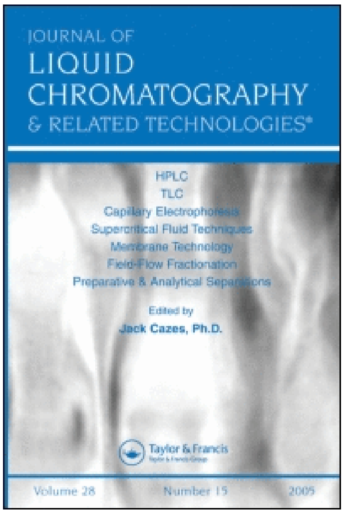

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POLYDISPERSITY STUDY OF NARROW POLYSTYRENE STANDARDS USING LIGHT SCATTERING

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ABSTRACT

Manufacturers of narrow polystyrene standards for use as column calibrants with size exclusion chromatography (SEC) often label the corresponding polydispersity (M_w/M_n) as < ~ 1.05. These values appear to reflect more the errors associated with the measurement than the true narrow polydispersity. Ideally, we would measure polydispersity by a light scattering measurement of M_w and a membrane osmometry measurement of M_w. The ratio of such measurements would be expected to have an error on the order of 5%– a number comparable to the difference of the measured polydispersity and unity! Multi-angle light scattering (MALS) allows the absolute determination of the rms radius independent of the separation technique and any other detector.

Using this information and following the method of Shortt,[1] column band broadening effects may be corrected and a far more precise determination of polydispersity made. A variety of standards from several manufacturers were measured, and the polydispersity of each was determined to be far smaller than stated, further confirming their suitability for column calibration purposes.

INTRODUCTION

Polystyrene standards have been used for column calibration in traditional size exclusion chromatography (SEC). A calibration curve is constructed by plotting the logarithm (base 10) of the peak molar mass (MM) of each selected standard against its corresponding elution volume. A curve is then fit to the data. This so-called "calibration" curve, is usually fit to a polynomial in elution volume, V, of order 3 to 5 and sometimes higher. The linear portion of such a curve for a typical column set is shown in Fig. 1.

Figure 2 shows the differential refractive index (DRI) detector chromatogram of a narrow 200K MM standard. Were we to compare each fraction against the calibration curve, we would seem to have a distribution of masses present no matter how narrow the standard actually was. Such broadening is due almost entirely to band broadening in the column. If we could correct adequately for this broadening, we should be able to calculate the actual sample polydispersity.

In a 1970 experiment which showed an improved resolution of a SEC column following a recycled injection, Waters[2] showed that on each pass of a sample through the column, in the limit of an infinite number of passes, the true MM variance could be calculated. For his sample, he obtained a polydispersity on the order of 1.002, considerably smaller than previously estimated. However, the recycle technique is time consuming and has been rarely used since then.

A great amount of theoretical speculation concerning means to correct for such broadening has filled the literature since Waters' experiments, but more recently, with the advent of multi-angle light scattering (MALS) combined with SEC,[3] a new experimental approach has been developed that, for many examples, may obviate the need to rely upon such sketchy theoretical approaches.

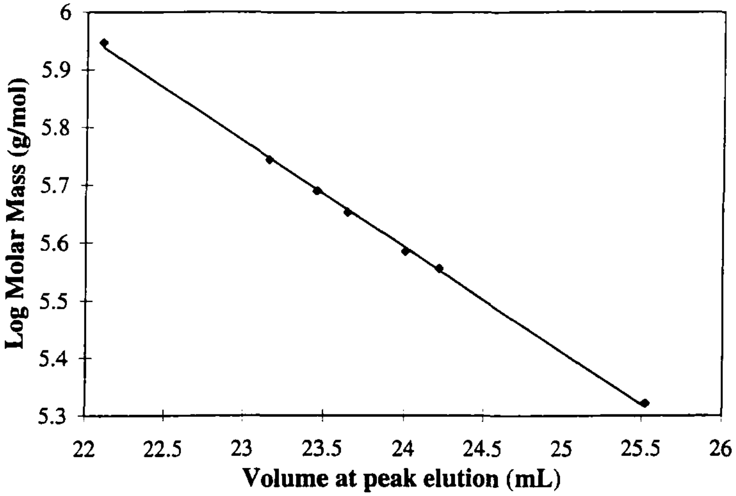

Figure 1. Typical SEC calibration curve in the vicinity of its linear behavior.

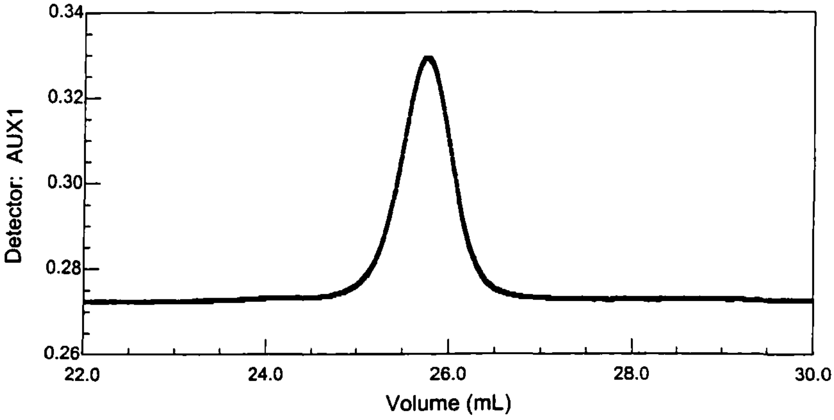

Figure 2. 200K narrow standard differential refractive index chromatogram.

Using MALS combined with SEC, it has become possible to measure MM directly without appeal to a calibration curve. In a recent paper by Wyatt[4] it was pointed out that in the limit of vanishingly small concentrations, the molecular root mean square (rms) radii, r_g can be determined directly from light scattering measurements for homopolymers independent of any concentration data. Appealing to the nearly flat variation of r_g versus elution volume, the paper stated that the standards must be much less polydisperse than currently estimated since the real MM must be directly proportional to some function of r_g and, therefore, should show a similar behavior with elution volume. The remaining question was, "Can this rms radius information be used to calculate more precisely the actual molar mass distribution?" A recent article by Shortt[1] showed how this could be achieved.

MEASUREMENT OF TRUE POLYDISPERSITY

The ideal determination of a sample's polydispersity would consist of measuring the weight average MM using light scattering and the number average MM using membrane osmometry. The polydispersity is defined as the ratio of these values, or

$$\text{Polydispersity} = \frac{M_w}{M_n}$$

Even under ideal conditions, the errors associated with M_w and M_n will be, at best, on the order of 2% and 4%, respectively. Accordingly, the error of the calculated polydispersities of a "narrow standard" based on such direct measurement will itself be comparable to the traditional manufacturer's stated deviation of the sample's polydispersity from unity, *viz.*, about 5%.

Shortt's paper, referenced above, described a new technique for determining M_w/M_n using a method based upon measurement of r_g from MALS coupled with SEC. This is the technique we follow in the present study to determine the polydispersity of various narrow polystyrene standards.

In his paper, Shortt assumed, at least for the central part of the band broadened peak, that the peak had a gaussian distribution. On this basis, he then showed that the polydispersity is given approximately by

$$\frac{M_w}{M_n} \cong 1 + 0.96 B^2 w_0^2 \qquad (1)$$

where B is the slope of the calibration curve in the linear region of the column set, *i.e.* where

$$\log_{10} M = A + BV \qquad (2)$$

Table 1

Reported Polydispersities of Various Standards

Manufacturer	Nominal Molar Mass (g/mol)	Reported Polydispersity
Pressure Chemical	2000,000	≤1.05
Pressure Chemcial	400,000	≤1.06
Pressure Chemical	575,000	≤1.06
Pressure Chemical	900,000	≤1.06
Polymer Labs	350,000	≤1.07
TSK	422,000	≤1.04
PSS	455,000	≤1.04

A is a constant, and w_0 is the full width at half maximum (FWHM) of the unbroadened peak. As it is impossible to measure this value directly, we examine the r_g calibration curve which, as mentioned earlier, may be established independent of a secondary detector. For r_g we have a relation similar to Eq. (2) involving the constants C and D,

$$\log_{10}r_g = C + DV \tag{3}$$

The slope D is related to the observed slope of the specific sample, D_{obs}, by the relation

$$D_{obs} = Dw_0^2/w_{obs}^2 \tag{4}$$

where w_{obs} is the measured broadened FWHM, w_0 is the unbroadened FWHM as before, D is the measured slope of the r_g calibration curve using the peak radius value for each standard. The observed slope of the r_g values across the peak for a given sample is D_{obs}. Equation (4) can then be solved to obtain a value for w_0, which can be substituted into Eq. (1) to obtain the true value of the polydispersity.

EXPERIMENTAL

Data were collected for various standards (the standards used as well as the values of their polydispersities as reported by the manufacturers are presented in Table 1) from a SEC/MALS system. The SEC system consisted of

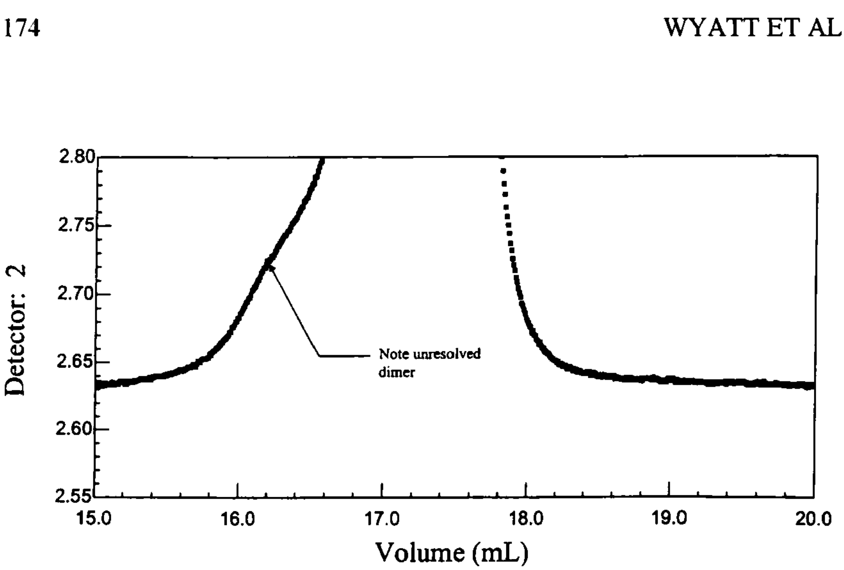

Figure 3. 200K polystyrene standard as separated by two columns (of 10^4Å and 10^6Å pore size). These columns were unable to resolve the dimer from the main peak.

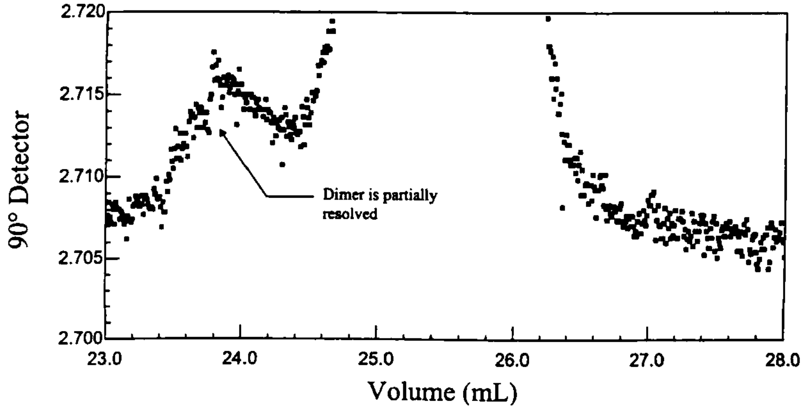

Figure 4. 200 K Polystyrene standard as separated by 3 columns (one additional 10^6Å pore size column was added on this run). The dimer is seen partially resolved from the main peak.

two Styragel columns (each 300 mm x 7.8mm) of 10^4 Å and 10^6 Å pore size, respectively. After each sample was separated, it passed through a Wyatt Technology mini-DAWN laser light scattering detector followed by Waters Model 410 differential refractometer. Mobile phase was toluene, 1 mL/min.

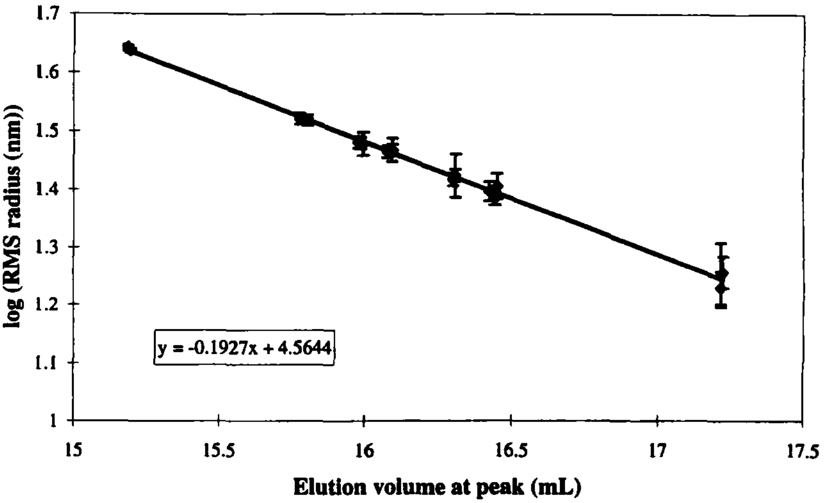

Figure 5. RMS radius calibration curve.

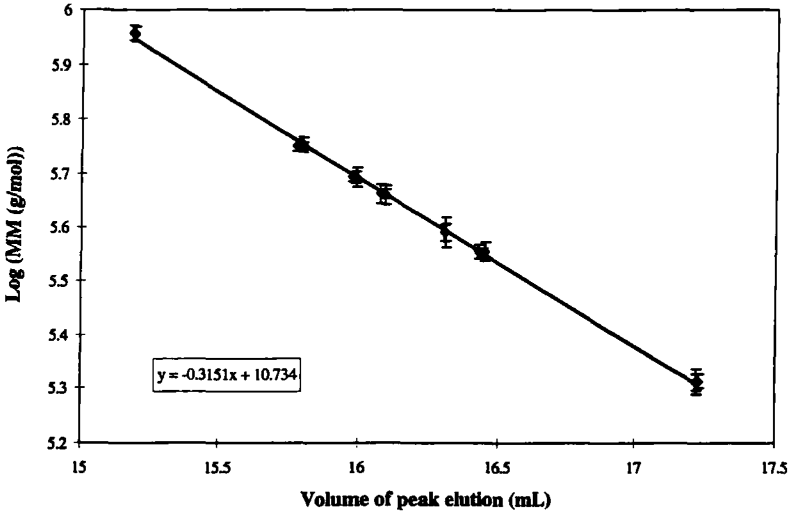

Figure 6. Molar Mass calibration curve. The slope value of the linear fit yields B.

The first two runs of standards were done with the two above mentioned columns, however these did not have the resolving power of those used by Shortt in his study. This configuration was unable to resolve the dimer (see Fig. 3) in some of the samples which led to larger values of D_{obs} and, thence, larger corresponding polydispersities. On the final run another 10^6 Å column was added, and the dimer can be seen partially resolved in Fig. 4 which allows for higher precision in the polydispersity calculation. Shortt was able to obtain baseline resolution of this aggregate component.

PROCEDURE FOR OBTAINING THE POLYDISPERSITIES

Data were collected and analyzed using Wyatt Technology's ASTRA® chromatography software to obtain the values of MM and r_g near the peaks for all of the standards used. The logarithm of these values were plotted against the elution volume for each peak value. A linear fit was applied to each of these curves to obtain calibration constants D and B. The calibration curves used for the second set of measurements (see RESULTS) are shown in Fig. 5 and Fig. 6.

Once values for B and D are obtained from the above plots, each data file must be analyzed individually to obtain unique values of w_{obs} and D_{obs} for each standard. This was done by measuring the 90° light scattering signal, as shown in Fig. 7, from which w_{obs} may be calculated directly. The FWHM is calculated by finding the maximum signal value from the LS chromatograph. This value is then halved and elution volume points with corresponding signal values are found to yield w_{obs}.

The weighted linear fit of the r_g values across the peak yields D_{obs} (Fig.8). Once values are obtained for D_{obs}, D and w_{obs}, one can solve Eq. (4) to find a value for w_0. Using the value of B previously obtained, Eq. (1) may now be evaluated and the polydispersity derived.

RESULTS

The samples measured all yielded values of polydispersity far lower than reported by their manufacturers. The resulting values were averaged over the three runs and are reported in Fig. 9 as a plot of $M_w/M_n -1$ against the manufacturer's reported nominal MM. The values for the individual runs are detailed in Table 2.

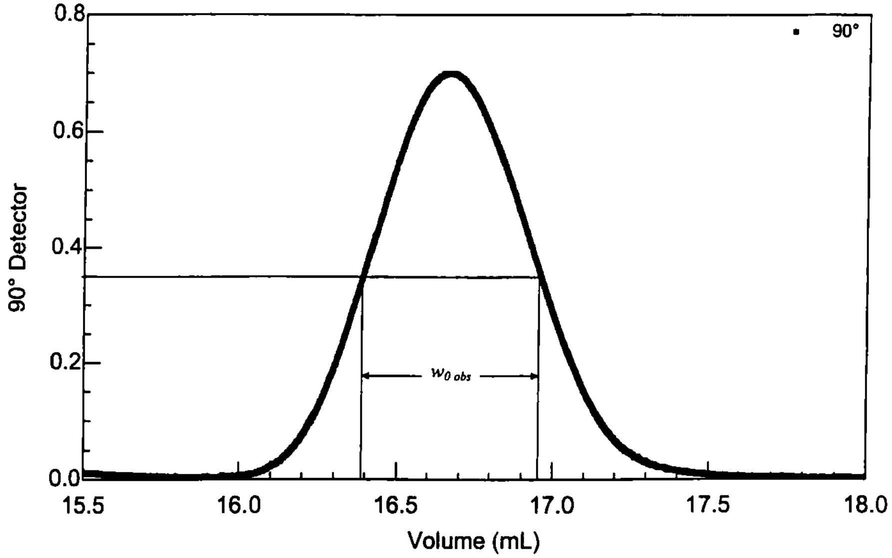

Figure 7. FWHM determination.

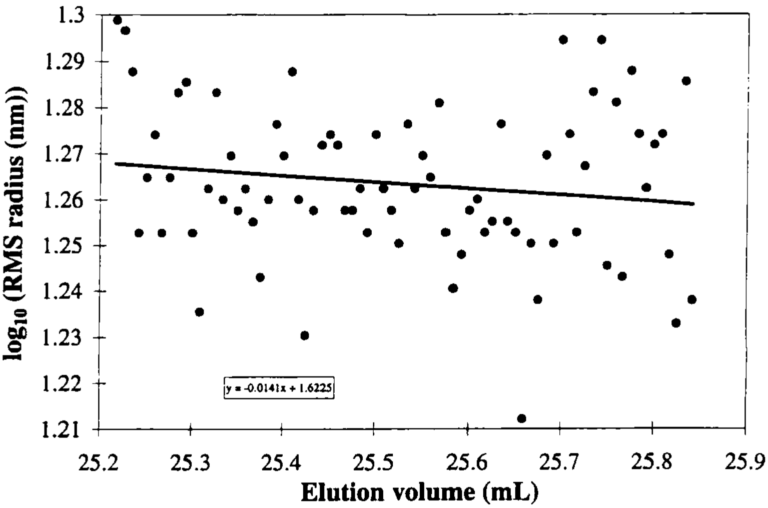

Figure 8. Curve for the determination of D_{obs}, the slope of the weighted linear fit.

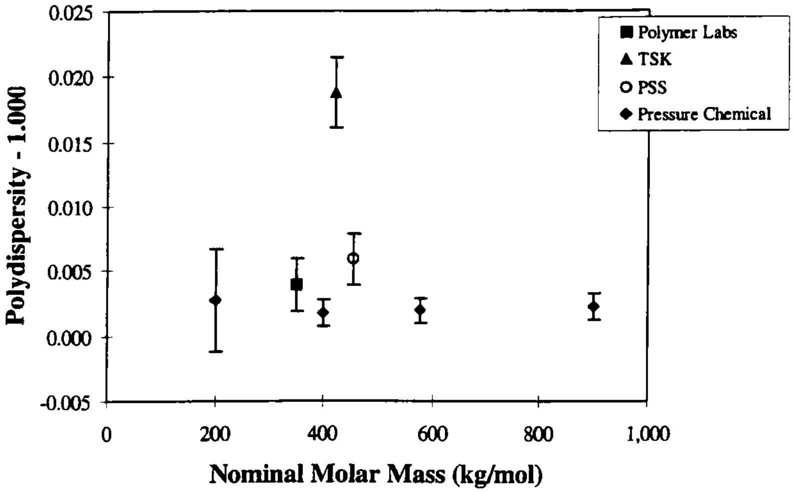

Figure 9. Polydispersity values with associated errors for various calibration standards.

Table 2

Polydispersities of Several Polystyrene Standards

Manufacturer	Nominal MM (kg/mol)	Run #1 Polydispersity	Run #2 Polydispersity	Run #3 Polydispersity
Pressure Chemical	200	1.003 ± 0.003	1.005 ± 0.005	1.001 ± 0.004
Pressure Chemical	400	1.003 ± 0.001	1.002 ± 0.002	1.000 ± 0.001
Pressure Chemical	575	1.003 ± 0.001	1.001 ± 0.001	1.002 ± 0.001
Pressure Chemical	900	1.003 ± 0.001	1.002 ± 0.001	1.001 ± 0.001
Polymer Labs	350	1.005 ± 0.002	1.004 ± 0.002	1.002 ± 0.002
TSK	422	1.025 ± 0.005	1.018 ± 0.002	1.014 ± 0.001
PSS	455	1.010 ± 0.002	1.006 ± 0.002	1.002 ± 0.002

CONCLUSIONS/DISCUSSION

Multiangle light scattering (MALS) is a powerful tool for the characterization of polymer mass and root mean square radius, r_g. For polymers of sufficient size ($r_g \sim 10$ nm or larger), r_g values can be determined in the limit of vanishingly small concentration independently of any concentration detector. (This is true for all homopolymers and homogeneous co-polymers.).

An important assumption of the method, at least in terms of simplifying the calculations, is that the samples are gaussian in their distributions. Most polystyrene standards include a contaminant, usually a dimer, associated with the termination process following polymerization. A good column set, such as selected by Shortt, will separate this aggregate from the main polymer peak leaving the latter indistinguishable from a gaussian and, thereby, assuming that treatment of the peak as a gaussian is valid.

It may be argued, however, that since the standard as provided by the manufacturer contains this termination contaminant, the stated polydispersity should include all of this fraction. We do not agree with this concept especially since the contaminant is often separated from the nominal peak during SEC. The polydispersity on the manufacturer's label invariably describes the product to be used for calibration.

Perhaps manufacturers might add a comment to the effect that a small residual dimer or aggregate is also present, but may be ignored. These dimer fractions are often found at fractions of less than 10^{-6} of the total, so their importance, especially for column calibration purposes, is negligible. Absent these contaminants, the appearance of the eluting standards peaks within the linear range of the column set is virtually indistinguishable from a gaussian.

Most of the analyzed standards were found to be very narrow and thus well suited for calibration purposes. Generally, polydispersities were found with values much lower than 1.01 over the full width at half maximum, which is the most important for calibration.

ACKNOWLEDGMENT

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