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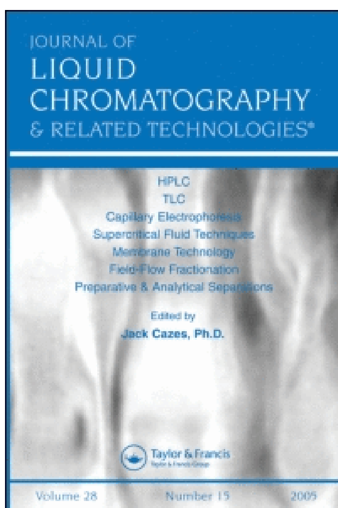

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Anthony R. Cooper[a]; David P. Matzinger[a]
[a] Dynapol, Palo Alto, CA

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CHARACTERIZATION AND PROPERTIES OF MACROMOLECULES. XII. NUMERICAL TREATMENT OF GEL PERMEATION CHROMATOGRAPHY DATA*

Anthony R. Cooper and David P. Matzinger
Dynapol
1454 Page Mill Road
Palo Alto, CA 94304

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ABSTRACT

Gel permeation chromatography provides a continuous recording of polymer concentration as a function of elution time. The latter may be converted by appropriate calibration into molecular weight. The chromatogram may be analyzed numerically to yield the various molecular weight averages. This process involves a number of assumptions and approximations. The magnitude of the errors introduced in the calculated molecular weight averages by the methods described in the literature are reported.

INTRODUCTION

Gel permeation chromatography, GPC, provides a continuous measure of polymer concentration as a function of elution volume. The latter must be calibrated for molecular weight in order to obtain information on the polymer molecular weight averages and molecular weight distribution (MWD). Several methods have been proposed to calculate the molecular weight averages from GPC data. The most common method of computation involves a measurement of

* Based on a transcript of a lecture delivered at the "Chromatography of Polymers Symposium," Chicago ACS Meeting, August 1977.

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peak heights at equal elution volume intervals. By appropriate summations of these data. all the molecular weight averages may be calculated. There are several assumptions in this treatment, and, in this paper, we have examined in detail the errors arising from this approach.

COMPUTATIONS

The calculations were performed ona Tektronix TEK 31 programmable calculator equipped with a digital plotter. Computer calculations were also performed, using a program developed by Pickett et al. *(1)*.

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THEORY

For the purpose of testing the accuracy of the computational methods, an idealized chromatogram was generated, using a log normal distribution of molecular weight, i.e.,

$$W(\ell n\ M) = \frac{\exp\ [-(\ell n\ M - \ell n\ \overline{M}_m)^2/2\sigma^2]}{(2\pi)1/2\sigma}$$

where $\overline{M}_m$ is the median value of the distribution,
σ is the standard deviation of ℓn M.

The molecular weight averages are related to these two parameters as follows:

Number average $\overline{M}_n = \overline{M}_m \exp\ (-\sigma^2/2)$

Weight average $\overline{M}_w = \overline{M}_m \exp\ (+\sigma^2/2)$

Z average $\overline{M}_z = \overline{M}_m \exp\ (+3\sigma^2/2)$.

Hence $\overline{M}_m = (\overline{M}_n\overline{M}_w)^{1/2}$

and $\overline{M}_w/\overline{M}_n = \exp\ \sigma^2$

Chromatograms were synthesized, having a desired $\overline{M}_w$ and $\overline{M}_n$ by application of these formulae. The chromatogram height, h_i, is equated to $W(\ell n\ M)_i$, and the elution volume, V_{e_i}, is obtained assuming a linear calibration of the form

$$V_{e_i} = 8.75 - 1.75 \log M_i$$

The molecular weight limits were set at $\pm 3\sigma$, which includes 99.73% of the area of the log normal distribution. At this point, it is pertinent to review the assumptions involved in our calculations.

1) The chromatogram height, h_i, is proportional to the solution concentration of species with molecular weight M_i, viz., there is no band broadening. The proportionality constant of chromatogram height to concentration is independent of molecular weight.
2) The calibration curve relating elution volume, V_e, to molecular weight is linear.

DISCUSSION

The calculation of molecular weight averages from peak height h_i at a given elution volume and its associated molecular weight M_i has been outlined previously *(2,3)*. Figure 1 shows the method: The chromatogram is divided into equal elution volume intervals, and the peak heights and molecular weights are tabulated.

The molecular weight averages are evaluted from

$$\overline{M}_n = \frac{\sum h_i}{\sum h_i/M_i} \quad (1)$$

$$\overline{M}_w = \frac{\sum h_i M_i}{\sum h_i} \quad (2)$$

There are two assumptions built into this approach. This approach implicitly integrates an area segment, and is the reason for which equal elution volume intervals are chosen. Thus, although the meas-

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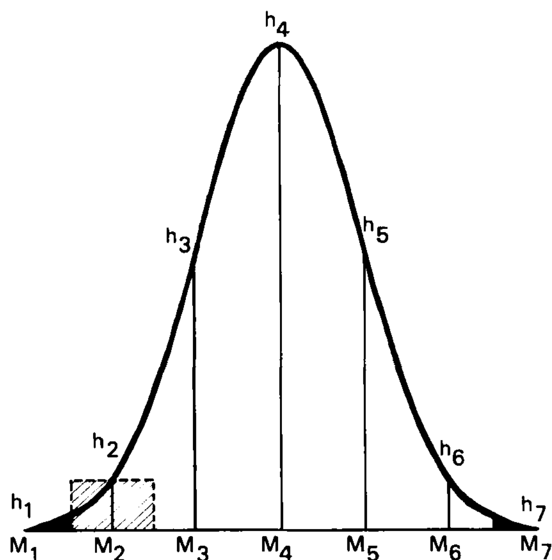

Figure 1 Illustration of Equal Elution Volume Method for Calculation of Molecular Weight Averages

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urement of h_i and the assignment of M_i is exact, the method is still capable of errors in the determination of segment area and the assignment of a single molecular weight to that area segment. This problem has been well-known in the treatment of fractionation data (*4*), where the polymer is fractionated into discrete fractions of weight, w_i. However, each fraction is usually characterized by only one molecular weight average, which is then assigned as the unique molecular weight of that fraction.

Consider the peak height h_2 in Figure 1, which implicitly represents the striped area extending ± 0.5 interval on either side of M_2. Thus the area is approximated by a rectangle, and an error is introduced which depends on the shape of the chromatogram in the region of interest. It should also be noted that two areas are not integrated in this treatment, shown by the shaded areas at the beginning and end of the chromatograms, since h_1 and h_7 are zero. The results are that $\overline{M}_n$ is too high because the very low molecular

weights are not integrated, and $\overline{M}_w$ is too low because the high molecular weight tail is neglected.

Boni et al. *(5)* previously recognized three sources of error in this approach. The first involved the assignment of the molecular weight at the center of one interval to the whole of that interval, and the second arose from the assignment of h to represent an area. A third involved the change of calibration curve slope within the interval. The first error is caused by the logarithmic variation of molecular weight within an interval. The assignment of the molecular weight at the interval center disproportionately weights the lower molecular weights in that interval. By calculation, it was found that reducing the interval size to 0.25 count (1 count = 5 ml), the error in calculating $\overline{M}_n$ could be eliminated for reasonable values of the calibration curve slope. Since their separating ranges were ~10 count, measurement of approximately 40 peak heights was required. Intervals of 1.0 count introduced errors of ~5% in $\overline{M}_n$. Essentially identical results were found for the second source of error. The third source also was capable of introducing errors up to 5%.

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RESULTS AND DISCUSSION

A simple calculator program was written to evaluate Equations 1 and 2 for various idealized chromatograms based on log-normal distributions of molecular weight and a linear calibration curve. The distributions had identical $\overline{M}_m$ values of 10,000, and $\overline{M}_w/\overline{M}_n$ values in the range 1.01 to 10.0. The program was written such that equal intervals were used as in Figure 1, as is usually done in GPC calculations. The calculations therefore ignore small area segments at the extremes of the chromatograph. The results are shown in Table I. For narrow MWD polymers, the correct averages and polydispersity are recovered independent of the number of data points used, which varied between five and 50. As the MWD increases, the recovered molecular weight averages and polydispersities are in error. The error increases with increasing number of data points

TABLE I

CALCULATED VALUES FOR $\overline{M}_w$ AND $\overline{M}_n$ USING EQUAL ELUTION VOLUME INTERVALS FOR VARIOUS SAMPLE POLYDISPERSITIES

THEORETICAL VALUES			CALCULATED VALUES			
$\overline{M}_w$	$\overline{M}_n$	$\overline{M}_w/\overline{M}_n$	NO. DATA POINTS	$\overline{M}_w$	$\overline{M}_n$	$\overline{M}_w/\overline{M}_n$
10050	9950	1.01	5	10050	9950	1.010
			10	10050	9950	1.010
			20	10050	9950	1.010
			50	10050	9950	1.010
10490	9535	1.1	5	10485	9540	1.099
			10	10480	9540	1.098
			20	10480	9540	1.098
			50	10480	9545	1.0975
12250	8165	1.5	5	12240	8170	1.498
			10	12220	8180	1.494
			20	12200	8200	1.489
			50	12180	8210	1.484
14140	7070	2.0	5	14120	7080	1.994
			10	14075	7105	1.981
			20	14025	7130	1.967
			50	13990	7150	1.957
17320	5775	3.0	5	17290	5785	2.989
			10	17160	5830	2.943
			20	17050	5865	2.907
			50	16980	5890	2.883
22360	4470	5.0	5	22290	4490	4.964
			10	21980	4550	4.831
			20	21750	4600	4.728
			50	21590	4630	4.663
31620	3162	10.0	5	31400	3185	9.859
			10	30630	3265	9.381
			20	30095	3320	9.065
			50	29750	3360	8.854

used, which is contrary to the predictions of Boni et al. *(5)*. A common pattern is evident, which is that $\overline{M}_w$ is always less than the theoretical value, and the error increases with increasing number of data points used. The converse is true for $\overline{M}_n$. The combined result is that $\overline{M}_w/\overline{M}_n$ is lower than the true values, and the error increases with increasing number of data points.

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Below $\overline{M}_w/\overline{M}_n$ values of 1.5, the recovered molecular weight averages are within 1% of the theoretical values. Figure 2 shows the percent deviation of $\overline{M}_n$ and $\overline{M}_w$ when $\overline{M}_w/\overline{M}_n = 2$. The percentage errors are approximately equal and opposite in sign, and approach 1% as the number of data points increases. In Figure 3, the results for the case where $\overline{M}_w/\overline{M}_n = 10$ are shown. Again, the errors are approximately equal and opposite in sign, and approach 6% at 50 data points.

Values of $\overline{M}_n$ higher than, and values of $\overline{M}_w$ less than, their theoretical values is consistent with the concept of the areas at the extremes of the chromatogram not being integrated. However, one would expect this to decrease as the number of points was increased and the areas which were omitted became smaller. Similarly, the first and second errors discussed by Boni et al. would intuitively be expected to decrease with increasing number of data points, i.e.,

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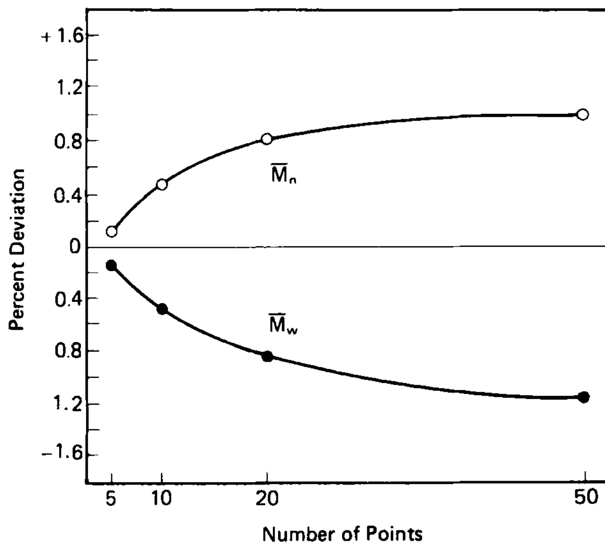

Figure 2 Percent Deviation of $\overline{M}_n$ and $\overline{M}_w$ from their Theoretical Values as a Function of the Number of Data Points Used. Sample $\overline{M}_m = 10{,}000$, $\overline{M}_w/\overline{M}_n = 2$

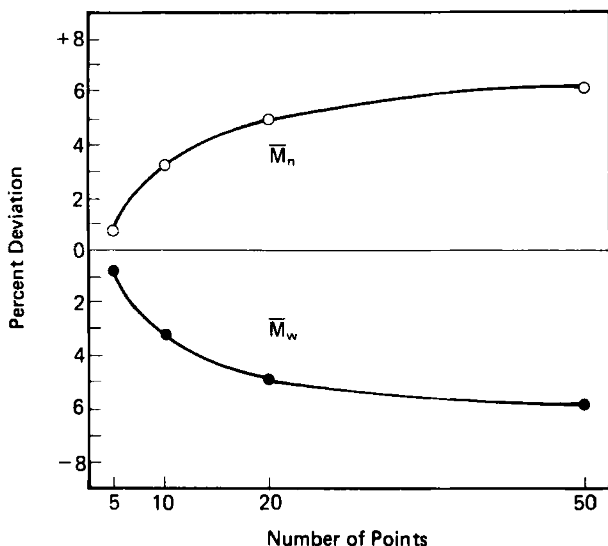

Figure 3 Percent Deviation of $\overline{M}_n$ and $\overline{M}_w$ from their Theoretical Values as a Function of the Number of Data Points Used Sample $\overline{M}_m$ = 10,000, $\overline{M}_w/\overline{M}_n$ = 10

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as the area and molecular weight range per area segment became smaller, and are better represented by h_i and M_i.

To investigate the sources of error further, we wrote a calculator program to calculate segment areas at unequal elution volume intervals to allow selective spacing of the area segments. No significant differences could be found in the cases tested between equal and unequal interval spacing. The peak area method integrates the segment area by multiplying the average height of the segment boundaries by the elution volume between them. As such, it integrates the small areas at the high and low ends of the chromatogram, which are neglected in the usual peak height approach. A comparison of the two methods is shown in Figure 4. Using a similar number of area segments, the peak area method produces larger errors than the peak height method. Similar trends are observed for error as a function of $\overline{M}_w/\overline{M}_n$.

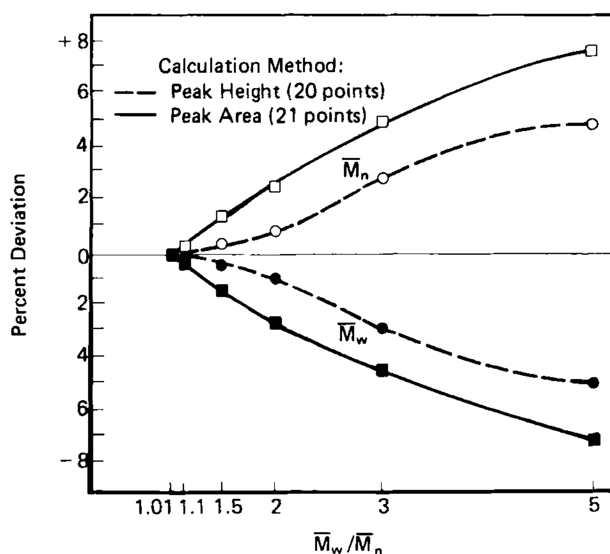

Figure 4 Comparison of Percent Deviations of $\overline{M}_w/\overline{M}_n$ from their Theoretical Values, Calculated by the Peak Height and Peak Area Methods

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The results from the calculator program which calculates peak areas were compared with those from a computer program *(1)*. The computer program determines the molecular weight distribution directly from the chromatogram, and then uses partial areas to calculate the molecular weight averages from the distribution. The results of the comparison are shown in Figure 5, and show similar trends. The differences are not large, and may be due to data-smoothing routines which are incorporated in the computer program.

CONCLUSIONS

Literature methods for calculation of molecular weight averages from GPC chromatograms have been shown to introduce errors. For high polydispersity samples, the error introduced by the calculation becomes significant. Surprisingly, at all polydispersities, the

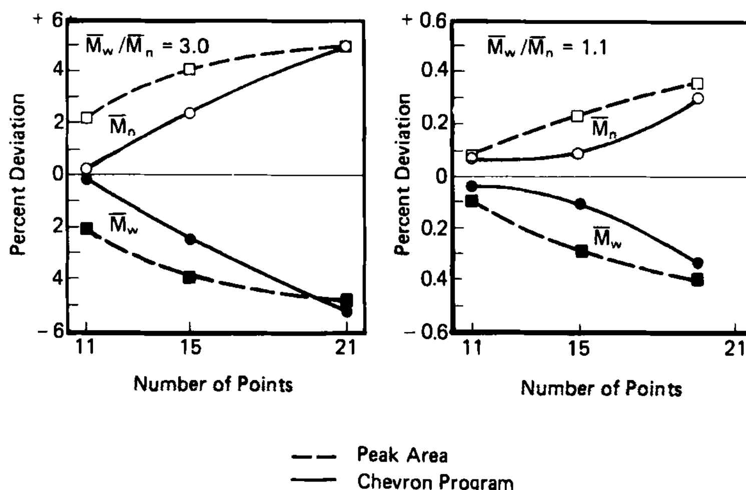

Figure 5 Comparison of Percent Deviation of $\overline{M}_n$ and $\overline{M}_w$ from their Theoretical Values as a Function of the Number of Data Points using the Peak Area and Computer (Chevron Program) Methods.

Sample $\overline{M}_n$ = 10,000, $\overline{M}_w/\overline{M}_n$ = 3.0 or 1.1

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error introduced by the calculation increases as the number of data points is increased.

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