Tips & Tricks: GPC/SEC
Do's and Don'ts in GPC/SEC Light Scattering

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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 became 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, requires knowledge of evaluation parameters and thorough set-up measurements.

Weigh in 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 for the molar mass determination.

The slice concentration can be measured with a concentration detector (e.g., an RI, 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 in 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 non-soluble sample parts are not affecting the slice concentration (and, therefore, the molar mass). It is nevertheless recommended to weigh in the sample precisely because then it is 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 the molecular concentration change, dc. The dn/dc of a sample depends (amongst 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 approx. 10000 Da.[2] Any error in the dn/dc results

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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 on-line dn/dc determination, it is required that the sample concentration is precisely known. Insoluble sample parts, water and/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 Check Out Samples and Validate Your System

Besides the sample-related evaluation parameters discussed above, GPC/SEC light-scattering data evaluation requires the knowledge of a number of system-related parameters. Proper data analysis can only be done 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 (MALLS detectors only).

If any of these parameters are wrong or have changed, the molar mass results will be wrong. Using a check out sample helps to identify accidental or yet unidentified changes in the set-up. Moreover, if the check out 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.

Don't Expect Accurate Results...

...for copolymers

The 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]

Moreover, GPC/SEC light-scattering experiments do not give any results on the copolymer composition or other

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

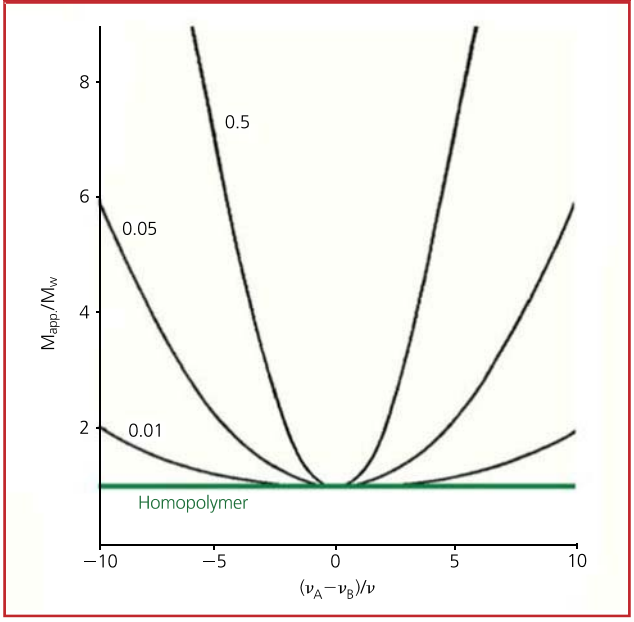
	Variation	M_w (Da)	Deviation (%)	M_n (Da)	Deviation (%)
Reference value	-	300000	-	150000	-
dn/dc	−5%	331000	10.3	165600	10.4
Concentration	−5%	315800	5.27	158000	5.33
Inter detector delay	5%	295100	−1.63	159500	6.33

important parameters such as homogeneity of the sample. For copolymers other characterization methods (e.g., copolymer characterization with dual detection, liquid adsorption chromatography (LAC) or 2D chromatography) provide more valuable information than an apparent molar mass only.[7]

...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 the way 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).



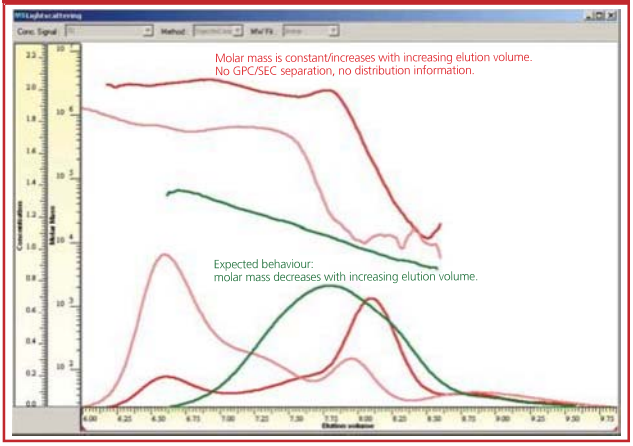
Don't Expect Accurate Molar Mass Distribution Information...

...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 on-line 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 MALLS detectors). All distribution

Figure 2: (Red) Examples of results from measurements with 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 behaviour is expected from the GPC/SEC separation principle. In addition to the molar masses the measured slice concentrations for the samples are shown.



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, where the expected behaviour is observed.

... 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/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 non-invasive 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 determined thoroughly and monitored over the complete system's life cycle.

References:

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