

Size Exclusion Chromatography of Standard Polystyrenes with a Wide Range of Molecular Weight Up to 7.45×10^6 on Monolithic Silica Capillary Columns

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Monolithic columns have recently received much attention as newly introduced technology for HPLC and capillary electrochromatography (CEC). These columns consist of a single piece of porous material (most often polymer or silica based) with a bimodal pore structure termed “throughpores” and “mesopores”. Typically, monolithic columns provide higher porosity compared to conventional columns packed with spherical particles leading to much lower column backpressure.^{1–3}

Ishizuka *et al.* prepared a continuous monolithic silica by the sol-gel process in a fused-silica capillary of 100 μm i.d. and 25 cm in length, and used as a column for capillary HPLC and CEC.^{4,5} A scanning electron micrograph of the column showed that uniform-sized, interconnected silica skeletons as large as *ca.* 2.2 μm and throughpores of up to *ca.* 8 μm were evenly distributed over the entire cross section of the capillary. The surface of the silica monolith was derivatized to C_{18} phase by the reaction with octadecyldimethyl-*N,N*-diethylaminosilane. External and total porosities of the C_{18} -covered monolithic silica capillary column were estimated to be 86 and 96%, respectively, from the retention volumes of polystyrene standard of 390,000 molecular weight and benzene in the size exclusion chromatography (SEC) using THF as mobile phase. This estimation was based on the assumptions that the polystyrene was totally excluded from mesopores of the silica monolith and that

benzene was totally permeated throughout the mesopores. The high external porosity together with the large throughpore size resulted in very low flow resistance (pressure drop, $\Delta P = 0.4 \text{ kg/cm}^2$), while achieving column efficiency of 43,000 plates/m at a linear velocity of the mobile phase 0.5 mm/s.⁵ However, the column seems to be too short to ensure high efficiency required for testing the assumptions mentioned above.

Efforts to improve the fabrication process of the column have made it possible to prepare monolithic silica with various sizes of throughpores (2–8 μm) in a fused-silica capillary (50–200 μm i.d. \times 100–200 cm).^{6,7} In this paper, we report separation of polystyrene standards with molecular weight ranging from 2,900 to 7,450,000 by the HPLC using monolithic silica capillaries with a dimension of 200 μm i.d. \times 150 cm. High separation efficiency achieved by the long columns has made it possible to fully assess the size exclusion effect brought about by the monolithic silica in capillary.

EXPERIMENTAL

Equipment

The chromatographic experiments were carried out using a micro HPLC pump MP681 (GL Sciences Inc., Tokyo, Japan), a micro valve injector with a 100 nL inner sample loop (Valco, Houston, USA), and a UV detector UV702 (GL Sciences Inc.) equipped with a

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Table I. Characteristics of the monolithic silica capillary columns^a

	Column		
	A	B	C
Median mesopore diameter (nm)	20	20	15
Silica surface	C ₁₈	bare	bare
Column efficiency (plates) ^b	103,000	52,000	101,000
Pressure drop (MPa) ^b	3.8	3.5	4.9

^aLength and inner diameter of the columns are 150 cm and 200 μ m, respectively. ^bData obtained at a flow rate of 1.0 μ L/min (eluent: THF).

fused-silica capillary flow-cell of a 18 nL detection volume. The system was operated at ambient temperatures (20–25 °C). Chromatograms were recorded on a SIC480 data station (System Instruments Co., Ltd., Tokyo, Japan) with a sampling rate of 400 points/min.

Columns

Monolithic silica capillary columns (A to C in Table I) were prepared by *in situ* hydrolysis and polycondensation of alkoxysilanes in a fused-silica capillary of 200 μ m i.d. and 150 cm in length by a method described previously.^{5,7} The silica surface of column A was derivatized to C₁₈ phase by the reaction with octadecyldimethyl-*N,N*-diethylaminosilane in a manner described previously.⁴ Columns of this type are commercially available from GL Sciences Inc. as MonoCap[®] High Resolution 750.

It was found difficult to determine the mesopore size of monolithic silica in a capillary by standard porosimetry measurements. Accordingly, the median mesopore diameter of monolithic silica prepared in bulk under the same conditions was determined instead by the nitrogen adsorption method. A Micromeritics ASAP2010 accelerated surface area and porosimetry analyzer was used for this purpose.

Chemicals

Seven polystyrene standards with peak top molecular weights (M_p 's) ranging from 2,900 to 2,890,000 were obtained from Tosoh Corp. (Tokyo, Japan). Polystyrene standard with M_p of 7,450,000 was purchased from Polymer Laboratories Ltd. (Shropshire, U.K.). Tetrahydrofuran (HPLC grade) was purchased from Kishida Chemical Co., Ltd. (Osaka, Japan).

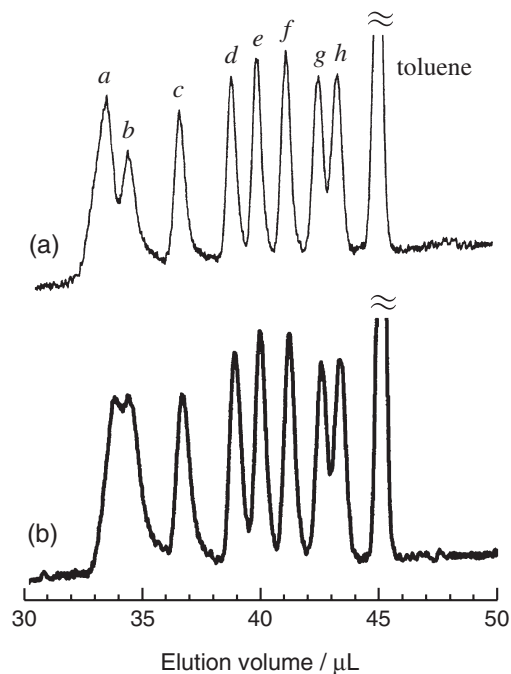
Procedures

A mixture of the 8 polystyrene standards (*a* to *h* in Table II) was dissolved at a concentration of 5 g/L in the mobile phase containing a tiny amount of toluene. For each measurement, 100 nL of the solution was injected into each column. The retention time was

Table II. Peak top molecular weight M_p , ratio of weight average molecular weight to number average molecular weight M_w/M_n , and hydrodynamic radius R_H in THF at 25 °C of polystyrene standards used in the present work

Sample	M_p	M_w/M_n	R_H^a (nm)
<i>a</i>	7,450,000	1.07	135.5
<i>b</i>	2,890,000	1.09	77.6
<i>c</i>	775,000	1.01	35.7
<i>d</i>	110,000	1.06	11.3
<i>e</i>	42,800	1.01	6.5
<i>f</i>	18,100	1.01	3.9
<i>g</i>	6,200	1.04	2.1
<i>h</i>	2,900	1.10	1.3

^aCalculated according to the method described by Van Kreveland and Van Den Hoed.⁸

**Figure 1.** Chromatograms of a mixture of polystyrene standards and toluene obtained by the use of monolithic silica capillary column A at flow rates of 0.5 (a) and 1.0 mL/min (b), respectively (UV detection, $\lambda = 225$ nm).

determined from the peak maximum and converted to the retention volume at a given flow rate.

RESULTS AND DISCUSSION

A mixture of 8 standard polystyrenes denoted by *a*–*h* (Table II) and toluene was chromatographed on the monolithic silica capillary column A (see Table I). Elution peaks due to each polystyrene standard were separated clearly, demonstrating high efficiency of this capillary column (Figure 1a). Pressure drop ΔP in the column was as low as 1.9 MPa at a flow rate of 0.5 μ L/min. When the flow rate was increased

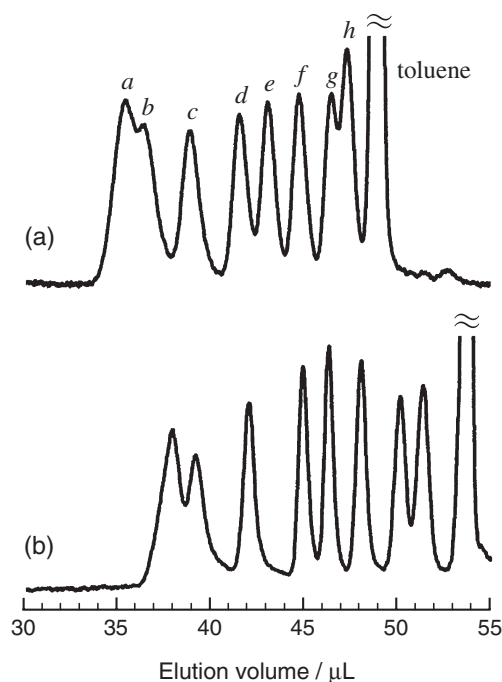


Figure 2. Chromatograms of a mixture of polystyrene standards and toluene obtained by the use of monolithic silica capillary columns B (a) and C (b) at a flow rate of 0.5 mL/min (UV detection, $\lambda = 254$ nm).

to 1.0 $\mu\text{L}/\text{min}$, the elution peaks broadened slightly (Figure 1b) and ΔP increased to 3.8 MPa. Retention volumes of polystyrenes *b–h* remained unchanged within experimental errors ($< 0.4\%$), except for that of polystyrene *a* with the highest M_p . The enlarged retention volume (+1.3%) of polystyrene *a* at 1.0 $\mu\text{L}/\text{min}$ may be ascribed to mechanical scission of long polymer chains during the chromatographic separation.

Figure 2a shows a chromatogram of the polystyrene mixture recorded by using column B at a flow rate of 0.5 $\mu\text{L}/\text{min}$. Though the chromatographic pattern was similar to Figure 1a, retention volumes of the polystyrenes increased by 6.3–9.5% as compared with those observed in Figure 1a. An essential difference between columns A and B is surface polarity of monolithic silica in these columns (column A: C_{18} , column B: bare), and thus the increase of retention volume may be partly ascribed to a more adsorptive nature of the bare monolithic silica surface. Another reason for the smaller retention volumes in column A than in column B would be a decrease in the total pore volume of silica monolith by the surface modification with octadecyldimethylsilyl groups. Somewhat poorer separation of elution peaks observed in Figure 2a is due to lower efficiency of column B. Column C with a smaller mesopore size also gave a chromatogram (Figure 2b) similar to Figures 1a and 2a. The shift of the elution peaks to larger retention volumes was

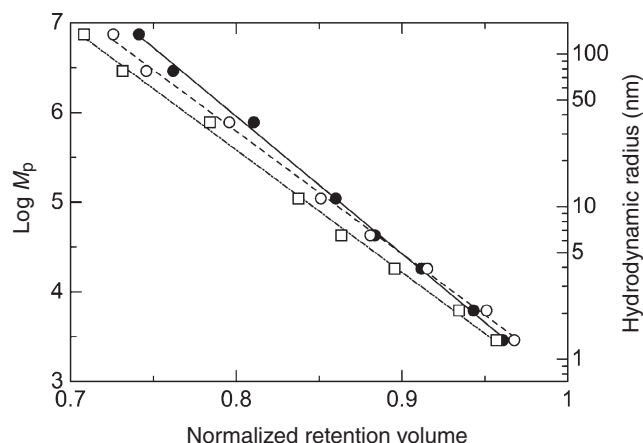


Figure 3. Plots of the logarithm of M_p of each polystyrene standard against its retention volume relative to the retention volume of toluene for the chromatography using columns A (●), B (○), and C (□) at a flow rate of 0.5 $\mu\text{L}/\text{min}$.

more noticeable (13.8–18.6%) as compared with that in the case for column B. This is attributable to a larger surface area and a larger total pore volume of silica monolith in column C than in column B.

For each column, the logarithm of M_p of each polystyrene standard was plotted against its retention volume relative to the retention volume of toluene (Figure 3). A notable feature of these plots is that the plots for each column can be fitted nicely to a straight line covering a wide range of molecular weight. Each line seems linear even for the molecular weight region exceeding 7.45×10^6 . According to the literature,⁸ hydrodynamic radius R_H of polystyrene standards *a–h* in THF at 25 °C are calculated to be in a range 1.3–135.5 nm (Table II). If maximum porosity of columns A–C in SEC were governed by the mesopore size of the monolithic silica, we should observe a kink in the $\log M_p$ versus retention volume plots at $\log M_p$ around 5.0 for columns A and B (median mesopore diameter 20 nm) and at around 4.7 for columns C (median mesopore diameter 15 nm).

Al-Bokari *et al.* have shown that the classical correlations between the bed properties (external porosity, permeability, column efficiency) and the particle size are no longer valid for monolithic columns from the inverse SEC studies using C_{18} -bonded monolithic silica rod columns with a dimension of 0.46 cm i.d. \times 10 cm. The separation of polystyrene according to molecular weight was suggested to occur not only in the mesopores of silica monolith (internal pore zone) but also at the outside (external pore zone). They also observed an obvious kink in $\log M_p$ versus retention volume plots at $\log M_p$ around 4.3.⁹

It has been recognized that there is a certain difference in morphology between the monolithic silica made as rod (S-A) and that made in capillary (S-B);

S-A possesses smooth cylindrical skeletons whereas S-B has the appearance of globule aggregates.² This morphological difference may account for the linearity of the $\log M_p$ versus retention volume plots in Figure 3 even in the molecular weight range higher than those expected from the mesopore size. The separation of polymers in the external pore zone of S-B is considered to be more effective than that of S-A, and as effective as the separation in the internal pore zone.

Obviously, the high column efficiency and the linear $\log M_p$ versus retention volume plots covering a wide range of molecular weight would be practically useful as a column for SEC. The small consumption of eluent by the use of capillary columns and the chemical and mechanical stabilities of the silica monolith will be advantageous for SEC using special and/or expensive eluent such as 1,1,1,3,3,3-hexafluoropropan-2-ol (HFIP). Studies are now in progress to extend the applicability of the present system toward the analysis of industrially important polymers such as nylon-6 and polyacetal, for which HFIP is known as a good eluent.

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