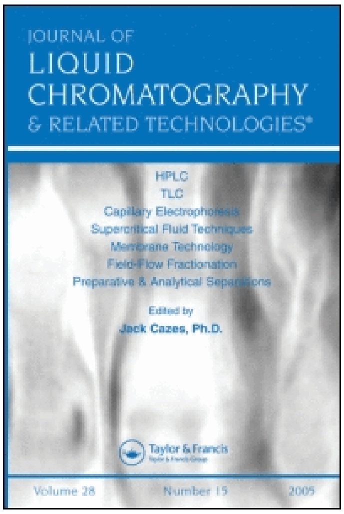

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SIZE EXCLUSION CHROMATOGRAPHY WITH LOW ANGLE LASER LIGHT SCATTERING DETECTION

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ABSTRACT

A review is given of the use of low angle laser light scattering (LALLS) detection in conjunction with size exclusion chromatography (SEC) to measure polymer molecular weight distributions without conventional SEC column calibration methods. A summary of light scattering theory is presented, and instrument configurations and principles of operation are described for two LALLS photometers. Also discussed are the overall performance of the SEC/LALLS technique and data from selected applications.

INTRODUCTION

Size exclusion chromatography (SEC) separates molecules of a polymer sample on the basis of hydrodynamic volume.(1) When the chromatograph is equipped only with a concentration-sensitive detector, i.e., conventional SEC, a molecular weight distribution (MWD) can be obtained from the chromatogram through use of a calibration function relating molecular weight and elution volume (V).(2) The calibration technique used in conventional SEC seldom gives the correct MWD, however. The molecular volume of a dissolved polymer depends on its molecular weight, chemical composition, molecular structure, and experimental parameters such as solvent, temperature, and pressure.(3) If the polymer sample and calibration standards differ in chemical composition the two materials probably will feature unequal molecular volume/weight relationships. Such differences also will persist between branched and linear polymers of identical chemical composition. Consequently, assumption of the same molecular weight vs. V relation for dissimilar calibrant and sample leads to transformation of the sample chromatogram to an apparent MWD. In some cases the relationship between polymer intrinsic viscosity ($[\eta]$) and molecular weight (M) of both sample and calibrant has been established for the SEC solvent and temperature conditions; i.e., the empirical Mark-Houwink(3) coefficients (K,a) in the equation

$$[\eta] = KM^a \tag{1}$$

have been determined. Under these circumstances the "universal" calibration approach can be utilized to calculate the correct MWD from the sample chromatogram.(1,4) However K,a values are not available for many samples.

A number of the limitations of conventional SEC can be overcome through use of a low angle laser light scattering (LALLS) detector attached in series with a concentration detector: SEC/LALLS.(5-7) Data from both detectors are used to obtain the absolute molecular weight at each point in a sample chromatogram. The SEC/LALLS technique is capable of quickly yielding the correct MWD without recourse to the approximate column calibration methods of conventional SEC.

This paper is a review of the principles of SEC/LALLS, LALLS detector design, SEC/LALLS sensitivity and accuracy, and selected applications of SEC/LALLS.

PRINCIPLES AND THEORY

The Rayleigh Factor

The measurement of molecular weight by light scattering methods makes use of the intensity of the light scattered at some angle, θ, as a function of the intensity impinging on the scattering volume. The ratio of these intensities may be put in a form(8)

$$R_\theta = J_\theta / I_0 V \tag{2}$$

where R_θ (cm^{-1}) is the Rayleigh factor and J_θ is the radiant intensity (watts/steradian) scattered at angle θ with respect to the illuminating beam of irradiance I_0 (watts/cm^2). The quantity V is the scattering volume, defined as the portion of the illuminated sample that is "seen" by the detector. In solution, light scattering data can be interpreted according to the fluctuation theory of light scattering.(3,9,10) The fluctuation theory dictates that light is scattered as a result of changes in the polarizability of the solution caused by assumedly random fluctuations in the solute concentration in small volume elements.

The MWD From SEC/LALLS

Figure 1 shows a schematic of a typical SEC/LALLS system. The sample effluent passes sequentially through the LALLS and concentration detectors and two chromatograms are obtained as shown in figure 2. As indicated in this figure, the concentration and LALLS detector responses will be used to obtain the absolute molecular weight at uniform increments through the chromatograms.

The Rayleigh factor at the i th increment ($R_{\theta,i}$) is the parameter obtained directly from scattering intensity measurements with the LALLS detector.

The quantity used to calculate the molecular weight in the i th interval, however, is the excess Rayleigh factor:

$$\bar{R}_{\theta,i} = R_{\theta,i(samp)} - R_{\theta,i\ (solv)} \tag{3}$$

Where $R_{\theta,i(samp)}$ and $R_{\theta,i(solv)}$ refer to the Rayleigh factors (at angle θ) of sample and solvent, respectively, in the i th interval of the chromatogram. At the low polymer concentration in the SEC effluent and for the low forward scattering angles (ca. 5^{o} to 7^{o}) used by LALLS detectors in most SEC/LALLS applications,(7) $\bar{R}_{\theta,i}$ is related to the molecular weight (M_i) in the i th interval through

$$\frac{Kc_i}{\bar{R}_{\theta,i}} = \frac{1}{M_i} + 2A_{2,i}c_i \tag{4a}$$

or,

$$M_i = \left[\frac{Kc_i}{\bar{R}_{\theta,i}} - 2A_{2,i}c_i\right]^{-1} \tag{4b}$$

In eqs 4 c_i is the sample concentration in i, K is an optical constant, and $A_{2,i}$ is the second virial coefficient. It should be emphasized that the linear form of these relationships results from the low angle configuration of the LALLS detectors; scattering

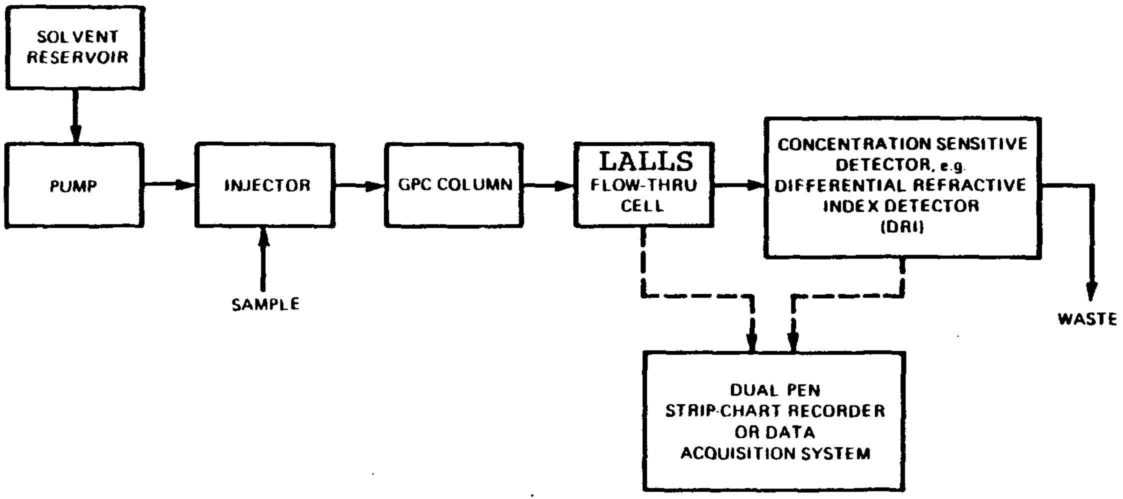


FIGURE 1 Block diagram of an SEC/LALLS system.

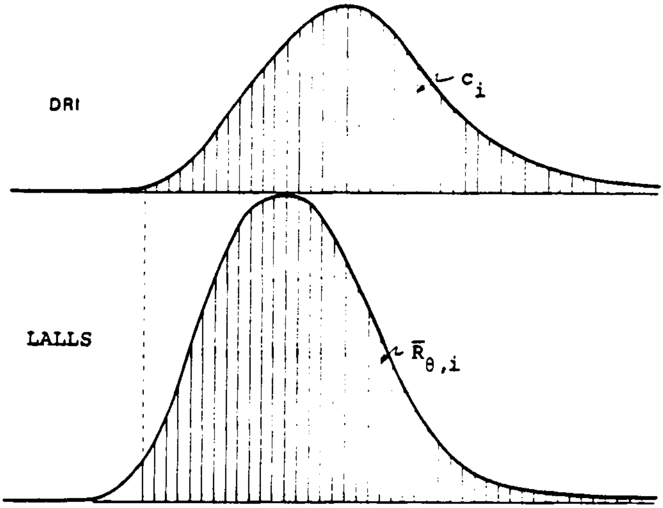


FIGURE 2 SEC/LALLS data analysis. Value of the excess Rayleigh factor ($\bar{R}_{\theta,i}$) and concentration (C_i) are evaluated at uniform intervals (i) and used to calculate the molecular weight at i.

measurements are carried out at such small angles that the scattering intensity is independent of polymer size or shape for most samples analyzed by SEC/LALLS.(11) The scattering intensity of the sample effluent can be continuously monitored and the data converted to absolute molecular weights through application of eqs 4. The concentration in i can be calculated from the concentration detector peak. From the injected mass (m), the effluent volume (V_i) passing through the detector cell during the i *th* interval, and the fraction $\frac{x_i}{\Sigma x_i}$ of the total peak area represented by the increment x_i

$$c_i = \frac{mx_i}{V_i \Sigma x_i} \qquad (5)$$

The optical constant K is defined, for the vertically polarized laser light source and annular collection optics of the LALLS detectors(8) described here, as:

$$K = \frac{(2\pi^2 n^2)}{(\lambda o^4 N)} \left(\frac{dn}{dc}\right)^2 (1 + \cos^2\theta) \qquad (6)$$

In eq 6 n, λo, and N represent the solution refractive index, *in vacuo* scattering wavelength, and Avogardro's number, respectively;

θ denotes the angle of observation of scattered radiation. The quantity dn/dc is the specific refractive index increment(12) and represents the incremental change in solution refractive index with sample concentration at the wavelength, temperature, and pressure of the SEC/LALLS measurements.

Since dn/dc reflects the different optical characteristics of the polymer and solvent, the value strongly depends on the chemical composition of both components.(12) However, differences in structure between species of identical composition, e.g., linear and branched polystyrene, have a relatively small effect on dn/dc. Extensive tabulations of dn/dc's have been published,(12,13) but when literature values are unavailable dn/dc can be measured with a differential refractometer.

Finally the second virial coefficient (A_2) is a quantity which depends in a complex manner on the temperature, pressure, molecular weight, size, and thermodynamics of polymer/solvent interactions.(3,10) The A_2 value can be found in the literature or determined from a static molecular weight analysis (below) of the polymer sample in the chromatographic solvent, and often $A_{2,i}$ can be assumed invariant with molecular weight without introducing significant uncertainty. However, high precision measurements on samples with wide MWD's require consideration of the variation of $A_{2,i}$ with molecular weight.(14) From static analysis of narrow MWD fractions of the polymer sample the constants α and β may be obtained, where

$$A_{2,i} = \beta M_i^{-\alpha} \tag{7}$$

This expression for $A_{2,i}$ then is substituted into the general LALLS equation (eqs 4) and the molecular weight analysis carried out. In many cases, however, it is adequate to estimate A_2 and set a constant value across the MWD, since at the low effluent concentrations encountered in SEC, $A_{2,i}c_i$ is usually small relative to the other terms of eqs 4. This will be illustrated further, below.

No column calibration methods are required when the SEC/LALLS technique is utilized. After K has been calculated and A_2 determined (or estimated), the LALLS and concentration detector responses can be used in eq 4b to calculate M_i at each interval of the chromatogram. In other words the concentration dector response is used to evaluate the concentration dependence of the LALLS detector signal. From the MWD found in this way the number -, weight -, and z-average moments can be calculated:(10)

$$\bar{M}_n = \frac{c_i}{\Sigma \frac{c_i}{M_i}} \tag{8a}$$

$$\bar{M}_w = \frac{c_i M_i}{\Sigma c_i} \tag{8b}$$

$$\bar{M}_z = \frac{c_i M_i}{\Sigma c_i M_i^2} \tag{8c}$$

Due to the substantial data handling task inherent in such analyses, data from both detectors in SEC/LALLS are usually collected and processed by a laboratory data system. Descriptions of data system hardware and software are given elsewhere, in addition to numerous applications examples.(6,7)

The Weight Average Molecular Weight ($\bar{M}_W$) from Static Analysis. Given a polymer solution of concentration c which is polydisperse with respect to molecular weight, the general LALLS equation becomes(11)

$$\frac{Kc}{\bar{R}_\theta} = \frac{1}{\bar{M}_w} + 2A_2c \tag{9}$$

Consequently, if K is known, measurement of the concentration dependence of $\bar{R}_\theta$ enables $\bar{M}_w$ and A_2 to be determined from the intercept and slope, respectively, of the linear dependence of $Kc/\bar{R}_\theta$ on c.

LALLS INSTRUMENTATION

Common Design Features. The two LALLS photometers described below have been designed with several common features, yet they are substantially different in terms of their range of applications. Both instruments allow absolute measurement of the Rayleigh factor of a liquid without use of calibrating solutions or standards. In other words from determinations of ratios of scattered to incident intensities at low forward angles, solution and solvent Rayleigh factors can be measured and used to determine absolute molecular weights of polymer samples (Principles and Theory).

The highly coherent HeNe laser light source (vertically polarized, 2 milliwatt, 6328Å) and optical configuration of the LALLS photometers allow scattering measurements to be made at angles less than 7° for most sample molecular weight measurements. This removes the need for the angular extrapolation procedure used in classical, high-angle light scattering experiments.(10,15) Futhermore, use of the laser light source produces a scattering volume of *ca.* $0.1 \mu\ell$; only single transient contaminants (e.g. dust particles) contribute to the sample scattering intensity and are readily distinguished from solution scattering. This reduces the sample clarification problems which have been associated with classical light scattering.(16

In each instrument a 5 mm wide flow-through sample cell body is held between two highly polished, fused-silica windows. The metal cell body has a $10 \mu\ell$ hole which contains the SEC column effluent. Incident laser light is focused to a beam diameter of 80 microns inside the sample cell to give the $0.1 \mu\ell$ scattering volume. When measuring the sample scattering intensity, a cone of scattered light at low forward angle θ to the incident beam is collected through an annular ring by a relay lens, focused through a field stop, and transmitted to the photomultiplier tube. Scattering from the air/glass interfaces is removed from view of the collection optics by the substantial length of the silica windows. Liquid/glass interface scattering is minimized by the highly polished glass surfaces.

<u>KMX-6</u>. The KMX-6 Low Angle Laser Light Scattering Photometer (Chromati is shown in Figure 3, with the design and optical configuration illustrated in Figure 4. The sample cell body/window assembly is held on a movable stage. The sample and cell windows can be examined by using a plunger to insert a microscope into the optical path. This is used to focus and align the cell after window cleaning. In place of the annulus, a hole is inserted in the axis of the optical path when measuring the incident beam intensity; movable attenuators diminish the intensity of the incident beam and insure measurement within the linear range of the photomultiplier tube.

The KMX-6 is compatible with high-temperature SEC systems; the flow-through sample cell body can be heated to 165° C. Several different annuli which allow scattering intensities to be measured from 2° to 7° are mounted on a movable wheel. Another wheel allows selection of a range of field stop diameters. A 15 mm thick Teflon cell body with a $150 \mu\ell$ sample volume is available for static $\bar{M}_w$ measurements and Rayleigh spectroscopy measurements (below). Acces-

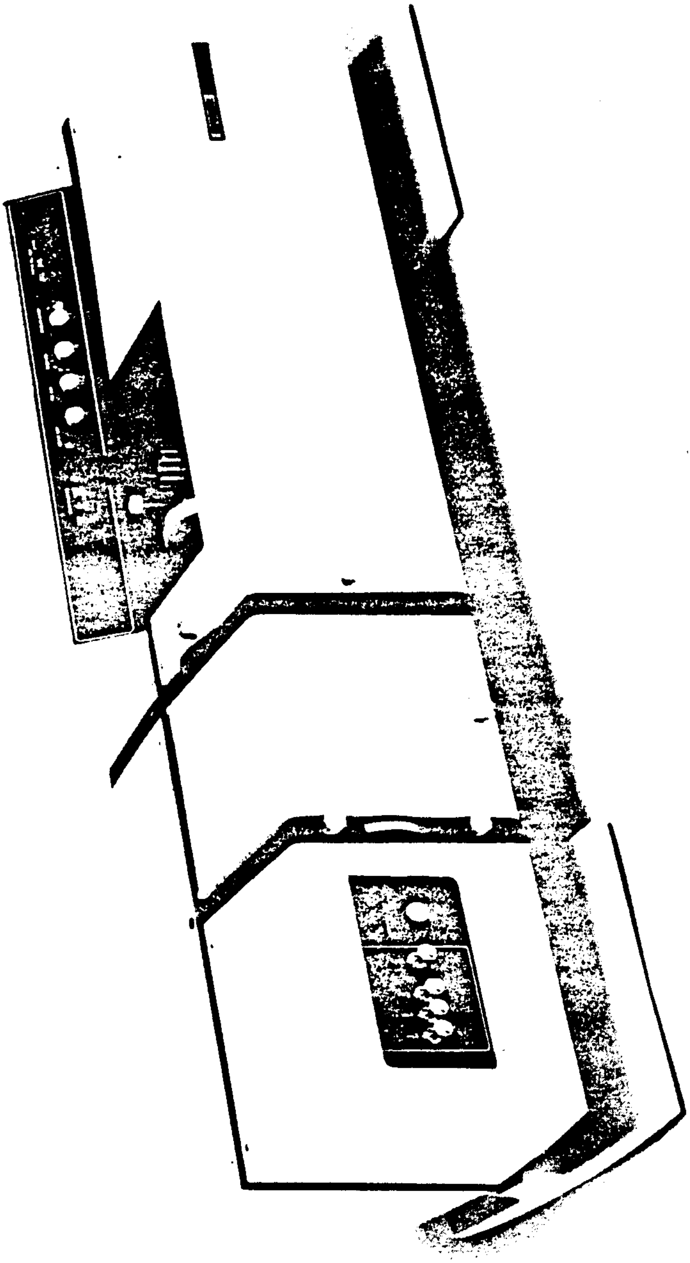


FIGURE 3 The KMX-6 Low Angle Laser Light Scattering Photometer.

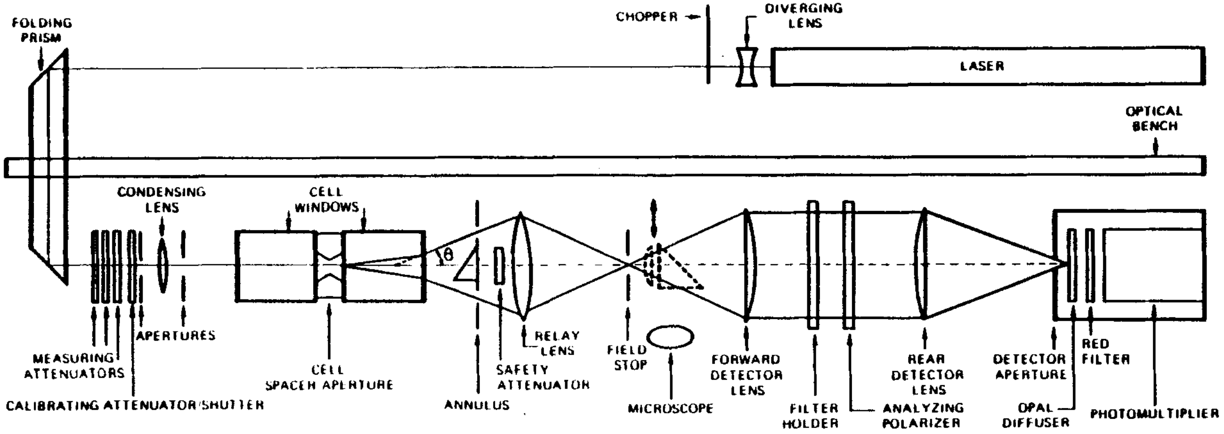


FIGURE 4 KMX-6 Optical diagram.

sories are available which allow scattering intensity measurements to be performed at angles of 90° and close to 180°. The KMX-6 can be used in Rayleigh spectroscopy(17,18) (i.e., quasielastic light scattering (QLS)) studies. With a photon counting interface and Model 64 Autocorrelator (Chromatix) the Doppler shifted frequency spectrum of scattered light from the sample can be analyzed.(19) Diffusion coefficient data and size-shape information about the sample can be obtained.(20)

The Rayleigh factor is obtained from two measurements: the first being a measurement of the signal, G_θ, from the photomultiplier caused by the light scattered from the sample at an angle θ; the second is a measurement of the signal from the photomultiplier caused by the illuminating beam transmitted through the sample, G_0. After measuring G_θ the annulus that defines the scattering angle is then removed, and the illuminating beam is permitted to pass directly through the sample and light-collecting optics to impinge on the photomultiplier, giving the G_0 signal. Since the scattered intensity is on the order of 10^{-5} times that of the incident intensity, a series of attenuators is inserted in the beam when measuring G_0. The only difference between the optical path in the G_θ measurement as compared to the G_0 measurement is the insertion of the attenuators. Thus, the Rayleigh Factor is obtained as the ratio of these two signals,

$$G_\theta = P_0 R_\theta \sigma' \ell' S_a T_\theta \tag{10a}$$

and

$$G_0 = P_0 D S_a T_0 \tag{10b}$$

Where P_0 is the radiant power incident on the sample, σ' is the solid angle over which the scattered light is viewed, ℓ' is the length parallel to the incident beam of the scattering volume, S_a is the anode sensitivity of the photomultiplier, D is the transmittance of the attenuators used in measuring the incident beam, and T_θ and T_0 are the transmittances, exclusive of the attenuators, for the scattered and incident beams. The terms σ' and ℓ' are dependent on the refractive index (n) of the scattering liquid. For any pair of G_0 and G_θ readings, S_a is held constant, and $T_\theta = T_0$. Therefore, eqs 10a and 10b yield

$$R_\theta = (G_\theta/G_0)\ D/\sigma'\ell' \tag{11}$$

Since the terms D, σ', and ℓ' are instrument constants, R_θ may be obtained without recourse to calibration standards, and eq 11 represents an absolute measurement of R_θ.

For SEC/LALLS, the KMX-6 is used to measure the excess Rayleigh factor at each interval i of the chromatogram. Typically, scattered light is collected at 6° - 7°, and the solvent (baseline) scattering is used, along with scattering from the eluting sample, to calculate the excess Rayleigh factor

$$\bar{R}_{\theta,i} = \frac{G_{\theta,i}\ (\text{smpl}) - G_{\theta,i}\ (\text{solv})}{G_0}\ D(\sigma'\ell')^{-1} \qquad (12)$$

Where $G_{\theta,i}$ (smpl) and $G_{\theta,i}$ (solv) denote the photomultiplier signals from sample and solvent, respectively. Measurement of G_0 is usually made before the sample run. The solvent value ($G_{\theta,i}$ (solv)) is typically calculated by constructing a baseline between G_θ (solv) readings on each side of the sample peak.

<u>LSD-100</u>. The LSD-100 Light Scattering Detector (Chromatix) is designed specifically for use in SEC; it is shown in Figure 5 with the design and dual-beam optical configuration illustrated in Figure 6. The SEC cell body/window assembly and its mount are "kinematically" configured. After removal for window-cleaning, the assembly is remounted without focusing or alignment by the operator. A thumbwheel moves the cell window faces in a plane perpendicular to the incident beam. No microscope/plunger is available, so the operator cannot inspect the cell contents during SEC/LALLS operation. The thumbwheel only is used to find a "clean" window position while monitoring the photomultiplier output with an analog recording device (e.g. strip chart recorder).

The LSD-100 has a fixed field stop and single annulus, where the latter collects scattered light at 5.1° - 6.1° (in air). Light from the source first is split into two beams: one passes through a fixed attenuator and bypasses the cell, while the other is incident on the cell. A chopper alternately allows the laser beam to follow the different pathways, but scattered and incident light impinge on the same photomultiplier detector. This continuous monitoring of incident light is used to compensate for fluctuations in laser intensity. A feedback circuit varies the photomultiplier voltage to maintain a constant incident intensity signal; thus no

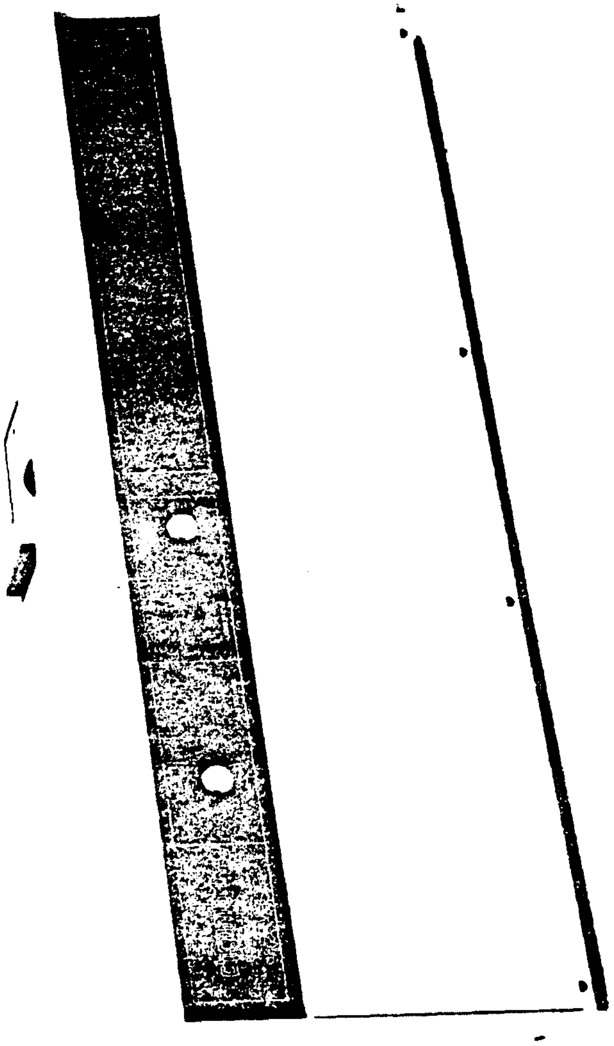


FIGURE 5 The LSD-100 Light Scattering Detector.

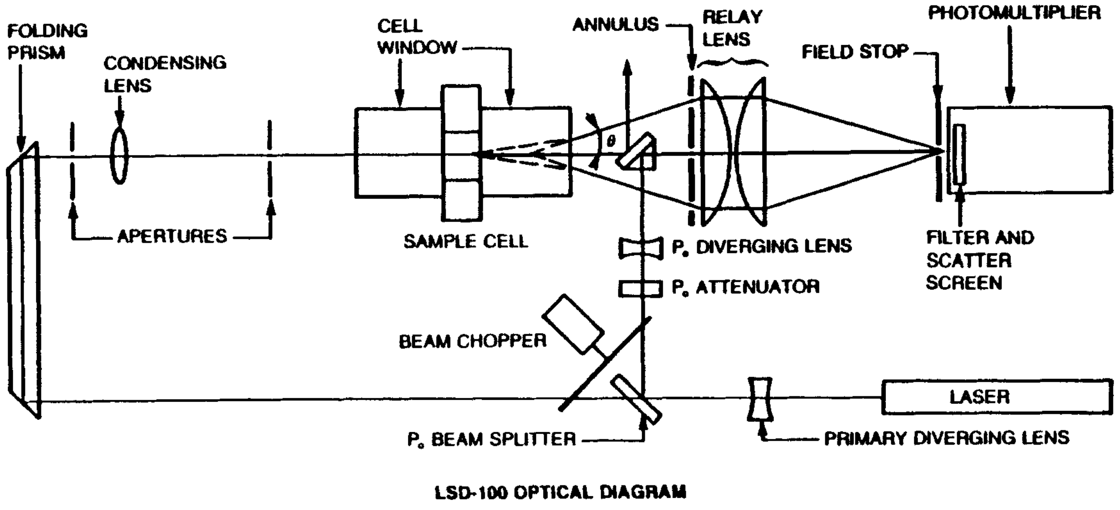


FIGURE 6 LSD-100 Optical diagram.

variations in scattering intensity result from changes in laser intensity. The operator uses a switch to select the photomultiplier signals P_0 and P_θ which result from the incident and scattered beams, respectively.

The Rayleigh factor is directly obtained following measurements of P_θ and P_0 but the "P_θ" and "P_0" optical paths (Figure 6) clearly differ. Therefore R_θ is related to the photomultiplier signals through

$$R_\theta = (P_\theta/P_0)\Phi Q(n) \tag{13}$$

where Φ is an instrument constant which takes account of the different transmittance and beam intensities along the two paths, and $Q(n)$ includes the refractive index (n) related terms $(\sigma'\ell')$ noted for the KMX-6 (above). The dependence of $Q(n)$ on n is calculated from the optical configuration and solvent refractive index; it is supplied as a listing with the instrument. The instrument constant Φ is determined at the factory from measurements on samples with known Rayleigh factors.

For SEC/LALLS, the LSD-100 is used to determine the excess Rayleigh factor at each interval i of the chromatogram from

$$\bar{R}_{\theta,i} = \left[\frac{P_{\theta,i}\ (\text{smpl}) - P_{\theta,i}\ (\text{solv})}{P_0}\right] \Phi Q(n) \tag{14}$$

Solvent values ($P_{\theta,i}$ (solv)) are calculated from a baseline which has been constructed from solvent scattering intensities on each side of the chromatogram. Measurement of P_0 and $P_{\theta,i}$ (smpl) during sample elution allows calculation of $\bar{R}_{\theta,i}$.

PERFORMANCE OF SEC/LALLS

Factors determining precision, sensitivity, and accuracy

The limiting factor in the precision of SEC/LALLS analyses is the signal to noise ratio (S/N) of the measured excess scattering intensity at each point in the chromatogram; i.e.
($G_{\theta,i}$ (smpl) - $G_{\theta,i}$ (solv) for the KMX-6, and
($P_{\theta,i}$ (smpl) - $P_{\theta,i}$ (solv)) for the LSD-100. The sensitivity and precision may be related by determining the minimum excess Rayleigh factor $\bar{R}_{\theta\text{-}min}$, at which the S/N achieves a specified value. Princi-

pal noise sources include particulate matter in the sample and homodyne beating of the Doppler-shifted scattered light.(18)

Homodyne noise is significantly reduced by the flowing conditions in SEC analyses.(5) The particulate concentration in SEC effluents is frequently much lower than encountered in static analyses. Thus $\bar{R}_{\theta-min}$ is generally lower for analyses than the value of $1 \times 10^{-6}\,cm^{-1}$ typically observed in static measurements for a S/N equal to 10.

The minimum detectable concentration, c_{min} may be related to $\bar{R}_{\theta-min}$ as

$$c_{min} = \frac{\bar{R}_{\theta-min}}{4.079 \times 10^{-6} \left(\frac{dn}{dc}\right)^2 n^2 \bar{M}_w} \qquad (15)$$

The terms in eq 15 are explained in the discussion of eqs 9 and 6, from which eq 15 is derived. The c_{min} term refers to a concentration in the LALLS detector flow-cell. To determine the minimum delectable concentration in the injected sample, c'_{min}, a calculation must be made based on the dilution of the sample during the chromatographic analysis. The extent of the dilution is dependent on the nature of the sample distribution and the analysis time, and will vary for different fractions of the sample. As a simple approximation, the dilution factor may be taken as the ratio of the elution width of the sample peak, V, to the injected volume, v. Thus,

$$c'_{min} = \frac{V}{v}\, c_{min} \qquad (16)$$

The SEC/LALLS chromatogram of 0.18 mg of a narrow MWD polystyrene standard SRM 705 (National Bureau of Standards) is shown in Figure 7. The KMX-6 LALLS photometer was set at nearly maximum gain to demonstrate the sensitivity and ratio of signal-to-noise obtained. The maximum usable photomultiplier output of the KMX-6 is reached at signals of 2.5 volts ($G_\theta = 2500$). Above this level, non-linear responses may occur. Thus a photomultiplier voltage was selected such that the total scattering from the solvent and solute did not exceed a G_θ of 2500.

For the analysis in Figure 7, the gain was set to yield a $G_{\theta\,-\,solvent}$ of 2000. The corresponding G_0 was 1236. The KMX-6 peak (B) maximum occurred at a $G_{\theta\,-\,solution}$ of 2310, i.e.,

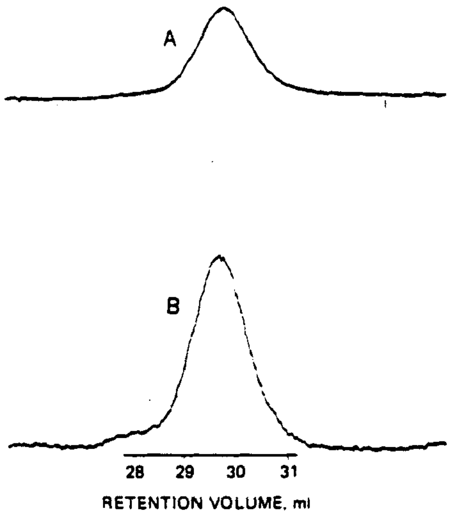


FIGURE 7 KMX-6/GPC Chromatogram of NBS 705 at near maximum KMX-6 gain. Top, DRI response, attenuation x4; bottom, KMX-6 response as G_θ. The $G_{\theta-solution}$ at the peak maximum was 2310, above 2500 non-linear response may occur. Conditions: Quantity injected: 0.18 mg NBS 705; mobile phase: toluene, 1 ml/min; $G_{\theta-solvent}$ = 2000, G_o = 1236; D = 1.208 x 10^{-8}, $(\sigma' \ell')^{-1}$ = 747.76; K = 1.099 x 10^{-7} mole-cm^2/g^2; 0.2 mm field stop, 6-7° annulus.

$\bar{G}_\theta$ = 310 and $\bar{R}_\theta$ = 2.27 x 10^{-6}. The $\bar{G}_\theta$ signal exceeded the peak-to-peak noise by a factor of 10 at an $\bar{R}_\theta$ of 5.85 x 10^{-7}, corresponding to a c_{min} of 2.66 x 10^{-5} g/ml, assuming a polystyrene sample of M_w = 200,000.

As given by eq 15 the sensitivity of the LALLS detector increases with increasing molecular weight. With samples of extremely wide molecular weight distribution, it may not be possible to obtain on-scale LALLS response at both high and low molecular weight with a single photomultiplier gain. In such cases the gain should be increased at low molecular weights. In addition to uncertainty in the determination of the excess scattering intensity, the accuracy of calculated molecular weights is affected by error in the KMX-6 and LSD-100 instrumental constants (D, σ',ℓ'), and (Φ, Q(n)), respectively. Also some error will be introduced from the measurement of the KMX-6 and LSD-100 incident intensities G_o and P_o, respectively. The probable molecular weight error propagated from these terms is less than 3%. It should be noted that for a single instrument this error is systematic, not random, and therefore does not affect reproducibility.

As seen from eq 15, for a particular molecular weight the minimum detectable concentration by LALLS depends of the magnitude of the square of dn/dc. Since this parameter is strongly solvent dependent, judicious choice of chromatographic solvent can considerably extend the molecular weight detectability range. This is illustrated by the polyol analysis shown in Figure 8. The value of dn/dc for THF and methanol is *ca.* 0.05 ml/gm and 0.15 ml/gm respectively Consequently the relatively low molecular weight material gives a light scattering signal which is 6X higher in methanol than THF. For higher molecular weight polyols, however, the light scattering response in THF may be sufficient for accurate SEC/LALLS analysis.

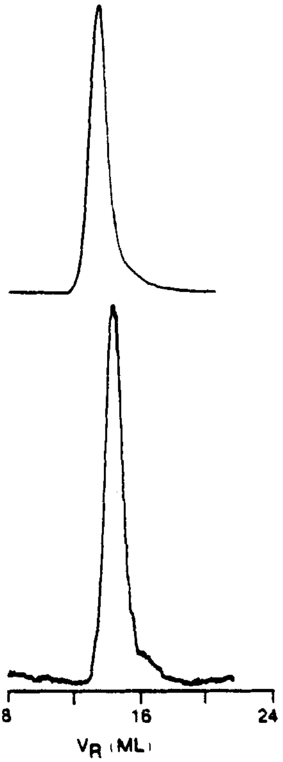


FIGURE 8 SEC/LALLS (KMX-6) analysis of a poly ethylene/propylene glycol sample. Sample load, 2.5 mg; Columns, Aquapore™ (OH-4000, OH-1000, OH-500, OH-300, OH-100); Conditions, methanol, 0.7 ml/min, optical constant (K) = 1.292×10^{-7} mole-cm^2/g^2. Results: $\bar{M}_w = 3060$, $\bar{M}_n = 2850$.

Comparison of SEC/LALLS data with that obtained from other methods.

Table 1 presents static $\bar{M}_w$ and $\bar{M}_w/\bar{M}_n$ data obtained by LALLS and SEC/LALLS, respectively, for some "standard" materials along with supplier's specifications and data from other methods. Although $\bar{M}_z$ and $\bar{M}_z/\bar{M}_w$ values are sometimes quoted by suppliers the $\bar{M}_w/\bar{M}_n$ ratio is most commonly used as a polydispersity index. While not deprecating their value in MWD analysis, $\bar{M}_z$'s and $\bar{M}_z/\bar{M}_w$'s have been omitted from the discussion here. The table primarily is presented to illustrate the range of applicability of the SEC/LALLS technique to polymer molecular weight analysis.

In most cases satisfactory agreement is shown between the $\bar{M}_w$ from static LALLS and $\bar{M}_w$ from SEC/LALLS. (The marked difference between the static LALLS and SEC/LALLS $\bar{M}_w$ for polyethylene SMR 1476 has been discussed elsewhere;(21) passage through the SEC columns may have removed high molecular weight material which was present during the static analysis). However, apparently SEC/LALLS usually gives a lower $\bar{M}_w/\bar{M}_n$ than that found by the supplier using other methods (e.g., conventional SEC, or classical light scattering and membrane osmometry). The nature of the SEC/LALLS measurement contributes to these discrepancies. Due to band-spreading and assorted hardware mixing of the sample, the LALLS detector cell contains a mixture which is polydisperse in molecular weight. As pointed out before,(5,14) the weight average of species in interval i ($M_{w,i}$) is calculated directly from the SEC/LALLS data; summation over all intervals i (eqs 8a and 8b) always yields the correct sample $\bar{M}_w$, but gives a $\bar{M}_n$ which is larger than the correct value. Of course, poor column resolution contributes towards low $\bar{M}_w/\bar{M}_n$ from SEC/LALLS. In conventional SEC band-spreading always raises $\bar{M}_w/\bar{M}_n$ above the correct value.

Recent experiments in our laboratory have examined this aspect of SEC/LALLS.(24) A band-spreading correction procedure(27) has been applied to SEC/LALLS data to compensate for molecular weight polydispersity in the LALLS detector cell. The effect of this approach was to increase the $\bar{M}_w/\bar{M}_n$ by 1% - 4% for narrow MWD polystyrene standards ($\bar{M}_w/\bar{M}_n < 1.2$), while a 5% increase in $\bar{M}_w/\bar{M}_n$ was noted for a broader MWD ($\bar{M}_w/\bar{M}_n = 1.7$) polystyrene standard. The latter material (National Bureau of Standards SRM 706) was also analyzed by conventional SEC: application of the band-spreading correction yielded congruence of the SEC and SEC/LALLS $\bar{M}_w$ and $\bar{M}_w/\bar{M}_n$. Errors of about 1% due to band-spreading in SEC/LALLS $\bar{M}_w/\bar{M}_n$ values were found by Basedow(25) during analyses of dextrans, whereas uncorrected and corrected (for band-spreading) conventional SEC $\bar{M}_w/\bar{M}_n$ values differ by as much as 22%.

TABLE 1

MATERIAL*	LALLS		SEC/LALLS			OTHER METHODS			SUPPLIER**
	$\bar{M}_w$	Ref.	$\bar{M}_w$	$\bar{M}_w/\bar{M}_n$	Ref.	$\bar{M}_w$†	$\bar{M}_w/\bar{M}_n$†	Methods	
PE SRM1475	54600^a	(21)	55800	2.94^a	(21)	52500	2.87	i	NBS
PE SRM1484	118000^a	(21)	109500	1.10^a	(21)	119600	1.19	ii	NBS
PE SRM1476	$*221000^a$	(21)	100400	3.59^a	(21)	105000(22)	4.2(22)	iii	NBS
PS SRM706	260000^b	(8)	275900	1.73^b	(6)	257800	2.1	iv	NBS
PS SRM705	178000^b	(8)	175800	1.02^c	(6)	179300	1.05	ii	NBS
PS PC61004	962^c	(23)	958	1.13^c	(23)	1024	<1.30	v	P.CH.
PS PC12C	2940^c	(23)	2930	1.10^c	(23)	2400	<1.06	v	P.CH.
PS PC116	3900^c	(23)	3800	1.03^c	(23)	3600	<1.10	v	P.CH.
PS PC41220	NA		17300	1.06^c	(24)	20400	<1.06	vi	P.CH.
DX 3	33900^d	(25)	35100	1.411^d	(25)	32300(25)	1.42(25)	vii	NA
DX 4	64100^d	(25)	67100	1.685^d	(25)	59200(25)	1.707(25)	vii	NA
DX 5	64900^d	(25)	65100	1.797^d	(25)	61950(25)	1.809(25)	vii	NA
DX T-10	NA		9260	1.49^e	(26)	9900	1.90	iii	PH.
DX T-40	NA		41000	1.28^e	(26)	41000	1.46	iii	PH.
DX T-70	NA		67900	1.37^e	(26)	64400	1.57	iii	PH.
DX T-500	NA		534000	2.10^e	(26)	517000	1.71	iii	PH.

** NBS = National Bureau of Standards; P.CH. = Pressure Chemical Co.; PHR = Pharmacia NA = Not available *Prefixes PE, PS, and DX denote polyethylene, polystyrene, and dextran, respectively. †References given when molecular weight data not that from supplier.

(a) Trichlorobenzene at 135° C (b) Toluene/ambient (c) THF/ambient (d) Water/ambient (e) 0.05 M aqueous phosphate buffer, pH7.

(i) $\bar{M}_w$ from classical light scattering; $\bar{M}_n$ from conventional SEC (ii) $\bar{M}_w$ from classical light scattering, $\bar{M}_n$ from membrane osmometry (iii) $\bar{M}_w$, $\bar{M}_n$ from SEC fractionation and summation (iv) $\bar{M}_w$ from classical light scattering; $\bar{M}_w/\bar{M}_n$ from viscometry of fractionated samples. (v) $\bar{M}_w$ from viscosity (=$\bar{M}_v$); $\bar{M}_w/\bar{M}_n$ from "method of preparation" and SEC data. (vi) $\bar{M}_w$ from light scattering; $\bar{M}_w/\bar{M}_n$ from "method of preparation" and SEC data. (vii) $\bar{M}_w$ and $\bar{M}_n$ from conventional SEC, corrected for band-spreading.

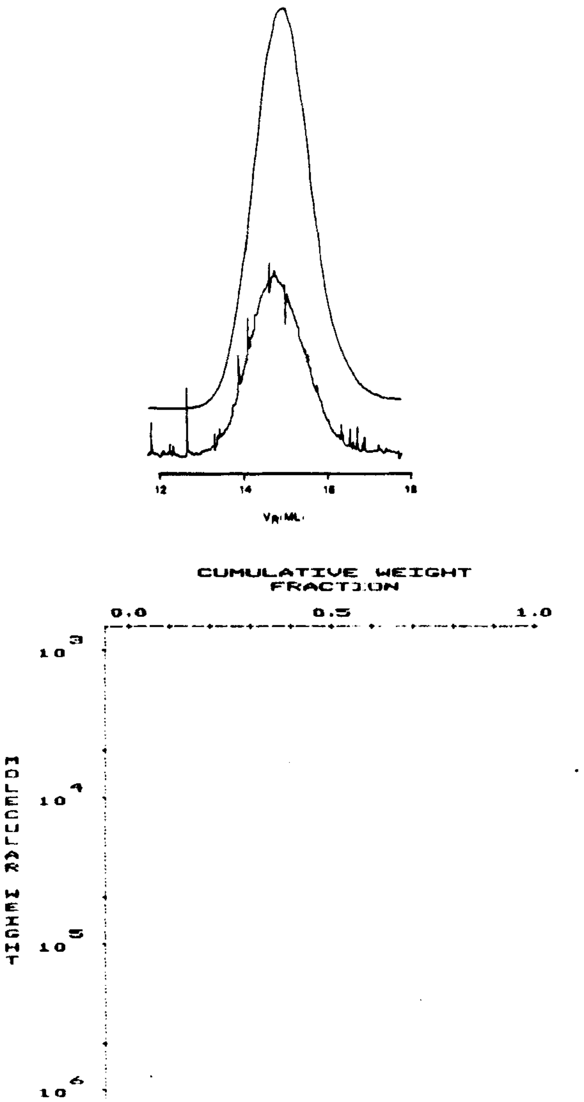
The technique of MWD analysis used by the supplier should, therefore, be carefully scrutinized. For example the supplier (Pharmacia) of some of the standard dextrans listed in Table 1 obtained $\bar{M}_w/\bar{M}_n$ from "gel filtration" fractionation; apparently no consideration was given to band-spreading effects. Figure 9 presents the chromatograms obtained from the SEC/LALLS analysis of one of the dextrans listed in Table 1; in addition the figure shows MWD information given by a computer analysis of the data.

POLYMER BRANCHING ANALYSIS

Qualitative Data.

As stated earlier (Introduction) conventional SEC analysis of branched polymers will usually lead to an apparent MWD. This results from the dissimilar molecular size/weight relationship of the branched sample and linear calibrant. The SEC/LALLS technique, however, gives the absolute molecular weight of the components at a particular retention value (V_R), and is capable of providing the correct MWD of branched samples. But the polydispersity in molecular weight at V_R can be significant, depending on the branching characteristics of the sample.(28) If linear polymers of low molecular weight elute at V_R with high molecular weight, densely branched polymers, the SEC/LALLS method gives the absolute weight average molecular weight of the polydisperse mixture at V_R (above).

However, in cases where branching is suspected, SEC/LALLS can be used to provide corollary or qualitative data. Figures 10a and 10b show SEC/LALLS chromatograms obtained for epoxy resin samples. The samples were suspected of containing different amounts of branche material. In Figure 10b (sample B), the distinct "shoulder" on the low retention volume (V_R) side of the concentration detector response curve suggests the presence of a high molecular weight component which is not apparent in Figure 10a (sample A). The shoulder may represent high molecular weight branched species. Comparison of the light scattering curves supports this analysis since LALLS depends primarily on concentration and molecular weight. Light scattering peak assymmetry in Figure 10b reflects the fact that material in the DRI peak shoulder gives a very high light scattering response, indicating high molecular weight material. Note also the four-fold difference in the weight average molecular weight ($\bar{M}_w$) of the samples Conventional SEC column calibration with linear standards would probably give a much lower and incorrect $\bar{M}_w$ for sample B. However, the



```
NUMBER AVG.    MW =  6.41688E+3
WEIGHT AVG.    MW =  9.09358E+3
Z-AVG.         MW =  1.35098E+4

RATIO:
     MZ        :       MW        :  MN
 2.10536E+0    :   1.41713E+0    :  1
```

FIGURE 9 Top Analysis of dextran with SEC/LALLS (KMX-6). The upper curve is the DRI response, lower curve is LALLS signal.
Sample: Dextran T-10 (Pharmacia), 2.5 mg;
Columns: Aquapore (OH-4000, OH-1000, OH-500, OH-300);
Conditions: 0.05 M phosphate, pH7.0, 0.7 ml/min.
Results: $\bar{M}_w = 9260$, $\bar{M}_n = 6220$

Bottom Cumulative weight fraction plot calculated from the data shown in the chromatograms, using an LDS-2 laboratory data system with MOLWT software (Chromatix). Note that the $\bar{M}_w$, $\bar{M}_n$ differ from those given above and in Table 1; the latter represent mean values of four runs.

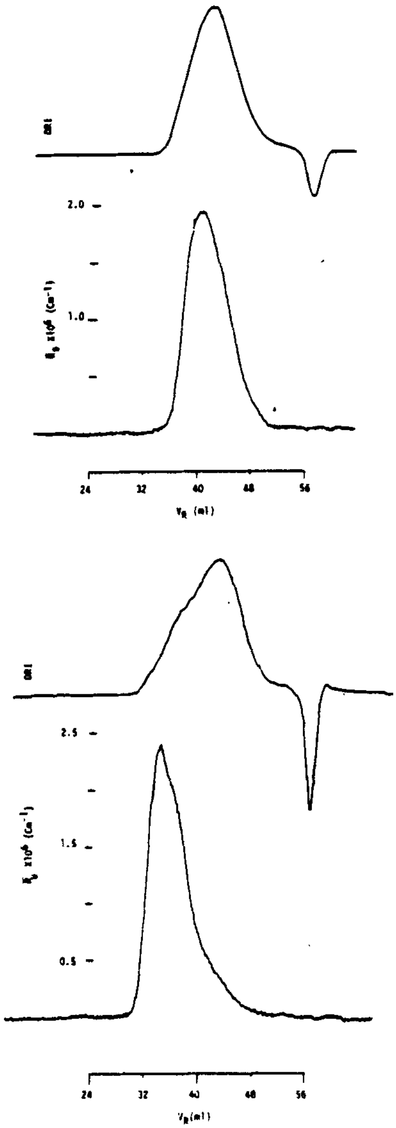


FIGURE 10 SEC/LALLS (KMX-6) analysis of epoxy resins. Columns μ-Styragel(R) (500, 10^3, 10^4, 10^5, 10^6 A°); Conditions: THF, 2 ml/min. (a) epoxy resin A, 4.02 mg., optical constant K = 2.819 x 10^{-7} mole-cm^2/g^2, $\bar{M}_w$ = 12600, $\bar{M}_n$ = 5490; (b) epoxy resin B, 1.17 mg, optical constant K = 2.858 x 10^{-7} mole-cm^2/g^2, $\bar{M}_w$ = 51700, $\bar{M}_n$ = 5690.

extent of mixing of branched and linear components in the detector cell is unknown, since no information is available about the branching characteristics of the epoxy; $\bar{M}_z$ and $\bar{M}_n$ values calculated from this data must be considered carefully.

Quantitative Branching Analysis. Quantitative analysis of polyethylene branching has been carried out using SEC/LALLS.(29) Other work has applied SEC/LALLS to the analysis of branching in polyvinylacetate and polychloroprene.(24) In the latter studies the universal calibration technique of SEC was used in conjunction with SEC/LALLS. More work in this direction is expected to accompany the increased use of SEC/LALLS in polymer molecular weight analysis.

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REFERENCES

(1) Z. Grubisic, P. Rempp, and H. Benoit, J. Polym. Sci. B, 5, 753(1967)

(2) W. W. Yau, J. J. Kirkland, and D. D. Bly, "Modern Size Exclusion Chromatography", John Wiley and Sons, New York, N. Y., 1979.

(3) P. J. Flory, "Principles of Polymer Chemistry", Cornell University Press, Ithaca, N. Y., 1953

(4) A. R. Weiss and E. Cohn-Ginsburg, J. Polym. Sci. B, 7, 379(1969)

(5) A. C. Ouano and W. Kaye, J. Polym. Sci. Polym. Chem. Ed., 12, 1151(1974)

(6) M. L. McConnell, Am. Lab., 10 (5), 63(1978).

(7) Application Notes LS-2, LS-3, LS-5, Chromatix, 1977-1979.

(8) W. Kaye and A. J. Havlik, Appl. Opt., 12 (3), 541(1973).

(9) K. A. Stacy, "Light Scattering in Physical Chemistry", Academic Press, New York, N. Y., 1956.

(10) C. Tanford, "Physical Chemistry of Macromolecules", John Wiley and Sons, New York, N. Y., 1961.

(11) Application Note LS-1, Chromatix, 1978.

(12) M. B. Huglin, in "Light Scattering from Polymer Solutions", M. B. Huglin, ed., Academic Press, New York, N. Y., 1972, p 165 ff.

(13) Applications Note LS-7, Chromatix, 1979.

(14) A. C. Ouano, J. Chromatogr., 118, 303(1976).

(15) B. H. Zimm, J. Chem. Phys., 16, 1093(1948).

(16) B. E. Tabor, in "Light Scattering from Polymer Solutions," M. B. Huglin, ed., Academic Press, New York, N. Y., 1973, p. 1 ff.

(17) B. Chu, "Laser Light Scattering", Academic Press, New York, N. Y., 1974.

(18) B. J. Berne and R. Pecora, "Dynamic Light Scattering", Wiley-Interscience, New York, N. Y., 1976.

(19) Application Note LS-8, Chromatix, 1978

(20) N. C. Ford, Chemica Scripta, 2, 193(1972)

(21) T. B. MacRury and M. L. McConnell, J. Appl. Polym. Sci., 24, 651(1979).

(22) H. L. Wagner and F. L. McCrackin, J. Appl. Polym. Sci., 21, 2833 (1977).

(23) Application Note LS-10, Chromatix, 1979

(24) M. L. McConnell and R. C. Jordan, in "Recent Advances in Size Exclusion Chromatography," ACS Symposium, 178th ACS National Meeting, Sept. 10-14, 1979, Washington, DC.

(25) A. Basedow, Deutsche Diskussionstagung fur Anwendug der HPLC, Vol. 2, Waters Associates, Konigstein/T Annus FRG, 1977, pp 175-200.

(26) Application Laboratory data, Chromatix, 1979.

(27) W. W. Yan, H. J. Stoklosa, and D. D. Bly, J. Appl. Polym. Sci., 21, 1911(1977).

(28) A. E. Hamielec, A. C. Quano, and L. L. Nebenzahl J. Liquid Chromatog., 1(4), 527(1978).

(29) D. E. Axelson and W. C. Knapp, J. Appl. Polym. Sci. (in press).