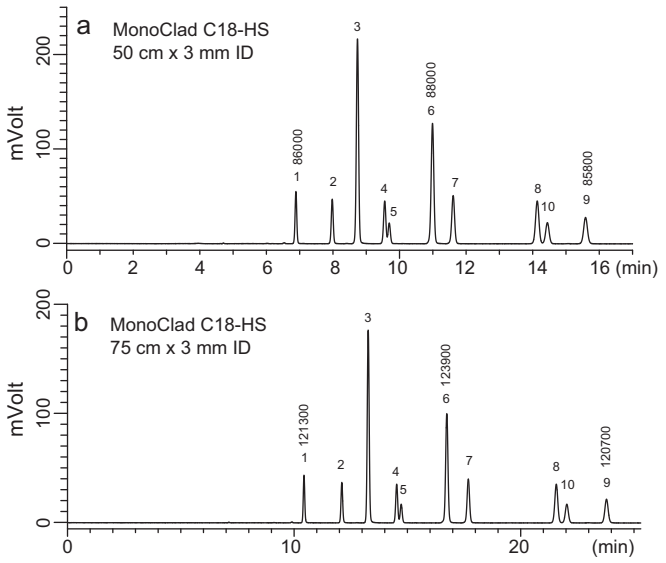

Fig. 4. Performance of two or three monolithic silica rod columns connected in series. Mobile phase: acetonitrile/water = 80/20 (v/v), 0.6 mL/min. Temperature: 40 °C. Solutes: PAH 1–10. (a) Column: MonoClad C18, 3 mmID, 25 cm long, two columns connected. (b) Three columns connected. The numbers of theoretical plates are indicated for peaks 1, 6, and 9.

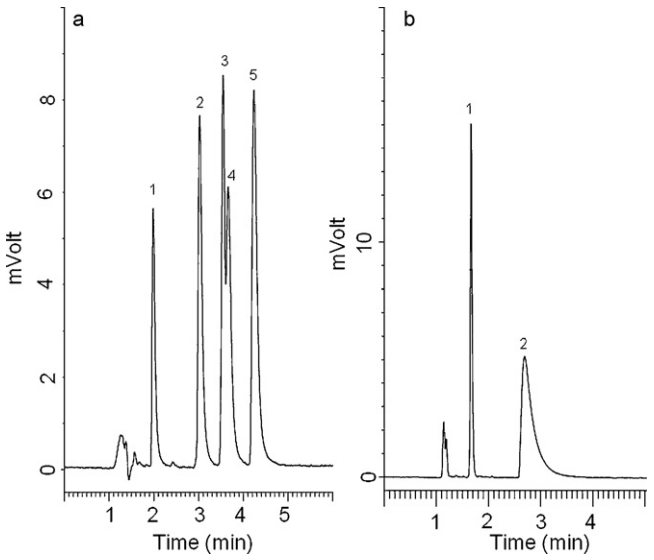

Fig. 5. Chromatograms of basic compounds obtained with MonoClad C18-HS. Column: 3 mmID, 10 cm long. Temperature: 40 °C. Detection: UV at 220 nm. (a) Separation of tricyclic anti-depressants. Mobile phase: acetonitrile/25 mM phosphate buffer (pH 7.0) = 60/40 (v/v), 0.4 mL/min. Solutes: 1. imipramine, 2. amitriptyline, 3. mianserin, 4. clomipramine, and 5. nortriptyline. (b) Elution of dextromethorphan. Mobile phase: acetonitrile/25 mM phosphate buffer (pH 7.0) = 40/60, 0.4 mL/min. Solutes: 1. phenol and 2. dextromethorphan.

line and some other antidepressants have been frequently used to characterize stationary phases for reversed-phase HPLC for their performance toward basic compounds [26,27]. The tricyclic antidepressants were eluted with small tailing, with a USP tailing factor of 1.8 for amitriptyline, as shown in Fig. 5a. While amitriptyline can be eluted with acceptable peak symmetry from most base-deactivated silica ODS columns, dextromethorphan is much more sensitive with respect to the silanol effect [28]. Most base-deactivated columns failed to elute this compound with good peak shape. The peak of dextromethorphan shown in Fig. 5b shows considerable tailing (USP symmetry factor 3.2), but still comparable with or better than most base-deactivated columns studied previously (USP symmetry factor 3–5) [28]. Tailing effect has been reported for monolithic silica C18 columns for basic compounds [12,29] as well as for PAHs [30]. Further improvement of monolithic silica columns for increased peak symmetry and efficiency is desirable.

3.6. Comparison with columns packed with superficially porous particles

It is of much interest to compare the performance of the monolithic silica rod column and columns packed with superficially porous particles. The latter have been attracting much attention, because they can provide the column efficiency of sub-2 μm particles, while actual pressure drop is based on the nominal size of the particles at 2.6–2.7 μm [4]. Acetonitrile gradient elution of pyridylamino derivatives of glucose oligomers (DP = 3–15) was carried out on Ascentis Express C18 with 2.7 μm superficially porous particles,

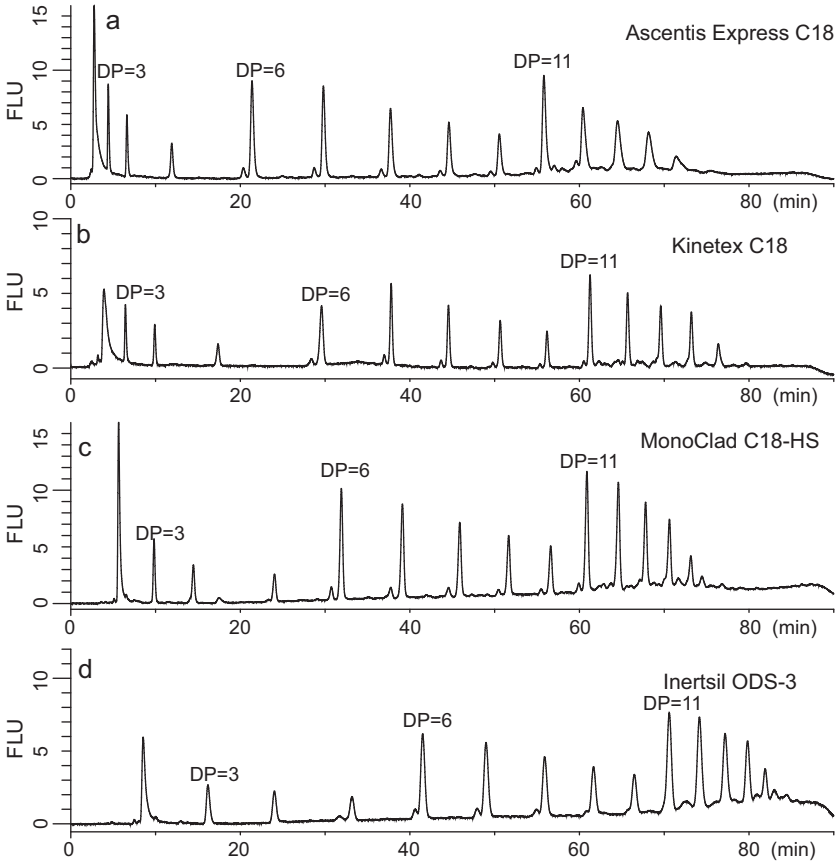

Fig. 6. Separation of pyridylamino derivaties of glucose oligomers (DP = 3–15) on particulate columns and a monolithic silica rod column. (a) Ascentis C18, (b) Kinetex C18, (c) MonoClad C18, and (d) Inertsil ODS-3. Column size: 3 mmID, 15 cm long in (a)–(d). Mobile phase: linear gradient from 100% mobile phase A to 90% A at 70 min, then to 85% A at 80 miin. Mobile phase A: water containing 0.02% trifluoroacetic acid, and mobile phase B: acetonitrile/water = 20/80 (v/v) containing 0.02% TFA. Flow rate: 0.3 mL/min. Detection: fluorescence with excitation at 310 nm, and emission at 380 nm. Temperature: 30 °C.

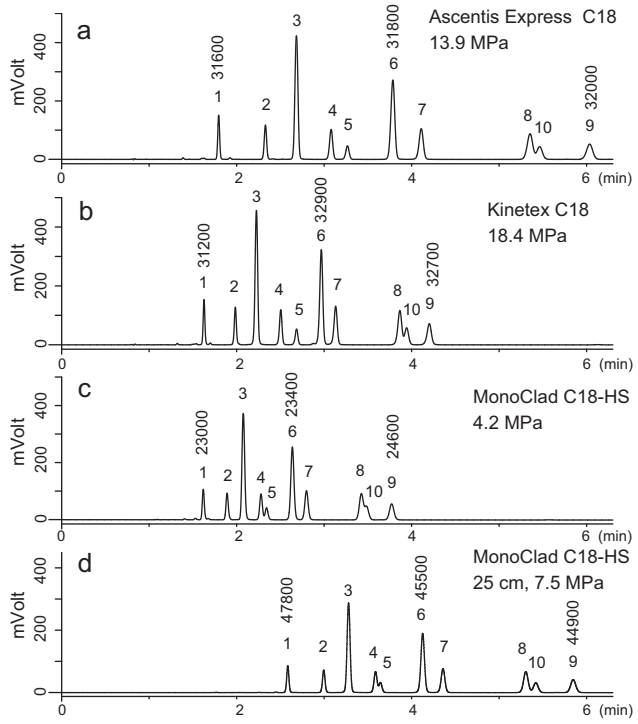

Fig. 7. Chromatograms of PAHs on monolithic silica rod and particulate columns packed with superficially porous particles. Column: (a) Ascentis C18, (b) Kinetex C18, (c) MonoClad C18-HS, (a–c) 3 mmID, 15 cm long, and (d) MonoClad C18-HS, 3 mmID, 25 cm long. Mobile phase: acetonitrile/water = 80/20 (v/v). Flow rate: 0.6 mL/min for (a–c), and 0.8 mL/min for (d). Solutes: PAH 1–10.

Kinetex C18 with 2.6 μm superficially porous particles, MonoClad C18-HS, as well as Inertsil ODS-3 with 3 μm totally porous particles. The results are shown in Fig. 6. The comparison was expected to provide examples of performance of these columns for the small or medium-sized, very hydrophilic species.

The peak capacity calculated for the elution range between DP = 3 and DP = 15 is 103 in 67 min for Ascentis, 133 in 70 min for Kinetex, 74 in 66 min for Inertsil ODS-3, and 108 in 63 min for MonoClad. The performance of MonoClad C18 was found to be much better than that of a column packed with 3-μm particles, and close to that of a column packed with superficially porous particles.

Fig. 6a shows that the Ascentis C18 column resulted in broader peaks for the later part of the chromatogram for species of higher molecular weights, while the earlier peaks showed excellent performance. Better performance of Kinetex than another type of superficially porous particles was observed for peptides and proteins [4]. The results were interpreted based on the smoothness of the outer surface of the particles and some possible secondary interactions. Pore size was reported to be 7.7 nm for Kinetex and 7.2 nm for Halo material after modification [4], while 10 nm pore size was stated for the materials by the manufacturers. It is not totally clear why Ascentis showed boarder peaks for the late-eluting pyridylamino derivatives of glucose oligomers. The columns packed with the superficially porous particles or the monolithic materials showed better performance than the column packed with 3 μm totally porous particles, although the two columns packed with the superficially porous particles resulted in the more severely tailed peak for the unretained solute, the derivatizing reagent.

The comparison of the performance of three columns, Ascentis C18, Kinetex C18, and MonoClad C18 (all columns having 3 mmID and 15 cm long) in the isocratic mode is shown in Fig. 7a–c for the separation of a PAH mixture (PAH 1–10). Retention of these aromatic hydrocarbons was greater on Ascentis C18 (retention factor of benz(a)pyrene, $k = 5.8$), than on Kinetex C18 ($k = 4.0$), and MonoClad C18 ($k = 2.4$). The results reflect the difference in the phase ratio, or the surface area and the surface coverage of the stationary phase of each material. The fraction of a superficially porous particle occupied by the core is greater with Kinetex (the fraction occupied by the solid core = 0.39) than with Ascentis (the fraction occupied by the solid core = 0.25). MonoClad C18 with greater total porosity showed smaller retention factors than the columns packed with these superficially porous particles.

While MonoClad C18 provided a fewer number of theoretical plates with a 15 cm column for the PAHs than with Ascentis or Kinetex, the 25 cm column provided the greater numbers of theoretical plates with back pressure half that of Ascentis or Kinetex. Thus MonoClad C18 could provide better separation between peaks 8 and 10, although the sensitivity will be lower because of the longer column leading to a greater dilution of solutes.

The separation between peaks 4 and 5 needs comments. The separation is dictated by the retention of ortho-terphenyl (peak 5), a bulky molecule, relative to those of surrounding peaks of planar solutes. The retention of the bulky solute tends to be small on a stationary phase having high surface coverage. The comparison of a separation factor, or the steric selectivity, for triphenylene (T, peak 6) and ortho-terphenyl (O, peak 5), having similar hydrophobic properties, can provide information on the density of alkyl groups on the silica surface [31]. The separation factor was found to be the smallest with Kinetex ($\alpha_{(T/O)} = 1.16$) compared to $\alpha_{(T/O)} = 1.21$ and 1.25 (with standard deviation <0.01) on Ascentis C18 and MonoClad C18, respectively, suggesting the higher density of octadecylsilyl moieties on the stationary phase of MonoClad C18. The retention of a bulky solute, o-terphenyl (peak No. 5), was significantly affected by the structure of the stationary phase [31].

3.7. Kinetic plot

Kinetic plots obtained for the three columns are shown in Fig. 8 together with calculated plots for columns packed with totally porous particles of 1.4–5 μm diameter. The column efficiency for benz(a)pyrene, eluting last among the PAHs studied, was used for evaluating the three columns in 80% acetonitrile. The plots were made with different pressure for the different types of columns to illustrate the performance under practical limits. The pressure limit was taken as 30 MPa for MonoClad (pressure limit for the column), 60 MPa for columns packed with superficially porous particles (a pressure limit for a column packed with certain superficially porous particles), 40 MPa for 5- and 3-μm totally porous particles (pressure limit of many conventional HPLC pumps), and 100 MPa for 2- and 1.4-μm particles (a typical pressure limit of ultrahigh pressure LC).

When compared with the performance of a column packed with 3 μm particles calculated at 40 MPa, the performance of MonoClad C18 calculated at 30 MPa was shown to be similar at 30,000–50,000 theoretical plates, and better for the range of the greater N. For separations in an N range of 10,000–100,000, columns packed with superficially porous particles operated at 60 MPa or 2 μm particles at 100 MPa are shown to provide faster separations.

The comparison at the same pressure drop, especially at low pressure, is more favorable for MonoClad. As shown in Fig. 8b, a MonoClad column can provide faster separations than columns packed with superficially porous particles in a range, $N > 50{,}000$–$60{,}000$ at 20 MPa, and than conventional particulate columns in a range, $N > 30{,}000$. Therefore the monolithic silica rod columns can show an advantage, when one attempts to generate a large number of theoretical plates by using conventional HPLC.

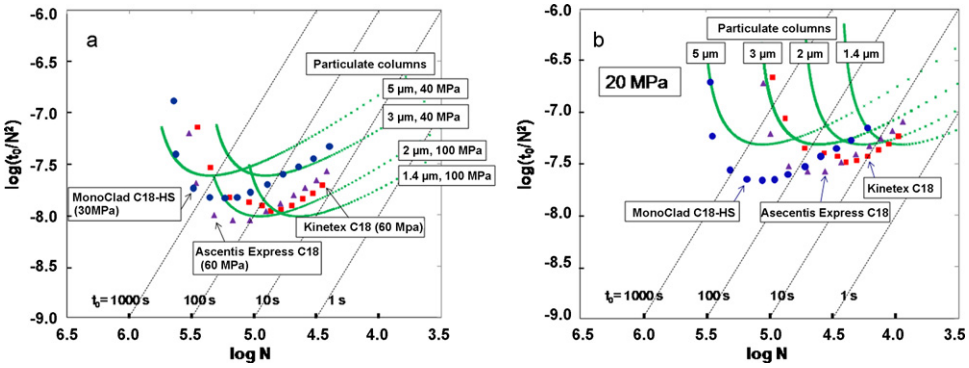

Fig. 8. Plots of $\log(t_0/N^2)$ against $\log(N)$ for MonoClad column (●) and the columns packed with Ascentis (▲) and Kinetex (■) particles obtained for benz(a)pyrene in acetonitrile/water = 80/20 (v/v) at 40 °C. The plots for the conventional particulate columns were created by assuming a flow resistance parameter of 700, the viscosity of the mobile phase 0.00041 Pa s, a diffusion coefficient of the solute $D_m = 2.22 \times 10^{-9}$ m^2/s, and a Knox equation, $h = 0.65\nu^{1/3} + 2/\nu + 0.08\nu$ (h: reduced plate height, ν: reduced velocity) [18]. (a) Pressure drop: 30 MPa for MonoClad column, 60 MPa for the columns packed with superficially porous particles, 40 MPa for columns packed with 5- and 3-μm totally porous particles, and 100 MPa for particulate columns packed with 1.4- and 2-μm particles. (b) Pressure drop: 20 MPa for all columns.

4. Conclusion

Monolithic silica rod columns, MonoClad C18 for reversed-phase HPLC can provide 40,000–45,000 theoretical plates with a 25 cm column with back pressure equivalent to 5 μm particles, allowing high efficiency separations with common HPLC equipment. Connecting two or three columns allows the generation of nearly 100,000 theoretical plates with back pressure below 25 MPa, although similar numbers of theoretical plates can be generated in a shorter time using columns packed with small superficially porous or totally porous particles at higher pressure. A column showing high permeability may also be beneficial, if ultrahigh-pressure operation is accompanied by significant effects of pressure on retention of hydrophilic solutes or ions that caused considerable change of selectivity in ultrahigh pressure LC [32].

The MonoClad C18 columns actually showed lower performance than a monolithic silica capillary column prepared from TMOS with increased phase ratios [18], and need improvement. As indicated in the study of the monolithic silica capillary columns, homogeneity of monolithic silica structure must be improved, along with the cladding procedure of silica rod columns, to correct peak fronting observed with the short monolithic silica column. An increase in pressure stability will also increase the utility of rod-type monolithic silica columns.

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