Number-Average Molecular Weight by Size Exclusion Chromatography

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SYNOPSIS

It is now theoretically possible to obtain absolute accurate values of number-average molecular weight of complex polymers (e.g., branched polymers or copolymers) using size exclusion chromatography (SEC) with only a detector that measures the difference between the eluting polymer solution viscosity and the viscosity of the pure mobile phase (a differential viscometer [DV] detector). However, both precision and accuracy of these "DV $\bar{M}_n$" values are of concern. In this work, the precision of NBS 706 polystyrene was found to be two to three times worse for the DV $\bar{M}_n$ than for the conventionally calculated $\bar{M}_n$. Also, regarding accuracy, the DV $\bar{M}_n$ values were affected by the location of the universal calibration curve along the retention volume axis (a problem intimately associated with the problem of specifying the correct interdetector volume), the sensitivity of the DV detector to low molecular weights present in the sample, and axial dispersion. Each of these sources of error are examined in turn and two methods of calculating $\bar{M}_n$ values are proposed. © 1994 John Wiley & Sons, Inc.

INTRODUCTION

Calculation of average molecular weights using SEC for polymers other than linear homopolymers is often a very uncertain procedure. For example, concentration detectors, such as a differential refractive index (DRI) detector, are generally sensitive to copolymer composition. So, the detector response reflects both composition and total polymer concentration at each retention volume. In addition to this detection problem, there is potentially an even more serious fractionation problem: Branched polymers and copolymers can exhibit more than one molecular weight at each retention volume because different combinations of branch length, branch frequency, and molecular weight for branched polymers or composition, sequence length, and molecular weight for copolymers can result in the same molecular size in solution. This means that there is then not a unique relationship between molecular weight and retention volume.

Until Goldwasser developed his equation,[1] the best recourse for such "complex" polymers appeared to be use of the light-scattering detector for true weight-average molecular weight for homopolymers and the differential viscometer (DV) detector for a true whole polymer intrinsic viscosity for any polymer. There was no satisfactory method of obtaining true, absolute $\bar{M}_n$ values for them. Many analysts treated every polymer as though it were polystyrene and calculated "polystyrene equivalent" number-average molecular weight from a DRI response alone. However, although $\bar{M}_n$ values obtained using the equation developed by Goldwasser often appear valid, three primary sources of error are present and can unexpectedly cause serious inaccuracies: the sensitivity of the DV detector to low molecular weights present in the sample, axial dispersion in the chromatograph, and accuracy of the universal calibration curve. In the next section, we present the equation, examine each of these sources of error in turn, and propose methods for assessing their effect on the final estimates of $\bar{M}_n$. We then show the results of an experimental investigation that attempts to apply these methods.

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THEORY

The DV $\bar{M}_n$ Equation

The equation developed by Goldwasser is as follows[1]:

$$\bar{M}_{n,J} = \frac{m}{\sum \frac{\eta_{sp,i}}{J_i} \Delta v_i} \quad (1)$$

A general derivation for the equation is shown in Appendix A. $\bar{M}_{n,J}$, the estimate obtained for $\bar{M}_n$ from this equation, is referred to here as the DV $\bar{M}_n$.

Equation (1) requires only the mass injected, m, the specific viscosity at each retention volume, $\eta_{sp,i}$, and the value of the ordinate of the universal calibration curve J_i ($= [\eta]_i M_i$) for any polymer at each v_i. The mass injected is obtained as the product of the concentration injected and the injection volume; the $\eta_{sp,i}$ values are obtained as the signal from the DV detector; and the J_i values are obtained by injecting a series of narrow polystyrene standards.

Goldwasser[1] showed that, by using eq. (1), $\bar{M}_n$ values of true absolute accuracy could be obtained for complex polymers so long as the polymer could be separated by hydrodynamic volume in the SEC and monitored using a DV detector.

Interdetector Volume

Interdetector volume needs to be determined when it is necessary to superimpose the output of the DV detector on that of the DRI. In application of the DV $\bar{M}_n$ [eq. (1)], this can occur when the universal calibration curve is based upon the peak retention volumes of narrow standards *as determined by the DRI detector.* Currently, there are many methods of determining the interdetector volume, with considerable controversy surrounding each of the methods.[2] One method of circumventing the problem is to read the peak retention volumes of the narrow standards from the DV detector chromatogram. The main concern in this approach is how accurately the position of the peak can be identified. The peak required is the peak of a chromatogram representing concentration vs. retention volume. It is that peak for which the molecular weight has been estimated for the standard. The DV chromatogram peak is the peak of $[\eta]_i c_i$ vs. retention volume. If the sample is not truly monodisperse, then there will be different values of $[\eta]_i$ and the peak of the DV chromatogram will not correspond to the point of maximum concentration.

A simple test for this situation is to examine the shapes of the DRI peak and the DV peak for each narrow standard. If the shapes are identical, then it may be safely assumed that this source of error is negligible and the peaks on the DV detector can be used to construct the universal calibration curve. If they are not identical, then either the samples are too polydisperse or significant axial dispersion is taking place between the two detectors.

Comparison of peak shapes is best accomplished by plotting the normalized chromatogram heights vs. retention volume, v_i, in each case. For the DRI, $W_{N,i}$ is the normalized chromatogram height:

$$W_{N,i} = \frac{W_i}{\sum W_i \Delta v_i} \quad (2)$$

where W_i is the base-line-corrected, concentration chromatogram height at retention volume v_i. For the DV, $\eta_{sp,N,i}$ is the normalized chromatogram height:

$$\eta_{sp,N,i} = \frac{\eta_{sp,i}}{\sum \eta_{sp,i} \Delta v_i} \quad (3)$$

where $\eta_{sp,i}$ is the base-line-corrected DV detector chromatogram height at retention volume v_i.

DV Detector Sensitivity

The response of the DV detector, $\eta_{sp,i}$, is the product of the intrinsic viscosity at each retention volume, $[\eta]_i$, and the concentration of polymer at each retention volume, c_i. This means that this detector may not respond to the low molecular weight tail of the distribution (since there we have low $[\eta]_i$ values at low c_i). Unfortunately, as is widely known, it is the low molecular weight tail that is vitally important for accuracy in the determination of $\bar{M}_n$.

Assessing the effect of this source of error on the $\bar{M}_n$ value obtained from eq. (1) depends upon the complexity of the polymer and the information available. The essential difficulty is that the effect is sample-dependent: It depends upon the low molecular weight tail present in a particular sample. The following cases can be defined when only a DRI and DV detector are available on the SEC:

Case 1: Linear Homopolymer

For this case, another estimate of $\bar{M}_n$ can be obtained in the usual way by using the DRI detector alone and the following equation:

$$\bar{M}_n = \frac{\sum W_i \Delta v_i}{\sum \frac{W_i}{M_i} \Delta v_i} \quad (4)$$

where, as above, W_i is the base-line-corrected, concentration chromatogram height at retention volume v_i, and M_i is the molecular weight at retention volume v_i. M_i values are obtained by some type of calibration (e.g., injection of narrow standards and plotting of their peak molecular weights vs. their peak retention times on a semilog plot).

Equation (4) can also be written as

$$\bar{M}_n = \frac{m}{\sum \frac{c_i}{M_i} \Delta v_i} \quad (5)$$

Comparing eqs. (1) and (5), it can be seen that the differences in the Goldwasser and the conventional approaches actually center about the quantity summed in the denominator. For eq. (1), the denominator is the area under a plot of $\eta_{sp,i}/J_i$ vs. v_i, whereas for eq. (5), the relevant area is under a plot of c_i/M_i vs. v_i. Furthermore, eq. (1) transforms to eq. (5) when we substitute $[\eta]_i c_i$ for $\eta_{sp,i}$ and $[\eta]_i M_i$ for J_i. What all this means is that, if there were no inaccuracies, these two plots would superimpose. They actually each represent different estimates of the number of moles of polymer present per milliliter of retention volume plotted vs. retention volume. Thus, an inspection of these two plots can provide the basis for assessment of the relative sensitivity of the two detectors and its impact on the calculated $\bar{M}_n$ value.

Complications that may need to be addressed include the effect of axial dispersion on the results and variation of the proportionality constant between concentration and the DRI detector response with molecular weight at low molecular weights.

Case 2: Branched Homopolymer

In this case, the DRI detector is providing a response that can be interpreted in terms of concentration of polymer at each retention volume. However, now, the DV detector must be used to obtain a calibration curve for use in eq. (4); i.e., the DV and DRI detectors together provide $[\eta]_i$ values across the chromatogram of the branched sample that are used with the universal calibration curve obtained from narrow polystyrene standards to provide molecular weight at each retention volume. Furthermore, this molecular weight value is now a local value of number-average molecular weight, $M_{n,i}$. Now, the relevant plots are $\eta_{sp,i}/J_i$ vs. v_i and $c_i/M_{n,i}$ vs. v_i with the same significance as before because the definition for J_i is $[\eta]_i M_{n,i}$.

However, now an additional complication is the need to determine the interdetector volume. Actually, as will be seen below, the problem of determining the interdetector volume actually enters the use of the eq. (1) in simpler cases also but to a lesser extent.

Case 3: Polymer Blends and Copolymers

Now, the response of the DRI detector is confounded by both composition of the molecules exiting at a particular retention volume and by their total concentration. Concentration at each retention volume cannot be calculated from the DRI response unless another detector, a composition detector such as a UV or IR detector, is added to the instrument. Once this is done, the investigation can proceed as for Case 2.

Axial Dispersion Effects

With regard to the eq. (1), it should be noted that although, in practice, the values used for m and J_i are "true values" not subject to axial dispersion effects, the values used for $\eta_{sp,i}$ are not. With axial dispersion present, the DV responds at each retention volume to the variety of molecules that it detects. Thus, the $\eta_{sp,i}$ values are affected by the degree of axial dispersion ("band spreading") in the chromatograph. We will now distinguish the specific viscosity attributed to a particular molecular size, $\eta_{sp,i}$, from that measured by the DV (the measurement of the mixture of different sizes in the detector cell as a result of axial dispersion effects), termed $\eta_{sp,i,axd}$, where the subscript "axd" means that the value is affected by axial dispersion.

Thus, in practice, it is not the "true" $\bar{M}_n$ value, $\bar{M}_{n,J}$, that is being obtained, but rather $\bar{M}_{n,J,unc}$, from

$$\bar{M}_{n,J,unc} = \frac{m}{\sum \frac{\eta_{sp,i,axd}}{J_i} \Delta v_i} \quad (6)$$

This situation is exactly analogous to the situation encountered in the more traditional method of obtaining $\bar{M}_n$, i.e., the use of the DRI chromatogram and eq. (4). The value of $\bar{M}_n$ obtained from eq. (4) is termed an "uncorrected" value because the chromatogram heights are affected by axial dispersion effects, whereas the M_i values are generally "true"

values. Thus, to reflect this situation, eq. (4) can be rewritten as

$$\bar{M}_{n,\text{unc}} = \frac{\sum F_i \Delta v_i}{\sum \frac{F_i}{M_i} \Delta v_i} \tag{7}$$

where the chromatogram heights are now denoted by F_i rather than by W_i to indicate that they are affected by axial dispersion.

Hamielec and Ray[3] showed that, with the assumption of a "linear" calibration curve over the range of the sample,

$$M_i = D_1 \exp(-D_2 v_i) \tag{8}$$

as well as constant, Gaussian spreading, a resolution correction factor that could be applied to the value of $\bar{M}_n$ obtained from eq. (1), $\bar{M}_{n,\text{unc}}$, was given by

$$\bar{M}_n = \bar{M}_{n,\text{unc}} \exp\left(\frac{\sigma^2 D_2^2}{2}\right) \tag{9}$$

where σ is the constant standard deviation of the Gaussian spreading function.

To derive a correction factor for the $\bar{M}_n$ value obtained from eq. (1), we first require that the values of $\eta_{sp,i}$ vs. retention volume can be treated exactly as we treat a concentration chromatogram, i.e., the specific viscosity values for different molecules must be additive at each retention volume. Appendix B shows reasoning supporting this assumption. Later in this article, we show some experimental verification.

Now, considering the specific viscosity as a continuous function with retention volume, $\eta_{sp}(v)$, just as we often do with concentration, we can consider the observed chromatogram from the DV detector as the sum of a series of overlapping chromatograms, one for each molecular size present in the sample. We then can parallel the derivation of Hamielec and Ray for the concentration detector with a similar derivation for the DV detector.

The resulting correction equation is (Appendix C)

$$\bar{M}_{n,J} = \bar{M}_{n,J,\text{unc}} \exp\left(\frac{\sigma^2 D_{2,J}^2}{2}\right) \tag{10}$$

The equation is almost identical to eq. (9). The main difference [other than the fact that it is to be applied to the $\bar{M}_n$ value obtained from eq. (1) rather than the one from eq. (4)] is that it includes the slope of the universal calibration curve, $D_{2,J}$, instead of the slope of the conventional molecular weight calibration curve, D_2. This is unfortunate because the former is always larger than the latter. For a linear homopolymer, e.g., $D_{2,J}$ is $(a + 1)$ times D_2 (or at least 1.5 times D_2).

EXPERIMENTAL

The SEC system using a low-angle-laser light-scattering (LALLS) detector, a DV and a DRI was configured as described previously.[4] Three 7.5 mm i.d. × 300 mm PLgel mixed-C columns (Polymer Laboratories, Amherst, MA) were coupled in series. Uninhibited HPLC-grade tetrahydrofuran (J. T. Baker) was used as received and continuously sparged with helium. The nominal flow rate was 1.0 mL/min. Flow was corrected using an internal flow marker. The column effluent was split approximately equally to a model 502A DV (Viscotek Corp., Porter, TX) and a KMX-6 LALLS photometer (LDC Analytical, Riviera Beach, FL). The DRI was connected to the outlet of the LALLS cell. The columns, DV, and DRI were controlled to 30.0 ± 0.5°C. Only data from the DV and DRI are reported in this study.

Narrow molecular weight distribution polystyrene standards were obtained from Polymer Laboratories. Narrow standard and universal calibration curves were constructed and evaluated as described in Ref. 5. The broad polystyrene standard (NBS 706) was obtained from the National Institute of Standards and Technology, Gaithersburg, MD, and the poly(methyl methacrylate) (PMMA) standards were synthesized at Eastman Kodak Co. All the broad standards (and their mixtures) were injected at a total concentration of 1.5 mg/mL.

Table I shows the various molecular weights of the standards and their blends. Values shown in this table are considered the "true" ones for later comparisons. For NBS 706, the "true" values were averages of over 400 analyses obtained in T. H. Mourey's laboratory at Eastman Kodak Co. over a 3 year period.[5,6] NBS 706 contains low molecular weight material that elutes near the solvent peaks. This material has not been included in the calculation of the "true" $\bar{M}_n$. Such extensive data were not available for PMMA. For the PMMA samples, "true" values were calculated using eq. (4) with both DV and DRI detectors. These values for PMMA have now been confirmed by light scattering and DV detection in both tetrahydrofuran and dimethylformamide mobile phases.

Table I Polymer Standards Used in the Study

Standard	$\bar{M}_n \times 10^{-3}$	$\bar{M}_w \times 10^{-3}$
NBS 706 (polystyrene)	122.0	275.0
PMMA 100K [poly(methyl methacrylate)]	44.5	89.0
PMMA 400K [poly(methyl methacrylate)]	158.0	426.0
NBS 706 + PMMA 100K[a]	65.2	182.0
NBS 706 + PMMA 400K[a]	137.7	350.5
PMMA 100K + PMMA 400K[a]	69.4	257.5

[a] Values for blends calculated from individual polymer blend component values assuming a 50 : 50 by wt blend.

RESULTS AND DISCUSSION

Accuracy and Precision of $\bar{M}_n$ Values

Figure 1 shows universal calibration curves determined from reading peak retention volumes of narrow standards on the DRI detector and from reading peak retention volumes of the same standards on the DV chromatograms. The main reason for the displacement of one curve with respect to the other is that the polymer molecules reach the DV and DRI detectors at different times. The displacement has been used to obtain an estimate of the "interdetector volume." However, when this estimate is used in conventional calculation of molecular weight averages from the combination of DRI and DV detectors via the universal calibration curve, incorrect values are generally obtained. This led to the development of an alternative method determining the interdetector volume by superimposing the $\log[\eta]$ vs. retention volume data from a broad standard on the plot obtained by plotting results for a series of narrow standards. This method is associated with what we term the "systematic approach" for multidetector SEC analysis,[7,8] and the interdetector volume estimate so obtained will be referred to in the remainder of this article as the "systematic approach estimate."

Table II shows a comparison of three calculated $\bar{M}_n$ values:

$\bar{M}_n$ from Eq. (4) and the DRI-based Universal Calibration Curve

In calculating $\bar{M}_n$ from eq. (4), an estimate for the molecular weight at each retention volume is needed.

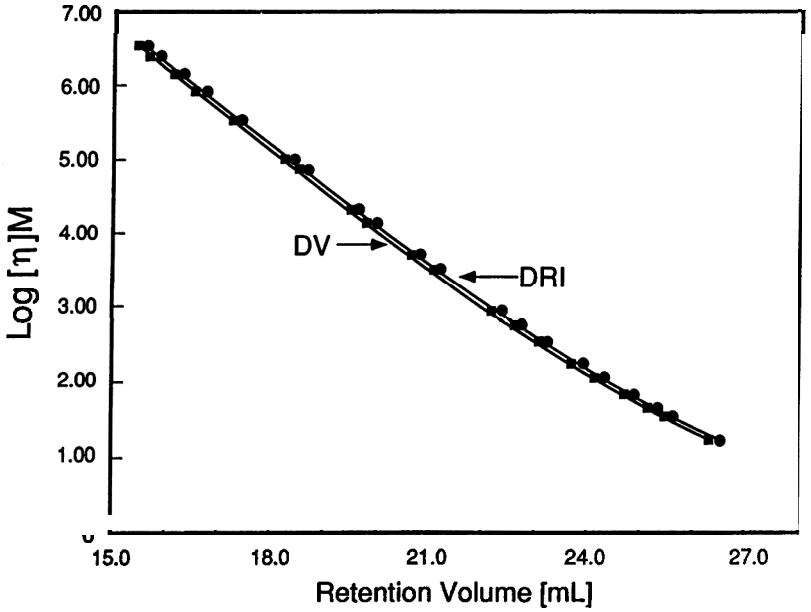

Figure 1 Universal calibration curves from peak retention volumes of narrow standards using the DRI and DV chromatograms.

Table II $\bar{M}_n$ Values for Standards and Mixtures of Standards

Standard: Description and True $\bar{M}_n$ Value $\times 10^{-3}$	$\bar{M}_n$ [Eq. (4), DRI-based Universal Calibration Curve)		$\bar{M}_n$ [Eq. (1), DRI-based Universal Calibration Curve)		$\bar{M}_n$ [Eq. (1), DV-based Universal Calibration Curve)	
	$\bar{M}_n \pm s^a \times 10^{-3}$	C.V.[b] (%)	$\bar{M}_n \pm s^a \times 10^{-3}$	C.V.[b] (%)	$\bar{M}_n \pm s^a \times 10^{-3}$	C.V.[b] (%)
NBS 706 ($\bar{M}_n$ = 122.0)	117.4 ± 6.5	5.5	131.3 ± 15.6	11.9	122.0 ± 15.5	12.7
PMMA 100K ($\bar{M}_n$ = 44.5)	44.5 ± 2.9	6.5	49.3 ± 2.1	4.4	45.4 ± 2.0	4.4
PMMA 400K ($\bar{M}_n$ = 158.0)	158.0 ± 7.5	4.7	173.7 ± 21.7	12.5	161.3 ± 20.5	12.7
NBS 706 + PMMA 100K ($\bar{M}_n$ = 65.2)	57.6 ± 3.1	5.4	66.4 ± 3.6	5.4	61.3 ± 3.4	5.5
NBS 706 + PMMA 400K ($\bar{M}_n$ = 137.7)	129.0 ± 10.7	8.30	136.7 ± 13.3	9.7	126.7 ± 12.5	9.9
PMMA 100K + PMMA 400K ($\bar{M}_n$ = 69.4	68.2 ± 2.7	4.0	78.2 ± 3.4	4.4	71.5 ± 3.4	4.8

[a] s is the sample estimate of the standard deviation of the measurements. The $\bar{M}_n$ value reported in each case is the average of n replicates, where n is 33 for NBS 706 and 3 for all other samples.

[b] C.V. is the coefficient of variation, which is defined as $(s/\bar{M}_n) \times 100$.

It was obtained in this case from the universal calibration curve constructed from the peak retention volumes of the DRI chromatograms of narrow polystyrene standards (this is henceforth referred to as the DRI-based universal calibration curve). The molecular weight calibration was generated each time a broad sample was injected by combining the intrinsic viscosity at each retention volume obtained from the DV and use of the DRI-based universal calibration curve. The systematic approach was used to obtain the interdetector volume necessary for this computation.[7-9]

When two different polymers are present, there are two sources of error in eq. (4). One source is specification of the molecular weight at each retention volume. The interpretation method used actually provides the $\bar{M}_n$ value at each retention volume. When the component chromatograms from the polymers present overlap, two different molecular weights can be expected at each retention volume (the difference between them will depend upon how different are the molecular weight calibration curves are for each of the polymers). The second source of error is the refractometer response. It is affected by both polymer composition and by concentration. If one polymer type causes a stronger response than the other, use of eq. (4) interprets this stronger response as a greater total polymer concentration. These sources of error are not present when eq. (1) is used.

$\bar{M}_n$ from Eq. (1) and the DRI-based Universal Calibration Curve

An estimate of molecular size at each retention volume is needed along with an estimate of the specific viscosity corresponding to this molecular size. In this case, the DRI-based universal calibration curve and the "systematic approach" estimate of the interdetector volume was used to obtain these respective values.

$\bar{M}_n$ from Eq. (1) and the DV-based Universal Calibration Curve

Now, the system is treated as though the DRI detector was not present. The molecular size estimate is obtained from a universal calibration curve constructed by using the peak retention volume of narrow standard DV chromatograms. This curve is henceforth referred to as the DV-based universal calibration curve. Since the DV chromatogram is

then on the same time axis as is the universal calibration curve, no interdetector volume estimate is necessary to find the value of specific viscosity corresponding to a certain molecular size. It is interesting to note that if a DRI detector was present and the DRI-based universal calibration curve was to be used, then this approach is synonymous with using the horizontal distance between the DRI-based and DV-based universal calibration curves as an estimate of the interdetector volume.

The following were the main observations:

i. Comparison of the various $\bar{M}_n$ values to the "true" values for the samples indicated that the highest accuracy was obtained by using eq. (1) with the DV-based universal calibration curve. For $\bar{M}_n$ values obtained from eq. (4), deviations from the "true" values ranged from 0% (as expected because of the origin of the true values for PMMA) to 11.7% for the NBS 706-PMMA 100K blend with an average deviation of 3.9% below the "true" value. For $\bar{M}_n$ values obtained from eq. (1) and the DRI-based universal calibration curve, deviations ranged from 0.73 to 12.7% with an average deviation of 7%. above the "true" value. For $\bar{M}_n$ values obtained from eq. (1) and the DV-based universal calibration curve, deviations ranged from 0 to 8.0% with an average deviation of 3.5% above the true value. Despite the known advantages of using eq. (1) over eq. (4) for polymer blends, because of the influence of other variables, no significant differences are evident here.

ii. Figure 2 shows a superposition of DV and DRI chromatograms of a narrow standard used in the calibration [using eqs. (2) and (3), respectively, to obtain the normalized heights for superposition]. The objective is to see if there is a significant change in the shape of the two chromatograms and not to determine interdetector volume. The retention volume of each peak was obtained by fitting the peak data points with a polynomial (the sampling rate does not adequately define the peak apices in Fig. 2), and the peaks were then superimposed based on their retention volumes. The different heights of the two normalized peaks indicate that the DV peak is broader than the one from the DRI. This result was evident in practically all other samples injected. The shape difference can be due to the imperfect monodispersity of the standards (thus causing a molecular weight effect on the DV detector response) or to extra-column band spreading. However, the effect does not appear to be severe and by itself would not discourage use of the DV-based universal calibration curve.

iii. Precision (i.e., reproducibility) of $\bar{M}_n$ using eq. (1) for NBS 706 was two to three times

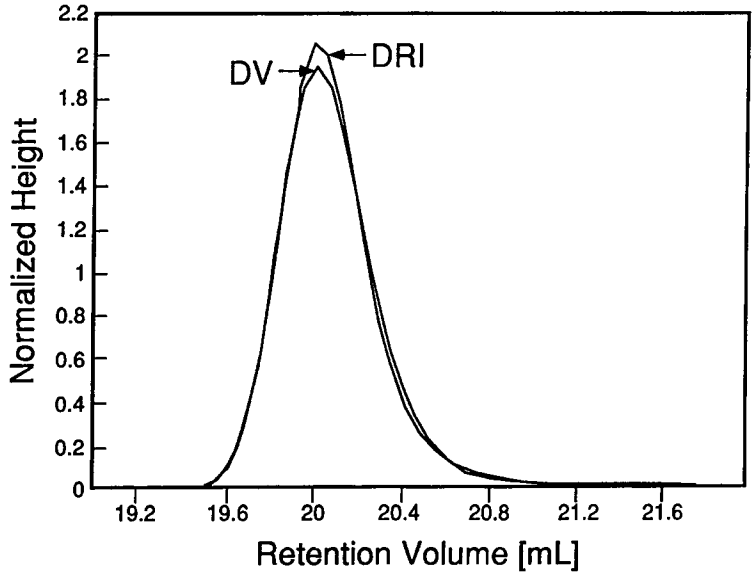

Figure 2 Normalized DV and DRI chromatograms of narrow standard polystyrene of MW 52,000. The DV chromatogram is shifted on the abscissa to superimpose on the DRI chromatogram.

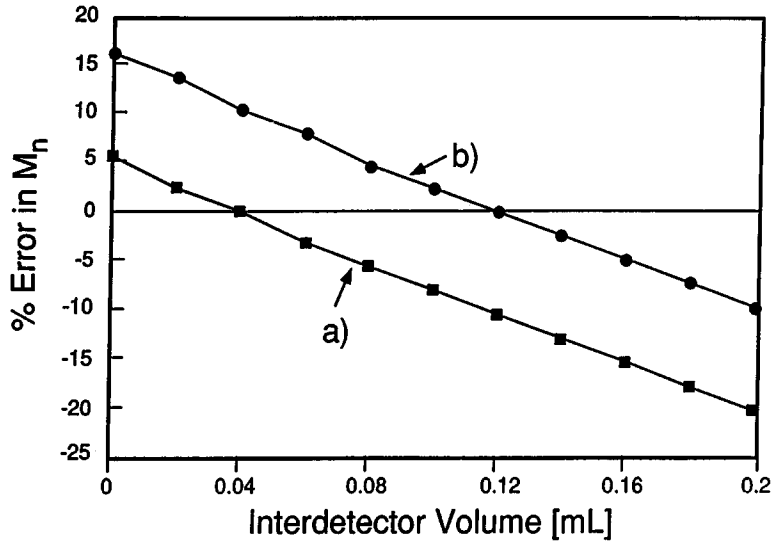

Figure 3 Effect of interdetector volume on the accuracy of $\bar{M}_n$ for NBS 706 calculated from (a) eq. (4) and the DRI-based calibration curve and (b) eq. (1) and the DRI-based calibration curve.

worse than the DRI equation [eq. (4)]. For other samples, the number of replicates was insufficient for us to comment on any trends in reproducibility.

Effect of Interdetector Volume

As mentioned above, the interdetector volume estimate was obtained from the "systematic approach."[7-9] Figure 3 shows the effect of the value of interdetector volume on the value of calculated $\bar{M}_n$. When $\bar{M}_n$ is obtained from eq. (4) and the DRI-based universal calibration curve, the interdetector volume determines the value of M_i via the universal calibration curve (since it determines the value of specific viscosity from the DV detector used with a particular value of concentration from the DRI detector, which, in turn, determines the value of intrinsic viscosity at a particular retention volume). When eq. (1) is used with the DRI-based universal calibration curve, the interdetector volume is the amount by which the DV chromatogram is shifted (in the direction of increased retention volume) in order for the specific viscosity values of the chromatogram to correspond to their respective molecular sizes on the universal calibration curve. In Figure 3, we see that at any particular value of interdetector volume, eq. (1) always provides a larger value than does eq. (4). However, values of interdetector volume could be chosen to enable either equation to provide the "correct" result—although they would be different values!

This ambiguity again reflects a need for a "systematic approach" in implementing quantitative analysis with multidetector SEC systems. Ideally, we would prefer to isolate each variable affecting results, find the "true" value for each variable, and then interpret the data by using all of these "true" values together. However, this can be impractical. For example, in the case of the coupled problems of accounting for the distance between detectors and correcting values of intrinsic viscosity at each retention volume for axial dispersion effects, considering the uncertainty involved in attempting to solve these two problems individually, it has proven much more practical to use an "effective" interdetector volume. To support this approach, we have shown the close mathematical relationship between the two purposes.[10] We also developed the "systematic approach" to avoid inconsistent application of the method and have shown it to be a useful approach in many cases. Estimating the DV $\bar{M}_n$ presents a new problem because, as we will see below, it is sensitive to detector sensitivity as well as to interdetector volume and axial dispersion effects.

Effect of Detector Sensitivity

Raw chromatograms of the DV and DRI detector responses (Fig. 4) show the difference in detector sensitivities at high retention volumes. The range of the DRI response exceeds that of the DV response by at least 2 mL. Since only the DV is used in calculating the DV $\bar{M}_n$, these polymer molecules at high

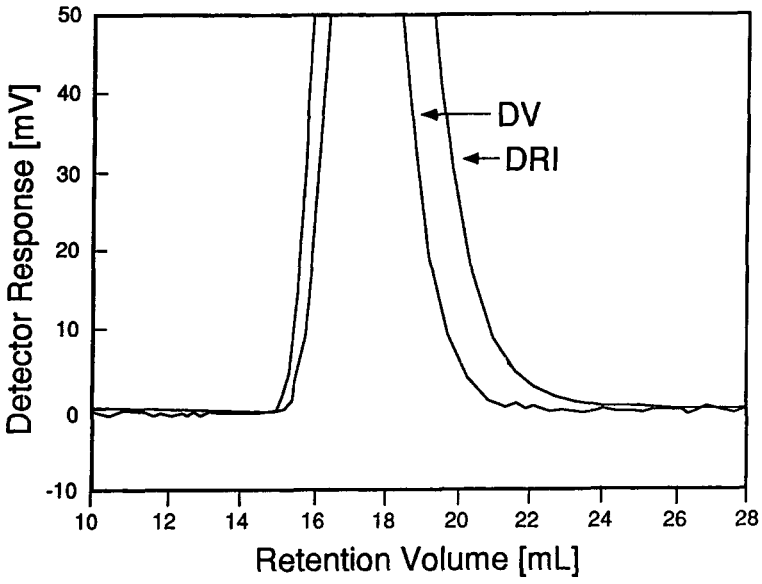

Figure 4 Raw chromatograms of NBS 706 polystyrene from the DV and DRI detectors.

retention volume are not counted in the calculation of $\bar{M}_n$. As mentioned previously, one way to examine this effect is to compare plots of the number of moles of polymer per milliliter of retention volume at each retention volume from the DV and DRI detectors, respectively. Figure 5 shows a plot of $\eta_{sp,i}/J_i$ vs. v_i superimposed upon a plot of c_i/M_i vs. v_i for NBS 706.

Notable characteristics observed are as follows:

i. The DV-based plot shows good superposition on the DRI-based plot only at the low retention volume end. At the higher retention volume end, the former plot abruptly cuts off at a significantly higher retention volume than does the DRI-based plot. The difference in area be-

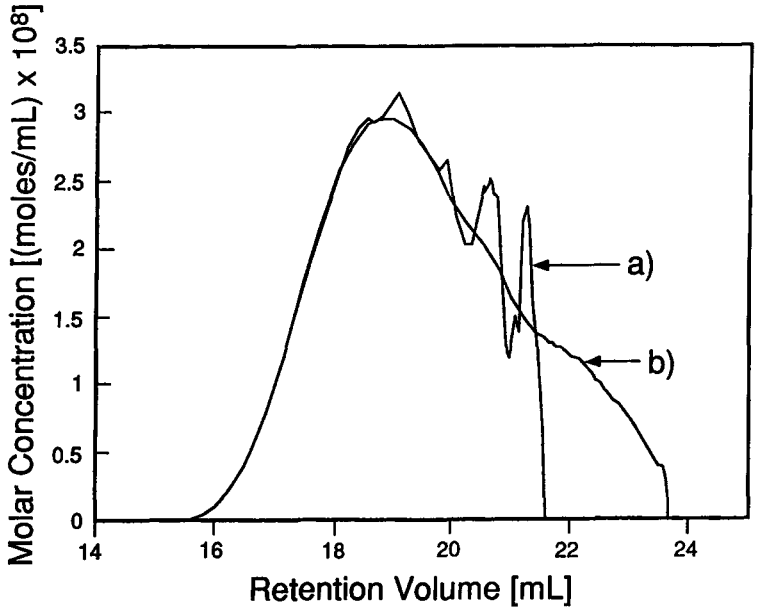

Figure 5 Molar concentration plots for NBS 706 polystyrene using the "systematic approach" estimate of the interdetector volume: (a) DV-based plot, $\eta_{sp,i}/J_i$; (b) DRI-based plot, c_i/M_i.

tween these two plots is a direct measure of the number of polymer molecules not counted by the DV detector compared to those counted by the DRI detector. This reduced area causes the value of the DV $\bar{M}_n$ result to be higher than it deserves to be because the area appears in the denominator of eq. (1).

ii. The high noise level for the DV-based plot at high retention volumes reflects the worsened reproducibility observed in the DV $\bar{M}_n$ values.

iii. Both plots shown in Figure 5 were calculated using the "systematic approach" estimate of the interdetector volume. The area under the c_i/M_i curve is thus known to provide a value of $\bar{M}_n$ very close to the true value. As shown in Figure 6, when the interdetector volume from the displacement of the DV-based calibration curve from the DRI-based calibration curve is used as the estimate [or, synonymously, when the DV-based calibration curve was used for in eq. (1)], superposition was poorer over the whole range. However, the area of the $\eta_{sp,i}/J_i$ was markedly increased and therefore was closer to the c_i/M_i vs. v area.

At this point, two methods for calculating the DV $\bar{M}_n$ are evident:

Method 1

In this method, the "systematic approach" interdetector volume is considered the value of the interdetector volume to be used in calculations. The impact of using this value of the interdetector volume for calculation of the DV $\bar{M}_n$ can be examined by writing eq. (1) as

$$\bar{M}_{n,J} = \frac{m}{\sum \frac{[\eta]_i c_i}{J_i} \Delta v_i} \tag{11}$$

We have previously shown that the effective interdetector volume corrects the local values of intrinsic viscosity, $[\eta]_i$, for axial dispersion effects.[7-9] Also, it is now well known that, for broad chromatograms, the values of concentration obtained from the DRI, c_i, are mostly unaffected by axial dispersion.[11-13] Inaccurate tail c_i values and the summation of small inaccuracies in c_i across the chromatogram remain of concern, but can probably be assumed negligible with high resolution columns. Thus, examining eq. (11), we see that the result obtained from the summation in the denominator would then be expected to be accurate so long as the systematic approach interdetector volume is used. Then, no other axial dispersion correction to the DV $\bar{M}_n$ value would be required.

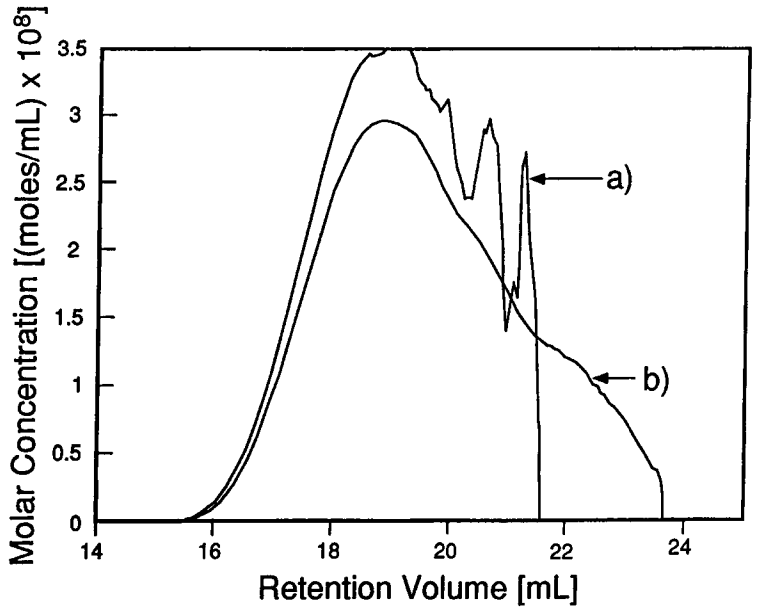

Figure 6 Molar concentration plots for NBS 706 polystyrene using the interdetector volume from the displacement of DV and DRI-based universal calibration curves: (a) DV-based plot, $\eta_{sp,i}/J_i$; (b) DRI-based plot, c_i/M_i.

Then, when the "systematic approach" interdetector volume estimate is utilized and (as seen in Table II) the values estimated from eq. (1) are too high, the reason is probably inadequate DV sensitivity. Efforts then can focus upon methods of estimating the missing number of molecules by various techniques. For example, experimentally, we can examine the effect of increasing the concentration injected.

Method 1 appears very feasible for broad molecular weight distribution polymers. However, for narrow distribution polymers, the systematic approach estimation procedure does not work. This leads us to the second alternative, Method 2.

Method 2

In this case, it is assumed that the displacement observed between the DRI and DV-based calibration curves actually provides the true value of interdetector volume. When this value is used, a separate axial dispersion correction is necessary. Although this means that the DV-based calibration curve can now be used in eq. (1) to obtain the DV $\bar{M}_n$ estimate, two questions arise: the effect of the low sensitivity of the DV detector on the low molecular weight polymer and the effect of axial dispersion (both intra- and extra-column band spreading). As we showed above, plots of the molar concentration of polymer vs. retention volume (e.g., Fig. 5) are useful in viewing detector sensitivity. However, now, axial dispersion correction of specific viscosity, concentration, and molecular weight (i.e., intrinsic viscosity) values at each retention volume may be necessary (depending upon the extent of extra-column axial dispersion).

The test of whether the correction is important is to examine the $\bar{M}_n$ resulting when the area under c_i/M_i vs. v is used in eq. (4). This is another way of saying that the $\bar{M}_n$ value obtained from the DRI–DV combination with universal calibration provides an accurate $\bar{M}_n$. In our case, it did not when the value of interdetector volume used was the displacement distance between the DV and DRI calibration curves. This has generally been found to be the case in the published literature. Thus, the axial dispersion correction would be considered necessary for two reasons: because our measure of detector sensitivity is affected (Fig. 5) and because axial dispersion directly affects the $\bar{M}_n$ value obtained.

An alternative method of implementing Method 2 is to devise a method of correcting the value of $\bar{M}_n$ obtained from eq. (1) when the DV-based calibration curve is used. The axial dispersion correction would tend to increase the value of $\bar{M}_n$. Any additional correction necessary would then be attributed to the detector sensitivity problem. In the next section, we present a method of accomplishing this axial dispersion correction.

Effect of Axial Dispersion

In the Theory section of this article we showed that a correction for axial dispersion for the DV $\bar{M}_n$ could

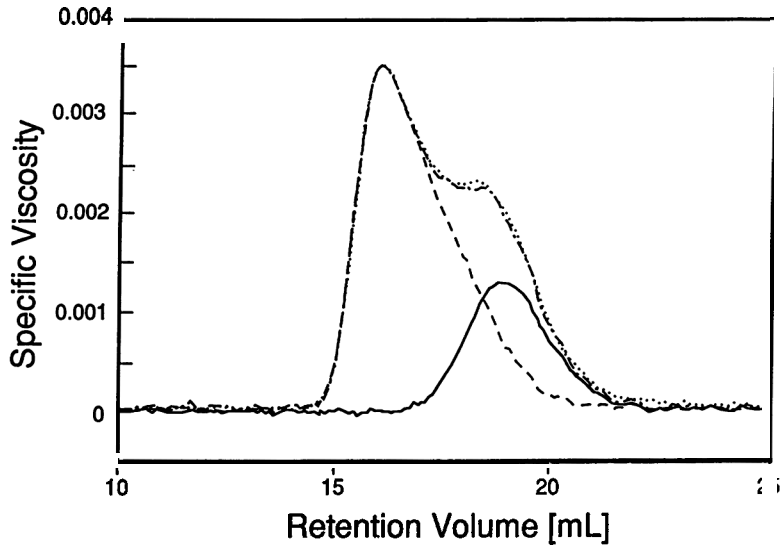

Figure 7 Specific viscosity chromatograms obtained from 100 μL injections of (a) (——) PMMA 100K, 1.5 mg/mL; (b) (– – –) PMMA 400K, 1.5 mg/mL; (c) (· · · · ·) PMMA 100K + PMMA 400K, 0.75 mg/mL each; (d) (– · – ·) curves (a) and (b) added together.

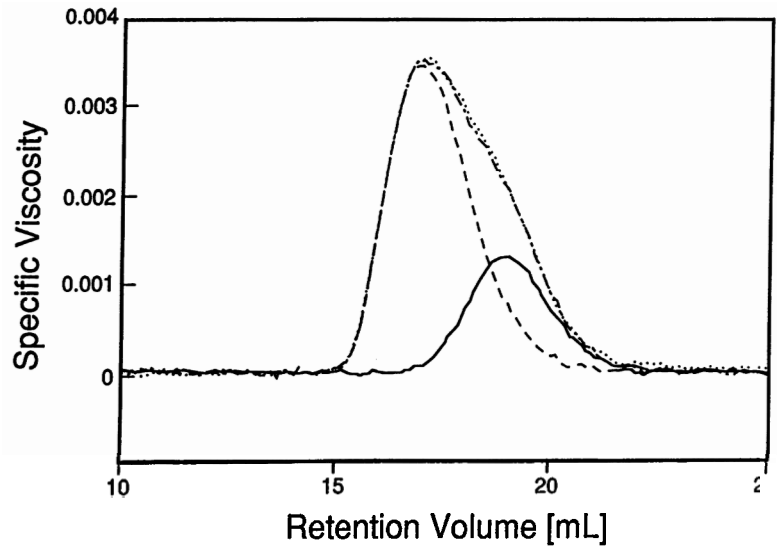

Figure 8 Specific viscosity chromatograms obtained from 100 μL injections of (a) (——) PMMA 100K, 1.5 mg/mL; (b) (– – –) NBS 706 polystyrene, 1.5 mg/mL; (c) (· · · ·) PMMA 100K + NBS 706, 0.75 mg/mL each; (d) (– · – ·) curves (a) and (b) added together.

be derived if the assumption that DV chromatograms were additive was valid. Figures 7–9 show tests of this assumption using polydisperse polystyrene and poly(methyl methacrylate) standards. Additivity appears excellent in all cases.

Figure 10 compares the values of D_2 obtained from the molecular weight, intrinsic viscosity, and universal calibration curves (denoted D_2, $D_{2\eta}$, and D_{2J}, respectively, and representing the slope of ln M vs. v, ln[η] vs. v, and ln J vs. v, respectively). As expected, the values for D_{2J} were approximately three times greater than the corresponding values for D_2. This means that the correction for axial dispersion would be much more significant for the DV $\bar{M}_n$ [eq. (1)] than for $\bar{M}_n$ obtained from the DRI alone [eq. (4)].

Values associated with J and [η] varied much more with retention volume than those associated with M, i.e., in these experiments, the universal calibration curve was more curved than was the mo-

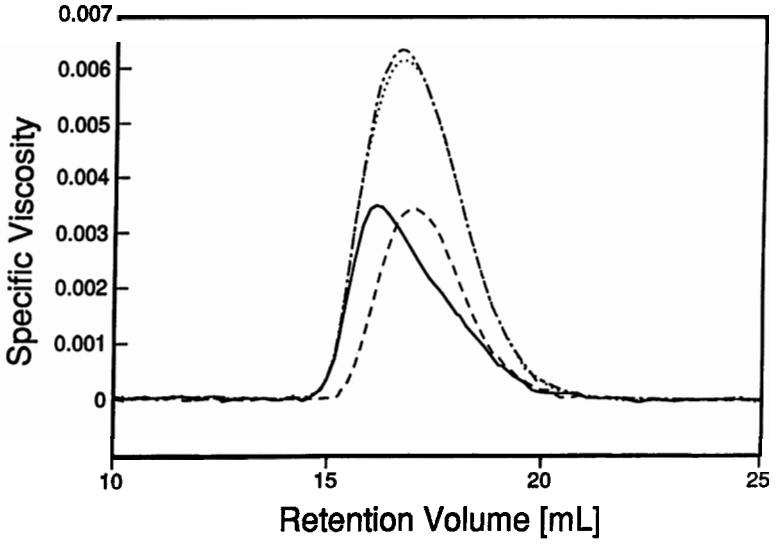

Figure 9 Specific viscosity chromatograms obtained from 100 μL injections of (a) (——) PMMA 400K, 1.5 mg/mL; (b) (– – –) NBS 706 polystyrene, 1.5 mg/mL; (c) (· · · · ·) PMMA 400K + NBS 706, 0.75 mg/mL each; (d) (– · – ·) curves (a) and (b) added together.

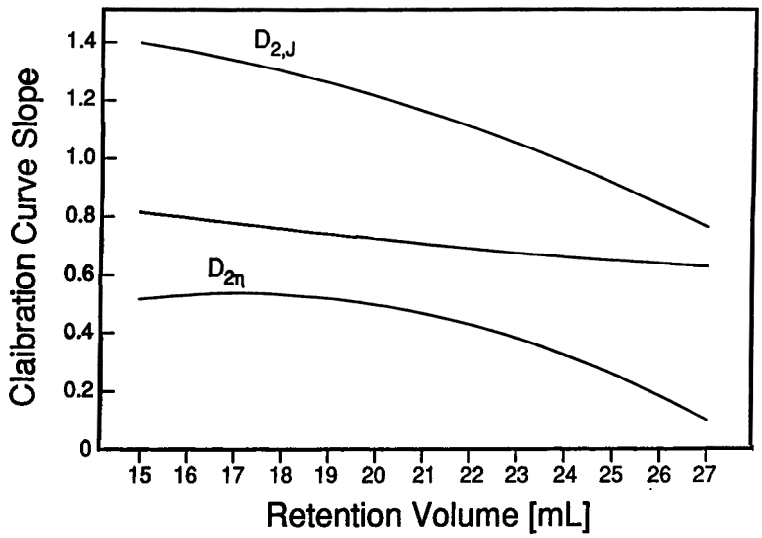

Figure 10 Calibration curve slope obtained from molecular weight (D_2), intrinsic viscosity ($D_{2\eta}$), and universal calibration ($D_{2,J}$) curves.

lecular weight calibration curve. This means that the assumption of constant D_2 would be more generally valid for eq. (9) than the assumption of constant $D_{2,J}$ for eq. (10).

The axial dispersion correction increases the estimate of the DV $\bar{M}_n$ by 4% in this example. This correction is small in comparison to the error introduced by the difference in detector sensitivities. Furthermore, since the sensitivity difference causes the $\bar{M}_n$ value to be already too high, the axial dispersion correction actually "corrects" in the opposite direction to that desired. If we have faith in the validity of the axial dispersion correction for Method 2, it means that we would at least then be able to attribute the increased discrepancy totally to the sensitivity problem. Ways would then need to be devised of assessing and correlating the sensitivity difference problem so that the sensitivity correction could be evaluated for unknown samples. As mentioned above, in Method 2, if plots of molar concentration of polymer vs. retention volume are to be used, axial dispersion of the quantities involved must then be considered.

CONCLUSIONS

Both precision and accuracy concerns arise with the values of $\bar{M}_n$ computed from eq. (1). Precision was found to be two to three times worse for NBS 706 polystyrene than when conventional calculation of $\bar{M}_n$ was conducted. Regarding accuracy, three primary variables are involved: the location of the universal calibration curve on the retention volume axis; the sensitivity of the DV detector to the smaller polymer molecules; and axial dispersion. Location of the universal calibration curve is closely tied to the problem of specifying interdetector volume between DRI and DV detectors. Using only a DV detector does not circumvent the question because the validity of the retention volume position of the resulting calibration curve is still in question.

In response to accuracy concerns, two methods of computing the $\bar{M}_n$ values were proposed:

Method 1 involved using the "effective value" of the interdetector volume to locate the universal calibration curve from the DRI-based calibration curve. Plots of molar concentration of polymer vs. retention volume computed from the DV detector response were compared to those obtained from the DRI response to estimate the detector sensitivity problem. It was proposed that detector sensitivity could be predicted by examining these plots as a function of injected concentration. This method relies on the interdetector volume to accomplish the required resolution correction.

Method 2 utilized the peak retention volumes obtained from the narrow standard chromatograms of the DV detector to obtain the universal calibration curve. A separate axial dispersion correction equation was derived for the $\bar{M}_n$ value. The issue of then determining the contribution of the detector sensitivity contribution was then more complex than

for Method 1 because of the axial dispersion influence on plots of the molar concentration of polymer vs. retention volume. Furthermore, considering beyond the problem of obtaining valid DV $\bar{M}_n$ values, this method requires that the axial dispersion correction be considered in calculation of other molecular weight averages and molecular weight distributions as well.

At this point, Method 1 is the superior option. Incorporating the troublesome axial dispersion correction effects into the interdetector volume has previously been shown to have some basis in theory and provides a much more reliable procedure than in Method 2. However, it must be pointed out that, currently, this method has only proven practical for polydisperse polymer samples. For narrow molecular weight distribution samples, the "systematic approach" procedure is unable to obtain an effective interdetector volume. This problem is likely associated with the much larger effect of axial dispersion on the local properties (concentration and specific viscosity at each retention volume) of narrow molecular weight distribution polymers.

NOMENCLATURE

c	concentration
C.V.	coefficient of variation (see note at bottom of Table II)
D_1, D_2	calibration curve constants [eqs. (8)–(10)]
F	concentration chromatogram height affected by axial dispersion
F_N	normalized concentration chromatogram height affected by axial dispersion
G	shape function giving the shape of a normalized, concentration chromatogram of a truly monodisperse sample
J	ordinate of the universal calibration curve
m	mass injected
M	molecular weight
M_n	number-average molecular weight
M_w	weight-average molecular weight
n	number of chromatogram heights
b	transform parameter (Appendix C)
s	sample estimate of the standard deviation of the measurements (Table II)
v	retention volume
w	weight fraction of polymer
W_N	normalized concentration chromatogram height (assuming perfect resolution) [eq. (2)]
W	concentration chromatogram height (assuming perfect resolution)
Δ	increment
$[\eta]$	intrinsic viscosity
η_{sp}	specific viscosity
$\eta_{sp,N}$	normalized differential viscometer chromatogram height [eq. (3)]
$\sum$	summation over all retention volumes
σ^2	variance of the concentration chromatogram of a truly monodisperse polymer

Subscripts

axd	value affected by axial dispersion
i	retention volume
J	calculated from eq. (1) (for M_n) or pertaining to the universal calibration curve (for $D_{2,J}$)
unc	value calculated using a mixture of values affected by axial dispersion and true values

Note

Overbar on a quantity (e.g., $\bar{M}$) indicates the "whole polymer" value (a wide variety of species are present). Quantities in curly brackets are bilateral LaPlace Transforms (e.g., $\{G(s)\}$) (see Appendix C).

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APPENDIX A: DERIVATION OF EQUATION (1)

By definition, the whole polymer number-average molecular weight, $\bar{M}_n$, can be expressed as the reciprocal of the sum of the weight fraction of the polymer at each retention volume increment (w_i), each divided by the number-average molecular weight of polymer within that volume increment, $M_{n,i}$, as follows:

$$\bar{M}_n = \frac{1}{\sum_i^n \frac{w_i}{M_{n_i}}} \tag{A.1}$$

The normalized chromatogram heights expressed as a continuous function of v, $F_N(v)$, are obtained from the raw chromatogram heights by dividing by the area under the chromatogram:

$$F_N(v) = \frac{F(v)}{\int_{-\infty}^{+\infty} F(v)\, dv} \tag{A.2}$$

Substituting into eq. (A.1) [since $F_N(v)dv$ is the weight fraction of polymer from v to $v + dv$],

$$\bar{M}_n = \frac{1}{\int_{-\infty}^{+\infty} \frac{F_N(v)}{M_n(v)}\,dv} \tag{A.3}$$

The concentration at any retention volume, $c(v)$, is obtained from

$$c(v) = mF_N(v) \tag{A.4}$$

where m is the mass injected. Therefore, eq. (A.3) can be written

$$\bar{M}_n = \frac{m}{\int_{-\infty}^{+\infty} \frac{c(v)}{M_n(v)}\,dv} \tag{A.5}$$

However, the ordinate of the universal calibration curve, expressed as a continuous function of v, is

$$J(v) = [\eta](v)M_n(v) \tag{A.6}$$

Therefore,

$$M_n(v) = \frac{J(v)}{[\eta](v)} \tag{A.7}$$

$$\eta_{sp}(v) = [\eta](v)c(v) \tag{A.8}$$

Substituting eqs. (A.7) and (A.8) into eq. (A.5) yields eq. (1).

APPENDIX B: ADDITIVITY OF SPECIFIC VISCOSITY CHROMATOGRAMS

We may consider the intrinsic viscosity within each retention volume increment, $[\eta]_{i,axd}$, to consist of the weighted sum of the intrinsic viscosities of each of the j different molecules present within that increment (of any molecular size or molecular weight), $[\eta]_{i,j}$, where the weighting factors are the weight fractions of each of the j different molecules within the increment, w_j, i.e.,

$$[\eta]_{i,axd} = \sum_{j=1}^{k} w_j[\eta]_{i,j} \tag{B.1}$$

Note that the subscript i is associated with the retention volume increment, dv_i, while the subscript j is associated with the k different molecules present at a particular retention volume increment dv_i.

At the dilute concentrations involved in SEC, eq. (B.1) becomes

$$\frac{\eta_{sp,i,axd}}{c_i} = \sum_{j=1}^{k} w_j \frac{\eta_{sp,i,j}}{c_{i,j}} \tag{B.2}$$

However,

$$w_j = \frac{c_{i,j}}{c_i} \tag{B.3}$$

Substituting eq. (B.3) into eq. (B.2), we obtain

$$\eta_{sp,i,axd} = \sum_{j=1}^{k} \eta_{sp,i,j} \tag{B.4}$$

The same result is obtained even for no axial dispersion effects (i.e., if the molecular variety within each slice is caused by different degrees of branching and different molecular weights yielding the same molecular size in solution).

APPENDIX C: RESOLUTION CORRECTION OF THE SPECIFIC VISCOSITY CHROMATOGRAM

Assuming that the individual specific viscosity chromatograms of different molecular sizes are additive, the Tung axial dispersion equation can now be applied to the overall specific viscosity chromatogram (as we do with the concentration chromatogram):

$$\eta_{sp,axd}(v) = \int_{-\infty}^{+\infty} \eta_{sp}(v)G(v - y)\,dy \tag{C.1}$$

Following Hamielec and Ray,[3] we can use the bilateral LaPlace transform and obtain

$$\{\eta_{sp,axd}(b)\} = \{\eta_{sp}(b)\}\{G(b)\} \tag{C.2}$$

where the curly brackets "{ }" denote the bilateral LaPlace transform and "b" is the transform parameter:

$$\{\eta_{sp}(b)\} = \int_{-\infty}^{+\infty} \eta_{sp}(v)\exp(-bv)\,dv \tag{C.3}$$

$$\{\eta_{sp,axd}(b)\} = \int_{-\infty}^{+\infty} \eta_{sp,axd}(v)\exp(-bv)\,dv \tag{C.4}$$

$$\{G(b)\} = \int_{-\infty}^{+\infty} G(v)\exp(-bv)\,dv \tag{C.5}$$

When $G(v)$ is Gaussian, we have

$$\{G(b)\} = \exp\left(\frac{b^2\sigma^2}{2}\right) \tag{C.6}$$

Now, we assume that the universal calibration curve is linear over the range of interest:

$$J_i = D_{1,J}\exp(-D_{2,J}v_i) \tag{C.7}$$

Then, eq. (1) can be written

$$\bar{M}_{n,J} = \frac{m}{\sum \eta_{sp,i} \dfrac{\exp(D_{2,J}v_i)}{D_{1,J}} \Delta v_i} \tag{C.8}$$

or, considering specific viscosity as a continuous function of v,

$$\bar{M}_{n,J} = \frac{m}{\displaystyle\int_{-\infty}^{+\infty} \eta_{sp}(v) \frac{\exp(D_{2,J}v_i)}{D_{1,J}} dv_i} \tag{C.9}$$

and using eqs (C.2)–(C.6) and setting $b = D_{2,J}$ then yields

$$\bar{M}_{n,J} = \frac{m}{\displaystyle\int_{-\infty}^{+\infty} \eta_{sp,axd}(v) \frac{\exp(D_{2,J}v_i)}{D_{1,J}} dv \exp\left(-\frac{D_{2,J}^2\sigma^2}{2}\right)} \tag{C.10}$$

which yields eq. (10) upon simplification.

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