

Gel Permeation Chromatography: The Effect of Flow Rate on Efficiency. II*

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Synopsis

The effect of flow rate on efficiency in gel permeation chromatography has been examined over wide ranges of flow rate, i.e., linear flow velocities using columns of different diameters using both organic and inorganic packings. The results indicate a considerable increase in efficiency at flow velocities below 0.02 cm/s. No lower limit to this increase could be determined due to experimental difficulties. Between flow velocities of 0.02–0.2 cm/s little efficiency is lost.

INTRODUCTION

The effect of flow rate in gel permeation chromatography (GPC) has been investigated by a number of researchers. In most of the early studies, low molecular weight compounds were used to evaluate the effects of solvent flow rate on GPC column efficiency. The results of a number of studies have been reviewed by Kelley and Billmeyer.¹ Since then several articles have appeared which report GPC column efficiency measurements for high molecular weight polymers. Yau, Malone, and Suchan² examined flow rates in the range of 1–11 mL/m and found that for lower molecular weight polymers the height equivalent to a theoretical plate (HETP) was linearly proportional to flow rate and that for samples with molecular weights near the exclusion limit there was a maximum in the HETP-flow-rate plot. Gudzinowicz and Alden³ and Hendrickson⁴ found that, within a relatively narrow flow-rate range, HETP was proportional to flow rate. Little et al.⁵ found that over a wide flow-rate range a plot of log HETP vs. $\log U d_p/D_m^2$ was linear, where U is the flow velocity, d_p is the particle diameter of the packing material, and D_m is the diffusion coefficient of the sample.

Several equations have been derived to relate the HETP and flow rate.^{6–12} Among these, the Giddings equation is used most often to compare theory and experiment. This equation is strictly valid only for monodisperse samples. Use of samples with appreciable molecular weight dispersity will cause HETP values to be overestimated. The equation is

$$\text{HETP} = \frac{4 D_m}{3 R U} + \frac{1}{20} R(1 - R) \frac{d_p^2 U}{D_m} + \sum \left(\frac{1}{2 \lambda_i d_p} + \frac{D_m}{W_i d_p^2 U} \right)^{-1} \quad (1)$$

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where D_m is the diffusion coefficient of the sample in the solvent mobile phase, R is the ratio of zone velocity to mobile phase velocity, U is the Mobile phase velocity, d_p is the particle diameter of the packing material, and W_i and λ_i are geometrical factors of the column. The first term in the equation is the contribution from the longitudinal diffusion of the sample. This term becomes important at low flow rates. The overall effect would be that HETP decreases with decreasing flow rate, reaches a minimum, and then increases again with further decrease in flow rate. Sliemers et al.¹² estimated that the minimum HETP would occur at flow velocities of about 2×10^{-4} cm/s for a polystyrene sample with molecular weight 125,000 in a column packed with 50- μ m particle diameter packing material. So far no studies have investigated such low flow velocities. The lowest values reported are about 4.4×10^{-3} cm/s.¹³

Experimental results have been reported in which samples were injected into the column and the flow of the eluent was stopped for some time. The elution curve obtained upon resuming the solvent flow was found to be identical to the curve for a noninterrupted experiment.^{4,14} Thus it seemed pertinent to examine the effect of longitudinal diffusion on GPC efficiency by studying low flow rate behavior. This study was designed to determine the minimum HETP as a function of flow velocity and to see if any empirical or theoretical equation could correlate the effect of flow rate upon GPC efficiency.

EXPERIMENTAL

A Varian Aerograph Liquid Chromatograph Series 4100 was used in this study. The experimental conditions are listed in Table I. All the experiments were carried out at room temperature, and the polymers used were narrow molecular weight distribution (MWD) polystyrene standards (Pressure Chemical Co., Pittsburgh, Pa.)

TABLE I
Experimental Conditions for Flow Rate Studies Using Bio-Glas Columns

Packing materials	Bio-glas 500 Å, < 325 mesh
Detector	UV 254 nm
Solvent	Chloroform containing 1% ethanol as stabilizer
Column A	1 m × 0.46 cm I.D. stainless steel tube
Sample solution	Mixture of narrow MWD polystyrenes with the following values of \bar{M}_w : 411,000, 97,200, and 20,400
Sample concentration	3 mg/mL
Injector	DuPont syringe injector, Part No. 820069
Injection volume	5 μ L
Column B1	33.3 cm × 2.22 cm I.D. stainless steel tube
Sample solution	Mixture of narrow MWD polystyrenes with the following values of \bar{M}_w : 411,000, 97,200, and 20,400
Sample concentration	1.5 mg/mL for each polymer
Injector	Waters Associates six-port injection valve
Injection volume	0.13 ml
Column B2:	50 cm × 2.22 cm I.D. stainless steel tube
Sample solution	Mixture of narrow MWD polystyrenes with the following values of \bar{M}_w : 411,000, 97,200, and 20,400
Sample concentration	2 mg/mL for each polymer
Injector	Water Associates six-port injection valve
Injection volume	0.13 mL

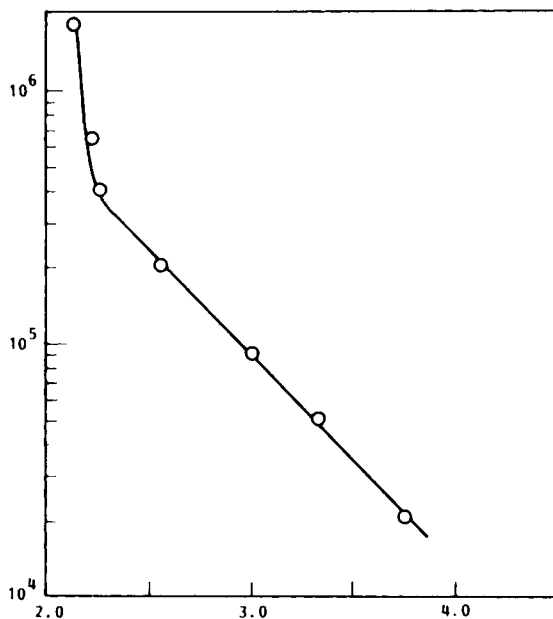


Fig. 1. Polystyrene calibration curve for 500-Å Bio-glas column A (1 m \times 0.47 cm ID) Established using chloroform, valid for all flow rates studied. Ordinate: polystyrene molecular weight \bar{M}_w . Abscissa: elution volume, counts.

The elution volume was measured by an automatic siphon. The discharge volume of the siphon was determined to be 2.742 mL. Because the flow rates are all below 40 mL/h, very little effect of flow rate on the siphon discharge volume was observed. Evaporation was prevented by using the method described by Yau et al.¹⁵

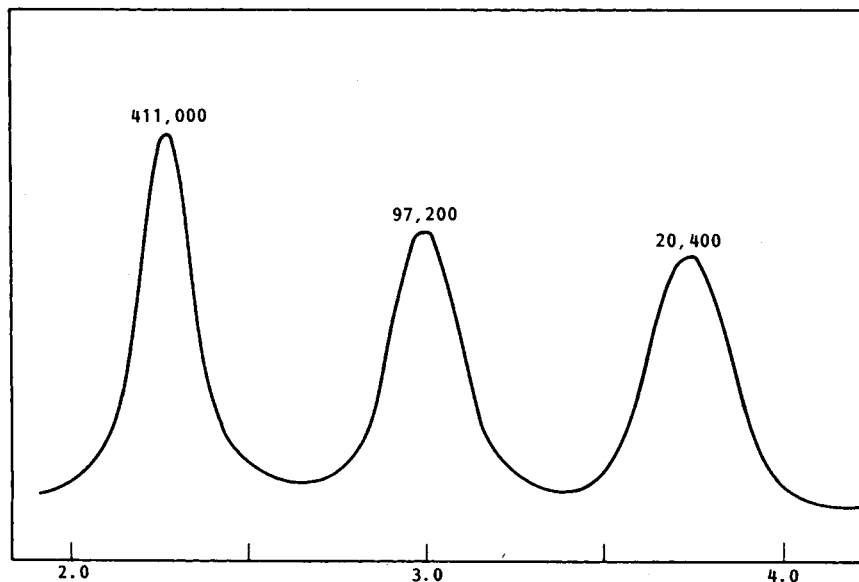


Fig. 2. Typical chromatograms of a ternary mixture of narrow MWD polystyrene solutes using column A operated at 1 mL/h. Polystyrene \bar{M}_w is indicated for each peak. Ordinate: absorbance, 254 nm. Abscissa: elution volume, counts.

The porous glass was deactivated by the method described earlier.¹⁶ During the packing of columns, small amounts of packing materials were added to the column with repeated tapping. This procedure was continued until the column was filled. Good packing density was obtained (about 0.7 g/mL). The ratio of interstitial volume to total volume of the column was 0.32. Because of the irregular shape of Bio-glas particles, a comparison of this value with the various packing models for uniform sized spheres is not appropriate.

RESULTS

The calibration curve for column A, length 1 m \times 0.47 cm ID packed with Bio-glas 500 Å, is given in Figure 1. This column has a large separation range. A typical elution curve of a ternary polymer mixture is given in Figure 2. The peaks obtained using this column are essentially Gaussian, for which the ratio of peak width at half the peak height to the peak width at the baseline should be 0.587. Using the chromatograms shown in Figure 2, this ratio was found to be 0.56 for the 411,000 \bar{M}_w polystyrene peak and 0.55 for the 97,200 \bar{M}_w polystyrene peak. In Table II the elution volumes and peak widths for three polystyrene standards are reported for column A at various flow rates. Because of the difficulty of injecting with a syringe into this column, which needed high inlet pressure at high flow rates, the flow rate range studied was kept below 40 mL/h. Similar results are summarized in Table III for columns B1 and B2.

The number of theoretical plates per foot, n , and the resolution R were calculated using the established equations:

$$n = (1/L)(4V/W)^2 \quad (2)$$

$$R = 2(V_1 - V_2)/(W_1 + W_2) \quad (3)$$

where V is the elution volume, W is the peak width at the baseline, and L is the column length in feet. The height equivalent to a theoretical plate (HETP) was calculated in appropriate units from the reciprocal of the number of theoretical plates. The values for n and HETP are also included in Tables II and III.

In Figure 3 the number of theoretical plates per foot is plotted against linear flow velocity. The figure shows that the efficiency of the column does not show large changes with change of the flow velocity when the flow velocity is high. When the flow velocity is low, the efficiency is very sensitive to changes in flow velocity.

It should be noted that the flow velocity at which the number of theoretical plates increases is ~ 0.020 cm/s. Most GPC work is done in $\frac{3}{8}$ -in. OD columns (ID = 0.775 cm); therefore, the flow rate corresponding to 0.020 cm/s flow velocity is 0.2 mL/m. Thus, ordinarily analytical scale GPC work is done 5–10 times faster than at this flow velocity. Similarly, resolution (R) does not increase until the flow velocity is < 0.02 cm/s (Fig. 4).

To extend the flow velocity range to lower values, the small-diameter column A could not be used. The baseline was not stable, and the elution curve was irregularly shaped at extremely low flow rates (< 0.2 mL/h). To circumvent this dilemma, viz., to increase the flow rate while achieving a lower flow velocity, a column B1 with a larger diameter was constructed. The column length chosen was 33.3 cm, and the internal diameter was 2.22 cm. If this larger diameter column had the same packing density, it would be expected that it could be used

TABLE II
 Elution Volume^a V and Peak Width^a W , Number of Theoretical Plates per Foot, n , and Height Equivalent to a Theoretical Plate HETP (mm) of Polystyrene Standards for Column A

Flow rate (mL/h)	Linear flow velocity (cm/s)	PS 411,000			PS 97,200			PS 20,400					
		V	W	n	HETP (mm)	V	W	n	HETP (mm)	V	W	n	HETP (mm)
40	0.209	2.254	0.459	118	2.6	3.000	0.672	97	3.1	3.750	0.759	119	2.6
30	0.157	2.257	0.462	116	2.6	3.000	0.666	99	3.1	3.748	0.737	128	2.4
20	0.104	2.253	0.461	116	2.6	3.000	0.615	116	2.6	3.772	0.705	140	2.2
10	0.052	2.250	0.420	140	2.2	3.000	0.551	145	2.1	3.750	0.636	170	1.8
5	0.026	2.267	0.391	164	1.9	3.000	0.495	180	1.7	3.749	0.586	200	1.5
4	0.021	2.262	0.365	187	1.6	3.000	0.467	200	1.5	3.756	0.571	210	1.4
3	0.016	2.240	0.356	193	1.6	3.002	0.467	200	1.5	3.754	0.540	236	1.3
2	0.010	2.258	0.340	215	1.4	2.986	0.461	205	1.5	3.756	0.515	260	1.2
1	0.005	2.250	0.321	240	1.3	2.990	0.422	245	1.25	3.730	0.469	310	0.99
0.2	0.001	2.268	0.250	401	0.76	2.914	0.363	314	0.97	3.787	0.340	604	0.51

^a In units of counts; 1 count equals 2.742 mL.

TABLE III
 Elution Volume^a V , Peak Widths^a W , Number of Theoretical Plates per Foot, n , and Height Equivalent to a Theoretical Plate, HETP, (mm) of Polystyrene Samples for the 2.22-cm ID Columns

Column	Column length (cm)	Flow rate (mL/h)	Linear flow velocity (cm/s)	PS 411,000			PS 97,200			PS 20,400					
				V	W	n	HETP (mm)	V	W	n	HETP (mm)	V	W	n	HETP (mm)
B1	33.3	100	0.022	16.075	1.917	1030	0.29	21.300	2.761	871	0.35	26.601	3.005	1147	0.26
		50	0.011	16.244	1.882	1090	0.28	21.786	2.500	1111	0.27	26.786	2.612	1540	0.20
B2	50.0	100	0.022	24.260	2.416	983	0.31	32.000	3.977	631	0.48	39.966	4.460	783	0.39
		80	0.018	24.296	2.551	885	0.34	32.089	3.857	675	0.45	40.000	4.451	788	0.39
		60	0.014	24.409	2.559	887	0.34	32.204	3.890	668	0.45	40.136	4.402	810	0.38
		40	0.009	24.503	2.374	1040	0.29	32.179	3.345	902	0.33	40.107	4.280	856	0.35

^a Measured in peak count units; 1 peak count = 2.742 mL.

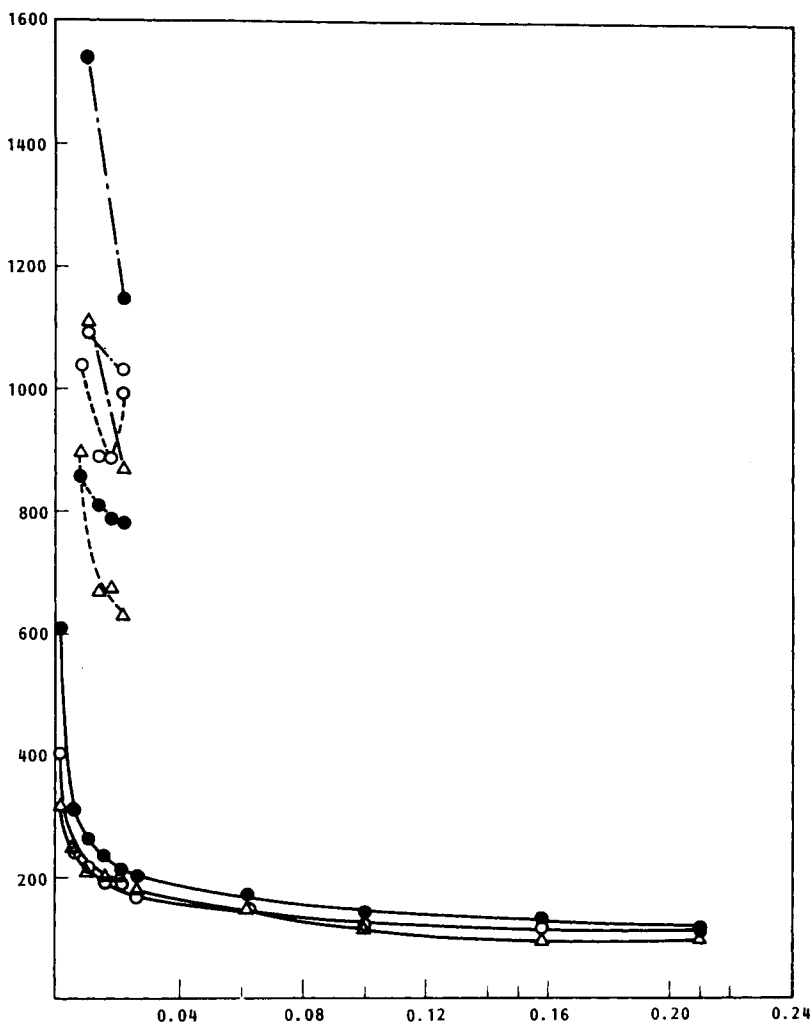


Fig. 3. Number of theoretical plates, n , per foot (ordinate) plotted against flow velocity U (cm/s) (abscissa). (—) Col A; (---) col B1; (· · ·) col B2; (●) PS 20,400; (Δ) PS 97,200; (○) PS 411,000.

to study the effect of the flow rate down to the range where the minimum HETP is predicted in a reasonable experimental time, about 10 days. The elution curves obtained with this column were not symmetrical, especially for the high molecular weight sample, PS 411,000.

The data for the polymer samples at the flow rates studied are given in Table III. The number of theoretical plates for column B1 was over 1,000, significantly higher than for column A, which is longer but has a smaller diameter. Previous studies have also demonstrated a higher efficiency for larger diameter columns^{17,18} compared with smaller diameter columns packed with the same material. Column B1 exhibited a higher efficiency at lower flow rates, as did the small-diameter column A. Poor elution curves resulted when the flow rate was reduced further. Repacking the column did not improve the situation. Because all the peaks showed the same type of distortion, it is possible that this was introduced by the end fitting of the column. Assuming this to be the case, an at-

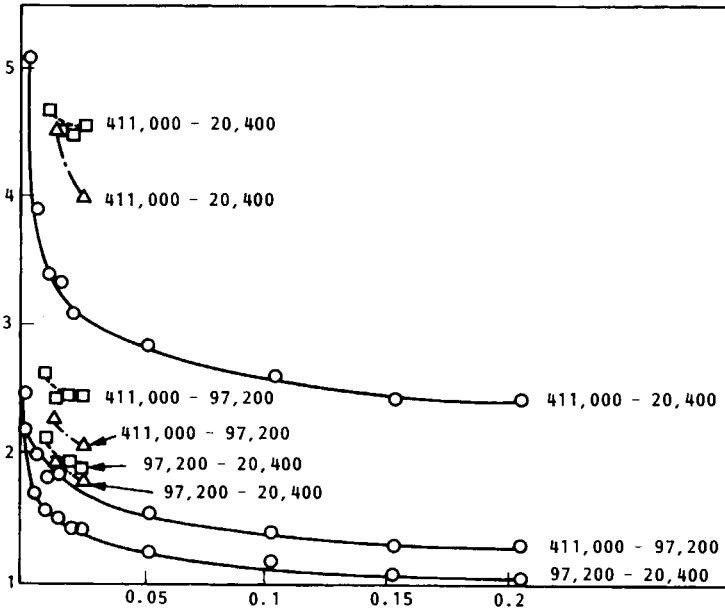


Fig. 4. The effect of flow rate on resolution. The polymer pair used to calculate resolution is indicated. Ordinate: resolution R . Abscissa: flow velocity (cm/s). (O—O) Col A; (Δ --- Δ) col B1; (\square --- \square) col B2.

tempt was made to reduce this by preparing a column with the same diameter but a longer length, 50 cm. The situation was the same—the tailing of the peaks still existed. The data for this longer column B2 are given in Table III.

The reason for the tailing of the elution curves when the large-diameter columns are used is still unknown. It is unlikely that flow rate instability is re-

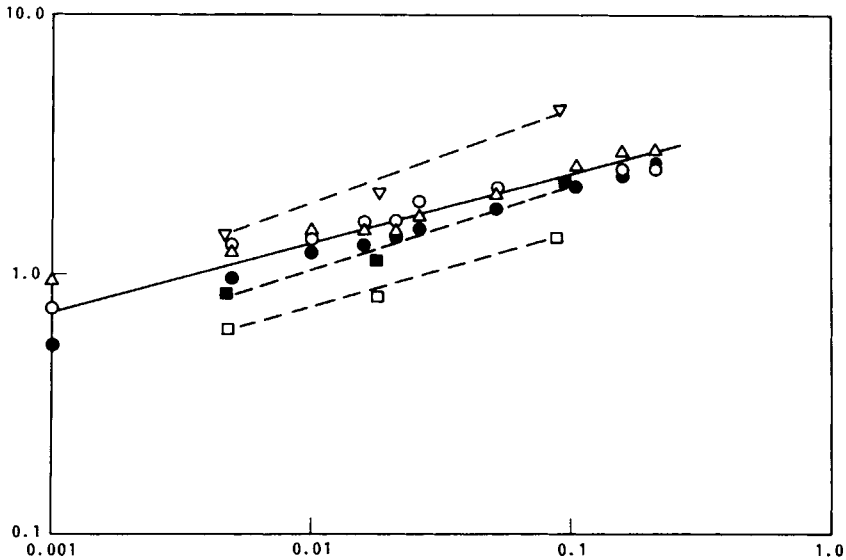


Fig. 5. Plot of log HETP (mm) (ordinate) versus log of flow velocity U (cm/s) (abscissa) for column A and a Styragel® column.¹³ (—) Col A: (●) 20,400; (Δ) 97,200; (○) 411,000. (---) Styragel® 10⁵ Å: (\square) 19,800; (■) 97,200; (∇) 411,000.

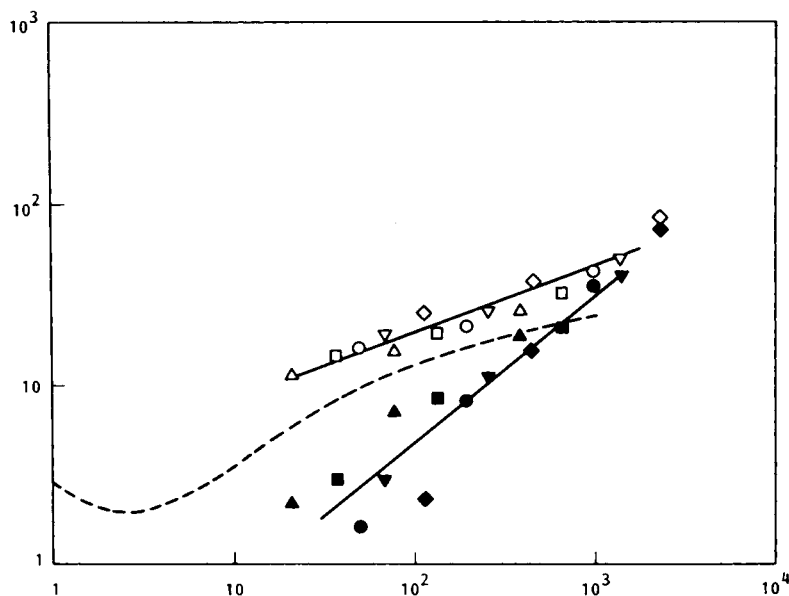


Fig. 6. Relationship between reduced plate Height h (ordinate) and reduced velocity v (abscissa) for polystyrene solutes eluting from a 10^5 -Å Styragel® column. (---) Theoretical curve; (Δ , \square , \circ , ∇ , \diamond) uncorrected results; (\blacktriangle , \blacksquare , \bullet , \blacktriangledown , \blacklozenge) corrected results, assuming $\bar{M}_w/\bar{M}_n = 1.06$; (Δ , \blacktriangle) 19,800; (\square , \blacksquare) 51,000; (\circ , \bullet) 97,200; (∇ , \blacktriangledown) 160,000; (\diamond , \blacklozenge) 411,000.

sponsible for the peak distortion, because considerably lower volumetric flow rates were used with the narrower column and no problems were encountered. Further study is needed to understand the process of separation for large-diameter gel permeation chromatographic columns.

TABLE IV
Reduced Plate Height and Reduced Velocity Data for Polystyrenes Eluting from Column A

Flow rate		Molecular weight of polystyrene	HETP (mm)	Reduced HETP h	Diffusion coefficient ^a $D_m \times 10^8$ (cm ² /s)	Reduced velocity v
(cm ³ /h)	(cm/s)					
40	0.209	411,000	2.6	74.3	22	3325
		97,200	3.1	88.6	51.3	1426
		20,400	2.6	74.3	124.4	588
20	0.104	411,000	2.6	74.3	22	1654
		97,200	2.6	74.3	51.3	710
		20,400	2.2	62.9	124.4	293
10	0.052	411,000	2.2	62.9	22	827
		97,200	2.1	60.0	51.3	355
		20,400	1.8	51.4	124.4	146
5	0.026	411,000	1.9	54.3	22	414
		97,200	1.7	48.6	51.3	177
		20,400	1.5	42.9	124.4	73
2	0.010	411,000	1.4	40.0	22	159
		97,200	1.5	42.9	51.3	68
		20,400	1.2	34.3	124.4	28
0.2	0.001	411,000	0.76	21.7	22	16
		97,200	0.97	27.7	51.3	6.8
		20,400	0.51	14.6	124.4	2.8

^a Assumed to be the same in chloroform as in toluene.

DISCUSSION

Waters et al.¹⁹ found that for low molecular weight samples the plot of log HETP vs. log flow rate exhibited a linear relationship. The relationship applied to our systems. The plot of log HETP vs. log flow velocity for column A is shown in Figure 5. Also included are some data for a Styragel® 10⁵-Å column taken from our previous work.¹³ Generally the slopes are similar, but for Styragel there is a molecular weight dependence of the HETP at a given flow rate. The Bio-glas results do not demonstrate this effect.

In order to reduce chromatographic data to a common plot, reduced variables⁶ have been introduced; $h = \text{HETP}/d_p$, where d_p is the packing particle diameter, and $v = U d_p/D_m$, where U is the flow velocity and D_m is the solute diffusion coefficient. During the course of this investigation an error in our previous paper¹³ was detected, namely that reduced velocities in the final columns of Tables II and V of Ref. 13 should not include the factor 10⁻² as indicated in the column heading. Unfortunately, the abscissa of Figure V of the same paper includes the error, so lines A and B should be displaced two log units to lower values. These data are correctly replotted here in Figure 6. In contrast to our previous statement (Ref. 13, p. 1388), these experimental data show reasonable agreement with the model. The h values calculated, assuming the polymer solutes are monodisperse, lie above the theoretical curve. When corrected for solute polydispersity, assuming $\bar{M}_w/\bar{M}_n = 1.06$, the data fall below the theoretical curve. The polydispersity of these polymers has not been determined accurately. If the true polydispersity values are less than 1.06, the data would be displaced towards the theoretical line.

In Table IV and Figure 7 the data from the present study are shown in terms of reduced parameters. The data lie above the theoretical curve and were calculated assuming a particle diameter of 35 μm . If extreme values for the particle

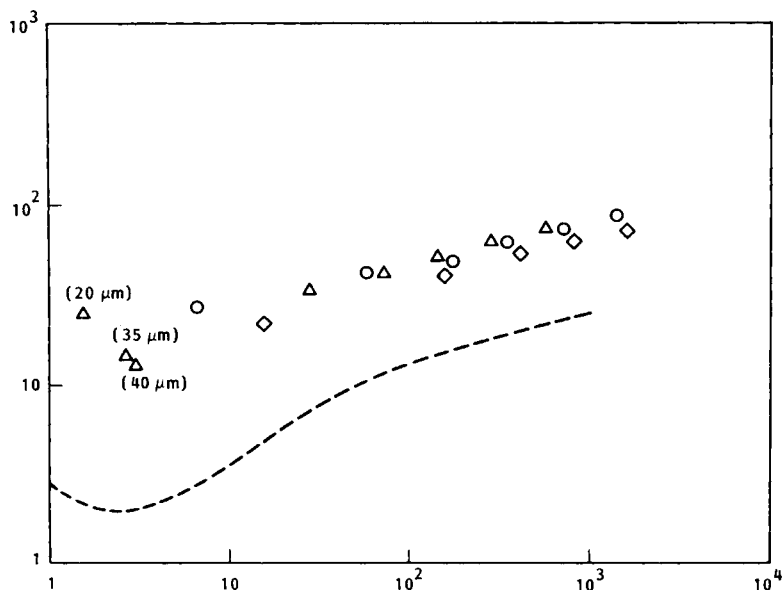


Fig. 7. Relationship between reduced plate height h (ordinate) and reduced velocity v (abscissa) for polystyrene solutes eluting from a 500-Å Bio-glas column A. (---) Theoretical curve; col A: (Δ) 20,400; (\circ) 97,200; (\diamond) 411,000.

diameter are substituted, viz., 20 μm or 40 μm , the agreement between theoretical and experimental is not improved, as indicated by the values for the 20,400 molecular weight data point in Figure 7. The experimental values would move toward the theoretical line if a correction were made for the actual polydispersities of the samples. However, these are not known with sufficient accuracy to allow a definitive comparison to be made.

CONCLUSIONS

Data from a series of columns with different internal diameters employing organic or inorganic column packings demonstrate that efficiency in GPC improves considerably below a flow velocity of 0.02 cm/s. The increase in efficiency persists at least up to the point predicted by Giddings theory, i.e., where the reduced velocity is ~ 2 –3. Therefore, efficiency and resolution are determined by the time available for each analysis. Above 0.02 cm/s, little efficiency is lost by using flow velocities up to 0.2 cm/s.

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