

Calibration and Molecular Weight Calculations in GPC Using a New Practical Method for Dispersion Correction—GPCV2

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Synopsis

The method of Balke, Hamielec, LeClair, and Pearce for GPC calibration and molecular weight computation is reviewed and evaluated. A new method, called GPCV2, is proposed which uses the single broad molecular weight distribution (MWD) standard for calibration developed by those workers, but which also incorporates the chromatographic dispersion σ to make the method more accurate in general use. Equations are presented for computing the relationship between actual MW and retention volume V_R under the chromatographically broadened curves. This relationship is then compared to those obtained by using the Balke et al. method and the peak position method.

INTRODUCTION

Calibration is necessary in size exclusion chromatography (gel permeation chromatography, GPC) for converting the raw data into molecular weight averages and/or distributions. Many approaches have been taken to solve the calibration/molecular weight calculation problem, with varying success. The literature to 1972 is documented in reference 1, pp. 36–40.

We have frequently used the Balke et al. method^{2,3} for calibration. (For convenience, we hereafter refer to this method as the Hamielec method in honor of Professor Hamielec, the senior investigator.) The outstanding advantage is that it permits calibration with one broad molecular weight distribution (MWD) standard ($\bar{M}_w/\bar{M}_n \sim 2$ or greater) of the polymer type of interest. It compensates for chromatographic dispersion, but, as the results here show, it is useful only when the sample and standard are similar in MWD and MW. Our method, called GPCV2 (our version “two” of the Hamielec method), permits the use of very dissimilar MWD standards and samples of the same polymer type while still effectively compensating for chromatographic dispersion. Both methods at this stage of development use the linear calibration curve approximation.

The exact formulations of \bar{M}_w and \bar{M}_n for linear calibration may be written as in eqs. (1) and (2):

$$\bar{M}_w = \Sigma_V W(V) D_1 e^{-D_2 V} \quad (1)$$

$$\bar{M}_n = 1/\Sigma_V [W(V)/(D_1 e^{-D_2 V})] \quad (2)$$

In these equations, $W(V)$ represents the true or theoretical chromatogram of the sample, and $D_1e^{-D_2V}$ is the linear portion of the calibration; D_1 is related to the intercept and D_2 , to the slope of the calibration lines. The Hamielec method uses these equations, but substitutes $F(V)$ to approximate $W(V)$, where $F(V)$ is the observed or experimental chromatogram which has been broadened by the chromatographic column dispersion process. Both $W(V)$ and $F(V)$ are normalized, i.e., $\Sigma_V W(V) = \Sigma_V F(V) = 1$.

In the calibration step of Hamielec's method, a standard sample is chosen with known \bar{M}_n and \bar{M}_w or two standards with any combination of two of the MW's known. $F(V)$ is determined by chromatographing the sample. (The review by Bly⁴ discusses data handling.) Equations (1) and (2) are then solved to determine the coefficients D_1 and D_2 for the calibration lines. (Equations (1) and (2) are presented in another form in Hamielec's original paper.² The transformation to the present form is described in ref. 5, p. 1384.) In actual fact, however, since $F(V)$ is substituted for $W(V)$, an "effective linear calibration" is obtained by the Hamielec method with coefficients D_1' and D_2' as shown by eqs. (3) and (4):

$$\bar{M}_w = \Sigma_V F(V) D_1' e^{-D_2' V} \quad (3)$$

$$\bar{M}_n = 1 / \Sigma_V [F(V) / D_1' e^{-D_2' V}] \quad (4)$$

Once the primed calibration coefficients are determined and the unknown samples are chromatographed, the $F(V)$ for the samples are inserted into eqs. (3) and (4) and the molecular weights are calculated according to the Hamielec method.

The effective linear calibration, $D_1' e^{-D_2' V}$, of Hamielec compensates for the GPC curve broadening [the difference between $W(V)$ and $F(V)$] and yields correct \bar{M}_n and \bar{M}_w when the standard and samples are similar. But it does not provide accurate MW results when they are different, and it also does not represent the true peak position calibration, i.e., the calibration line which would be obtained if narrow MWD standards of the polymer were used.

Development

We have retained the true calibration $D_1 e^{-D_2 V}$ by using equations which correct the chromatographic dispersion effect in $F(V)$. This provides much more accurate \bar{M}_n and \bar{M}_w values (Table I).

Our formulations which form the basis of GPCV2 are derived in Appendices I and II and are given by eqs. (5) and (6). These equations have also been derived by Hamielec^{5,6} and Provder and Rosen⁷ using another mathematical approach and purpose:

$$\bar{M}_w = e^{-1/2(D_2\sigma)^2} \cdot \Sigma_V [F(V) D_1 e^{-D_2 V}] \quad (5)$$

$$\bar{M}_n = e^{1/2(D_2\sigma)^2} / \Sigma_V \frac{F(V)}{D_1 e^{-D_2 V}} \quad (6)$$

The procedures for making the calibration and MW computations are the same as described above for the Hamielec method. The correction term employs the third variable, σ , the peak standard deviation caused by column dispersion, which is measured to a first approximation as the experimental σ of a very narrow-

TABLE I
Effect of GPC Calibration Method on Accuracy

Method	Sample 1			Sample 2			Sample 3			Sample 4		
	\overline{M}_w^a	\overline{M}_n^a	Ave. % error	\overline{M}_w	\overline{M}_n	Ave. % error	\overline{M}_w	\overline{M}_n	Ave. % error	\overline{M}_w	\overline{M}_n	Ave. % error
A. μ -Styragel Columns (N = 13,000, Toluene; $\sigma = 0.7$ ml, 4A)												
Calcd./rep. value	64	44	—	39	28	—	288	137	—	20	20	—
Peak position	74	34	19	47	22	21	454	107	40	23	17	15
Hamielec	64	44	—	46	32	16	210	107	25	30	26	40
GPCV2	64	44	—	42	30	7	314	129	7	23	23	15
B. Vit-X Columns (N = 3,500, Toluene; $\sigma = 1.05$ ml, 4A)												
Calcd./rep. value	64	44	—	39	28	—	288	137	—	20	20	—
Peak position	86	42	20	51	26	21	333	136	8	24	18	15
Hamielec	64	44	—	44	31	13	166	105	33	27	23	25
GPCV2	64	44	—	38	27	2	247	143	9	18	19	8

^a See experimental section. Values listed are $\times 10^{-3}$.

MWD polystyrene standard. At $\sigma = 0$, GPCV2 reduces to the Hamielec method (see Appendix III).

The much improved results of GPCV2 shown in Table I were obtained by using a single σ value. Since σ has a small dependence on the GPC retention volume,⁸ it would be best to account for σ variation in the method, but the functionality depends on the system, and the correction is small relative to not using σ at all. We are studying this further as well as a formulation to compensate for the skewness of GPC column dispersion.

In working with the Hamielec method, we observed several effects which caused further study. The effective calibration line rotates counterclockwise relative to the peak position calibration line and the extent of rotation increases with increasing dispersion of the column and with decreasing polydispersity of the standard used. The rotation also occurs about an anchor point located near the average retention volume of the standard. This explains why the sample and the standard must be similar for accurate results with the Hamielec method.

Realizing these effects, we sought to examine the true change in molecular weight with retention volume for an experimental GPC elution curve by incorporating the effect of the chromatographic dispersion into the formulations. The derivations of eqs. (7) and (8) for $\overline{M}_w(V)$ and $\overline{M}_n(V)$ (the actual \overline{M}_w and \overline{M}_n of an infinitesimal fraction at retention volume V) are presented in Appendix I:

$$\overline{M}_w(V) = \frac{F(V - D_2\sigma^2)}{F(V)} e^{1/2(D_2\sigma^2)} M_t(V) \quad (7)$$

$$\overline{M}_n(V) = \frac{F(V)}{F(V + D_2\sigma^2)} e^{-1/2(D_2\sigma^2)} M_t(V) \quad (8)$$

All symbols are the same as previously defined; $M_t(V)$ is the peak position molecular weight as a function of retention volume; and $D_2\sigma^2$ is an incremental volume unit.

The variations of MW with retention volume for experimental chromatograms as predicted by eqs. (7) and (8) were then compared to the predictions made by the Hamielec method and the peak position method [$M_t(V)$, designated as $\langle V_R \rangle$]

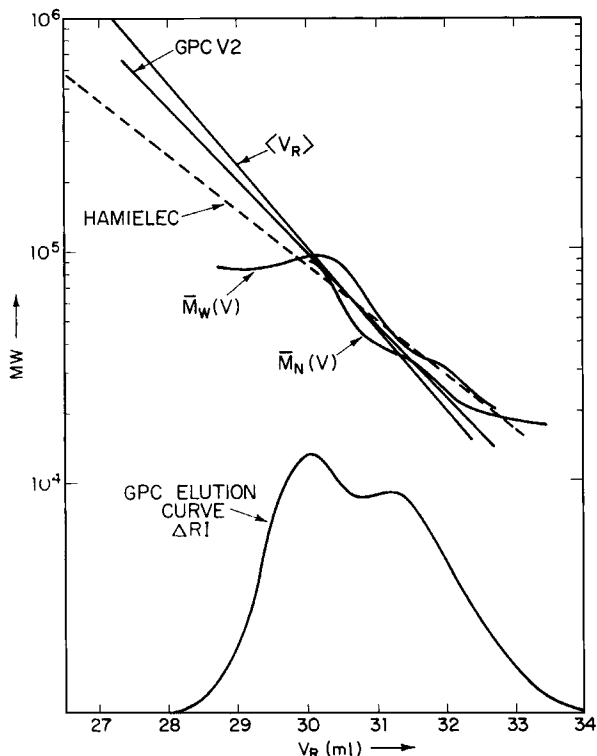


Fig. 1. Comparison of true molecular weight curves and calibration lines: sample 1, the standard.

in the figures] in Figures 1–4. As shown in Appendix II, eqs. (7) and (8) led to eqs.(5) and (6), the principal formulations for the GPCV2 method.

EXPERIMENTAL

Polystyrene samples were prepared using blends of commercially available characterized standards (Pressure Chemical Co., Pittsburgh, Pa.). Sample 1 was a blend of 3 parts PS 4A, 2 parts 7B, and 1 part 2A. Sample 2 was a blend of 1 part 4A, 2 parts 7B, and 3 parts 2A. Sample 3 was NBS 706 polystyrene, and sample 4 was the narrow polystyrene standard 2A alone. Narrow standards 4A, 7B, and 2A have reported polydispersity values of less than 1.06 and reported MW values of 97,200, 37,000, and 19,800, respectively. The calculated and/or reported MW values of the blends and NBS 706 are listed in Table I.

In Figures 1–4 and in Table I, the peak position calibration (actual calibration is done using the average retention volume $\langle V_R \rangle$) was made from the individual narrow standard peaks (4A, 7B, and 2A, respectively). The Hamielec and our GPCV2 calibration lines (GPCV2 line shown in Fig. 1 only) were calculated by using sample 1 as the calibration standard. For the data in Table I, the \bar{M}_n and \bar{M}_w calculations were made using the appropriate calibration, $F(V)$, and, where applicable, σ values. For Figures 1–4, $\bar{M}_w(V)$ and $\bar{M}_n(V)$ were calculated from eqs. (7) and (8). The value of σ was determined from a separate peak of standard 4A in our computer program using the procedure of James and Martin.⁹ The

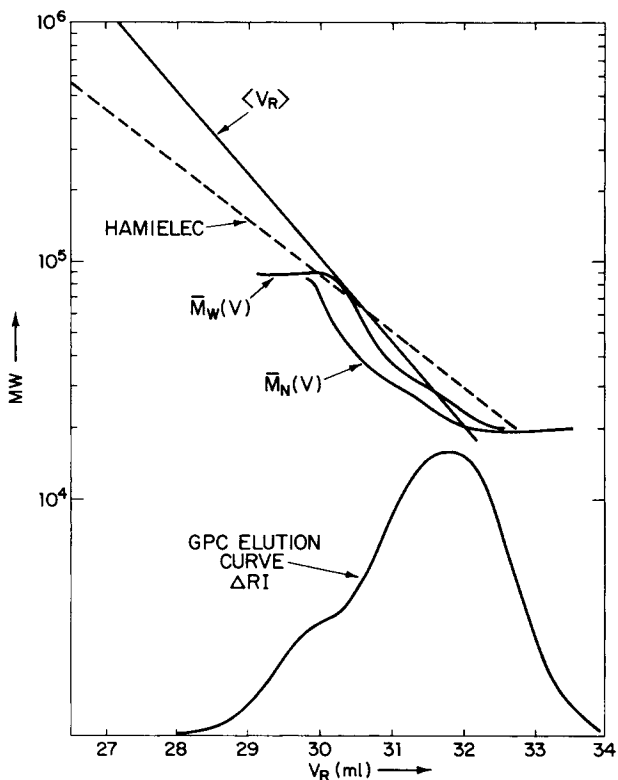


Fig. 2. Comparison of true molecular weight curves and calibration lines: sample 2, a polystyrene mixture.

pertinent equation is $\sigma = (A/H)/\sqrt{2\pi}$, where H is peak height and A is peak area.

The experimental chromatograms were obtained on a high-speed GPC assembly composed of a Model R401 refractive index detector and a Model 6000 solvent delivery system (Waters Associates, Milford, Mass.). The samples were injected using a high-pressure Model CV-6UHPa-C-20 valve with an external loop (Valco Instruments Co., Houston, Texas). Four 30 cm \times 0.76 cm I.D. columns of MW exclusion limits of 10^2 , 10^3 , 10^5 , 10^6 μ -Styragel (Waters Associates) or 84, 171, 660, 1933 \AA Vit-X (Perkin-Elmer Co., Norwalk, Conn.) were used in series as indicated. The GPC data were collected by computer by the du Pont Experimental Station Real Time System.¹⁰ Computer programs for handling the equations were developed by H.J.S.

RESULTS

The results of using different methods for calibration in GPC are shown in Table I, where the per cent error in MW for various samples is related to the calibration method. In the table, sample 1 was chosen as the calibrating standard for the Hamielec method and for our GPCV2 method, while the individual components of the sample were used to obtain the peak position calibration (see experimental section). The errors are minimum for the GPCV2 method. Note

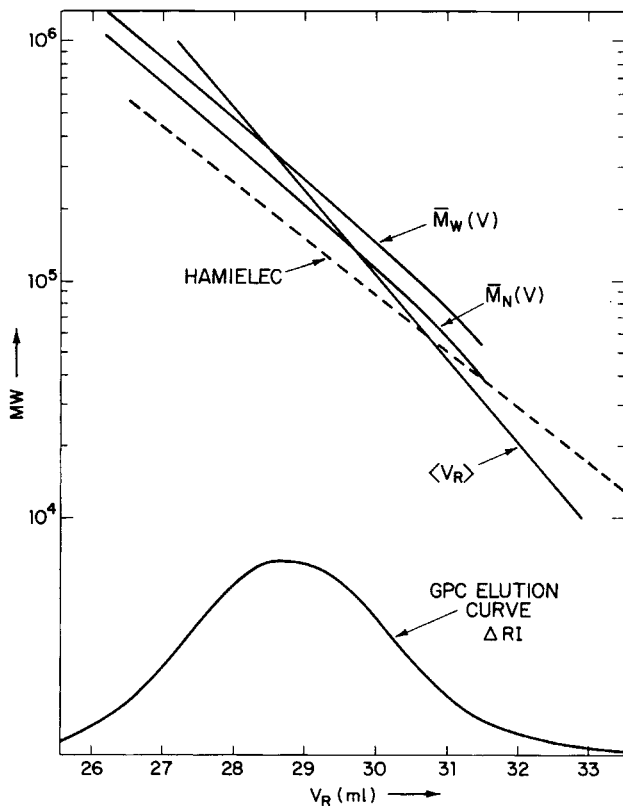


Fig. 3. Comparison of true molecular weight curves and calibration lines: sample 3, a broad-MWD sample.

that the errors in the data in Table I are caused by the summation of all error-producing effects, i.e., column and detector dispersion, skewing, errors in reported MW values for the samples, flow instability, detector noise, etc. The residual error in the GPCV2 method is within expectations compensating for dispersion. Although somewhat obscured by the experimental precision, GPCV2 also gives improved MW accuracy over the peak position method even though the peak position method is optimum with these specific experiments. For polymers other than polystyrene, the true peak position calibration is not possible due to lack of narrow standards.

As shown in Figure 1, GPCV2 has considerably reduced the difference between the Hamielec and the peak position calibration curves. For polymers where there are only broad MWD standards available, GPCV2 is the preferred method to approximate the true GPC calibration.

The features of the conventional-peak position and Hamielec calibrations are compared to the true MW variations $\bar{M}_w(V)$ and $\bar{M}_n(V)$ in Figures 1-4. Figure 1 shows how the Hamielec calibration is rotated counterclockwise away from the peak position calibration at the anchor point (for this sample) of about $M = 50,000$. The Hamielec line encompasses the true MW values over most of the MW range since they were computed from the same sample used as standard. MW values computed by GPCV2 or the Hamielec method for this sample will give accurate results.

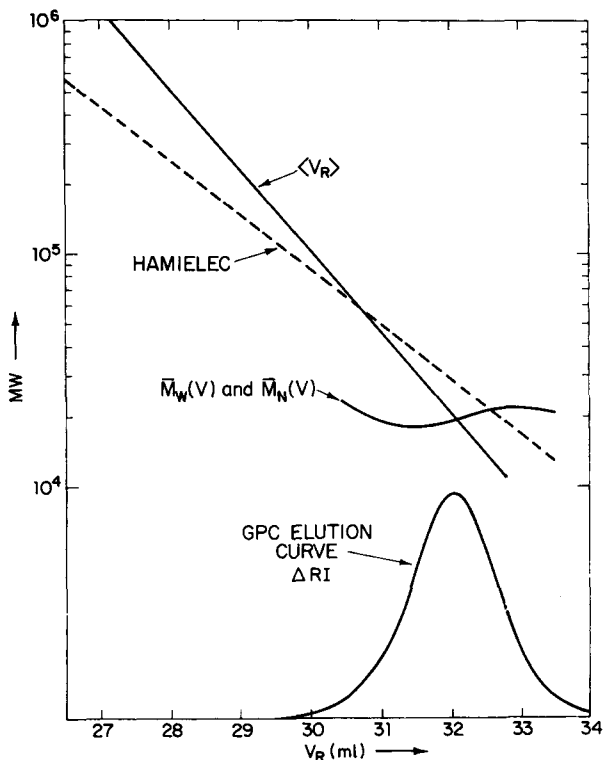


Fig. 4. Comparison of true molecular weight curves and calibration lines: sample 4, a narrow-MWD sample.

However, Figure 2 illustrates that the true MW values begin to differ from the Hamielec and peak position lines when another sample of different MWD is considered. In Figures 1–4, the calibrations were made via sample 1, and are the same as in Figure 1, but the true $\bar{M}_w(V)$ and $\bar{M}_n(V)$ changes across the experimental GPC curves were calculated according to eqs. (7) and (8). As the sample differs more and more from the standard, the Hamielec line becomes a poorer estimate of the true MW behavior. For example, in Figure 3, neither $\bar{M}_w(V)$ nor $\bar{M}_n(V)$ ever intersect with the Hamielec line; and in Figure 4, the respective slopes are nearly at right angles. Figure 4 is the chromatogram of a very narrow-MWD sample, and most of the curve profile is caused by peak broadening. Therefore, it is expected that MW should vary only little with V_R .

The fact that the $\bar{M}_w(V)$ and $\bar{M}_n(V)$ curves vary as a function of column dispersion and the shape of the sample MWD led to the prediction that the GPC elution curve profiles as monitored by continuous viscometer or light-scattering detectors¹¹ are similarly affected by column dispersion and sample MWD. These effects should be considered in interpreting the results of these MW-specific GPC detectors.

The authors wish to acknowledge stimulating discussions held with Dr. A. E. Hamielec on portions of this work.

Appendix I

Actual MW Across the Experimental GPC Elution Curve, $\overline{M}_w(V)$ and $\overline{M}_n(V)$

In a GPC experiment for a broad-MWD sample, the molecular species detected at any particular retention volume is not truly monodispersed in MW. Neighboring molecules of different MW are dispersed to the same retention volume by column dispersion. The actual MW at each retention volume is, therefore, not accurately described by the true calibration curve of the GPC columns, except under the hypothetical condition of infinite column resolution.

We let $\overline{M}_w(V)$ and $\overline{M}_n(V)$ be the weight- and the number-average MW of the MW mixture within the infinitesimal fraction at retention volume V . If there were no column dispersion, i.e., at infinite resolution, the weight concentration and the MW at any GPC retention volume, y , are $W(y)$ and $M_t(y)$, respectively, where $M_t(y)$ is the true calibration relationship. If we let a Gaussian function $G(V - y)$ describe the fraction of the species at y which gets spread over to V , we can consider the MW mixture at any V to be made up by species coming from all different y retention volumes. The MW of these species are $M_t(y)$, and their weight concentrations detected at V are $W(y)G(V - y)$.

By the definition of the weight-average \overline{M}_w , we can, therefore, write $\overline{M}_w(V)$ of the MW mixture at V as follows:

$$\overline{M}_w(V) = \frac{\int_{-\infty}^{\infty} [W(y)G(V - y)]M_t(y)dy}{\int_{-\infty}^{\infty} [W(y)G(V - y)]dy} \quad (\text{A1})$$

where

$$G(V - y) = \frac{1}{\sigma\sqrt{2\pi}} e^{-(V-y)^2/2\sigma^2} \quad (\text{A2})$$

and

$$M_t(y) = D_1 e^{-D_2 y} \quad (\text{A3})$$

The denominator of eq. (A1) gives the overall weight concentration detected at the retention volume V , which is simply $F(V)$, or

$$F(V) = \int_{-\infty}^{\infty} W(y)G(V - y)dy \quad (\text{A4})$$

The integral in the numerator of eq. (A1) can be reduced as follows:

$$\begin{aligned} \text{numerator} &= \int_{-\infty}^{\infty} W(y) \left(\frac{1}{\sigma\sqrt{2\pi}} \right) D_1 e^{-1/2\sigma^2[(V-y)^2 + 2D_2\sigma^2 y]} dy \\ &= \int_{-\infty}^{\infty} W(y) \left(\frac{1}{\sigma\sqrt{2\pi}} \right) D_1 e^{-1/2\sigma^2[(V-D_2\sigma^2)-y]^2 - D_2 V + 1/2(D_2\sigma)^2} dy \\ &= D_1 e^{-D_2 V} e^{1/2(D_2\sigma)^2} \left\{ \int_{-\infty}^{\infty} W(y)G(V - D_2\sigma^2 - y)dy \right\} \\ &= M_t(V) e^{1/2(D_2\sigma)^2} F(V - D_2\sigma^2) \end{aligned} \quad (\text{A5})$$

By substituting (A4) and (A5) into (A1), we thus derived eq. (7) in the text:

$$\overline{M}_w(V) = \frac{F(V - D_2\sigma^2)}{F(V)} e^{1/2(D_2\sigma)^2} M_t(V) \quad (\text{A6})$$

Similarly, we derived $\overline{M}_n(V)$ as follows:

$$\overline{M}_n(V) = \frac{\int_{-\infty}^{\infty} W(y)G(V - y)dy}{\int_{-\infty}^{\infty} W(y)G(V - y)/M_t(y)dy} \quad (\text{A7})$$

where the denominator

$$\begin{aligned}
 &= \int_{-\infty}^{\infty} W(y) \left(\frac{1}{\sigma\sqrt{2\pi}} \right) D_1^{-1} e^{-1/2\sigma^2[(V-y)^2-2D_2V\sigma^2]} dy \\
 &= \int_{-\infty}^{\infty} W(y) \left(\frac{1}{\sigma\sqrt{2\pi}} \right) D_1^{-1} e^{-1/2\sigma^2[(V+D_2\sigma^2)-y]^2+D_2V+1/2(D_2\sigma^2)^2} dy \\
 &= D_1^{-1} e^{D_2V} e^{1/2(D_2\sigma^2)^2} \left\{ \int_{-\infty}^{\infty} W(y) G(V+D_2\sigma^2-y) dy \right\} \\
 &= \frac{1}{M_t(V)} e^{1/2(D_2\sigma^2)^2} F(V+D_2\sigma^2)
 \end{aligned} \tag{A8}$$

By substituting (A4) and (A8) into (A7), we obtained eq. (8) in the text:

$$\bar{M}_n(V) = \frac{F(V)}{F(V+D_2\sigma^2)} e^{-1/2(D_2\sigma^2)^2} M_t(V) \tag{A9}$$

Appendix II

Derivation of Sample \bar{M}_w and \bar{M}_n Formulations from $\bar{M}_w(V)$ and $\bar{M}_n(V)$

We can consider the entire sample chromatographed by GPC to be the sum of the fractions actually eluted at different retention volumes V . The weight concentration of each fraction is $F(V)$; and we know from Appendix I that each fraction has the $\bar{M}_w(V)$ and $\bar{M}_n(V)$ values as described by eqs. (A6) and (A9).

We can, therefore, write \bar{M}_w and \bar{M}_n of the entire sample as follows in accordance with the definition of the weight- and the number-average MW:

$$\bar{M}_w = \frac{\int_{-\infty}^{\infty} F(V) \bar{M}_w(V) dV}{\int_{-\infty}^{\infty} F(V) dV} \tag{A10}$$

and

$$\bar{M}_n = \frac{\int_{-\infty}^{\infty} F(V) dV}{\int_{-\infty}^{\infty} F(V) / \bar{M}_n(V) dV} \tag{A11}$$

The integrals in these equations are evaluated as follows:

$$\begin{aligned}
 \text{The numerator of (A10)} &= \int_{-\infty}^{\infty} F(V - D_2\sigma^2) e^{1/2(D_2\sigma^2)^2} D_1 e^{-D_2V} dV \\
 &= \int_{-\infty}^{\infty} F(V) e^{1/2(D_2\sigma^2)^2} D_1 e^{-D_2(V+D_2\sigma^2)} dV \\
 &= D_1 e^{-1/2(D_2\sigma^2)^2} \int_{-\infty}^{\infty} F(V) e^{-D_2V} dV
 \end{aligned} \tag{A12}$$

$$\begin{aligned}
 \text{The denominator of (A11)} &= \int_{-\infty}^{\infty} F(V + D_2\sigma^2) e^{1/2(D_2\sigma^2)^2} D_1^{-1} e^{D_2V} dV \\
 &= \int_{-\infty}^{\infty} F(V) e^{1/2(D_2\sigma^2)^2} D_1^{-1} e^{D_2(V-D_2\sigma^2)} dV \\
 &= D_1^{-1} e^{-1/2(D_2\sigma^2)^2} \int_{-\infty}^{\infty} F(V) e^{D_2V} dV
 \end{aligned} \tag{A13}$$

The substitutions of (A12) and (A13) into (A10) and (A11) give

$$\bar{M}_w = D_1 e^{-1/2(D_2\sigma)^2} \frac{\int_{-\infty}^{\infty} F(V) e^{-D_2 V} dV}{\int_{-\infty}^{\infty} F(V) dV} \quad (\text{A14})$$

$$\bar{M}_n = D_1 e^{1/2(D_2\sigma)^2} \frac{\int_{-\infty}^{\infty} F(V) dV}{\int_{-\infty}^{\infty} F(V) e^{D_2 V} dV} \quad (\text{A15})$$

By replacing the integrals with discrete summations of equal retention volume increment, we derived eqs. (5) and (6) in the text:

$$\bar{M}_w = e^{-1/2(D_2\sigma)^2} \sum_V [F(V) D_1^{-D_2 V}] \quad (\text{A16})$$

$$\bar{M}_n = e^{1/2(D_2\sigma)^2} / \sum_V \frac{F(V)}{D_1 e^{-D_2 V}} \quad (\text{A17})$$

with $\sum_V F(V) = 1$.

Appendix III

A single broad standard calibration program has been widely distributed by Dr. A. E. Hamielec. This program uses a single-variable search (Fibonacci algorithm¹²) to provide the "effective" linear calibration curve from a single sample. To update this program to correct for dispersion, it is necessary to incorporate σ into the AVEMWS subroutine. This can be accomplished through a number of methods, one being a named COMMON statement. In the AVEMWS subroutine, the exponential factors of our eqs. (5) and (6) are calculated. The procedure for calculation of the summations remains unaltered. The summation results are then multiplied by the appropriate exponential factor before the return from AVEMWS. This procedure provides the dispersion corrected molecular weights with a minimum of programming effort. Similar incorporation of appropriate exponential factors can be performed to provide dispersion corrected results for unknown samples.

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Received April 9, 1976

Revised May 28, 1976