Low-Angle Light Scattering (LALS) for Molecular Weight Determinations by GPC/SEC

Why Closer is Better

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Background

The use of laser-light-scattering detectors to measure molecular weight in gel permeation chromatography (GPC) experiments is now commonplace. The attraction for most users is that the molecular weight is measured from the light-scattering (LS) detector signal directly, avoiding the need to use polymer or protein standards to calibrate the GPC elution volume. In addition, the molecular weight measured by the LS detector is independent of polymer type and structure. For these reasons, molecular weights measured by GPC-LS are often erroneously referred to as absolute. In reality, all GPC-LS detectors require calibration and most of the LS detectors in current usage obtain the molecular weights either by data extrapolation or data correction – far from absolute!

This application note explains the theory behind this issue and explains why low-angle light scattering (LALS) is the only LS technique that actually measures a scattered light intensity which is directly proportional to molecular weight.

Light Scattering Theory

To begin, we need to look at the relationship between the measured quantity (scattered light intensity) and the desired result (weight average molecular weight). These two quantities are linked by the well known Rayleigh equation:

$$\frac{KC}{R_o} = \frac{1}{M_w} + 2A_2C$$

Where R_0 is the Rayleigh Ratio at zero scattering angle,
M_w is the weight-average Molecular Weight,
C is the concentration of the solution,
K is an optical constant that includes dn/dc,
A_2 is the 2nd virial coefficient.
For solutions of low concentration (e.g., typical GPC conditions) the concentration dependant term can be ignored and the equation rearranged into the simpler form:

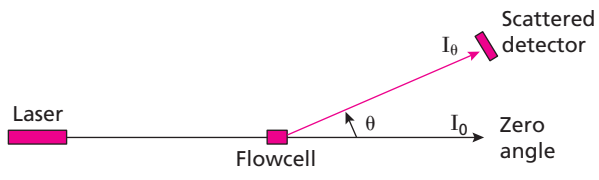
So here is the basis of the method — we have an expression

$$R_o = KCM_w$$

that equates scattered light directly to M_w. However, you will notice that the equation is expressed in terms of scattered light at zero angle (i.e., 0°). It is, of course, impossible to measure the scattered light at 0° because of the presence of the incident laser beam. So, therefore, we have to measure the scattered light at some other angle(s), (Figure 1). Unfortunately, this introduces a complication into the process, as the amount of scattered light varies significantly with both the scattering angle and the size of the molecules being measured. Only in the case of very small molecules can these angular dependence effects be ignored. For molecules of any significant size (>12 nm) a solution to the problem must be found.[1]

The theory tells us that there are fundamentally three ways of

Figure 1: Presence of incident laser beam passing straight through the flowcell prevents measurement of scattered light at zero angle (I_0). Instead, the scattered light must be measured at an angle to the beam (I_θ).

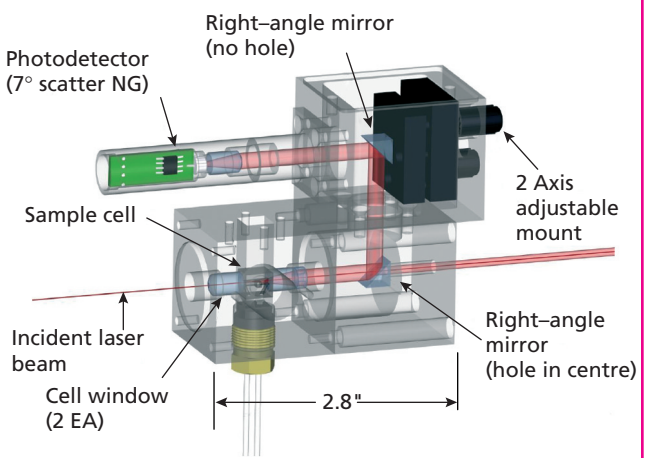


overcoming the problem:

1. Multi-angle light scattering (MALS). Measure the scattered light at two or more angles and extrapolate the data back to the zero angle.
2. Right-angle light scattering/viscometry (RALS/viscometry). Measure the scattered light at 90° and use viscosity data to correct to the zero angle.
3. Low-angle light scattering (LALS). Measure at a low angle — close to zero — where the angular effects are negligible. No correction needed.

Many hundreds of instruments using the first two techniques are in use world-wide, but neither measures the molecular weight directly. The multi-angle approach always relies on the extrapolation of data and the RALS approach needs a correction of the data for larger molecules using a viscometer.

Figure 2: Drawing showing the optical pathway of the Viscotek LALS detector.



All of the multi-angle instruments work on the same principle of measuring the scattered light at two or more angles and then extrapolating the measured values back to zero angle. Unfortunately, many users believe that they are measuring absolute molecular weight.

Historically, the LALS approach has always, quite rightly, been regarded as the most accurate method as, after initial calibration, it measures the molecular weight directly avoiding the assumptions and data manipulations of the first two methods. The benchmark commercial LALS detector was the Chromatix KMX6 produced from the late 1970s to late 1980s. Unfortunately, it was never developed and there was no commercial LALS detector available for the entire 1990s. In 2001 the new Viscotek LALS detector became available and re-opened the possibility for GPC-LS users to measure molecular weights directly again.

Why the multi-angle approach is flawed

All of the multi-angle instruments work on the same principle of measuring the scattered light at two or more angles and then extrapolating the measured values back to zero angle. Unfortunately, many users believe that they are measuring absolute molecular weight. However, this is not true, as all MALS instruments require calibration and normalization procedures and, in addition, the molecular weights then measured are derived from the extrapolation of data rather than a direct measurement.

This, indeed, is the primary and fundamental weakness in the MALS method — which type of data plot or extrapolation fit do I need to use for each sample? For smaller molecules a linear data fit yields the most accurate values. As the molecules get larger, the

Figure 3: Chromatograms of two polymers, showing differences in response for LALS and RALS (see text for details). (a) is polyethylene oxide; (b) is hyaluronic acid.

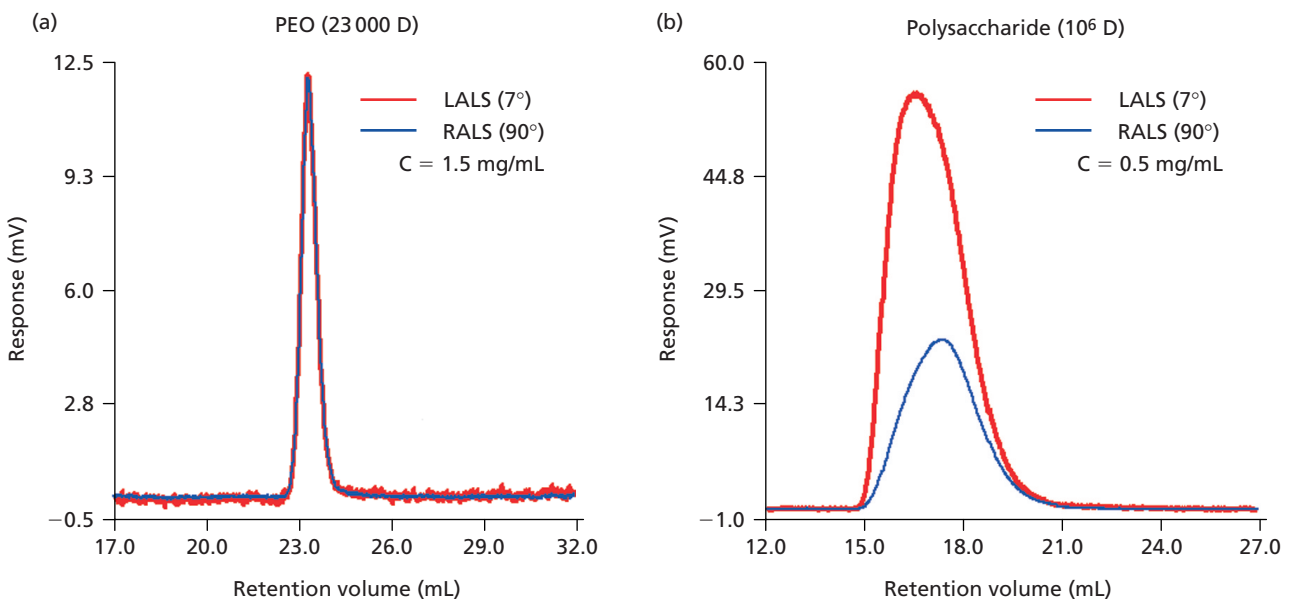
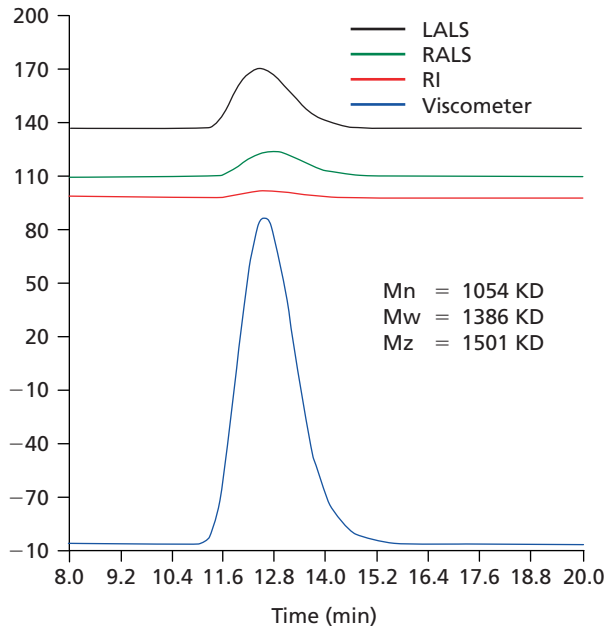


Figure 4: Direct measurement of molecular weight by low-angle light scattering applied to a sample of hyaluronic acid.



required data fit changes significantly.[2] For larger molecules the data plots are highly curved and the resulting extrapolated molecular weight will depend highly on the data fit chosen. This means for broad samples, where there will be both small and large molecules, the order of fit chosen is always a compromise.

The second but linked problem of the multi-angle approach is a lack of data in the all important low-angle region. This is because multi-angle detectors are designed to collect data from many angles so the cell is, in practice, incapable of collecting high-quality data from the low angles. This exacerbates the problems of achieving both accuracy and precision in molecular weight determinations, as the extrapolation accuracy (the 'goodness' of fit) depends highly both on how close to zero the lowest angle data point is, and the quality of that signal.[3] This lays bare the perceived wisdom of 'more angles is better.' It is clear that in fact 'closer is better' when measuring scattered light.

The new LALS detector

The new LALS approach avoids all of the extrapolation and data fitting issues by measuring the scattered light as close as possible to the desired zero angle, in this instance at 7°. Figure 2 is a cut-away schematic of the optical path of the detector. The novel design cleverly separates the incident beam from the scattered light.[4] This allows the 7° scattered light to be collected with excellent signal to noise.

Figure 3 shows a comparison of signal to noise for small and large polymers at 90° (RALS) and 7° (LALS). Note that in Figure 3(a), both detectors give the same signal for the low molecular weight polyethylene oxide sample as there is no angular dependence. This chromatogram gives a direct comparison of the signal to noise of the LALS compared with the 90° signal, which is always the highest quality signal. The comparison shows that the LALS has excellent signal to noise.

Of course, the importance of the LALS direct measurement becomes most apparent when the analysis involves large molecules such as polysaccharides. These type of molecules show very high angular dependence on the scattered light (see Figure 3(b)) and cause severe problems with both the RALS and, particularly, the multi-angle method. Direct measurement of the scattered light at 7° eliminates the need for any extrapolation or data fitting and yields accurate molecular weights. Figure 4 shows an example of data obtained by the LALS method on a sample of hyaluronic acid.[5]

Conclusion

The availability of a state-of-the-art LALS detector allows GPC practitioners to measure the molecular weight of their samples with certainty. The LALS avoids any issues of data extrapolation or data fitting. In addition, the small size and simplicity of the new LALS allows it to be easily used in integrated multidetector systems with viscometers,[6] allowing both molecular weight and molecular structure to be determined simultaneously.[7,8]

References

1. GPC MasterClass™, Viscotek (1998-2003).
2. S. Mori and H. Barth, *Size Exclusion Chromatography,* (Springer-Verlag Berlin, Heidelberg, Germany, 1999).
3. M. Anderson, K-G Wahlund and Bengt Wittgren, *Separation and Characterisation of Natural and Synthetic Macromolecules,* (Amsterdam, February 2003).
4. M. Haney and D. Stone, Waters International GPC Symposium, 2000.
5. M. Haney, *Separation and Characterisation of Natural and Synthetic Macromolecules,* (Amsterdam, February 2003).
6. Triple Detector Array (TDA) product brochure, Viscotek (2001-2003)
7. R. Walkenhorst, *LC-GC Europe,* **14**(10), 2001.
8. P. Clarke, *LC-GC Europe Applications Book,* September 2002, 37-39.