

Size-Exclusion Chromatography with Two-Angle Laser Light-Scattering (SEC-TALLS) of High Molecular Weight and Branched Polymers

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SYNOPSIS

The accuracy and precision of results obtained from light-scattering detection at two angles (TALLS) for size-exclusion chromatography (SEC) are examined for linear narrow molecular weight distribution polystyrenes between 1,290,000 and 20,000,000 MW and for branched polyesters. The ratio of light-scattering intensities at 15° and 90° is used to calculate weight-average molecular weight, \bar{M}_w , and an average root-mean-square radius, \bar{r}_{gu} , equivalent to the *z*-average radius. A shape for the polymer molecule is assumed and an analytical relationship for the particle-scattering function is required. It is shown that analysis of the data using the particle-scattering function for a random coil is valid for both high molecular weight, linear polystyrenes and long-chain branched polyesters. The radius, \bar{r}_{gu} , is determined with high precision by using the ratio of light-scattering signals, which is insensitive to errors in sample concentration and changes in the eluent flow rate. The correct average radius for the whole polymer is obtained despite using low-efficiency, large-particle diameter SEC columns; however, axial dispersion significantly affects molecular weights and radii calculated at each retention volume that can limit the utility of plots used to deduce polymer conformation. © 1995 John Wiley & Sons, Inc.

INTRODUCTION

Two (or three)-angle laser light-scattering (TALLS) has recently been distinguished as a subset of the family of elastic light-scattering methods for size-exclusion chromatography (SEC) that includes low-angle (LALLS), right-angle (RALLS), and multiangle MALLS laser light-scattering. These SEC detectors can all be used for the measurement of molecular weight distributions. TALLS is distinguished from the other detectors by a different method of data analysis at large polymer sizes that requires an assumption of the shape of the molecule.¹⁻³ Using the particle-scattering function for a random coil, accurate molecular weight averages and root-mean-square radii were determined by TALLS for linear polystyrenes with molecular weights up to

2,280,000 and root-mean-square radii up to 72 nm in a previous study.² Although encouraging, the general applicability of this data analysis method to polymers of larger sizes has not been demonstrated. Also, application to polymers that are not linear has not been verified experimentally. A review of some basic ideas for SEC light-scattering detectors followed by a more detailed examination of the TALLS data analysis method reveals why these are points of concern.

THEORY

Light-scattering detectors measure excess Rayleigh scattering $R_{\theta i}$ at angle θ for each retention volume v_i of an SEC chromatogram. This scattering intensity is related to the concentration at each retention volume, c_i , which is measured by a separate concentration-sensitive (e.g., differential refractive index or DRI) detector:

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$$\frac{Kc_i}{R_{\theta_i}} = \frac{1}{M_{wi}P(\theta)_i} + 2A_{2i}c_i + \dots \quad (1)$$

The particle-scattering function $P(\theta)_i$ defines the angular variation of scattered light. M_{wi} is the molecular weight at retention volume i and is a weight-average if the slice contains molecules of more than one molecular weight. A_{2i} is the second virial coefficient at retention volume i and K is an optical constant that contains the refractive index of the solvent, wavelength, and the specific refractive index increment, dn/dc , of the sample.

We wish to calculate M_{wi} from the light-scattering detector and concentration detector signals. If possible, it is also desirable to calculate $P(\theta)_i$, from which the average spatial extension (typically the root-mean square radius or "radius of gyration") of the molecule may be calculated. Calculation of the molecular weight requires extrapolation of c_i/R_{θ_i} values to infinite dilution, although at the low polymer concentrations employed in SEC the extrapolation is often unnecessary and the concentration term on the right-hand side of eq. (1) is assumed negligible. For this case,

$$M_{wi} \approx \frac{R_{\theta_i}}{Kc_iP(\theta)_i} \quad (2)$$

The data can then be treated in different ways depending on the angle(s) of observation and the size of the molecules. If the molecules are small compared to the wavelength of incident light, $P(\theta) \approx 1$ for any angle of observation. These molecules are isotropic scatterers and, with laser light sources greater than 600 nm, correspond to linear polymers that are less than $\sim 50,000$ MW. $P(\theta)$ is also close to unity at low angles of observation. This simplifies the calculation of molecular weights by LALLS in which scattering intensities are measured at observation angles of 7° or less. Accurate molecular weights can be measured for polymers with molecular weights of several million; however, no information is obtained on the size of the molecule from light-scattering intensity at a single low angle. MALLS instruments that collect light scattered by large polymer molecules at multiple, higher angles of observation measure scattering intensities for which $P(\theta) \neq 1$. The scattering data at zero concentration are extrapolated to zero angle to obtain M_{wi} and the size of the molecule. The method is unique because the angular variation of scattering intensities provides a measure of the root-mean-square radius *independent* of polymer shape (e.g., random coil, rod, sphere), provided

that the data can be accurately extrapolated to zero angle. A variety of graphical methods are available for calculating M_{wi} and r_{gi} , with the most familiar being reciprocal scattering plots related to the method developed by Zimm.⁴

The classical graphical methods of extrapolating multiangle light-scattering data to zero angle cannot be applied with the same level of confidence to data obtained at only two or three angles. The uncertainty arises not only from the fact that fewer data points are available for the extrapolation, but also from the nonlinearity observed in many light-scattering plots because of the significance of higher-order terms in the particle-scattering function, particularly when the polymer molecules are large. An alternative is to measure the *ratio* of scattering intensities at angles θ_1 and θ_2 , which is directly proportional to the ratio of particle-scattering functions at the same two angles:

$$Z_i = \frac{P(\theta_1)_i}{P(\theta_2)_i} = \frac{R_{\theta_1 i}}{R_{\theta_2 i}} \quad (3)$$

Values of r_{gi} and $P(\theta)_i$ as a function of Z_i can be obtained by iterative solution of the ratio of particle-scattering functions at the two angles. However, a shape must be assumed and an analytical relationship for $P(\theta)_i$ must be available. The ratio of light-scattering intensities symmetric about 90° is commonly known as a dissymmetry measurement. Most frequently, the angles chosen are 45° and 135° , and the method has been used for obtaining sizes of particles for several years. However, the two angles need not be symmetric about 90° for eq. (3) to apply.

Analytical relationships for particle-scattering functions are available for several common shapes. The Debye function for random coils is commonly used for polymer molecules:⁵

$$P(\theta) = \frac{2}{x^2} [e^{-x} + x - 1] \quad (4)$$

where

$$x = \frac{16\pi^2 n^2}{\lambda_0^2} r_g^2 \sin^2 \frac{\theta}{2} \quad (5)$$

where n is the solvent refractive index, and λ_0 , the wavelength of light in a vacuum. The solution for spheres given by Rayleigh and Gans^{6,7} might be more suitable for globular proteins or highly branched, unswollen polymers such as dendrimers.

A method of data analysis for TALLS described previously² proceeds as follows:

1. Measure the ratio, Z_i , of the excess Rayleigh scattering at each retention volume from the light-scattering responses at two different angles.
2. Substitute the appropriate particle-scattering function into eq. (3). For example, the expression for random coils would be

$$Z_i = \frac{\frac{2}{x_{\theta_1}^2} [\exp(-x_{\theta_1}) + x_{\theta_1} - 1]}{\frac{2}{x_{\theta_2}^2} [\exp(-x_{\theta_2}) + x_{\theta_2} - 1]} \quad (6)$$

3. Find the value of r_g that satisfies eq. (6), where x is given by eq. (5). This can be accomplished by iteration (2) or by using the series expressions given by Frank et al.³ This value is r_{gi} , the root-mean-square radius at retention volume i .
4. Calculate $P(\theta)_i$ from r_{gi} , using eq. (4). The particle-scattering function can be calculated at both 15° and 90°, although only the 15° data are used in step 5.
5. Calculate M_{wi} using eq. (1) [or eq. (2)] and the excess Rayleigh-scattering and particle-scattering function at 15°.

Average properties of the whole polymer may then be calculated from values of r_{gi} and M_{wi} at each retention volume and the concentration at each retention volume, c_i , obtained from the DRI. However, the weight-average molecular weight of the whole polymer, \bar{M}_w , and the corresponding z -average root-mean radius, \bar{r}_{gz} , are unique because they can be calculated from the light-scattering detector alone (no DRI). Methods that require only the light-scattering detector are significant because problems associated with the time lag between detectors (interdetector volume) are avoided. These quantities are also unaffected by imperfect resolution, which can be particularly important with columns designed for high molecular weight polymers; many of these columns use large particles for the column packing to minimize shear degradation of samples and to extend the upper molecular weight range that can be fractionated, but have low chromatographic efficiency.

From the light-scattering detector alone, \bar{M}_w is calculated from the sample mass injected, m , and the volume increment between data points, Δv_i :

$$\bar{M}_w = \frac{1}{Km} \sum \frac{R_{\theta_i}}{P(\theta)_i} \Delta v_i \quad (7)$$

A previous article on this method² also demonstrated that the ratio of the light-scattering chromatogram areas,

$$\bar{Z} = \frac{\sum R_{\theta_i} \Delta v_i}{\sum R_{\theta_i} \Delta v_i} \quad (8)$$

calculates a value \bar{r}_{gu} for the whole polymer using eq. (6) that is equivalent to the z -average radius. This method of calculation also uses only the light-scattering detector and provides the most precise estimate of \bar{r}_{gz} by TALLS.

Choosing a particle shape is central to the TALLS data analysis method. The preliminary study^{1,2} suggested that this requirement is less restrictive than it might seem for linear polystyrene; the monodisperse, random coil model worked well for \bar{r}_{gu} up to 72 nm (2,300,000 MW). For $\bar{r}_{gu} < 50$ nm (< 1,000,000 MW), the radii and molecular weights calculated for a sphere and a random coil were indistinguishable. However, a polymer molecule of even larger size can be fractionated by SEC. For example, branched polymers often contain large molecules that influence physical and melt rheological properties. At large sizes, the particle-scattering functions for shapes diverge and the choice of model becomes important.

This study addresses these issues by examining polystyrene samples with root-mean-square radii up to 200 nm and some branched polyesters of known molecular weight and size. In addition, it touches on the effects of axial dispersion and the significance of interdetector volume (the volume between the light-scattering and concentration detector).

EXPERIMENTAL

A PD2000W light-scattering detector (Precision Detectors, Amherst, MA) installed in a Waters 410 DRI immediately before the differential refractive index cell was used to simultaneously measure scattering intensities at 15° and 90°. The light source is a plane-polarized solid-state laser emitting at 670 nm. Three 7.5 mm i.d. × 300 mm PLgel 15 μm mixed-A columns were thermostated to 30.0°C. Uninhibited tetrahydrofuran (THF) at a nominal flow-rate of 0.5 mL/min was the eluent. Narrow distribution polystyrene standards obtained from Polymer Laboratories (Amherst, MA) and American Polymer Standards (Mentor, OH) were injected at

a volume of 100 μL with concentrations ranging from 0.02 to 0.1 mg/mL, depending on molecular weight. Branched polyester samples were synthesized as described previously.⁸ Sample concentrations of branched polyesters were 1.5 mg/mL. Both polystyrene and polyester sample solutions were prepared by dissolving the samples without agitation for 24 h. Flow rates were corrected using the retention volume of acetone as a flow marker.

The specific refractive index increments of polystyrene (PS) and polyester samples at 670 nm are 0.180 and 0.110 mL/g, respectively. Calibration factors to convert light-scattering detector voltage to $R(\theta)$ at 15° and 90° were calculated using eq. (7) for $P(\theta) = 1.0$ from the response of multiple injections of PS 26,700, PS 19,400, and PS 22,000.

RESULTS AND DISCUSSION

Accuracy and Precision

Excess Rayleigh-scattering chromatograms at 15° and 90° for PS 8,420,000 are shown in Figure 1. The ratio of the curves is most reliable in the middle of the chromatograms (~ 17 –18 mL) where light-scattering signals at both angles are strong and substantially different from each other. The tails of the distribution are less reliable because the ratio of two small, similar numbers is taken.

The simplest quantities to measure are “whole polymer” averages calculated from the light-scattering detector alone. The weight-average molecular weight \bar{M}_w is calculated from eq. (7) from the Rayleigh scattering at 15°. Results given in Table I are from assuming that $P(\theta) = 1$ (column 3) and by using

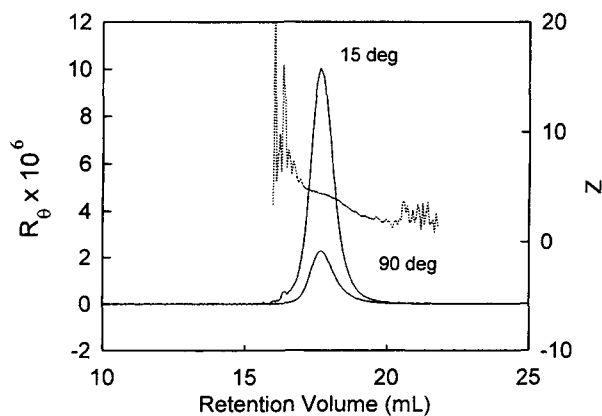


Figure 1 Light-scattering chromatograms at 15° and 90° of polystyrene 8,420,000 converted to excess Rayleigh scattering. Dotted line is the ratio of 15° and 90° scattering.

the particle-scattering function for a random coil at each retention volume (TALLS calculation, column 2). Similar to previous results,^{1,2} the assumption that $P(\theta) = 1$ increasingly underestimates \bar{M}_w with increasing molecular weight. Above $r_g \sim 30$ nm ($\sim 500,000$ MW polystyrene in THF), the difference between 0° and 15° becomes significant. Molecular weights calculated by the TALLS data analysis method are in reasonable agreement with vendor values except for PS 20,000,000 and 13,900,000. This difference is attributed to shear degradation of these samples during the SEC analysis. Light-scattering intensity increases and peak shapes change as the flow rate is decreased for these samples, and the results suggest a practical limit for these columns of PS MW $\sim 8,000,000$.

The reproducibility of \bar{M}_w values calculated using eq. (7) is comparable to that reported for SEC-LALLS.⁹ The precision of \bar{r}_{gu} values is considerably better, excluding the two highest samples that shear degrade during the experiment. Calculation of \bar{M}_w requires the injected sample mass, m , and the volume increment between adjacent data points, Δv_i , whereas the calculation of \bar{r}_{gu} does not. Sample mass is calculated from the sample concentration and injection volume, and Δv_i is determined from the flow rate. Thus, errors in sample preparation and fluctuations in the eluent flow have a direct influence on the reproducibility of \bar{M}_w values. In contrast, the ratio of the light-scattering chromatograms is constant regardless of the mass injected or the flow rate. Provided that there is reasonable signal-to-noise, a highly reproducible measurement of peak areas and peak heights is obtained, which, in turn, results in highly reproducible measurement of \bar{r}_{gu} .

Values of \bar{r}_{gu} calculated by TALLS using the particle-scattering function for a random coil are consistent with previously published values for z -average root-mean-square radii of polystyrene in tetrahydrofuran^{10,11} (Fig. 2). The relationship between molecular weight and root-mean square radius for polystyrene data presented in Table I is

$$\bar{r}_{gu} = (0.0123 \pm 0.0002)\bar{M}_w^{0.59 \pm 0.01} \quad (9)$$

The z -average root-mean square radii from this study and the previous results for polystyrenes below 2,000,000 MW (Refs. 1 and 2) scale with molecular weight according to

$$\bar{r}_{gu} = (0.0156 \pm 0.0008)\bar{M}_w^{0.58 \pm 0.01} \quad (10)$$

The results indicate that the TALLS data analysis method using the particle-scattering function for a monodisperse, random coil is applicable to the large-

Table I Narrow Distribution Polystyrene Whole Polymer Averages

Vendor \bar{M}_w	TALLS \bar{M}_w	\bar{M}_w if $P\theta = 1$	Z	\bar{r}_{gz} (nm) ^a
20,000,000	12,900,000 ($\pm 11.19\%$) ^b	10,500,000 ($\pm 5.68\%$)	6.278 ($\pm 7.85\%$)	197.0 ($\pm 5.09\%$)
13,900,000	10,700,000 ($\pm 7.77\%$)	9,380,000 ($\pm 7.63\%$)	5.257 ($\pm 0.10\%$)	175.6 ($\pm 1.21\%$)
8,420,000	8,320,000 ($\pm 4.31\%$)	7,610,000 ($\pm 4.64\%$)	4.259 ($\pm 0.67\%$)	153.4 ($\pm 0.42\%$)
6,770,000	6,860,000 ($\pm 4.58\%$)	6,310,000 ($\pm 4.93\%$)	3.672 (± 0.65)	139.5 ($\pm 0.42\%$)
5,480,000	5,960,000 ($\pm 0.86\%$)	5,580,000 ($\pm 0.78\%$)	3.262 ($\pm 0.90\%$)	129.0 ($\pm 0.61\%$)
4,480,000	5,100,000 ($\pm 3.84\%$)	4,870,000 ($\pm 3.73\%$)	2.705 ($\pm 0.70\%$)	112.8 ($\pm 0.53\%$)
3,864,000	4,120,000 ($\pm 1.46\%$)	4,020,000 ($\pm 3.32\%$)	2.407 ($\pm 0.67\%$)	103.0 ($\pm 0.54\%$)
2,370,000	2,320,000 ($\pm 3.47\%$)	2,280,000 ($\pm 3.14\%$)	1.634 ($\pm 0.27\%$)	71.8 ($\pm 0.31\%$)
1,296,000	1,290,000 ($\pm 5.88\%$)	1,280,000 ($\pm 5.66\%$)	1.308 ($\pm 1.42\%$)	50.5 ($\pm 2.96\%$)

^a z-average radius obtained from ratio of light-scattering peak areas.

^b Sample standard deviation/mean $\times 100$ for five trials.

est linear polystyrenes that can be analyzed by our SEC system without shear degradation.

Branched Polyesters

There is little difference in the value of r_g calculated for a sphere and random coil at values of $Z = R_{15}/R_{90}$ less than 1.5 (Fig. 3). It is possible for the high

molecular weight end of a branched polymer distribution to have Z values of 6–9, making the choice of an appropriate particle shape more important. Lightly branched, highly swollen polymers are expected to be more like random coils. Highly branched, compact, or collapsed structures might be better described by a sphere. The first case is tested by examining two long-chain branched polyesters characterized previously by multiangle elastic light-scattering.

The root-mean-square radius and particle-scattering function at each retention volume are calculated from the ratio of light-scattering signals at 15° and 90°. Then, the molecular weight at each retention volume is calculated using eq. (2) from the 15° light-scattering signal, the particle-scattering function, and the concentration, c_i , obtained from the DRI. The predicted scaling relationship between r_{gi} and M_i for a branched polymer in a good solvent is¹²

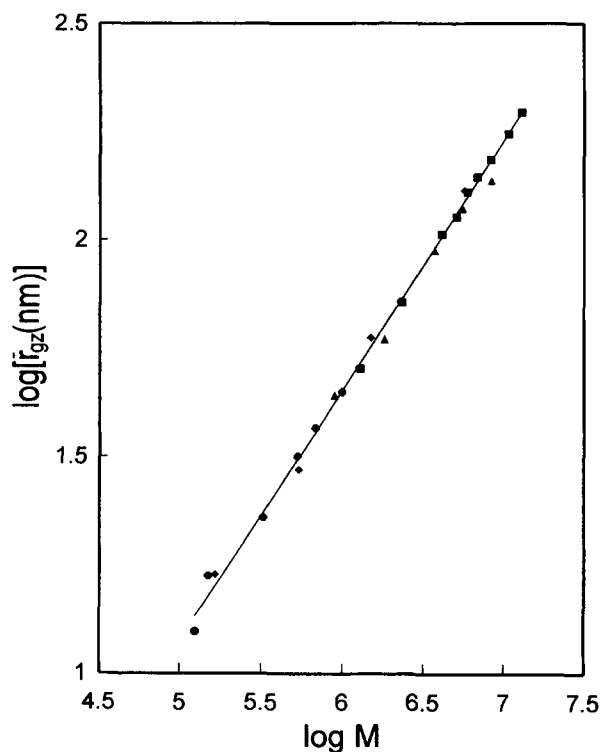


Figure 2 Polystyrene z-average root-mean-square radii in tetrahydrofuran: (■) TALLS data, this study; (●) TALLS data from Ref. 2; (◆) light-scattering data from Ref. 10; (▲) light-scattering data from Ref. 11.

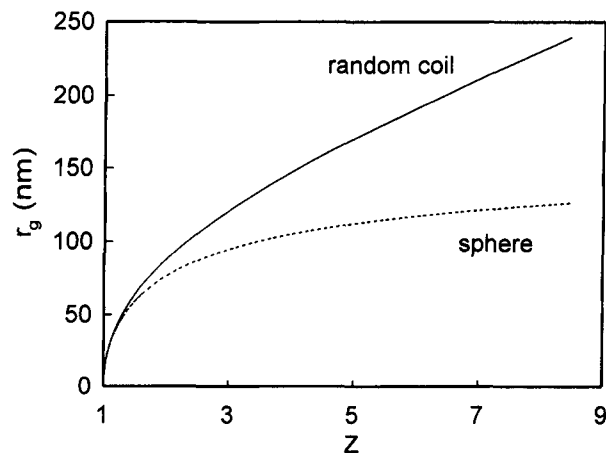


Figure 3 Root-mean-square radius as a function of Z for a random coil and sphere.

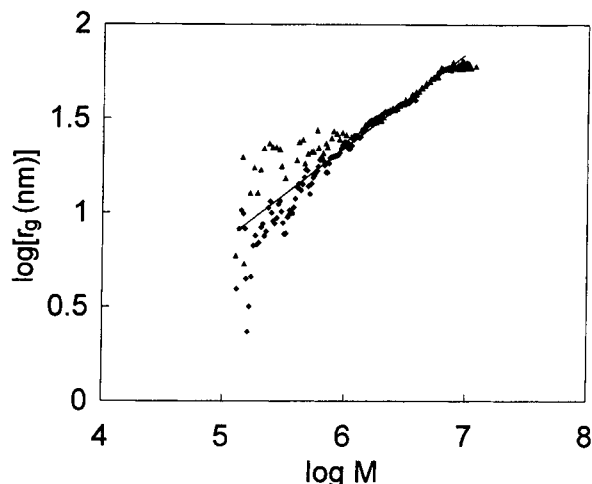


Figure 4 Branched polyesters: (◆) sample 125; (▲) sample 91F. Solid line is slope of 0.5.

$$r_{gi} \sim M_i^{0.5} \quad (11)$$

An exponent for these polyesters of 0.49 was reported in Ref. 8. Data measured by TALLS are consistent with the prediction of an exponent of 0.5, although it is apparent from Figure 4 that reliable data are limited to the molecular weight range between 1×10^6 and 7×10^6 . Also, it was shown for broad polystyrene standards that the reproducibility of the slope of these conformation plots obtained by TALLS is about $\pm 20\%$, making it difficult to distinguish between linear and branched conformations.² However, the whole polymer averages obtained by TALLS, assuming a random coil shape, agree with the previously published values⁸ for these samples (Table II). Although limited, these results are encouraging for the application of the random-coil particle-scattering function for long-chain branched polymers in a good solvent.

Axial Dispersion

None of the data presented have been corrected for imperfect resolution. \bar{M}_w calculated from the light-

scattering signals alone using eq. (7) is unaffected by axial dispersion—it measures the integrated light-scattering intensity for the entire sample and does not require separation of the molecules by size. Results from this study indicate that values of \bar{r}_{gu} calculated from the light-scattering signals alone are also unaffected by axial dispersion. The most convincing evidence for this is the agreement of \bar{r}_{gu} values from TALLS with literature values of \bar{r}_{gz} (Fig. 3) and a scaling relationship that is consistent with theoretical predictions [eqs. (9) and (10)].

This is not to say that imperfect resolution is unimportant. The columns used in this study are packed with large particles designed for high molecular weight polymers, but are inefficient compared to most modern SEC columns. With perfect resolution, the molecular weight measured by light-scattering at each retention volume of a chromatogram should superimpose on a calibration curve made from narrow standards of the same chemical composition. In one method for optimizing multi-detector SEC systems, this is purposely obtained by searching for the value of the interdetector volume that provides the best superposition.^{13,14} The interdetector volume obtained by this method is an effective value which also partially corrects for imperfect resolution. The true interdetector volume can be measured by several other methods.¹⁵ In this study, we used peak onsets of the light-scattering and concentration detectors. The molecular weights of a broad polystyrene standard measured by light-scattering at each retention volume using this interdetector volume clearly do not superimpose on the corresponding narrow standard calibration curve (Fig. 5). This is caused by axial dispersion, and it creates molecular weight distributions that are too narrow. It should also be noted that the choice of interdetector volume also rotates plots as in Figure 5 and can be misinterpreted as evidence for axial dispersion. A similar plot can be made for the root-mean-square radius (Fig. 6). The narrow standard calibration data in Figure 6 are obtained by converting molecular weights of the standards to root-

Table II Branched Polyester Whole Polymer Averages

Sample	TALLS \bar{M}_w	\bar{r}_{gu} (nm) ^a	\bar{M}_w (lit. ^b)	\bar{r}_{gz} (nm) (lit. ^b)
91F	345,000 ($\pm 4.8\%$) ^c	47.1 ($\pm 1.6\%$)	380,000	49
125	116,000 ($\pm 6.5\%$)	26.7 ($\pm 1.1\%$)	114,000	27

^a z-average radius obtained from ratio of light-scattering peak areas.

^b Ref. 8.

^c Sample SD/mean $\times 100$.

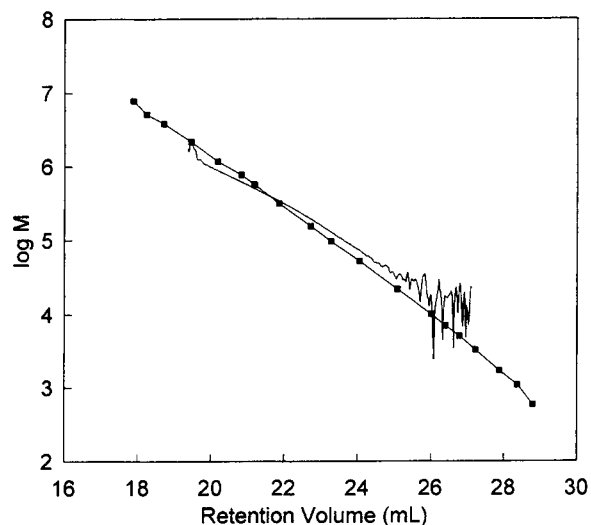


Figure 5 NBS 706 polystyrene molecular weights at each retention volume measured by (solid line) TALLS and (■) polystyrene narrow standard log M calibration curve.

mean-square radii using the scaling relationship in eq. (10). Root-mean-square radii measured at each retention volume for broad polystyrene NBS 706 do not require an interdetector volume because r_g is obtained from the ratio of the two light-scattering signals measured in the same detector cell. The effects of axial dispersion are less obvious because sizes are measured over a narrow retention volume range. The results are less reliable at small sizes; however, for data greater than $\log r_g = 1.18$ in Figure 6, the

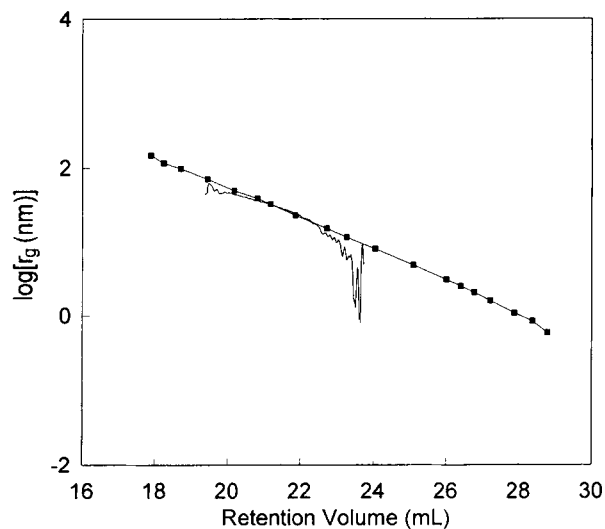


Figure 6 NBS 706 polystyrene root-mean-square radii at each retention volume measured by (solid line) TALLS and (■) polystyrene narrow standard $\log \bar{r}_{gz}$ calibration curve.

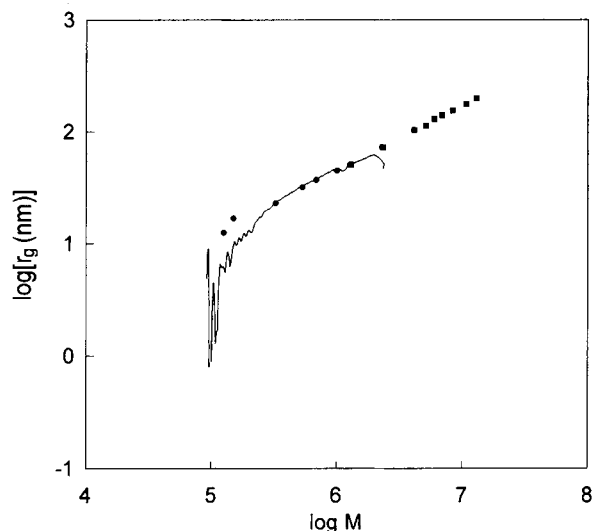


Figure 7 NBS 706 polystyrene measured by (solid line) TALLS and (■) narrow polystyrene standards from this study and (●) Ref. 2.

tendency is again for the size distribution to be narrower than the true distribution. Both the molecular weight and root-mean-square radius measured at each retention volume are average values representing detector cell contents that have been imperfectly resolved by these SEC columns. However, the relationship between r_g and molecular weight is close to true, as shown in Figure 7. The most reliable data are at large sizes, and here the scaling relationship measured on this broad polystyrene sample is close to that for narrow standards. At smaller sizes, the superposition is poor, suggesting that effects of axial dispersion are not totally lost. Provided that the true interdetector volume is used, the scaling relationship between molecular weight and root-mean-square radius is obtained for part of the polymer molecular weight distribution, despite imperfect resolution.

CONCLUSIONS

By assuming a random coil shape and by applying the associated particle-scattering function in the analysis of SEC light-scattering data at two angles, we obtained accurate molecular weights and root-mean-square radii at the largest sizes that can be separated practically on current high-performance SEC columns. The precision of the z -average root-mean square radius, \bar{r}_{gz} , measured from the ratio of light-scattering peak areas at 15° and 90° is exceptional ($\pm 0.5\%$) at sizes between about 70 and 150 nm because calculation from the light-scattering

detector alone is insensitive to errors in sample preparation and fluctuations in the flow rate. Preliminary results indicate that the random coil model is a good approximation for high molecular weight linear polystyrenes and also for long-chain branched polyesters, and the scaling relationship between weight-average molecular weight and root-mean-square radius can be obtained accurately from part of the molecular weight distribution even on columns that exhibit significant axial dispersion.

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