

Exclusion Chromatography Using Controlled-Porosity Glass. I. Comparison with Styrene Gels

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Synopsis

The instrumental parameters influencing the calibration of a gel permeation chromatograph (GPC) using column packings of Corning CPG-10 porous glass have been evaluated. The porous glass system used a set of columns $1.6\times$ greater in length and $0.6\times$ smaller in diameter than the Styragel system, which was used for comparison. The dependence of retention volume of polystyrene calibrants on eluent flow rate and on sample size was similar in both systems. Samples of poly(methyl methacrylate), poly(vinyl chloride), and poly(4,4'-isopropylidene-diphenylene 1,1,3-trimethyl-3-phenylindan-4',5-dicarboxylate) fractions were examined, and the molecular weights, obtained by using the hydrodynamic volume concept, were in satisfactory agreement with the light-scattering data for both chromatographic systems. Corrections for axial dispersion, for the polyester fractions and polystyrene samples, were probably equivalent in these two chromatographic systems.

INTRODUCTION

In the course of our studies, a chromatograph for exclusion chromatography has been developed which uses controlled-porosity glass (CPG) as the stationary phase. It seems appropriate to compare the performance of the CPG system for molecular weight distribution analyses with a commercial chromatograph that uses Styragel as the stationary phase. The comparison of controlled porosity glasses and Styragel for performing molecular weight distribution (MWD) analyses has been intentionally conducted under nonidentical chromatographic conditions. The primary objective of this work was to establish a reference for subsequent reports that will describe the use of these porous glasses in aqueous media. This present study may enable others to refine our apparatus and further demonstrate the utility of controlled porosity glasses for performing MWD analyses.

Measurements of the molecular weight distribution (MWD) of synthetic hydrophobic polymers are most commonly performed by gel permeation chromatography (GPC). This form of exclusion chromatography uses a porous, "inert" support consisting of polystyrene crosslinked to varying degrees with divinylbenzene, as described by Moore et al.¹⁻³ The advantageous physical and mechanical properties of porous glasses have prompted investigations of the pore structure⁴ and suitability of these materials for GPC applications.⁵⁻⁷ Detrimental solute-substrate interactions, i.e., adsorption effects, can be diminished or removed by a surface treatment of the glass.⁸ These deactivated substrates are

useful for high-speed (reduced analysis time) GPC.^{9,10} Pacco,¹¹ as well as Kelley and Billmeyer,^{12,13} has shown that MWD data can be obtained using porous glass.

The performance of Styragel and CPG-10 column-packing materials has been evaluated under conditions of similar chromatographic resolution. One might expect^{7,8,11} that comparable separations could be achieved through the use of column sets of porous glass longer than those commonly used in Styragel systems. The present evaluation used porous glass and Styragel column sets 25 and 16 ft long, respectively, each chromatographic system using columns of overlapping pore sizes. Axial dispersion effects were expected^{12,13} to be appreciably larger than those encountered in Styragel systems. It was hoped that a large decrease in column cross-sectional area (while keeping the particle size small) and attempting to minimize the broadening effects outside the columns (fittings, tubing, etc.) would diminish these dispersion effects to an acceptable level.

It will be shown that the molecular weight range covered by the porous glass system is not as great as that typically obtained in Styragel systems. (The molecular weight range of the CPG system has been limited to the region in which hydrophilic calibrants, to be described in a subsequent report, are available.) In principle, an equivalent molecular weight range for study can be achieved in the CPG system by further increasing the column length with glasses of the appropriate pore size. The operational variables (eluent flow rate, sample size, etc.) influencing the calibration of the CPG-10 system are reported. A comparison of molecular weight determinations using CPG-10 and Styragel is presented.

EXPERIMENTAL

The Styragel studies were performed with a Waters Associates M-100 chromatograph employing tetrahydrofuran (THF) as the solvent at a flow rate of 1.0 ± 0.1 ml/min. Four Styragel columns of 10^6 -, 10^5 -, 10^4 -, and 10^3 -Å (nominal) porosity were used in series, each being 4 ft long with 0.30-in. i.d.

The porous glass system is shown schematically in Figure 1. Five columns, 5 ft \times 0.17-in. i.d., were packed (dry) with CPG-10 glasses of 1250-, 700-, 500-, 190-, and 75-Å porosity as received by the manufacturer. A Waters Associates differential refractometer (R 401) was used as a detector, and the retention

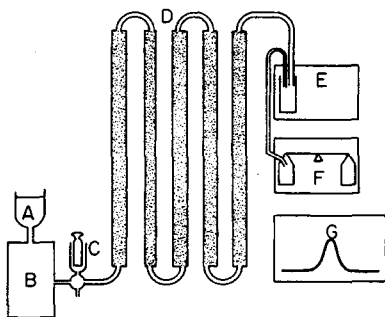


Fig. 1. Schematic arrangement of the CPG-10 chromatographic system: A, eluent supply; B, positive displacement pump; C, sample injection valve; D, five 5' \times 0.017" i.d. columns; E, differential refractometer; F, automatic balance; G, strip-chart recorder.

volume, V_R , was monitored by an automatic balance (F). THF was the eluent; its flow rate was 0.70 ± 0.03 ml/min.

All measurements were performed at ambient temperature, and 1 ml of sample was injected from a standard 2-ml sample valve. Polymer concentrations were 0.3% (w/v) or less in all cases. Calibration curves were obtained with commercially available polystyrene samples (Pressure Chemical Co., Pittsburgh, Pa.) of narrow MWD.

The poly(methyl methacrylate) (PMMA) samples were obtained from the "polymer bank" administered by the Brooklyn Polytechnic Institute. Poly(vinyl chloride) (PVC) samples were obtained commercially (Pressure Chemical Co.). The POLA polyester fractions [poly(4,4'-isopropylidenediphenylene 1,1,3-trimethyl-3-phenylindan-4',5-dicarboxylate)] were prepared in our laboratory by the column elution-temperature gradient technique.^{14,15}

RESULTS AND DISCUSSION

Operational Variables

The reproducibility of the peak retention volumes for the polystyrene standards is shown in Table I for the porous glass-chromatographic system. The data were collected daily by injecting blends of four or five components. Several samples were examined individually (shown in parentheses in Table I); they gave the same retention volumes as they did when they were examined as a blend. The data in Table I demonstrate that the retention volumes of these materials can be measured reliably to ± 0.2 ml. Our past experience indicates that this reproducibility is slightly poorer than that obtained in comparable studies using Styragel (± 0.1 ml). This difference is due, at least in part, to the instruments used to monitor the retention volume, the siphon system being somewhat more reliable than the balance arrangement used in this study.

The dependence of retention volume on sample size was evaluated by injecting a five-component blend of polystyrene standards at several concentrations. The results, Figure 2, demonstrate that the concentration dependence of V_R is equivalent to that reported for similar studies using Styragel.^{3,16} One might have anticipated a larger effect using the porous glass system, since the smaller-diameter columns used could exhibit a greater tendency to become overloaded.¹⁷⁻¹⁹

TABLE I
Reproducibility of V_R for Porous Glass Chromatograph

Polystyrene standard $M_w \times 10^{-4}$	V_R , ml				
	Run 1	Run 2	Run 3	Run 4	Average
210.0	44.0	43.9	43.8	43.9	43.9 \pm 0.1
68.0	48.0		(48.2)		
50.0	49.6	49.8	49.4	49.6	49.6 \pm 0.2
20.0	55.5		(55.3)		
11.0	58.6	59.0	58.6	58.8	58.8 \pm 0.2
3.7	66.4				
2.0	70.5	70.3	70.2	69.7	70.2 \pm 0.3
0.4	79.2		(79.0)		
0.2	80.3	80.6	80.4	79.9	80.3 \pm 0.3

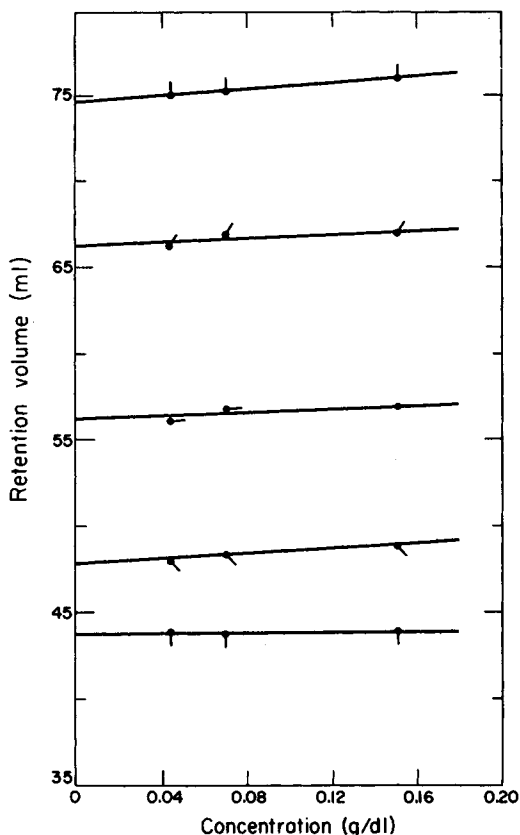


Fig. 2. Dependence of V_R of the polystyrene calibrants on polymer concentration for the porous glass system: ▽, $M = 2 \times 10^3$; △, $M = 2 \times 10^4$; ●, $M = 1.1 \times 10^5$; ◄, $M = 5 \times 10^5$; ▲, $M = 2.1 \times 10^6$.

The variation of V_R with volumetric flow rate at constant concentration (0.14 g/dl) for the polystyrene blend is shown in Figure 3. A negligible dependence of V_R on flow rate is observed in these porous glass studies. Considerably greater effects have been reported for Styragel,^{3,16} a result that we attribute to variations in the amount of solvent transferred by the siphon as a function of eluent flow rate. Our studies using a 1-ml siphon have shown that the volume of THF delivered by the siphon increases linearly by 2.5% over the range of flow rates 0.2 to 1.8 ml/min. Corrections of V_R due to siphon effects show a small dependence of peak elution volume on volumetric flow rate in these Styragel studies (Fig. 4). The porous glass and Styragel systems now show a negligible dependence of V_R on flow rate in the range routinely used here.

The respective calibration curves for the porous glass and Styragel chromatographs are shown in Figure 5. The large difference in the elution volumes between the two calibration curves is due, primarily, to the significant differences in cross-sectional areas of the columns used in the two chromatographic systems. This factor of ~ 4 is somewhat compensated for by the longer column length used in the study of porous glass. Several polystyrene calibrants were examined in each system, and the calculated molecular weight values are in reasonable agree-

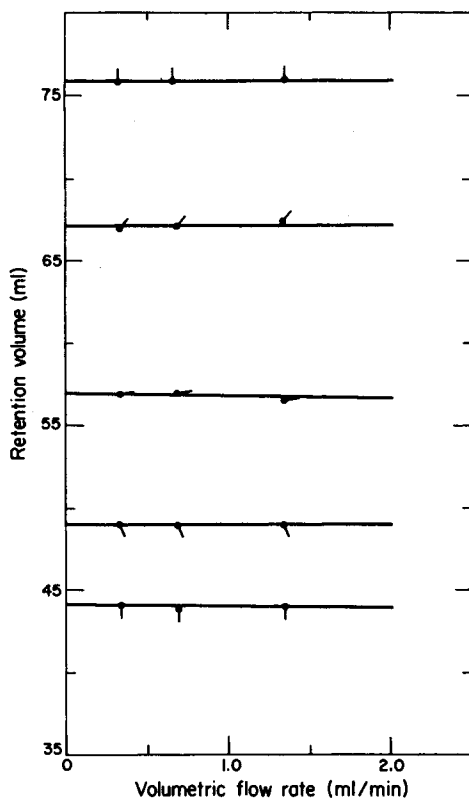


Fig. 3. Variation of V_R of the polystyrene calibrants with volumetric flow rate for the porous glass chromatograph. Symbols as in Figure 2.

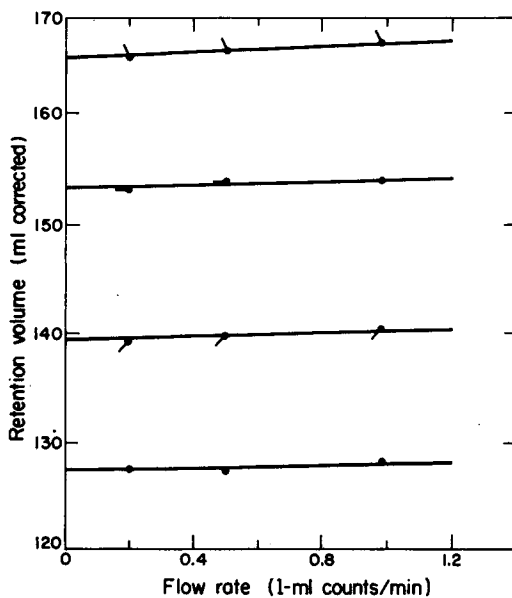


Fig. 4. Dependence of V_R , corrected for siphon effects, on volumetric flow rate for the Styragel system: \searrow , $M = 5 \times 10^3$; \dashrightarrow , $M = 2 \times 10^4$; \nearrow , $M = 9.7 \times 10^4$; \bullet , $M = 4.1 \times 10^6$.

TABLE II
 GPC Results for Polystyrene Standards

Sample ^a	$\bar{M}_w \times 10^{-4}$			\bar{M}_w/\bar{M}_n	
	Reported ^a	Styragel	CPG-10	Styragel	CPG-10
PS 11a	0.46	0.45	0.44	1.10	1.6
PS 8a	1.02	0.98	0.98	1.14	1.5
PS 1c	20.0	19.0	17.8	1.13	1.16
PS 13a	67.0	66.4	62.0	1.26	1.23

^a Pressure Chemical Co., Pittsburgh, Penna.

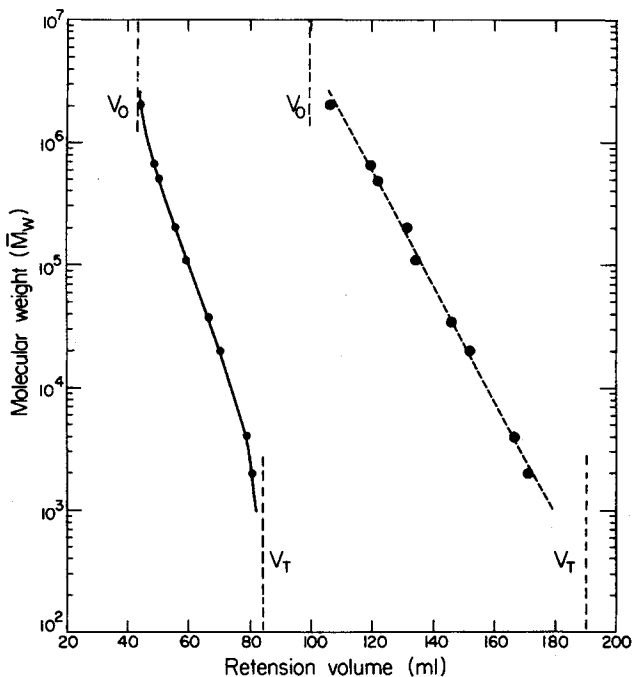


Fig. 5. Calibration curves of log molecular weight vs. V_R for the two chromatographic systems: (—) porous glass; (---) Styragel.

ment with the reported values as shown in Table II. These data demonstrate that the data reduction analyses, i.e., recorder trace to molecular weight values, are conducted properly. The anomalous values of \bar{M}_w/\bar{M}_n of polystyrenes 11a and 8a obtained with the porous glass system reflect the practical difficulties encountered when the calibration curve changes very rapidly with retention volume, i.e., $V_R \sim 75-85$ in Figure 5. Otherwise, the estimates of the polydispersity \bar{M}_w/\bar{M}_n shown in Table II are in good agreement, although corrections for axial dispersion have not been applied in either study. It is important to note that the polydispersities of these polystyrene standards are equivalent as measured by these two chromatographic systems. This finding was unexpected in view of previous studies¹³ and the results that will be presented show a marked difference in efficiency between these two chromatographic systems.

Resolution and Efficiency

The terms resolution, $R_{1,2}$, separation factor, S , and height equivalent to a theoretical plate, H , have been described in detail elsewhere^{20,21} and will be used as defined by Bly et al.²²

The efficiency of a chromatographic system can be estimated by computing H , although Bly has shown that such computations using polystyrene samples of narrow MWD are not appropriate for quantitatively describing the efficiency in GPC separation.²³

Polystyrene samples of 6.7×10^5 and 1×10^4 molecular weight (nominal) were used to evaluate $R_{1,2}$ and S . It was found that $R_{1,2} = 2.5$ and 2.6 , whereas $S = 0.11$ and 0.08 for the porous glass and Styragel systems, respectively, indicating that the two systems do have comparable resolution for these samples. Extending the resolution of the CPG system to cover a wider range of molecular weights might be accomplished by further increases in the column length and pore size distribution of the CPG system. This would necessarily increase the analysis time for these MWD studies.

Calculations of H using low molecular weight compounds, e.g., benzene or water, yield values of 0.4 mm and 3–5 mm for the Styragel and porous glass systems, respectively. Studies by Otocka¹⁰ and others^{9,13} have demonstrated the importance of the particle size and particle size distribution on H . This factor should be considered when the parameters in the chromatographic system are being optimized and accounts in part for the large difference found here, since the porous glass particles were relatively large, i.e., 75–125 μm in diameter.

Another comparison of the polystyrene calibration curves for the Styragel and porous glass systems can be made despite the differences in the number of columns used and their respective diameters. The calibration curves can be normalized using the term K_{av} , as defined by Laurent and Killander²⁴:

$$K_{av} = \frac{V_R - V_0}{V_t - V_0} \quad (1)$$

where V_R = retention volume of the solute molecule, V_0 = interstitial volume (measured with a very high molecular weight polystyrene sample), and V_t = total volume of the gel matrix (measured with benzene). We have chosen this expression since K_{av} is independent of the geometry and packing density of the column; also, the other parameters are easily and reliably measured.²⁵

The comparison of the Styragel and porous glass calibration curves is shown in Figure 6, in which the logarithm of the molecular weight has been plotted as a function of K_{av} . Since the calibration curves have now been normalized through the use of the parameter K_{av} , it is apparent from Figure 6 that the porous glass system can separate molecules of similar molecular weight over a wider range of K_{av} than that which can be achieved in the Styragel system.

Molecular Weight Determinations

Several methods that use GPC and viscometry for the determination of the weight-average molecular weight, \bar{M}_w , have been evaluated recently.²⁶ The simplified hydrodynamic method²⁷ of Funt and Hornof²⁸ was found to give accurate molecular weights for several types of polymers. A number of these materials were examined with the CPG-10 chromatograph. A comparison of the

TABLE III
Molecular Weight Determinations by GPC and Viscometry

Sample	$\bar{M}_w \times 10^{-4}$			\bar{M}_w/\bar{M}_n	
	Reported	Styragel	CPG-10	Styragel	CPG-10
PMMA 6038	4.92 ^a	4.26	4.96	1.9	2.2
PMMA 6036	11.5 ^a	11.8	10.8	1.9	2.1
PMMA 6041	26.7 ^a	25.0	22.8	1.8	2.1
PVC II	6.86 ^b	6.88	7.08	2.1	2.0
PVC III	11.8 ^b	10.8	11.6	1.9	1.8
PVC IV	13.2 ^b	12.7	15.1	1.9	1.8
POLA 1	2.60 ^c	2.32	2.52	1.15	1.15
POLA 3	6.80 ^c	6.78	7.83	1.2	1.3
POLA 6	21.8 ^c	20.3	18.8	1.3	1.4

^a Rohm and Haas Co., Philadelphia, Penna.

^b Arro Laboratories, Joliet, Ill.

^c Reference 15.

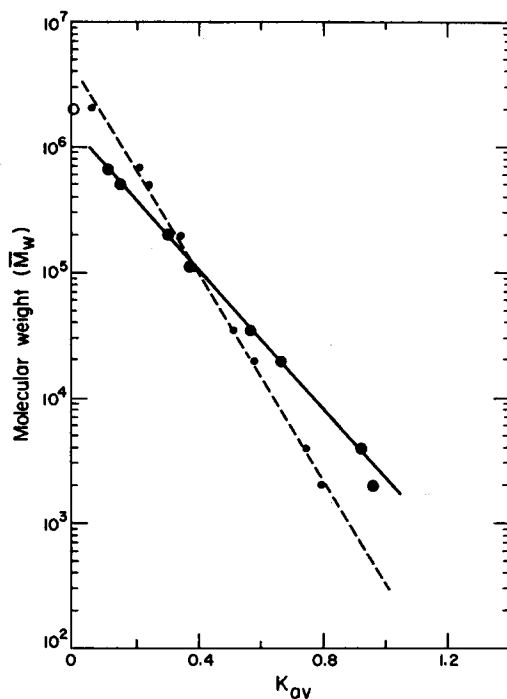


Fig. 6. Comparison of CPG-10 and Styragel calibration curves. $\log \bar{M}_w$ vs. the reduced retention volume parameter, K_{ov} : (—) porous glass; (---) Styragel.

calculated molecular weights with those determined by similar Styragel investigations is shown in Table III.

The porous glass and Styragel studies yield \bar{M}_w values that are in reasonable agreement with those obtained by light scattering. The Styragel results are in slightly better agreement with the light-scattering data (± 5 – 10%). However, it is important that estimates of \bar{M}_w/\bar{M}_n using porous glass and Styragel are in good agreement, particularly in the case of materials of narrow MWD, i.e., poly-

styrene and POLA samples. The CPG-10 porous glass system is nearly as effective in estimating \bar{M}_w and \bar{M}_w/\bar{M}_n as the Styragel system. The data in Tables II and III suggest that the corrections for axial dispersion are similar for the two chromatographic systems.

CONCLUSIONS

The dependence of V_R on sample size and the variation of V_R with flow rate in the CPG-10 system are equivalent to those observed when Styragel is used as support. A CPG-10 system has been designed to yield resolution comparable to that obtained with Styragel over a limited range of molecular weight. The lower column efficiency observed in the porous glass system could be improved by using smaller particles of more uniform size.¹⁰ The universal calibration concept²⁸ can be used with CPG-10 porous glass studies to give molecular weight values in reasonable agreement with those obtained by light scattering. While better agreement was achieved in similar studies using Styragel, CPG-10 can be used effectively to determine \bar{M}_w and MWD. Cooper et al.^{29,30} have reached similar conclusions in their studies using Porasil. (Porasil is available from Waters Assoc., Framingham, Mass.) The porous glass system might be better than Styragel for the study of semicrystalline polymers that generally require chromatographic operating temperatures higher than 100°C. Also, MWD measurements may now be performed using solvents that are not compatible with Styragel.

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