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Measurement of narrow-distribution polydispersity using multi-angle light scattering

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Abstract

Molecular radius information obtained with multi-angle laser light scattering combined with gel permeation chromatography was used to obtain an upper limit for the polydispersity $\bar{M}_w/\bar{M}_n$ of a narrow-distribution polystyrene standard dissolved in toluene. The radius obtained by light scattering is independent of any other detector and so is not affected by interdetector delay volumes or interdetector broadening. The value obtained for $\bar{M}_w/\bar{M}_n$ was 1.00096 ± 0.00004, much closer to unity than previously reported. Column broadening was found to be responsible for nearly all the observed peak width.

1. Introduction

The molecular mass distribution (MMD) of samples prepared by termination-free chain addition polymerization is theoretically close to a Poisson distribution, in which the molecular mass polydispersity $\bar{M}_w/\bar{M}_n$ (where $\bar{M}_w$ is the weight-average molecular mass and $\bar{M}_n$ is the number-average molecular mass) is predicted to be very close to unity [1–3]. In practice, the methods available to measure whole-polymer $\bar{M}_w$ and $\bar{M}_n$ directly (for example, light scattering and osmometry, respectively) are only accurate to 1–2%, making determination of polydispersities smaller than about 1.03 impossible. Gel permeation chromatography (GPC) allows samples to be separated by molecular size and then detected, typically by a differential refractive index (DRI) detector. In traditional GPC one injects a series of narrow-distribution polymer standards of known molecular masses M to create a calibration curve [4] of log M vs. elution volume V. The DRI chromatogram for an unknown sample is then used along with the calibration curve to determine the molecular mass averages and MMD for the unknown [5]. A number of factors affect the accuracy of this determination, among them conformation differences between the calibration standards and the unknown, and band broadening in the chromatographic system. When studying narrow-MMD samples, band broadening is a particularly important issue and has been discussed by many researchers [6–8]. The presence of broadening makes calculating the polydispersity of very narrow distribution samples difficult. In this paper we shall use the term "column broadening" to refer to any broadening prior to the first detector in the system, and "interdetector broadening" to refer to subsequent broadening occurring in various detectors' flow cells and/or connecting tubing. Interdetector broadening is

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particularly important when signals from various instruments are compared.

With the use of molecular mass-sensitive techniques such as light scattering (LS) combined with GPC it is possible to determine molecular masses directly [9]. Since the LS signal is proportional to the product of molecular mass and concentration, one can determine the molecular mass by taking the ratio of the LS and DRI signals. This technique allows accurate molecular mass and radius determination for each slice across a sample peak without column calibration. When using GPC–LS, the effect of broadening is to make the calculated molecular mass values more constant than they should be, decreasing the calculated polydispersity of the sample. In addition, interdetector broadening which occurs between the LS and DRI detectors causes the calculated molecular mass values to be incorrect because one signal is broadened more than the other. The presence of interdetector broadening makes determination of polydispersities less than 1.01 very difficult, even when corrections are applied. However, by using thermal field flow fractionation to correct for interdetector broadening, Giddings [10] showed that a polystyrene sample with a nominal polydispersity of 1.06 actually had a value closer to 1.003.

Furthermore, in order to combine properly the signals from multiple instruments, it is important to determine the interdetector delay volume accurately. The effect of changes in assumed delay volume on molecular mass determinations with LS has been discussed in detail by Wyatt and Papazian [11]. The effect is relatively small; it does not affect the calculation of $\bar{M}_w$ at all, and it changes M_n by only a few percent. Unfortunately, when we are attempting to calculate accurately the polydispersity of a narrow-MMD peak, an effect of even a few percent makes molecular mass measurements unsuitable for this purpose.

Multi-angle laser-light scattering (MALLS) provides not only the molecular mass but also the mean square molecular radius. At the low concentrations typical of GPC separations, the calculated molecular radius values are independent of the DRI detector, so they depend neither on the interdetector delay volume nor on interdetector broadening. Thus molecular radius is an excellent way to study the polydispersity of narrow-MMD samples. This paper describes a new technique which uses the molecular radius values obtained by GPC–MALLS to calculate the polydispersity of a narrow-MMD polystyrene standard, taking proper account of column broadening effects. First a model of a simple chromatographic system with Gaussian peaks and Gaussian broadening is presented. Effects of interdetector delay volume and broadening on the calculation of molecular mass and radius are discussed. Data from narrow-MMD polystyrene standards are presented, and the radius information is used to place a new upper limit on the polydispersity of one of these narrow standards.

2. Theory

2.1. Model of a simple chromatographic system

Let us consider a simple chromatographic system. We shall assume a single Gaussian peak. This means the normalized concentration signal is given by

$$h(V) = 2\left(\frac{\ln 2}{\pi}\right)^{1/2} \cdot \frac{m_0}{w_0} \cdot \exp\left[-4 \ln 2(V - V_0)^2 / w_0^2\right] \quad (1)$$

where m_0 is the mass of solute in the peak, V_0 is the peak elution volume and w_0 is the full width at half maximum (FWHM) of the true, unbroadened peak. We further assume that the column separation is log–linear according to molecular mass as

$$M(V) = 10^{A + BV} \quad (2)$$

where A and B are constants and $M(V)$ is the molecular mass at elution volume V. Likewise, if the separated molecules have a conformation (random coil, rigid rod, sphere, etc.) which is independent of molecular mass, then the column separation in root mean square (rms) radius $\langle r^2 \rangle^{1/2}$ follows a similar relation:

$$\langle r^2 \rangle^{1/2}(V) = 10^{C+DV} \quad (3)$$

where $\langle r^2 \rangle^{1/2}(V)$ is the rms radius of the molecules at elution volume V.

The molecular mass polydispersity is equal to the ratio $\bar{M}_w/\bar{M}_n$, where $\bar{M}_n$ and $\bar{M}_w$ are given by [12]

$$\bar{M}_n = \frac{\int h(V)\,dV}{\int h(V)/M(V)\,dV}$$

$$\bar{M}_w = \frac{\int M(V)h(V)\,dV}{\int h(V)\,dV} \quad (4)$$

The limits of integration are taken to be $-\infty$ to $+\infty$. Integrating these relations using Eqs. 1 and 2 gives

$$\bar{M}_w/\bar{M}_n = \exp[(\ln 10)^2 B^2 w_0^2/8 \ln 2] \quad (5)$$

For a suitably narrow-distribution sample, $Bw_0 \ll 1$, and we have the following approximation:

$$\bar{M}_w/\bar{M}_n \cong 1 + (\ln 10)^2 B^2 w_0^2/8 \ln 2 \cong 1 + 0.96 B^2 w_0^2 \quad (6)$$

Thus the polydispersity depends only on the product Bw_0. Even though real chromatographic peaks differ somewhat from Gaussian, peaks from narrow-distribution samples can be fit quite successfully by a Gaussian line shape, and so this relation provides a reasonable estimate of the polydispersity.

2.2. Light scattering

The following equation [13] describes the Rayleigh–Gans–Debye (see [14]) approximation for the Rayleigh ratio $R(\theta)$ of light scattered at an angle θ by macromolecules having a weight-average molecular mass $\bar{M}_w$, in the limit of small concentrations c:

$$\frac{R(\theta)}{K^* c} = \bar{M}_w P(\theta) - 2A_2 \bar{M}_w^2 P^2(\theta) c \quad (7)$$

Here K^* is an optical constant given by

$$K^* = 4\pi^2 (dn/dc)^2 n_0^2 / N_A \lambda_0^4 \quad (8)$$

for vertically polarized incident light, where n_0 is the solvent refractive index, N_A is Avogadro's number, λ_0 is the vacuum wavelength of the incident light, and dn/dc is the refractive index increment. The scattering function $P(\theta)$ describes the angular dependence of the scattered light and will be discussed in more detail below. Here it suffices to remark that $P(\theta \to 0) = 1$ for any molecular conformation or size satisfying the Rayleigh–Gans–Debye approximation. The quantity A_2 is the second virial coefficient which describes solvent–solute interactions and can usually be ignored for the low concentrations common in GPC. Furthermore, in chromatography we assume a separated sample so that at each elution volume V only one molecular mass species is present, implying $\bar{M}_w = M$. Thus for GPC we have the following useful expression:

$$R(\theta) = K^* c M P(\theta) \quad (9)$$

In the low-angle limit $R(\theta \to 0) = K^* cM$. We shall explore the implications of imperfect chromatographic separation in Section 2.4 below.

We define $LS(V)$ to be the measured light-scattering signal [proportional to $R(\theta)$] in the limit $\theta \to 0$. Thus $LS(V)$ is proportional to the product of the molecular mass $M(V)$ and the concentration signal $h(V)$:

$$LS(V) \propto M(V)h(V) \propto \exp[\ln 10\,(A + BV) - 4 \ln 2\,(V - V_0)^2/w_0^2] \quad (10)$$

which can be rewritten as

$$LS(V) \propto \exp\left[-4 \ln 2 \left(V - V_0 - \frac{\ln 10}{8 \ln 2} Bw_0^2\right)^2 \Big/ w_0^2\right] \quad (11)$$

ignoring constant additive terms in the exponential. This implies that the LS signal is itself a Gaussian with the same FWHM but shifted with respect to $h(V)$ by a volume $(\ln 10)Bw_0^2/8 \ln 2$. Since B, the slope of the molecular mass calibration curve, is typically negative in GPC, the shift is to smaller elution volumes. When analyzing data, we measure $LS(V)$ from the light-scattering instrument, $h(V)$ from the DRI detec-

tor, and we take the ratio $LS(V)/h(V)$ to recover $M(V)$.

2.3. Size information from multi-angle light scattering

Multi-angle light scattering allows determination of the molecular size as well as the molecular mass. The angular dependence of the scattered light is described by the scattering function $P(\theta)$, where θ is the scattering angle. For molecules smaller than about $\lambda/20$, where λ is the wavelength of the scattered light in solution, $P(\theta)$ can be well approximated by

$$P(\theta) = 1 - \frac{16\pi^2}{3\lambda^2} \cdot \langle r^2 \rangle \sin^2(\theta/2) \tag{12}$$

where $\langle r^2 \rangle$ is the mean square radius of the molecules. For larger molecules, more terms containing higher powers of $\sin^2(\theta/2)$ are required. Alternatively, one can use a specific model for $P(\theta)$ which assumes a certain molecular conformation such as random coil, rigid rod, or sphere. For example, ideal random coils [15] scatter according to

$$P(\theta) = 2(e^{-x} - 1 + x)/x^2 \tag{13}$$

where

$$x = (4\pi/\lambda)^2 \langle r^2 \rangle \sin^2(\theta/2) \tag{14}$$

In any case, the mean square radius $\langle r^2 \rangle$ is proportional to the low-angle derivative of $P(\theta)$ with respect to $\sin^2(\theta/2)$. For any given slice, $R(\theta) \propto P(\theta)$ as long as c is small enough that the A_2 term in Eq. 7 can be neglected. Therefore, to calculate $\langle r^2 \rangle$ for a particular slice one fits the angular LS data $R(\theta)$ to one of the above models. For example, using Eqs. 9 and 12 gives the complete model

$$R(\theta) = a\left[1 - \frac{16\pi^2}{3\lambda^2} \cdot \langle r^2 \rangle \sin^2(\theta/2)\right] \tag{15}$$

where a is equal to K^*cM. Since λ is known, Eq. 15 is a model having two parameters, a and $\langle r^2 \rangle$. Note that a serves as a scaling factor; if we arbitrarily double all the $R(\theta)$ values, a doubles but $\langle r^2 \rangle$ remains unchanged. Thus the value of $\langle r^2 \rangle$ determined from the model is independent of the value of a, and therefore of c, and hence independent of the concentration detector and interdetector delay volume. Even if we had no concentration signal, we would still obtain $\langle r^2 \rangle$ correctly (of course, in this case we would obtain a meaningless value of M). The independence of $\langle r^2 \rangle$ from c is extremely important, for it will allow us to make meaningful statements about the polydispersity of narrow-distribution standards without the errors associated with delay volume inaccuracy or interdetector broadening.

2.4. Effect of peak broadening on molecular masses and radii calculated from light-scattering data

We shall assume Gaussian broadening [4] characterized by a FWHM of w_G. Then the normalized (i.e., unit area) broadening kernel is

$$G(V) = \left(\frac{\ln 2}{\pi}\right)^{1/2} \cdot \frac{2}{w_G} \cdot \exp(-4 \ln 2 V^2/w_G^2) \tag{16}$$

If we have a concentration chromatogram $h(V)$, the broadened chromatogram $F(V)$ is then given by Tung's convolution integral [16]

$$F(V) = \int h(y)G(V-y)\,dy \tag{17}$$

where the limits of integration are taken to be $-\infty$ to $+\infty$. It is not difficult to show that the convolution of two Gaussian peaks is itself Gaussian and that the peak widths add in quadrature. For example, the observed FWHM of $F(V)$ is given by

$$w_{obs}^2 = w_0^2 + w_G^2 \tag{18}$$

The LS signal is also broadened, and we must account for the fact that when multiple molecular mass species are present, the amount of light scattered is proportional to the weight average of those species. The observed molecular mass as measured by the LS detector is the ratio of the broadened LS signal to the broadened concentration signal:

$$M_{obs}(V) = \frac{\int M(y)h(y)G(V-y)\,dy}{\int h(y)G(V-y)\,dy} \quad (19)$$

Integration of Eq. 19 is tedious but straightforward; the result is that $M_{obs}(V)$, like $M(V)$, is log–linear but with a slope B_{obs} given by the simple expression

$$B_{obs} = Bw_0^2/w_{obs}^2 \quad (20)$$

Details of the derivation of Eq. 20 are given in the Appendix. Thus the effect of broadening on the combination of DRI and LS detectors is to reduce the magnitude of the apparent slope of the calibration curve, making the peak appear to have a smaller polydispersity. For a broad distribution peak $w_{obs} \approx w_0$ and $B_{obs} \approx B$. For a very-narrow-distribution peak, however, $w_{obs} >> w_0$ and B_{obs} approaches zero. Therefore any estimate of the polydispersity of a narrow-distribution peak must include the effects of broadening.

A similar analysis may be performed to obtain the observed rms radius $\langle r^2\rangle_{obs}^{1/2}(V)$. In this case, however, it is the so-called z-average mean square radius, not the weight average, to which the LS detector responds [17]. The result for the observed rms radius is

$$\langle r^2\rangle_{obs}^{1/2}(V) = \left[\frac{\int \langle r^2\rangle(y)M(y)h(y)G(V-y)\,dy}{\int M(y)h(y)G(V-y)\,dy}\right]^{1/2} \quad (21)$$

which is again log–linear with a slope D_{obs} given by an expression directly analogous to Eq. 20:

$$D_{obs} = Dw_0^2/w_{obs}^2 \quad (22)$$

Details of this derivation are given in the Appendix. This relation allows us to recover the unbroadened width w_0 if we know the observed width w_{obs}, the observed rms radius calibration slope D_{obs}, and the true calibration slope D. The quantity w_{obs} is easy to obtain from a sample's chromatogram, and D_{obs} can be obtained by measuring the rms radius at each slice across the sample peak. The true calibration slope D can be obtained by injecting a series of narrow standards and constructing the rms radius calibration curve. Once w_0 has been determined, it can be used in Eq. 6 to obtain the polydispersity. This is the procedure we shall use below.

In calculating the effect of broadening on molecular mass we have assumed that the peak undergoes no additional broadening in passing from one instrument to the other. If some interdetector broadening occurs, the ratio of LS to DRI gives calculated molecular masses which do not lie on a log–linear curve, and the results depend on which detector is connected first in line. Typically the LS detector is first, followed by the DRI detector. In this case the DRI signal is broadened more than the LS signal, and the calculated molecular masses are too large at the peak center and too small on either side [18]. This effect, like the delay volume effect discussed by Wyatt and Papazian [11], is small but is more pronounced for narrow peaks. Interdetector broadening depends heavily on the particular flow design of the various instruments and may be far from Gaussian. The calculated radius is not affected by interdetector broadening since the DRI signal is not used. This is another reason why radius measurements, if available, are superior to molecular mass measurements when studying the polydispersity of narrow-distribution samples.

3. Experimental

Data were collected from a chromatograph made up of an LDC Analytical Model 396-57 pump, two columns (300 mm × 8 mm; MZ Analysentechnik, Mainz, Germany; styrene–divinylbenzene; 5 μm particle diameter; 10^6 and 10^4 Å pore diameter; ambient temperature), a Wyatt Technology Model DAWN DSP-F laser photometer and a Waters Model 410 differential refractometer. The mobile phase was toluene and the flow-rate was 1.0 ml/min. The delay volume between the LS instrument and the DRI detector was measured to be 184 μl, meaning that the DRI detector's cell was 184 μl down-

stream of the LS detector's cell. Stainless-steel tubing with I.D. 0.25 mm was used for all connections. A series of Pressure Chemical polystyrene standards of nominal molecular masses 30, 65, 130, 200, 400 and 950 kg/mol was injected. The injection volume for all samples was 100 μl. Column loading ranged from 0.6 mg for the 30 kg/mol sample to 0.03 mg for the 950 kg/mol sample. Data points were collected every 1.0 s. Molecular masses and rms radii were calculated independently for each sample using Wyatt Technology's ASTRA chromatography software version 1.1.5 for Macintosh.

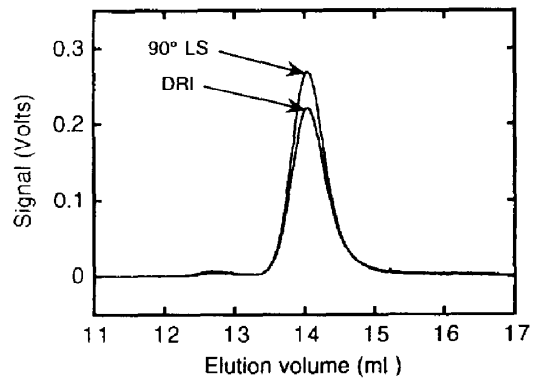

Fig. 1. 90° LS and DRI signals for nominal 400 kg/mol polystyrene in toluene.

4. Analysis and results

First, all the data were analyzed to verify that reasonable values are obtained for both molecular mass and radius for the samples of interest. Table 1 shows the results for the samples used in this study. Molecular mass and radius values in Table 1 were calculated for slices near the tops of the respective peaks. Since the polydispersity calculations will depend on precise radius measurements, it is important to use data with a high signal-to-noise ratio. The nominal 400 and 950 kg/mol samples gave the highest relative precision for the rms radius. Although column calibration is not necessary to determine molecular masses or sizes from LS data, in order to calculate the polydispersity of narrow standards it will prove necessary to measure the slope of log $\langle r^2 \rangle^{1/2}$ vs. V and log M vs. V to obtain the coefficients B and D in Eqs. 2 and 3. We expect these coefficients to be functions of V since the calibration curves are not exactly log–linear. Thus it is important to measure B and D at the elution volume of interest. For these reasons, the 400 kg/mol sample was chosen for polydispersity analysis, with the 200 and 950 kg/mol samples providing additional data used to determine B and D.

Fig. 1 shows the 90° LS and DRI signals for the 400 kg/mol sample as a function of elution volume. The FWHM w_{obs} of the peaks is 0.54 ± 0.01 ml. There is no statistically significant difference in the FWHM for the two peaks, although a small amount of additional tailing can be seen in the DRI signal on close examination. Peaks for the other samples were of similar quality. The slopes of the molecular mass and radius calibration curves were determined by fitting the data in Table 1 for the 200, 400 and 950 kg/mol samples. The best-fit slope of log M vs. V, which

Table 1
Peak molecular mass and rms radius results for six polystyrene standards in toluene

Nominal molecular mass (kg/mol)	Peak elution volume (ml)	M (kg/mol)	$\langle r^2 \rangle^{1/2}$ (nm)
30	18.92	29.9 ± 0.8	6 ± 1
65	17.51	65 ± 1	9.7 ± 0.4
130	16.46	133.0 ± 0.4	14.2 ± 0.3
200	15.48	206.0 ± 0.4	18.0 ± 0.3
400	14.04	406 ± 1	26.8 ± 0.2
950	12.30	954 ± 2	45.1 ± 0.2

The nominal values are supplied by the manufacturer. Values for M and $\langle r^2 \rangle^{1/2}$ are the values for slices at the center of the peaks.

is B in Eq. 2, is -0.209 ± 0.002 ml^{-1}. The best-fit slope of log $\langle r^2 \rangle^{1/2}$ vs. V, or D in Eq. 3, is -0.128 ± 0.002 ml^{-1}.

If no broadening were present in the chromatograms of Fig. 1, then the observed peak width w_{obs} would be due only to differences in molecular mass across the peak, and $w_{obs} = w_0$. We could then use w_0 along with the calibration slope B to calculate $\bar{M}_w/\bar{M}_n$ via Eq. 6. The above values for w_0 and B give $\bar{M}_w/\bar{M}_n = 1.0122 \pm 0.0005$. According to Eq. 18 the presence of broadening makes the observed peak wider than with no broadening. Because the true width is always smaller than the observed width, this simple calculation therefore puts a rough upper bound on the sample's polydispersity.

Fig. 2 shows the rms radius as a function of elution volume across the peak of nominal 400 kg/mol polystyrene. The 90° LS signal is superimposed. At the edges of the peak where the signal-to-noise ratio is low, the rms radius is uncertain. But across the center FWHM of the peak, the rms radius exhibits little variation. Since the radius calculation is independent of the DRI signal, delay volume and interdetector broadening are not factors. This makes the molecular radius an excellent parameter to observe effects of column broadening. The observed flatness in Fig. 2 could have two causes: (i) the sample is extremely monodisperse, and the observed width of the peak is due entirely to column broadening; (ii) the sample has significant polydispersity but has been broadened so much by the columns (in accordance with Eq. 22) that the observed slope is near zero. To calculate the true polydispersity it is thus necessary to determine the amount of column broadening present.

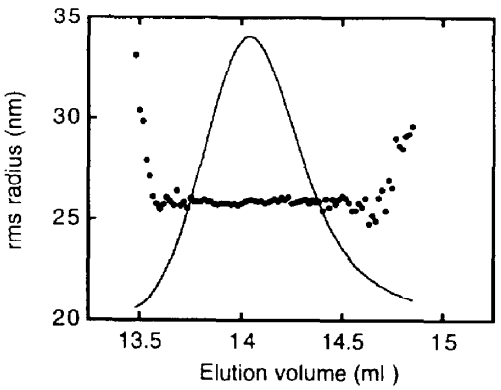

Fig. 2. Calculated rms radius as a function of elution volume for the sample of Fig. 1. The 90° LS signal is overlaid.

The unbroadened width w_0 for a narrow-distribution sample can be calculated from Eq. 22 if we determine both D and D_{obs} as well as w_{obs}. The value of D was shown above to be $-0.128 + 0.002$ ml^{-1}. As determined above, $w_{obs} = 0.54 \pm 0.01$ ml. The value of D_{obs} can be obtained by fitting the radius data from Fig. 2 and finding the slope. The typical uncertainty in the radius measurements for each slice is about 0.3 nm, obtained from statistically analyzing the fluctuation in the LS data for the slices within the FWHM of Fig. 2. These uncertainties, combined with the radius data within the FWHM, yield $D_{obs} = 0.004 \pm 0.005$ ml^{-1}. Analysis of a second injection of the same sample gives $D_{obs} = 0.0008 \pm 0.003$ ml^{-1}. In other words, D_{obs} is zero to within its uncertainty, a result consistent with visual inspection of the FWHM region of Fig. 2. To be conservative, let us take two standard deviations of the slope with the larger uncertainty, or 0.01 ml^{-1}, as an upper bound on the magnitude of D_{obs}. Mathematically, this means $|D_{obs}| < 0.01$ ml^{-1}. Using two standard deviations gives a 95% confidence interval in the results. We then find from Eq. 22 that $w_0 < 0.151 \pm 0.003$ ml. Note that the unbroadened width is smaller than w_{obs} by at least a factor of 3.6. Thus almost all the observed width of 0.54 ml is due to broadening. The limit on $|D_{obs}|$ is an upper bound, with 95% confidence, and so the value of w_0 is also an upper bound.

Finally, having determined we can apply Eq. 6 to calculate the polydispersity. The result is $\bar{M}_w/\bar{M}_n < 1.00096 \pm 0.00004$. This is a surprisingly small polydispersity. To gain an understanding of why the result is so small, note that Eq. 6 implies that $\bar{M}_w/\bar{M}_n - 1$ is proportional to w_0^2. Earlier in this section we saw that assuming $w_0 = w_{obs}$ implies a polydispersity of about 1.012. In fact w_0 is smaller than w_{obs} by at least a factor of 3.6 due to column broadening, and $\bar{M}_w/\bar{M}_n - 1$ is therefore smaller by the square of this factor, or

about 13. Thus the true polydispersity must be at least 13 times closer to unity than 1.012, or roughly 1.001. The theoretical lower limit for the polydispersity of the Poisson distribution is $1 + x^{-1}$, where x is the degree of polymerization [3]. For a 400 000 g/mol polymer with a repeat unit mass of about 100 g/mol, x is about 4000. The theoretical lower limit is then 1.00025, about four times closer to unity than measured. Thus the polydispersity measured here, even though it is small, is consistent with the polymerization mechanism.

The values for $\bar{M}_w/\bar{M}_n$ calculated from Eq. 6 depend on the assumption of a Gaussian peak shape, but not strongly. Deviations from Gaussian peaks, for example extra peak tailing, show up as a somewhat different coefficient of $B^2w_0^2$ in Eq. 6. A large change in this coefficient, even a factor of two, does not significantly alter the conclusions of this paper. The expression in Eq. 22 for the change in apparent slope D depends on the peak and the broadening being Gaussian. Again, the dependence is not a strong one; as long as the peaks and broadening are approximately Gaussian, the conclusions presented are valid.

In the case of Gaussian broadening, Hamielec and Ray [19] derived correction factors for the various molecular mass moments, assuming refractometer chromatograms are used. These expressions assume a linear calibration curve but do not assume a Gaussian peak shape. Their results show that the necessary polydispersity correction factor is $\exp[-(\ln 10)^2B^2w_G^2/8 \ln 2]$, or 0.9890 ± 0.0004. Applying this factor to the value 1.0122 obtained from the calibration curve and the DRI signal gives a corrected polydispersity of 1.0011 ± 0.0006, a value consistent with the results presented in this paper. This approach requires high precision in the knowledge of B and w_G in order to determine the polydispersity of a narrow standard. In contrast, the method presented in this paper requires placing an upper bound on the radius calibration slope from which small polydispersities may be calculated with relatively high precision.

To check whether the broadening was due to the columns or to the LS flow cell, observed peak widths were compared for two configurations: (i) the standard configuration used for the experiments described above, and (ii) the same configuration with an additional LS flow cell inserted in line between the columns and the LS instrument. The observed peak width w_{obs} increased by only about 5% in the second configuration, indicating that the great majority of the broadening occurred in the columns and not in the LS instrument.

5. Conclusions

A new technique was demonstrated using molecular radius values obtained by GPC–MALLS across a peak to calculate the polydispersity of narrow-distribution standards. The sensitivity of the technique can approach the theoretical limit of the Poisson distribution. The upper limit obtained for a narrow polystyrene in toluene is much closer to unity than previously measured but still consistent with the polymerization mechanism.

Appendix

In this appendix we derive Eqs. 20 and 22. We begin with the expression for $M_{obs}(V)$ in Eq. 19:

$$M_{obs}(V) = \frac{\int M(y)h(y)G(V-y)\,dy}{\int h(y)G(V-y)\,dy} \qquad \text{(A1)}$$

Substituting the expressions for $h(y)$, $M(y)$, and $G(V-y)$ from Eqs. 1, 2 and 16, respectively, we obtain

$$M_{obs}(V) = \frac{\int 10^{-(1+B)y} \exp[-4 \ln 2\,(y-V_0)^2/w_0^2] \exp[-4 \ln 2\,(V-y)^2/w_G^2]\,dy}{\int \exp[-4 \ln 2\,(y-V_0)^2/w_0^2] \exp[-4 \ln 2\,(V-y)^2/w_G^2]\,dy} \qquad \text{(A2)}$$

Noting that $10^x = \exp(x \ln 10)$ and combining the exponentials gives

$$M_{obs}(V) = \frac{\int \exp\{\ln 10(A + By) - 4 \ln 2[(y - V_0)^2/w_0^2 + (y - V)^2/w_G^2]\}\,dy}{\int \exp\{-4 \ln 2[(y - V_0)^2/w_0^2 + (y - V)^2/w_G^2]\}\,dy} \quad (A3)$$

The exponentials in the integrals are quadratic polynomials in y, so we can use the standard expression

$$\int_{-\infty}^{+\infty} \exp(-ay^2 + by + c)\,dy = \sqrt{\frac{\pi}{a}} \exp(b^2/4a + c) \quad (A4)$$

with proper choices of a, b and c for the numerator (num) and denominator (den). These quantities are:

$$a_{den} = a_{num} = a = 4 \ln 2 \cdot \left(\frac{1}{w_G^2} + \frac{1}{w_0^2}\right)$$

$$b_{den} = 8 \ln 2 \cdot \left(\frac{V}{w_G^2} + \frac{V_0}{w_0^2}\right)$$

$$b_{num} = b_{den} + B \ln 10$$

$$c_{den} = -4 \ln 2 \cdot \left(\frac{V^2}{w_G^2} + \frac{V_0^2}{w_0^2}\right)$$

$$c_{num} = c_{den} + A \ln 10 \quad (A5)$$

Our goal is to form the ratio

$$M_{obs}(V) = \frac{\sqrt{\frac{\pi}{a}} \cdot \exp(b_{num}^2/4a + c_{num})}{\sqrt{\frac{\pi}{a}} \cdot \exp(b_{den}^2/4a + c_{den})} = \exp\left(\frac{b_{num}^2 - b_{den}^2}{4a} + c_{num} - c_{den}\right) \quad (A6)$$

Substituting values from Eq. A5 and cancelling yields

$$M_{obs}(V) = \exp\left[\frac{(Vw_0^2 + V_0w_G^2 + Bw_0^2w_G^2 \ln 10/16 \ln 2)B \ln 10}{w_0^2 + w_G^2} - A \ln 10\right] \quad (A7)$$

which can be rewritten as

$$\log[M_{obs}(V)] = \frac{(Vw_0^2 + V_0w_G^2 + Bw_0^2w_G^2 \ln 10/16 \ln 2)B}{w_0^2 + w_G^2} + A \quad (A8)$$

This expression is clearly linear in V with a slope B_{obs} given by

$$B_{obs} = Bw_0^2/(w_0^2 + w_G^2) = Bw_0^2/w_{obs}^2 \quad (A9)$$

which is the required expression.

For the mean square radius $\langle r^2\rangle_{obs}(V)$, the derivation is analogous, and we obtain

$$\langle r^2\rangle_{obs}(V) = \exp\left(\frac{b_{num}^2 - b_{den}^2}{4a} + c_{num} - c_{den}\right) \quad (A10)$$

The quantities a, b_{den} and c_{den} are the same as in Eq. A5. The quantities b_{num} and c_{num} are given by

$$b_{num} = b_{den} + B \ln 10 + 2D \ln 10$$

$$c_{num} = c_{den} + A \ln 10 + 2D \ln 10 \quad (A11)$$

Substituting yields

$$\log[\langle r^2\rangle_{obs}(V)] = \frac{2[Vw_0^2 + V_0w_G^2 + (B + D)w_0^2w_G^2 \ln 10/8 \ln 2]D}{w_0^2 + w_G^2} + A + 2C \quad (A12)$$

which is linear in V with a slope given by $2Dw_0^2/(w_0^2 + w_G^2)$. Note that this is the slope for the mean square radius; the slope for the rms radius is one-half this value, or

$$D_{obs} = Dw_0^2/(w_0^2 + w_G^2) = Dw_0^2/w_{obs}^2 \quad (A13)$$

which is the required result.

References

[1] P.J. Flory, *J. Am. Chem. Soc.*, 62 (1940) 1561.
[2] P.J. Flory, *Principles of Polymer Chemistry*, Cornell Univ. Press, Ithaca, NY, 1953.

[3] M. Morton, *Anionic Polymerization: Principles and Practice*, Academic Press, London, 1983.
[4] W.W. Yau, J.J. Kirkland and D.D. Bly, *Modern Size-Exclusion Liquid Chromatography*, Wiley-Interscience, New York, 1979.
[5] N.C. Billingham, *Molar Mass Methods in Polymer Science*, Halsted Press, New York, 1977.
[6] W.W. Yau, H.J. Stoklosa and D.D. Bly, *J. Appl. Polym. Sci.*, 21 (1977) 1911.
[7] A.E. Hamielec, in J. Janca (Editor), *Steric Exclusion Liquid Chromatography of Polymers* (*Chromatographic Science Series*, Vol. 25), Marcel Dekker, New York, 1984, Ch. 3.
[8] G.R. Meira, in H.G. Barth and J.W. Mays (Editors), *Modern Methods of Polymer Characterization*, Wiley, New York, 1991, Ch. 2.
[9] P.J. Wyatt, *Anal. Chim. Acta*, 272 (1993) 1.
[10] J.C. Giddings, *J. Appl. Polym. Sci.*, 33 (1987) 117.
[11] P.J. Wyatt and L.A. Papazian, *LC·GC*, 11 (1993) 862.
[12] D.W. Shortt, *J. Liq. Chromatogr.*, 16 (1993) 3371.
[13] B.H. Zimm, *J. Chem. Phys.*, 16 (1948) 1093.
[14] H.C. van de Hulst, *Light Scattering by Small Particles*, Dover, New York, 1981.
[15] P. Debye, *J. Phys. Coll. Chem.*, 51 (1947) 18.
[16] L.H. Tung, *J. Appl. Polym. Sci.*, 10 (1966) 375.
[17] P. Kratochvíl, in M.B. Huglin, *Light Scattering from Polymer Solutions*, Academic Press, London, 1972, p. 344.
[18] P.J. Wyatt, *J. Chromatogr.*, 648 (1993) 27.
[19] A.E. Hamielec and W.H. Ray, *J. Appl. Polym. Sci.*, 13 (1969) 1319.