Size-Exclusion Calibration Curves from Light-Scattering Detection: Application to Poly(ethylene terephthalate)

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SYNOPSIS

A simple method is proposed for using a size-exclusion chromatograph equipped with both concentration and light-scattering detectors to calibrate other chromatographs having only concentration detectors. The method is developed and demonstrated for poly(ethylene terephthalate) in methylene chloride/dichloroacetic acid. It is shown that, in addition to circumventing the need for a light-scattering instrument on other chromatographs to be used for the analysis of PET, precision and accuracy of results are improved over those obtainable with light-scattering detection. The method uses averaging of the light-scattering detector data to establish a correlation between the molecular weight of the polymer of interest and the molecular weight of polystyrene at each retention volume. This correlation can then be used for other instruments employing the same mobile phase and only a concentration detector. Although the method assumes the validity of the universal calibration curve, the actual curve is not required, nor are Mark–Houwink constants or intrinsic viscosities. © 1994 John Wiley & Sons, Inc.

INTRODUCTION

In size-exclusion chromatography (SEC), when light-scattering detection is used with a concentration detector, the weight-average molecular weight at each retention volume across a chromatogram is obtained. Thus, each sample generates its own calibration curve. Despite this attribute, a single concentration detector with a conventional calibration curve (i.e., one determined from injecting a series of narrow-distribution polymer standards) is often preferred for routine analysis. There are two main reasons:

Precision

Conventional calibration is usually superior for making sample-to-sample comparisons, especially over long-term use. There is a substantial difference in the relative sensitivities of the concentration and light-scattering detectors at low molecular weights. As a result, when a light-scattering detector is used, the low molecular weight end of the distribution is often obtained by extrapolation methods similar to those described for viscometry detection.[1] These methods can adversely affect precision and accuracy of number-average molecular weights. Noise from the light-scattering detector, ratioing of the light-scattering and concentration detector signals, and the need for an accurate measure of interdetector volume, for which several methods have been proposed,[2,3] all introduce uncertainties that affect the reproducibility (as well as the accuracy) of molecular weight distributions obtained from light-scattering detection.

Simplicity

Apart from the additional cost, a light-scattering detector must be calibrated and maintained in addition to the concentration detector. Also, SEC columns must not shed particles, the eluent must be well filtered, the output of the light source must be

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constantly monitored, attenuators or detectors must be calibrated, and cell windows are susceptible to contamination. In comparison, constructing a narrow-standard SEC calibration curve is an infrequent event provided that the columns are maintained properly and the flow rate is monitored accurately. In fact, conventional narrow-standard calibration has been proposed as a prerequisite for obtaining accurate results from SEC with light-scattering detection using a "systematic approach."[4,5]

The disadvantage of conventional calibration is the need for standards of the same composition as that of the samples. Sometimes such standards are not available. One solution is to use light-scattering detection to establish a conventional calibration curve and then to employ this calibration curve with only the concentration detector for routine analysis. However, this calibration curve remains specific for the column and detector combination used to establish it and will not be useful for other SEC systems.

This restriction can be partially addressed by using light-scattering detection and a universal calibration curve to calculate Mark–Houwink–Sakurada constants K and a.[6,7] Knowledge of these constants eliminates the need to measure intrinsic viscosities of the unknown; however, it requires accurate values of intrinsic viscosity for the polymer standards to construct the universal calibration curve. This is difficult for new solvent systems for which K and a values of polymer standards are not available.

We employ a much simpler approach that does not require calculation of the Mark–Houwink constants and can even accommodate situations where the Mark–Houwink constants vary with molecular weight. The approach is well founded theoretically and effectively links the results of light-scattering detection for the polymer of interest on one SEC system to the conventional SEC calibration curve established using polystyrene (or other commonly available narrow standards) on other SEC systems. Furthermore, the approach is really the further evolution of previously published methods directed at converting "equivalent" molecular weight averages to "absolute" molecular weight averages. In particular, early work in this direction by Mori[8] actually employs the proposed method in its simplest form, and more recent studies show its utility in the analysis of PET[9] and polyamides.[10]

The method assumes that universal calibration is valid, i.e.:

$$[\eta]_1 M_1 = [\eta]_2 M_2 \qquad (1)$$

The Mark–Houwink–Sakurada (MHS) relation between viscosity and molecular weight is

$$[\eta] = KM^a \qquad (2)$$

which upon substitution into eq. (1) and rearranging provides the familiar relationship often used for converting an "equivalent" molecular weight M_1 to absolute molecular weight M_2 for the polymer of interest:

$$\log M_2 = \frac{1}{1 + a_2} \log \frac{K_1}{K_2} + \frac{1 + a_1}{1 + a_2} \log M_1 \qquad (3)$$

Application of eq. (3) requires measurement of intrinsic viscosities and molecular weights of both narrow standards and samples to obtain K and a if these values are not available.

If we are not interested in the specific values of K and a, they can be grouped into two constants, β_1 and β_2 (this approach has been successfully used previously in "calibration curve search" techniques[11,12]). Then, eq. (3) can be written to show that it really represents a simple linear relationship between the molecular weight of one polymer and the molecular weight of another at the same retention volume, i.e.:

$$\log M_2 = \beta_1 + \beta_2 \log M_1 \qquad (4)$$

where β_1 and β_2 are the intercept and slope of a straight line plot of log M_2 vs. log M_1. In the most basic version of the method proposed here, the constants in eq. (4) are obtained by plotting the values of M_2 obtained from a light-scattering detector applied to the polymer of interest (polymer 2) vs. the values of M_1 for polystyrene (polymer 1) determined from the conventional calibration curve obtained by the injection of polystyrene fractions. This M_2–M_1 plot can then be used when required with the conventional polystyrene calibration curves for other SEC systems (not equipped with a light-scattering detector) to generate a calibration curve for M_2.

A more general version of the method is obtained by removing the assumption of constant K and a. In eq. (3), it is generally assumed that K and a are constant across the molecular weight distribution. This assumption can be a source of error.[13] Furthermore, the assumption is unnecessary; it is evident that if K and a do vary with molecular weight and if the different values of K and a are known, they could be used in eq. (3) at different molecular weights. So, eq. (3) can be written with the various K's and a's as functions of M_1 or M_2:

$$\log M_2 = \frac{1}{1 + a_2(M_2)} \log \frac{K_1(M_1)}{K_2(M_2)} + \frac{1 + a_1(M_1)}{1 + a_2(M_2)} \log M_1 \quad (5)$$

and now eq. (4) can be written

$$\log M_2 = \gamma_1(M_1, M_2) + \gamma_2(M_1, M_2) \log M_1 \quad (6)$$

where now the constants denoted by β_1 and β_2 have been replaced by functions of M_1 and M_2 denoted by $\gamma_1(M_1, M_2)$ and $\gamma_2(M_1, M_2)$. This equation shows that the relationship between M_2 and M_1 can be nonlinear and provides the justification for fitting the correlation with a higher-order polynomial rather than with a straight line:

$$\log M_2 = \beta_3 + \beta_4 \log M_1 + \beta_5 \log^2 M_1 + \cdots \quad (7)$$

where, again, the β's are constants.

In this work, light-scattering detection is applied to poly(ethylene terephthalate) (PET), a polymer that contains cyclic and oligomeric materials that scatter very little light and are difficult to measure reproducibly by light-scattering. Equations (4) and (7) are used to relate the PET molecular weights at each retention volume to polystyrene molecular weights at the same respective retention volumes. This then enables absolute PET molecular weight values to be obtained from SEC systems equipped with only a concentration detector.

EXPERIMENTAL

Poly(ethylene terephthalate) (PET) samples were synthesized at Eastman Chemical Co. (Kingsport, TN). PET 39 K was obtained from American Polymer Standards (Mentor, OH). Narrow-molecular-weight distribution polystyrene standards were obtained from Polymer Laboratories (Amherst, MA). The eluent was prepared at a volumetric ratio of 80 : 20 methylene chloride/DCAA, containing 0.01 M tetrabutylammonium acetate (TBAA), and was continuously sparged with a light stream of helium. The nominal flow rate was 1.0 mL/min, and samples were injected in a volume of 100 μL. A Spectroflow 757 UV detector, LDC Analytical KMX-6 low-angle laser light-scattering (LALLS) photometer, and a Waters Model 410 DRI were connected in series after three Polymer Laboratories 7.5 × 300 mm Mixed-B columns. The SEC columns and DRI were thermostated to 30.0°C. The UV and LALLS detectors were operated at room temperature. All light-scattering intensities were measured at 6–7° with an aperture of 0.15 mm. The specific refractive index increment of PET at 632.8 nm is 0.148 mL/g.

Further details, including the dissolution of samples before SEC analysis and measurement of specific refractive index increments, are available in a previous article on the development of this eluent for PET and related materials.[14]

RESULTS AND DISCUSSION

As shown in Figure 1, the light-scattering detector response for PET is considerably noisier than is the UV response. Also, there is no light-scattering response in the cyclic trimer and oligomer region. Molecular weights of PET at each retention volume are calculated from these two responses. Plots of these data as log M vs. retention volume are shown in Figure 2 for the highest and lowest molecular weight PET samples. Also shown in Figure 2 is the conventional calibration curve for polystyrene. In contrast to the polystyrene curve, the light-scattering curve shows significant noise at both high and low retention volume ends. At the low retention volume end of the chromatogram, the insensitivity of the UV detector is the source of noise, and at the high retention volume end, the light-scattering detector is the limiting factor. Also, the light-scattering curves encompass a comparatively small portion of the elution range for each sample (note in Fig. 2 that only approximately half of a concentration detector's chromatogram is represented by a light-scattering calibration curve). Thus, the low molecular weight region, and to a much lesser extent the high molecular weight region, must be defined by extrapolation. To attempt to improve this situation, more than one broad molecular weight distribution PET sample can be used to establish a plot of log M vs. retention volume, i.e., samples can be chosen so that their combined elution ranges extend over as broad a region as possible. Also, averaging calibration curves improves precision in the noisy regions at high and low retention volumes. In this study, only the highest and lowest molecular weight PET samples of Table I are used. A plot of log M vs. retention volume is obtained in duplicate for each. Then, the average log M at each retention volume is calculated from these four sets of data. There is an improvement in the precision of the high and low retention volume ends of the PET curve in Fig-

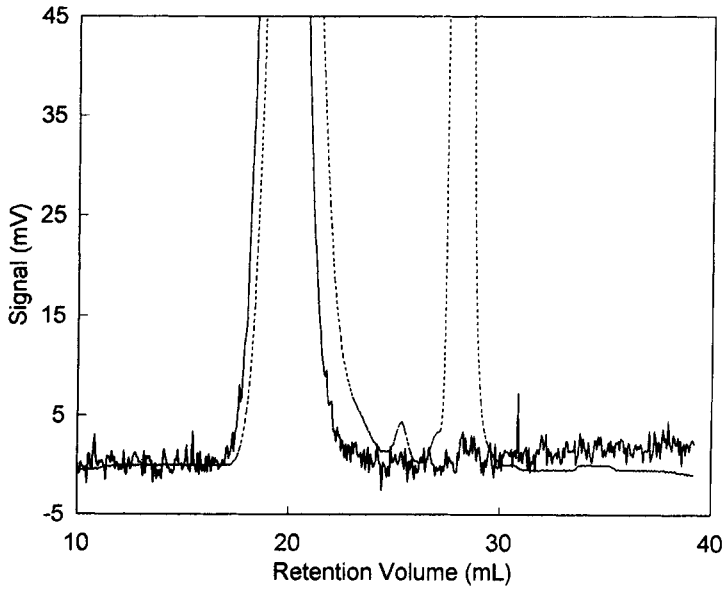

Figure 1 (——) LALLS and (----) UV chromatograms of PET 9902.

ure 3, and the calibration curve covers a slightly broader range of retention volumes than does a single broad standard alone. This, in turn, shortens and improves the accuracy of the extrapolations required (dashed lines). In our particular case, using all the samples provides the same results as using only the PET samples with the highest and lowest molecular weights. In general, however, the more samples averaged, the more precise would be the averaged calibration curve and the more reliable the resulting extrapolations.

From Figure 3 it can be seen that for each selected value of retention volume, a value of log M for PET and for polystyrene can be determined. Figure 4 shows these values of log M for PET plotted vs. log M for polystyrene. Now, these data can be fit by either a straight line [eq. (4), assuming constant K and a] or by a polynomial [eq. (7), allowing for

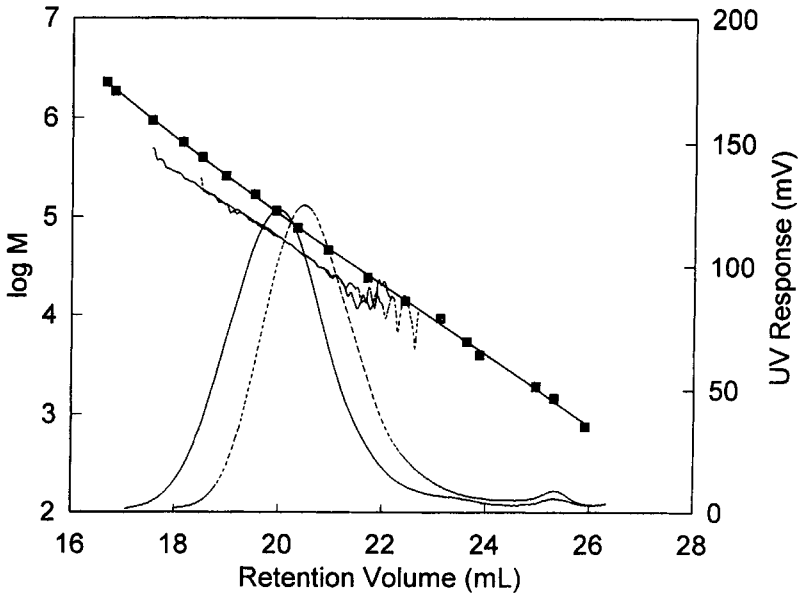

Figure 2 UV chromatograms and log M vs. retention volume: (——) PET 10388; (----) PET 39K; (— ■ —) polystyrene narrow standard calibration curve.

Table I

PET	LALLS M_n	LALLS M_w	LALLS M_z	Narrow Standard Eq. (8) M_n	Narrow Standard Eq. (8) M_w	Narrow Standard Eq. (8) M_z	Narrow Standard Eq. (9) M_n	Narrow Standard Eq. (9) M_w	Narrow Standard Eq. (9) M_z	LALLS Trimer MW	Eq. (9) Trimer MW
10388	24,100	71,700	117,000	21,700	68,400	129,000	22,300	70,000	118,000	903	575
	(30.8%)[a]	(2.0%)	(3.8%)	(7.1%)	(1.5%)	(3.0%)	(7.9%)	(1.6%)	(2.2%)	(102%)	(1.0%)
39K	14,300	34,800	53,700	12,100	35,500	57,600	12,300	38,300	59,700	1238	576
	(29.2%)	(3.1%)	(4.9%)	(3.7%)	(0.8%)	(1.2%)	(3.7%)	(0.79%)	(1.0%)	(83%)	(1.2%)
7352	17,500	47,200	72,600	16,200	45,200	76,700	16,600	48,000	76,500	742	572
	(22.3%)	(2.5%)	(3.0%)	(6.6%)	(2.1%)	(1.8%)	(7.0%)	(2.0%)	(1.5%)	(59%)	(1.0%)
9902	19,200	62,600	93,800	19,500	56,400	101,000	20,100	58,800	96,000	708	571
	(35.6%)	(2.2%)	(2.3%)	(5.8%)	(1.3%)	(1.7%)	(6.3%)	(1.2%)	(1.3%)	(102%)	(1.0%)

[a] Error reported as $100(\sigma/\bar{x})$, where σ is the sample standard deviation and $\bar{x}$ is the mean of 10 trials.

variation of K and a with molecular weight]. The two respective fits obtained are

$$\log M_2 = -0.2884 + 1.0061 \log M_1 \quad (8)$$

and

$$\log M_2 = 2.5679 - 1.1693 \log M_1 + 0.60611 (\log M_1)^2 - 0.068517 (\log M_1)^3 + 0.002353 (\log M_1)^4 \quad (9)$$

A polystyrene calibration curve (values of M_1) can now be converted to a PET calibration curve using eq. (8) or (9) because these equations permit each log M value to be converted to a PET log M value (Fig. 5). Each PET calibration curve so obtained is applied to the chromatograms of PET samples and the values of molecular weight averages calculated, making the assumption that the UV response is not a function of molecular weight.

The precision and values of the weight-average molecular weights (M_w) measured by light-scattering detection (LALLS) given in Table I are comparable to whole polymer values calculated from the light-scattering detector alone, reported on these samples earlier.[14] These weight-average molecular

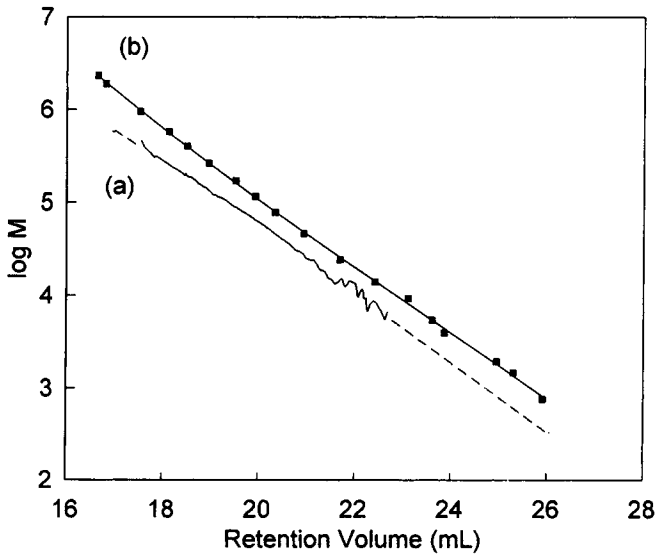

Figure 3 (a) PET log M vs. retention volume obtained from combining duplicate data sets of PET 10388 and PET 39K; (b) polystyrene narrow standard calibration curve. Dashed lines denote extrapolated region.

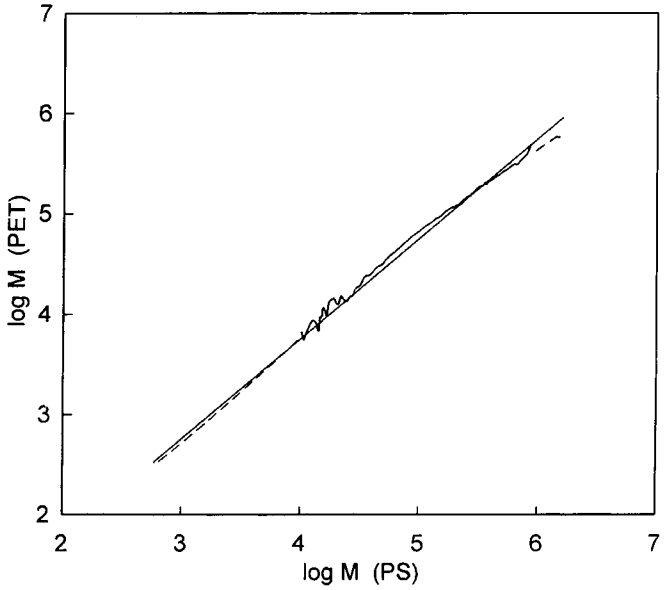

Figure 4 First-order fit of PET and polystyrene log M data.

weights are accepted as the "true" values. Weight-average molecular weights calculated using eq. (8) or (9) are within ~ 10% of the true values. There are only small differences in results calculated using the linear [eq. (8)] and fourth-order [eq. (9)]; eq. (9) increases accuracy on three samples and worsens accuracy for one (39K). In all cases, the precision of weight-average molecular weights calculated by either narrow standard calibration method [eq. (8) or (9)] is better than that obtained by LALLS. This is consistent with expectations based on previous experience: The precision of molecular weight averages calculated by narrow standard calibration methods is generally better than the precision of molecular weight averages calculated by LALLS.[15] This is more clearly seen in the precision of number-average molecular weights. The poor precision of M_n (~ ±30%) obtained from LALLS results from the lack of light-scattering signal at low molecular weights and the uncertainties in extrapolating mo-

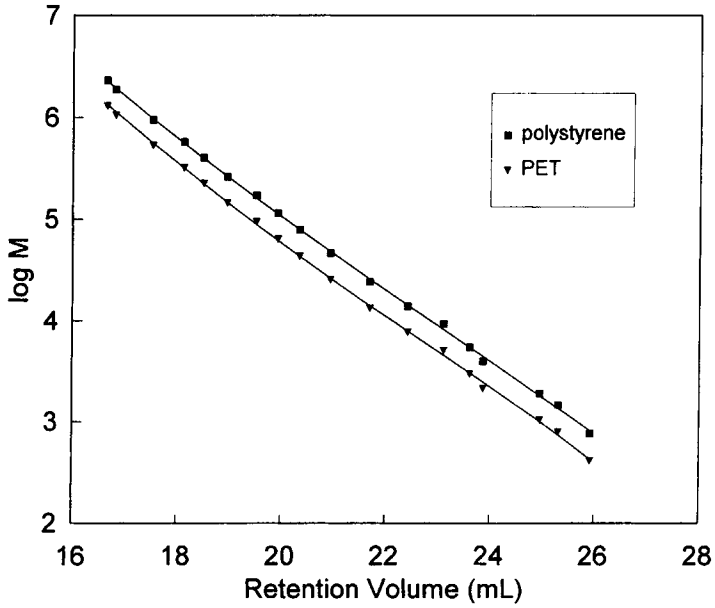

Figure 5 Polystyrene and corresponding PET calibration curve using linear fit [eq. (8)].

lecular weights in this region. Precision is clearly improved by either narrow standard method used. Even more dramatic are the differences in molecular weights calculated for the cyclic trimer peak. The molecular weight of the cyclic trimer is obtained by extrapolation of the log M vs. retention volume curves in the LALLS method. Reproducibility is very dependent on noise in the chromatograms (particularly LALLS), among other factors. Narrow-standard calibration methods offer an improvement in precision of approximately a factor of 100. The accuracy of the cyclic trimer molecular weight calculated by narrow-standard methods may be fortuitous; others have reported apparent cyclic trimer molecular weights between 275 and 2500.[16,17]

CONCLUSIONS

A simple method for obtaining absolute molecular weights from size-exclusion chromatographs equipped with only concentration detectors has been proposed and demonstrated using the analysis of PET as an example. The method utilizes one SEC system equipped with a light-scattering/concentration detector combination to relate PET molecular weight to polystyrene molecular weight at the same retention volume. Selection of different PET samples and averaging of log M vs. retention volume data improves the results of the light-scattering. This correlation is then used to convert conventional polystyrene calibration curves to PET calibration curves on SEC systems equipped only with a concentration detector and using the same mobile phase. Thus, PET molecular weight information from these systems is then based upon the accuracy of light-scattering but exhibits the precision associated with the use of a conventional polystyrene calibration curve. The advantages of the method then include an improvement in both the precision and accuracy of molecular weight averages over those obtainable with light-scattering/concentration detector combinations, improved estimates of the molecular weight of low molecular weight components, and the simplicity associated with the use of only a single concentration detector. This makes the method particularly attractive for routine analyses. Mark–Houwink constants, a universal calibration curve, and intrinsic viscosities are not required, although the method does assume the validity of universal calibration. The method is not limited by variation of Mark–Houwink constants across the molecular size distribution.

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