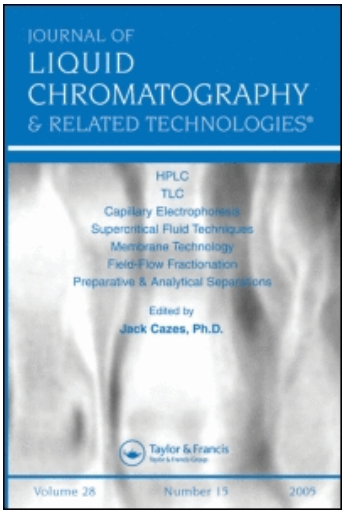

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Differential Molecular Weight Distributions in High Performance Size Exclusion Chromatography

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DIFFERENTIAL MOLECULAR WEIGHT DISTRIBUTIONS IN HIGH PERFORMANCE SIZE EXCLUSION CHROMATOGRAPHY

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ABSTRACT

The definition and derivation of differential molecular weight distribution functions are thoroughly discussed. Common errors appearing in the literature are revealed and corrected. For high performance size exclusion chromatography (HPSEC), the importance of the calibration curve and its slope is discussed, and the application to gel permeation chromatography (GPC) is illustrated with simulations and real data.

1. INTRODUCTION

The differential molecular weight distribution (MWD) of polymers or macromolecules is of great importance to the field of high performance size exclusion chromatography (HPSEC) or gel permeation chromatography (GPC). A number of papers and texts cover the subject as part of the more general field of polymer characterization. Unfortunately, there is some confusion in the literature on this subject, and it is the task of this paper to present clearly the definition and derivation of the differential distribution functions as well as to relate them to measured quantities. Section 2 covers the definitions and conventions of the

differential MWD and the moments of the distribution. Section 3 relates the MWD to measured quantities. Finally, Section 4 contains several examples for both broad and narrow distributions, using both simulations and real data.

2. DEFINITIONS AND CONVENTIONS

We adopt the following definitions:

(*i*) The cumulative number fraction $N(M)$ is defined as the *number* fraction of molecules having molecular weight less than M. Thus $N(M)$ is the number of molecules in the sample having molecular weight less than M, divided by the total number of sample molecules.

(*ii*) The cumulative weight fraction $W(M)$ is defined as the *weight* fraction of molecules in the sample having molecular weight less than M. Thus $W(M)$ is the mass of sample having molecular weight less than M, divided by the total sample mass.

Since the quantities $N(M)$ and $W(M)$ are pure fractions, they are dimensionless.

The corresponding differential distributions, or MWD's, which we shall denote by the lowercase symbols $n(M)$ and $w(M)$, are defined by the relations

$$n(M) = \frac{d\,N(M)}{dM}$$
$$w(M) = \frac{d\,W(M)}{dM}. \tag{1}$$

The distributions $w(M)$ and $n(M)$ possess the following properties:

(*i*) The quantity $n(M)\,dM$ is the *number fraction* of molecules in the sample having a molecular weight between M and $M + dM$. Thus $n(M)$ is the *number of sample molecules* having molecular weight between M and $M + dM$, divided by the total number of sample molecules, and divided by dM, in the limit as $dM \to 0$.

(*ii*) The quantity $w(M)\,dM$ is the *weight fraction* of sample having a molecular weight between M and $M + dM$. Thus $w(M)$ is the *mass* of sample having molecular weight between M and $M + dM$, divided by the total sample mass, and divided by dM, in the limit as $dM \to 0$.

The definitions of Eq. (1) lead to the following normalization conditions for $n(M)$ and $w(M)$:

$$\int_0^\infty n(M)\,dM = 1$$
$$\int_0^\infty w(M)\,dM = 1. \tag{2}$$

These conditions are necessary because both $N(M)$ and $W(M)$ approach unity as $M \to \infty$. Put another way, the conditions are a consequence of taking $n(M)$ and $w(M)$ to be fractions: if we add up all the fractions, we must obtain unity. In the following derivations we shall take all integrals to have the limits 0 and ∞ unless otherwise stated. Also note that both $w(M)$ and $n(M)$ have dimensions of inverse molecular weight.

To convert between $w(M)$ and $n(M)$, note that for any molecular weight M, $M\,n(M)$ is proportional to the mass of sample having molecular weight between M and $M + dM$, and therefore proportional to $w(M)$. Simply normalize to obtain

$$w(M) = \frac{M\,n(M)}{\int M\,n(M)\,dM}. \tag{3}$$

The inverse relation is

$$n(M) = \frac{w(M)\,/\,M}{\int \left[w(M)\,/\,M\right] dM}. \tag{4}$$

Because we may easily obtain $n(M)$ from $w(M)$ or vice versa, only one of these is really necessary. As explained below, $w(M)$ corresponds more closely to quantities actually measured in the laboratory. Therefore we shall use $w(M)$ rather than $n(M)$.

There is another common way to write a differential MWD, one used by a great many researchers. It is

$$x(M) = \frac{d\,W(M)}{d\,(\log_{10} M)}\,. \tag{5}$$

We call $x(M)$ a "differential log MWD" to indicate the derivative with respect to the logarithm of molecular weight. Since $d\,(\log_{10} M) = (\log_{10} e)\,dM\,/\,M$, we see that

$$\begin{aligned} x(M) &= \frac{M}{\log_{10} e}\,\frac{d\,W(M)}{dM} \\ &= \frac{M}{\log_{10} e}\,w(M)\,. \end{aligned} \tag{6}$$

Historically, $x(M)$ has been used because many samples contain fractions of very different molecular weights, making a logarithmic M scale convenient, and the necessary derivatives could be taken directly from the appropriate graphs. With the advent of GPC, in which the molecular weight of typical eluting fractions decreases roughly exponentially with elution volume, a logarithmic molecular weight scale is particularly appropriate. However, the distribution $x(M)$ has properties quite different from those of $w(M)$. First, it obeys a different normalization condition, namely

$$\int x(M)\,d\,(\log_{10} M) = 1. \tag{7}$$

Physically, $x(M)\,d\,(\log_{10} M)$ is proportional to the weight fraction of sample having a log molecular weight between $\log_{10} M$ and $\log_{10} M + d\,(\log_{10} M)$. We shall calculate both $x(M)$ and $w(M)$ and compare them for a number of examples later in this paper.

We can easily express the various moments of the distribution in terms of $w(M)$ or $x(M)$. Using $w(M)$ we can write the moments generally as

$$\bar{M}_k = \frac{\int w(M)\, M^k\, dM}{\int w(M)\, M^{k-1}\, dM} \tag{8}$$

where $k = 0, 1, 2$ corresponds to the number-, weight-, and z-average moments, written $\bar{M}_n$, $\bar{M}_w$, and $\bar{M}_z$, respectively. For different values of k we obtain the following explicit expressions for the various moments:

$$\bar{M}_{k=0} = \bar{M}_n = \frac{\int w(M)\, dM}{\int [w(M)/M]\, dM} = \frac{1}{\int [w(M)/M]\, dM} \tag{9a}$$

$$\bar{M}_{k=1} = \bar{M}_w = \frac{\int w(M)\, M\, dM}{\int w(M)\, dM} = \int w(M)\, M\, dM \tag{9b}$$

$$\bar{M}_{k=2} = \bar{M}_z = \frac{\int w(M)\, M^2 dM}{\int w(M)\, M\, dM} = \frac{\int w(M)\, M^2 dM}{\bar{M}_w} \tag{9c}$$

Moments beyond z are labeled $z + 1$, $z + 2$, etc. To write the moments in terms of $x(M)$, first combine Eqs. (1) and (5) to obtain

$$w(M)\, dM = x(M)\, d(\log_{10} M)\ . \tag{10}$$

Then replace $w(M)\, dM$ in Eqs. (9a)–(9c) with $x(M)\, d(\log_{10} M)$.

3. RELATION TO MEASURED QUANTITIES

Collecting data in GPC involves measuring, at each elution volume V, the molecular weight M as well as a concentration-sensitive signal. Typically, M is determined either by calibrating columns with standards or by using an absolute instrument such as a light scattering detector, and the concentration is determined with a refractive index (RI) or ultraviolet (UV) detector. It is almost universally assumed that the column separation is ideal, meaning that each volume contains a monodisperse fraction and thus possesses a unique value of M. However, it is important to realize that knowledge of M at each volume, and hence the determination of the MWD, is only as good as the separation of the columns. If different molecular weight species are present at each volume, the value of M we derive will be some sort of average (for example, light scattering gives the weight-average $\overline{M}_w$). This could also happen, for example, if the sample contains molecules of differing conformations and the same effective size. Most often the separation approximates the ideal, but it is advisable to be aware of the assumptions implicit in the calculations.

The presence of axial dispersion can cause difficulties in interpreting the signals from molecular weight and concentration-sensitive detectors. If the detector data are used in conjunction with a standard molecular weight calibration curve or with a universal calibration curve, correction for axial dispersion is necessary, particularly for samples with narrow MWD's. Such samples are quite common (in fact, they are often used for column calibration), but their uncorrected elution profiles are incompatible with a previously measured calibration curve due to dispersion in the columns or in the detector cell itself. An uncorrected detector signal will yield an unacceptably broad MWD if the width of the eluting peak is due mostly to column and/or detector broadening. The concentration signal must be corrected first.

If one uses an on-line molecular weight sensitive detector, such as light scattering or viscosity, the situation is somewhat different. In this case, one must take the ratio of signals from the weight sensitive detector and the concentration detector. Assuming that interdetector broadening is not dominant, a narrow distribution sample yields a nearly constant ratio across the peak, correctly indicating a narrow distribution. Thus the sample can be determined to be monodisperse without correcting for dispersion. In the case of light scattering, the ratio of detector signals yields molecular weight directly, and the MWD can be

calculated. (It should be noted that interdetector broadening is often not negligible and can easily be revealed by taking the ratio of detector signals for a monodisperse standard). If the data are to be used with standard or universal calibration, corrections to both detectors may be necessary. A number of methods exist to correct for axial dispersion for both narrow and broad distribution samples, and are well presented in the literature.[1–6] In the discussion and examples which follow, it is assumed that such corrections have been applied where necessary.

How do we convert measurements of molecular weight and concentration into a MWD? Begin by letting $h(V)$ denote the concentration-sensitive detector response after subtraction of the baseline, where V is the elution volume. If $h(V)$ is measured with a RI or UV detector, we assume that $h(V)\,dV$ is proportional to the mass of the sample in the elution volume V to $V + dV$. Next, let $F(V)$ be the weight fraction of sample eluted up to retention volume V. Then for each incremental change in volume, the quantity $h(V)\,dV$ is proportional to the incremental change in $F(V)$:

$$h(V)\,dV \propto d\,F(V) \tag{11}$$

or

$$h(V) \propto \frac{d\,F(V)}{dV}\,. \tag{12}$$

These expressions could be made equalities if $h(V)$ were normalized, *i.e.*, if $\int h(V)\,dV = 1$. Thus we simply divide by the integral to insure normalization and obtain the desired relation:

$$\frac{h(V)}{\int h(V)\,dV} = \frac{d\,F(V)}{dV}\,. \tag{13}$$

Furthermore, since in GPC greater elution volumes correspond to smaller molecular weights, the cumulative quantities $F(V)$ and $W(M)$ are implicitly related by

$$F(V) = 1 - W(M). \tag{14}$$

Equation (1) then yields

$$w(M) = -\frac{d\,F(V)}{dM} . \tag{15}$$

The differential distribution $w(M)$ is thus the negative rate of change of the weight fraction of sample with respect to molecular weight. Remember that V is an implicit function of M through the "calibration curve" for the GPC column set. We know F as a function of V, not of M, but using the chain rule we can perform this change of variables and write

$$w(M) = -\frac{d\,F(V)}{dV}\frac{dV}{d\,(\log_{10} M)}\frac{d\,(\log_{10} M)}{dM} . \tag{16}$$

We choose this apparently arbitrary combination of factors because $d\,(\log_{10} M)\,/\,dV$ is the slope of the calibration curve for the column. The third factor on the right-hand side of Eq. (16) can be written as $(\log_{10} e)\,/\,M$, and so we have, using Eq. (13),

$$w(M) = -\frac{\log_{10} e}{M\left[d\,(\log_{10} M)\,/\,dV\right]}\frac{h(V)}{\int h(V)\,dV} . \tag{17}$$

Note that $d\,(\log_{10} M)\,/\,dV$, the slope of the calibration curve, is negative because larger volumes correspond to lower molecular weights. Thus $w(M)$ will be positive as required. Also, a mathematical word of caution is in order. Some texts, particularly older ones, use the symbol "log x" to indicate the natural or base e logarithm rather than the common or base 10 logarithm. For this reason (apparently) some presentations neglect the factor $\log_{10} e$ in Eq. (17), which then makes the equations true only if the logarithms are taken to the base e. Since column calibrations are traditionally plotted as $\log_{10} M$ vs. V, it is prudent to include the base explicitly if properly normalized values of $w(M)$ are required.

To calculate $x(M)$ from GPC data, we write an equation analogous to Eq. (16):

$$x(M) = -\frac{d\,F(V)}{dV}\frac{dV}{d\,(\log_{10} M)} . \tag{18}$$

Applying Eq. (10) and rearranging gives

$$x(M) = -\frac{1}{d(\log_{10} M)/dV} \frac{h(V)}{\int h(V)\, dV}. \tag{19}$$

It is worth mentioning here that a number of errors have appeared in the literature concerning Eqs. (17) and/or (19). Yau *et. al.*[3] derive an equation analogous to Eq. (19) which is in error by a factor of dV. Their worked example is saved by the fact that they take dV exactly equal to 1 ml. They also make the $\log_{10} e$ error discussed above; their MWD graph (their Fig. 10.4) integrates to about 0.4. Finally, the molecular weight axis of the graph is incorrect. Analysis of their data shows that the molecular weight axis is reversed with respect to the data which is plotted; it should run from 10^3 to 10^7 daltons, not 10^7 to 10^3. Billingham[7] and Styring and Hamielec[5] also make the $\log_{10} e$ error if it is assumed that their logarithms are to the base 10.

A more serious error is committed by those who ignore the necessity of including the slope of the calibration curve in the calculation of $w(M)$ and $x(M)$. It is reported[8] that some researchers (and commercial software!) calculate the weight fraction $h(V)\, dV$ at each volume, transform V into M via the calibration curve, and plot the weight fraction against $\log_{10} M$. In cases where the calibration curve is quite linear [*i.e.*, $d(\log_{10} M)/dV$ is a constant, independent of V], this omission results in MWD's which have the correct shape but are in error by a constant factor; this type of error would not affect any calculation of moments of the distribution by Eqs. (9). If the calibration curve is not linear, the errors can be extreme, as pointed out clearly by Yau and Fleming.[9] This type of error renders the calculations quite inappropriate for any quantitative conclusions or for any long-term comparisons of data.

An advantage of the log MWD formalism implied by Eq. (18) is that in the linear region of the calibration curve, $x(M)$ is just a rescaled version of the elution curve $h(V)$. The MWD $w(M)$, on the other hand, is proportional to $h(V)/M$. Thus $w(M)$ is more sensitive to fractions having lower molecular weights, while $x(M)$ is sensitive to higher molecular weights. However, if the calibration curve is not linear, neither $w(M)$ nor $x(M)$ resemble $h(V)$. Today's desktop computers have rendered both $w(M)$ and $x(M)$ quite easy to calculate, and the

researcher may choose the distribution most suitable to the problem at hand, *as long as he or she is aware of which distribution the software is calculating.* Researchers should keep in mind that both distributions appear in the literature, although $x(M)$ is more common. From a theoretical viewpoint, $w(M)$ is probably more appropriate, but from a practical perspective, $x(M)$ often more closely resembles $h(V)$. In the following section $w(M)$ and $x(M)$ will be calculated and compared for several examples.

4. WORKED EXAMPLES

To clarify the preceding analytical discussion, it is useful to consider several detailed examples. First we shall consider two examples based on computer-simulated, rather than measured, data. Next we shall perform similar analysis on real data.

A. Simulation — Broad Distribution

First we need a column calibration curve, *i.e.*, a plot of $\log_{10} M$ vs. elution volume V, as well as the concentration-sensitive signal $h(V)$. Figure 1 shows such a plot. In an actual experiment the calibration curve would be obtained either by using standards of known molecular weight or by an absolute measurement such as light scattering. For clarity in the final results, the curve in Fig. 1 covers a wide range of molecular weights and is linear over a large volume interval. The concentration signal $h(V)$ is a Gaussian centered at $V = 10$ ml. Of course, h is never measured as a continuous function of V. Instead it is sampled at discrete time intervals proportional to the elution volume in each interval. We might more appropriately label the concentration-sensitive signal h_i, where the subscript labels the i^{th} interval. The integral in Eq. (13) becomes a sum:

$$\int h(V)\, dV \rightarrow \sum_i h_i\, \Delta V_i \tag{20}$$

where ΔV_i is the i^{th} volume interval.

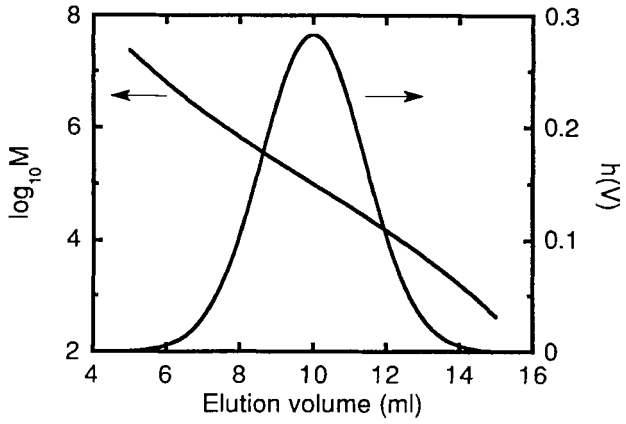

FIGURE 1. Calibration curve and concentration response for a computer-simulated broad-distribution sample.

Note that the distribution implied by Fig. 1 is intentionally very broad to illustrate differences between $w(M)$ and $x(M)$. The number-average molecular weight is 38,000 daltons, while the weight-average is 260,000, giving a polydispersity of 6.8.

Our goal is to implement Eq. (17) for $w(M)$ and Eq. (19) for $x(M)$. To accomplish this, we need the derivative $d(\log_{10} M)/dV$, the slope of the calibration curve. Since the calibration curve for this example was determined analytically, it is easy to find the slope at any volume exactly. For real data this is more difficult; various techniques are discussed below. Once we have $h(V)$ or h_i and the calibration slope $d(\log_{10} M)/dV$ for each volume, we calculate the differential distribution $w(M)$ and the differential log distribution $x(M)$ by Eqs. (17) and (19). The results appear in Figs. 2 and 3. Figure 2 shows the two functions plotted with a logarithmic M axis, while Fig. 3 has a linear M axis. Usually, one would plot $w(M)$ against M and $x(M)$ against $\log_{10} M$. In order to compare the calculations, however, in this paper we plot both functions against both axes.

Note that $x(M)$ has a maximum at $M = 10^5$, the same molecular weight at which $h(V)$ is a maximum. As discussed earlier, to the extent that the calibration slope is a constant, corresponding to a linear calibration curve, $x(M)$ is just a

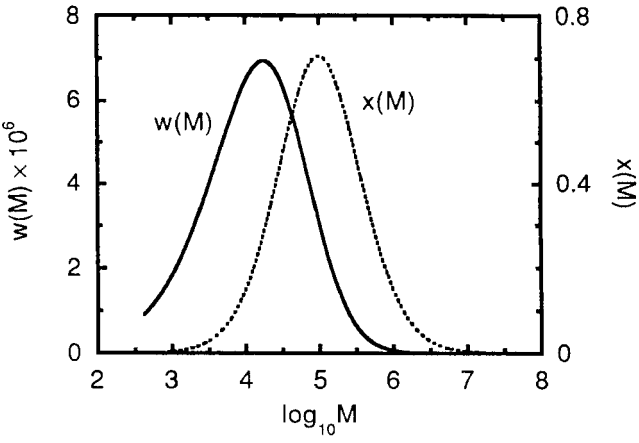

FIGURE 2. Differential MWD and log MWD for the sample in Fig. 1, plotted against $\log_{10} M$.

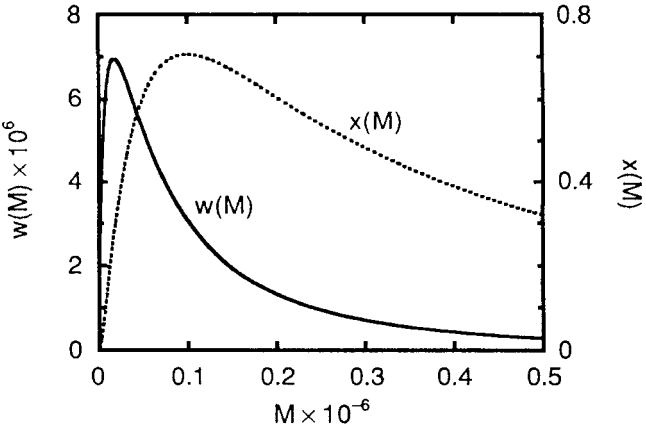

FIGURE 3. Differential MWD and log MWD for the sample in Fig. 1, plotted against M.

rescaled plot of $h(V)$ [see Eq. (19)]. The differential MWD $w(M)$, however, is proportional to $h(V) / M$ [see Eq. (17)]. The difference is particularly evident in Fig. 3, which shows that $x(M)$ has a large high molecular weight tail not present in $w(M)$.

The factor of M between $w(M)$ and $x(M)$ causes the two distributions to have maxima at quite different molecular weights. The maximum of $w(M)$ occurs at $M \approx 18{,}000$, different by more than a factor of five from the maximum of $x(M)$. The difference will be greater for polydisperse samples such as the one simulated here. This point is troubling, since many researchers use the peak of the differential distribution as a rough estimate of the "molecular weight" of their sample. It is even more important to know which differential distribution is being plotted if hard quantitative conclusions are to be drawn. For example, if the distribution is used to calculate any of the molecular weight moments, the correct equations must be employed. In other words, it is necessary to know whether to integrate over dM or $d(\log_{10}M)$. Remember that both distributions occur in the literature.

One clue as to which distribution has been calculated is to note the actual distribution values. For typical columns and samples (elutions occurring over several milliliters with molecular weights between 10^4 and 10^6 daltons), $x(M)$ tends to have peak values on the order of 0.1–10. This is because $x(M)$ is normalized on a logarithmic scale [see Eq. (7)]. In contrast, $w(M)$ tends to have peak values in the 10^{-6}–10^{-4} range due to its normalization condition [Eq. (2)]. Caution is advised, however, since experience shows that the scaling and normalization of these functions in the literature is sometimes in error, and ordinate values often fail to appear on graphs. The situation is further complicated because even though $w(M)$ is normalized by integrating over M, it is sometimes plotted against $\log_{10} M$. It is customary, however, to use abscissas appropriate to the normalization of the distribution — that is, M for $w(M)$ and $\log_{10} M$ for $x(M)$. As mentioned above, for the sake of completeness these examples will illustrate both functions plotted on both axes.

B. Simulation — Narrow Distribution

Let us turn now to a second example involving two narrow elution peaks. We shall also use this example to investigate the effect of linear approximation to the

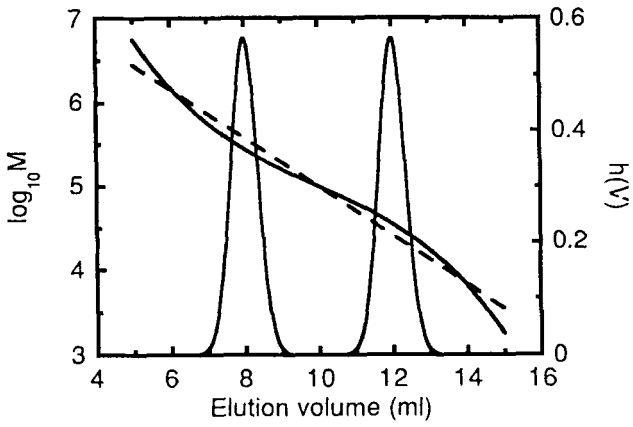

FIGURE 4. Calibration curve and concentration response for a computer-simulated sample with two narrow peaks. The dashed line is a linear fit to the calibration curve.

calibration curve $\log_{10} M$ vs. V. Figure 4 shows a calibration curve which is somewhat more non-linear than the one in Fig. 1. The dashed line is a linear fit to the curve. Also plotted is a concentration signal containing two narrow peaks of equal injected mass. For each of these two peaks the polydispersity is about 1.05. The peak positions have been chosen to be at volumes where the linear fit to the calibration curve is poor.

The distributions $w(M)$ and $x(M)$ appear in Figs. 5 and 6. Note that once again $x(M)$ looks very much like the elution curve (two equal-height peaks), while $w(M)$ is weighted toward low molecular weights. If the calibration curve is exactly linear (meaning that $\log_{10} M$ is proportional to the volume V), then two peaks of $x(M)$ having equal injected mass must have equal areas, when plotted against $\log_{10} M$. The calibration curve here is not particularly linear, but the slopes near the peaks are roughly equal, and as a result the peak areas of $x(M)$ in Fig. 5 are nearly equal. This is an important consequence of the logarithmic normalization of $x(M)$. On the other hand, because $w(M)$ is a differential distribution over M, not $\log_{10} M$, the low molecular weight peak must have a larger maximum. It is Fig. 6, plotted against M, in which the two peaks of $w(M)$ have equal areas. Also note that the molecular weights of the maxima of

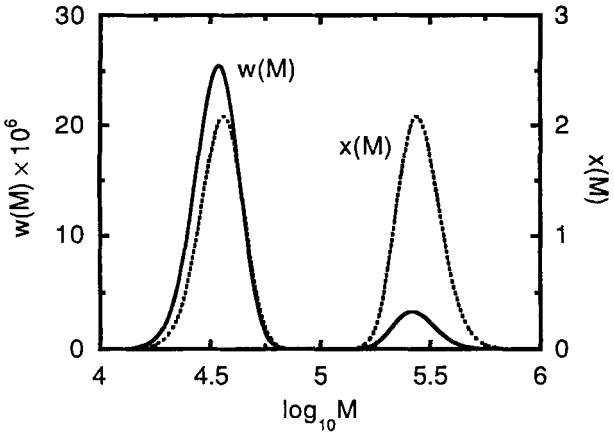

FIGURE 5. Differential MWD and log MWD for the sample in Fig. 4, plotted against $\log_{10} M$.

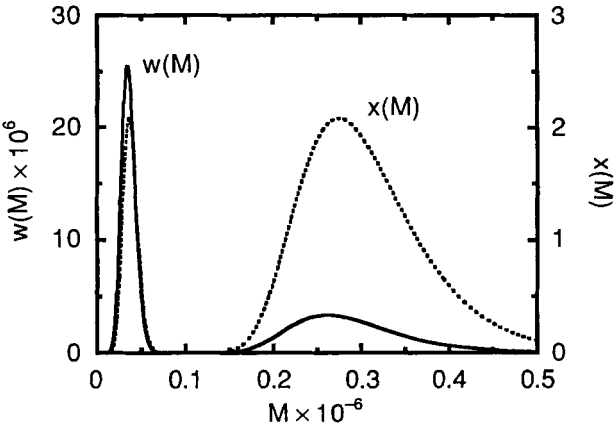

FIGURE 6. Differential MWD and log MWD for the sample in Fig. 4, plotted against M.

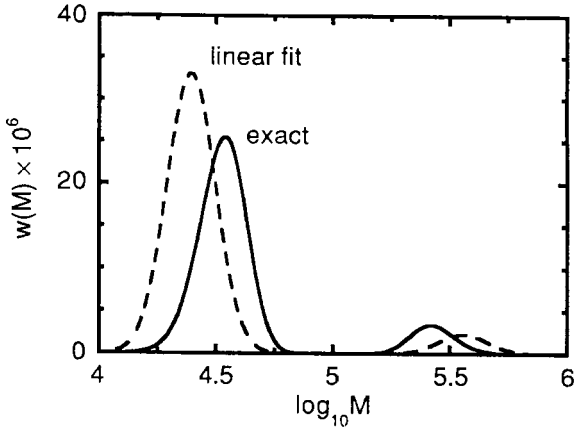

FIGURE 7. Comparison of linear fit with exact calibration curve in the calculation of $w(M)$.

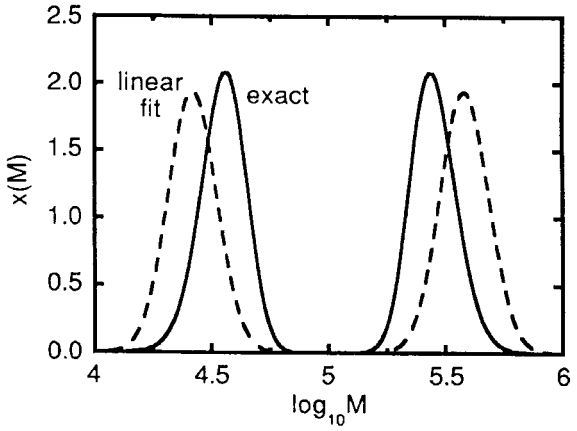

FIGURE 8. Comparison of linear fit with exact calibration curve in the calculation of $x(M)$.

$w(M)$ and $x(M)$ agree much more closely for these narrow peaks than for the first example. This is to be expected, since M is more nearly constant across each peak.

Figures 5 and 6 were both calculated using the exact calibration slope. If instead we use a linear fit to the calibration curve (the dashed line in Fig. 4), the results are quite different. Figures 7 and 8 compare the linear fit to the exact slope for both $w(M)$ and $x(M)$. The linear approximation to the calibration curve is poor near both peaks, too high for the first and too low for the second. This pushes the peaks of the distributions away from the molecular weight at the inflection point. Although the linear fit in Fig. 4 looks fairly benign, the logarithmic scaling is misleading, resulting in molecular weights of the MWD maxima which are in error by about 25–30%. If these curves are used in turn to calculate the various molecular weight moments [Eq. (9)], the moments will contain similar errors. Few researchers would be likely to accept the linear fit in Fig. 4; it is obviously in error. However, a fit three times as good might seem acceptable but would still give molecular weights nearly 10% off! The lesson is that an accurate determination of the calibration curve is essential in order to obtain accurate differential MWD's and their moments. It is highly desirable to use a method which provides many good calibration points, allowing a reliable fit to be obtained. Many researchers use a cubic (or spline) fit, requiring at least four calibration points, although ten to fifteen points are much preferred. Alternatively, a method such as light scattering automatically provides a calibration curve for every sample, since the molecular weight is determined independently for each elution volume.

C. Real Data

To determine the MWD for a real sample we need the slope of a real calibration curve. The calibration curve consists of discrete points either taken from elution volumes for known standards or from some independent molecular weight-sensitive measurement such as light scattering. Thus for real data we need to (*i*) approximate the derivative at each point from the data or (*ii*) fit the calibration curve to a model and use the derivative of the model. Method (*i*) tends to amplify errors in the calibration data. The preferred technique is to use method (*ii*) with either a

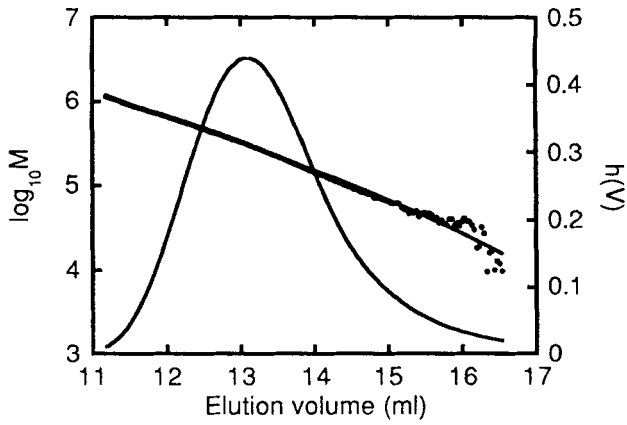

FIGURE 9. Calibration curve data and concentration response for the polystyrene standard NBS 706.

linear or cubic polynomial or a spline. A cubic fit allows the nonlinearities at low and high volumes to be reasonably well-approximated. Furthermore, fitting the calibration curve to a model smooths noisy calibration data somewhat. Another practical factor is that the molecular weight standards used for calibration are often quite widely spaced in molecular weight; a fit is necessary in order to interpolate between the calibration points. On-line light scattering measurements remove this restriction, since a calibration curve is automatically determined for each injection. Even so, a polynomial fit to the calibration curve has proved to be the best choice.

To study the differential MWD of an actual sample, let us examine the results for a broad molecular weight standard, NBS 706. The sample was dissolved in toluene at ambient temperature. Separation was performed with two PSS SDV columns (10^4 and 10^6 Å). Light scattering measurements were made with a Wyatt Technology DAWN model F multiangle laser light scattering photometer operating at a wavelength of 488 nm, and concentrations were determined by a Waters 410 differential refractometer. The results appear in Figs. 9–11. Figure 9 shows the overlaid refractometer and the calibration curve determined by light scattering. The calibration curve data are plotted along with a cubic fit used to calculate the slope at each volume. The resulting differential distributions appear in Figs. 10 and 11. A logarithmic molecular weight scale is used in Fig. 10 and a linear scale in Fig. 11.

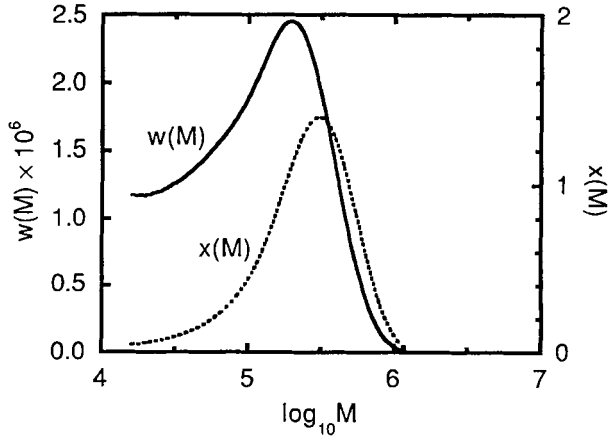

FIGURE 10. Differential MWD and log MWD for NBS 706, plotted against $\log_{10} M$.

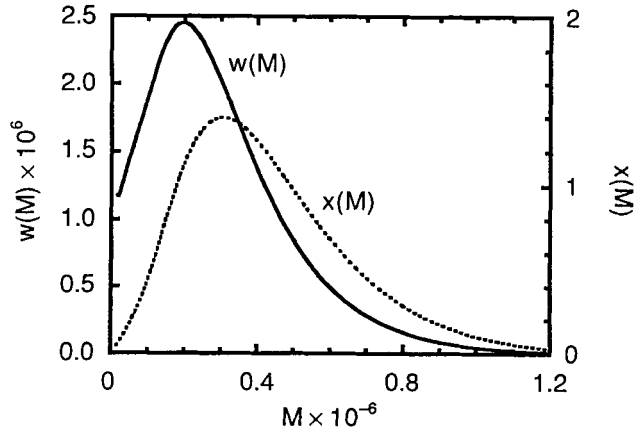

FIGURE 11. Differential MWD and log MWD for NBS 706, plotted against M.

As observed earlier for the broad molecular weight simulation, the maxima of the two distributions occur at quite different molecular weights: about 200,000 daltons for $w(M)$ and 300,000 for $x(M)$.

Note that $w(M)$ does not seem to approach zero in the limit of low molecular weights. At large volumes (low molecular weights), $h(V)$ approaches zero. But M is also decreasing approximately exponentially. Thus $w(M)$, which requires the ratio $h(V) / M$, does not approach zero as quickly as $x(M)$. A plot on a linear scale (Fig. 11) shows that $w(M)$ is indeed approaching zero at low molecular weights.

5. CONCLUSIONS

This paper has attempted to convey a clear, concise, and correct derivation of the differential MWD and its application to a number of specific examples. It was demonstrated that knowledge of the slope of the calibration curve is essential in determining an accurate MWD. In addition, care with logarithms is necessary. Common errors occurring in the literature were identified and corrections noted.

The differential MWD is obviously one of the most important functions one can calculate from GPC data. The computing power available today allows a researcher to construct MWD's far more quickly than he or she can collect data, a quantum improvement from the situation just a few years ago. As Billingham[7] wisely writes, however, "It is necessary to use any computer programme for GPC analysis with considerable care since the computer print-out gives the results an air of authority which may be misleading."

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REFERENCES

[1]L.H. Tung, *J. Appl. Polym. Sci.*, **10**, 375 (1966).

[2]W.W. Yau, H.J. Stoklosa, and D.D. Bly, *J. Appl. Polym. Sci.*, **21**, 1911 (1977).

[3]W.W. Yau, J.J. Kirkland, and D.D. Bly, *Modern Size-Exclusion Liquid Chromatography* (Wiley, New York, 1979).

[4]A.E. Hamielec, "Correction for Axial Dispersion," in *Steric Exclusion Liquid Chromatography of Polymers*, Chromatographic Sci. Ser., Vol. 25, J. Janca, ed. (Dekker, New York, 1984).

[5]Mark G. Styring and Archie E. Hamielec, "Determination of Molecular Weight Distribution by Gel Permeation Chromatography," in *Determination of Molecular Weight*, Anthony R. Cooper, ed. (Wiley, New York, 1989).

[6]Gregorio R. Meira, "Data Reduction in Size Exclusion Chromatography of Polymers," in *Modern Methods of Polymer Characterization*, Howard G. Barth and Jimmy W. Mays, eds. (Wiley, New York, 1991).

[7]N.C. Billingham, *Molar Mass Measurements in Polymer Science* (Wiley, New York, 1977).

[8]L.A. Papazian, private communication.

[9]W.W. Yau and S.W. Fleming, *J. Appl. Polym. Sci.*, **12**, 2111 (1968).

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