Evaluation of Light-Scattering Detectors for Size Exclusion Chromatography. I. Instrument Precision and Accuracy

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SYNOPSIS

A systematic evaluation of two types of light-scattering detectors for size exclusion chromatography (SEC) was completed. The two detectors were the low-angle laser light scattering photometer (LALLS) and the multiangle laser light-scattering photometer (MALLS). Instrument evaluations were performed at both room (30–40°C) and high (135–145°C) temperatures using the polystyrene standard, NBS 706, at room temperature and the polyethylene standard, SRM 1476, at high temperature. Results of the evaluation showed that when experimental uncertainties were taken into account LALLS and MALLS demonstrated equivalent precision and accuracy for molecular weight determination. The main source of inaccuracy found (particularly for SRM 1476) was the sensitivity difference between the light-scattering and the concentration (DRI) detectors; i.e., the DRI detector was unable to measure very low concentrations of very high molecular weight material present in SRM 1476, whereas the light-scattering detectors respond strongly. It was shown that for LALLS the overall weight-average molecular weight $(\bar{M}_w)$ for the whole polymer calculated using an equation that did not require the DRI detector output circumvented this sensitivity problem while assuming that the low angle used was sufficiently close to zero. Use of this equation for MALLS is possible by extrapolating data from all angles used to obtain a light-scattering chromatogram at zero angle. However, this possibility was not examined here. A particular advantage of MALLS over LALLS is that MALLS can provide the z-average root mean square radius (commonly referred to here and in other light-scattering literature as the "radius of gyration") values from the same data as those used to obtain molecular weight values. Although the radius of gyration values at each retention volume were not as precise as the corresponding weight-average molecular weights, at room temperature, precision was better than 2% for a significant portion of the chromatogram. © 1993 John Wiley & Sons, Inc.

INTRODUCTION

In this study, two instruments that utilize light scattering to measure molecular properties were examined. Both of these light-scattering photometers are used as detectors in a size exclusion chromatograph (SEC) system.

The older of these two instruments is the low-angle laser light-scattering photometer (LALLS).

It measures the light scattered from a single low angle. The combination of SEC and LALLS can yield information on molecular weight directly without SEC column calibration.[1] The newer, competing instrument is the multiangle laser light-scattering photometer (MALLS). MALLS was first coupled with SEC in 1988 and was shown capable of continuously monitoring the light scattered from a polymer solution up to 15 different angles simultaneously. This detector can provide information on both molecular weight and radius of gyration distributions when used with SEC.[2,3] Radius of gyration is particularly desired for branching evaluation.[4]

However, there is currently some discussion regarding both the accuracy and reproducibility of this new instrument.[5,6] Complicating matters is the fact that MALLS produces much more data than does LALLS and involves many more options in data interpretation than does LALLS. Furthermore, a significant fact that has not yet been recognized in the published literature is that the error in the molecular weight obtained is probably not independent of the error in the radius of gyration when the two quantities are estimated from the intercept and slope of a fitted line (or, in general, from the coefficients of a fitted equation). A correlation is to be expected because of an interaction of the mathematical fitting process with the experimental error in the data. This aspect will be further discussed in Part II of this series.

The objective of this paper is to compare these two instruments in terms of both accuracy and precision. Precision here refers to the repeatability of a measured quantity. To estimate precision, standard deviations from 10 repeated SEC runs were used. Accuracy is defined as the "closeness to the truth" and is assessed by comparison with literature values.

THEORY

Light-scattering Methods

Light-scattering detectors in SEC can eliminate the need for conventional calibration using narrow molecular weight standards by providing a direct measurement of weight-average molecular weight at each retention volume across the chromatogram. They also are much more sensitive than the differential refractometer detector to the presence of high molecular weight species. However, there are well-known complications. For example, the drastic difference in sensitivities between the DRI detector and the light-scattering detector becomes a liability when interpretation demands both detector outputs simultaneously.[7] Also, if light-scattering detectors are to be used for copolymers where variations in dn/dc (the change in refractive index of the solution with polymer concentration) occur with retention volume, then dn/dc must be determined and used in the interpretation.[8] Axial dispersion effects and the need to determine the correct interdetector volume between different detectors can also cause difficulties.

In this work, only linear and branched homopolymers are used. Axial dispersion effects are assumed negligible and interdetector volume is determined by the most frequently used method (noting the difference in peak retention times as a narrow molecular weight distribution polymer is transported from the light-scattering detector to the DRI). The following sections summarize the equations used to interpret the data.

Low-angle Laser Light Scattering

When measurement of scattered light is done at one very small angle, as in LALLS, weight-average molecular weight can be directly calculated from the excess Rayleigh ratio, R_θ, and the polymer concentration. It is assumed that there is no need for extrapolation to zero angle to remove the angular dependence of scattered light. Local values of $M_w(v)$ are calculated by superimposing the light-scattering chromatogram data points [$R_\theta(v)$ vs. v], on the DRI chromatogram [$c(v)$ vs. v], and since the term involving the second virial coefficient is negligible at injected concentrations,

$$M_w(v) = \frac{R_\theta(v)}{Kc(v)} \tag{1}$$

where K is the optical constant:

$$K = \frac{4\pi^2 n_0^2 (dn/dc)^2}{\lambda_0^4 N} \tag{2}$$

where n_0 is the refractive index of the pure solvent; λ_0, the wavelength of the incident light; and N, Avogadro's number. It should be noted that eq. (2) is written for vertically polarized incident light, the light used in the multiangle laser light scattering (MALLS) instrument. The K value for LALLS is one-half that given by eq. 2 (assuming that the random polarized light source is equivalent to an unpolarized source).

Multiangle Laser Light Scattering

Multiangle laser light scattering (MALLS) provides as many as 15 chromatograms from measurement of light scattered at each of 15 different angles, typically ranging from 13° to 160°. Theoretically, both weight-average molecular weight, $M_w(v)$, and radius of gyration, $r_g(v)$,[†] can be determined as a function of retention volume, v.

[†] $M_w(v)$ and $r_g(v)$ are termed "local values" of the properties because they are the values as a function of v. They are also written M_{wi} and r_{gi} or M_w and r_g (see Nomenclature section). These local values should be distinguished from $\bar{M}_w$ and $\bar{r}_g$, which are overall values for the whole polymer (i.e., when all molecules are examined together regardless of when they exit from the SEC).

Conventional interpretation of MALLS to obtain $M_w(v)$ and $r_g(v)$ depends upon construction of a Debye plot[9] at each retention volume (a plot of R_θ/Kc vs. $\sin^2(\theta/2)$) based upon

$$\frac{R_\theta(v)}{Kc(v)} = P(\theta)M_w(v) \qquad (3)$$

where

$$P(\theta) = 1 - \frac{16\pi^2 n_0^2 r_g^2(v)\sin^2(\theta/2)}{3\lambda_0^2}$$

$$+ \; higher \; terms \quad (4)$$

for each of the 15 detector angles.

By neglecting the higher terms in eq. (4) for now, linear regression can be used to determine $M_w(v)$ and $r_g(v)$ at any specific retention volume by minimizing Q over the n_a angles at retention volume v:

$$Q = \sum_{j=1}^{n_a} w_j\left[\left(\frac{R_{\theta j}(v)}{Kc(v)}\right)_{exp} - \left(\frac{R_{\theta j}(v)}{Kc(v)}\right)_{calc}\right]^2 \quad (5)$$

where w_j is a weighting factor generally considered as the reciprocal of the error variance of the quantity $[R_{\theta j}(v)/(K\,c(v)]_{exp}$. If all the w_j are set equal to unity, then the implicit assumption is that error variance is not a function of angle. This is the assumption employed in this paper and in the commercial software, ASTRA version 1.15, offered by Wyatt Technology Corp., Santa Barbara, CA. Other equations and expressions for the weighting factors are examined in Part II of this work.

Overall Averages from SEC/LS

When coupled with a SEC, the overall molecular parameters obtainable from light scattering are calculated from the local values as follows:
Number-average molecular weight:

$$\bar{M}_n = \frac{\sum c_i}{\sum (c_i/M_{wi})} \qquad (6)$$

Weight-average molecular weight:

$$\bar{M}_w = \frac{\sum c_i M_{wi}}{\sum c_i} \qquad (7a)$$

For LALLS, M_w can also be obtained from

$$\bar{M}_w = \frac{\sum R_{\theta,i}\Delta v_i}{K(injected\ mass)} \qquad (7b)$$

where Δv_i is the retention volume increment for each slice of the chromatogram.

Wyatt[5] pointed out that eq. (7b) could also be used with MALLS by extrapolating the light-scattering signal to a zero angle. If the extrapolation can be carried out reliably (e.g., if it is a straight-line extrapolation), then the result may well be superior to the value obtained from LALLS because it utilizes a result for the zero angle rather than assuming that the lowest angle experimentally utilized is sufficiently close to zero.

The z-average molecular weight:

$$\bar{M}_z = \frac{\sum c_i M_{wi}^2}{\sum c_i M_{wi}} \qquad (8)$$

For the multiangle light-scattering instrument, additional size information for the whole polymer may also be determined using the following three equations:

Number-average root mean square radius of gyration:

$$\bar{r}_{gn} = \left[\frac{\sum \left(\frac{c_i}{M_{wi}}\right)r_{g_i}^2}{\sum \left(\frac{c_i}{M_{wi}}\right)}\right]^{1/2} \qquad (9)$$

Weight-average root mean square radius of gyration:

$$\bar{r}_{gw} = \left[\frac{\sum c_i r_{gi}^2}{\sum c_i}\right]^{1/2} \qquad (10)$$

z-average root mean square radius of gyration:

$$\bar{r}_{gz} = \left[\frac{\sum c_i M_{wi} r_{gi}^2}{\sum c_i M_{wi}}\right]^{1/2} \qquad (11)$$

EXPERIMENTAL AND COMPUTATIONAL OPTIONS USED

Apparatus

Low-Angle Laser Light-Scattering Detector

In LALLS, scattered light was collected in an angle between 6°–7° to the incident laser beam using the Chromatix KMX-6 instrument. A 2 mW helium–neon laser (632.8 nm wavelength) provides the source irradiation. Light scattered from a 0.1 μL scattering volume is measured via a photomultiplier.

Table I   Experimental Conditions

SEC Conditions	Room Temperature		High Temperature	
	LALLS	MALLS	LALLS	MALLS
Temperature (°C)	30	40	135	145
Solvent	Tetrahydrofuran		Trichlorobenzene	
Polymer standard used	PS : NBS 706		LDPE : SRM 1476	
SEC flowrate (mL/min)	1	1	0.5	1
Detector angle (°)	6–7	20.2–160	6–7	13.4–159
Injected mass (g)	1.12×10^{-4}	1.24×10^{-4}	5.84×10^{-4}	5.84×10^{-4}
SEC column specifications	Three 5 μm particle diameter, PLGel mixed-bed columns	Three 5 μm particle diameter, PSS SDV-Gel5 columns: 10^{6}, 10^{4}, 10^{2} Å pore size	Four Shodex 802–805s columns	Five 5 μm PL Labs columns: 50, 500, 10^{3}, 10^{4}, 10^{6} Å pore size

Multiangle Laser Light-Scattering Detector

For MALLS, the model DAWN-F detector from Wyatt Technology was used. In this design, individual photodiodes are placed at fixed positions around the sample cell. Up to 15 detector angles to be used simultaneously during a run can be selected from a range of 5°–175° depending upon the solvent/glass refractions. The same 632.8 nm wavelength He–Ne laser source used in LALLS is also used in MALLS. However, the MALLS utilized a 5 mW laser of smaller diameter than that of the 2 mW source of the LALLS. Light scattered from the center of the cell is refracted according to Snell's law to one of the fixed detectors surrounding the cell.

## Evaluation of Light-Scattering Detectors

Procedure

Experimental work dealing with assessment of the two light-scattering detectors was performed at four different laboratories. The room-temperature LALLS data were obtained at Eastman Kodak Company, Rochester, NY, and the corresponding MALLS data, at Wyatt Technology Corp., Santa Barbara, CA. The high-temperature LALLS data were obtained at Exxon Chemical Company, Linden, NJ, and the corresponding MALLS data, at the University of Toronto. Refer to Table I for details of the experimental conditions used at each of the sites.

The procedure for sample preparation depended upon the standard used. The polyethylene standard,

SRM 1476, was heated at 170°C in trichlorobenzene for 3 h before being transferred to the heated SEC injector compartment (145 or 135°C; refer to Table I) for at least another 3 h prior to injection in order to avoid poor dissolution and aggregates.[10] The room-temperature polystyrene standard, NBS 706, was dissolved overnight in tetrahydrofuran prior to injection.

The eluent from the SEC columns first passed through the light-scattering detector before going through a Waters model 410 differential refractometer (DRI). Figure 1 shows a schematic of the SEC system. NBS 706 polystyrene was analyzed at room temperature using a 100 μL injection volume and a concentration of 1.2 mg/mL. The high-temperature standard, SRM 1476 polyethylene, was analyzed using a 200 μL injection volume and a concentration of 1.6 mg/mL. Ten repeated injections of the same sample were made. Three such sets of 10 injections were made over a period of 1 week, and the best set of 10 was used for the statistical treatment.
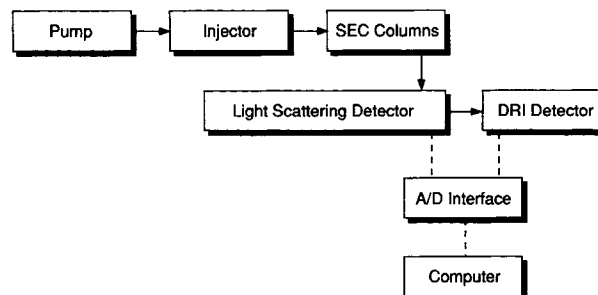
Figure 1   Schematic diagram of the SEC system.

Computations

All raw data files were processed at the University of Toronto using software developed there. The refractive index increments used were provided by Exxon Chemical Company, Linden, NJ, for the high-temperature LALLS study and by Eastman Kodak Company, Rochester, NY, for the room-temperature study. The differential refractive index used was -0.104 mL/g for polyethylene in trichlorobenzene at 135°C and 0.184 mL/g for polystyrene at 30°C (also at 40°C). For polyethylene in trichlorobenzene at 145°C, the value of the differential refractive index increment used was -0.098 mL/g based on work done by Grinshpun.[10]

Reproducibility of the detector signal is expressed in terms of the percent deviation:

$$\% \text{ Deviation } = \frac{s}{Y_{avg}} \times 100\% \qquad (12)$$

where s is the sample estimate of the standard deviation of the characteristic of the detector signal of interest and Y_{avg} is the average value of the characteristic. Percent deviations can also be calculated for the derived parameters, M_w, r_g, $\bar{M}_w$, and $\bar{r}_g$.

RESULTS AND DISCUSSION

Precision of Raw Detector Response: Room Temperature

Definition of the precision of the raw detector response is of fundamental importance to the detector assessment. Both the light scattering and the DRI
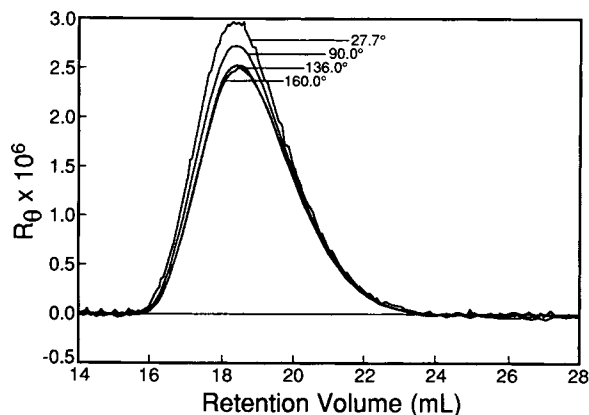

Figure 2 Typical room-temperature MALLS data (SEC/MALLS, NBS 706 polystyrene).
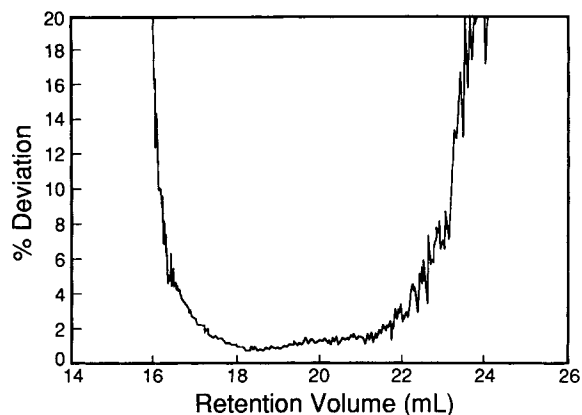

Figure 3 MALLS detector precision at room temperature (SEC/MALLS, 90° scattering angle, NBS 706 polystyrene).

detectors must be considered. These detector response error variances are propagated into the error variances of calculated values (such as molecular weight averages) through the equations used to compute those values.

Typical chromatograms from SEC/MALLS analysis of the polystyrene standard, NBS 706, are displayed in Figure 2. The angular dependence of R_θ is evident. The light-scattering signal at 160° is only 80% of the signal at 27.7°. However, the lower the angle, the more likely the signal will show light scattered by impurities or dust; hence, the noise level at lower angles tends to be higher. An example of MALLS detector precision is shown in Figure 3. The percent deviations for the MALLS detectors at room temperature were reasonably consistent; the main peak region had a percent deviation of 1%. However, the range of elution volume (molecular weights) at which the percent deviations were below 2% was reduced for lowest and highest angles. The corresponding chromatogram from the DRI detector in the SEC/MALLS system is shown in Figure 4. The actual distribution of molecular weights for the NBS 706 standard from the DRI detector was broader than it would appear from studying the chromatogram from the light-scattering detector alone. No appreciable signal was discerned in the light-scattering response beyond 24 mL (a molecular weight of approximately 26,000), although from the DRI detector, it can be seen that the concentration response remained significant at about 0.4×10^{-5} g/mL. The percent deviations of the concentration values across the central portion of the chromatogram were 1–2% (Fig. 5). Although the light-scat-
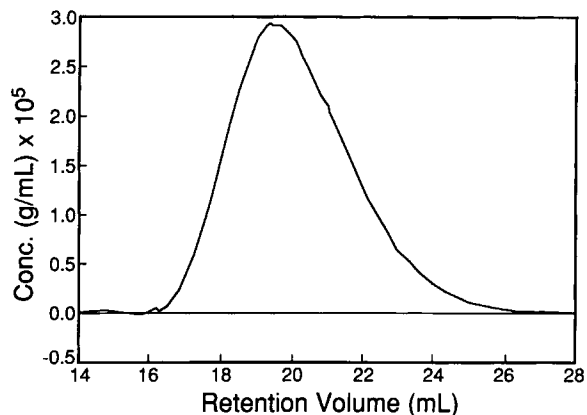
Figure 4 Typical DRI room-temperature data (SEC/MALLS, NBS 706 polystyrene).
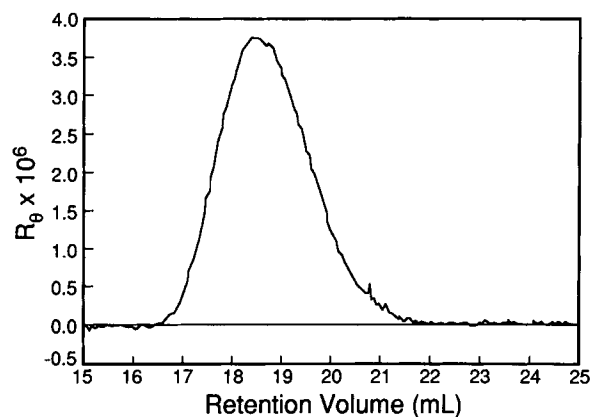

Figure 6 Typical LALLS room-temperature data (SEC/LALLS, NBS 706 polystyrene).

tering signal was slightly more precise at the high molecular weight end (i.e., at 16 mL or a molecular weight of 1.46 million), the precision of the DRI detector was superior elsewhere and extended over a wider range of molecular weights.

The chromatogram from the LALLS instrument is shown in Figure 6. The percent deviation for the main portion of the elution volume was approximately 1% (Fig. 7). But similar to MALLS, the LALLS signal tailed off rapidly at low molecular weights (between 21 and 22 mL on the chromatogram, or molecular weights of 64,000 to 36,000 for the columns used). Figure 8 is a plot of the corresponding concentration values from the DRI detector for the NBS 706 sample analyzed using SEC/LALLS. The DRI revealed a longer low molecular weight tail than that of the light scattering. The percent deviation plot (Fig. 9) showed poor precision for the concentration values at the high molecular

weight tail (16.5–17 mL or approximately 1.5 million in molecular weight).

Thus, at room temperature, both light-scattering detectors, LALLS and MALLS, demonstrated high precision, but over a narrower molecular weight range of the chromatogram than did the DRI detector. The reproducibility of the DRI was as good or better than either light-scattering detector except for the high molecular weight tail.

Precision of Raw Detector Response: High Temperature

In this evaluation, the precision of high-temperature analyses was expected to be inferior to that of room-temperature runs. One reason for this is that the solvent at high temperature, trichlorobenzene, scatters more light than does the room temperature solvent, tetrahydrofuran. Thus, the light scattered due
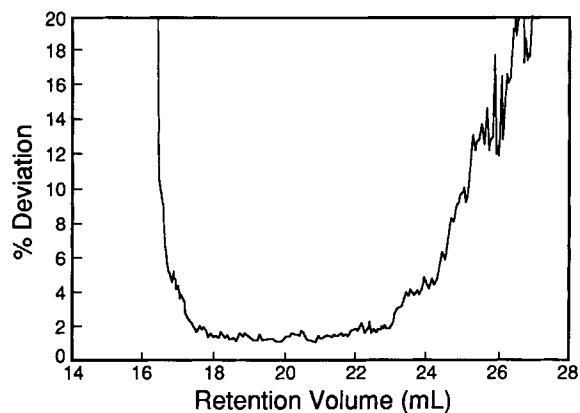

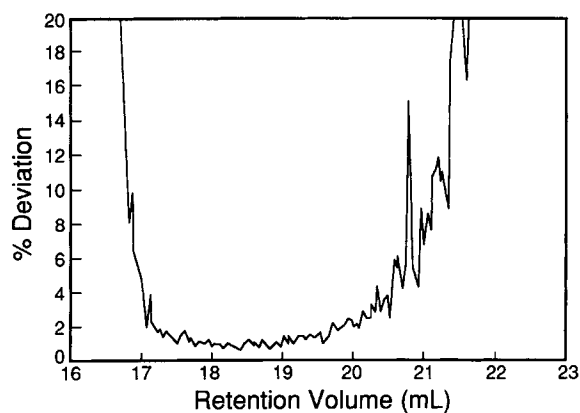**Figure 5** DRI detector precision at room temperature (SEC/MALLS, NBS 706 polystyrene).

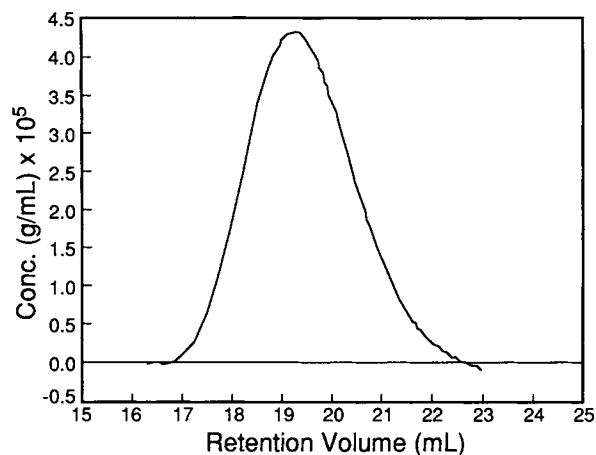**Figure 7** LALLS detector precision at room temperature (SEC/LALLS, NBS 706 polystyrene).

Figure 8 Typical DRI room-temperature data (SEC/LALLS, NBS 706 polystyrene).

Figure 10 Typical MALLS high-temperature data (SEC/MALLS, SRM 1476 low-density polyethylene).

to the polyethylene, above that of trichlorobenzene at high temperature, was less than the difference in scattered light intensity in the polystyrene/tetrahydrofuran system studied at room temperature. Furthermore, light scattered by polystyrene dissolved in trichlorobenzene has an even weaker signal than that of polyethylene in trichlorobenzene at 145°C. Thus, the base-line scattering level is high for trichlorobenzene and the dn/dc is much lower than that obtained using the room-temperature system with tetrahydrofuran. Hence, to improve precision, in this evaluation, the mass of the SRM 1476 polyethylene standard injected was four times that of the NBS 706 polystyrene.

Typical chromatograms from SEC/MALLS analysis of the polyethylene standard, SRM 1476, are shown in Figure 10. This standard is clearly bimodal: A sharp peak was detected just before the main polymer peak in the chromatogram. The an-
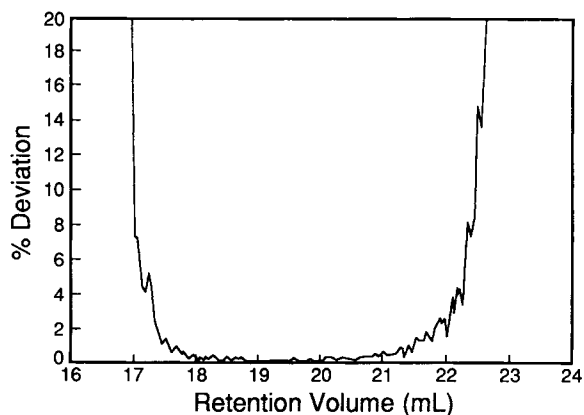
gular dependence of R_θ is the strongest at the pre-peak (at approximately 31 mL), indicative of very high molecular weight material. An example of MALLS detector precision is shown in Figure 11. The percent deviations for the MALLS detectors at high temperature increased at decreasing scattering angle. At 28.2°, the percent deviation across the bulk of the elution volume was about 5%, but at 115.2°, the precision was improved to approximately 3%. The corresponding chromatogram from the DRI detector in the SEC/MALLS system did not reveal the bimodal nature of the SRM 1476 standard (Fig. 12). However, the actual molecular weight distribution for the SRM 1476 standard from the DRI detector was significantly broader than it appeared in the light-scattering chromatogram. No appreciable light-scattering signal was observed after 38 mL (at a molecular weight of approximately 24,000 for the columns used), although from the DRI detector
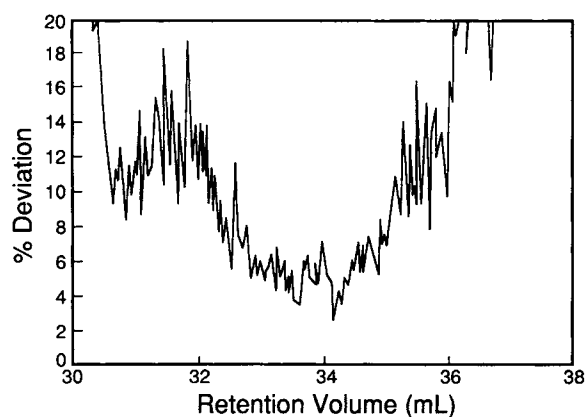

Figure 9 DRI precision at room temperature (SEC/LALLS, NBS 706 polystyrene).

Figure 11 MALLS detector precision at high temperature (SEC/MALLS, 90° scattering angle).

Figure 12 Typical DRI high-temperature data (SEC/MALLS, SRM 1476 low-density polyethylene).
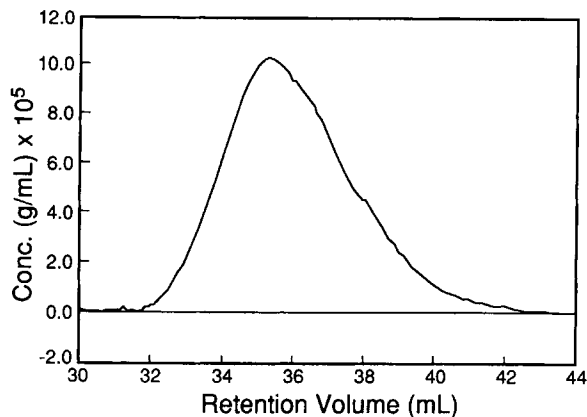

Figure 14 Typical LALLS high-temperature data (SEC/LALLS, SRM 1476 low-density polyethylene).

it can be seen that the concentration was still significant (0.45×10^{-5} g/mL). The percent deviation of the concentration values across the chromatogram was generally between 1 and 2% (Fig. 13). Whereas the light-scattering signal was much more precise at the high molecular weight prepeak (between 30 and 32 mL or molecular weights in the range of 7 million), the precision of the DRI detector was considerably better for the main polymer peak.

The chromatogram for SRM 1476 from the LALLS instrument is shown in Figure 14. The height of the prepeak observed was significantly higher than that observed previously using MALLS, even if the angular dependence of R_θ was taken into account. (The resulting molecular weight of the prepeak was about 30 million from LALLS. However, in retrospect, an important difference between the LALLS and MALLS data may have been the lower mobile phase flow rate used for the former.
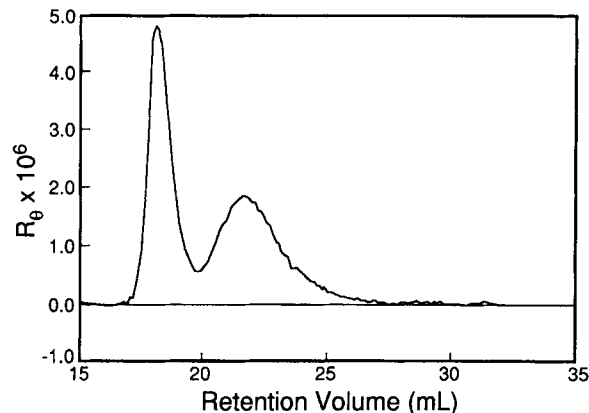
Extremely high molecular weights may have been shear degraded by the 1 mL/min flow rate used for the MALLS. Also, higher flow rates can accelerate column packing breakup, which can, in turn, increase noise in the light-scattering signal.) The percent deviation for the main portion of the elution volume was 1–4% (Fig. 15). But similar to the MALLS instrument, the light-scattering signal tailed off rapidly between 25 and 27 mL (corresponding to molecular weights between 27,000 and 16,000). Figure 16 is a plot of the corresponding concentration values from the DRI detector in the SEC/LALLS system. There was a very small baseline deviation prior to the main polymer peak matching the high molecular weight prepeak observed in the light-scattering chromatogram. The DRI again revealed a much longer low molecular weight tail than that of the light scattering. The precision of the concentration values near the pre-
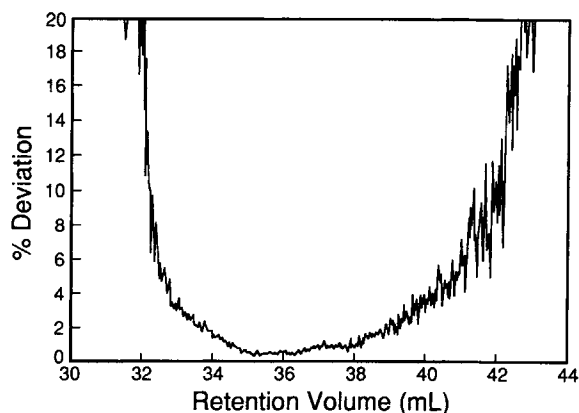

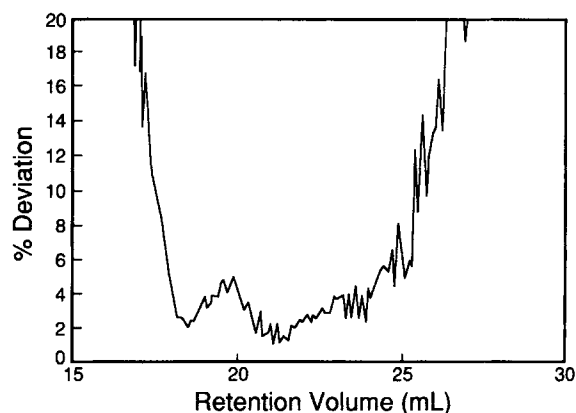**Figure 13** DRI Precision at high temperature (SEC/MALLS, SRM 1476 low-density polyethylene).

Figure 15 LALLS precision at high temperature (SEC/LALLS, SRM 1476 low-density polyethylene).
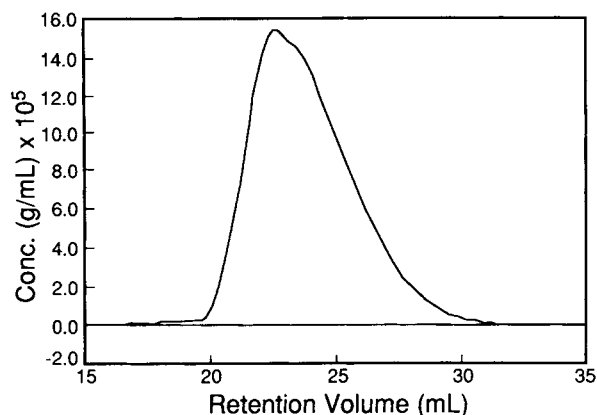
Figure 16 Typical DRI high-temperature data (SEC/ LALLS, SRM 1476 low-density polyethylene).

peak was still poor, at only 16% (Fig. 17). The concentration percent deviation for the main polymer peak at only 1% was superior to the precision of LALLS.

Precision of Local Property Values: Room Temperature

The precision of the molecular weight and radius of gyration distributions, $M_w(v)$ and $r_g(v)$, was determined for LALLS and MALLS. For LALLS, eq. (1) was used to calculate the M_w at each elution volume. For MALLS, the Debye equation [eq. (3), linear in $\sin^2(\theta/2)$] was used. Standard deviations were determined based upon 10 repeated SEC analyses.

Since both the light-scattering and the DRI detectors were used in estimation of the molecular weight and size parameters, the sensitivity difference between the two detectors is of concern. However,
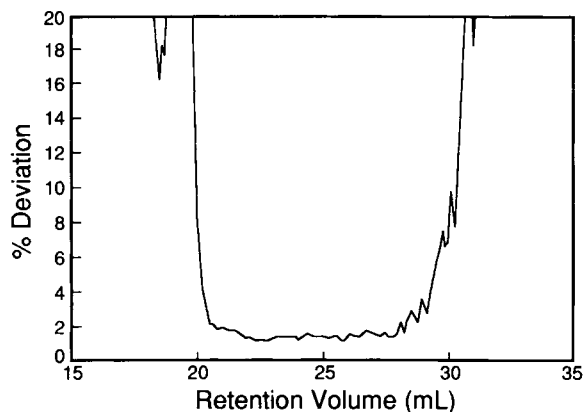
Wyatt[11] recently pointed out that, in practice, since concentration is a constant within a slice as well as being consistently low in value for SEC and since only the slope of the line is required for the radius of gyration, the correct value for the radius of gyration (but not molecular weight!) can be obtained even when an incorrect concentration value is used in the determination. This does not hold for whole polymer averages requiring concentration as a weighting factor [eqs. (9) and (10)]. The z-average radius of gyration [eq. (11)] is expected to remain unaffected by the DRI because the light-scattering signal is directly proportional to the product of concentration and molecular weight.[11]

Figure 18 shows the superposition of chromatograms from LALLS and the DRI detectors, respectively, for the room-temperature SEC/LALLS system. When the percent deviation of M_w is plotted (Fig. 19), it becomes obvious that the range of reliable M_w was limited by the sensitivity of the DRI at the high molecular weight tail, and by LALLS, at the low molecular weight tail. However, for the bulk of the elution volume of NBS 706 (i.e., molecular weight ranging from 1.35 million to 49 thousand), the precision was quite high with an uncertainty of between 1 and 4% for 1 standard deviation.

The room-temperature SEC/MALLS system showed the same repeatability as that of the SEC/ LALLS system for the M_w of the NBS 706 standard analyzed. A similar difference in detector sensitivity between the DRI and the MALLS detector was observed. The multiple detector angles in MALLS allowed both r_g and M_w to be calculated at each elution volume. The percent deviations for both r_g and M_w are plotted in Figure 20. Excellent precision, percent deviations of 1–6%, was obtained for high molecular
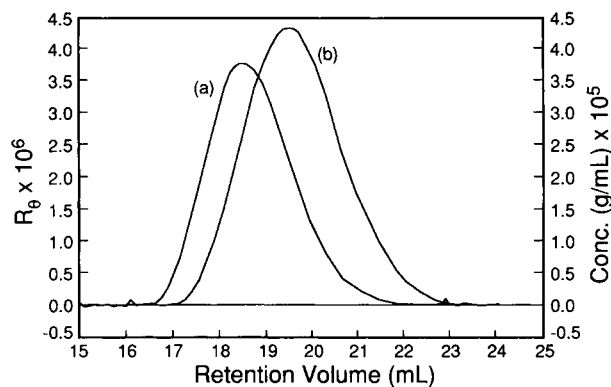

Figure 17 DRI precision at high temperature (SEC/ LALLS, SRM 1476 low-density polyethylene).

Figure 18 (a) LALLS chromatogram; (b) DRI chromatogram showing sensitivity difference at room temperature (SEC/LALLS, NBS 706 polystyrene).
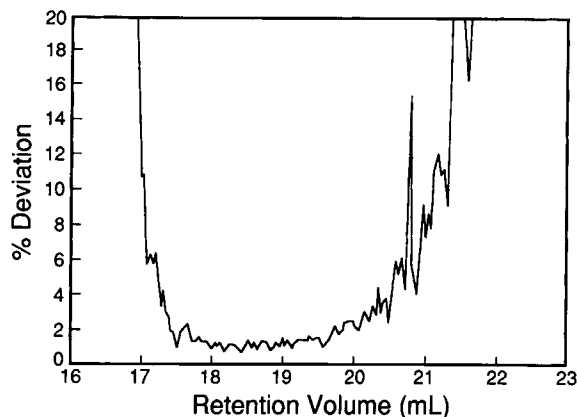
Figure 19 Variation of precision of local weight-average molecular weight with retention volume (room-temperature SEC/LALLS, NBS 706 polystyrene).

Figure 21 (a) LALLS chromatogram and (b) DRI chromatogram showing sensitivity difference at high temperature (SEC/LALLS, NBS 706 polystyrene).

weight and large molecular sizes: M_w ranging from 1.3 million to 26 thousand and r_g ranging from 42 to 13 nm. However, the repeatability of r_g decreased much earlier than that for M_w (21 mL [r_g of 13.2 nm and M_w of 134,000] vs. 24 mL [r_g of 8.4 nm and M_w of 26,000]).

Precision of Local Property Values: High Temperature

As noted in the previous discussion on precision of detectors, the sensitivity difference between the light-scattering and the DRI detectors is a serious problem when analyzing the molecular weight of the polyethylene standard, SRM 1476. Figure 21 shows the overlay of chromatograms from LALLS and the DRI detectors respectively for SRM 1476 analyzed
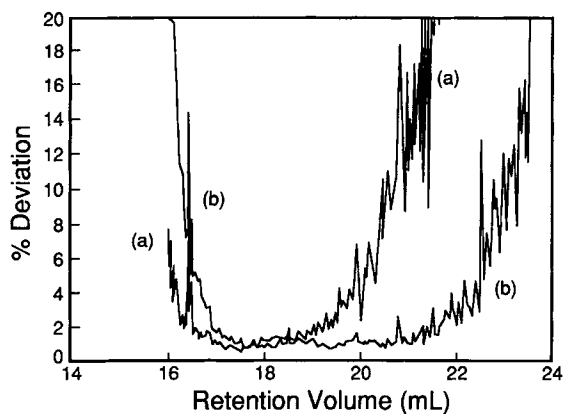
using the high-temperature SEC/LALLS system. The percent deviation of M_w is plotted in Figure 22. Although the light-scattering signal was strongest at the prepeak (M_w in the tens of million), the reliability of M_w is limited by the sensitivity of the DRI, resulting in a poor percent deviation of 20%. At the low molecular weight tail, the most significant source of error came from the LALLS detector. A reasonable precision in M_w, percent deviation of less than 5%, was achieved for the main polymer peak of SRM 1476 (i.e., molecular weight ranging from 1.36 million to 17 thousand).

Similar differences in detector sensitivity between the DRI and the MALLS detector were observed as that between the DRI and LALLS detectors. The percent deviations for both r_g and M_w are plotted in Figure 23. The high-temperature SEC/MALLS system showed repeatability comparable to the SEC/LALLS system for M_w of the SRM 1476 stan-

Figure 20 Variation of (a) local radius of gyration and (b) local weight-average molecular weight with retention volume (room-temperature SEC/MALLS, NBS 706 polystyrene).
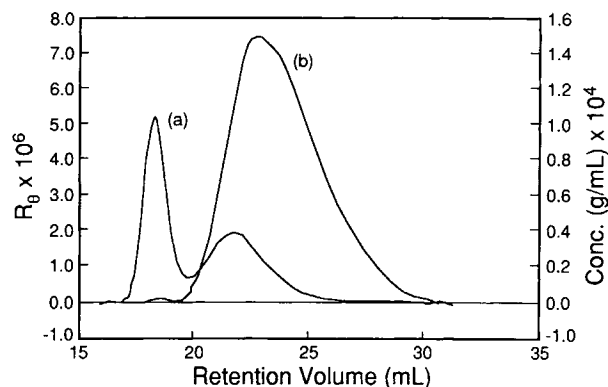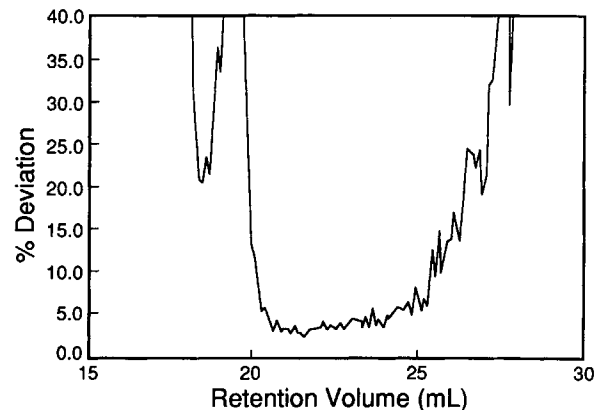

Figure 22 Variation of precision of local weight-average molecular weight with retention volume (high-temperature SEC/LALLS, SRM 1476 low-density polyethylene).
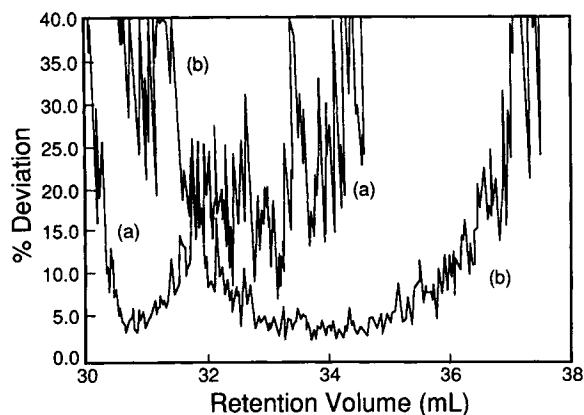
Figure 23 Variation of (a) local radius of gyration and (b) local weight-average molecular weight with retention volume (high-temperature SEC/MALLS, SRM 1476 low-density polyethylene).

dard. For the bulk of the elution volume, percent deviation of M_w was approximately 5% for molecular weights from 0.9 million to 18 thousand. However, precision of r_g was much poorer than that of the corresponding M_w. The precision of r_g was less dependent on the precision of the DRI than was M_w, because the r_g was calculated from the slope of the Debye plot. For small molecules, the slope is near zero; thus, the most reproducible r_g occurred at the high molecular weight prepeak, where the r_g was 40 nm. The radius of gyration can be determined reasonably (within 20% deviation) from 42 to 10 nm, corresponding to M_w ranging from 10 million to 75 thousand.

Precision of Overall Averages: Room-temperature Study

Results of the SEC/LALLS and SEC/MALLS analysis of the polystyrene standard, NBS 706, at room temperature are summarized in Table II. Precision of SEC/LALLS in the present study compares well with other published values. Assessment of the effect of coupled molecular weight detectors (LALLS and viscometer) showed the reproducibility (the percent deviation) of the molecular weight parameters for NBS 706 to be $\bar{M}_n \pm 9.8\%$, $\bar{M}_w \pm 3.8\%$, and $\bar{M}_z \pm 3.5\%$.[12] The most reliable molecular weight average from either LALLS or MALLS, in this work, is the weight-average molecular weight with a percent deviation precision of 1%. The overall average calculated from integration of the LALLS chromatogram also showed a 1% precision. Considering the many factors involved that can affect the run-to-run precision, such as temperature fluctuations, column particulates, and base-line drifts, both LALLS and MALLS performed exceptionally well in this case. Light scattering, in general, is not particularly sensitive to the low molecular weight end (less than 10^4). Thus, precision of the number-average molecular weight (which depends upon the low molecular weight end) is expectedly poor. In this case, the LALLS instrument showed an apparently good reproducibility at 3.9%, but the precision of $\bar{M}_n$ from the SEC/MALLS system was only 44.7% because of two runs with extremely poor low molecular end data. If those two runs were discarded, the standard deviation of $\bar{M}_n$ from the SEC/MALLS system becomes 14.8%. The reproducibility of the overall $\bar{M}_z$, which relies strongly on the extreme high molecular weight end of the distribution, is excellent with both detectors: The percent deviations are both under 10%.

The different radii of gyration for the whole polymer obtained from SEC/MALLS are also tabulated in Table II. In general, the radius of gyration values are less precise than are the corresponding molecular weight averages. Again, the number-average value is not very precise ($\pm 21\%$) because of the poor sensitivity of the light-scattering instrument at the low molecular weight end. Both the weight-average and z-average $\bar{r}_g$ values have reasonable reproducibility at room temperature. However, the most reliable

Table II Summary of Overall Averages for NBS 706

	LALLS*	MALLS*
$\bar{M}_n$	$1.636 \times 10^5 \pm 3.9\%$	$1.464 \times 10^5 \pm 44.7\%$
$\bar{M}_w$	$2.770 \times 10^5 \pm 1.0\%$	$2.866 \times 10^5 \pm 1.0\%$
$\bar{M}_z$	$4.216 \times 10^5 \pm 7.6\%$	$4.216 \times 10^5 \pm 1.3\%$
$\bar{M}_w$ (peak integration)	$2.785 \times 10^5 \pm 1.0\%$	—
$\bar{r}_{gn}$ (nm)	—	$21.1 \pm 21.8\%$
$\bar{r}_{gw}$ (nm)	—	$23.5 \pm 11.2\%$
$\bar{r}_{gz}$ (nm)	—	$25.1 \pm 1.38\%$

* Values are shown as "estimate $\pm$ % deviation."

overall average is $\bar{r}_{gz}$, the one less dependent upon the response for smaller molecules.

Precision of Overall Averages: High-temperature Study

Precision [described in terms of percent deviation defined in eq. (12)] of molecular parameters determined from SEC using only the DRI[13] was found to be as follows: $\bar{M}_n \pm 3.9\%$, $\bar{M}_w \pm 3.5\%$, and $\bar{M}_z \pm 9.5\%$ for polyethylene. Results from the current study on SEC/LALLS and SEC/MALLS analysis of the polyethylene standard, SRM 1476, at high temperature are summarized in Table III. Precision of the overall weight-average molecular weight, $\bar{M}_w$, at 4–5%, in the present study generally agrees with the published values from SEC alone. As previously mentioned, at high temperatures, the many factors that are not present at room temperature aggravate the run-to-run precision problem (e.g., temperature fluctuations, column degradation, and low light-scattering intensity).

The overall average calculated from integration of the LALLS chromatogram without using the DRI signal for local concentration [eq. (7b)] showed an improved precision (standard deviation of 2.8% vs. 3.9%) over the method that required the concentration chromatogram [eq. (7a)]. This indicates that the fluctuations in the local concentration are important factors in the analysis of SRM 1476. The individual chromatograms from light scattering and the differential refractive index detectors indicated a significant difference in the sensitivity of these two detectors for the SRM 1476 sample (refer to Fig. 21). Precision of both number- and z-average molecular weights were poor when compared with those obtained from high-temperature SEC using only the DRI. In this case, the lack of precision at the low molecular weight end of the light-scattering instrument and the similar problem at the high molecular weight end for the DRI were major obstacles

in the characterization of broad molecular weight samples with a low concentration of high molecular weight material, such as the SRM 1476.

The different radii of gyration for SRM 1476 obtained from SEC/MALLS are also tabulated in Table III. Because of the sensitivity discrepancy in the two detectors, the number-average $\bar{r}_g$ could not be determined (a negative value would be obtained because of the noise at the low molecular weight end). As in the case of room-temperature results, the radii of gyration values are less precise than the corresponding molecular weight averages with the most precise average being $\bar{r}_{gz}$.

Accuracy of Calculated Values

Accuracy requires comparison of computed values to known values for standards. Thus, in assessing the accuracy of the SEC/LALLS and SEC/MALLS analyses, the $\bar{M}_w$'s for NBS 706 and SRM 1476 from the present study were compared with accepted literature values. There are many sources of error that can affect the accuracy of the $\bar{M}_w$ determined from SEC coupled with a light-scattering detector. First, the difference in sensitivity between the light-scattering and DRI detectors was significant (see Figs. 18 and 21). The dilution effect in the SEC columns lowered the concentration at the tails of the chromatogram to below 1×10^{-5} g/mL (refer to Figs. 4, 8, 12, and 16). However, the light-scattering detector response is proportional to the molecular weight, creating a large discrepancy in the magnitude of the response at the high and low molecular weight ends. Normally, when calculating the $\bar{M}_w$ of the whole polymer, it is necessary to know the molecular weight distribution and, hence, the concentrations at each retention volume. In this case, i.e., when eq. (7a) is used, the accuracy of $\bar{M}_w$ will be highly susceptible to the sensitivity difference between the two detectors at the tail ends of the chromatogram. This problem can be overcome when only $\bar{M}_w$ is of interest

Table III   Summary of Overall Averages for SRM 1476

	LALLS*	MALLS* (13 Angles)
$\bar{M}_n$	$4.078 \times 10^4 \pm 8.7\%$	$4.677 \times 10^4 \pm 19.6\%$
$\bar{M}_w$	$1.761 \times 10^5 \pm 3.9\%$	$1.144 \times 10^5 \pm 5.3\%$
$\bar{M}_z$	$1.112 \times 10^6 \pm 21.8\%$	$1.735 \times 10^6 \pm 38.9\%$
$\bar{M}_w$ (peak integration)	$2.137 \times 10^5 \pm 2.8\%$	—
$\bar{r}_{gn}$ (nm)	—	—
$\bar{r}_{gw}$ (nm)	—	$32.2 \pm 37\%$
$\bar{r}_{gz}$ (nm)	—	$25.1 \pm 15\%$

* Values are shown as "estimate $\pm$ % deviation."

by using eq. (7b). Since the mass injected is known (either from the concentration and volume of the sample injected or from integrating the DRI chromatogram), $\bar{M}_w$ can be calculated directly from the LALLS R_θ. [As previously mentioned, the equation may also be used with MALLS.[5] However, the software to accomplish this was unavailable at the time of the study. Thus, eq. (7b) was used only with LALLS.] Equation (7b) means that the $\bar{M}_w$ can be found completely independent of the DRI detector, neatly circumventing the sensitivity problem, while taking full advantage of the light-scattering method for detecting high molecular weight material.

Second, error in the ancillary measurements such as the differential refractive index increment (dn/dc) and refractive index of the solvent are often significant. Off-line measurement is necessary in order to obtain these parameters. In particular, the dn/dc may vary with retention volume. Since dn/dc is squared in the light-scattering optical constant [eq. (2)], a 5% error in dn/dc would give a 10% difference in the $\bar{M}_w$. Third, polymer degradation both during sample preparation and in the SEC columns may occur. This is a particular problem for very high molecular weight material, which, because of the higher solution viscosity, will experience more shear in the SEC columns and, hence, degrade to lower molecular weights. But the problem of analysis

of high molecular weight material does not stop at the possibility of degradation. High molecular weight material is more difficult to dissolve, and improper sample preparation will cause formation of aggregates. If these relatively large aggregates have not been filtered out prior to SEC analysis, they will appear as spikes in the light-scattering chromatogram. This is of particular concern in the analysis of SRM 1476 where there is a high molecular weight prepeak. Also, although NBS standards were used in the present study, there may still be heterogeneity between the pellets and sampling errors, since only a very small amount of polymer is required for SEC analysis.

In 1972, Evans[14] stated that an overall accuracy of 8–10% for derived parameters, $\bar{r}_g$ and $\bar{M}_w$, should be considered good in view of the uncertainties described above. With improvements to instrumentation, clarification techniques, and methods of data processing, the accuracy may be improved to approximately 3–5%.[10]

Accuracy of $\bar{M}_w$ for Polystyrene Standard, NBS 706

A comparison of the published values for the $\bar{M}_w$ of NBS 706 is shown in Table IV. Agreement between

Table IV   Accuracy of $\bar{M}_w$ for NBS 706

Reference	Method	$\bar{M}_w$ (10^5)	% Deviation from SEC/LALLS Present Work	% Deviation from SEC/MALLS Present Work
Present work	SEC/LALLS[a]	2.77 ± 0.029	—	−3.5
Present work	SEC/LALLS[b]	2.79 ± 0.029	0.7	−2.8
Present work	SEC/MALLS	2.87 ± 0.029	3.6	—
Jordan and McConnell[15]	SEC	2.751, 2.654[c]	−0.7, −4.2	−1.4, −7.5
	SEC/LALLS	2.622	−5.3	−8.6
NBS	SEC/LS	2.578	−6.9	−10.0
	Sed. equilibrium[d]	2.881	−4.0	0.4
Mori[16]	SEC	2.59–2.78	−6.5–0.4	−9.8 to −3.1
Alfredson et al.[17]	SEC	2.76	−0.4	−3.8
		2.80	−1.1	−2.4
Haney and Armonas[18]	SEC	2.72	−1.8	−5.2
Kato et al.[19]	SEC	2.578	−6.9	−10.0
Mourey et al.[12]	SEC	2.65 ± 0.026	−4.3	−7.7
		2.74 ± 0.027	−1.1	−4.5
		2.60 ± 0.089	−6.1	−9.4
		2.77 ± 0.090	0.0	−3.5
	SEC/LALLS	2.77 ± 0.105	0.0	−3.5

[a] Equation (7a).
[b] Equation (7b).
[c] Corrected for band-spreading.
[d] Sedimentation equilibrium.

the two different light-scattering detectors in the present work, LALLS and MALLS, is excellent. The 3.4% difference in the $\bar{M}_w$ is well within the systematic variations that exist between the two different laboratories at which the work was performed. For example, the different column sets used, flow-rate fluctuations, length of connections (axial dispersion), and different operators were all factors. The values obtained in the present study also compare well with other published values, including those provided by the National Bureau of Standards (NBS). The SEC/LALLS value fall within 5% of most published results and that from SEC/MALLS are within 10%. For the analysis of NBS 706, the sensitivity difference, uncertainties in the ancillary measurements, polymer degradation, and dissolution were not serious problems. Although NBS 706 is a broad standard, it does not have a very long high molecular weight tail. For polystyrene at room temperature, off-line measurements of dn/dc can be performed easily and it is well studied in the literature. Polymer dissolution was not of concern because polystyrene dissolves easily even at room temperature. Thus, for the room-temperature SEC analysis of NBS 706, both LALLS and MALLS provide equivalent accuracy for molecular weight determination within the expected experimental variations.

Accuracy of $\bar{M}_w$ for the Polyethylene Standard, SRM 1476

A wide range of values has been reported for the $\bar{M}_w$ of the low-density polyethylene standard, SRM 1476 (see Table V). In the present study, the value obtained from SEC/LALLS differs significantly from that from SEC/MALLS. The previously mentioned difference in sensitivity of light scattering and DRI (shown in Fig. 1) along with the long high molecular weight tail of SRM 1476 is an important reason for the diversity of reported values in Table V. Analyzed using SEC/LALLS, the value of $\bar{M}_w$ from eq. (7b) exceeded that from eq. (7a) by 22%. Also, because of the extremely high molecular weight involved (in excess of 10^7), how well the "prepeak" was analyzed would greatly affect the molecular weight averages. As mentioned previously, high molecular weight polymer is also extremely sensitive to shear and could be degraded as it elutes from the columns. It could also be filtered out during sample preparation or during the actual SEC run through the internal filters due to formation of aggregates. Although appropriate sample preparation procedures were taken to minimize these effects in the present study, these could be the reasons behind the relatively low molecular weights reported in literature (Table V). The most obvious indication of these problems is the

Table V Accuracy of $\bar{M}_w$ for SRM 1476

Reference	Method–Solvent	$\bar{M}_w$ (10^5)	% Deviation from SEC/LALLS Present Work	% Deviation from SEC/MALLS Present Work
Present work	SEC/LALLS–TCB[a]	1.76 ± 0.069	—	−10 to 54
Present work	SEC/LALLS–TCB[b]	2.14 ± 0.069	22	87
Present work	SEC/MALLS–TCB	1.14 ± 0.061	−54	—
MacRury and McConnell[1]	LALLS/OL–TCB	2.21 ± 0.09	26, 3.3*	93
	SEC/LALLS–TCB	1.00	−43	−13
Wagner and McCrackin[20]	CLS/OL–CN	1.40	−20, −35*	22
	SEC/VIS–TCB	0.91–1.05	−48 to −40	−20 to −8
Stejskal et al.[21]	CLS/OL–TCB	2.15	22, 0.5*	88
	CLS/OL–CN	2.54	44, 19*	122
Grinshpun et al.[10]	LALLS/OL–TCB	2.14	22, 0*	87
	SEC–TCB	0.74–0.98	−58, −44	−35, −14
	SEC/LALLS–TCB	0.93	−47	−19
Wild et al.[22]	SEC/VIS–TCB	0.83–0.85	−53 to −52	−27 to −26
Axelson and Knapp[23]	SEC/LALLS–CN	0.76	−57	−34

OL = off-line/batch; TCB = trichlorobenzene; CLS = classical multiangle light-scattering (mercury lamp light source); VIS = intrinsic viscometry; CN = 1-chloronaphthalene.
[a] Equation (7a).
[b] Equation (7b).

discrepancy between the off-line analysis using the LALLS alone and using the SEC/LALLS system: As shown in Table V, deviations approach 50%. SEC/MALLS analysis of SRM 1476 in the present study also yielded a lower molecular weight average [calculated from eq. (7a)]. However, the size of the high molecular weight "prepeak" in the chromatogram was not as pronounced as that from the SEC/LALLS study, even taking into account the angular dependency of the light-scattering signal (the lower the observation angle, the higher the scattering intensity). Pellet-to-pellet variations could also be a problem here because the fraction of very high molecular weight material in SRM 1476 is very small. As mentioned earlier, the fact that shear degradation may have occurred because of the higher flow rate used for the high-temperature MALLS data is also a possibility. At any rate, the SEC/MALLS value still falls within the range of reported values in the literature and cannot be disregarded because of the many sources of experimental variations.

Thus, the high-temperature SEC/LS analysis of SRM 1476 was subject to more sources of error than was the room-temperature study of NBS 1476. Accurate determination of $\bar{M}_w$ for SRM 1476 is suspect when both DRI and light-scattering detectors were used because of the exaggerated sensitivity problem at the high molecular weight prepeak. In this case, more accurate and precise values of $\bar{M}_w$ can be determined utilizing eq. (7b).

CONCLUSIONS

- In general, when all sources of variability in results are taken into account, LALLS and MALLS showed equivalent precision and accuracy of molecular weight determination. Both instruments were effectively used at both room (30–40°C) and high (135–145°C) temperatures to analyze the polystyrene standard, NBS 706, at room temperature and the polyethylene standard, SRM 1476, at high temperature. It must also be noted that the experimental work for this study utilized a 1989 version of the multiangle laser light-scattering instrument. Continual improvements in hardware and software have been advertised for this instrument since that time. Several of the hardware improvements have been directed at reducing noise levels in high-temperature measurements.

- Precision of the raw detector responses, local weight-average molecular weight, and (for MALLS only) local radii of gyration varied across the chromatogram with percent deviations of less than 2% in the central portion. Overall weight-average molecular weight showed percent deviations of 5.3% or less. However, overall number-average molecular weight ranged from 3.9 to 19.6%. Overall radius of gyration (obtainable only from MALLS) showed percent variations of 1.38 to 37%, with best results for the z-average value at room temperature.

- Accuracy of overall weight-average molecular weights was defined by comparison with literature values. At room temperature, results from LALLS and MALLS were quite close and corresponded well with what we considered the best literature values. However, at high temperatures, LALLS and MALLS deviated by as much as 54% from each other while still lying within the range of acceptable literature values. The perverse nature of the SRM 1476 molecular weight distribution (notably a very high molecular weight tail) was considered an important reason for the uncertainty.

- The main source of inaccuracy found (particularly for SRM 1476) was the sensitivity difference between the light-scattering and the concentration (DRI) detectors. For LALLS, the overall $\bar{M}_w$ for the whole polymer calculated from LALLS using an equation that did not require the DRI detector output circumvented this sensitivity problem by assuming that the low angle used was sufficiently close to zero. The same approach can be used with MALLS if the light-scattering chromatogram at zero angle can be obtained using extrapolation.

- A particular advantage of MALLS over LALLS is that MALLS can provide the radius of gyration values from the same data as those used to obtain molecular weight values.

NOMENCLATURE

c_i	concentration at retention volume, v_i
$c(v)$	concentration as a function of retention volume, v
dn/dc	differential refractive index increment
K	optical constant defined by eq. (2)
M_w	local weight-average molecular weight at some retention volume
M_{wi}	local weight average molecular weight at retention volume, v_i

$M_w(v)$ local weight-average molecular weight as a function of retention volume

$\bar{M}_n$ whole polymer number-average molecular weight

$\bar{M}_w$ whole polymer weight-average molecular weight

$\bar{M}_z$ whole polymer z-average molecular weight

N Avogadro's number

n_0 refractive index of the pure solvent

$P(\theta)$ scattering function [eq. (4)]

Q objective function defined by eq. (5)

R_θ excess Rayleigh ratio

$R_\theta(v)$ excess Rayleigh ratio as a function of retention volume

$\bar{r}_g$ whole polymer z-average root mean square radius of gyration

r_g local z-average root mean square radius of gyration at some retention volume

r_{gi} local z-average root mean square radius of gyration at retention volume, v_i

$r_g(v)$ local z-average root mean square radius of gyration as a function of retention volume

$\bar{r}_n$ whole polymer number-average root mean square radius of gyration

$\bar{r}_w$ whole polymer weight-average root mean square radius of gyration

$\bar{r}_z$ whole polymer z-average root mean square radius of gyration

v retention volume

λ_0 wavelength of the incident light

Subscripts

calc   calculated value
exp   experimental value
i retention volume
j angle

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