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Principles of Triple Detection GPC/SEC, Part 2: The Low Angle Light Scattering Detector

Gel Permeation Chromatography (GPC), also known as Size Exclusion Chromatography (SEC), is a popular analytical tool for characterizing natural and synthetic polymers and proteins. Rapid developments in industrial, biomedical, pharmaceutical and food technologies have provided a strong push to extend the capability of conventional GPC beyond relative MW measurements. In response, many users have implemented the technique of Triple Detection, which refers to the combination of concentration, viscosity, and light scattering detectors, to obtain absolute MW plus information on structure and conformation. This article, the second in a series of three, will explore the principles of measurement and operation of the Low Angle Light Scattering detector (LALS), which is the simplest and most powerful light scattering detector in use today.

Theory

The fundamental equation for scattering of light from polymer solutions is the Zimm equation.

$$[1] \quad \frac{KC}{R_{\theta}} = \frac{1}{MP_{\theta}} + 2A_2C$$

M is the molecular weight of the polymer sample and C is the sample concentration

A_2 is the second virial coefficient of the solution, which corrects for the interaction of polymer molecules with each other. A_2 may be calculated from the concentration dependence of the light scattering signal. However, that is beyond the scope of this discussion.

R_{θ} is the excess Rayleigh scattering ratio of the solution above that of the pure solvent, measured at angle θ with respect to the incident beam.

$$[2] \quad R_{\theta} = k \cdot \frac{I_{\theta}}{I_0} = k \cdot \frac{Lals}{I_0}$$

I_0 is the irradiance of the incident laser. I_{θ} is the excess intensity of the scattered light above that of the pure solvent at angle θ ; in the present case it is the baseline-corrected LALS signal. k is an instrument constant related to the scattered light collection efficiency. A schematic of the optical arrangement of the LALS is shown in Figure 1.

P_{θ} is the particle scattering factor. It is a measure of the angular dissymmetry of the scattered light and is related to the size and the angle at which the scattering is determined. Much of light scattering science is devoted to determination of P_{θ} but the beauty of LALS is that it can be ignored.

- P_{θ} is exactly equal to unity for all molecules when θ is zero.
- P_{θ} is 0.98 for molecules with radius of gyration (RG) of 150 nm when θ is 7 degrees.
- R_G of 150 nm constitutes the upper limit of separation of GPC.

Therefore, with LALS multiple angle measurements are unnecessary because extrapolation or correction for angular dissymmetry is unnecessary. This reduction to a single angle greatly simplifies the processing of data from multiple detectors.

K is a composite of optical and fundamental constants.

$$[3.] \quad K = \frac{2p(\pi \cdot n_0 \cdot v)^2}{N_A \cdot \lambda_0^4}$$

n_0 is the refractive index of the solvent,

v is the refractive index increment of the polymer solution,

N_A is Avogadro's number,

λ_0 is the wavelength of the incident light in vacuum.

p is an integer equal to 2 for vertically polarized incident light, 1 for un-polarized.

The latter three parameters (N_A , λ_0 , and p) are instrument constants and can be merged with the detector constants k and I_0 in equation 2 to form a new constant which we will call Lals.Cal. Equation 1 can now be rearranged to separate the variables of molecular weight and concentration from the other parameters.

$$[4.] \quad MC = \frac{1}{\frac{n_0^2 v^2}{\text{Lals.Cal} \cdot \text{Lals}} - 2A_2}$$

The 2nd virial coefficient A_2 is typically much smaller than the first term in the denominator, so equation 4 can be simplified by neglecting the A_2 term.

$$[5.] \quad MC \cong \frac{\text{Lals.Cal} \cdot \text{Lals}}{n_0^2 v^2}$$

Equation 4 is the general equation but equation 5 will be used in the present discussion for purposes of simplification.

Elution Profiles

The elution profile consists of successive fractions of the eluant sampled at equally spaced time intervals i . Each fraction will be characterized by its molecular weight M and concentration C_i . C_i is determined from the following equation derived in part 1 of this series, *The Deflection Refractometer*.

$$[6.] \quad C_i = \frac{RI_i \cdot n_0}{RI.Cal \cdot v}$$

RI_i is the signal from the RI detector at interval i , and $RI.Cal$ is the detector calibration constant. Substituting equation 6 into equation 5 yields the elution profile of molecular weight.

$$[7] \quad M_i \cong \frac{\text{Lals.Cal} \cdot \text{Lals}_{i-\delta}}{n_0^2 \cdot v^2 \cdot C_i} = \frac{\text{Lals.Cal} \cdot \text{RI.Cal} \cdot \text{Lals}_{i-\delta}}{n_0^3 \cdot v \cdot \text{RI}_i}$$

Note that the array index for the LALS signal is offset from that of the RI detector by an amount δ . This *detector offset* reflects the fact that the two detectors do not measure each fraction simultaneously. They have a time (volume) separation between them corresponding to the volumes of the two detector plus the volume of the interconnecting tubing. Equation 7 reveals that molecular weight elution profile is proportional to the ratio of the LALS detector signal to the RI detector signal.

An elution profile for molecular weight on a broad distribution polystyrene sample is shown in Figure 2, overlaid with the RI and LALS signals. Notice that the M_i profile has more noise at each end than in the middle. This is due to the RI and LALS signals being lower in magnitude near the ends of the peaks so the noise is relatively higher. In fact, the calculation of M_i becomes so unreliable on the ends that the calculation must be truncated at some point and M_i is obtained for the rest of the distribution by extrapolation. The extrapolation is shown in Figure 2 as the dashed line and is much more extensive on the low molecular weight end.

Determination of Instrument Constants (Calibration)

The instrument constants RI.Cal, Lals.Cal, and δ are determined from the chromatograms of a narrow distribution polymer standard, for example, polystyrene standard 90K shown in Figure 3. The offset δ is easily determined as the difference in the peak positions. Figure 4 shows the same chromatograms after the offset is applied. The RI calibration constant is determined from the RI peak area and dn/dc as shown previously in Part 1. The LALS calibration constant is determined from the LALS peak area, which is directly proportional to the weight-average molecular weight as shown in the section below.

Molecular Weight Distribution

The molecular weight distribution can be represented in several ways; the most important being the number-average molecular weight M_N and the weight-average molecular weight M_W . M_N is defined as the average molecular weight (molar mass) over the successive fractions of the sample with the statistical weight of each fraction being the number of molecules, or molar concentration N_i . The molar concentration is simply the ratio of the weight concentration C_i and molar mass M_i , which are determined by equations 6 and 7, respectively.

$$[8] \quad N_i = \frac{C_i}{M_i}$$

The number-average molecular weight is defined therefore as follows.

$$[9] \quad M_N = \frac{\sum N_i M_i}{\sum N_i} = \frac{\sum C_i}{\sum \frac{C_i}{M_i}}$$

The weight-average molecular weight is defined as the average molecular weight (molar mass) over the successive fractions of the sample with the statistical weight of each fraction being the mass of molecules, or weight concentration C_i .

$$[10.] \quad M_w = \frac{\sum C_i M_i}{\sum C_i}$$

The summations in equations 9 and 10 are directly related to peak areas in the GPC chromatogram.

- The sum of C_i is proportional to the area of the RI detector peak, per equation 6.

$$[11.] \quad \sum C_i = \frac{n_0 \sum RI_i}{RI.Cal \cdot v} \propto RI \text{ Peak Area}$$

- The sum of $C_i M_i$ is proportional to the area of the LALS detector peak, per equation 5.

$$[12.] \quad \sum C_i M_i = \frac{Lals.Cal \sum Lals_i}{n_0^2 \cdot v^2} \propto Lals \text{ Peak Area}$$

Combining equations 10-12 reveals a very simple relationship between M_w and the chromatographic peak areas.

$$[13.] \quad M_w \propto \frac{Lals \text{ Peak Area}}{RI \text{ Peak Area}}$$

- The sum of C_i/M_i is proportional to the area of the molar concentration peak per equation 8. So M_N can also be considered a ratio of peak areas as follows.

$$[14.] \quad M_N \propto \frac{RI \text{ Peak Area}}{\text{Molar Conc Peak Area}}$$

However, molar concentration is a derived function, not a detector signal, so M_N does not have the same type of simple relationship to detector peak areas as does M_w . The derivation of molar concentration is often results in greater error for determination of M_N than for M_w . The reason for this is illustrated in Figure 5 where the relevant peaks are overlaid for a broad distribution sample, polystyrene in THF. M_w can be determined very precisely because it is simply proportional to the ratio of the LALS and RI peak areas, both of which have excellent signal/noise. M_N is determined by the peak area of molar concentration, which has considerable noise on the long elution side of the peak. The excessive noise is inherent in a broad distribution sample and arises from the fact that molar concentrations are higher where the molar mass, and hence the LALS signal, is lower. M_N will therefore be determined by light scattering with substantially less precision than will M_w for broad distributions. Narrow distribution samples do not exhibit this noise problem.

Figure 1. Schematic of Low Angle Light Scattering (LALS) Detector. « Back

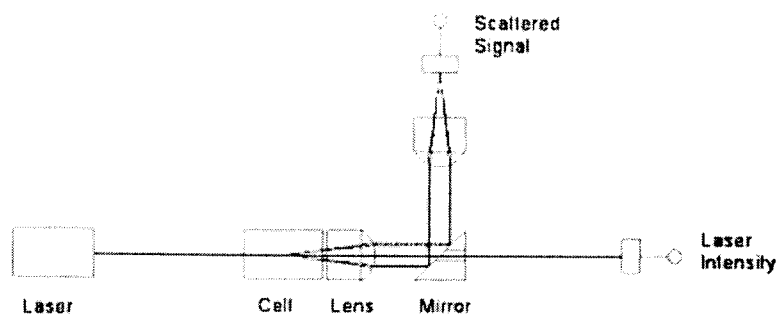


Figure 2. Log M elution profile overlaid with RI and LALS chromatograms for a Broad Distribution Polystyrene Sample. « Back

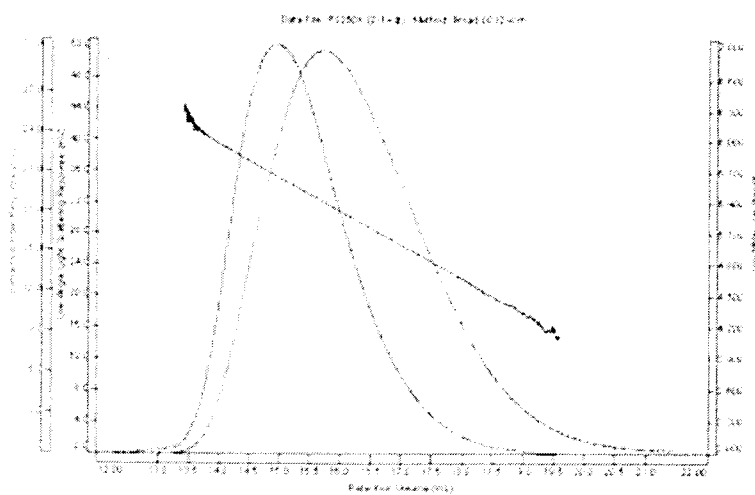


Figure 3. RI and LALS Chromatograms of a Narrow Distribution Polystyrene Standard. « Back

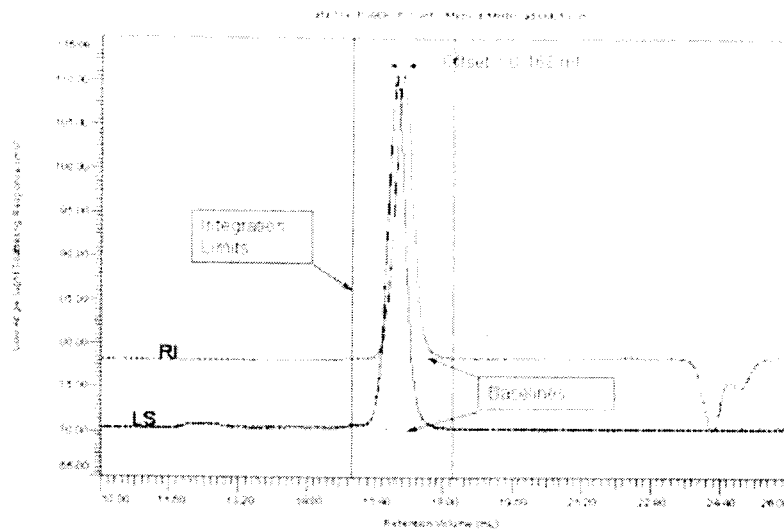


Figure 4. RI and LALS Chromatograms of a Narrow Distribution Polystyrene Standard with Detector Offset Applied. [« Back](#)

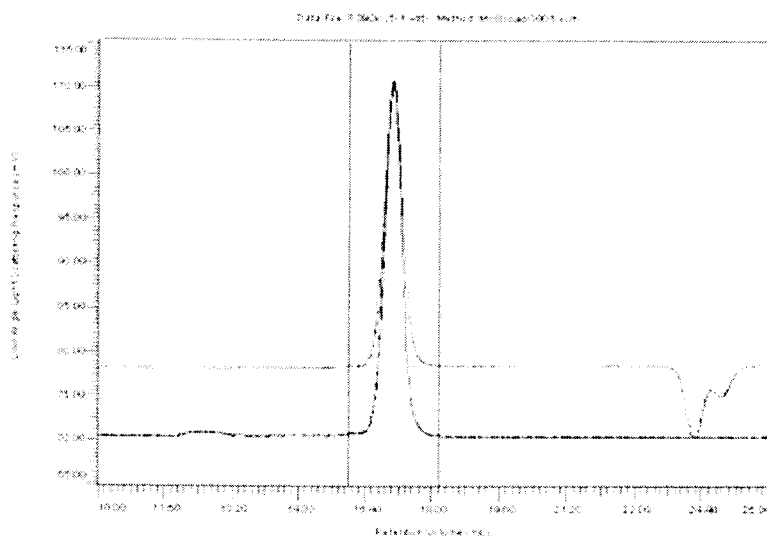
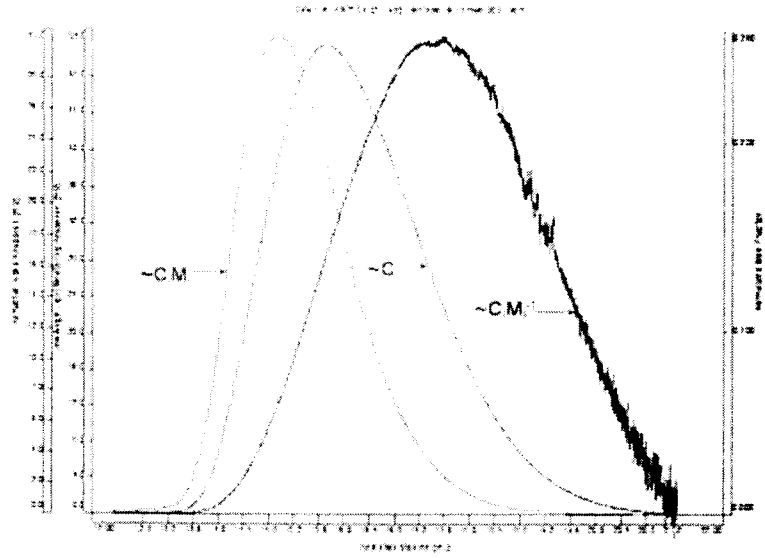



Figure 5. Peaks Used in Calculation of M_w and M_n . Broad Distribution Polystyrene Sample. [« Back](#)



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SEC-VISCOMETRY-RALLS

In this triple detector method, the angular dissymmetry in the scattered intensity is accounted for by using the combined measurements of intrinsic viscosity at 90° light scattering intensity (RALLS: Right Angle Laser Light Scattering) to determine both molecular size and the molecular weight. Size determination of small polymer molecules is possible with this new method, even when there is no observable multi-angle LS dissymmetry.

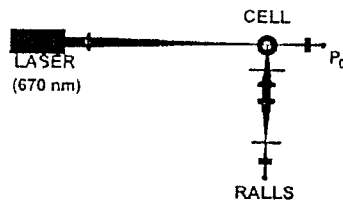


Figure 12 RALLS Schematic

Angular light scattering dissymmetry is not a factor for many important commercial random-coil type polymers having molecular weight values in the order of 100,000 or less, like most polycondensation, Nylon, or Dacron type polymers. For higher MW samples, a proper account of LS dissymmetry is highly desirable to assure a better than 5% accuracy in the molecular weight determination. In Viscotek's software, the following algorithm is used to account for LS dissymmetry automatically to guarantee accuracy:

- 1) Initially, the particle scattering function at 90°, $P(90^\circ)$, is assumed a value of 1.0 and a molecular weight estimate M_{est} is calculated directly from the RALLS intensity R_{90} from Equation [A.3-10], that is:

$$[A.3-16] \quad M_{est} = \frac{R_{\theta=90^\circ}}{KC}$$

where K is the optical constant defined earlier.

- 2) A Flory-Fox radius R_{FF} is then calculated from the above M_{est} and the experimental intrinsic viscosity value $[\eta]$:

$$[A.3-17] \quad R_{FF,est} = \left(\frac{1}{6}\right)^{1/2} \left(\frac{[\eta]M_{est}}{\Phi}\right)^{1/3}$$

where Φ is the Flory viscosity constant defined earlier in Equations [A.3-12] and [A.3-13] that take into account the different degrees of solvent expansion factor for the polymer molecules.

- 3) An improved 90° dissymmetry value of $P(90^\circ)_{est}$ is then calculated from a specific particle scattering function that fits either the rigid-rod, hard-sphere, or random coil type; the following Debye $P(\theta)$ function is chosen to update the $P(90^\circ)_{est}$ value.

$$[A.3-18] \quad P(90^\circ)_{est} = \frac{2}{X^2} (e^{-X} - (1-X))$$

where,

$$[A.3-19] \quad X = \frac{4\pi n_0}{\lambda_0} R_{FF,est}$$

where n_0 is the solvent refractive index, and λ_0 is the wavelength of the incident light.

- 4) A new estimate of the molecular weight is calculated from:

$$[A.3-20] \quad \text{New } M_{est} = \frac{M_{est}}{P(90^\circ)_{est}}$$

- 5) Steps of 2 and 4 are repeated using the new estimate of molecular weight until the molecular weight and R_{FF} values no longer change. This usually takes three iterations or less.

MALLS detector

The signal $R_\theta =$:

$$\lim_{\theta \rightarrow 0} R_\theta = K * M_w * P(\theta) - 2 * A2 * [C] * M_w^2 * P(\theta)^2$$

(when $A2$ very small)

$$\lim_{\theta \rightarrow 0} R_\theta = K * c * M_w * P(\theta)$$

Therefore the signal is proportional to $[C]$ and M_w

For LMT $[C]$ and M_w are very small \Rightarrow bad signal

SEC viscosimetry RALLS (Viscotek)

See joint document provide by Viscotek

- Only one angle is used for M_w measurement (90°C) therefore same defaults that MALLS measurement with in addition no A_2 determined (assumed equal to 0).
- Viscosity is only used for $P(\theta)$ determination (assumption we have a random coil and that Flory equation can be used (is it correct for polyelectrolytes?) with the same defaults than for all viscosity detector
- Iterative process used
- error of computation especially for LMT where signal is noisy

The three types of error must probably be added

Differential pressure detector

The signal is a specific viscosity

For random coil polysaccharide, it is known that

$$\text{Log}(\eta_{sp}) = a \cdot \log(c^*[\eta])$$

With $a = 1.4$ for diluted solution and 3.3 for concentrated solutions (master curve)

Therefore the signal is proportional to $[C]^{1.4}$ and $[\eta]^{1.4}$

For LMT $[C]$ and M_w ($[\eta] = K \cdot M^\alpha$) are very small \Rightarrow very bad signal