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GEL PERMEATION CHROMATOGRAPHY: DATA TREATMENT

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SUMMARY

Since the advent of gel permeation chromatography it has been difficult to compare molecular weight distributions of samples analyzed on different gel columns or under different experimental conditions. Corrections are described herein which convert gel permeation chromatographic curves to absolute molecular weight distributions. Good agreement of corrected molecular weight distributions for several samples analyzed on different column sets was obtained.

INTRODUCTION

Because gel permeation chromatographic (GPC) curves are graphs of Δ refractive index/Δ volume *vs.* elution volume, it is virtually impossible to superimpose GPC curves for identical samples from two different column sets. Even if the area under the curves is normalized, it is invalid to merely substitute molecular weights (from the calibration curves) for elution volumes. Furthermore the instrument spreading will most likely vary from instrument to instrument. To reiterate, in most cases there will be three significant differences between the chromatograms for the same sample analyzed under widely divergent experimental conditions, as follows: (1) identical elution volumes will not correspond to identical molecular weights, (2) the degree of instrument spreading will be different for each instrument or column set, and (3) polymer peaks will be of different size.

The first step in the correction process is the establishment of a calibration curve. Elution volumes of the peaks of twelve narrow distribution polystyrene standards were graphed *versus* the logarithm of the molecular weight at the peak. This calibration curve was fitted by a least-squares procedure to a fifth-order polynomial function. As a check of the validity of the calibration curve, the weight- and number-average molecular weights were calculated for the polystyrene standards for comparison with the reported values.

The values of the instrument spreading factors, σ_{GPC}, were determined by also using the polystyrene standards and the method proposed by HENDRICKSON[1]. The relationship $\sigma_{GPC} = (\sigma_{obs}^2 - \sigma_{dist}^2)^{1/2}$ was employed, where σ_{obs}, the observed spreading, is one-half the volume between the chromatogram peak and the baseline intercept of a tangent (theoretically the tangent at the first inflection point) to the leadside of the chromatograms, and where σ_{dist} is the volume (obtained from the calibration curve) corresponding to the difference between the molecular weight at the peak and the molecular weight (Table I) at the first inflection point.

The chromatograms were corrected for spreading using a third generation version of SMITH's[2] computer program. This correction procedure assumes a molecular weight component at each particular volume increment. The chromatogram is then considered to be an envelope of the sums of the individual components, each of which has a gaussian shape due to spreading. The spreading factors, σ_{GPC}, can vary with molecular weight or can be unequal (for unsymmetrical, skewed peak shapes). Initially the amount of each component at each particular elution volume is set proportional to the chromatogram height at that particular elution volume. Then an iterative procedure is begun in which an envelope of the sums of the individual components is calculated, considering the contribution of neighboring components to each other due to gaussian spreading. The amount of each component is then adjusted by a ratio of the height of the chromatogram to the height of the calculated envelope. This procedure is repeated for a specified number of times or until the chromatogram and the calculated envelope agree within specified ("gate value") limits. Frequently CHANG AND HUANG's[3] smoothing subroutine is used in conjunction with the spreading correction to smooth spurious baseline noise in the original chromatogram.

The replot technique proposed by YAU AND FLEMING[4] was used to correct for non-linear calibration curves. The weight fraction per volume increment (obtained from the chromatogram) is multiplied by the reciprocal of the slope of the calibration curve to obtain the corrected value of weight fraction per log molecular weight increment ($dW/d(\log M) = (dW/dV) \times dV/d(\log M)$). Finally the molecular weight distributions are normalized so that there are equal areas under every distribution.

After the chromatograph has been calibrated and all the various corrections made to a chromatogram, the inevitable question arises, just how accurate is the molecular weight distribution that one obtains? A good check of the validity of the corrections would be to analyze several duplicate samples on different chromatographs using different columns, apply the various corrections, and then compare the MWD from each chromatograph for identical samples.

EXPERIMENTAL

Results were obtained using Waters Associates Gel Permeation Chromatographs. Two instruments were used in this study, GPC-4 and GPC-3. Both chromatographs had been modified for operation at high temperature, for automatic injection, and for column reversing. GPC-4 is a Model 100 with a null-balance differential refractometer operating at 135° using the solvent 1,2,4-trichlorobenzene (TCB) and a flow rate of 1 ml/min. GPC-4 utilized three 4 ft. columns in series (2500 plates/ft.) of permeabilities 10^4, 2×10^5, and 10^6 Å. GPC-3 is a Model 200 with an R-4 differential refractometer operating at 145° also using the solvent TCB and a flow rate of 1 ml/min. GPC-3 utilized four 4 ft. columns in series (3000 plates/ft.) of permeabilities 10^4, 2×10^5, 2×10^5, and 10^6 Å.

All samples were injected onto the columns 46.5 sec. Below mol. wt. 300000 all calibration samples were 1.0 mg/ml. Above mol. wt. 300000 all calibration samples were 0.5 mg/ml and fresh samples made the day they were injected. Above mol. wt. 10^6 polystyrene solutions in TCB appear to degrade upon long standing, as the peaks broaden and elute later. Above mol. wt. 10^6 the concentrations may be slightly overloaded. However, due to the limits of instrument sensitivity, the concentration could not be reduced further.

RESULTS AND DISCUSSION

Calibration curves were constructed for GPC-3 and GPC-4 by graphing log molecular weight *vs.* elution volume for twelve narrow distribution polystyrene standards. Smooth curves were drawn through the twelve points. Readings every milliliter were taken from this curve and the readings were fitted by a least-squares procedure to a fifth-order polynomial (log $M = a + bv + cv^2 + dv^3 + ev^4 + fv^5$, where v = volume) by computer program CALJAM. The values of spreading factors were determined from the leadside of the polystyrene standards using values by MOORE[5] (Table I).

TABLE I

MOLECULAR WEIGHT VALUES[a] FOR DETERMINATION OF SPREADING DUE TO THE DISTRIBUTION (σ_{dist})

Standard	*Peak molecular weight*	*Molecular weight at 1st inflection*
PS4190042	11 700	14 290
PS4190039	19 500	24 400
PS4190041	51 200	57 800
PS41984	170 600	178 200
PS4190037	411 000	428 000
PS4190038	990 000	1 042 000
PS61970	2 000 000	2 150 000

[a] See ref. 5.

Then, using the analytical calibration curve and the experimentally-determined spreading factors, weight- and number-average molecular weights as well as the polydispersity ratios ($\bar{M}_w/\bar{M}_n$) were calculated for the polystyrene standards using computer program JAMGPC. In general the calculated molecular weights were zero to ten percent low. The calibration curve was then shifted upwards the corresponding zero to ten percent and a revised calibration curve calculated. An example of the agreement between the twelve experimental points and the revised analytical calibration curve is shown in Fig. 1. In order to check the spreading correction, the reported polydispersities were compared with the calculated polydispersities. The reported polydispersities were less than the calculated polydispersities which were uncorrected for spreading, but more than the calculated polydispersities which were corrected for spreading. This indicated that the experimentally-determined spreading factors, σ_{GPC}, were too large. Fig. 2 shows the experimental variation of spreading factors with elution volume for GPC-3 and GPC-4. Lesser arbitrary values of spreading factors, also shown in Fig. 2, gave better agreement with reported polydispersity data and were used in all subsequent calculations. Table II includes reported polydispersities, weight-, and number-average molecular weights of the polystyrene standards, as well as the calculated values from GPC-3 and GPC-4 using the revised calibration curves and spreading factors.

Having established valid calibrations for GPC-3 and GPC-4, as evidenced by the good agreement of reported and calculated molecular weight values for the standards, it is possible to calculate valid molecular weight distributions from GPC-3 and

GPC-4. Fig. 3 is a comparison of MWD for a medium broad distribution ($\bar{M}_w/\bar{M}_n$ = 1.95) polystyrene sample obtained from GPC-3 and GPC-4. The chromatograms were smoothed prior to spreading corrections in order to minimize baseline noise. The agreement of MWD from different chromatographs (Fig. 3) appears to be about as good as the agreement of MWD from repeat analysis on the same chromatographs (Fig. 4). The agreement even seems better when one realizes that the area under the curves in Figs. 3 and 4 is approximately six times the area of the original chromatograms, which has the effect of magnifying the differences approximately six times.

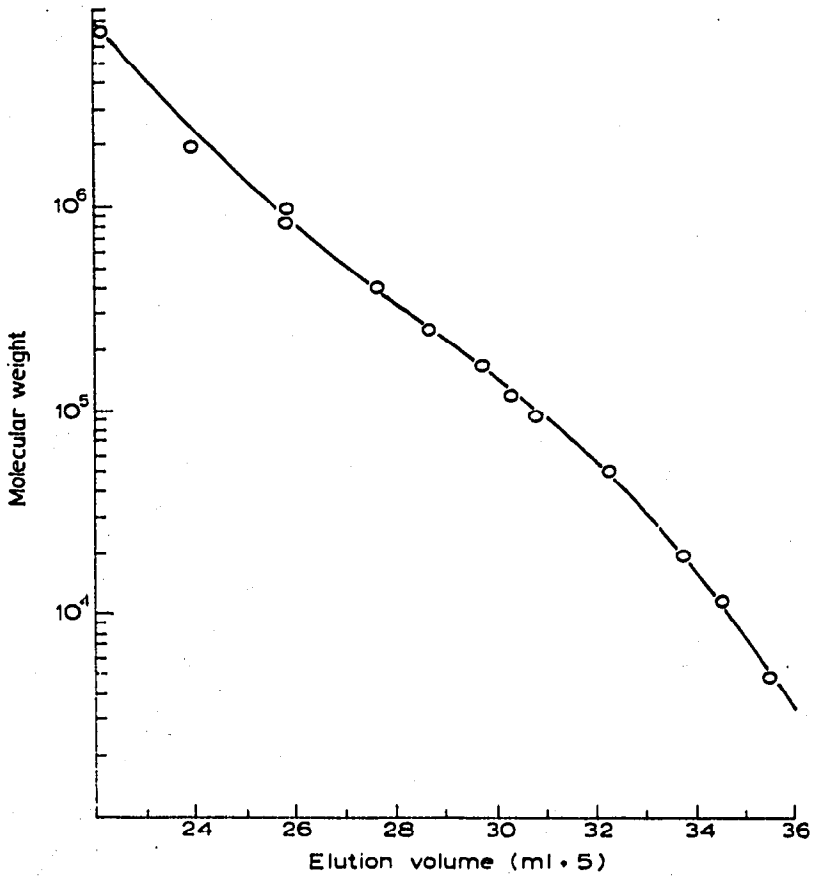

Fig. 1. Calibration curve for the polystyrene standards. The line represents the revised analytical function.

Prior computer programs give accurate spreading corrections when the molecular weight distributions are broad and the spreading is relatively small. However, when the distributions are narrow and spreading is relatively large, most programs over-accentuate baseline noise and suffer from artificial oscillations, especially on the leading and trailing edges. Several of the prior-published spreading corrections oscillate badly and yield negative values for weight fraction of polymer at times. Most of these computer programs can not adequately handle multipeaked distributions. DUERKSON AND HAMIELEC[6] have done a good review of the currently used computer programs. Fig. 5 shows the chromatograms from GPC-3 and GPC-4 of a blend of five narrow distribution polystyrene standards which was prepared to rigorously test our present correction program. The two chromatograms are similar in shape, but cannot

TABLE II

POLYSTYRENE STANDARDS DATA

No.	Sample	Reported			GPC-3			GPC-4		
		$\bar{M}_w$	$\bar{M}_n$	$\bar{M}_w/\bar{M}_n$	$\bar{M}_w$	$\bar{M}_n$	$\bar{M}_w/\bar{M}_n$	$\bar{M}_w$	$\bar{M}_n$	$\bar{M}_w/\bar{M}_n$
a	PS4190040	5 000	4 600	1.08	4 630	4 310	1.075	4 905	4 660	1.053
b	PS4190042[a]	10 790	9 970	1.083	10 710	9 960	1.076	11 080	10 180	1.089
c	PS4190039[a]	20 340	19 410	1.048	19 860	18 800	1.056	17 446	14 550	1.199
d	PS4190041[a]	52 770	51 860	1.018	52 450	50 840	1.018	50 660	48 234	1.050
e	PS41995	98 200	96 200	≤1.06	100 190	98 540	1.017	98 322	95 730	1.027
f	PS103	125 000	119 000	1.05	123 396	112 900	1.093	—	—	—
g	PS41984[a]	169 020	167 170	1.011	164 880	162 000	1.018	164 660	160 270	1.027
h	PS4190037[a]	404 850	383 390	1.056	404 620	395 090	1.024	412 070	394 080	1.046
i	PS4190038[a]	870 530	778 780	1.118	886 540	849 840	1.043	839 800	790 510	1.062
j	PS61970[a]	1 667 000	1 149 910	1.459	1 656 000	1 137 000	1.45	2 021 300	1 815 800	1.113
k	PS, blend of c, d, e, f, and PS108	114 590	54 460	2.10	102 810	50 850	2.02	99 140	46 950	2.11

[a] Ref. 5.

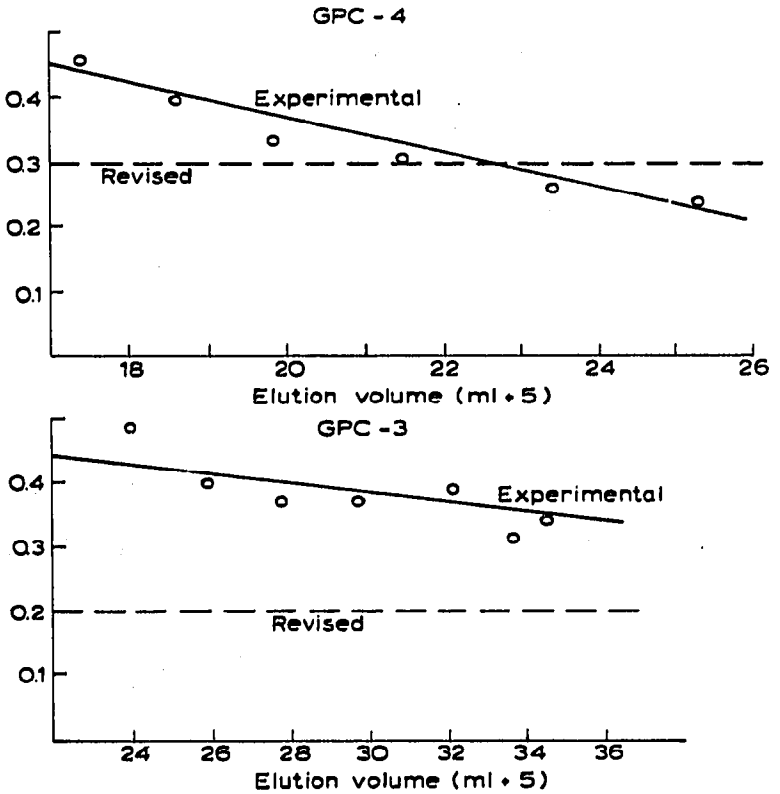

Fig. 2. Spreading factors *vs.* elution volume for GPC-3 and GPC-4. The revised spreading factors (dashed lines) gave better agreement with the reported values of $\bar{M}_w/\bar{M}_n$.

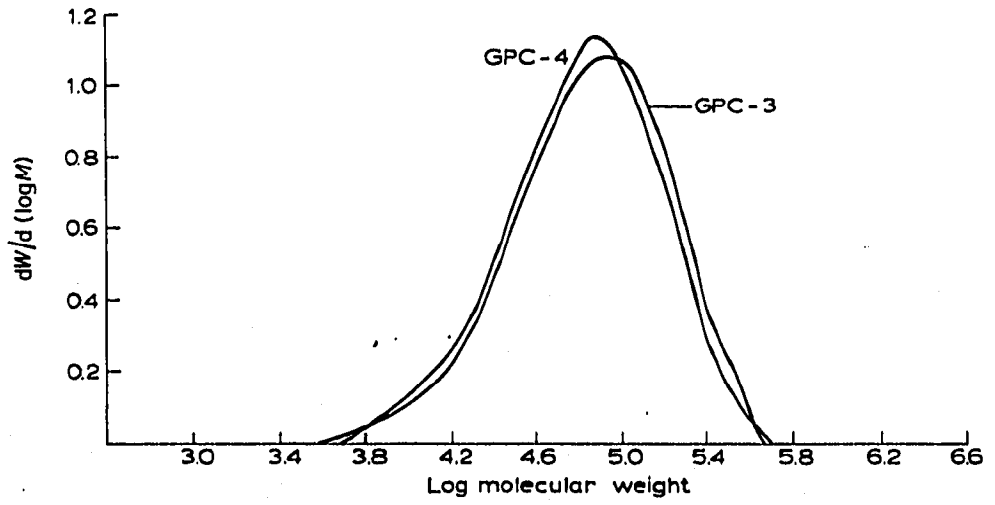

Fig. 3. Comparison of molecular weight distributions for polystyrene sample PS-6 obtained from GPC-3 and GPC-4.

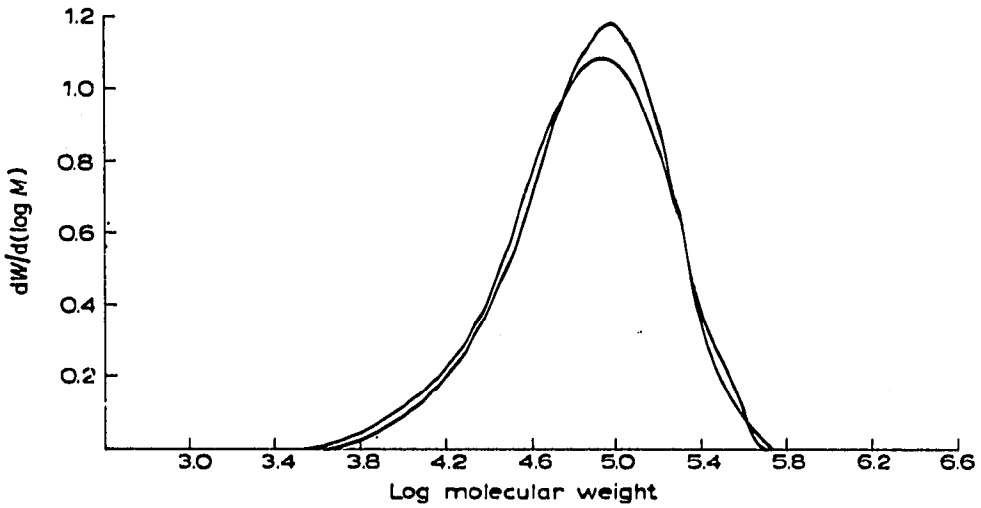

Fig. 4. Repeat analysis of polystyrene sample PS-6 on GPC-3.

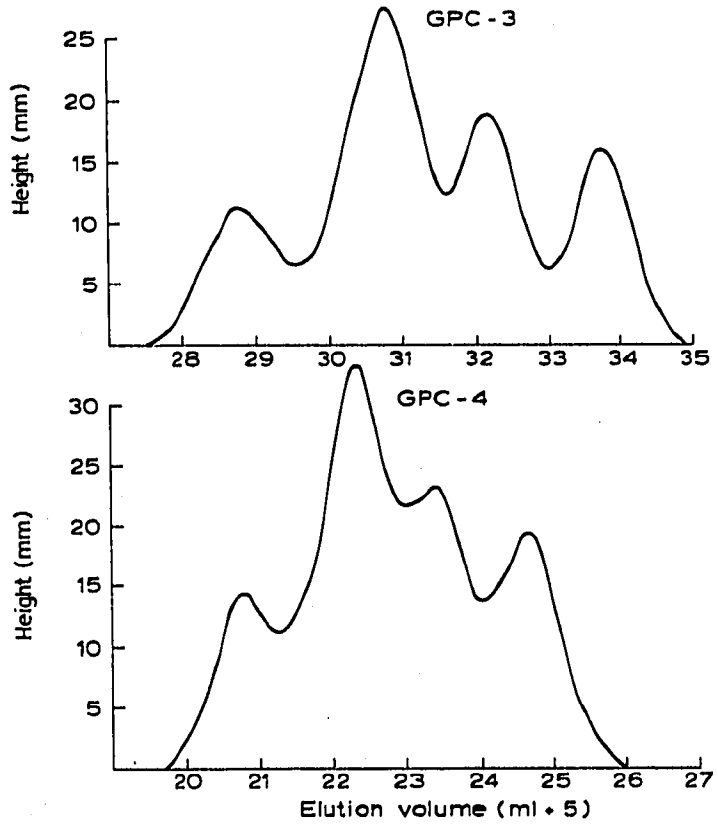

Fig. 5. Chromatograms of a five-component blend run on GPC-3 and GPC-4.

be readily visually compared. However, after these chromatograms have been corrected and plotted (Fig. 6) as weight fraction per log M increment *vs.* log mol. wt., the MWDs are very nearly the same. The area under the curves in Fig. 6 is approximately four times the area under the original chromatograms. The data were not smoothed prior to spreading corrections for Fig. 6. MWD (using smoothed data)

obtained from GPC-3 and GPC-4 for another blend of five narrow polystyrene standards is shown in Fig. 7. In this case the five peaks are so close together that the blend could not be resolved by the chromatographs into separate peaks.

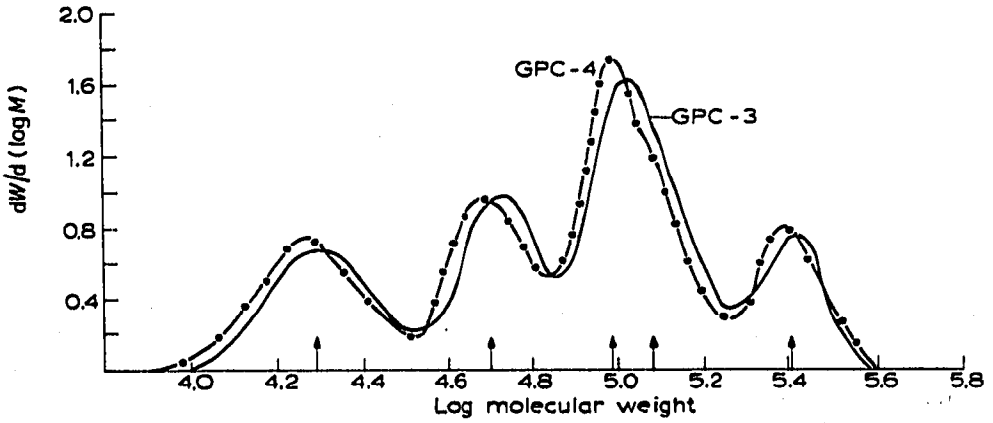

Fig. 6. Molecular weight distribution of the blend shown in Fig. 5. The arrows represent the peak elution volumes of the five components.

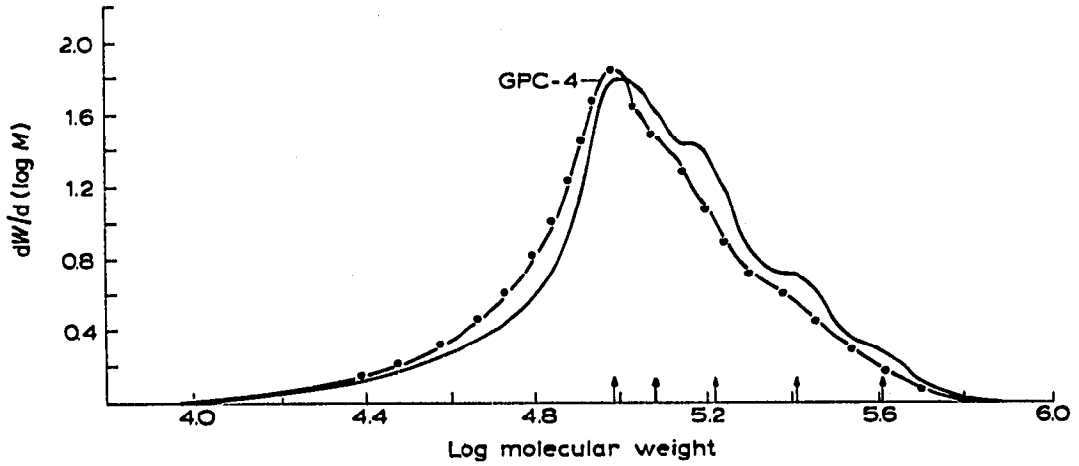

Fig. 7. Molecular weight distribution for a second blend run on GPC-3 and GPC-4. The arrows represent the peak elution volumes of the five components.

With respect to the spreading correction, there are two important questions. First, how large should the "gate value" be and second, when should the data be smoothed to remove baseline noise? If the gate value is too large, the spreading will not be corrected for. If the "gate value" is too small, the iterative program will overcorrect, magnifying anomalies and attempting to place a peak at every minor inflection or bump in the chromatogram. This can be disastrous if there is any baseline noise or short-term baseline drift. The best value for the "gate value" seems to be a value corresponding to the precision with which one can measure the curve height, or a value corresponding to the magnitude of baseline noise. For the present spreading corrections, the value of 1 mm was used as the "gate value", discounting smaller inflections as inconsequential. The smoothing of data can be used in most instances. The difference between smoothed and unsmoothed data in most cases was less than

1 mm. However, when few data points are taken and peaks are sharp, the smoothing routine will round off peaks and destroy essential detail. This loss of detail is a result of the inherent nature of the smoothing procedure. The smoothed height at a particular elution volume is the height at that elution volume of the best cubic curve through the point and the three data points on each side of the considered point, or in other words, a seven point cubic smoothing function is used. Therefore, if enough points are not taken and a smooth cubic curve cannot be placed through any seven neighboring points, the smoothing routine should not be used. The data shown in Fig. 5 were not smoothed because data was collected every milliliter, and therefore the peaks and valleys would have been rounded off.

In conclusion, a good procedure has been described for obtaining accurate molecular weight distributions, independent of the columns and experimental conditions (assuming the columns are good and of the applicable porosity range). However, when very minute and subtle differences are to be examined between two samples, the easiest and best method still is to chromatograph the samples consecutively under the same conditions and merely compare the chromatograms. Nevertheless, instances arise when one has earlier chromatograms of samples, but none of the samples to chromatograph consecutively.

CONCLUSIONS

1. Good agreement of corrected molecular weight distributions for identical samples analyzed on different column sets was found.

2. Narrow, multipeaked, as well as broad, simple molecular weight distributions were dealt with successfully in peak spreading corrections.

3. Criteria for the use of data smoothing prior to the spreading correction, and criteria for choosing a "gate value" in spreading corrections were discussed.

4. When extremely small differences in molecular weight distributions between two samples are to be examined, it is still best to chromatograph the samples on the same columns and compare the uncorrected chromatograms.

REFERENCES

1 J. G. HENDRICKSON, *J. Polym. Sci. A-2*,6 (1968) 1903.
2 W. N. SMITH, *J. Appl. Polym. Sci.*, 11 (1967) 639, modified by J. C. MOORE AND J. A. MAY, Jr.
3 K. S. CHANG AND R. Y. M. HUANG, *J. Appl. Polym. Sci.*, 13 (1969) 1459.
4 W. W. YAU AND S. W. FLEMING, *J. Appl. Polym. Sci.*, 12 (1968) 2111.
5 J. C. MOORE, private communication. Molecular weight values were from calculations by MOORE on data from L. H. TUNG.
6 J. H. DUERKSON AND A. E. HAMIELEC, *J. Polym. Sci. C*, 21 (1967) 83.