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Fast Gel Permeation Chromatography*

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Summary

A systematic study of the major factors influencing fast analysis by gel permeation chromatography is presented. The study included the effects of (a) solvent flow rate [1–35 ml/min], (b) sample concentration [0.05–0.5%], (c) sample molecular weight [41–411,000 mol wt] and (d) particle size of column packing [10–42 μ]. The effect of the operating temperature at the high flow rates was also investigated.

INTRODUCTION

In a previous paper (*1*) we reported the effect of several operational parameters on separations by gel permeation chromatography (GPC). This study included the effects of (a) solvent flow rate, (b) sample concentration, (c) sample molecular weight, and (d) particle size of column packing. In the present study, we extended the upper pressure limit of the system to 2200 lb/in.^{2} and evaluated these effects on column performance. The effect of the operating temperature at the high flow rates was also investigated.

EXPERIMENTAL

In these studies a modified Waters Associates Gel Permeation Chromatograph, Model 200—operated at room temperature and 80°C—was used with toluene as the solvent (see Fig. 1). The GPC-200 was

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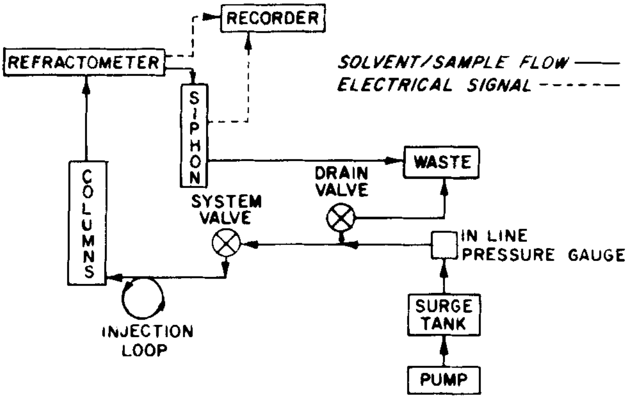

FIG. 1. Modified GPC-200 flow diagram.

modified to operate at an upper pressure limit of 2200 lb/in.^{2} by (a) replacing the standard sample and in-line gauges with 3000 lb/in.^{2} gauges; (b) filling the reference side of the detector with toluene and then closing the reference valve; (c) inserting a tee with two Hoke-needle valves after the surge tank, so that one line leads to the column system and the other to a drain; (d) substituting a variable relief valve (1600–2400 lb/in.^{2}) for the one in the instrument, and (e) disconnecting both injection port valve and the column switching valve. For the fast flow rates (up to 35 ml/min), a Milton-Roy Industrial Mini-pump with ¼ in. diameter plunger was used.

Samples were injected through a Swagelok fitted loop of 4 ft of 0.040-in. i.d. tubing (1 ml volume) inserted into the system ahead of the columns. A high-speed recorder was used for the high flow rates.

When operating at 80°C, an auxiliary preheater was used which consisted of three empty 4 ft × 0.305 in. i.d. stainless steel GPC columns installed in the oven and placed just before the sample injection loop. This ensured that the toluene solvent would be at the operating temperature (80°C) when passing through the analytical test columns. The sample injection loop was mounted outside the oven and connected to the system through two holes in the oven wall.

The procedure for injecting samples was the same as described previously (*1*). The siphon was not used at the high flow rates, since continuous siphoning occurred. Elution volumes and peak widths of test samples were determined by measuring the flow rate of solvent with a graduated cylinder and relating this to measured distances on the chart paper record.

Two sets of Styragel columns—as described in Table 1—were used in these tests.

TABLE 1

GPC Test Column Characteristics

Test column sets	Number of columns	Column dimensions		Styragel packing	
		Length (feet)	i.d. (inches)	Porosity (Å)	Particle size (μ)
1	2	4	0.305	1.5×10^6	10–15
2	2	4	0.305	1.5×10^6	37–42

Samples in concentrations of 0.05 and 0.5% (w/v) were prepared by dissolving polystyrene standards (mol wt 411,000 and 19,850), acetonitrile (CH_3CN) and orthodichlorobenzene (ODCB) in toluene. The molecular weight of these test samples ranged from 41 to 411,000. A constant sample volume of 1 ml was used for all test samples.

RESULTS AND DISCUSSION

Effect of Flow Rate on Elution Volume

Elution volume was found to be independent of flow rate from 0.1 to 35 ml/min (see Fig. 2). The data for flow rates up to 12.5 ml/min are taken from our earlier study (*1*). Thus, the effect of permeation remained invariant to flow for both large and small molecules [polystyrene (mol wt = 411,000) and acetonitrile, respectively]. At the highest flow rate (35 ml/min), there appears to be a slight tendency for the more concentrated samples (0.5%) to be eluted earlier than the dilute samples (0.05%).

The data here and that in our earlier study are in disagreement with the work of Yau et al. (*2*), who found that elution volumes of the high molecular weight compounds decrease with increasing flow rate. They attributed this to a nonequilibrium distribution of the polymer molecule between the mobile and stationary phases at high flow rates (up to 10 ml/min).

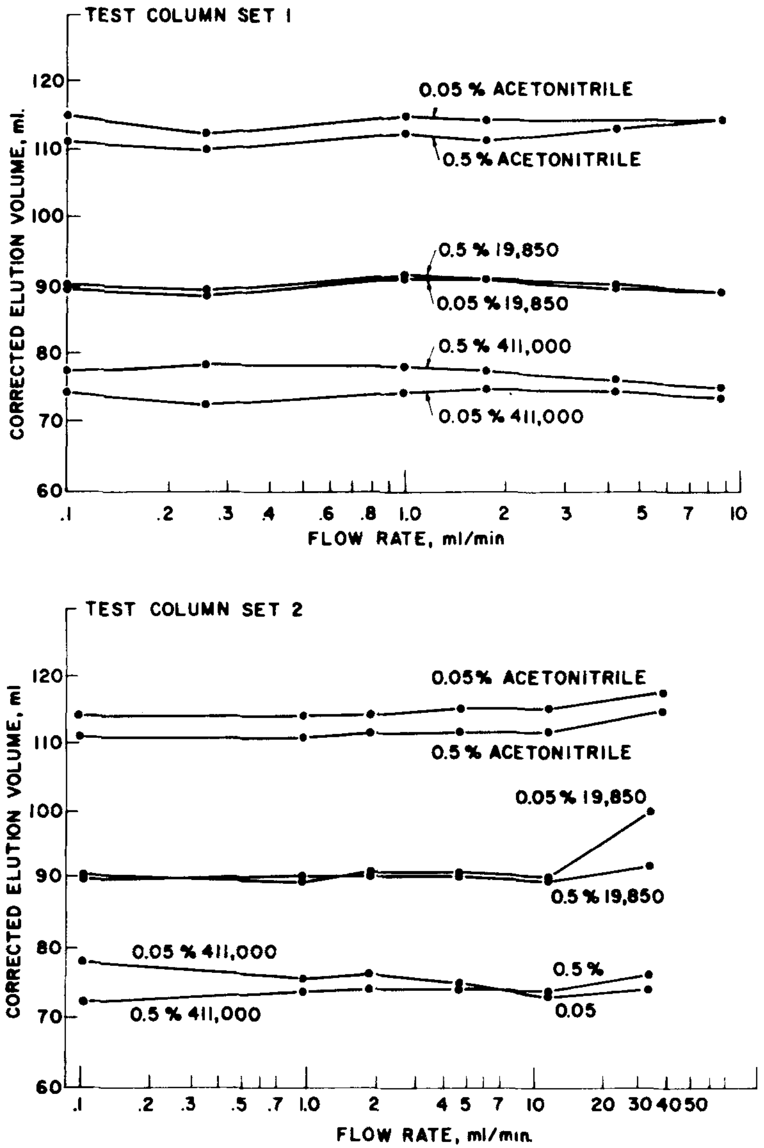

FIG. 2. Elution volume as a function of flow rate and column-packing particle size. (Acetonitrile and polystyrene standards used in all tests under same operating/analytical conditions.)

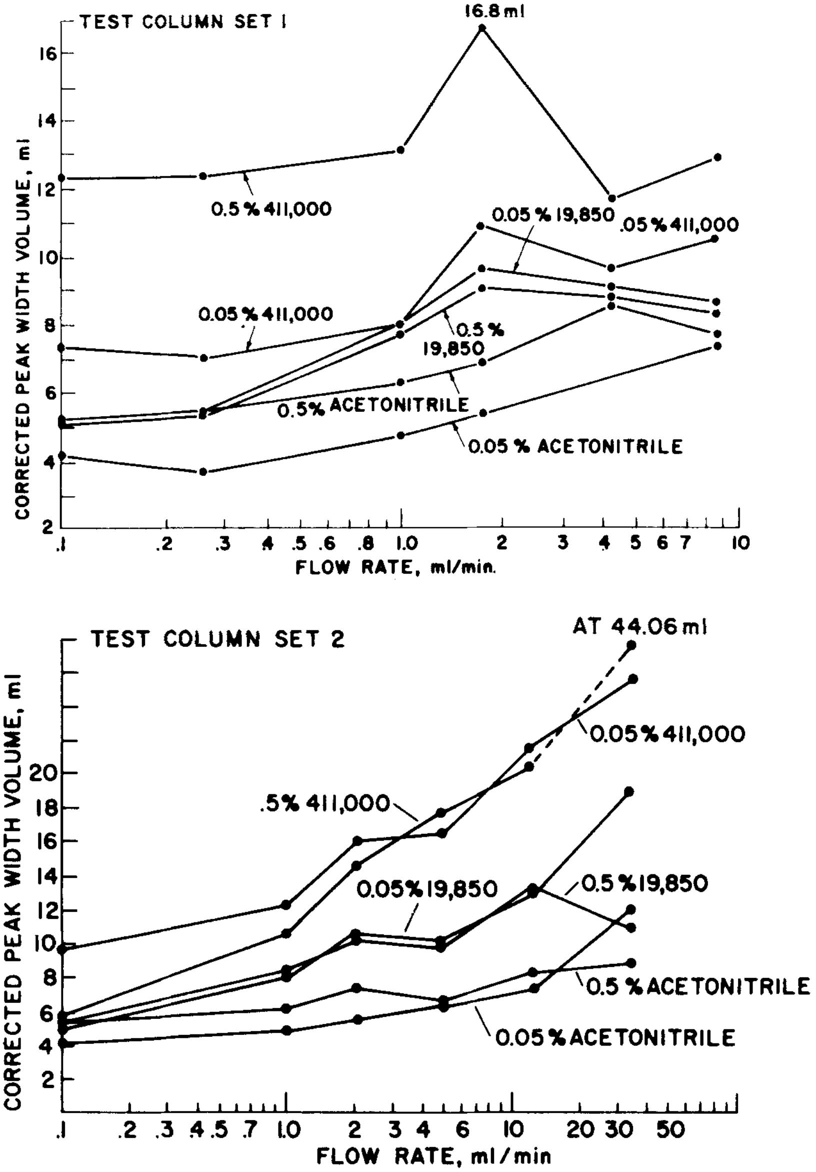

FIG. 3. Peak width as a function of flow rate and column packing particle size. (Acetonitrile and polystyrene standards used in all tests under same operating/analytical conditions.)

Effect of Flow Rate on Peak Width

The increase in peak width with flow rate was found to be less than would be anticipated from the Van Deemter equation (*3*, *4*) (see Fig. 3). With Column Set 1, the peak widths of all samples only doubled, as the flow rate increased 87 times (0.1 to 8.7 ml/min). On Column Set 2, the sample peak widths increased approximately 3–4 times as the flow rate increased 350 times. Since the height equivalent to a theoretical plate (H) is a function of the square of the peak width, the value of H increased 10–16 times as the flow rate increased 350 times. Two of the data points (0.05% 19,850 and 0.05% 411,000) at the 35 ml/min flow rate were calculated from chromatograms with a sloping baseline and may be in error by as much as 20%.

As reported earlier (*1*), "viscous fingering" of high molecular weight compounds apparently does not occur at high flow rates. At 35 ml/min the high molecular weight compounds at concentrations of 0.5% gave symmetrical peak shapes and without delayed elution.

The results here extend the workable upper pressure limit of the Styragel column packing material to 2200 lb/in.^{2}. At 2200 lb/in.^{2} there was no change observed in column permeability with pressure and no indication that the gel network was collapsing or compacting.

Effect of Flow Rate on Resolution

Mixtures of polystyrene 411,000 and 19,850 were injected at 35 ml/min. The resolution obtained is shown in Table 2 and compared with

TABLE 2

GPC Resolution as a Function of Flow Rate and Sample Concentration (Column Set No. 2 and Same Operating/Analytical Conditions Used Previously)

Test sample[a]	Sample concentration (%)	Flow rate (ml/min)	Resolution
411,000 + 19,850	0.05 + 0.05	1.0	1.75
		5.0	1.33
		12.5	0.99
		35.0	0.66
411,000 + 19,850	0.50 + 0.50	1.0	1.43
		5.0	1.19
		12.5	0.89
		35.0	0.63

[a] Mixture of two polystyrene standards with weight-average molecular weights of 411,000 and 19,850, respectively. Test samples dissolved in toluene.

that found previously at slower flow rates. A 30% loss in resolution was observed when the flow rate was tripled (from 12.5 to 35 ml/min). Also, resolution did not decrease at a faster rate when the more concentrated (0.5%) mixture was used.

Effect of Temperature

Column Sets 1 and 2 were tested at 80°C using flow rates of 8.7 and 35 ml/min, respectively. These flow rates were the maximum attainable at room temperature and 2200 lb/in.^{2}. A comparison of the elution volumes and peak widths of the samples is shown in Table 3. Elution volumes of the test compounds were found to decrease at the higher temperature. The compounds may have a tendency to relax or uncoil at the higher temperature and occupy a larger volume. Therefore, a greater proportion of the pores may exclude the compounds and give earlier elution. Peak width volumes were also found to decrease

TABLE 3

Comparison of Elution Volumes and Peak Widths for Standards at 23 and 80°C

Sample	80°C elution volume	Peak width	23°C elution volume	Peak width
Test Set No. 1, 10–15 μ column set, flow rate 8.7 ml/min				
0.05% ODCB	97.95	5.04	100.98	5.95
0.5% ODCB	98.64	6.47	100.64	6.62
0.05% Acetonitrile	104.48	11.25	114.83	7.69
0.5% Acetonitrile	103.82	7.48	114.47	7.74
0.05% 19,850	87.03	8.97	89.42	8.70
0.5% 19,850	87.43	9.46	89.26	8.36
0.05% 411,000	71.30	10.30	73.88	10.60
0.5% 411,000	74.94	10.70	75.40	12.90
Test Set No. 2, 37–42 μ column set, flow rate 35 ml/min				
.05% ODCB	102.45	7.80	105.00	11.70[a]
0.5% ODCB	102.10	6.74	103.25	8.26
.05% Acetonitrile	109.19	7.09	117.73	12.39[a]
0.5% Acetonitrile	111.67	7.09	115.32	8.95
.05% 19,850	91.46	15.60	101.90	11.02[a]
0.5% 19,850	85.79	15.24	92.26	19.62
.05% 411,000	73.03	24.82	76.42	44.1[a]
0.5% 411,000	69.48	23.11	74.30	25.8

[a] Calculated from a sloping baseline.

TABLE 4

Resolution as a Function of Flow Rate and Temperature on Test Column Set No. 2

	Elution volume	Peak width volume	Resolution
A. 12.6 ml/min, 23°C, 775 lb/in.^{2}			
Mixture 0.5% 411,000	74.37	22.25	0.89
0.5% 19,850	90.52	14.21	1.88
0.5% Acetonitrile	112.12	8.81	
B. 35 ml/min, 23°C, 2200 lb/in.^{2}			
Mixture 0.5% 411,000	76.77	26.16	.63
0.5% 19,850	92.95	25.47	1.30
0.5% Acetonitrile	115.32	8.95	
C. 35 ml/min, 80°C, 1350 lb/in.^{2}			
Mixture 0.5% 411,000	75.1	26.8	.84
0.5% 19,850	92.0	13.47	1.83
0.5% Acetonitrile	110.2	6.38	

at the higher temperature. This would result from decreased viscosity of the test compounds and solvent at 80°C, resulting in better mass transfer.

The combined effects of temperature and flow rate on resolution for Column Set 2 are shown in Table 4. As shown, resolution dropped 30% on increasing the flow rate from 12.5 to 35 ml/min. By raising the temperature from 23 to 80°C, the original resolution is almost achieved while decreasing the analysis time by a factor of 3. Figure 4 shows the chromatogram of the mixture run at 35 ml/min and 80°C.

The combined effects of particle size and flow rate on resolution for

TABLE 5

Resolution as a Function of Particle Size and Temperature for Test Column Sets Nos. 1 and 2

System		Time (min)	R	Pressure (lb/in.^{2})
A. 37–42 μ, 8.7 ml/min room temp	411,000 19,850	12	1.16	400
B. 10–15 μ, 8.7 ml/min room temp	411,000 19,850	12	1.56	2200
C. 10–15 μ, 8.7 ml/min 80°C	411,000 19,850	12	1.66	2200

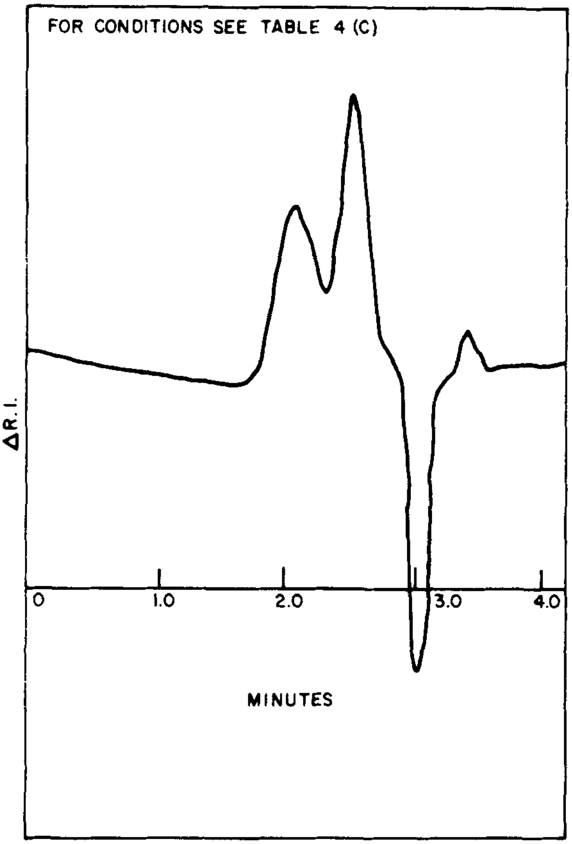

FIG. 4. Fast GPC chromatograms.

both column sets are shown in Table 5. At the same flow rate (i.e., same analysis time) and temperature, Column Set 1 (10–15 μ) gave better resolution (35%), but at the sacrifice of operating at over 5 times the pressure as Column Set 2 (37–42 μ). Only a 6% improvement in resolution was obtained when Column Set 1 was operated at 80°C. The much lower flow rate and smaller particle size of Column Set 1 tends to decrease the magnitude of the temperature effect.

Correlation of Data

The data obtained in this study and in the previous study represents a flow rate range from 0.1–35 ml/min, a particle size range from 10–

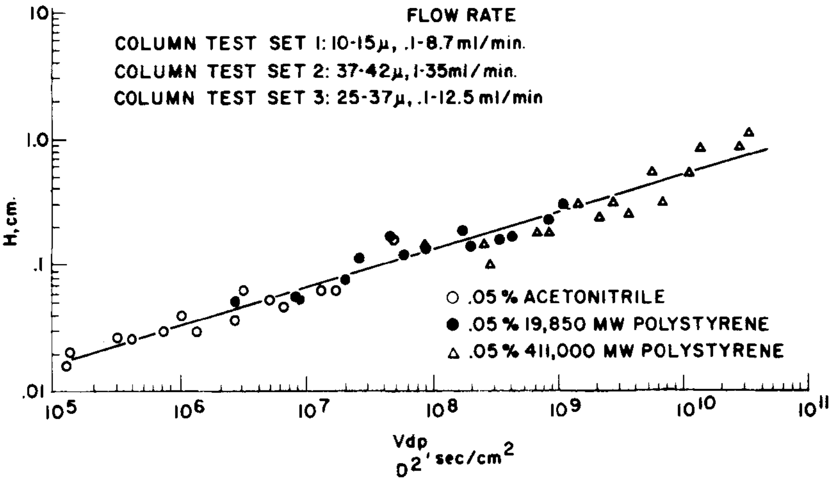

FIG. 5. H as a function of (Vdp/D^2).

42 μ and test samples with diffusion coefficients from 10^{-5} to 10^{-7} cm^2/sec. Several theoretical models were considered in an attempt to correlate the data. Coupek and Heitz (*5*), using poly(vinyl acetate) and low cross-linked polystyrene gels, were able to correlate their data by plotting the reduced plate height (H/dp) versus the reduced velocity (Vdp/D). Our data did not fit this model, as the ordinate (reduced velocity) appeared to be particle size independent. Kelley and Billmeyer (*6*), using nonpermeating systems, found their data to fit a plot of plate height (H) versus reduced velocity (Vdp/D). A plot of our data as log H vs. log (Vdp/D) showed some correlation (the data formed a band), but a definite dependence on diffusion coefficient could be observed. We found that the square of the diffusion coefficient greatly improved the correlation and a plot of log (H) vs. log (Vdp/D^2) is shown in Fig. 5.

It is interesting to speculate as to why this model describes the data. In the Kelley and Billmeyer model (H vs. Vdp/D), for non permeating systems, the diffusion coefficient is to the first power. In their systems, only diffusion and spreading can occur in the interstitial spaces. In our work, this also occurs, but there is the added effect of permeation into and out of the gel pores. Thus, the data obtained here appears to show that when both processes are present, the square of the diffusion coefficient best describes the data.

CONCLUSIONS

In our studies of operational variables for fast gel permeation chromatography, we have found the following: (a) elution volumes are independent of flow rate for flow rates up to 35 ml/min; (b) for flow rates up to 35 ml/min, peak width volumes were found not to be excessive and were much less than predicted by Van Deemter's equation; (c) the effect of "viscous fingering" decreased at high flow rates resulting in improved peak symmetry; (d) loss of resolution at high flow rates is much less than expected from the Van Deemter equation; (e) increasing the operating temperature decreased peak widths and improved resolution; (f) a new model (H vs. Vdp/D^2) was found to best correlate the data.

Nomenclature

R = resolution
H = height equivalent of a theoretical plate (cm)
v = linear velocity (cm/sec)
dp = particular diameter (cm)
D = diffusion coefficient of sample in solvent (cm^2/sec)

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