Tips & Tricks: GPC/SEC
How to Choose a Static Light-scattering Technique for Molar Mass Determination

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Light scattering is one of the few absolute methods for the characterization of macromolecules and biopolymers. On-line light-scattering detectors are used in GPC/SEC systems to measure molar masses, the radius of gyration and to identify high molar mass content at low concentrations. There are several detectors available that differ in the provided light-scattering technique. It is necessary to select the correct technology for a specific application and to do this users need to know the basic principles.

What is the Principle of Static Light Scattering from Polymer Solutions?
In light scattering from polymer solutions, detectors measure the intensity of light scattered from the dilute sample solution. The light source is a laser; the scattered light is measured at one or more fixed detector angles (compare Figure 1). These angles can be, but must not be the true scattering angles. This depends on the cell design and geometry:

• If the intensity is measured directly in a cell with cylindrical geometry the scattering angle and the detector angle are always the same.
• If glass cells are used the interface solution/glass is responsible for the fact that all detector angles besides the 90° angle need a correction to obtain the true scattering angle (compare Figure 2).

The term “static” does not refer to performing an on-line (GPC/SEC) or batch (stand-alone) light-scattering experiment, but to the fact that the time averaged scattering intensity is measured. “Dynamic” light scattering measures the light intensity fluctuations and can also be done in an on-line and batch experiment.

Why is One Angle (e.g., at 90°) for Light Scattering Not Enough?
If the dimensions of the particle are small compared to the wavelength of the incident beam this particle can be
seen as a point scatterer. This is the case if the maximum distance between two points of a particle is smaller than about \(\lambda/20\), where \(\lambda\) is the wavelength of the laser. However, many macromolecules with a higher molar mass have solute dimensions of many tens of nanometres up to a few hundred. In this instance, the light scattered from different points is coherent and, therefore, capable of interference (compare Figure 3). The intensity of the scattered light of a large particle is reduced compared to the scattering intensity from all the individual mass points. This results in an angular dependence of the scattered light intensity, meaning that it is not sufficient to measure at 90° when molecules are too large.\(^1\)

Figure 4 shows the signals for an RI detector and a MALLS detector with seven signals where the angles at 35°, 90° and 145° are shown. The apparent shift in the RI and MALLS signals is because of the fact that RI is a concentration detector while the MALLS is a molar mass sensitive detector.\(^2\) The apparent signal shift in the light-scattering signals is as a result of the angular dependence of the scattering signal showing that the detector angle is important.

90° LS results will only be accurate for random chain linear polymers with molar masses up to 200000 Da, globular proteins with molar masses up to 1000000 Da or branched polymers over 200000 Da (depending on the branching density).\(^3\)

However, to be sure that accurate results for unknown samples are obtained it is not recommended to rely on 90° light-scattering results only. If the user can be absolutely sure that the molar masses are low (which cannot be judged by estimated molar mass averages only)\(^4\) or the structure is very compact (e.g., as it is for proteins) 90° LS is a good option because of the very good signal-to-noise ratio that can be obtained with this technique.

If one low angle is used higher molar masses can be measured (see below). However, Figure 5 shows that the

**Figure 2(a) and (b):** Comparison of typical MALLS detector cells with a cylindrical index matching cell, where scattering and detector angle are the same, and a glass cell (top view).

**Figure 1:** Light scattering of polymers in solution (schematic) with \(J_0\): intensity primary beam, \(\lambda\): wavelength of primary beam and scattered light (elastic), \(r\): distance scattering centre to detector and, \(\Theta\): scattering angle.

**Figure 3:** Light scattering of large particles with A, B: scattering centres, l: path length distance (at \(\Theta = 0\), l = 0).
experimental (and, therefore, also the molar mass) error is highest for low angles. Therefore, the result accuracy can be improved when more than one angle is used.

**What Does LALLS, RALLS and MALLS Mean?**
These are abbreviations for low angle, right angle and multi-angle laser light-scattering techniques and detectors. The difference between the detectors is the number and the position of the angles detected. LALLS and RALLS instruments measure at just one angle, either at a low angle (e.g., 6–7°) or at a right angle (90°).

MALLS detectors measure simultaneously at several angles.

RALLS, 90° light scattering, has the advantage of the most accurate signal with the lowest influence of stray light and dust particles, that might be present in the solution and disturb the signal. However, the molar mass range that can be measured accurately is very limited (see above).

LALLS has the advantage that the observed intensities can virtually be identified with intensities at a scattering angle of 0°. 0° angle is not affected by interference and is a direct measure of the molar mass of large particles. Unfortunately, LALLS presents the most experimental difficulties. Complex optics and a very clean system are required to measure the signals. The method requires strong signal processing (e.g., in the detector or the software) making it often impossible to see the true raw data. In some chromatographic systems, for example, systems for aqueous GPC/SEC, the requirement of a clean system can almost never be fulfilled.

MALLS instruments use an extrapolation to the scattering angle of 0° to measure an accurate molar mass. They have the advantage that besides the molar mass the radius of gyration can be measured. The disadvantages are the high costs because of the fact that a MALLS detector is, in general, several detectors in one.

**What Does Triple Detection Really Mean?**
The term “triple detection” has become very popular over the last few years as an alternative to MALLS. Unfortunately, it is often not clear what is meant when people use this expression and there is no well defined definition any more:

a) Some people use this term to describe the determination of intrinsic viscosity using a viscometer and molar mass using light scattering at a single angle. In this instance

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**Figure 4:** RI and 3 light scattering signals of a 7 angle MALLS detector for a poly(styrene) with a broad molar mass distribution. The detector delay is corrected, the apparent shift is because of the angular dependence of the scattering intensity and the fact that RI and LS detectors are based on different principles.

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**Figure 5:** Comparison of the error for different scattering angles and for the molar mass determination when using single- or multi-angle.
molar masses are obtained from the combination of light scattering and concentration detection, while intrinsic viscosities are measured using a viscosity and a concentration detector.

b) The original term “triple detection” was and is used for a system composed of a viscosity, a 90° single angle light scattering and a concentration detector where the signals are processed using a special algorithm. This algorithm includes several assumptions and, therefore, results in severe limitations. For each unknown sample it needs to be known a priori that the sample is run under theta conditions (not GPC/SEC conditions), that the sample is linear and that it can be represented by a chain model (coil, rod, sphere). Thus, the uncritical use of triple detection might result in severe misinterpretation of experimental results. However, if these requirements are fulfilled, triple detection allows users to overcome the limitations for 90° light scattering.

The following steps are part of the algorithm for molar mass determination:

1. In a first step, a molecular weight (M1) will be calculated from the light-scattering signal without consideration of the angular dependence. Generally, this will underestimate the molecular weight.
2. From the calculated M1 and the experimental intrinsic viscosity (η), the radius of gyration of the molecule can be deduced by the Flory-Fox equation.
3. Using the estimated radius of gyration, the scattering function at the given scattering angle can be calculated based on the assumption of a chain model.
4. Now a new molecular weight can be calculated from the intensity of the light-scattering instrument and the estimated scattering function. The steps 2–4 will be iterated until stable values for radius of gyration and molecular weight are obtained.

Is SELS an Option in GPC/SEC?

SELS is a newly introduced acronym for solvent enhanced light scattering. It uses the above discussed techniques and recommends that samples are dissolved in other solvents than the mobile phase to allow analysis under conditions where the sample contrast (dn/dc) is higher. This approach is not universal and should be tested for every application so that the users are sure that the samples (or parts of it) do not precipitate when injected into the mobile phase. This could block capillaries and lead to an overall pressure increase in the GPC/SEC system. Additionally, users of this approach should be aware of selective sorption (also referred to as preferential solvation), which is a typical effect in solvent mixtures. As a result of the different solubility parameters of the solvent components, the solvent composition in the vicinity of the macromolecule will be different from the bulk composition. If the macromolecule is a copolymer, has different degrees of substitution or end groups, the local solvent composition will be different for each molecule. Therefore, the sample dn/dc in solvent mixtures is often unknown leading to inaccurate results. An approach for mixed solvents would be to measure dn/dc with solution and mixed solvent in osmotic equilibrium.

Why Can’t ELSDs be Used for Absolute Molar Masses?

Evaporative light-scattering detectors (ELSDs) measure the light scattered from the solid solute particles remaining after nebulization and evaporation of the mobile phase. This is in contrast to the above mentioned light-scattering detectors, where the polymer is still in solution. ELSDs are used to measure concentration profiles and molar masses based on a calibration curve when RI detectors, for example, cannot be used (e.g., as a result of the use of solvent mixtures or low sample concentrations). Their signal intensity is not influenced by the molar mass, therefore, they cannot measure absolute molar masses.

References

(b) G.C. Berry and P.M. Cotts, Static and dynamic light scattering, in Experimental methods in polymer characterization, Wiley & Sons Ltd (1998).

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