CHROM. 3389

EVALUATING DISPERSION IN GEL PERMEATION CHROMATOGRAPHY

II. THE INJECTION–DETECTION SYSTEM*,**

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(Received December 29th, 1967)

SUMMARY

The standard injection–detection system in the Waters Gel Permeation Chromatograph can lead to significant contributions to peak broadening and retention time when single columns or short columns are used in the gel permeation chromatography of macromolecular solutes. Use of a micro refractometer cell and elimination of all excess tubing, including the heat exchanger, led to significant reductions in contributions to peak width at half-height (0.065 to 0.019 in. at standard recorder-chart speed and a flow rate of 1 cc/min) and to retention time (0.166 to 0.040 in.) from the injection–detection system. Anomalies in the chromatograms such as spurious peaks and severe tailing were also eliminated. HETP data as a function of flow rate agreed with theory (Part I of this series); HETP for a nonpermeating macromolecular solute was always greater than that for a permeating low-molecular-weight solute (1.4×10^{-3} *vs.* 0.8×10^{-3} ft. at 1 cc/min). Values of D/Ud_p were approximately constant near 5.0 for Reynolds' numbers between 0.03 and 0.3.

INTRODUCTION

Gel permeation chromatography (GPC), as introduced by MOORE[2], has become in a very short time one of the most powerful analytical tools available to polymer chemists. A wide variety of polymer types covering a molecular-weight range from that of monomers to several million has been successfully analyzed. GPC offers a convenient method for on-stream analysis in commercial production units. The introduction of a preparative-scale unit[3] should make available gram-size quantities of narrow-distribution polymer fractions for research purposes. Detailed information on the molecular-weight distribution of a sample, which previously would have taken weeks to obtain, can usually be obtained by GPC in a matter of hours. Not since the introduction of the light-scattering method of DEBYE[4] has a new technique had such an impact on polymer science in the first few years of its existence.

* Presented in part at the *4th International Seminar on Gel Permeation Chromatography, Miami Beach, Fla., May 22–24, 1967*, and at the *154th National Meeting, American Chemical Society, Chicago, Ill., Sept. 10–14, 1967* (*Polymer Preprints, Am. Chem. Soc. Div. Polymer Chem.*, 8, No. 2 (1967) 1259).

** For Part I of this series, see ref. 1.

The GPC process is basically a liquid–solid chromatographic method in which the separation is carried out by passing a dilute polymer solution through a column containing a rigid, but highly porous, crosslinked polystyrene gel packing. The solute molecules permeate the gel according to their volume in solution[5–7]. Since the higher-molecular-size species have less pore volume to permeate, they are eluted first. As is true with all chromatographic processes, dispersion during flow through the packed column and associated tubing and detector causes a spread in retention times about the mean. This peak spreading is a major factor limiting the accuracy of GPC analyses of polydisperse polymers.

One great advantage of GPC is its speed compared to that of other characterization methods. As GPC becomes adapted for on-stream analysis in commercial production units, speed of analysis will become increasingly important. For shorter column lengths, corrections for extra-column broadening can also become important. To evaluate accurately dispersion occurring within the column, and to apply the theory presented in Part I of this series[1], it is necessary to apply corrections for the finite width of the injection–detection pulse and for broadening arising from extra-column contributions. In this paper, the nature and importance of these contributions is examined. The injection and detection systems of the Waters Gel Permeation Chromatograph were studied without a column and with both standard and micro refractometer cells. After proper correction for extra-column effects, data were obtained and are presented for the dispersion of both permeating and nonpermeating samples, determined as a function of flow rate using a single column.

EXPERIMENTAL

A Waters Associates (Framingham, Mass.) Model 100 Gel Permeation Chromatograph was used with tetrahydrofuran as the solvent at room temperature. Recorder chart speeds up to 60 in./h were used to allow accurate measurement of peak widths at half-height. The instrument was used without a column to study the characteristics of the injection–detection system; both a standard refractometer cell (volume 70 μl) and a micro cell (volume 10 μl) were used, with a 1/16-in. null glass. Dispersion was studied with a column designated by Waters as 250 Å. The minimum molecular weight of polystyrene eluted at the interstitial volume of this column (21.94 cc) should be approximately 10,000. As determined with *o*-dichlorobenzene (ODCB) at a flow rate of 1 cc/min, this column had approximately 1200 plates/ft. In the dispersion studies, ODCB was used as a permeating solute and a narrow-distribution polystyrene (PS) (Pressure Chemicals Co., Pittsburgh, Pa.; $\bar{M}_w$ = 160,000; $\bar{M}_w/\bar{M}_n$ = 1.06) as a nonpermeating macromolecular solute.

Modifications made to the Chromatograph during study of the injection–detection system are described in the next section.

RESULTS

The injection–detection system

To evaluate the extra-column contributions to peak broadening, samples were injected through the sample loop directly into the refractometer cell, with no column in the system. The sample loop furnished with the instrument, consisting of 81.18 in.

of 1/16-in. O.D. hypodermic tubing, contains approximately 2 cc. It is common practice to inject from half to all of this material (injection times of 1–2 min at a flow rate of 1 cc/min) when using multiple-column assemblies for analytical purposes. Solute concentrations normally range from 0.25–1 wt. %.

When operating under these conditions with a 0.5 % PS solution, we observed considerable tailing (asymmetry) in the injection–detection pattern with no column in the system. As the flow rate was lowered, a double peak was produced. Injection–detection patterns for ODCB showed considerably less tailing. Similar effects have been noted by others[8–10].

To minimize these effects, we made several changes in the chromatograph flow system, *viz.*

(1) The size of the injection loop was reduced from 81.18 in. to 5.18 in. of 1/16-in. O.D. hypodermic tubing, corresponding to a volume of 0.13 cc.

(2) All excess tubing (5–6 ft.) between the sample-injection loop and the refractometer was eliminated.

(3) The sample and reference inlets to the refractometer were interchanged because we observed that this improved the pattern.

These changes reduced peak broadening in the injection–detection system considerably but improved the tailing only slightly. As shown in Fig. 1, the peaks appeared to approach symmetrical (Gaussian) shape at high flow rates, but showed considerable tailing at low flow rates and severe anomalies in an intermediate region

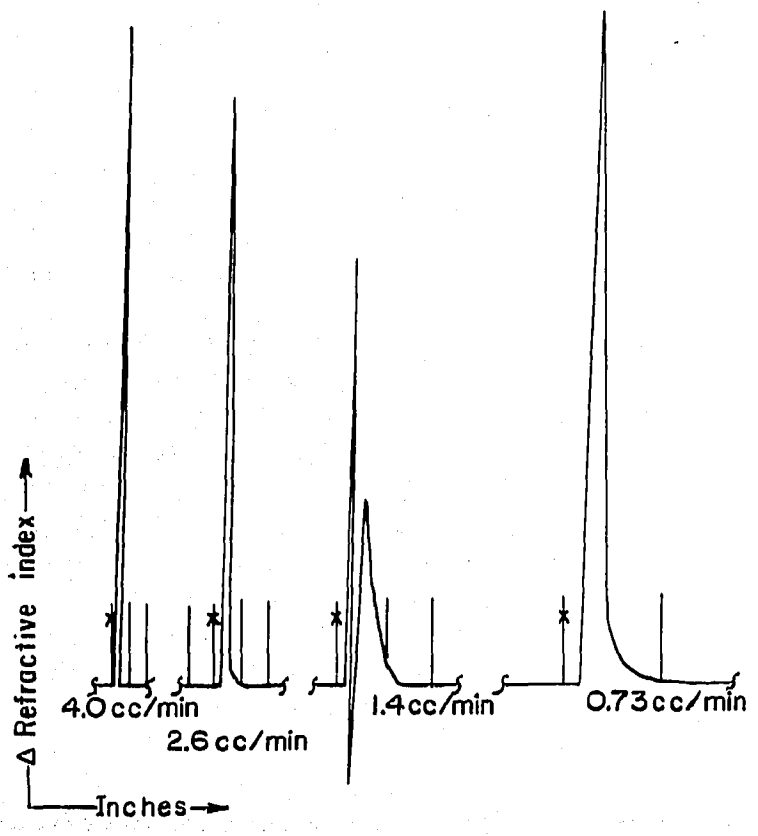

Fig. 1. Injection–detection patterns with standard refractometer cell, at the indicated flow rates. Flow system modified as described in the text; 0.1 % PS in THF at room temperature; standard recorder-chart speed: 6 in./h.

(1.1–1.6 cc/min). With ODCB, however, peaks were nearly symmetrical at all flow rates (Fig. 2).

To insure that these phenomena were not peculiar characteristics of our own instrument, we repeated the experiments on another chromatograph belonging to Waters Associates, with essentially the same results. No appreciable tailing was observed with ODCB as solute at any flow rate, whereas solutions of PS showed both tailing and other anomalies, including a sharp negative deflection just before the peak, a characteristic also seen by OSTERHOUDT AND RAY[8].

It seems probable, particularly in view of the difference in behavior between the two sides of the refractometer cell, that these anomalies result from the flow pattern and mixing characteristics in the cell. We believe that at high flow rates, sufficient mixing occurs in the cell to prevent severe distortion of the peak. At lower flow rates with the more viscous solutions of polymeric solutes, poor mixing causes the tailing and other anomalies. With ODCB as the solute, these are not observed because of its lower viscosity and higher diffusivity.

Since the results of these flow disturbances can still be seen with single columns in the instrument, it is essential to minimize them and correct for them if dispersion in the column is to be studied accurately. (It should be noted that these flow disturbances are usually insignificant when the Waters Instrument is used with more than two columns.) Following a suggestion of MOORE AND HENDRICKSON[11], we explored the use of a micro refractometer cell as a means of reducing both the breadth and the tailing of the injection–detection peak.

The Waters micro refractometer cell has an internal volume of 10 μl compared to 70 μl for the standard cell, and a flow pattern more nearly duplicating that in capillary tubing. In addition to installing such a cell, we removed several feet of capillary tubing included in a heat-exchanger block located between the columns and the refractometer. While this heat exchanger is required to achieve temperature

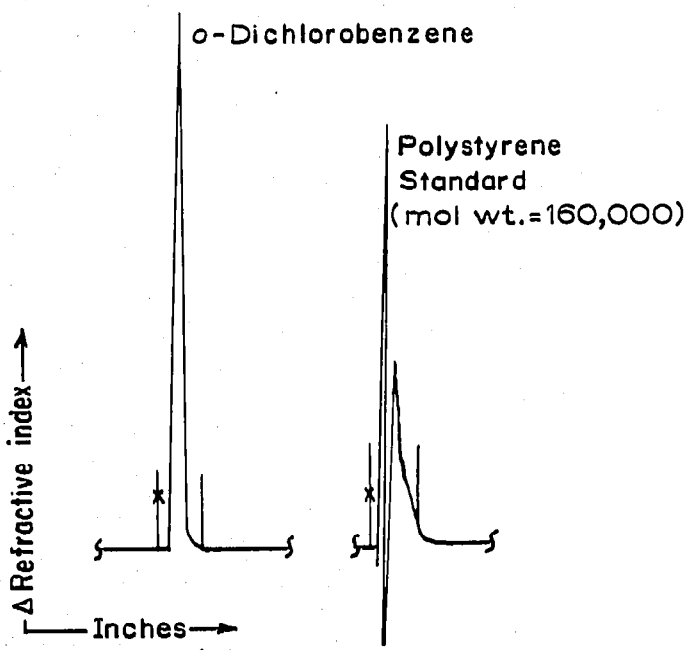

Fig. 2. Injection–detection patterns with standard refractometer cell. Experimental conditions as in Fig. 1; samples: 0.5% ODCB and 0.1% PS; flow rate: 1.45 cc/min.

equilibration in the sample and reference streams when operating at elevated temperature, it has proved unnecessary in our work at room temperature. We passed the liquid stream into the micro refractometer cell from either the column or our shortened injection loop with the shortest possible length of capillary tubing. Where necessary, the location of the injection valve and loop was changed to keep tubing lines no more than a few inches long.

Injection patterns obtained with the chromatograph modified in this way are shown in Fig. 3. It can be seen that tailing was greatly reduced, and other anomalies eliminated, over the entire range of flow rates used in this work. In other experiments, we found that the micro cell gave approximately three times the sensitivity and one half the peak width obtained with the standard cell.

Column end-fittings

The effect of column end-fittings on peak broadening was assessed separate from that of the remainder of the system by preparing a "column" by joining two such fittings together with a minimum of space between them. The use of these end-fittings increased peak broadening slightly. More experiments are planned in this area since we have not yet determined whether the flow pattern is identical with that at the ends of a packed column.

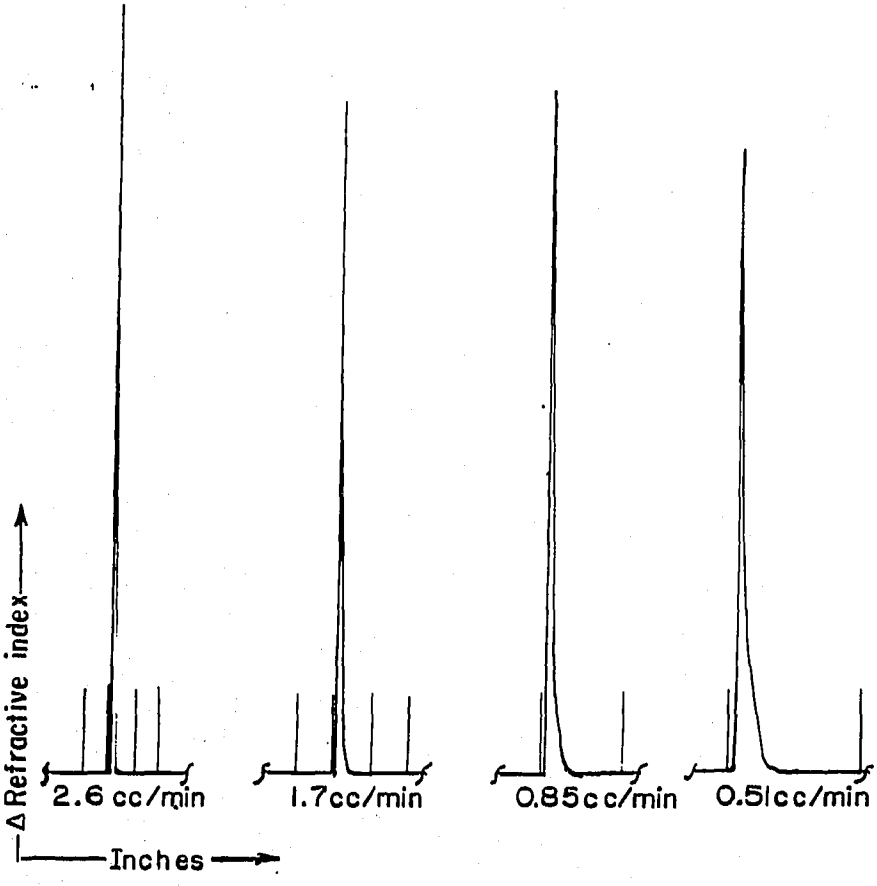

Fig. 3. Injection–detection patterns with micro refractometer cell, at the indicated flow rates. Flow system modified as described in the text; 0.1 % PS in THF at room temperature; standard recorder-chart speed: 6 in./h.

Corrections for extra-column effects

Fig. 4 shows the variation with reciprocal flow rate of the width at half-height of the injection–detection pulse. The relationships are linear within experimental error, with the broadening greater for ODCB than for PS, and the end-fittings increasing the broadening slightly.

The average residence time of the pattern was also found to increase linearly with reciprocal flow rate, as shown in Fig. 5. The end-fittings and associated tubing almost doubled the residence time at a given flow rate for PS. We do not yet understand why slightly longer residence times appear to be observed for ODCB.

From the curves of Figs. 4 and 5, accurate corrections to any chromatogram can be made for residence time and peak broadening associated with the injection–detection system.

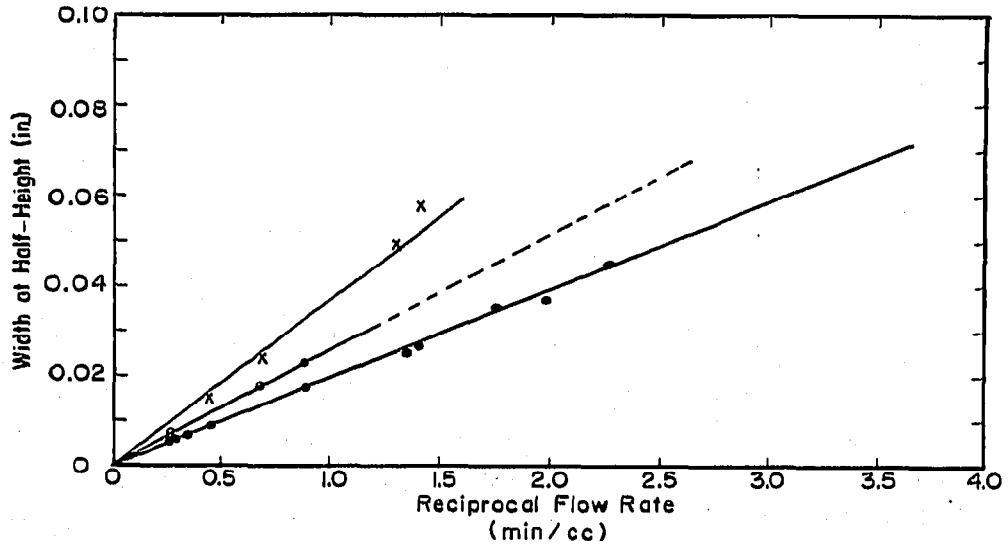

Fig. 4. Extra-column contributions to peak broadening. (●) 0.1 % PS, end-fittings not included; (○) 0.1 % PS, end-fittings included; (×) 0.5 % ODCB, end-fittings included. Conditions same as in Fig. 3.

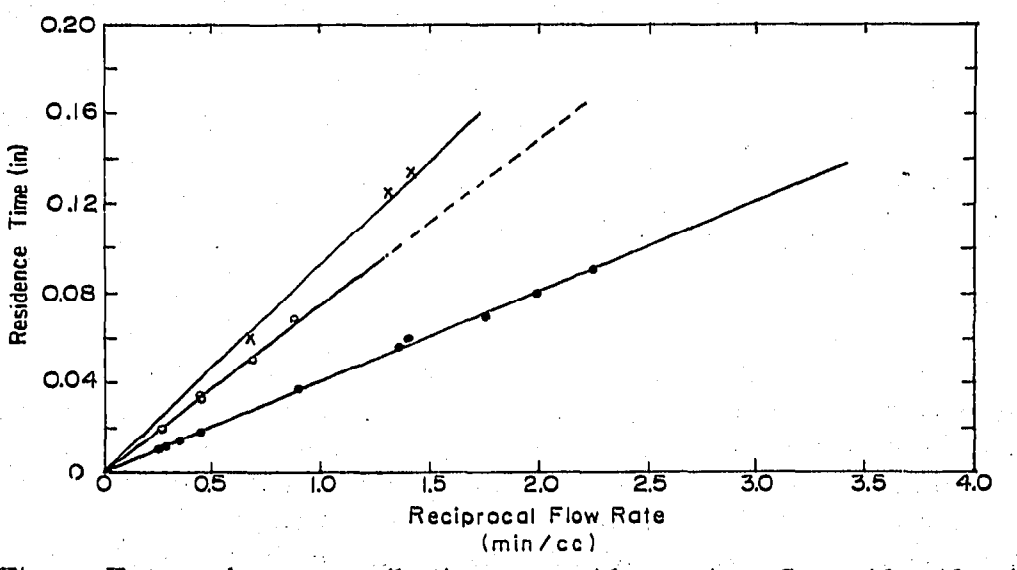

Fig. 5. Extra-column contributions to residence time. Same identifications as for Fig. 4; other conditions same as in Fig. 3.

Dispersion data

Dispersion data were obtained with a single 250 Å column, using ODCB as a permeating solute and PS as a nonpermeating macromolecular solute. At each flow rate, retention time and peak width at half-height were corrected for extra-column effects using the data of Figs. 4 and 5. Values of height equivalent to a theoretical plate (HETP) were calculated from the average residence time (location of peak), peak breadth and column length as described in Part I[1]. These results are given in Fig. 6.

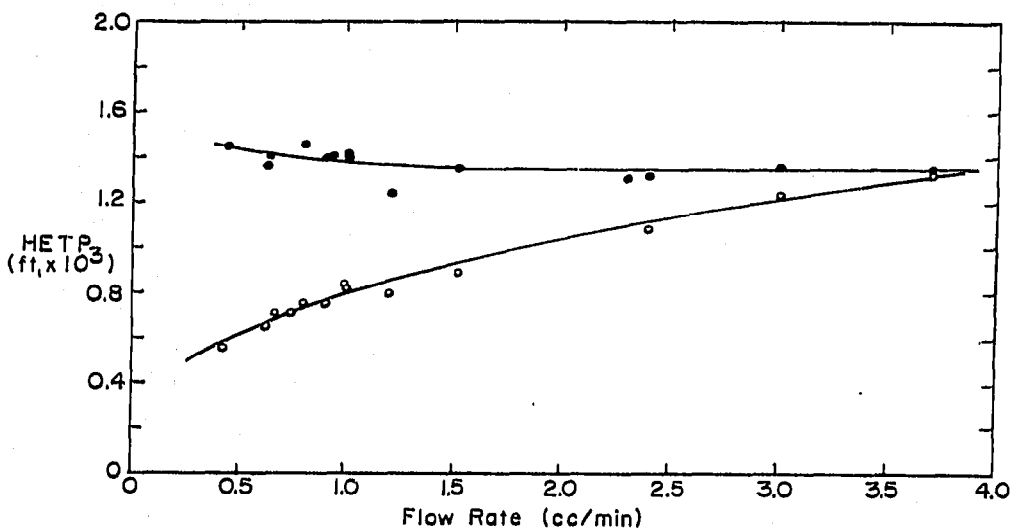

Fig. 6. Effect of flow rate on HETP for permeating (○, 0.5 % ODCB) and nonpermeating (●, 0.1 % PS) solutes. Single 250 Å column; other conditions same as in Fig. 3.

For the nonpermeating solute PS, the effective longitudinal-dispersion coefficient was calculated from the relation (Part I) HETP $= 2D/U$ where U is the flow rate. Numerical values leading to this calculation are given in Table I. It can be seen from Table I that, with the modified flow system, corrections for extra-column peak broadening were indeed negligible.

The dispersion characteristics of the high-efficiency column used in these experiments can be illustrated by plotting D/Ud_p (the inverse of the Peclet number), where d_p is the particle diameter of the column-packing gel, against the Reynolds number. Such a plot is shown in Fig. 7; the value of d_p was taken as 40 μ, which is in the range of the actual particle size. The region designated as typical for liquid axial dispersion was taken from LEVENSPIEL's work[12] on the axial dispersion of solutes in packed beds of uniform-size spherical particles.

DISCUSSION

Our results show that accurate dispersion data can not be obtained from experiments with single GPC columns using a standard refractometer cell in the Waters Chromatograph and retaining the large amount of hypodermic tubing incorporated in the normal flow system. Flow anomalies associated with this injection–detection system were, however, virtually eliminated in our instrument by changing to a micro refractometer cell, minimizing the amount of tubing in the system (including elimination of the heat exchanger), using short injection times (5–10 sec at a

TABLE I

DISPERSION CHARACTERISTICS OF A MACROMOLECULAR SOLUTE (PS) WITH A 250 Å COLUMN[a]

Flow rate (*cc/min*)	*Residence time*[b] (*in.*)	*Variance*[b] (*in.*$^2 \times 10^4$)	*HETP* (*ft.* $\times 10^3$)		*U* (*ft./min*)	*Reynolds number*[c]	*D*[d] (*ft.*^{2}/*min* $\times 10^4$)
			Uncorrected	*Corrected*			
0.43	5.015	91.186	1.46	1.45	0.080	0.029	0.580
0.62	3.476	41.300	1.38	1.37	0.115	0.041	0.788
0.63	3.425	41.413	1.42	1.41	0.116	0.042	0.818
0.79	2.714	26.937	1.47	1.46	0.147	0.053	1.073
0.90	2.439	20.856	1.41	1.40	0.164	0.059	1.148
0.92	2.320	19.002	1.42	1.41	0.172	0.062	1.212
0.98	2.204	17.235	1.43	1.42	0.181	0.065	1.285
1.00	2.156	16.175	1.40	1.39	0.186	0.067	1.292
1.20	1.771	9.640	1.24	1.23	0.226	0.081	1.390
1.52	1.418	6.820	1.36	1.35	0.282	0.101	1.903
2.29	0.922	2.758	1.30	1.30	0.434	0.155	2.821
2.39	0.886	2.617	1.34	1.33	0.451	0.161	2.999
2.99	0.700	1.662	1.35	1.35	0.571	0.204	3.854
3.68	0.565	1.076	1.35	1.34	0.708	0.253	4.743

[a] 0.1% PS in THF at room temperature; micro refractometer cell and modified flow system as described in the text.
[b] Data corrected for extra-column effects and converted to standard recorder-chart speed.
[c] Calculated assuming $d_p = 40\ \mu$, solution density 0.89, solution viscosity 0.50 cP.
[d] Calculated assuming $d_p = 40\ \mu$.

flow rate of 1 cc/min), and reducing solute concentration. For example, the injection–detection system gave rise to a residence time of 0.166 in. and a peak width at half-height of 0.065 in. for 0.1 % PS at 1 cc/min, using the standard refractometer cell and normal flow system; these figures were reduced to 0.040 in. and 0.019 in., respectively, with the micro cell and other modifications to the system. The effect of column end-fittings will increase both sets of figures somewhat, but the comparison will not change.

Both the correction factor for width at half-height and that for residence time, plotted as functions of flow rate in Figs. 4 and 5, respectively, are greater for ODCB

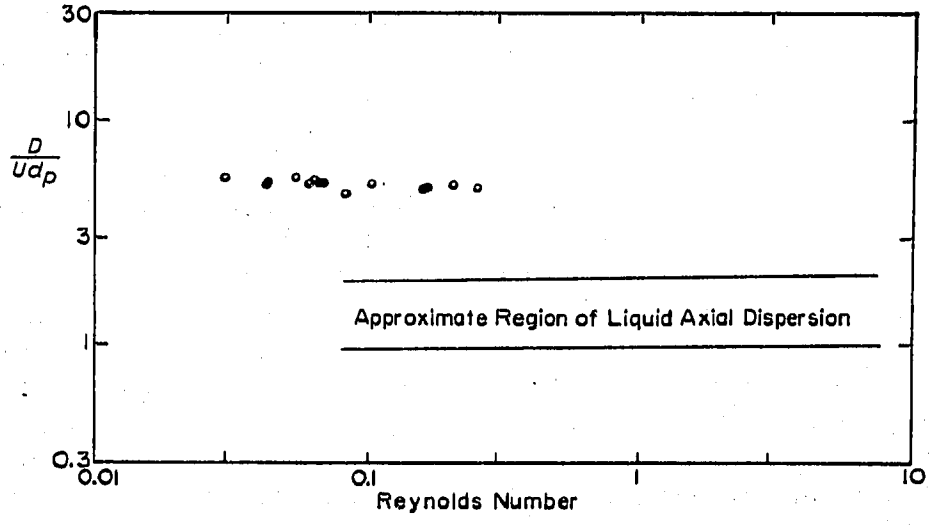

Fig. 7. D/Ud_p *vs.* Reynolds' number for nonpermeating solute (0.1 % PS); conditions as in Fig. 6.

than for PS. We believe this reflects the larger diffusivity of ODCB as compared to the macromolecular solute, giving rise to better radial mixing with ODCB. Most of the effect of column end-fittings, also shown in these figures, lies in the larger residence time associated with the fittings and additional tubing required; they contribute little to the peak broadening.

Dispersion data corrected for the injection–detection system characteristics, Fig. 6, show that HETP varies little with flow rate for a nonpermeating solute, as predicted by the theory presented in Part I[1]. For a permeating solute, ODCB, HETP drops with flow rate decrease in a concave-downward curve observed by others[7,9] using multiple-column assemblies and consistent with our approach[1] and that of GIDDINGS[13].

We believe it important to note that HETP for the nonpermeating solute is substantially higher than for the permeating solute at corresponding flow rates. This may result in part from the larger volume (interstitial plus pore volume) available to a permeating solute, and its correspondingly longer retention time. Even if the two values of HETP could be compared at equal elution volumes, however, we feel that a second effect, arising from the higher viscosity and lower diffusivity of a macromolecular solute, would be important.

The form of the relation for HETP,

$$\text{HETP} = (2D/U) + CU$$

where C describes the permeation effect, would suggest that HETP should be higher for a permeating solute if the two species had similar values of D. But, as indicated in Part I, D is made up of a molecular-diffusivity term (usually quite negligible in liquid systems), an eddy-diffusivity term, and a velocity-profile term. The velocity-profile term is inversely proportional to a radial-dispersion number, D_r. This number should be relatively large for low-molecular-weight solutes, and thus much larger for ODCB than for PS. In other words, velocity-profile effects are expected to be much more important for high polymers than for low-molecular-weight solutes in flow through packed beds.

Since D_r is large for ODCB, the second term in, and thus the value of, D is smaller for ODCB than for PS, leading to the observed lower values of HETP for this solute. This analysis assumes that the eddy-diffusivity term does not outweigh the velocity-profile term for macromolecular solutes. As yet we have no evidence on this point; its elucidation is one objective of our continuing studies.

From Fig. 7 it appears that values of D/Ud_p for a nonpermeating macromolecular solute are much higher than those predicted from classical dispersion studies in packed beds[12]. We believe this effect is also associated with the high values of D for macromolecular solutes, reflecting the importance of velocity-profile effects. Segregation effects may also be significant since the column-packing gels used in GPC contain a range of particle sizes. It seems likely that with more uniform column packings impermeable to nonviscous solutes such as ODCB, values of D/Ud_p would be more nearly within the region of liquid axial dispersion.

Clearly, the key to any successful treatment of gel permeation chromatography lies in separating and examining individually each process contributing to retention time and peak broadening. Any single column-efficiency parameter, such as HETP, of

necessity combines these individual contributions, and obscures an assessment of their relative importance.

ACKNOWLEDGEMENTS

This work is supported jointly by the Texas Division of The Dow Chemical Company and Waters Associates, Inc. It was carried out at Rensselaer's Materials Research Center Laboratory, a facility supported by the National Aeronautics and Space Agency. We are grateful to JOHN C. MOORE and GLENN W. JOHNSON for encouragement and many helpful discussions.

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