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High-Speed Gel Permeation Chromatography. A Study of Operational Variables

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Synopsis

Systematic studies of operational variables affecting GPC separation using μ-Styragel of nominal porosity 10^6, 10^5, 10^4, and 10^3 Å are reported. The dependences of (1) flow rate, (2) injection volume, and (3) concentration on elution volume (V_e), the theoretical plate number (N), and peak width at half-height were examined. The values of V_e changed with changing (1), (2), and (3). The values of N were inversely proportional to the root of flow rate. The relation between sample load and N showed that lower concentration and larger injection volume were desirable for N than the opposite. The concentration dependance for high-speed GPC was significantly higher than classical low-speed GPC. The methods of minimizing the effects produced by the individual variables and the optimum operational conditions are discussed. Recommended operational conditions are as follows: flow rate, 1 to 2 ml/min; injection volume, 0.1 ml; sample concentration, below 0.4% (or if injection volume 0.5 ml, sample concentration below 0.1%).

INTRODUCTION

As a result of new advances in high-pressure solvent transport systems, high-sensitivity detectors of low cell volume, and polystyrene gel bead processing technology, high-speed gel permeation chromatography (GPC) is rapidly becoming the method of choice for determining molecular weight distributions and other related characteristics of polymers. Recent data for high-speed GPC have been obtained in less than 20 min using high-resolution GPC columns packed with polystyrene gel beads of very small diameter obtained commercially with a high-speed liquid chromatograph, in contrast to the 2 to 4 hr normally required using a conventional GPC apparatus and columns. Because of small diameters of the gel particles, fluid frictional resistance in the elution process is assumed to be very high, and optimum conditions for high-speed GPC must be different from those for conventional GPC.

Little et al.[1,2] determined elution volume, peak width, and peak symmetry as a function of flow rate ranging from 0.1 to 12.0 ml/min by using a conventional GPC apparatus. Gudzinowicz and Alden[3] obtained good column efficiency under high flow rate using porous glass beads sieved into 44- to 50-micron-diameter particles as a packing material. Kato et al.[4] investigated the effects of column packing particle size, solvent flow rate, and column length on the separation efficiency. Limpert et al.[5] commented the effects of particle size and its distribution of packing materials on the number of theoretical plates in high-speed GPC.

In this study, the effects of operational variables were investigated to establish optimum conditions for high-speed GPC using Waters Associates μ-Styragel columns.

EXPERIMENTAL

A home-made high-speed GPC apparatus assembled from component parts which were available from commercial suppliers was used with toluene as the solvent. The apparatus consisted of a high-pressure pump (Waters Associates Model C-6000), a loop injector (a Kyowa Seimitsu high-pressure six-port valve), columns, and a detector (a Waters Associates differential refractometer Model R-401). This instrument was operated at room temperature. Nominal porosity of μ-Styragel was 10^6, 10^5, 10^4, and 10^3 Å and it was packed in columns dimensions of which were 0.305 in. I.D. and 1 ft. length.

For the measurement of operational variables, the polystyrene standard of 200,000 molecular weight was dissolved in toluene in concentrations from 0.1% to 0.8% (w/v), and elution volume, peak width at half-height, and theoretical plate number were determined at flow rates from 0.5 to 4.0 ml/min by injecting the sample solution in the range of 0.1 to 1.0 ml. A six-port injection valve permitted sample injections of constant volume ranging from 0.1 to 1.0 ml by selecting the proper sample loop volume. Measurement of chromatograms was done in triplicate.

The dependence of operational variables on molecular weight was evaluated by injecting the polystyrene standards of several molecular weights at concentrations of 0.1%, 0.2%, and 0.4% with an injection volume of 0.25 ml and flow rate of 2.0 ml/min. Calibration curves were obtained at these concentrations and extrapolated to infinite dilution. Weight- and number-average molecular weights of polystyrene NBS 706 at concentration of 0.4% were calculated in the usual manner for GPC and in the special method[6] for correcting concentration effects, by dividing the GPC chromatogram at 0.5-ml intervals and by using these calibration curves. The polystyrene standards were obtained commercially (Pressure Chemical Co.).

As the pump used in this system had a dial to adjust the flow rate manually at 0.1 ml/min increments, it was easy to obtain a correct and reproducible flow rate by setting the dial and measuring the time required to fill a 25-ml measuring flask with solvent eluted from the system (this procedure was added to ensure correct flow rate). The elution volume V_e could then be obtained by measuring chart length between an injection point and a peak maximum and by knowing flow rate and chart speed. As the chart speed of a recorder could be adjusted from 10 mm/min to 60 mm/min as the flow rate increased, it was easy to interpret small differences in elution volume up to 0.01 ml.

RESULTS AND DISCUSSION

Determination of Operational Variables

Measurement of elution volume was done in triplicate. The reproducibility of the system was checked using the polystyrene standard of 200,000 molecular weight at a flow rate of 1 ml/min by injecting 0.1 ml of a 0.4% solution. The

relative standard deviation of elution volume after ten analyses was 0.43%, and that of $W_{1/2}$ was 4.0%. The reproducibility of N was a function of those of V_e and $W_{1/2}$.

Bly[7] has pointed out that the theoretical plate number N could be calculated from chromatograms of polymers if the curve widths were normalized for polydispersity and that the resulting N value was useful for comparison purposes. Though plate counts using low molecular weight compounds would probably be more appropriate,[8] the object of the present study was not the estimation of column performance, but the variation in separation efficiencies of polymers with flow rate, concentration, and so on, and the use of monodisperse polymers for the measurement of N was useful for a measure of the efficiencies afforded by the columns to polymers. Inasmuch as the same polymer was used for the measurement of operational variables, the value N which is not normalized is of value.

Effect of Flow Rate

The flow rate dependence of elution volume (V_e), the theoretical plate number (N), and peak width at half-height ($W_{1/2}$) was investigated by determining V_e for the polystyrene standard of 200,000 molecular weight at several flow rates. Sample concentration was varied from 0.1% to 0.8% with injection volume of 0.1 or 0.25 ml.

An example of flow rate dependence of V_e is shown in Figure 1. The observed V_e passed through a minimum as flow rate was increased. The flow rate corresponding to the minimum occurred at approximately 1.0 ml/min. The dependence of peak elution volume on flow rate has been reported by several authors.[1-3,9-11] If the GPC unit collects the effluent liquid in a siphon for the purpose of monitoring the flow and the elution volume, errors will arise from solvent evaporation in the siphon chamber and from solvent continuing to flow into the siphon during discharge. With the siphon calibration established for the various flow rates investigated, Yau et al.[10] observed the flow rate dependence expressed in corrected elution volumes in the range from 0.1 to 10.0 ml/min. In their study, the elution volume of the polystyrene peaks decreased with increasing flow rate. Little et al.[1,2] on the other hand, found V_e was independent of flow rate in the range of 0.1 to 10 ml/min. The system of the present study did not include a siphon, so that problems arising from it could be neglected. Spatorico[11] reported that a negligible dependence of V_e on flow rate was observed in porous glass packings studies, whereas a small increase in V_e was observed with increasing flow rate in the range of 0.2 to 1.0 ml/min in polystyrene gel studies. The results of the present study were in agreement with those reported by Yau et al. at flow rates below 1.0 ml/min and with the observations made by Spatorico over 1.0 ml/min.

Viscosity effects might be predominant at higher flow rate for high-speed GPC because of high fluid frictional resistance. Thus, an increase in flow rate produces an increase in viscosity effects as secondary result. Similarly, an increase in concentration of sample solution[9] and a decrease in column temperature[12] bring about an increase in viscosity effects, and as a result, elution volume will increase. The results at lower flow rate are in agreement with the work by Yau et al., because viscosity effects are negligible.

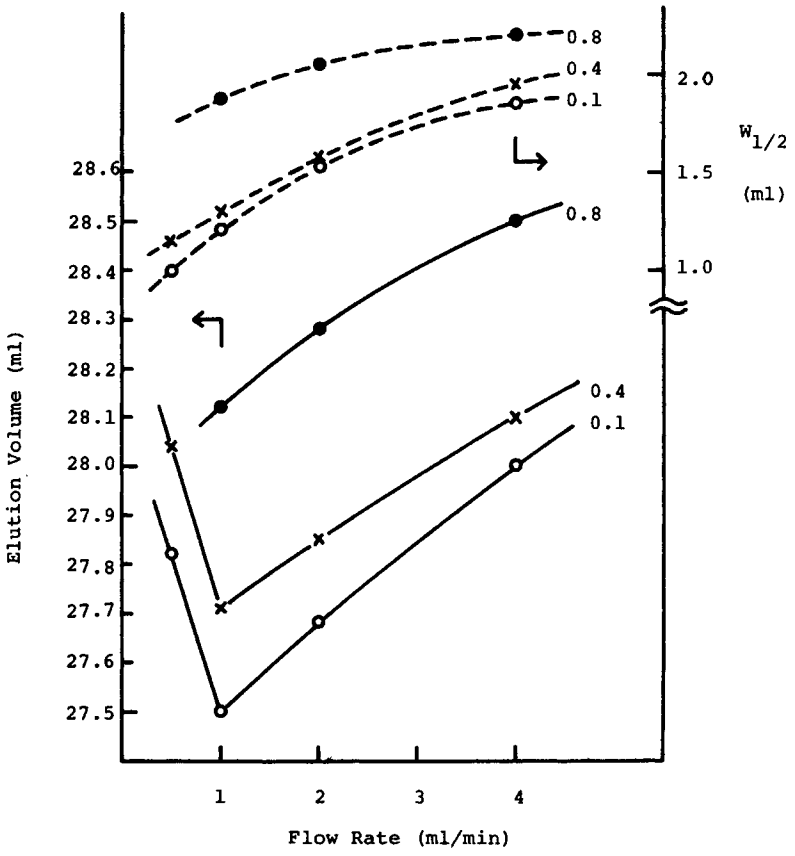

Fig. 1. Dependence of elution volume and peak width at half-height on flow rate at 0.10 ml injection: (——) elution volume vs. flow rate; (- - -) $W_{1/2}$ vs. flow rate. Numbers on curves refer to sample concentration in %.

Examples of variation of N and $W_{1/2}$ with flow rate are shown in Figures 1 and 2. The values of N were approximately inversely proportional to the root of flow rate. With increasing flow rate, the values of N decreased and peak width at half-height increased in the entire system. These results were in agreement with the work of Little et al.[1,2] and Gudzinowicz and Alden.[3]

Limpert et al.[5] showed plots of flow rate versus N for 5-, 10-, and 20-μm polystyrene gels in high-speed GPC and observed that there was an optimum flow rate as the van Deemter equation predicts when 5- and 10-μm polystyrene gels were used. The results for 20-μm polystyrene gel were similar to those in the present study. According to the manufacturer's bulletin, particle size of gels in μ-Styragel columns supposedly is 10 μm, and the main reason for the difference in the results between Limpert et al. and the present study might be that the theoretical plate numbers were measured using polystyrene in the present study.

Effect of Injection Volume

The effect of injection volume on V_e, N, and $W_{1/2}$ were investigated, and the results are shown in Figures 3 and 4. Elution volumes increased and N decreased

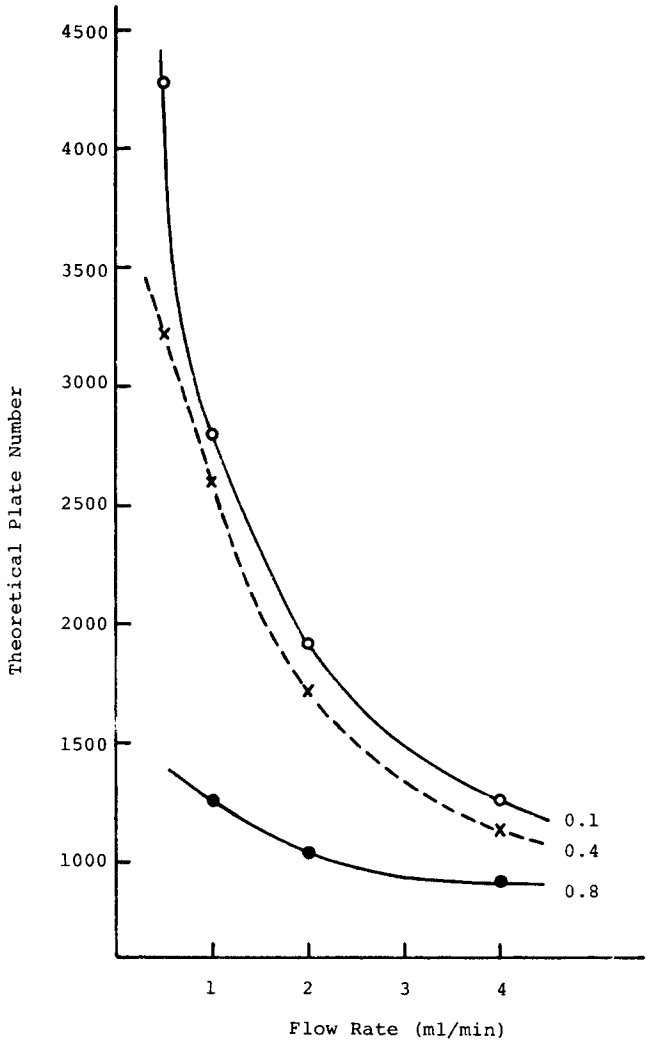

Fig. 2. Theoretical plate number as a function of flow rate and sample concentration at 0.10 ml injection volume. Numbers on curves refer to sample concentration in %.

as injection volume increased. The increment of V_e between 0.1 ml and 0.25 ml was larger than that between 0.25 ml and 1.0 ml, suggesting that precise or constant injection is required if the injection volume is smaller. The injection volume-versus-N plots showed that these relations were different at different concentrations and flow rates. The observed shift in N was smaller at injection volumes of 0.25 to 0.5 ml and at flow rates of 4 ml/min.

Boni et al.[9] observed an increased V_e with increased injection volume, and subtraction of one half the injection volume from the observed elution volumes produced an essentially constant value. The results in Figure 3 showed that increments of V_e were larger than those of injection volume, except for the cases at 0.1% concentration in the range of 0.25 to 1.0 ml injection volume. In view of the large effect of injection volume on V_e, it is important to use the same injection volume for the sample of interest as when constructing the calibration curve.

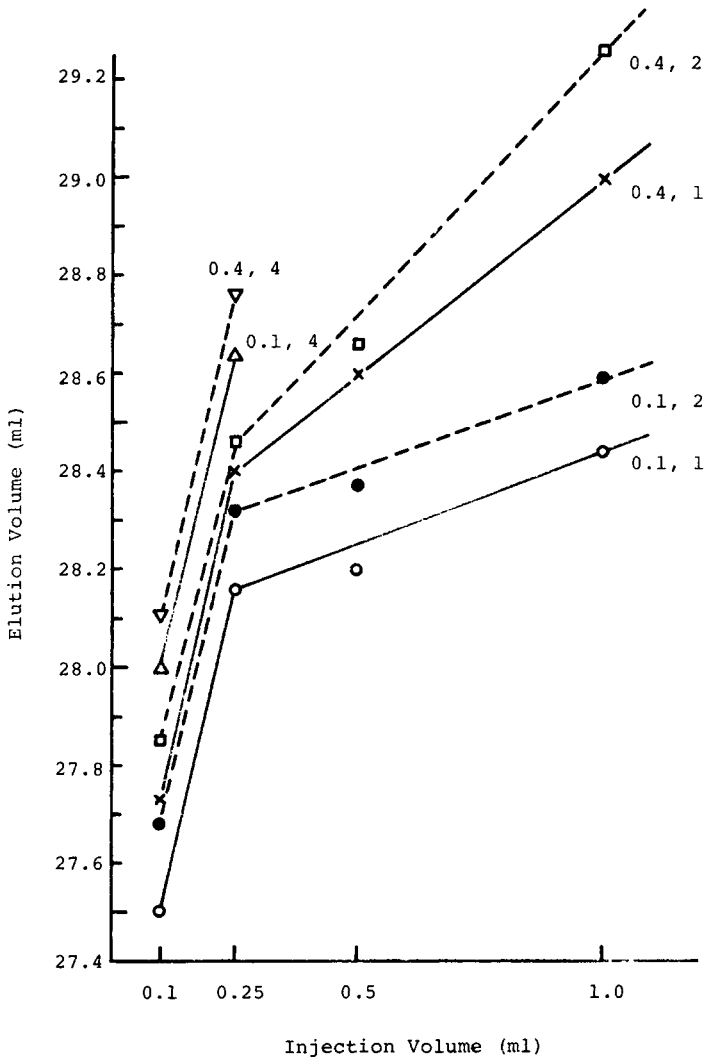

Fig. 3. Effect of injection volume on elution volume at different concentrations and flow rates. Numbers on curves refer to sample concentration in % and flow rate in ml/min in this order.

The effect of injection volume on measured peak width depended on sample concentration. Spreading was independent on injection volume in case of 0.1% concentration, except for injection where the increment in $W_{1/2}$ corresponded to that of the injection volume. The increase in peak width, more than that of injection volume, was found even at small injection volume in case of 0.4% concentration. These findings indicate that 0.4% concentration was too high and 1 ml injection volume was too much.

Effect of Concentration

Elution volume increased with increasing sample concentration, which was similar to that reported previously using conventional GPC columns.[9,11] The relation between V_e and concentration could be expressed as a linear equation

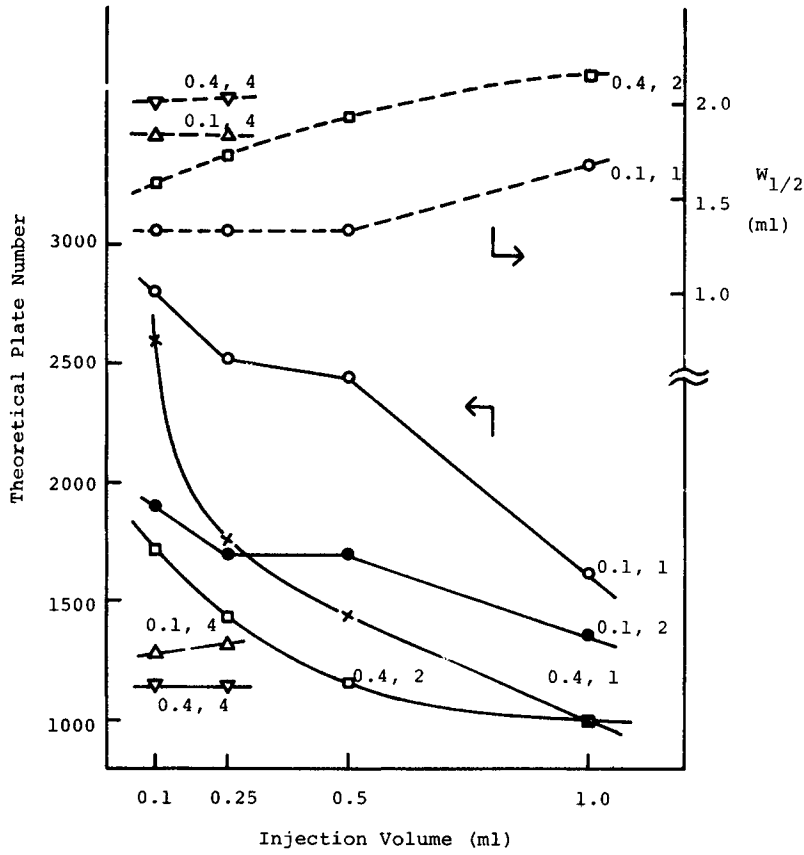

Fig. 4. Effect of injection volume on theoretical plate number and peak width at half-height: (——) N vs. flow rate; (- - -) $W_{1/2}$ vs. flow rate. Numbers on curves refer to sample concentration in % and flow rate in ml/min in this order.

of concentration in the range studied. The slope of the plots became steeper with increasing injection volume and/or with decreasing flow rate, indicating that the concentration dependence of V_e decreased. The increment of V_e per 0.1% concentration, ΔV_e, which is proportional to the slope of the plots, is shown

TABLE I
Variation of Elution Volume Against Increase in Concentration and its dependence of Injection Volume and Flow Rate

Injection volume, ml	Flow rate, ml/min	Increment of elution volume per 0.1% concentration, (ΔV_e), ml	Elution volume extrapolated to zero, $[(V_e)_0]$, ml	$[\Delta V_e/(V_e)_0]$
0.1	1	0.07	27.43	0.00255
	2	0.0575	27.62	0.00208
	4	0.0325	27.97	0.00116
0.25	1	0.08	28.08	0.00285
	2	0.065	28.28	0.00230
	4	0.035	28.62	0.00122
0.5	1	0.15	28.15	0.00534
	2	0.10	28.35	0.00353

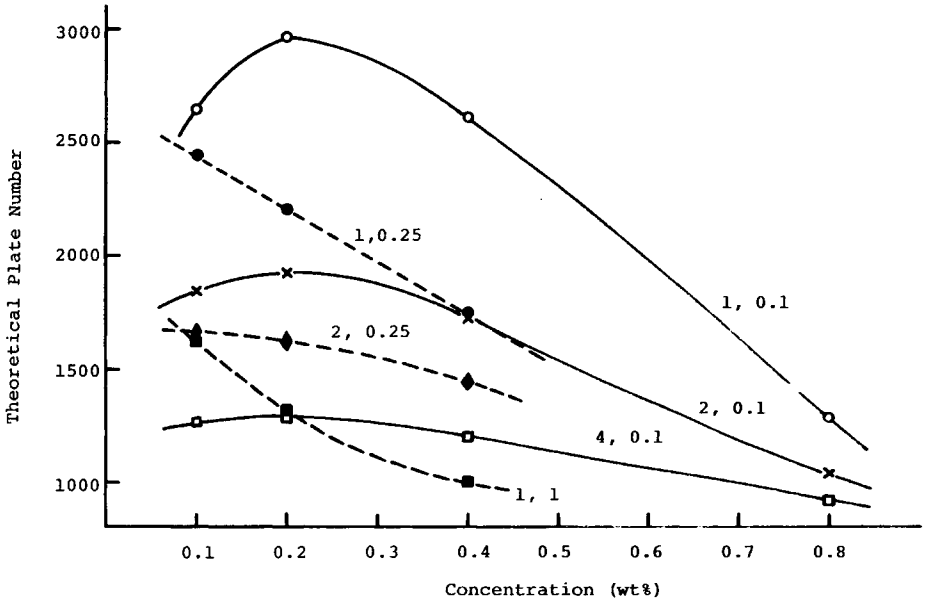

Fig. 5. Theoretical plate number as a function of concentration, flow rate, and injection volume. Numbers on curves refer to flow rate in ml/min and injection volume in ml in this order.

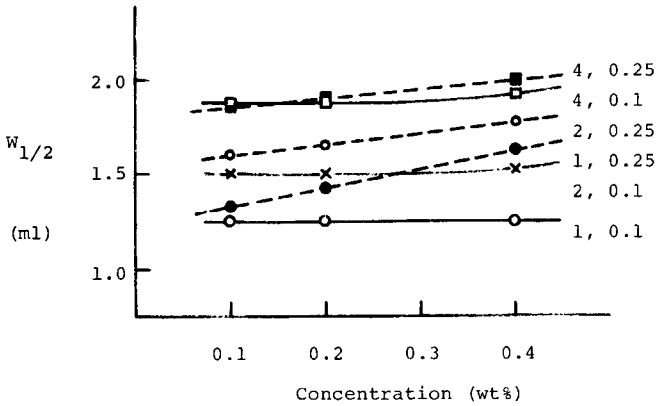

Fig. 6. Peak width as a function of concentration, flow rate, and injection volume. Numbers on curves refer to flow rate in ml/min and injection volume in ml in this order.

in Table I. The dependence of ΔV_e on injection volume and flow rate can be seen from Table I.

The example of the concentration dependence of N is shown in Figure 5. The observed N for 0.1 ml injection passed through a maximum as concentration increased, and the concentration corresponding to the maximum occurred at approximately 0.2%. The N values for over 0.25 ml injection volume decreased with increasing sample concentration. The slope of the plots for N and concentration decreased with increasing flow rate.

As is shown in Figure 6, the concentration dependence of the peak width was observed over 0.25 ml injection, whereas the peak width at injection volume of

0.1 ml was found to be independent of concentration. The variation of peak width for concentration decreased with increasing flow rate.

Chuang and Johnson[13] observed that little or no effect of sample size on elution curves was obtained for molecular weights up to 200,000 and that, above this molecular weight, increases in V_e and peak width were observed with increasing concentration. The plot of N and concentration of molecular weight of 200,000 in their study showed that the relation was almost unchanged between 0.05% and 0.8% concentrations and N increased at 0.0125%. They used porous glass CPG-10 as packing materials, and the system was classical low-speed GPC, which might have been responsible for these differences.

Relation Between Sample Load and N

The values of theoretical plate number increase in general with decreasing flow rate, injection volume, and sample concentration. However, by using smaller injection volume and sample concentration, we also sacrifice detector sensitivity. Smaller flow rate consumes analytical time, but it is preferable from the standpoint of the lifetime of the columns used. Consequently, a compromise among flow rate, injection volume, and sample concentration should be made.

Table II shows the changes of the values of N at equal sample load with different flow rates (1 ml/min and 2 ml/min). From Table II, it can be seen that at equal sample load (sample of equal weight was injected), lower concentration, and larger injection volume are more desirable for N than the opposite.

Dependence of Molecular Weight on Operational Variables

Significant increases in the values of V_e, N, and $W_{1/2}$ with increasing concentration, for the higher molecular weight polystyrenes, generally affect the calculation of molecular weight averages and the correction of peak broadening effects. Comparisons of the theoretical plate number and peak width at half-height versus concentration for some polystyrenes of different molecular weights are shown in Table III. In this table, N was normalized for polydispersity[7] by simply multiplying by d^2. The d value is the sample polydispersity. The con-

TABLE II
Relation Between Sample Load and Values of Theoretical Plate Number for Polystyrene (M = 200,000)

Injection volume, ml	Concentration, %	ml × %	N at flow rate of 1 ml/min	N at flow rate of 2 ml/min
0.25	0.4	0.10	1740	1440
0.50	0.2	0.10	2000	1660
1.00	0.1	0.10	1670	1330
0.10	0.8	0.08	1270	1050
0.10	0.2	0.020	2960	1930
0.25	0.1	0.025	2520	1690
0.10	0.4	0.04	2600	1720
0.25	0.2	0.05	2200	1620
0.50	0.1	0.05	2450	1700

centration dependence on operational variables increases with increasing molecular weight of solute.

Table IV shows the increment of molecular weight with increasing concentration by 0.1%, for different molecular weight polystyrenes, at the same elution position. Porous glass beads, CPG-10, were used for comparison.[6] The concentration dependence for high-speed GPC was significantly higher than classical low-speed GPC. Here, "classical low-speed GPC" means that three to four 4-ft × ⅜-in. columns of varying porosities were used, and analysis times were 2 to 3 hr. If Styragel of 30- to 80-μm particle size was used as packing material, it would be named conventional GPC.

TABLE III
Theoretical Plate Number and Peak Width at Half-Height as a Function of Molecular Weight and Concentration[a]

MW of PST	Concentration, %	N_0	$W_{1/2}$, ml
1,800,000	0.4	520	3.00
	0.2	780	2.40
	0.1	860	2.24
670,000	0.4	1190	2.02
	0.2	1380	1.86
	0.1	1670	1.66
200,000	0.4	1620	1.76
	0.2	1820	1.66
	0.1	1900	1.62
97,200	0.4	2180	1.60
	0.2	2250	1.56
	0.1	2280	1.56
20,400	0.4	2960	1.52
	0.2	2940	1.52
	0.1	2790	1.56
2,100	0.4	3160	1.76
	0.2	3160	1.76
	0.1	3030	1.80

[a] Determined at flow rate of 2.0 ml/min at injection volume of 0.25 ml. N_0 means N which was normalized polydispersity ($N_0 = 5.54\ (V_e/W_{1/2})^2 d^2$); d is polydispersity of the polymer.

TABLE IV
Influence of Concentration on Molecular Weight

	Increment of molecular weight against 0.1% concentration increase	
MW of PST	High-speed GPC (μ-Styragel®)	Low-speed GPC[6] (CPG-10)
1,800,000	350,000	150,000
670,000	60,000	25,000
200,000	7,000	5,000
97,200	3,700	2,000
20,400	400	300

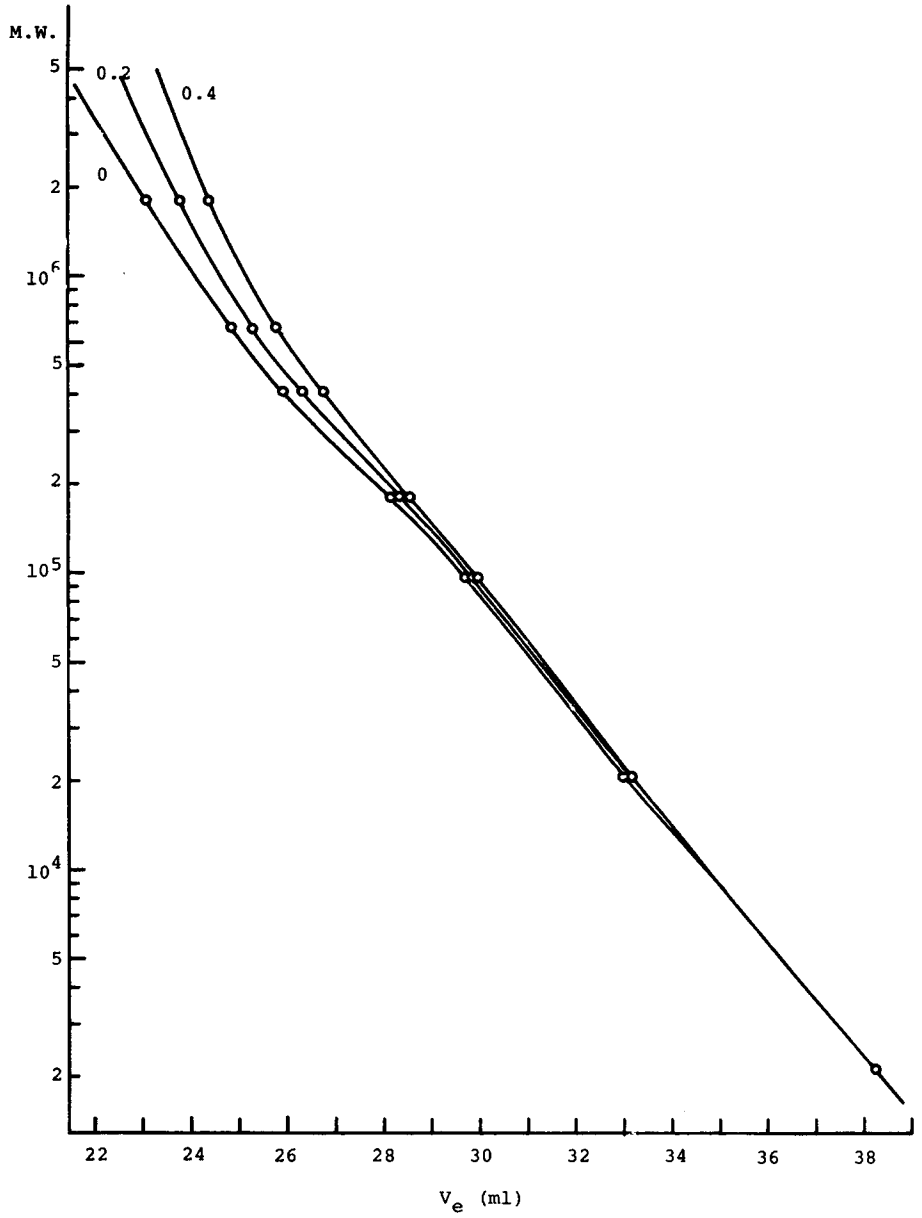

Fig. 7. Calibration curves at different concentrations. Numbers on curves refer to concentration in %.

Calculation of Molecular Weight Averages of NBS 706 Polystyrene

Calibration curves for 0.2% and 0.4% concentration and that extrapolated to zero were constructed at a flow rate of 2 ml/min and injection volume of 0.25 ml and are shown in Figure 7. The magnitude of concentration dependence on V_e was higher for higher molecular weight polystyrenes than lower ones. For example, solutes of 7.8×10^5 and 1.1×10^6 molecular weights appeared at elution

volume of 25 ml when sample concentrations were 0.2% and 0.4%, respectively.

Weight- and number-average molecular weights of NBS 706 polystyrene measured at 0.4% concentration were 3.84×10^5 and 1.52×10^5, which were far from the values measured by classical methods. As was discussed, 0.4% concentration was overconcentration, so that these results were not surprising. By using a concentration correction method,[6] weight- and number-average molecular weights of NBS 706 were reduced to 2.85×10^5 and 1.37×10^5, which was in close agreement with the values from classical methods.

In conclusion, for high-speed GPC, a lower concentration such as 0.1% is preferable. Tetrahydrofuran is a better solvent for high-speed GPC; also, the differential refractive index of polystyrene and tetrahydrofuran is higher than that of polystyrene and toluene, which makes possible the use of sample solution of lower concentration. However, as many other polymer–solvent systems have lower differential refractive indexes, it is sometimes necessary to prepare a sample solution of high concentration. In this case, a concentration correction method[6] will be very valuable.

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