GPC/SEC eBook Series

Static Light Scattering in GPC/SEC

What you should know when you need to analyze polymers, biopolymers, and proteins

Agilent

Trusted Answers

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About this eBook series

GPC/SEC *Tips & Tricks* articles have been published in more than 60 editions of LC/GC's digital magazine *The Column* over the course of 10 years. These *Tips & Tricks* are designed to support GPC/SEC users in their daily work, providing comprehensive overviews on different aspects of this powerful technique.

This eBook series was created to have all published topics at a glance.

Each eBook contains five to eight different *Tips & Tricks* publications that have been updated with the latest information, new examples, and figures.

To allow new users to GPC/SEC a continued reading experience, content has been edited, resulting in some differences compared to the original publications.

Nevertheless, the original spirit is maintained. So, the publications are independent references that allow users to read only the dedicated publication of interest.

Introduction to GPC/SEC –light scattering

Light scattering is one of the few absolute methods for the characterization of macromolecules and biopolymers. Online 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 make this selection, users need to know the basic principles.

The term "static" does not refer to performing an online (GPC/SEC) or batch (standalone) 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 online and batch experiments.

In contrast to static light scattering, where the macromolecule is measured in solution, evaporative light scattering detectors (ELSDs) measure the light scattered by particles that remain after nebulization and evaporation of the mobile phase. ELSDs are used to measure concentration profiles and molar masses based on a calibration curve when, for example, RI detectors cannot be used (for example, due to 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.

1. How to choose a static light scattering technique for molar mass determination

What is the principle of static light scattering from polymer solutions?

Scattered light is the result of the interaction of light with matter or molecules. This forms the basis of studying absorption, light scattering, and luminescence. The most important types include elastic and inelastic light scattering. Elastic light scattering means that the wavelength of the scattered light will not be altered during the scattering process.

In light scattering from polymer solutions, detectors measure the intensity of light scattered from the dilute sample solution. The light source is a laser, and the scattered light is measured at one or more fixed detector angles (Figure 1). These angles can be, but might 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 (Figure 2).

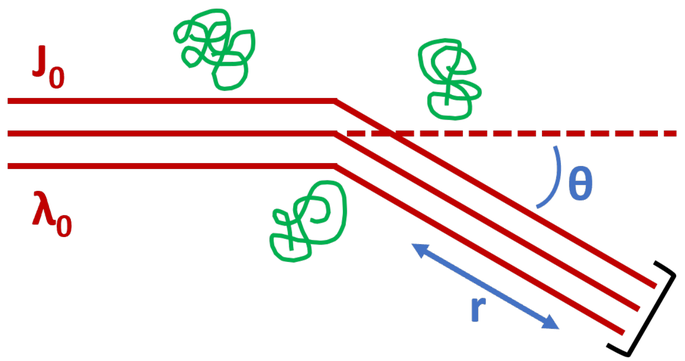

Figure 1. Light scattering of polymers in solution (schematic). J_0 = intensity of the primary beam; λ_0 = wavelength of the primary beam and scattered light (elastic); r = distance of the scattering center to the detector; θ = the scattering angle.

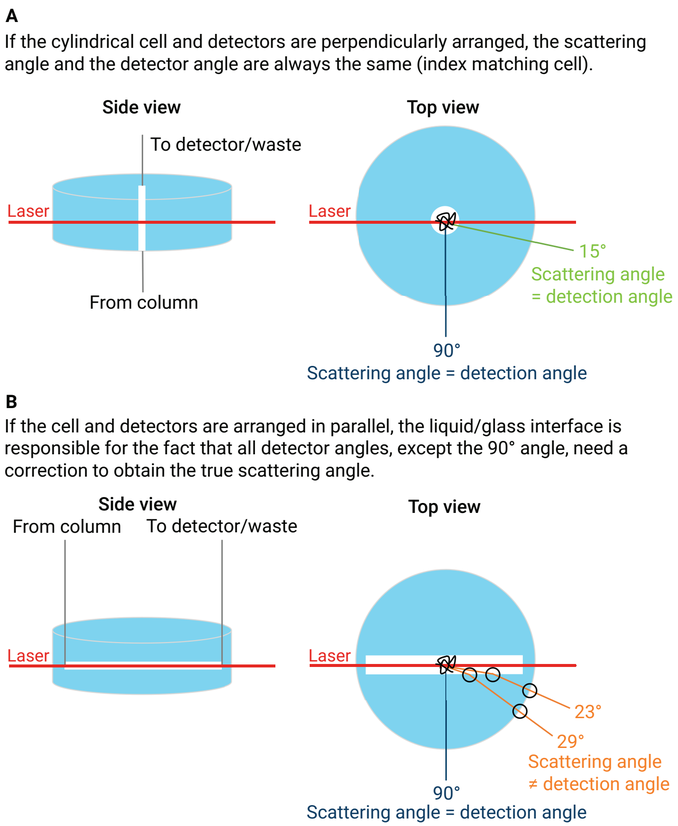

Figure 2. (A) and (B): Comparison of typical MALS detector cells with a cylindrical index matching cell, where scattering and detector angle are the same, and a glass cell (top view).

Why is one angle (for example, 90°) for light scattering not enough?

If the dimensions of the molecules are small compared to the wavelength of the incident beam, this molecule can be described as a point scatterer or isotropic scatterer. This is the case if the maximum distance between two points of a molecule is smaller than approximately λ/20, where λ is the wavelength of the laser. However, many macromolecules with a higher molar mass have solute dimensions of many tens of nanometers up to a few hundred. In this instance, the light scattered from different points is coherent and, therefore, capable of interference (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]

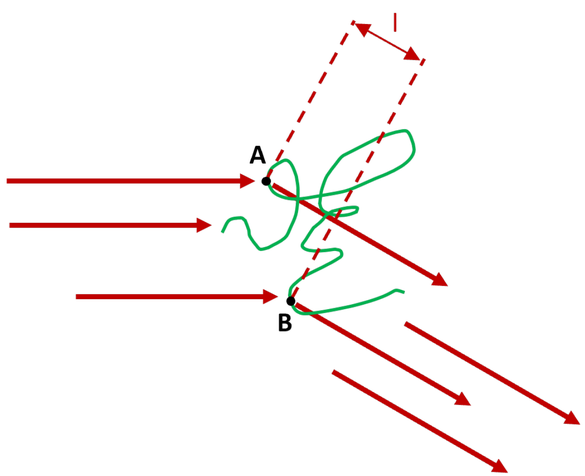

Figure 3. Light scattering of large particles. A, B = scattering centers; I = path length distance (at θ = 0, I = 0).

Figure 4 shows the signals for an RI detector and a MALS detector where the angles at 35°, 90°, and 145° are shown. The apparent shift in the RI and MALS signals is because RI is a concentration detector while the MALS is a molar mass sensitive detector.[2] The apparent signal shift in the light scattering signals is a result of the angular dependence of the scattering signal, showing that the detector angle is important.

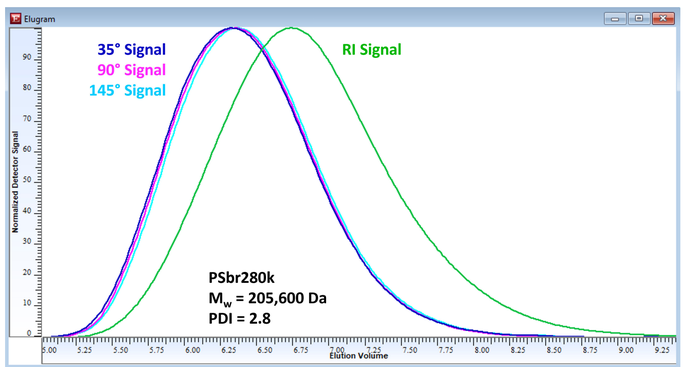

Figure 4. RI and three light scattering signals of a seven-angle MALS detector for a poly(styrene) with a broad molar mass distribution. The detector delay is corrected; the apparent shift is because of the angular dependence of the scattering intensity and the fact that RI and LS detectors are based on different principles.

LS results using an angle of 90° will only be accurate for random chain linear polymers with molar masses up to 200,000 Da, globular proteins with molar masses up to 1,000,000 Da, or branched polymers over 200,000 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 sure that the molar masses are low (which cannot be judged by estimated molar mass averages only)[4], or the structure is very compact (for example, 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 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.

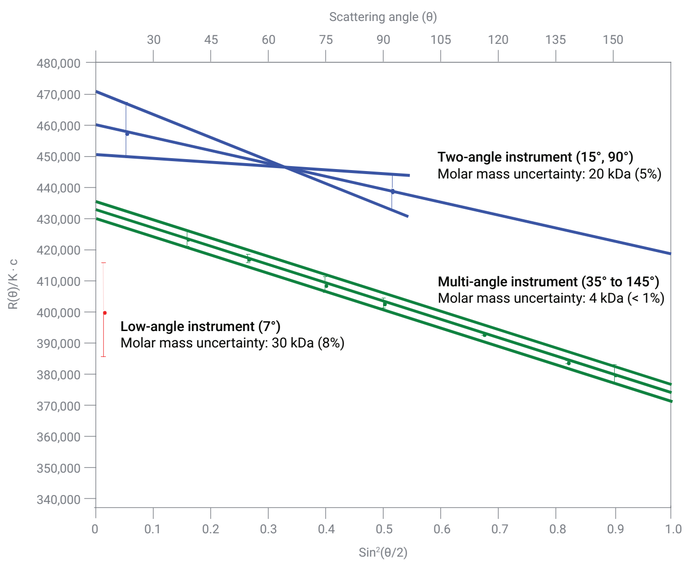

Figure 5. Comparison of the error for different scattering angles and for the molar mass determination when using single- or multi-angle.

What do LALS, RALS, and MALS mean?

These are abbreviations for low-angle (LALS), right-angle (RALS), and multi-angle (MALS) laser light scattering techniques and detectors. The difference between the detectors is the number and the position of the angles detected. LALS and RALS instruments measure at just one angle, either at a low angle (e.g., 6 to 7°) or at a right angle (90°).

LALS has the advantage that the observed intensities can virtually be identified with intensities at a scattering angle of 0°. A 0° angle is not affected by interference and is a direct measure of the molar mass of large particles. Unfortunately, LALS 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.

RALS, 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).

MALS detectors measure simultaneously at several angles. These instruments use an extrapolation to the scattering angle of 0° to measure an accurate molar mass. They have the advantage that in addition to the molar mass, the radius of gyration can be measured. The disadvantage is the higher cost, because a MALS detector is, in general, several detectors in one.[5]

What does triple detection really mean?

The term "triple detection" has become popular over the last few years as an alternative to MALS. Unfortunately, it is often not clear what is meant when people use this expression and there is no well-defined definition anymore:

a. Some people use this term to describe the determination of intrinsic viscosity using a viscometer and molar-mass-using light scattering detector at a single angle. In this instance, 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 viscometer, a 90° single-angle light scattering detector, and a concentration detector where the signals are processed using a special algorithm.[6] This algorithm includes several assumptions and, therefore, results in severe limitations. For each unknown sample, it needs to be known first 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.[7] 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. Steps 2 to 4 will be iterated until stable values for the 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 techniques discussed in this chapter and recommends that samples are dissolved in solvents other 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 them) 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. Also, 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, or 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.[1,8,9]

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2. Do's and don'ts in GPC/SEC–light scattering

Light scattering (LS) is one of the few absolute, theoretically founded methods for the determination of molar mass and molecular size of polymers and biopolymers. Light scattering instruments became commercially available in the late 1950s. LS detectors for GPC/SEC appeared in the mid-1970s. Since then, they have become an important tool to investigate macromolecules. Expectations are high when the method is implemented in the laboratory. However, even an absolute method is not suitable for all types of macromolecules, and requires knowledge of evaluation parameters and thorough setup measurements.

Weigh your samples precisely

While GPC/SEC does not require the accurate knowledge of the mass in a chromatographic slice, GPC/SEC–light scattering does. To calculate the molar mass from the scattering intensity, the sample concentration (injected mass) in the chromatographic slice must be known.[1] An error in the slice concentration results in an identical error in the molar mass determination.

The slice concentration can be measured with a concentration detector (e.g., an RI or UV detector). Two different methods can be used:

- Method 1 requires the accurate knowledge of the bulk sample concentration. The slice concentration is calculated from the batch concentration assuming that 100% of the sample elutes from the column and that the injection system works properly. For this method, the sample needs to be weighed accurately using an analytical balance.
- For method 2, the refractive index increment (dn/dc) of the sample and the concentration detector calibration factor must be known. The calibration factor can be measured using a reference substance (e.g., poly(styrene) or pullulan) with accurately known dn/dc and known concentration(s) (method 1 needs to be applied for the reference substance to determine the detector response). If the detector calibration factor is determined precisely, method 2 produces more accurate results. Errors resulting from deviating injection volume, adsorption on the column, or nonsoluble sample parts do not affect the slice concentration (and, therefore, the molar mass). It is nevertheless recommended to weigh the sample precisely, as it is then possible to quantify the sample recovery. This helps to identify systematic errors or malfunctioning system components early.

Be aware of the dn/dc

The dn/dc of the sample must be known to calculate the molar mass from the scattering intensity. The dn/dc is the change, dn, of the solution's refractive index n with respect to the molecular concentration change, dc. The dn/dc of a sample depends (among others) on the chemical nature of the sample, the composition, the solvent, the temperature, and the wavelength of the light source used. It also depends on the molar mass but can be assumed to be constant above approximately 10,000 Da.[2] Any error in the dn/dc results in a substantial error for the molar mass determination. Table 1 shows the influence on typical GPC/SEC–light scattering results for inaccurate dn/dc, concentration, and inter-detector delay. The data of a theoretical Schulz-Flory distribution have been used to investigate the influence of the evaluation parameters on the final results.[3] Inaccurate evaluation parameters have been used to calculate the molar mass averages and to quantify the deviations.[4]

The most precise and accurate method for dn/dc determination is a batch experiment using dedicated dn/dc instrumentation. However, it is common practice that the dn/dc is measured within the GPC/SEC–light scattering experiment using the concentration detector (normally an RI). If this method is used, it is inevitable to use method 1 for the determination of the slice concentration. For both batch and online dn/dc determinations, it is required that the sample concentration is precisely known. Insoluble sample parts, water, or salt content must be taken into account to achieve reliable results. Measurements should be made at the same temperature and at the same wavelength to minimize errors.

Use checkout samples and validate your system

Besides the sample-related evaluation parameters, GPC/SEC–light scattering data evaluation requires the knowledge of a number of system-related parameters. Proper data analysis can only be carried out if the following are known:

- The inter-detector delay between the light scattering and the concentration detector
- The detector constant of this specific light scattering detector
- The detector constant of this specific concentration detector
- The normalization coefficients (MALS detectors only)

If any of these parameters are wrong or have changed, the molar mass results will be wrong. Using a checkout sample helps to identify accidental or yet unidentified changes in the setup. Moreover, if the checkout sample is selected cleverly, it can be used to determine the actual system-related parameters. This helps to minimize the time loss.

System validation is always required, even when working with an absolute detector. Light scattering validation standards or certified reference materials can be used to check the detector performance and to prove that the system is in good working condition. They also allow systematic errors to be identified and the mode of operation to be verified.

Do not expect accurate results...

...for copolymers: An accurate dn/dc is required for accurate molar mass determination. Because dn/dc depends on the sample type and on the composition, the dn/dc for copolymers is often not constant within the copolymer. Unfortunately, it is not enough to measure the batch (average) dn/dc for the copolymer and to use this value. For proper data analysis, it is necessary to know the dn/dc in every chromatographic slice. However, there is no method that allows this value to be measured and, therefore, GPC/SEC–light scattering molar mass results for copolymers are not accurate. An exception to this rule is block copolymers with a narrow molar mass distribution. In this instance, the measured molar masses can be assumed to be close to the true molar masses.[5] The difference between the measured apparent M_w and the true value increases with the width of the copolymer chemical composition distribution and with the difference for the homopolymer dn/dc values (Figure 1).[6]

Besides, GPC/SEC–light scattering experiments do not give any results on the copolymer composition or other important parameters such as the homogeneity of the sample. For copolymers, other characterization methods (e.g., copolymer characterization with dual detection, liquid adsorption chromatography (LAC), or ^{2}D chromatography) provide more valuable information than an apparent molar mass only.[7]

Table 1. Influence of evaluation parameters on the accuracy of molar mass averages for GPC/SEC–light scattering.

	Variation	M_w (Da)	Deviation (%)	M_n (Da)	Deviation (%)
Reference Value	–	300,000	–	150,000	–
dn/dc	–5%	331,000	10.3	165,600	10.4
Concentration	–5%	315,800	5.27	158,000	5.33
Interdetector Delay	5%	295,100	–1.63	159,500	6.33

...in mixed solvents: Selective sorption (also referred to as preferential adsorption) is a typical effect in solvent mixtures. Since the polymer has a higher affinity for one of the solvents, the concentration of this solvent is higher close to the polymer chain than in the polymer-free solvent.[6] This has an influence on the dn/dc and how it should be determined. An approach for mixed solvents is to measure dn/dc with solution and mixed solvent in osmotic equilibrium. Without this, the dn/dc is inaccurate and, again, apparent molar masses are measured that differ from the true molar mass.

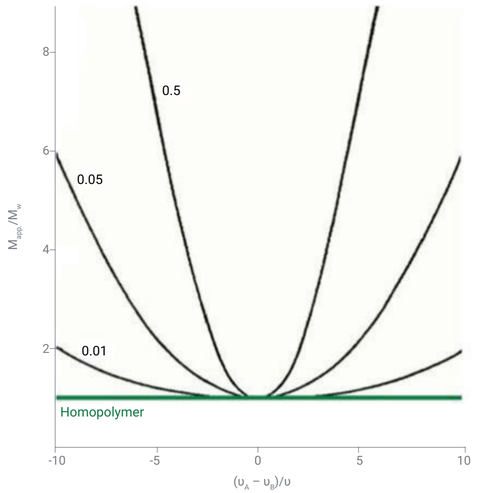

Figure 1. Variation of apparent molar mass and true molar mass for a copolymer with heterogeneous chemical composition (data from reference 6).

Do not expect accurate molar mass distribution information...

...without a properly developed GPC/SEC method: Light scattering allows the M_w of a sample to be measured. Only by combining this technique with a fractionating method (such as GPC/SEC) can other molar mass averages, the polydispersity index PDI, and the molar mass distribution be measured. This is because the chromatographic slices are assumed to be monodisperse (only one molar mass is present). The light scattering detector can then be used to measure the online calibration curve for the sample. This calibration curve is often fitted and then used to derive the molar mass distribution from the concentration detector signal in the usual way.

If the separation step fails and the slices are not monodisperse, this overall approach will fail. The only valuable result from the GPC/SEC–light scattering experiment will be the weight-average molecular weight (and the z-average of the radius of gyration for MALS detectors). All distribution information will be lost if the GPC/SEC method is not working properly. The information obtained from the light scattering detector can give hints as to whether the GPC/SEC method is working. Figure 2 shows two examples for separations where a method check is recommended. Here the molar mass does not decrease with the elution volume. There are even parts where the molar mass increases with the elution volume, which is in total contrast to the separation mechanism of GPC/SEC. In addition, the figure shows an example in which the expected behavior is observed.

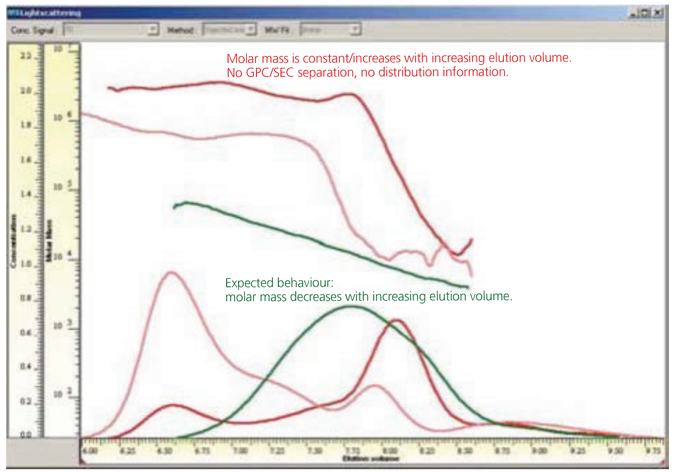

Figure 2. (Red) Examples of results from measurements with an unsuitable GPC/SEC method. The molar masses measured with the light scattering detector show that the molar mass is constant or even increasing with increasing elution volume. (Green) The molar mass decreases with increasing elution volume. This behavior is expected from the GPC/SEC separation principle. In addition to the molar masses, the measured slice concentrations for the samples are shown.

...when the column is overloaded: Distribution information is also not available when the column is overloaded. An often-heard recommendation for samples with low molar masses (and low dn/dc) is to increase the concentration and/or the injection volume so that higher signals and better signal-to-noise ratios are obtained. However, when the concentration is increased so much that the column is overloaded, the separation step is lost and the only parameter that can be measured accurately is M_w. In this instance (and if enough sample is available), a better recommendation is to perform a batch light scattering measurement.

Summary

GPC/SEC–light scattering is ideal for identifying high molar mass content at low concentrations and for investigating aggregates. It is a powerful noninvasive technique to measure the molar mass distributions of homopolymers if:

- The dn/dc value is known or can be measured
- The GPC/SEC method is developed properly
- Experimental parameters are thoroughly determined and monitored over the complete system's life cycle

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3. Improving the reliability of GPC/SEC–light scattering and viscometry results

Online light scattering and viscometer detectors increase the information depth of GPC/SEC data and allow absolute molar masses and structures to be measured. However, a setup with advanced instrumentation is not parameter-free and the inter-lab comparison of results is often sobering. Sometimes even the comparison of inter-lab results or the reproducibility is not acceptable. In most instances, the reason for these deviations can be related to a wrong determination of the slice concentration. In contrast to conventional GPC/SEC, hyphenation with molar mass sensitive detection requires the precise and accurate knowledge of the mass in every chromatographic slice.

Why do setups with an LS detector or viscometer require a concentration detector?

GPC/SEC detectors can be classified according to the dependence of the signal intensity on the physical properties. The signal intensity of concentration detectors (refractive index (RI), UV/DAD, ELSD, and others) is described in Equation 1.

Equation 1.

$$\text{Signal intensity} = K_{detector} \cdot k_{sample} \cdot m_{injected}$$

With $K_{detector}$ = detector constant, k_{sample} = sample-related constant (e.g., refractive index increment (dn/dc) for RI) and $m_{injected}$ = injected mass. Many chemists replace the injected mass in this equation with the concentration, which is, of course, related to $m_{injected}$ and the injection volume and more commonly used in chemistry.

For molar mass sensitive detectors, another equation is used to describe the signal intensity:

Equation 2.

$$\text{Signal Intensity} = K_{detector} \cdot k_{sample} \cdot m_{injected} \cdot M^{x}$$

With M = molar mass, x = 1 for light scattering detectors (RALS, LALS, MALS), and x = Mark Houwink α for viscometers.

Figure 1 shows the difference between the two detector types. While a molar mass sensitive detector is ideal for high molar masses, a concentration detector can also detect low molecular weights with significant concentration.

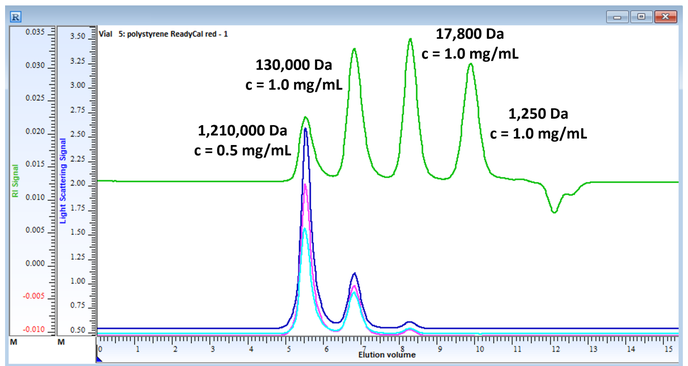

Figure 1. RI signal (green) and light scattering signal (blue) for four polystyrene samples with different molar masses and a narrow molar mass distribution.

Equations 1 and 2 are valid for the whole peak as well as for every chromatographic slice, i.

$K_{detector}$ and k_{sample} can be determined using reference materials or dedicated instrumentation. Therefore, when working with molar mass sensitive detectors, there are two sample-related unknowns left: the mass in every slice (m_{slice}) and the molar mass (M_{slice}).

An additional concentration detector can now be used to measure m_{slice} so that the combination of both detectors allows the molar mass to be determined.

In a second step, often after fitting the measured molar masses against the elution volume, the molar mass distribution and the averages are obtained by evaluating the concentration detector signal.

Which methods are used to determine the slice concentration?

In general, there are two independent methods that are used to determine the slice concentration or mass from the concentration detector signal.

Method A:

For the first method, Equation 1 is used:

$$\text{Signal intensity} = K_{detector} \cdot k_{sample} \cdot m_{injected}$$

First, $K_{detector}$ is determined. This is done using a reference material with a known k_{sample} and with known injected mass (known concentration). In the case of RI detectors, k_{sample} is the specific refractive index increment, dn/dc, which is also required for light scattering data evaluation. Often, the $K_{detector}$ is determined with just one concentration, however, it is, of course, more accurate and precise to use several concentrations. This procedure is the same as in HPLC when response factors are measured.

To determine the slice mass for unknown samples, the only unknown in Equation 1 is k_{sample}. In the case of RI detectors, this can be measured using dedicated (offline) instrumentation or be found in the literature.

Method B:

For the second method, it is assumed that the injected mass elutes completely from the column. This requires a precisely and accurately working injection system as well as an adsorption-free chromatographic method. In addition, all sample parts should be soluble or, at least, the number of insoluble parts should be known.

When using this method sample, preparation needs to be done extremely carefully. It is recommended to weigh in the sample and the solvent, taking the density of the solvent into account.

The mass in the chromatographic slice is now calculated using the rule of three. The total area under the concentration detector signal equals the total injected mass while the area in the chromatographic slice equals a proportion of it (Figure 2).

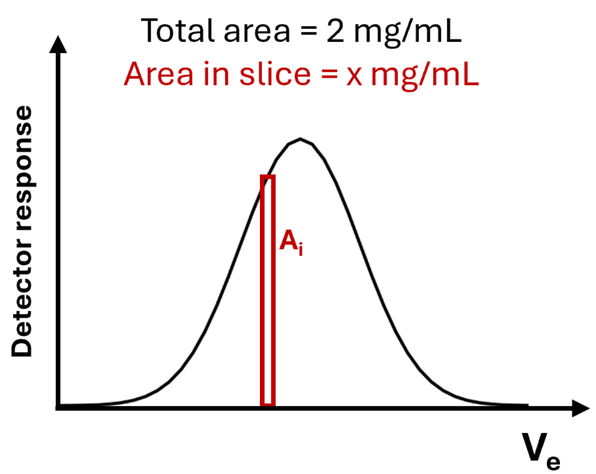

Figure 2. Rule of three for the determination of the slice concentration from the overall concentration, assuming that 100% of the sample elutes from the column in this peak.

If this method is used, it is possible to determine unknown parameters in Equation 1 with some preconditions. If, for example, $K_{detector}$ is known, k_{sample} can be determined. This method is used when the dn/dc of unknown samples is determined online within a GPC/SEC–light scattering setup. If k_{sample} is known, for example, $K_{detector}$ can be determined. This method is used when the detector response is determined (see method A).

What is the influence of a wrong concentration on the results?

Since the slice concentration influences the measured molar mass and other results, a wrong concentration leads to wrong molar mass averages and molar mass distributions. The effect depends on the detection applied, has been investigated using theoretical and experimental data, and is discussed in more detail in the literature.[1]

In GPC/SEC–light scattering, a lower-than-expected concentration leads to higher M_n and M_w values. The radius of gyration that can be additionally determined when using multi-angle light scattering (MALS) is not affected by a wrong concentration.

In GPC/SEC–viscometry, a lower-than-expected concentration leads to lower M_n and M_w values and higher intrinsic viscosity (bulk and slice value). The Mark-Houwink coefficient α is not affected by a wrong concentration.

Since the same concentration error leads to opposite effects on the molar mass determination when using GPC/SEC–LS or GPC/SEC–viscometry, it is now understandable why there are so often deviations between these two methods.

The use of triple detection does not help to identify a problem with the injected (or eluted) mass. In the case of 90° light scattering, where the viscosity data are only used to measure true light scattering molar masses, the effect of a wrong concentration is similar to that of a GPC/SEC–light scattering experiment only.

In triple detection systems where the methods are used independently (e.g., MALS or LALS plus viscometry), the large differences between the results of the different methods is at least indicating a problem.

What are the recommendations when working with LS and/or viscometry detectors?

Since the accurate concentration is of such importance for the reproducibility and accuracy of the molar mass determination, it is recommended that the sample solutions are carefully prepared.

Best results are obtained if the sample itself is available as a pure solid without residual solvent or monomer and if it is completely soluble.

Sample solutions should always be prepared freshly with weighing of the sample and the solvent. In the instance of highly volatile solvents (e.g., THF or DCM), the amount of added solvent should be double-checked prior to sample injection. Multiple injections from the same vial are acceptable if the injections occur as repeats. If the sequence needs to be repeated, it is good practice to prepare fresh samples.

Practical experience shows that the reproducibility of results is better when method A is used for the determination of the injected mass. This means $K_{detector}$ and k_{sample} should be known. However, if possible, the accurate concentration of the samples should also be known using a good analytical balance. It is then possible to calculate the mass recovery, which gives hints about the reliability of the method.

If method A cannot be used because the k_{sample} cannot be determined (e.g., because of too many different samples or a lack of dedicated instrumentation), method B together with a known $K_{detector}$ is often applied.

The use of checkout samples is always recommended when working with molar mass-sensitive detectors. If the molar masses of the checkout sample can be determined correctly, it can be assumed that the injection system is working properly and that the $K_{detector}$ of the concentration detector has not changed. Deviations in mass recovery for unknown samples are then most likely due to chromatographic method problems. If the molar masses of the checkout samples are not correct, the data help to identify which parameters have changed or which system parts are not working reliably.

What are the recommendations when working with manual injectors?

Autosamplers must not be better than manual injection. When following a few precautions, it is possible to work precisely and accurately with manual injectors in molar mass-sensitive detection. If the amount of sample solution is sufficient, it is recommended to flush the sample loop three to five times with the sample solution before filling the loop. For most manual injection systems, the needle should be left with the syringe in the port while the valve position is turned to injection. Of course, a well-maintained injector and regular replacement of the seals are required.

Reference

1. Held, D.; Kilz, P. *In* Quantification in LC and GC – A Practical Guide to Good Chromatographic Data, H. J. Kuss, S. Kromidas (Eds.), Wiley-VCH; **2009**.

4. GPC/SEC–light scattering: garbage in, garbage out

This section discusses the influence of analytical parameters on gel permeation chromatography/ size-exclusion chromatography–light scattering (GPC/SEC–LS) results when artificially wrong parameters reflecting typical experimental errors are used.

Hyphenation of GPC/SEC with light scattering can help both methods to overcome the limitations of each single method. Light scattering benefits from the separation power of GPC/SEC, and when combined with GPC/SEC, it is possible to determine the complete molar mass distribution of a sample and not just the weight average molecular weight. GPC/SEC benefits from the absolute detection principle of the light scattering detector, which reduces the need to rely on elution volume and calibration with narrow standards.

However, when combining two techniques, the prerequisites of both methods need to be fulfilled. Data obtained using an inappropriate GPC/SEC method will provide false distribution information. Results obtained using the wrong parameters or settings for light scattering detection will result in the wrong molar mass distributions.

Why is precise and accurate concentration needed for GPC/SEC–light scattering measurements?

The scattering intensity of a substance is a product of an instrument-specific constant (light scattering detector constant), a sample-specific constant (refractive index increment, $[dn/dc]^2$), the molar mass, and the concentration. Both parameters contribute—the higher the molar mass and/or concentration, the higher the signal intensity. If the molar mass needs to be determined, the concentration needs to be known.

Batch light scattering experiments (only light scattering, no GPC/SEC fractionation prior to detection) are easy to evaluate because the sample concentration can be determined using a balance. In this case, all parts of the sample are present at the same time in the cell and contribute to the measured intensity of the scattered light.

This is different for online experiments. Although the complete sample will be injected with a known concentration, the sample parts (molecules of different sizes) are fractionated in the column. They reach the light scattering detector cell at different times and the light scattering data are obtained for fractions with unknown concentrations.

Concentration determination for GPC/SEC–LS measurements

A typical solution to overcome the concentration problem is to use a concentration detector (typically refractive index, RI, or UV/DAD) in combination with the light scattering detector. This allows the concentration of each single slice or chromatographic fraction to be measured online.

There are two approaches to determine the slice concentration, c_i:

1. **100% Mass recovery/injected mass:** Here, it is assumed that the area underneath the signal peak corresponds to the injected mass (concentration and injection volume-dependent). This means that all parts of the sample are soluble, that everything is injected properly, and that everything elutes from the column. The parameters that mostly influence the results are dn/dc, concentration, and injection volume. If these parameters are wrong, the obtained molar masses are also wrong.

2. **Calibrated concentration detector and known sample-specific constant for the concentration detector:** In this case, a response factor/detector constant is determined for the concentration detector, similar to high-performance liquid chromatography (HPLC) data evaluation processes. In the case of RI detection, this constant and the dn/dc of the sample are used to determine the slice concentration from the signal intensity of the concentration detector. The parameters that mostly influence the results are dn/dc and the detector constants of the concentration detector and the light scattering detector. If these parameters are wrong, the obtained molar masses are also wrong. Note that the detector constant of the light scattering detector will influence the results of the 100% mass recovery method in the same way. The concentration detector constant will show no influence.

Influence on results from dn/dc and concentration determination

Using a calibrated concentration detector and the matching sample-specific constant is the more robust method and leads to less deviation and higher reproducibility of the results.

Table 1 and the corresponding Figure 1 compare results for a polystyrene (PS) sample evaluated using artificially wrong evaluation parameters for the method 100% mass recovery.

Table 1. Results for the evaluation of a PS reference material with a GPC/SEC–light scattering, concentration detection method "100% mass recovery" with correct and artificially wrong parameters.

	M_w Light Scattering (Da)	M_n Light Scattering (Da)
All parameters correct	87,500	85,600
dn/dc (–1.6%)	90,400 (+3.2%)	88,500 (+3.4%)
Injection volume (–11%)	97,200 (+11%)	95,200 (+11%)
Concentration (–2.4%)	89,600 (+2.4%)	87,700 (+2.5%)
All parameters wrong	103,000 (+18%)	100,600 (+18%)

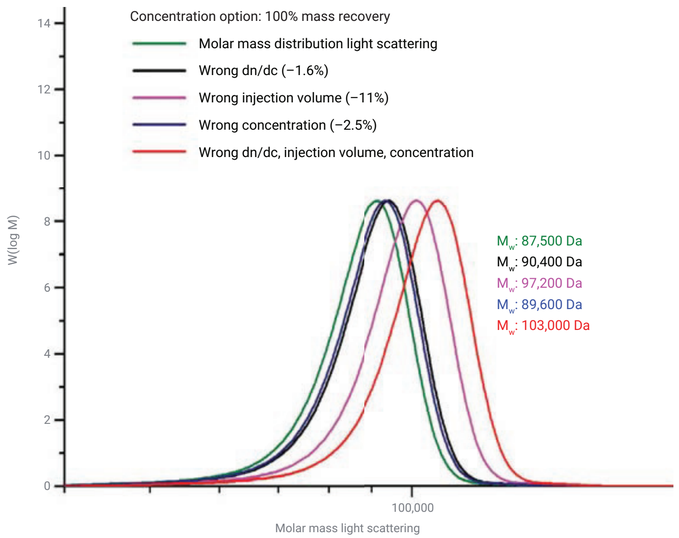

Figure 1. Molecular weight distributions obtained using light scattering detection with correct and artificially wrong parameters for slice concentration detection based on 100% mass recovery.

Table 2 and the corresponding Figure 2 compare results for the same PS sample evaluated using artificially wrong evaluation parameters for the method-calibrated RI and known dn/dc.

Table 2. Results for the evaluation of a PS reference material with GPC/SEC–light scattering, concentration detection method "calibrated RI and dn/dc known" with correct and artificially wrong parameters.

	M_w Light Scattering (Da)	M_n Light Scattering (Da)
All parameters correct	87,500	85,600
dn/dc (−1.6%)	88,900 (+1.6%)	87,000 (+1.6%)
Wrong RI constant (+5%)	83,300 (−4.8%)	81,600 (−4.9%)
Wrong LS constant (−5%)	83,100 (−5.0%)	81,400 (−5.2%)
All parameters wrong	80,400 (−8.1%)	78,800 (−8.0%)

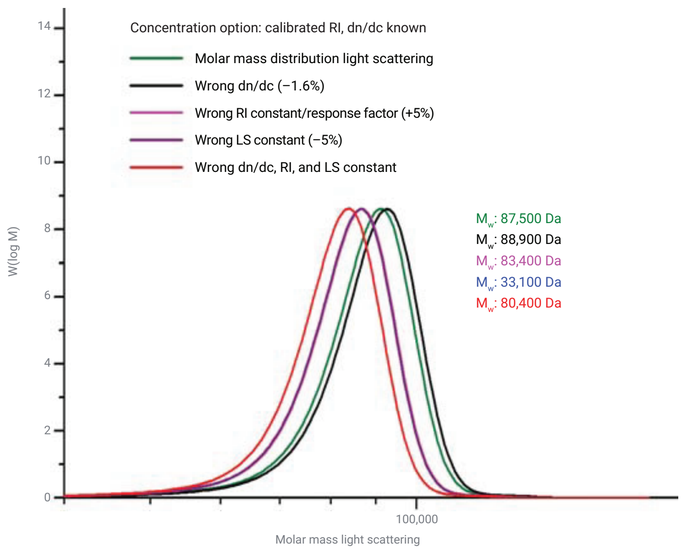

Figure 2. Molecular weight distributions obtained using light scattering detection with correct and artificially wrong parameters for slice concentration detection based on a calibrated RI and known dn/dc.

Comparison of the data shows that if all parameters are correct and chromatographic conditions are good, both concentration detection methods will yield the same results within experimental error. This would not be the case if, for example, the wrong parameters are used, parts of the sample are insoluble, parts of the sample are retained by frits or on the column, or in the case of unreliable injection.

In general, the influence of a wrong dn/dc on the results is less for the method "Calibrated concentration detector and known sample-specific constant". Also, this method is more robust with respect to the most common experimental error of an incorrectly determined injected mass or incompletely eluted mass. It is good practice to prepare samples in a way that it is possible to work with both concentration detection options. This means that all samples and solvents should be weighed to ensure a precise and accurate concentration. It is also then possible to check sample recovery.

Comparison to conventional GPC/SEC and GPC/SEC–viscometry (universal calibration)

One advantage of GPC/SEC alone is that absolute sample concentration does not influence the results. Changing any of the parameters above will not influence the results. Molar mass averages and molar mass distribution results will be reproducibly the same for all the curves shown if only standard detection is used. Even the constant for the concentration detector does not influence the results.

In contrast, the majority of the parameter changes above will also influence the results obtained with GPC/SEC–viscometry. As with GPC/SEC–light scattering, this method also relies on slice concentration determination. However, the shift of GPC/SEC–viscometry results will be in the opposite direction to GPC/SEC–light scattering. If a wrong concentration parameter decreases the molar mass for light scattering evaluation, it will increase the molar mass for viscometry/universal calibration. One of the most common experimental problems therefore leads to a strong deviation between results for the two methods that should measure true molar masses. Understanding the influence of analysis parameters is therefore essential for users of advanced multidetection systems.

Summary

- While conventional GPC/SEC results are not affected by imprecise and inaccurate concentrations, GPC/SEC–light scattering results show a dependence.
- Two independent concentration detection methods can be applied for GPC/SEC–light scattering. The method to measure the slice concentration using a calibrated detector and a known sample-specific constant is more robust. This method is also less affected by a wrong dn/dc.
- dn/dc Needs to be considered every time that light scattering detection is applied for GPC/SEC. The dn/dc should be high enough and constant over the evaluated fractions, which limits the applicability of GPC/SEC–light scattering for copolymers. GPC/SEC–light scattering results and GPC/SEC–viscometry results are influenced in opposing directions by the same experimental error.

5. Column issues with light scattering detectors

The addition of a light scattering detector dramatically increases the capabilities of gel permeation chromatography/size-exclusion chromatography (GPC/SEC) analysis. However, the complexity of the system also increases.

Detectors that measure the intensity of scattered light in solution are very popular additions to GPC/SEC instruments because they add true value.[1] The light scattering detector models differ mainly in the position where the scattered light is measured and in the number of simultaneously acquired signals. These detectors should never be confused with evaporative light scattering detectors (ELSD)[2], which measure scattered light from analyte droplets after mobile phase evaporation.

Light scattering detectors are molar mass-sensitive detectors. Their signal response depends on both the concentration and the molar mass. The higher the molar mass and the concentration, the higher the measured signal intensities. The sample itself also plays an important role: the higher the polarizability of the sample (compared to the solvent), the higher the signal intensity. This sample–solvent parameter is addressed as the refractive index increment, dn/dc.

To obtain the desired results, LS detectors need to be combined with at least one concentration detector, such as a differential refractive index (RI) detector or a UV detector. To gain precise, accurate, and reliable results, impurities that interfere with the LS detector need to be reduced to a minimum.

What are the challenges when working with light scattering detectors?

LS detectors respond very sensitively to high molar masses and large sizes and particles, not only from the sample of interest, but also from any contamination in the system. They are able to detect large analytes at concentrations that are below the detection limit of concentration detectors, such as RI or UV, and therefore using them for analysis is very challenging because they require very clean systems.

An advantage of GPC/SEC–LS compared to batch LS experiments is that the columns can act as a huge filter so that extensive solvent filtration (typical for batch application) can be avoided. On the other hand, columns could unintentionally be a source of contamination.

While the concentration detector shows a stable baseline, that is, it is balanced to the solvent itself, an increased LS baseline level (also referred to as high background) and a noisy LS signal are reported when a molar mass sensitive LS detector is connected for the first time to an existing system or when new columns are installed. Initial bleeding of gel particles eluting from the column may cause a permanent scattering noise level as a result of their high molar mass.

The severity of the problems caused depends on the analytical conditions, such as the stationary and mobile phase used, the sample molar mass, the dn/dc, and the detection angle.

As a guideline:

- The lower the detection angle, the greater the likelihood of problems. The 90° angle is not so greatly affected, but low angles are especially prone to a decrease in baseline stability and increase in noise.
- The smaller the dn/dc and the molar mass, the lower the signal. Therefore, a higher noise level can be more problematic for low molar mass or low dn/dc samples, while high molar mass and high dn/dc samples can still be analyzed because of a better signal-to-noise (S/N) ratio.
- Aqueous systems are more complicated than systems operated in organic solvents.
- In organic systems, some organic solvents are more challenging than others.

Figure 1 shows the typical problems. While the RI signal is well-suited for evaluation, the two light scattering detector signals are not. For the low angle, it is impossible to detect a peak because of the high background, the noise, and the drift.

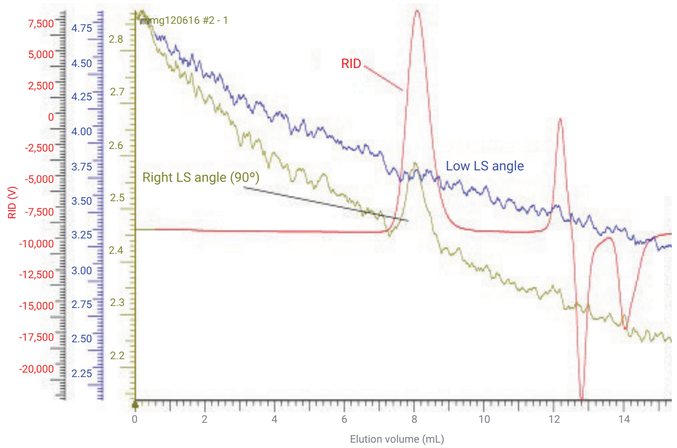

Figure 1. Chromatogram of polymethyl methacrylate (PMMA) 100K Da in DMAc on a freshly installed (new) column using an RI (red trace) and an LS detector (blue trace low angle and yellow trace 90° angle). While the RI signal shows a stable baseline, the LS detector signals suffer from a high background (see corresponding axis, worse for the low angle) with a strong drift and a high noise.

Influence of the column stationary phase

Two different types of stationary phases are required to cover the wide range of GPC/SEC applications: silica-based and polymer-based materials.

Compared to polymer-based columns, silica-based materials have a much higher pressure stability. They are often used for protein separations. Polymer-based columns are less prone to interaction problems. In addition, they can cover much wider molar mass ranges. As a result of their less steep exclusion and total permeation limits, they can be combined to column sets.

The challenges in GPC/SEC–LS are the same for both column types. The reason for this is the particular structure of the stationary phase. Column packing materials are typically made of particles in the range of < 2 to 20 µm. Particles in the nanometer range are formed as well. These nanometer-sized particles and the particle fragments formed during postpolymerization processes (Figure 2) cause the problems described. They cannot be retained by the frits installed in the columns or by externally installed frits because they are generally much smaller than the mesh size of the frits.

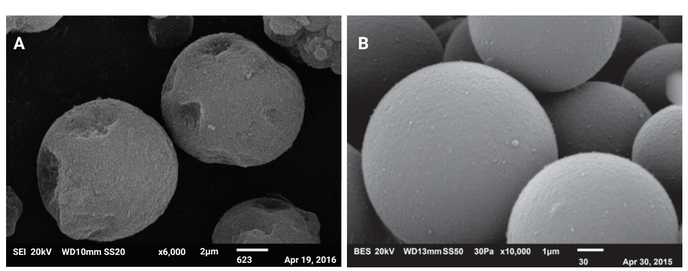

Figure 2. SEM showing particles (A) with and (B) without fragmentation.

Standardized cleaning and purification after manufacturing, which is sufficient to allow for clean columns generating high-quality results with RI, UV, or viscometers, does not ensure LS applicability of the column.

Influence of the mobile phase

The mobile phase can affect the contamination dramatically. While the same stationary phase works fine in one solvent, operation in another solvent can be difficult. This is observed for polymer-based stationary phases comparing tetrahydrofuran (THF) and dimethylformamide/dimethylacetamide (DMF/DMAc). Silica-based materials behave differently if the mobile phase is changed, for example, from THF to hexafluoroisopropanol (HFIP). While the column works perfectly for the GPC/SEC–LS application in THF, it needs an additional cleaning step after switching to HFIP. It is therefore important that the proper solvent is chosen when the column is ordered from the supplier.

How to overcome these problems

To overcome column-related problems in GPC/SEC–LS, one must distinguish between three different scenarios: (a) The problems are always present, independent of the time the columns are in use; (b) The problems occur suddenly, the column used to work perfectly before; (c) The problems occur only when new columns are installed. "Older" columns do not cause any problems.

In (a), the column used is probably not designed to be compatible for light scattering. In a few cases, a filter installed after the columns but before the light scattering

detector can be a solution. This filter should be replaced regularly. However, if the filter needs to be exchanged often because of blocking or pressure increase, and mobile phase quality and sample preparation can be eliminated as a problem source, a column replacement is unavoidable. Please note that filters will not help if the impurities are smaller than the mesh size. In addition, filter usage should be monitored carefully because sample parts can be removed by the filter, too.

In (b), the columns need to be inspected more carefully—either the column bed is damaged or additional impurities elute as a result of solvent or temperature change. The column performance can be tested by measuring the plate count, asymmetry, and resolution.[3]

For scenario (c), where the columns are generally light scattering-applicable but not ready to use yet, fragments and particle fines can be removed by flushing the columns. In this case, it is recommended to ask for a cleaning or purification process, if possible, from the supplier. While doing the purification step, the column should not be connected to any type of detector to avoid detector contamination. However, this process may take several column volumes to remove the fine parts from the huge surface of the macroporous column material.

Some column manufacturers have responded to this tedious, time-consuming challenge by offering stationary phases specifically optimized for light scattering detection. These columns are pretreated to reach the steady state faster and to show less noise so that they are ready to use directly after installation. During the pretreatment, the noise level goes down constantly and the S/N increases with the reduced noise level. This is shown in Figure 3. Here, the same sample has been injected on the same column, which is halfway through the cleaning process. The baseline level of the light scattering detector signal has decreased significantly (compare the two right Y-axes in Figure 1) and the noise has also been reduced.

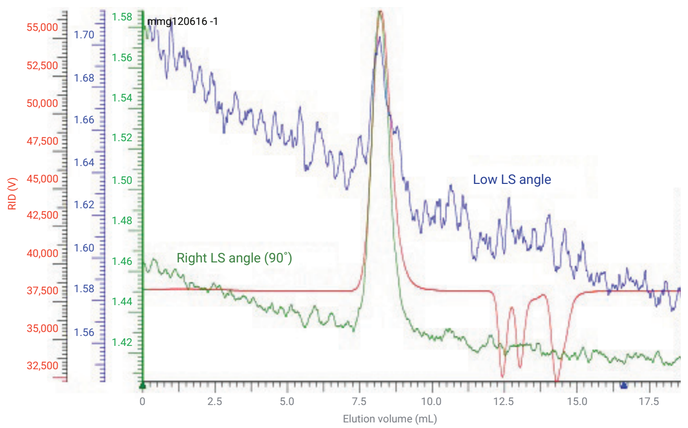

Figure 3. Pretreatment of an Agilent GRAM column; the reduction of the noise is shown as a function of time and observation angle.

Figure 4 shows the final result after the pretreatment. The noise level has significantly decreased for both LS angles. The polymer signals correspond to a polymethyl methacrylate (PMMA) and a polystyrene (PS) of the same molar mass in one of the most challenging mobile phases, DMAc. The middle injection shows butylated hydroxytoluene (BHT) injected for column testing. The PS signal has a higher intensity because of the higher dn/dc compared to PMMA.

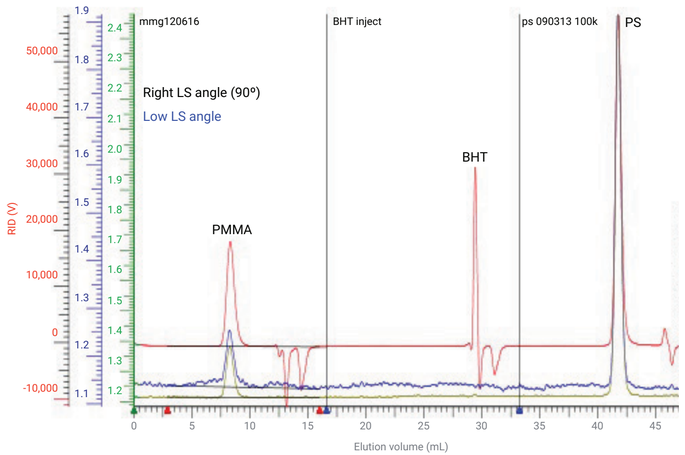

Figure 4. Performance of an Agilent GRAM column after completing the pretreatment. Polystyrene (PS) and PMMA data in one of the most challenging mobile phases (DMAc) are shown. The difference in response is a result of the different dn/dc values.

Summary

- While a column can be sufficiently clean for a concentration detector, this may not be the case for LS detectors.
- Light scattering detectors respond to low concentration and large size contaminants, resulting in increased baselines and higher noise for systems that are not ultraclean.
- Small, nanometer-sized particles and particle fragments need to be removed for both porous polymer- and porous silica-based particles.
- Pretreated light scattering columns available from the supplier show a low noise level after a short period of time.

References

1. Held, D; Kilz, P. *The Column* **2009**, *5(4)*, 28–32.
2. Held, D. *The Column* **2013**, *9(18)*, 2–5.
3. Gores, F.; Held, D. *The Column* **2014**, *10(14)*, 7–10.

6. How to determine dn/dc values

The refractive index increment (dn/dc) is an essential parameter when analyzing light scattering (LS) data, independent of the LS method applied.

Light scattering is an absolute method for measuring molar mass and is therefore an important tool when analyzing polymers, biopolymers, and proteins. Many R&D and QC labs combine light scattering detectors with separation techniques such as gel permeation chromatography/size-exclusion chromatography (GPC/SEC). However "absolute" does not mean parameter-free, rather the opposite. One of the most important parameters for light scattering is the refractive index increment (dn/dc), because this sample and method-related parameter determines if light scattering can be applied successfully, and calculates accurate results.

What is the dn/dc?

The dn/dc refers to the rate of change of the refractive index with the concentration of a solution for a sample at a given temperature, a given wavelength, and a given solvent:

Equation 1.

$$\frac{dn}{dc_{c=0}} = \lim_{c \to 0} \left(\frac{n - n_0}{c} \right)$$

Above a certain molar mass, the influence of end groups can be neglected and the dn/dc of homogeneous polymers is constant and can be considered as a contrast factor. A high dn/dc indicates higher signal intensity and therefore higher accuracy and precision. This can be easily understood by looking at Equation 2, which shows the dependency of the signal intensity for LC detectors from the most important instrumental and experimental parameters:

Equation 2.

$$\text{Signal Intensity} = K_{detector} \cdot k_{sample} \cdot \text{concentration} \cdot M^{x}$$

In addition to the detector constant ($K_{detector}$) and the concentration (or injected molar mass), signal intensity also depends on a sample related constant (k_{sample}) and may depend on the molar mass (M).

The molar mass exponent x equals 1 for a molar mass sensitive detector (such as a light scattering detector) and is 0 for a typical concentration detector (such as a refractive index detector [RI]). When using either an RI or a light scattering detector, k_{sample} is equal to the dn/dc.

The consequences can be best explained when looking at typical detector signals.

Figures 1 and 2 show a raw data comparison of mixtures of four different molar masses for polystyrene (PS) and polymethyl methacrylate (PMMA) in tetrahydrofuran (THF) for an RI detector (Figure 1) and a light scattering detector (Figure 2, MALS, 90° only). While concentration and injection volume are the same for both mixtures and the molar masses are comparable, the dn/dc values are very different. Under these conditions, PS has quite a high dn/dc of 0.184 mL/g, while the PMMA dn/dc is much smaller at 0.089 mL/g. It is clear from the figures that the signal intensities and peak areas for the PS RI signals are much higher than for PMMA. This is even more pronounced for the light scattering signal because this signal depends on dn/dc.[2] It is also important to note that as the dn/dc gets higher, the lower the molar masses that can be detected (under the same analytical conditions).

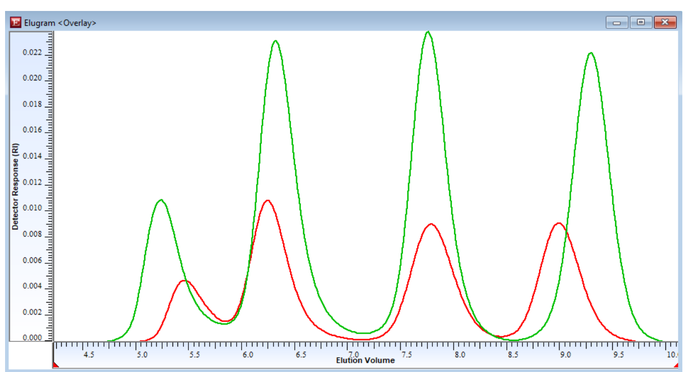

Figure 1. RI detector signals for a mixture of four different polystyrene molar masses (green trace, dn/dc = 0.187 mL/g) and four different polymethyl methacrylate molar masses (red trace, dn/dc = 0.087 mL/g). Conditions: peak 1: 0.5 mg/mL, peaks 2 to 4: 1 mg/mL.

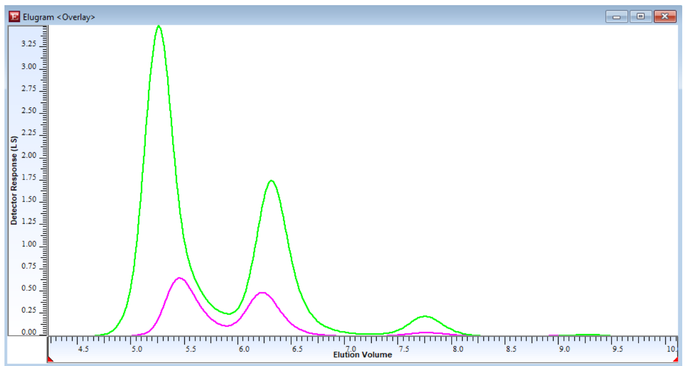

Figure 2. MALS 90° detector signals for a mixture of four different polystyrene molar masses (green trace, dn/dc = 0.184 mL/g) and four different polymethyl methacrylate molar masses (pink trace, dn/dc = 0.089 mL/g). Conditions: peak 1: 0.5 mg/mL, peaks 2 to 4: 1 mg/mL. Molar masses PS/PMMA: 1.2/1.0 MDa, 130/202 kDa, 17.6/18.7 kDa, 1,250/2,380 Da.

The dn/dc values can also be zero or negative, meaning that it is not possible to perform detection with RI/light scattering (dn/dc = 0, sample is isorefractive) or that the RI signal is negative. A well known example of an isorefractive system is polydimethyl siloxane (PDMS) in THF. In this example, only small RI signals can be measured because of an end group effect, and typical light scattering detection is not possible. One solution is to either replace the RI with an evaporative light scattering detector (ESLD)[2] or to switch to using toluene as the solvent and mobile phase. In this case, the dn/dc will be less than zero, resulting in negative RI traces.

Determining dn/dc values

There are a number of ways to determine dn/dc, and analysts select a method based on the accuracy and precision required as well as the applicability in the laboratory. The three different general approaches are as follows:

Approach 1: Literature data can be used (if they are available), but it is important that experimental data such as mobile phase, temperature, and wavelength (for example, 635 nm) are identical to the experimental conditions. Good sources for dn/dc data are manuals for light scattering detectors or general literature.[3–5] It is always good practice to verify literature data.

Approach 2: A more accurate method is to measure the dn/dc online with the sample using the peak area(s) detected by the RI of the liquid chromatography (LC) equipment. This method is often applied because it is fast, easy-to-use, and does not consume additional sample. This is explained in more detail in the following section.

Approach 3: The most accurate method is to use dedicated batch/standalone instrumentation and to measure different concentrations. This requires more time and sample than the online method, but is not influenced by chromatographic parameters. Again, there is a detailed description in this chapter.

Approach 2: online dn/dc determination

In this approach, the measured RI signal area is used to get a dn/dc value, but it can be problematic if the general assumptions that 100% of the sample mass is injected and elutes from the column fail. Also, coelution of system peaks or additives—because of inefficient separation—can be a problem. According to Equation 2, the RI area depends on the sample concentration, the RI detector constant, and the dn/dc value.

The first step is to determine the RI detector constant by injecting at least one sample with a precisely known concentration and dn/dc. It is a prerequisite that all of the sample reaches the detector cell. With this detector constant, and again assuming that all of the injected sample mass reaches the RI detector, the dn/dc can now be determined. The strategies adopted by laboratories are very different, with some using only one sample concentration to save time and sample volume, while others inject several concentrations to minimize the experimental error.

Figure 3 shows an experiment with five different concentrations where the injected mass is plotted versus area. The dn/dc is determined from the slope, which reflects the detector constant multiplied by the dn/dc. For this approach, typical RI detectors are very often used independently of the detector light source type. While there are some RI detectors with a fixed wavelength on the market, many laboratories also use RI detectors successfully with a white light source.

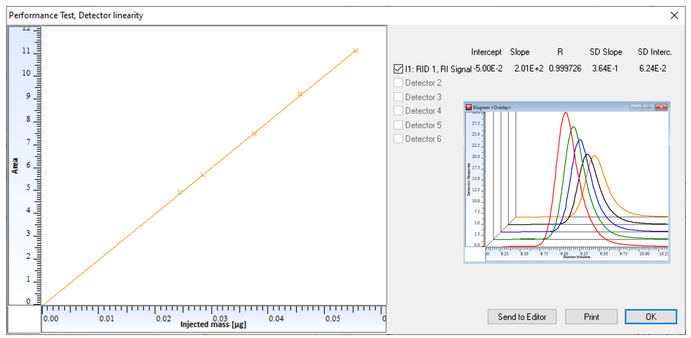

Figure 3. RI peak area plotted versus the injected mass (concentration, injection volume) for five different concentrations and double injections. The dn/dc can be obtained from the slope, which depends on the RI detector constant and the dn/dc. Inset: raw data RI detector for five concentrations.

Approach 3: batch dn/dc determination with dedicated instrumentation

Dedicated standalone instrumentation can be used for highly accurate and precise dn/dc values. Most commercial instruments today are deflection-type instruments optimized for convenient batch mode operation, and are available with different wavelengths (red, green, or blue light sources) to allow for interpolation of other wavelengths.

The procedure is comparable to the one using an RI detector. The first step is to calibrate the instrument (determine the detector constant), which is performed using NaCl or KCl solutions. Afterwards, the sample dn/dc can be measured by manually injecting five or more concentrations ranging from 0.1 mg/mL to 10 mg/mL.

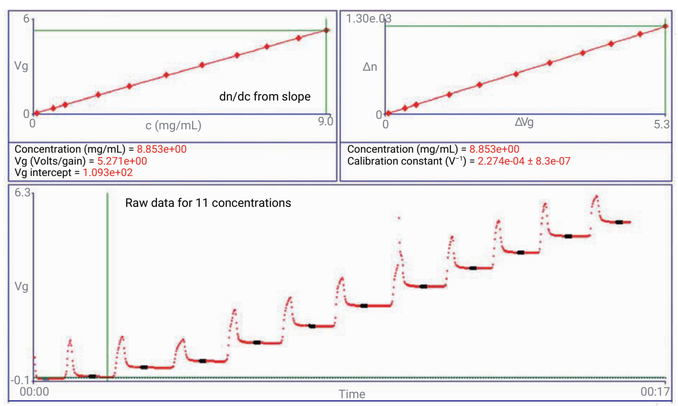

Figure 4. Determination of dn/dc with a dedicated deflection type batch dn/dc instrument (620 nm). Raw data for 11 concentrations and automatic dn/dc determination from slope.

Summary

The dn/dc is a crucial parameter for the analysis of standalone or online light scattering data independent of the method applied (LALS, MALS, RALS).

dn/dc Values can be taken from the literature if the experimental conditions match; however, a verification of the value is highly recommended.

dn/dc Values can be measured online with an RI detector, and also in a hyphenated GPC/SEC/gel filtration chromatography (GFC) light scattering setup. This is a fast approach that often does not require additional sample. It is only applicable if 100% of the sample elutes from the column and requires accurate sample concentrations.

Most accurate is the offline approach to measure dn/dc values using dedicated instrumentation, but it also requires more time and sample.

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Glossary

BHT	Butylated hydroxytoluene
c_i	Slice concentration
Da	Dalton (g/mol)
DAD	Diode array detector
DCM	Dichloromethane
DMAc	Dimethylacetamide
DMF	Dimethylformamide
dn/dc	Refractive index increment
ELSD	Evaporative light scattering detector
η	Intrinsic viscosity (eta)
FRCM	Flow rate correction marker (internal standard)
GFC	Gel filtration chromatography
GPC	Gel permeation chromatography
HFIP	Hexafluoroisopropanol
λ	Wavelength of light (lambda)
LAC	Liquid adsorption chromatography
LC	Liquid chromatography
LiBr	Lithium bromide
LiCl	Lithium chloride
LS	Light scattering
$K_{detector}$	Detector constant
k_{sample}	Sample-related constant
J_0	Primary beam intensity
MALS	Multi-angle laser light scattering
M_n	Number-average molar mass
Mobile phase	Liquid phase used in a chromatography system
m_{slice}	Slice mass
M_{slice}	Slice molar mass
M_w	Weight-average molar mass
M_z	z-Average molar mass
NaCl	Sodium chloride
NaN_3	Sodium azide
QC	Quality control
PDI	Polydispersity index ($D = M_w/M_n$)
PDMS	Polydimethylsiloxane
PMMA	Polymethyl methacrylate
PS	Polystyrene
RG	Radius of gyration
RI	Refractive index (detection/detector)
SDV	Styrene-divinylbenzene
SEC	Size exclusion chromatography
SELS	Solvent-enhanced light scattering
Solvent	Liquid in which a solute is dissolved to create a solution
Stationary phase	Solid phase in a separation device on which materials will be separated
TCB	Trichlorobenzene
THF	Tetrahydrofuran
UV	Ultraviolet (detection/detector)
V_e	Elution volume

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