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Polymer Reactors and Molecular Weight Distribution. VII. Further Development of Gel Permeation Chromatography

JOHN H. DUERKSEN* and ARCHIE E. HAMIELEC, *Chemical Engineering Department, McMaster University, Hamilton, Ontario, Canada*

Synopsis

The results of an investigation into molecular weight and resolution calibration of a gel permeation chromatograph are reported. Effects of sample amount and solvent flow rate were observed. Severe skewing and tailing of the standard chromatograms were observed at 2.0 ml/min flow rate, relative to 1.0 and 3.0 ml/min. Resolution calibration by flow reversal was inadequate when skewing and tailing or sample impurity effects were significant. Differential molecular weight distributions (MWD) of broad-distribution polystyrenes were compared for four methods of resolution correction. Inconsistent oscillations were observed in the MWD's at low resolutions and higher molecular weights. None of the methods was completely adequate in accounting for skewing and tailing.

INTRODUCTION

Part III of the previous papers in this series[1] reported a preliminary evaluation of four methods[2–5] of correcting for the imperfect resolution of the gel permeation chromatograph (GPC).[6] Molecular weight averages calculated by the four methods for different column combinations were compared. The samples analyzed were broad-distribution polystyrenes produced by free-radical polymerization.[7,8] This paper reports the results of an investigation into molecular weight and resolution calibration and makes a further evaluation of the four methods of resolution correction. Differential molecular weight distributions (MWD) are compared for both low and high molecular weight polystyrenes (up to $\bar{M}_w = 10^6$) for different column combinations and a range of GPC operating conditions.

GPC COLUMN COMBINATIONS

The thirteen column combinations tried in this investigation are described in Table I. The maximum rated porosity of each column is the

* Present address: Chevron Research Company, Richmond, California 94802.

TABLE I
Description of GPC Column Combinations[a]

Col. combin. code no.	No. of columns in series	Max. rated porosities, straight-chain (for column no.), Å					Flow rate, ml/min	Combin. plates/ft (ODCB)[b]
		1	2	3	4	5		
1	4	10^6	10^4	900	400	—	1.0	380
2	5	10^4	900	800	400	45	1.0	595
3	5	10^4	10^4	900	800	800	1.0	873
4	3	10^5	10^4	400	—	—	1.0	—
5	3	10^5	10^4	800	—	—	1.0	615
6	4	10^6	10^5	10^4	800	—	1.0	580
7	1	10^5	—	—	—	—	1.0	—
8	2	10^5	800	—	—	—	2.0	470
9	2	10^5	800	—	—	—	3.1	614
10	3	10^5	10^4	800	—	—	2.0	364
11	3	7×10^6	10^6	10^5	—	—	2.0	—
12	5	7×10^6	10^6	10^5	10^4	800	2.0	—
13	3	10^5	10^4	800	—	—	3.0	467

[a] The GPC operating temperature for all column combinations was 24 ± 2°C.
[b] Orthodichlorobenzene.

extended or straight chain length of the polymer molecule that would just be excluded from the largest pore in the gel. Thus, it is not a true measure of the maximum pore size, since the polymer molecule would be coiled in solution and have a dimension much smaller than its extended chain length. It has been shown[9-11] that the hydrodynamic diameter is the molecular dimension that determines whether a polymer molecule will be excluded from a gel pore.

The plates-per-foot values in Table I refer to the number of theoretical plates per foot of column length. The total number of theoretical plates is given by[12,13]

$$N = (4V_e/w)^2$$

where V_e is the peak elution volume and w is the baseline width between lines drawn tangent to the chromatogram at its inflection points. Although the theoretical-plate concept is a staged concept, it can be a useful tool for characterizing the efficiency of the continuous process in the GPC columns. It has been used to test the effect of operating variables on column efficiency.[10,13-15] The number of theoretical plates depends on the solute injected. The values in Table I are for orthodichlorobenzene (ODCB). It is interesting to note that for column codes 8 and 9 and codes 10 and 13 the number of plates increases with increasing flow rate, contrary to expected behavior.[13]

MOLECULAR WEIGHT CALIBRATION

For conversion of the GPC chromatograms to MWD's each column combination was calibrated for molecular weight versus elution volume by using the narrow-distribution polystyrene standards described in Table II. Since the standards are not truly monodisperse, $\bar{M}_n \neq \bar{M}_v \neq \bar{M}_w$, and the problem arises of which molecular weight average, if any, corresponds to the peak position of each standard chromatogram. Since all but the high molecular weight standards have relatively narrow MWD's ($\bar{M}_w/\bar{M}_n$ less than 1.1), any error introduced in the choice of average is small. Waters Associates recommend the use of root-mean-square average, defined as $\bar{M}_{\text{rms}} = (\bar{M}_n \times \bar{M}_w)^{1/2}$. This implies that the chromatogram peak position corresponds to a molecular weight between $\bar{M}_n$ and $\bar{M}_w$.

Since the GPC molecular weight calibration uses standards with molecular weight averages measured by other methods (osmometry, viscometry, and light scattering), any inaccuracies in these methods will lead to inaccuracies in the calibration curve. Where provided, the reproducibility limits of the averages for the standards are indicated in Table II.

The calibration curves for codes 1, 2, 3, 5, 8, and 9 have already been presented,[1,7,16] but codes 5, 8, and 9 are also presented here for purposes of comparison. Since codes 4 and 7 were not used for sample analysis, their calibrations are not presented. The calibration curves for codes 5, 6, and 8–13 are presented in Figures 1–6.

TABLE II
Suppliers' Data for Anionically Prepared Polystyrene Standards

Supplier	Designation	$\bar{M}_n$	$\bar{M}_n$ tech.[a]	$\bar{M}_v$[b]	$\bar{M}_w$	$\bar{M}_w$ tech.[c]	$\bar{M}_w/\bar{M}_n$
Polymer corp.	styrene	104.15	—	—	104.15	—	1.0
Pressure chem.	S 13a	927 ± 7%	VOS	1,220 ± 7%		—	<1.10
		1,060 ± 7%	VOS				
		1,130 ± 7%	VOS				
		912	CRYO				
		1,032	CRYO				
Pressure Chem.	S 12a	2,070 ± 5%	VOS	2,070 ± 5%	2,200 ± 10%	kinetics	<1.10
Pressure Chem.	S 11a	5,200 ± 5%	VOS	4,700 ± 5%	—	—	<1.10
		5,200 ± 7%	MOS				
Pressure Chem.	S 8a	10,900 ± 5%	MOS	10,500 ± 4%	10,000 ± 10%	LS	<1.06
Pressure Chem.	S 2a	19,800 ± 3%	MOS	19,600 ± 3%	19,800 ± 2%	LS	≤1.06
Waters Assoc.	S 4190039	19,650	—	—	19,850	—	1.01
Pressure Chem.	S 7a	50,100 ± 5%	MOS	51,000 ± 3%	50,500 ± 4%	LS	≤1.06
Pressure Chem.	S 4a	97,600 ± 5%	MOS	98,200 ± 3%	96,200 ± 2%	LS	≤1.06
Dow	S 103	—	—	—	127,000	UC	—
H. W. McCormick		118,000	—	—	124,000	—	1.05
		109,000	MOS	—	117,000	LS	1.07
		—	—	128,000	126,000	UC	—

Pressure Chem.	S 1a	—	—	160,000 ± 3%	—	—	≤1.06
Waters Assoc.	S 41984	164,000	—	—	173,000	—	1.06
Dow	S 108	247,200	MOS	—	267,000	LS	1.08
H. W. McCormick		236,000	MOS	—	242,000	LS	1.03
		—	—	264,000	253,000	UC	—
Pressure Chem.	S 3a	392,000 ± 5%	MOS	411,000 ± 3%	394,000 ± 2%	LS	≤1.06
Pressure Chem.	S 5a	404,000 ± 5%	MOS	498,000 ± 3%	507,000 ± 4%	LS	≤1.20
Pressure Chem.	S 6a	735,000 ± 5%	MOS	842,000 ± 3%	862,000 ± 2%	LS	1.17
		773,350	FR	—	867,000	FR	1.12
Waters Assoc.	S 4190038	773,000	—	—	867,000	—	1.12
Pressure Chem.	S 14a	1.61×10^6	FR	$1.7 \times 10^6 \pm 4\%$	1.9×10^6	FR	1.18
		—	—	—	$1.7 \times 10^6 \pm 4\%$	LS	—
Waters Assoc.	S 61970	1.78×10^6	—	—	2.145×10^6	—	1.20
G. C. Berry Mellon Inst.	A-82	—	—	—	$4.04 \times 10^6 \pm 10\%$	EST	—
G. C. Berry, Mellon Inst.	A-82-F	—	—	—	4.04×10^6	LS	—
G. C. Berry, Mellon Inst.	A-30	—	—	—	4.40×10^6	LS	—

[a] VOS = vapor pressure osmometry, MOS = membrane osmometry, CRYO = cryoscopy, FR = fractionation.

[b] All viscosity-average molecular weights were determined by intrinsic viscosity measurements.

[c] LS = light scattering, EST = estimated, UC = ultracentrifugation.

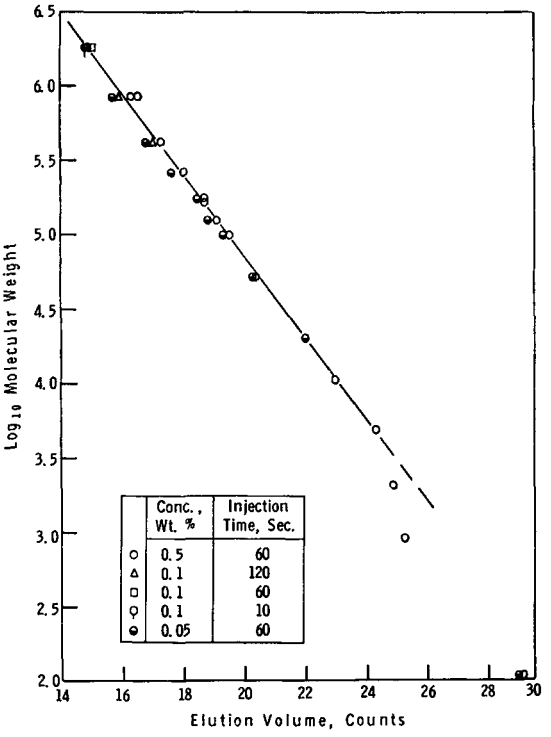

Fig. 1. Molecular weight calibration curve for code 5.

There are several possible sources of error or variation in the calibration curves in addition to the two already discussed. Variations with time and solvent are possible, but sufficient calibration checks can account for these variations. Two more serious sources of variation are sample concentration or amount and solvent flow rate.

Variation with Sample Concentration or Amount

At sufficiently high concentration levels an increase in sample concentration or amount injected causes a shifting of the chromatogram peak to a higher elution volume, as shown in Figure 7. Several explanations of this behavior have been proposed.[9] The direction of the shift indicates that it is due to the viscosity of the polymer solution.[9] When the solution enters the first GPC column, it creates a zone of higher viscosity. The extra pressure drop caused by the sample viscosity permits the eluting solvent to push through at some weak point in the sample zone, causing an uneven velocity profile at that point, until considerable dilution of the sample has occurred. This phenomenon has been called "viscous fingering."[12] It causes the sample to have a longer residence time (i.e., greater elution volume) and causes distortion and tailing in the sample chromatogram. Since viscosity increases with molecular weight as well as with

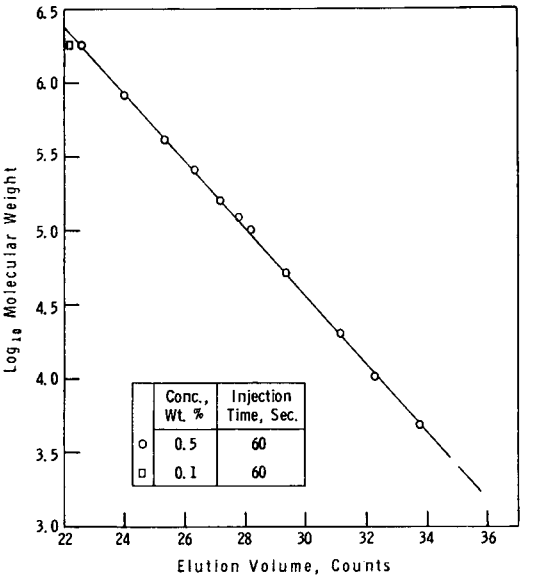

Fig. 2. Molecular weight calibration curve for code 6.

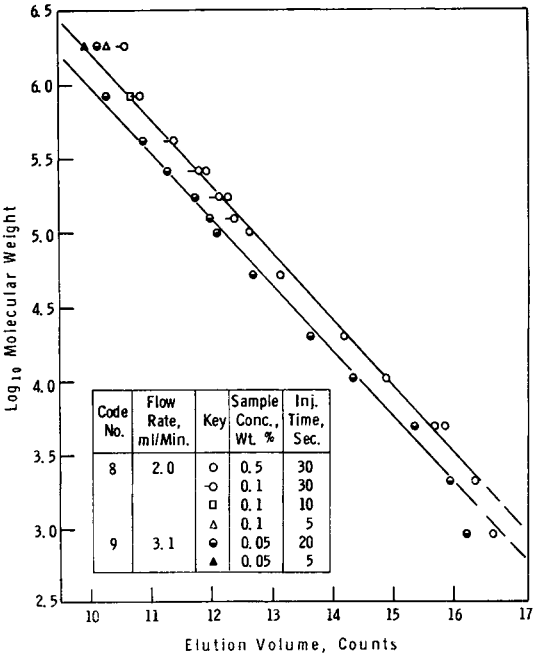

Fig. 3. Molecular weight calibration curves for codes 8 and 9.

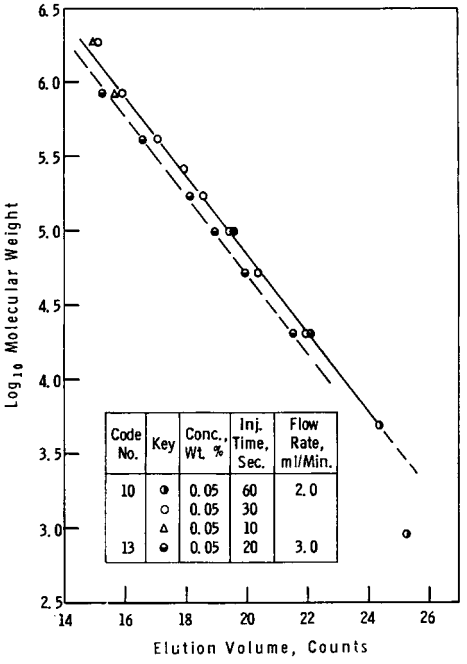

Fig. 4. Molecular weight calibration curves for codes 10 and 13.

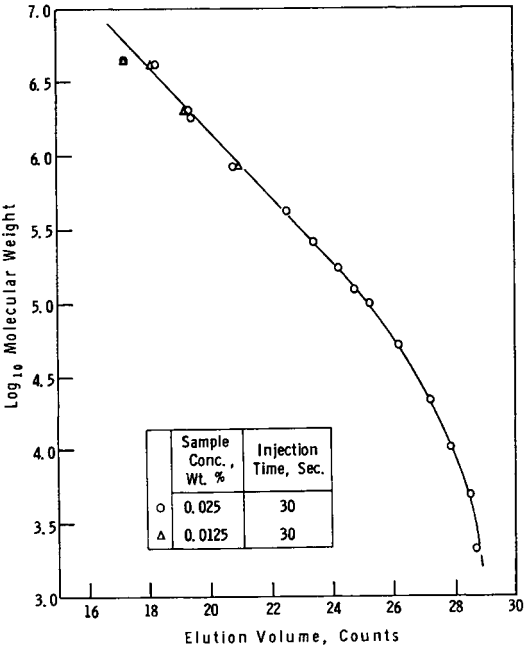

Fig. 5. Molecular weight calibration curve for code 11.

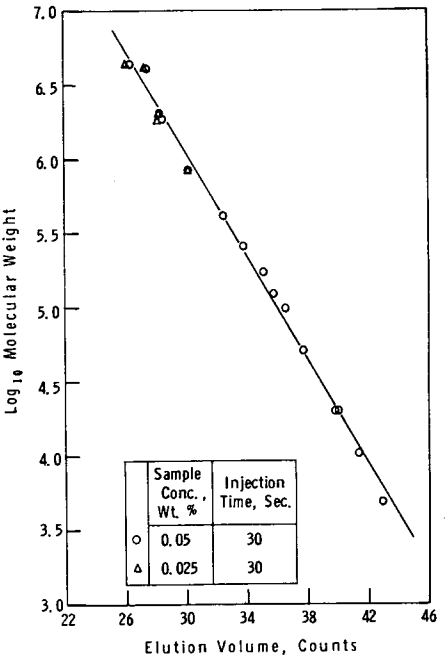

Fig. 6. Molecular weight calibration curve for code 12.

concentration, the effect should be more pronounced with the higher molecular weight standards, as was observed in Figure 7. Figure 7 indicates that the magnitude of the peak shift may also depend on the number of columns used.

Several workers[9,13] have shown that for many polymers the calculated GPC $\bar{M}_n$ and $\bar{M}_w$ decrease with increasing concentration or amount injected. However, in calculating $\bar{M}_n$ and $\bar{M}_w$ they used a single calibration curve. This may not be a valid procedure, since the calibration curve itself can be a function of concentration, as shown in Figures 1–6. The question then arises what calibration curve should be used for a particular sample concentration.

The GPC sample concentration should be in the region where the calibration curve has no concentration dependence. Because of the limitations of the GPC detector this may not always be possible, especially at high molecular weights. Boni et al.[17] have proposed extrapolation of the calibration curve to zero concentration. They argue that the use of the extrapolated curve is justified because the concentration in the effluent for a broad-distribution sample is significantly lower than for the narrow-distribution calibration standards, especially at the high molecular weight end of the chromatogram. Their approach would be more valid if both the calibration curve and the sample chromatogram were extrapolated to zero concentration. The added time and effort involved in injecting the

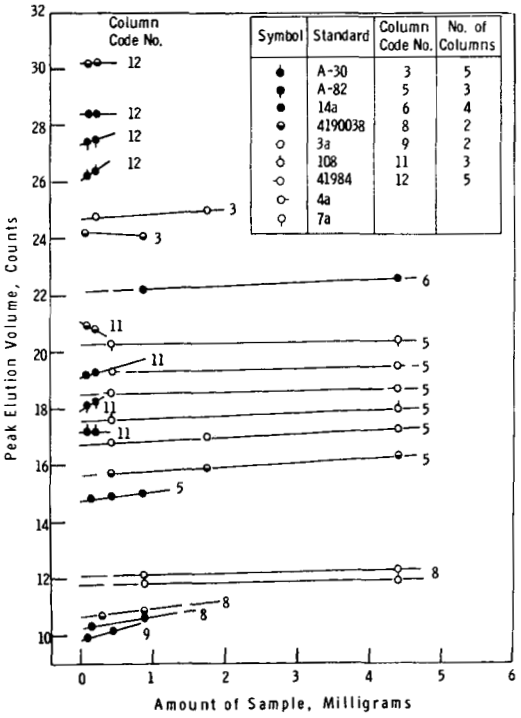

Fig. 7. Variation in peak elution volume with amount of sample injected.

sample at several concentrations and extrapolating would reduce the advantage of rapid analysis time.

A computational method of accounting for the concentration dependence might be possible. Calibrating for molecular weight over a range of concentrations would yield a functional relationship, possibly linear[17] (Fig. 7), between elution volume and concentration for each molecular weight standard. From the sample chromatogram the concentration at each elution volume would be known, and the corresponding molecular weight could be calculated. In effect, this would provide a calibration curve for each sample analyzed. Use of a computer would minimize the time and effort required.

In this investigation the calibration curve was usually an estimated best straight line through the points for the calibration standards (Figs. 1–6). The use of a straight line simplified the computation of MWD. A significant effect of sample concentration or amount can be observed in the calibration curves for codes 5 and 8 (Figs. 1 and 3). The effect generally becomes noticeable above a molecular weight of 100,000.

Variation with Solvent Flow Rate

Several workers[9,13–15,17–22] have investigated the effects of solvent flow rate on peak elution volume and column efficiency (i.e., number of

theoretical plates or height equivalent to a theoretical plate). Some of their results and the results from this investigation are shown in Figures 8 and 9.

In addition to extra column effects[21,22] variations in elution volume and column efficiency can be due to a flow rate effect on (*1*) size of the polymer molecule in solution, (*2*) interstitial volume of the gel, (*3*) pore size of the gel, (*4*) distribution of each polymer species between the gel pores and interstitial volume (i.e., nonequilibrium diffusion), and (*5*) velocity profile in the column.

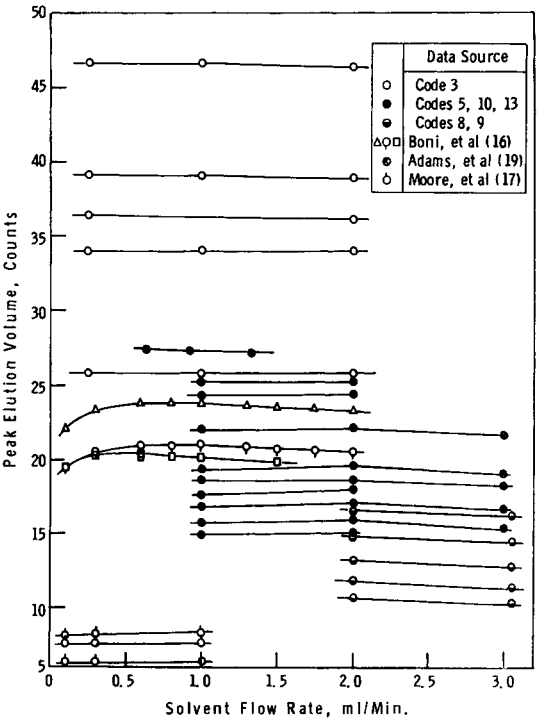

Fig. 8. Variation in peak elution volume with solvent flow rate: (○) code 3; (●) codes 5, 10, and 13; (◒) codes 8 and 9; (△, ♀, □) Boni et al.;[17] (⊗) Adams et al.;[20] (♁) Moore and Arrington.[18]

Boni et al.[17] observed significant variations and a maximum in the peak elution volumes of two polystyrene standards over a flow range of 0.1–2.0 ml/min, both in THF at 23°C and trichlorobenzene (TCB) at 130°C. They concluded that (*1*) shear rate effects on molecular dimensions were negligible, (*2*) nonequilibrium diffusion effects were negligible, and (*3*) changes in elution volume were due mainly to variations in interstitial volume and pore size distribution caused by variation in pressure drop across the columns with flow rate. The latter conclusion was supported by the much smaller variation observed by Moore and Arrington,[18] using a

rigid, porous, glass packing (Fig. 8). Boni et al.[17] did not consider variations in velocity profile with changing flow rate.

Although the results of this investigation are less extensive, they do not show the marked variations in peak elution volume observed by Boni et al.[17] except at flow rates above 2.0 ml/min (Fig. 8). With five columns in

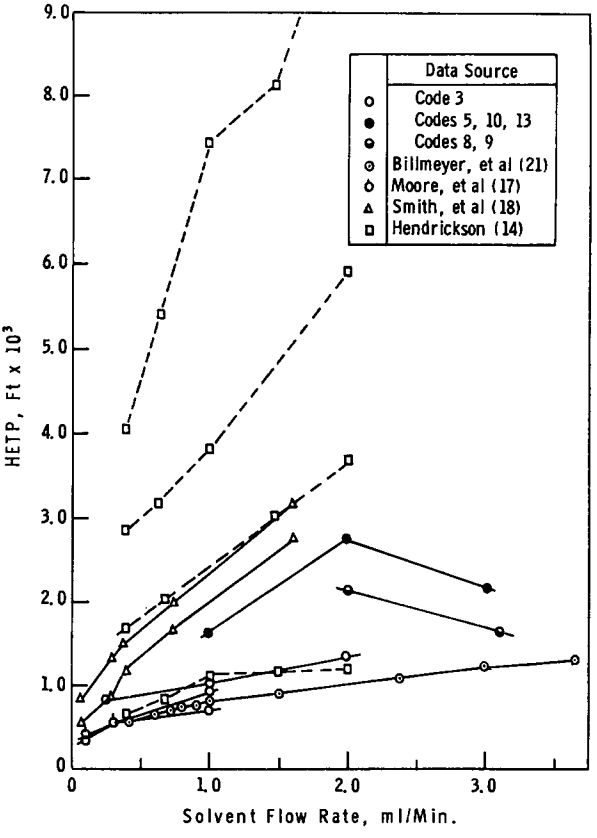

Fig. 9. Variation in HETP with solvent flow rate: (○) code 3; (●) codes 5, 10, and 13; (◒) codes 8 and 9; (⊙) Billmeyer et al.;[22] (◊) Moore and Arrington;[18] (△) Smith and Kollmansberger;[19] (□) Hendrickson.[15]

series (code 3) and equal concentrations and amounts injected the variations in peak position between 0.25 and 2.0 ml/min were negligible. With three columns in series (codes 5, 10, and 13) there was either no significant change in peak elution volume at low molecular weights or a slight increase at high molecular weights at rates of 1.0 to 2.0 ml/min but a significant decrease for all molecular weights at rates of 2.0 to 3.0 ml/min. The magnitude of this decrease (0.5 count) was apparently independent of molecular weight. With two columns in series (codes 8 and 9) a decrease

of approximately the same magnitude was observed at rates of 2.0–3.1 ml/min.

For flow rates ordinarily used in the GPC (about 1.0 ml/min) the fractionating mechanism is assumed to involve diffusional equilibrium of the polymer species; that is, the polymer molecules are assumed to have time enough to distribute themselves among the interstitial volume and available gel pores.[12] At sufficiently high flow rates the rate of polymer transport through the columns is of the same order of magnitude as the molecular diffusion rate, and some fraction of the pores available to each species will not be used, causing the species to appear in the effluent earlier than expected. This could explain at least some of the decrease in peak elution volume observed at flow rates above 2 ml/min.

From equations in the literature[12] it is possible to estimate the importance of nonequilibrium diffusion. One estimate can be obtained by comparing the time required for a zone of polymer to move past a gel particle with the half-time of the polymer species for self-diffusion through the spherical gel particle, called the half-time of diffusion equilibrium, $t_{0.5}$.[12] If the time it takes the solute zone to pass the gel particle is much greater than $t_{0.5}$, nonequilibrium diffusion effects should be negligible.

For a sample volume of 1 ml and from the column dimensions used in this investigation a zone length of 5 cm was estimated. The time required for the zone to move half its length[12] is 15 sec at 2 ml/min and 10 sec at 3 ml/min.

Vermeulen[12] derived the following equation for estimating half-times of diffusion equilibrium:

$$t_{0.5} = 0.03(r^2/\bar{D})$$

where r is the radius of the particle and $\bar{D}$ (cm^2/sec) is the diffusion coefficient in the gel. Waters Associates indicate that most of the gel particles should have a radius of about 0.0025 cm. Diffusion coefficients for polystyrene in various solvents are given in the literature.[23] No values were found for polystyrene in THF, but Hendrickson[15] indicates that such values should be only about 10% greater than the values in toluene. On this basis an estimate of the free diffusion coefficient D for a molecular weight of 100,000 is 5×10^{-7} cm^2/sec. If it is assumed that $\bar{D} = D$, then $t_{0.5}$ is 0.4 sec, which is only a small fraction of the time required for the zone movement.

Altgelt and Moore[12] point out that $t_{0.5}$ may be less than calculated, since the large polymer molecules will normally have access only or mainly to the outer shell of the gel particles, and their traveling distance will be smaller than r. On the other hand, $\bar{D}$ may be significantly smaller than D, owing to the restricted diffusion of the molecules in the irregular gel pores.[9,12] If it is assumed that $\bar{D} = D/10$,[12] then $t_{0.5}$ is about 4 sec, which is a significant fraction of the time required for zone movement.

A second estimate of the nonequilibrium diffusion effects can be obtained from an equation for the concentration equilibration of a solute diffusing

into a gel. It was derived by Vink and modified by Altgelt and Moore[12] to give

$$\Delta C/\Delta C_0 = \exp\{-18\bar{D}t/r^2\}$$

where ΔC_0 and ΔC are the differences in concentrations inside and outside the gel at time $t = 0$ and $t = t$. For $\bar{D} = D/10 = 5 \times 10^{-8}$ cm^2/sec and $r = 0.0025$ cm a deviation from diffusion equilibrium of 12% is calculated for $t = 15$ sec (2 ml/min) and 24% for $t = 10$ sec (3 ml/min).

These estimates indicate that at least part of the decrease in peak elution volume with increasing flow rate above 2 ml/min could be due to the effects of nonequilibrium diffusion. Since the diffusion coefficient decreases with increasing molecular weight,[23] the molecular weight independence of the peak shift (Figs. 3 and 4) is unexpected. A possible explanation is that with increasing molecular weight the effect of decreasing diffusion coefficient is just balanced by the decrease in travel distance into the pores. The reasons for the apparent independence of peak shift on column length also are not obvious.

Variation of the velocity profile in the columns with flow rate might also cause changes in peak elution volume and column efficiency.[9] At a flow of 2 ml/min in this study severe skewing and tailing toward higher elution volume was observed in the chromatograms of the higher molecular weight standards. At 1 and 3 ml/min the tailing was much less severe. Similar behavior was observed by Billmeyer and Kelley,[14] using low-angstrom columns and a nonpermeating polystyrene standard and solvent flow rates ranging from 0.52 to 3.6 ml/min. They proposed that the tailing was due to stagnant areas in the columns. In subsequent studies[21,22] they showed that some of the tailing could be caused by extra column effects in the refractometer cell. They pointed out that this extra column contribution is usually insignificant when more than two columns are used.

Although the variation in velocity profile with viscosity difference between sample and solvent[9] may be regarded as a concentration effect, there may be an interaction between the effect of viscosity difference and solvent flow rate.

If the variation of the column pressure drop with flow rate was causing a change in pore size and interstitial volume and, hence, elution volume, the resolution-corrected molecular weights of the same sample run at two flow rates should agree if the calibration curve for each flow rate is used.[17] Table III compares such data for codes 5, 10, and 13 for Tung's method of resolution correction.[2] The poor agreement between code 10 and codes 5 and 13 probably is due to the inadequacy of the assumed gaussian shape in Tung's method.[1] The good agreement between codes 5 and 13, where the standard chromatograms were close to gaussian and the column pressure drops were significantly different, indicates that the change in peak elution volume with flow rate could be due partly to a pressure drop effect on the gel packing.

TABLE III
Tung's Resolution-Corrected Molecular Weight Averages for One Column Combination and Different Flow Rates and Pressure Drops[a]

Sample no.	$\bar{M}_n$ ($\times 10^{-4}$) for code no.			$\bar{M}_w$ ($\times 10^{-4}$) for code no.		
	5	10	13	5	10	13
1	5.32	4.76	4.55	8.25	8.04	8.13
2	4.00	3.06	3.72	6.23	5.32	6.20
3	4.53	3.77	4.41	7.41	6.44	7.15

[a] Flow rates were 1.0, 2.0, and 3.0 ml/min, and pressure drops were 90, 160, and 240 psig, for codes 5, 10, and 13, respectively.

Further investigation is required over a larger range of flow rates to determine the relative importance of viscosity difference, velocity profile, nonequilibrium diffusion, and pressure drop effects on elution volume.

RESOLUTION CALIBRATION

Since the resolution of the GPC is not infinite,[2-5] a resolution correction must be made to the chromatogram to obtain absolute MWD's. The causes of imperfect resolution or undesirable chromatogram spreading have been discussed in the literature.[9,15,21,22] The causes can be external to the GPC columns[21,22] as well as internal.[9,15]

Two of the four available methods of resolution correction[2,3] use a predetermined resolution factor[1] h to describe the undesirable chromatogram spreading. At present there are three methods of determining h: (*1*) a fitting technique,[24] (*2*) a flow reversal technique,[25] and (*3*) a once-through technique.[15]

The fitting technique requires accurate data for the MWD's of narrow-distribution polymer standards. It converts the MWD to chromato-

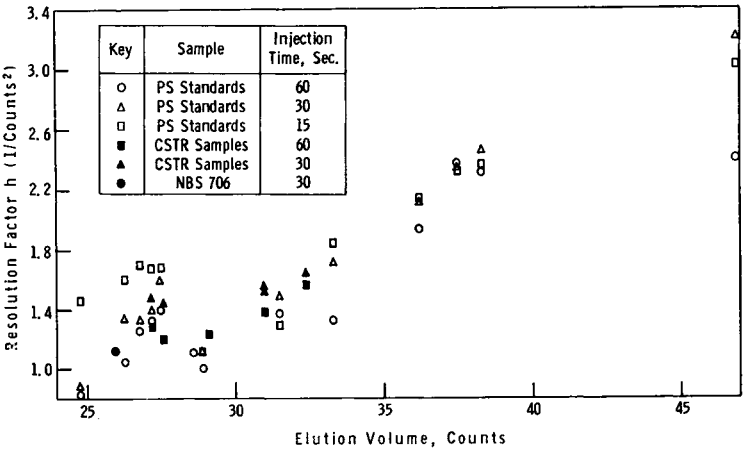

Fig. 10. Resolution calibration curve for code 3 by flow reversal.

grams by using various assumed values of h. These chromatograms are then compared with the experimental chromatogram for the same standard. The correct value of h is that which gives the best fit between the predicted and experimental chromatogram. An optimum search method[26]

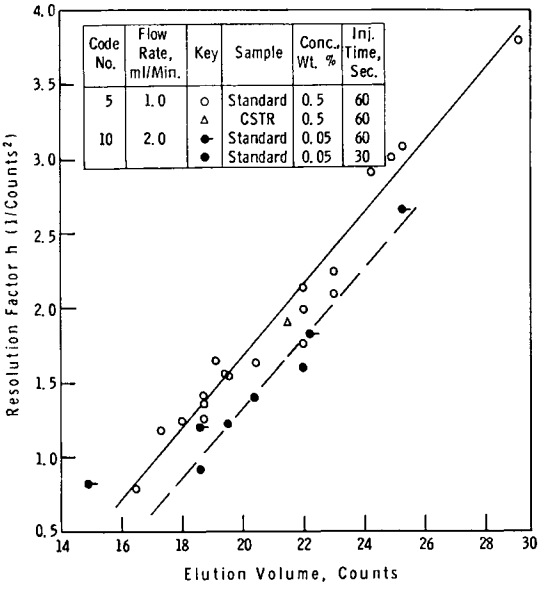

Fig. 11. Resolution calibration curves for codes 5 and 10 by flow reversal.

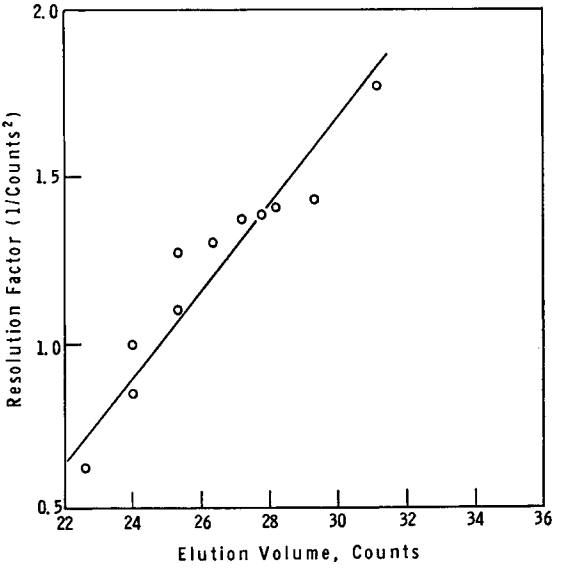

Fig. 12. Resolution calibration curve for code 6 by flow reversal.

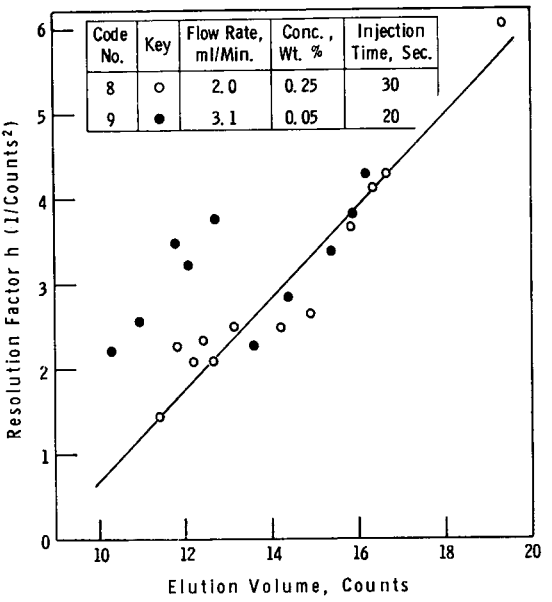

Fig. 13. Resolution calibration curves for codes 8 and 9 by flow reversal.

could be used to do the fitting. This technique assumes that h is a constant over the molecular weight span of each of the standards and that the undesirable chromatogram spreading is gaussian.

The flow reversal technique has already been adequately described.[15,25] It is based on the assumption that spreading due to molecular size difference is completely reversible, so that if a sample is allowed to proceed to some part of the column and then the direction of the solvent flow is reversed, the chromatogram of the eluant reflects only the undesirable spreading. It also assumes that the undesirable spreading is gaussian.

If the spreading due to molecular inhomogeneity is assumed to be negligible, the normal once-through chromatogram of the standards can be treated by the method of moments[25] to yield h. If the chromatograms are skewed, they can be treated as log normal or as two unequal gaussian halves.[27] However, any contribution from molecular inhomogeneity will result in h values that are too low.[1]

A more accurate once-through technique, which accounts for molecular inhomogeneity, has been proposed by Hendrickson.[15] It requires that flow reversal studies first be done to determine the molecular weight range over which the standards would elute if no undesirable peak spreading occurred. If a specific shape is assumed, these ranges can then be used by other investigators to subtract the contribution of molecular inhomogeneity from the once-through chromatogram to obtain the undesirable spreading contribution. Since reverse flow chromatograms do not exhibit significant skewing, this once-through technique would be preferred when the once-through chromatograms are skewed.

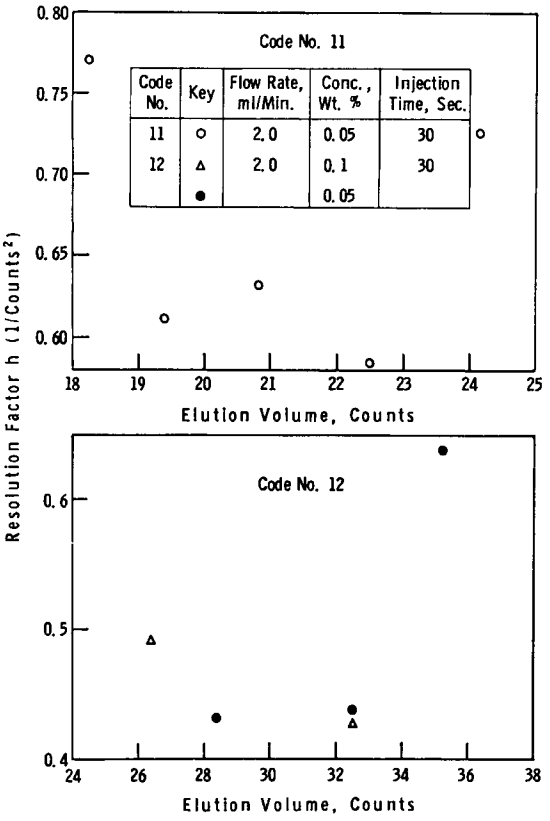

Fig. 14. Resolution calibration curves for codes 11 and 12 by flow reversal.

Pickett et al.[5] have developed a technique that subtracts the contribution of molecular inhomogeneity from the chromatograms of narrow standards. These reshaped chromatograms could be used to obtain h values.

In this study flow reversal was the technique generally used to determine h. The resulting resolution calibration curves for codes 3, 5, 6, and 8–12 are shown in Figures 10–14. When skewing was significant, the once-through chromatograms were divided into two halves at the peak position, and the gaussian h for each half was determined with no correction for molecular inhomogeneity. A typical curve is shown in Figure 15. The following sections will discuss possible sources of variation and scatter in the h curves.

Effect of Impurities

In flow reversal calibration a chromatogram due only to the polymer injected is desired. However, by reversing the flow any impurities in the sample that had become separated from the polymer peak would again

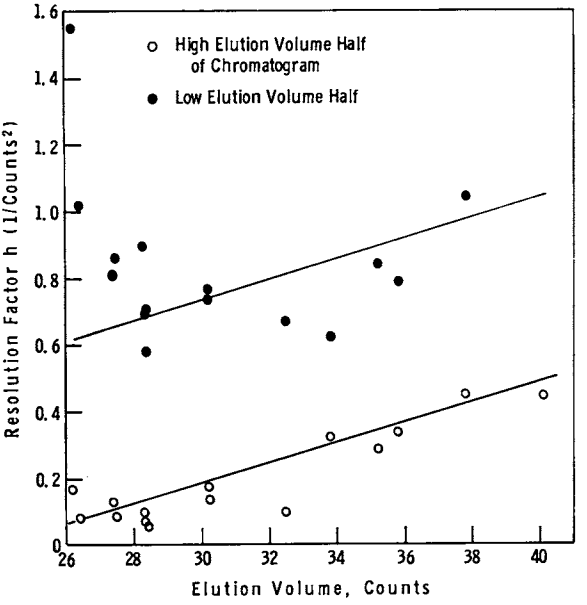

Fig. 15. Typical resolution calibration curves, assuming two gaussian halves for the once-through chromatogram.

come together with the polymer, and the resulting chromatogram would be a superposition of the polymer peak and the impurity peak.

With studies in THF solvent Segal[28] showed that the impurity peaks from air and water are negative relative to the instrument baseline. The polystyrene peak is positive. When impurity effects were significant, a bimodal reverse-flow chromatogram (Fig. 16) was caused by a superposition of the negative impurity peak on the positive polystyrene peak.

The use of a special sample-preparation technique[1] appeared to eliminate most of the impurity distortions in the codes 9 and 10 chromatograms (Fig. 16), except for the higher molecular weight standards. For the high molecular weight standards used in codes 11 and 12 secondary peaks and shoulders were observed even with the special sample-preparation technique (Fig. 16). This indicates that an even more stringent sample-preparation technique is required to make the impurity effects negligible at low sample concentrations (0.1 wt-% and lower).

The bimodal reverse-flow chromatograms for the high molecular weight standards were, in general, quite unsymmetrical (Fig. 16). The shape indicates that the positive polymer peak eluted later than the negative impurity peak. The location of the impurity peak relative to the polymer peak will determine whether the resulting h is higher or lower than the true value. Thus, when significant impurity effects are observed or suspected, the h values calculated from flow reversal can only be considered estimates of the true values.

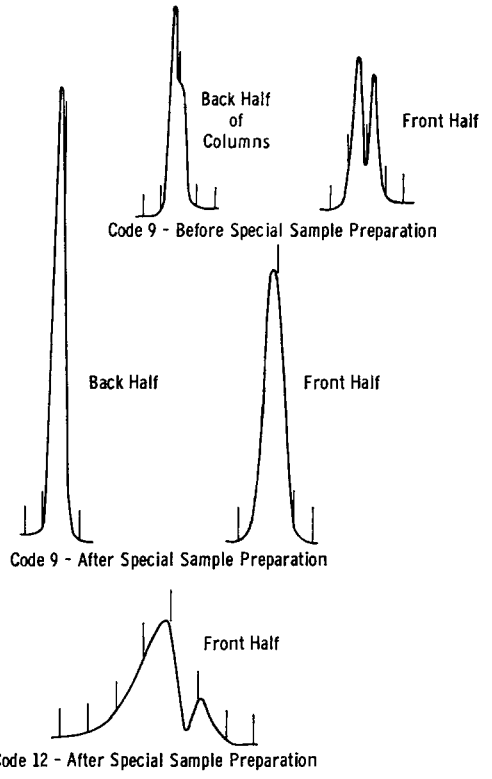

Fig. 16. Typical flow reversal chromatograms.

Effect of Amount Injected

The amount of sample injected can be varied by changing the sample concentration or injection time. For code 3 injection times of 60, 30, and 15 sec were used (Fig. 10). Because of possible impurity effects and scatter, positive conclusions cannot be drawn. There appears to be an increase in h with decreasing injection time at 0.5% sample concentration. However, this effect was not observed for code 10 (Fig. 11) at 0.05% concentration. There is also an unexpected hump in the codes 3 and 9 curves (Figs. 10 and 13) at 28 and 13 counts, respectively. This could be an impurity effect.

Effect of Flow Rate

Figures 11 and 13 show data for the same column combinations and different flow rates. However, since sample concentrations and injection times were also different, no conclusions can be drawn regarding the effect of flow rate. Since the shape (i.e., skewing and tailing) of the once-through chromatograms was strongly influenced by flow rate, it would also be expected to influence the shape of the reverse flow chromatogram and

the resulting h value. As already mentioned, resolution calibration by flow reversal is considered inadequate when the once-through chromatograms are significantly nongaussian, since it only yields a single h value for an assumed gaussian shape.

Effect of MWD

For codes 3 and 5 (Figs. 10 and 11) polystyrenes having narrow and broad MWD's were used for resolution calibration by flow reversal. The narrow standards (Table II) generally had M_w/M_n ratios of less than 1.1. The one broad standard (NBS 706) had a ratio of about 2.0. The other broad samples were experimental polystyrenes[7,8] with ratios of about 1.5 for code 3 and 2.8 for code 5. Considering the scatter in the data, the broad-distribution samples gave about the same h values as did the narrow standards. This indicates that the assumption of reversibility of spreading due to molecular size is reasonably valid over the range studied. However, this does not imply that the spreading due to other factors is necessarily the same in each flow direction. For example, the effect of viscosity difference[9] should be more prominent in the flow direction being used during injection.

Further investigation is required to clarify the limitations of the various methods of calibrating the resolution and to elucidate the effects of sample characteristics and GPC operating conditions.

MOLECULAR WEIGHT DISTRIBUTIONS

Low and intermediate molecular weight averages for the four methods of resolution correction[2–5] were previously reported.[1] Corresponding MWD data and higher molecular weight results are presented and discussed here. Owing to the large volume of data, only typical MWD's are presented. For the methods of Tung[2] and Smith[3] MWD's were obtained for low, intermediate, and high molecular weight ranges ($\bar{M}_n$'s of 10^4 to 5×10^4, 5×10^4 to 10^5, and 10^5 to 10^6, respectively); for the methods of Hess and Kratz[4] and Pickett et al.[5] only low and intermediate range MWD's were calculated. For the MWD's reported here the $\bar{M}_w/\bar{M}_n$ ratios were between 1.5 and 2.0.

Tung's Polynomial Expansion Method

Typical MWD's for the low and intermediate molecular weight ranges are compared in Figures 17–20. Figure 17 compares MWD's from codes 3, 5, and 8 for a low molecular weight sample ($\bar{M}_n$ = 14,000). Codes 3 and 5 agree well, but code 8 shows a small deviation, indicating that even at this low molecular weight there was sufficient skewing and tailing to cause a significant deviation from the assumed gaussian shape for the single molecular species.[2]

Figure 18 compares MWD's for codes 3, 5, and 8 for an intermediate molecular weight sample ($\bar{M}_n$ = 50,000). All three MWD's exhibit

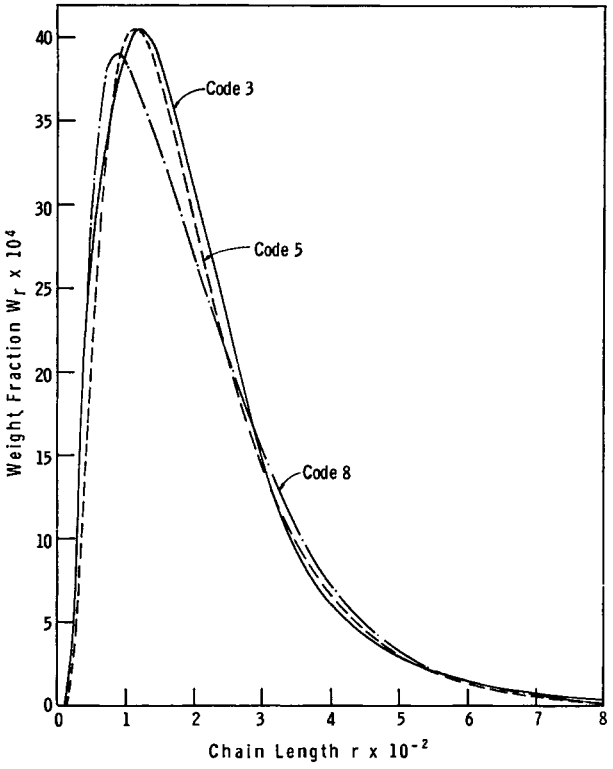

Fig. 17. Typical MWD's for a low molecular weight polystyrene by Tung's method, for codes 3, 5, and 8.

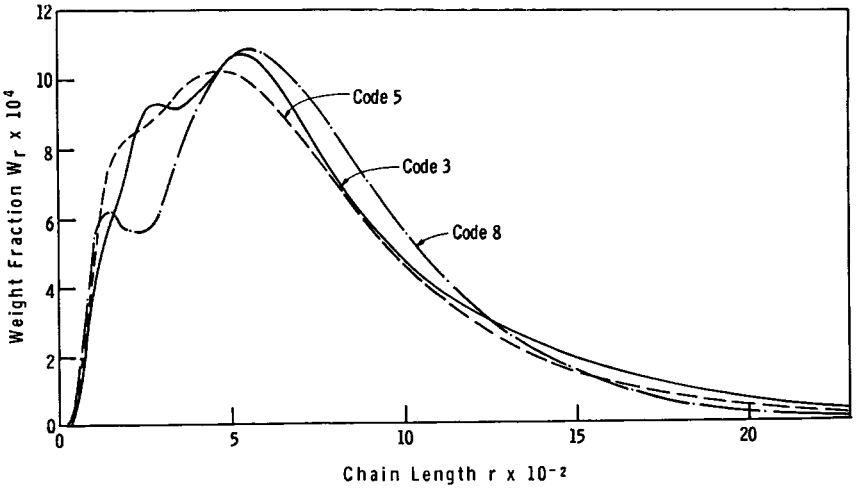

Fig. 18. Typical MWD's for an intermediate molecular weight polystyrene by Tung's method, for Codes 3, 5, and 8.

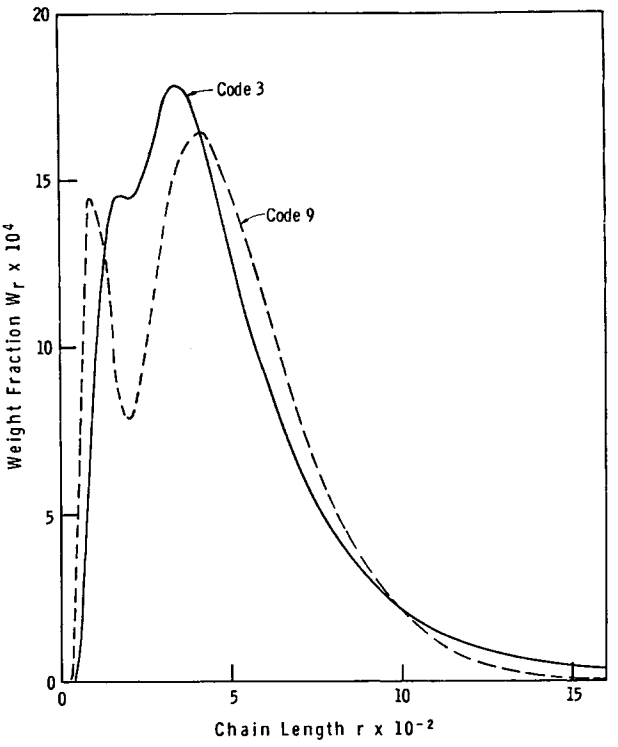

Fig. 19. Typical MWD's for a low molecular weight polystyrene by Tung's method: effect of flow rate, codes 3 and 9.

oscillations, and since these are not identical for each code, they appear to be mathematically generated rather than real. The code 8 MWD again shows the poorest agreement among the three codes. The MWD for this sample was also calculated from Tung's equation by means of a relatively simple mathematical method recently reported by Pierce and Armonas.[29] It eliminates the polynomial representation of the chromatogram and, hence, avoids arbitrary selection of the number of terms to be used in the polynomial. The molecular weight averages agreed well with Tung's method, but the oscillations in the MWD were even more severe.[30] These were considerably reduced by selecting a larger volume interval between heights read off the chromatogram.[31] This suggests that some of the oscillations may be due to noise or inaccuracies in the measured chromatogram heights.

Figure 19 compares MWD's for codes 3 and 9 for a low molecular weight sample ($\bar{M}_n$ = 33,000). The code 3 MWD exhibits a small oscillation, whereas the code 9 MWD exhibits a relatively large oscillation. The code 9 oscillations ranged from small at low molecular weights to very large at intermediate molecular weights. Thus, even though the codes 3 and 9 average molecular weights agreed well;[1] i.e., the lower

moments of the MWD agreed; the MWD's differed significantly in detail.

Figure 20 compares typical MWD's for codes 5, 10, and 13 for a low molecular weight sample ($\bar{M}_n$ = 45,000), where the only difference was

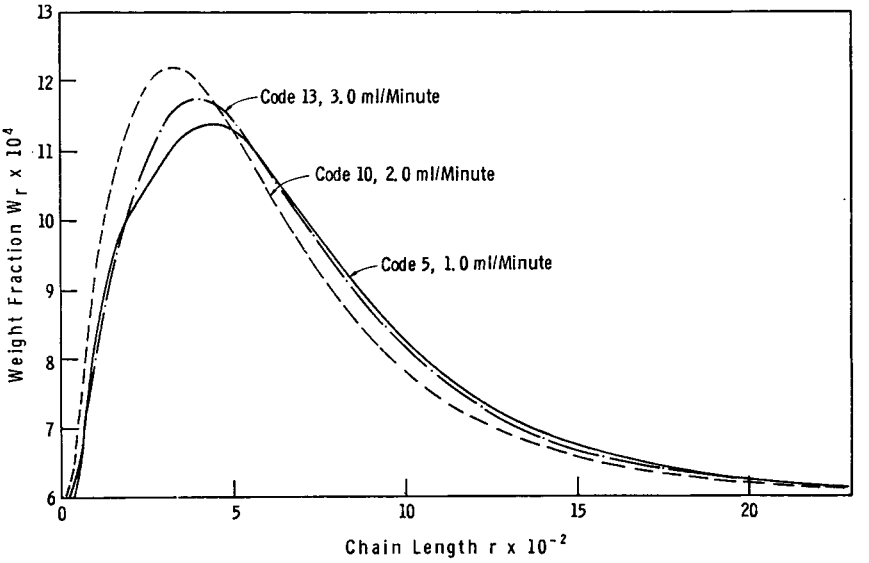

Fig. 20. Typical MWD's for a low molecular weight polystyrene by Tung's method: effect of flow rate, codes 5, 10, and 13.

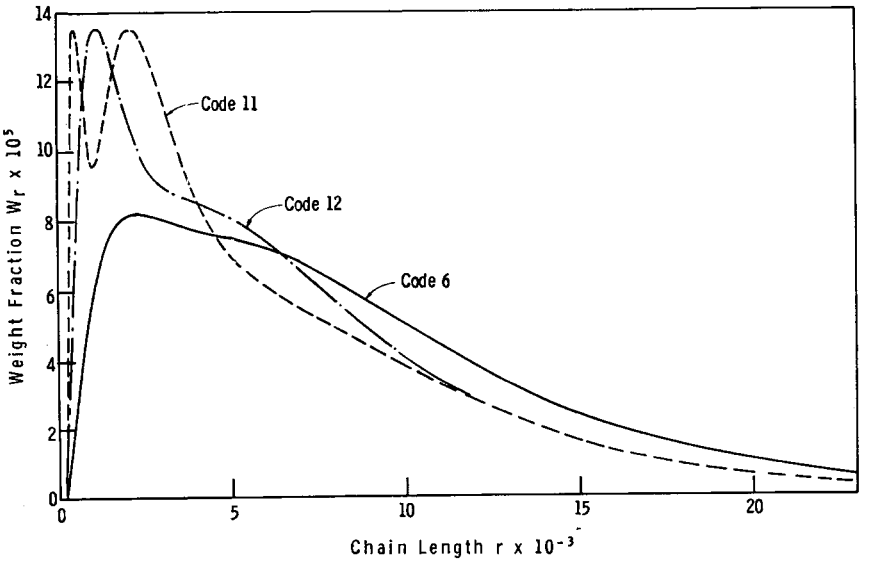

Fig. 21. Typical MWD's for a high molecular weight polystyrene by Tung's method, for codes 6, 11, and 12.

solvent flow rate. Sample concentration was 0.1%, and the calibration curves were determined by means of 0.05% concentration of the standards. The MWD's for 1.0 and 3.0 ml/min (codes 5 and 13) agree reasonably well, whereas the 2.0 ml/min MWD (code 10) is significantly shifted in the direction of low molecular weight. This is in accordance with the direction of the observed tailing in the standard chromatograms at 2.0 ml/min. This is further evidence that 2.0 ml/min is an undesirable GPC flow rate. Except for a slight shoulder in the code 5 MWD, the MWD's exhibit no oscillations. This indicates that it may be possible to minimize oscillations in Tung's method by operating with low sample concentrations, high flow rates, and a sufficient number of columns.

High molecular weight polystyrenes were produced by thermal polymerizations in a continuous stirred tank reactor.[32] They were analyzed on codes 6, 11, and 12. In view of the marked skewing and tailing observed in the chromatograms of the high molecular weight standards, even at 1.0 ml/min (code 6), the assumption of gaussian shape would not be expected to give consistent MWD's and averages. This is evident in the comparison of MWD's in Figure 21 and averages in Table IV.

The averages for codes 11 and 12 (2.0 ml/min, 0.05%, and 0.1% sample concentration) were significantly lower than for code 6 (1.0 ml/min, 0.5% concentration). This behavior is in accordance with the greater tailing observed in the codes 11 and 12 standard chromatograms, indicating that tailing was due more to flow rate than high sample concentration. The

TABLE IV
Tung's Resolution-Corrected Molecular Weight Averages for High Molecular Weight Polystyrenes[a]

Sample no.	$\bar{M}_n$ ($\times 10^{-4}$) for code no.			$\bar{M}_w$ ($\times 10^{-4}$) for code no.		
	6	11	12	6	11	12
1	38.9	28.8	23.7	77.0	54.6	56.4
2	45.5	27.0	30.4	82.9	63.3	66.1
3	39.3	28.3	26.2	74.8	64.1	59.6

[a] Flow rates were 1.0, 2.0, and 2.0 ml/min, sample concentrations were 0.5, 0.05, and 0.1 wt-%, and injection times were 60, 20, and 20 sec, for codes 6, 11, and 12, respectively.

TABLE V
Effect of Sample Concentration and Amount on Tung's Code 6 Resolution-Corrected Molecular Weight Averages for High Molecular Weight Polystyrenes

Concn., wt-%	Inject. time, sec	Amt. inject., mg	$\bar{M}_n$ ($\times 10^{-4}$)	$\bar{M}_w$ ($\times 10^{-4}$)
0.5	60	5	45.5	82.9
0.25	120	5	44.1	85.8
0.1	120	2	50.8	98.1

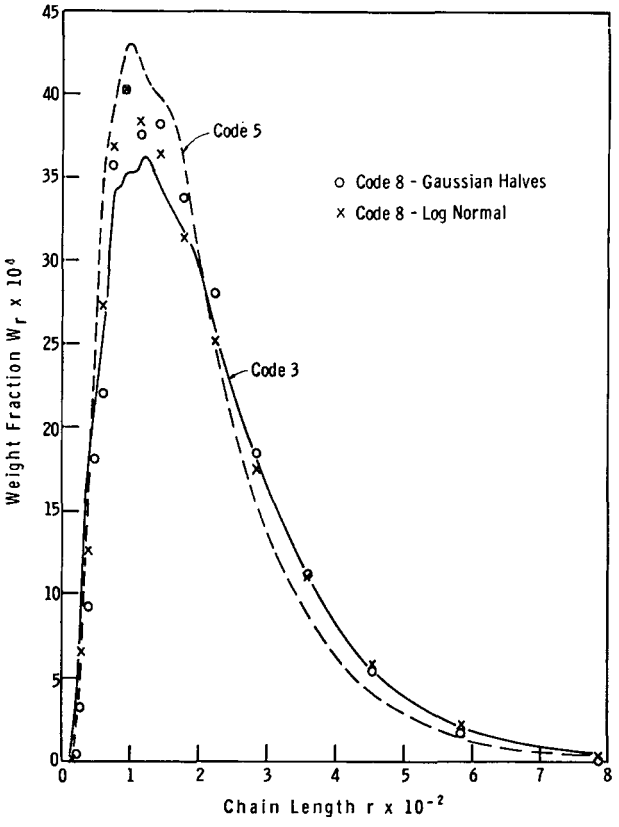

Fig. 22. Typical MWD's for a low molecular weight polystyrene by Smith's method, for codes 3, 5, and 8.

effect of sample concentration and amount in code 6 is shown in Table V, where the averages were calculated with a single calibration curve.

Since Tung's method assumes a gaussian shape for the chromatogram of a single molecular species, it can be used with confidence only when there is no significant skewing or tailing of the standard chromatograms.

Smith's Method

The original version of this method[3] assumed a gaussian shape for the single molecular species. Subsequent modifications[1,27] allowed the use of nonsymmetrical shapes to account for skewing and tailing. The MWD data presented here were calculated by assuming a log normal shape and a shape consisting of two gaussian halves, each with its own standard deviation.

Smith has also modified his method to eliminate the assumption that his proportionality constant k_j is the same for all species contributing to the refractive index $f(x_j)$ at elution volume x_j.[3] This involves using the initial

set of k values to calculate the sum of height contributions of all species at each point read off the chromatogram. If the sums do not agree with the observed chromatogram heights, the k's are adjusted by the ratio of the observed to the calculated heights, and the calculation is repeated until the desired agreement is obtained. The method also adjusts the resolution factors, if necessary, so that the sum of the species areas is usually between 100 and 101% of the chromatogram area.

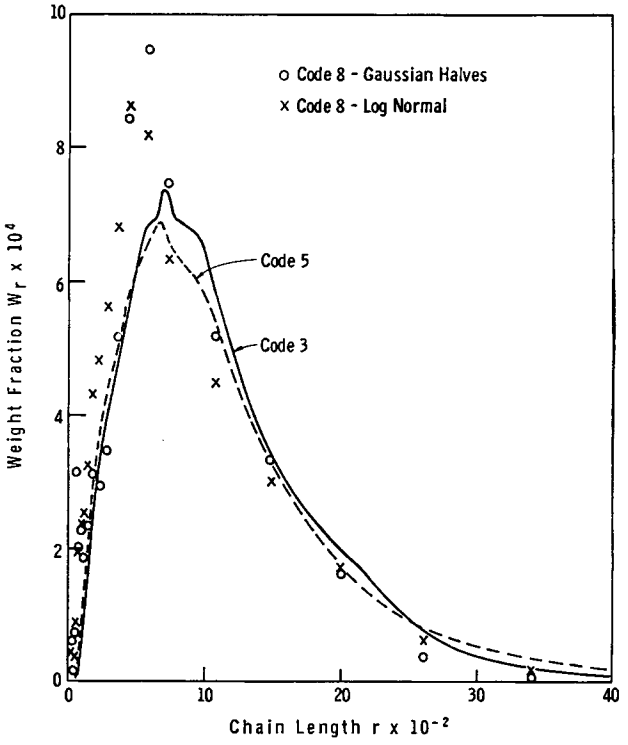

Fig. 23. Typical MWD's for an intermediate molecular weight polystyrene by Smith's method, for codes 3, 5, and 8.

Typical MWD's from codes 3, 5, and 8 for the low and intermediate molecular weight ranges are compared in Figures 22 and 23. Except for small oscillations, the agreement among the low MWD's ($\bar{M}_n$ = 14,000) is quite good. The agreement for the intermediate MWD's ($\bar{M}_n$ = 75,000) is good between codes 3 and 5, but code 8 shows a marked deviation for both the log normal shape and the shape consisting of two gaussian halves.

Typical MWD's from codes 6, 11, and 12 for the high molecular weight range are shown in Figure 24 ($\bar{M}_n$ = 400,000). These were calculated by using a single-species shape made up of unequal gaussian halves, in an attempt to account for the observed skewing and tailing. There are large deviations among the MWD's and large oscillations in each MWD.

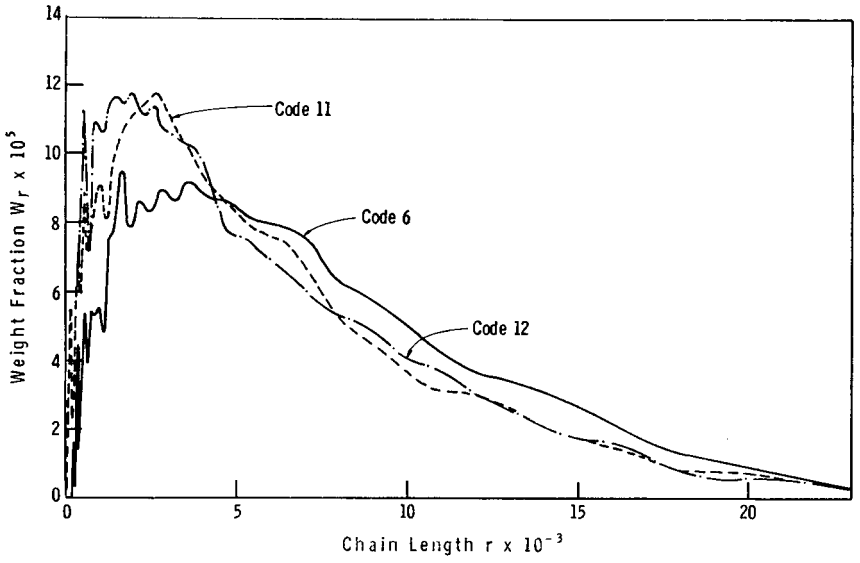

Fig. 24. Typical MWD's for a high molecular weight polystyrene by Smith's method, for codes 6, 11, and 12.

These comparisons of MWD's and corresponding molecular weight averages[1] indicate the problems involved in accounting for skewing and tailing by using nonsymmetrical shapes to represent the single species. One problem is the variation of shape with molecular weight. The shape can range from a gaussian one at low molecular weights to a severely skewed one at high molecular weights. Thus, the use of a fixed shape would not be expected to give an adequate representation of the single species over the entire molecular weight range. Another problem is the variation of shape with concentration. The concentration of the standards used to determine the shape or resolution factor usually is significantly higher than the concentration of the equivalent "species" in the unknown sample. A significant difference in shape could result, especially at high molecular weights. In view of these problems it would be desirable to select sample concentrations and GPC operating conditions that gave a gaussian shape over the entire molecular weight range of interest.

The Method of Hess and Kratz

The computational difficulties associated with this method have been previously discussed.[1] The successful code 5 solutions generally yielded weight fractions for only six species, which were insufficient to define accurately the MWD.

The Method of Pickett, Cantow, and Johnson

This method has been described in a recent publication.[5] It does not assume a specific shape for the chromatogram of a single molecular species.

Instead, it makes use of the observable shape of narrow-distribution polymer standards of known molecular weight to reshape the raw chromatograms into a resolution-corrected chromatogram.

Typical MWD's from codes 5 and 8 for the low and intermediate molecular weight ranges are compared in Figures 25 and 26 ($\bar{M}_n$ = 10,000 and 50,000, respectively). Although the codes 5 and 8 molecular weight averages for these samples (i.e., their lower moments) showed good agreement, the MWD's differ significantly in detail. Large oscillations were

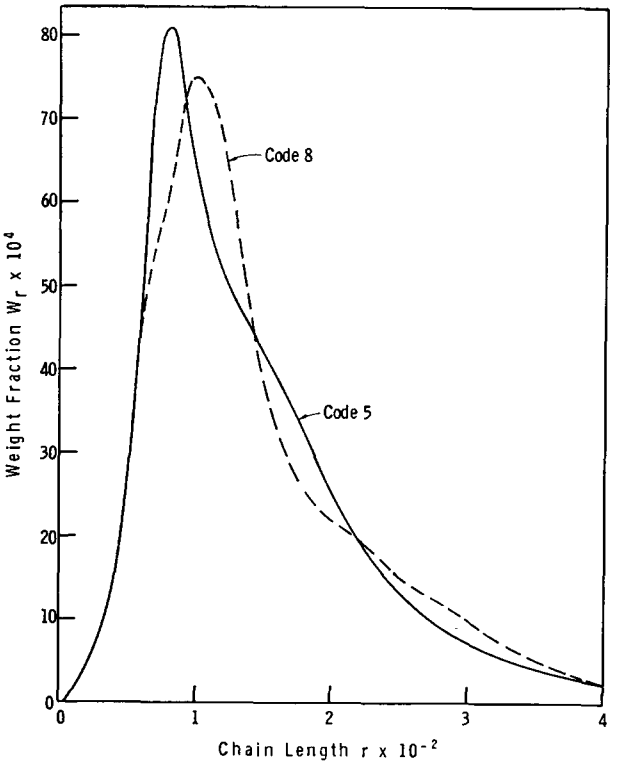

Fig. 25. Typical MWD's for a low molecular weight polystyrene by the method of Pickett et al.,[5] for codes 5 and 8.

observed in many of the codes 5 and 8 MWD's, even at low molecular weights. If the oscillations represented real peaks in the sample, they should be the same for different column combinations. As with the methods of Tung and Smith, this is not the case. Further investigation is required to establish whether the oscillations are due to inaccuracies in the GPC itself or whether they are generated mathematically within the methods. Since detailed knowledge of the MWD is required to correlate with variations in the physical properties of the polymer, any artificial oscillations in the MWD must be eliminated to make the methods completely effective.

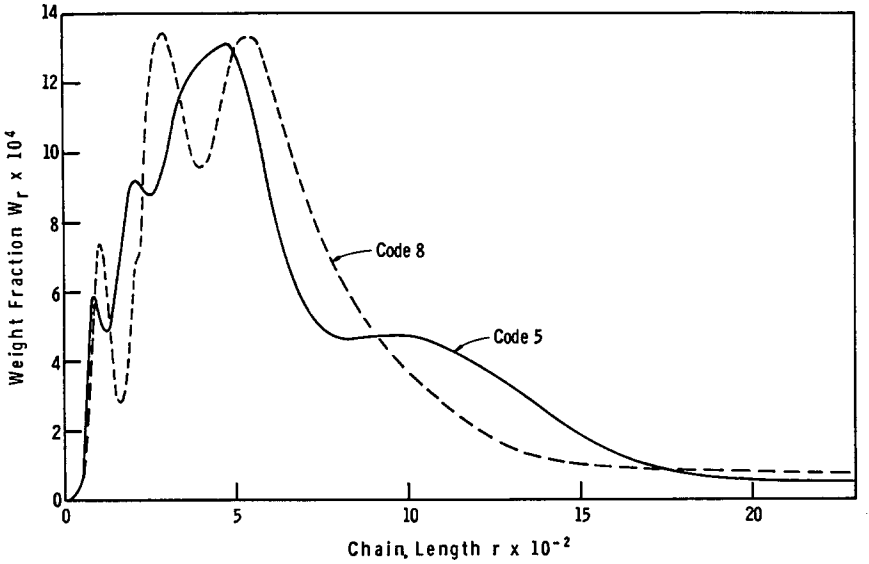

Fig. 26. Typical MWD's for an intermediate molecular weight polystyrene by the method of Pickett et al.,[5] for codes 5 and 8.

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