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SEMI-MICRO SIZE-EXCLUSION CHROMATOGRAPHY OF POLYMERS CALIBRATION OF COLUMNS AND COMPARISON WITH CONVENTIONAL COLUMNS

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SUMMARY

Stainless-steel tubes having inside diameters of 1.5 mm and 1.8 mm were packed with polystyrene gels of particle diameter $10 \pm 2 \mu\text{m}$. Two 50 cm \times 1.8 mm I.D. packed columns, connected in series, were calibrated and molecular-weight averages of polystyrene NBS 706 were measured, the results coinciding with the data of the National Bureau of Standards. The peak widths of polystyrenes of narrow molecular-weight distributions in both semi-micro column (four 25 cm \times 1.5 mm I.D.) and conventional column (two 50 cm \times 8 mm I.D.; packed by the manufacturer) systems were determined at different mobile-phase velocities, and the minimum peak width in the latter system was obtained at the velocity of 0.2 mm/sec, which was higher than that for the semi-micro system. The interstitial volume was higher and the inner volume was lower for the semi-micro column system (1.8 mm I.D.) than those for the conventional one, which means that semi-micro columns were packed less densely, resulting in a steep calibration curve. The peak height of a solute was proportional to the cell length of an ultraviolet detector if the sample load was proportional to the cross-sectional areas of columns having the same column efficiency. Although conventional size-exclusion chromatography has many advantages in respect of velocity, calibration curve and sample peak height, semi-micro size-exclusion chromatography still holds some merits such as low consumption of gels and of mobile-phase solvents.

INTRODUCTION

In our previous report¹, we showed how to pack polystyrene (PS) gels into 1.8 mm I.D. or 1.5 mm I.D. columns and obtain high-quality columns, with *e.g.*, 36,000 plates/m at a flow-rate of 30 $\mu\text{l}/\text{min}$ by using PS gels of particle diameter $10 \pm 2 \mu\text{m}$. This value was comparable to that of conventional size-exclusion chromatography (SEC) columns, which contained PS gels of the same particle diameter ($10 \pm 2 \mu\text{m}$) and were packed by a manufacturer. The effects of mobile-phase velocity, injection volume and concentration on the number of theoretical plates (N) and retention volume were also discussed.

In this report, the column system is calibrated and molecular-weight averages of polystyrene NBS 706 are calculated. Then, several parameters for semi-micro SEC are compared with those for conventional SEC.

EXPERIMENTAL

The SEC measurements were performed on a Jasco TRIROTAR-V® high-performance liquid chromatograph (Japan Spectroscopic, Hachioji, Tokyo 192, Japan), which has a triple piston pump for smooth delivery of 10 $\mu\text{l}/\text{min}$ to 9.9 ml/min of solvent at a 10- μl interval in the semi-micro mode and at a 0.1-ml interval in the normal liquid-chromatographic mode. Detectors were an ultraviolet (UV) absorption detector Model UVIDEC-100 IV (Jasco) with a semi-micro flow-cell (5 \times 0.5 mm I.D.; cell volume, 1 μl) and a differential refractometer Model SE-11 (cell volume, 8 μl), the latter being used for comparison purposes. The sample-injection volume was regulated by time by using a variable loop Model VL-611. Columns for semi-micro SEC (50 cm \times 1.8 mm I.D. and 25 cm \times 1.5 mm I.D.) were packed with a mixture of PS gels of nominal exclusion limits of 10^3 , 10^4 , 10^5 and 10^6 Å in our laboratory¹ and those for conventional SEC were Shodex A 80M HPSEC columns.

Calibration of semi-micro SEC column systems was made by injecting 4 μl of 0.05 or 0.025% PS solutions. These PS, which have narrow molecular-weight distributions, were purchased from Pressure Chemical (U.S.A.) and Toyo Soda (Japan). The mobile phase was tetrahydrofuran at a flow rate of 40 $\mu\text{l}/\text{min}$. The UV detector was set to 254 nm and attenuation was \times 0.08 a.u.f.s. The SEC was performed at room temperature.

Molecular-weight averages of a standard PS NBS 706 were calculated by a conventional method. A 4- μl portion of the 0.2% solution was injected. Peak widths at half height for several PS of narrow molecular-weight distributions were measured on both semi-micro and conventional columns at different mobile-phase velocities.

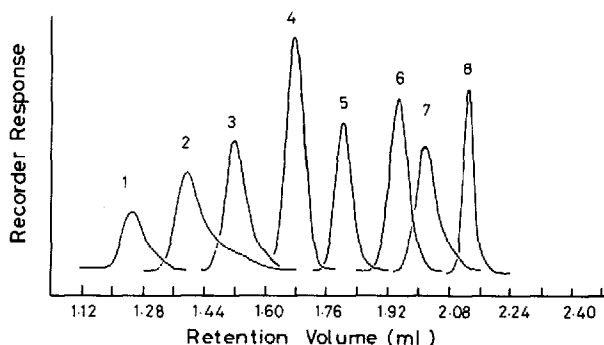


Fig. 1. Semi-micro size-exclusion chromatograms of standard PS of narrow molecular-weight distributions. Columns: two 50 cm \times 1.8 mm I.D. Sample load: 0.05%, 4 μl except samples 1 and 2; 0.025% for samples 1 and 2. UV detector at 254 nm, \times 0.08 a.u.f.s. Sample: (1) PS [molecular-weight average (M) = $8.42 \cdot 10^6$]; (2) PS (M = $1.8 \cdot 10^6$); (3) PS (M = $6.7 \cdot 10^5$); (4) PS (M = 97,200); (5) PS (M = 20,400); (6) PS (M = 2100); (7) PS (M = 600); (8) *n*-butylbenzene.

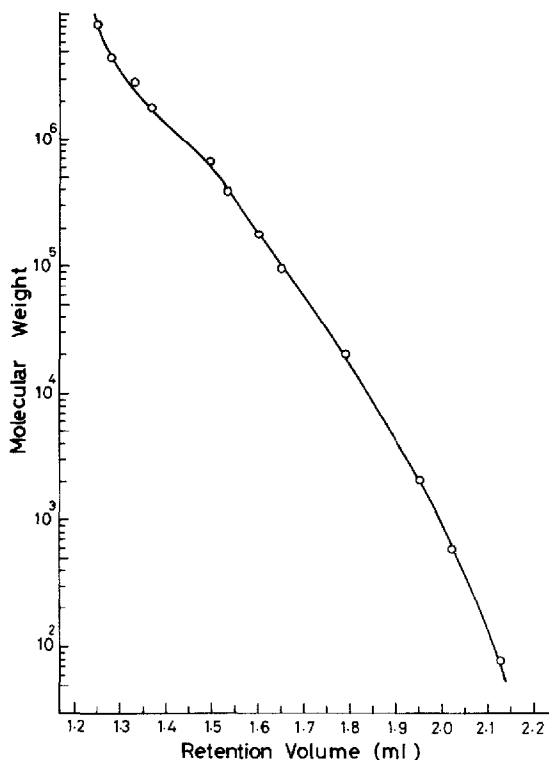


Fig. 2. Calibration graph for two 1.8 mm I.D. columns. Conditions as for Fig. 1.

RESULTS AND DISCUSSION

Size-exclusion chromatograms of standard PS of narrow molecular-weight distributions on two 1.8 mm I.D. columns connected in series are shown in Fig. 1. Symmetrical peaks have been obtained for each sample except PS of higher molecular weights. A calibration graph for the column system was prepared by plotting log molecular weight vs. retention volume of these PS (Fig. 2).

A chromatogram of standard PS NBS 706 was measured on two coupled 1.8 mm I.D. columns by injecting 4 μ l of a 0.1% solution (Fig. 3). Molecular-weight averages were calculated by a conventional method and were obtained as $\bar{M}_w = 2.55 \cdot 10^5$ and $\bar{M}_n = 1.32 \cdot 10^5$. These values were nearly equal to the standard values ($\bar{M}_w = 2.58 \cdot 10^5$; $\bar{M}_n = 1.37 \cdot 10^5$) published by NBS. Kever *et al.*² reported similar experiments in which silica gels were packed into a 0.6 mm I.D. poly(tetrafluoroethylene) column, the molecular-weight averages obtained being somewhat higher than the NBS data.

In the previous paper¹, the effect of the mobile-phase velocity on the height equivalent to a theoretical plate (HETP) for benzene has been investigated, where the minimum HETP was obtained at 0.4 mm/sec for semi-micro SEC and at *ca.* 1.5 mm/sec for conventional SEC (8 mm I.D. column). In this paper, it is shown in Figs. 4 and 5 that the linear velocity required for obtaining the minimum peak width at

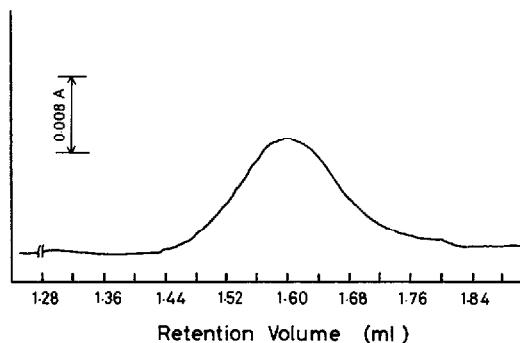


Fig. 3. A semi-micro size exclusion chromatogram of standard polystyrene NBS 706. Sample concentration, 0.1%; injection volume, 4 μ l; UV detector, $\times 0.08$ a.u.f.s. Column: two 50 cm \times 1.8 mm I.D.

half height decreased with increasing molecular size of a solute. For example, the minimum peak width at half height of dinonyl phthalate was obtained at 0.4 mm/sec for conventional SEC as shown in Fig. 5. As a matter of course, the HETP is proportional to the square of peak width at half height.

Similarly, peak widths at half height for several PS of narrow molecular-weight distributions decreased by decreasing the mobile-phase velocity, the minimum values being obtained at 0.2 mm/sec (flow-rate 0.27 ml/min) for conventional SEC. The case of PS of molecular weight $1.8 \cdot 10^6$ was exceptional, a pronounced decrease in peak width at half height being observed below 0.4 mm/sec. The velocity that gave the minimum peak width for conventional SEC was higher than that for semi-micro SEC.

Column parameters for semi-micro SEC were compared with those for conventional SEC and the results are shown in Table I. The interstitial volume (V_0) was estimated as the retention volume of PS of $8.5 \cdot 10^6$ molecular weight minus the dead volume, which was 0.033 ml for semi-micro SEC and 0.17 ml for conventional SEC. The dead volume was measured by connecting the injection valve and the detector directly after inserting a column between the pump and the injection valve in order

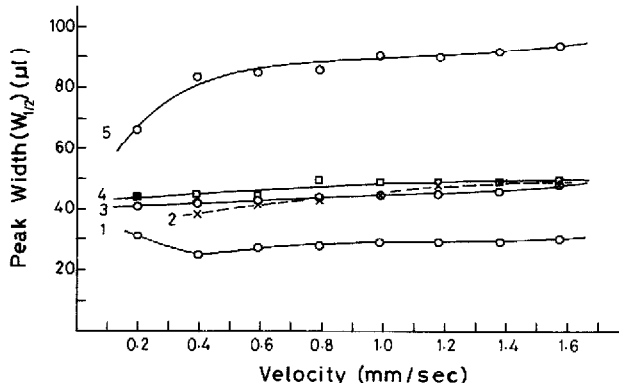


Fig. 4. Effect of mobile phase velocity on peak width at half height for semi-micro SEC. Column: four 25 cm \times 1.5 mm. Sample: (1) benzene; (2) PS ($M = 1.8 \cdot 10^5$); (3) PS ($M = 20,400$); (4) PS ($M = 2100$); (5) PS ($M = 1.8 \cdot 10^6$). Concentration, 0.2%; injection volume, 4 μ l; UV detector $\times 0.32$ a.u.f.s.

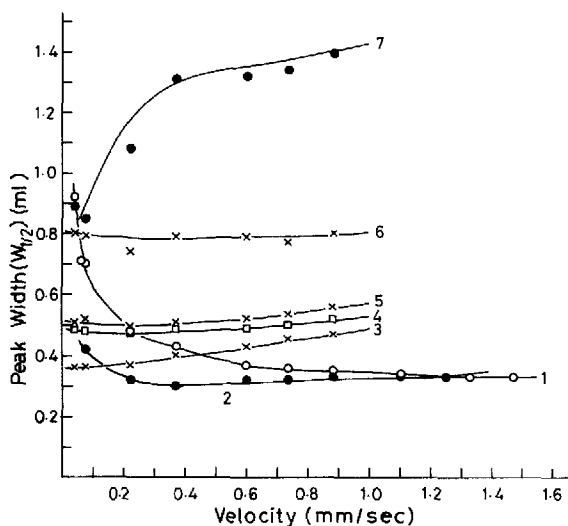


Fig. 5. Effect of mobile-phase velocity on peak width at half height for conventional SEC. Column: two 50 cm \times 8 mm I.D. Sample: (1) benzene; (2) dinonyl phthalate; (3) PS ($M = 20,400$); (4) PS ($M = 1.8 \cdot 10^5$); (5) PS ($M = 2100$); (6) PS ($M = 6.7 \cdot 10^5$); (7) PS ($M = 1.8 \cdot 10^6$). Concentration, 0.2%; injection volume, 50 μ l; refractive-index detector, $\times 16$.

to prevent the pulsation of flow, followed by injecting 1% benzene solution, the retention volume at peak position being assumed to be a dead volume. The inner volume (V_i) was estimated as the difference between the retention volumes of benzene and of PS of $8.5 \cdot 10^6$ molecular weight. The similar values of V_i/V_g in both SEC systems suggest that the pore volume in a gel particle is equal in both columns. In other words, the compressibilities of gels in both columns are equal. On the other hand, the differences in the ratio of V_i/V_0 and the percentage of V_g between both SEC systems exhibit the difference of packing density in both columns, gels in semi-

TABLE I

COMPARISON OF COLUMN PARAMETERS BETWEEN SEMI-MICRO AND CONVENTIONAL SEC

V_0 = Interstitial volume; V_i = inner volume; V_g = gel matrix volume; V_t = column volume. $V_t = V_0 + V_i + V_g$.

Parameter	Semi-micro*		Conventional**	
	Vol. (ml)	(%)	Vol. (ml)	(%)
V_0	1.217	47.9	22.67	45.1
V_i	0.875	34.4	18.18	36.2
V_g	0.451	17.7	9.39	18.7
V_t	2.543	100	50.24	100
V_i/V_0	0.70		0.80	
V_i/V_g	1.940		1.936	

* Two 50 cm \times 1.8 mm I.D. columns connected in series.

** Two 50 cm \times 8 mm I.D. columns connected in series.

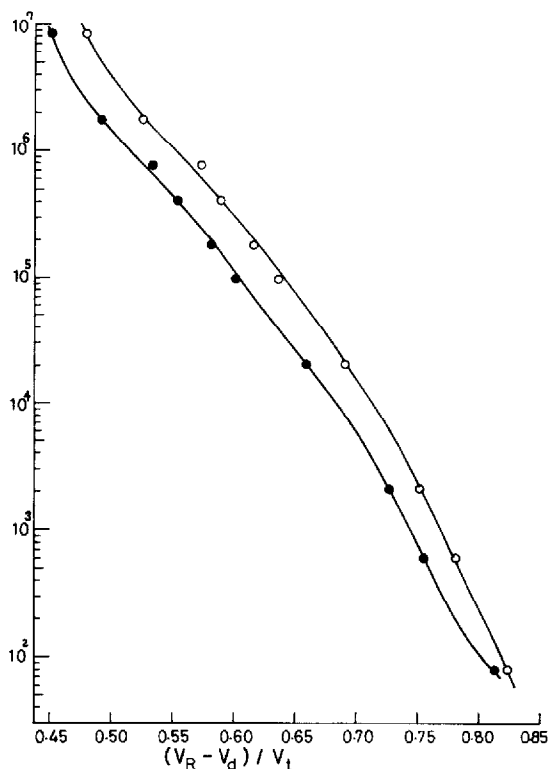


Fig. 6. Comparison of calibration curve for semi-micro SEC columns with that for conventional SEC columns. (○) Semi-micro (two 50 cm \times 1.8 mm I.D.); (●) conventional (two 50 cm \times 8 mm I.D.). V_R = Retention volume; V_d = dead volume; V_t = total volume in columns.

micro columns being packed less densely than in conventional columns; this may imply that semi-micro columns can increase in N , if the columns can be packed more densely.

Another comparison for semi-micro SEC and conventional SEC was made on the PS calibration curves by normalizing the retention volume as shown in Fig. 6. The abscissa in Fig. 6 is the ratio of the retention volume (minus the dead volume) to the total column volume. All solutes except benzene elute earlier in conventional SEC columns than in semi-micro SEC columns at a constant volume interval, which corresponds to the difference in the interstitial volumes between both column systems. The retardation of elution of benzene in conventional SEC may be attributed to its increased adsorption on the gels due to the higher packing density.

In Table II, dimensions and operational variables of both systems are listed. Injection volume of a sample solution can be increased in proportion to the cross-sectional area of a column; *e.g.*, injection volume in a 8 mm I.D. column can be twenty times larger than that in a 1.8 mm I.D. column. The peak height of a solute will be proportional to the cell length of a UV detector if the injection volume is proportional to the cross-sectional area of the column and the number of theoretical plates is equal in both systems. As the cell length of a UV detector in semi-micro

TABLE II

COMPARISONS OF SYSTEM DIMENSIONS AND OPERATIONAL PARAMETERS BETWEEN SEMI-MICRO AND CONVENTIONAL SEC

<i>Parameter</i>	<i>Semi-micro</i>		<i>Conventional</i>
Column I.D. (mm)	1.8	1.5	8
Ratio of cross section	1	0.69	19.8
	1.44	1	28.4
UV cell volume (μ l)		1	8
Cell length (mm)		5	10
Cell I.D. (mm)		0.5	1
Injection volume of 1% benzene (μ l)		2	50
Minimum HETP (μ m)	26.2	32.8	21.9
Linear velocity of mobile phase at minimum HETP (mm/sec)	0.41	0.39	1.47
Flow rate at minimum HETP (μ l/min)	30	20	2000
Peak width (σ) of benzene at $N = 30,000$ (μ l)	10.4	8.3	204
Peak height of benzene at $N = 30,000$ (arbitrary)	9.6		19.2

SEC is half that in conventional SEC, peak height in the latter system is twice as high than in the former, as shown at the foot of Table II. Similarly, peak width is proportional to the cross-sectional area of a column, if the injection volume is also proportional to it. Minimum HETP in conventional SEC was obtained with a linear velocity of mobile phase of *ca.* four times higher than in semi-micro SEC. At the same HETP (*ca.* 26 μ m), the linear velocity in conventional SEC was twice that in semi-micro SEC. These results suggest that the time required for analysis in conventional SEC is half that in semi-micro SEC at the same HETP, or one-fourth at the minimum HETP.

In a comparison of both systems, conventional SEC has many advantages over semi-micro SEC. However, consumption of gels and of solvents used as the mobile phase in the latter system is less than one-twentieth than in the former system, so that the practical merits of semi-micro SEC can be enhanced by optimization of the operating conditions.

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