

Tips & Tricks GPC/SEC: Quantify and Get More Than Molar Mass Averages

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Gel permeation chromatography/size-exclusion chromatography (GPC/SEC) is used to determine molar mass averages and the complete molar mass distribution with just one injection. This is possible because GPC/SEC is a fractionating technique. The fractionation power allows the higher molar mass fraction to be characterized and more about low molar mass compounds such as oligomers, additives, or residual educts to be learned.

Gel permeation chromatography/size-exclusion chromatography (GPC/SEC) is a liquid chromatographic fractionating technique that separates molecules based on their size in solution. The fractionation occurs in the pores of particles, which have been packed into a separation column. Molecules of larger sizes cannot enter the pores and therefore elute first, while the smaller ones penetrate the pores and elute later.

A typical GPC/SEC calibration curve (see Figure 1), where the logarithm of the molar mass is plotted versus the elution volume, illustrates this behaviour nicely and helps to distinguish between three regions. In region 1, the region between the injection volume and the exclusion limit, nothing can elute. At the exclusion limit, very large molecules of the sample, independent of their size, elute at the same elution volume because the

molecules (compared to the pores) are too large to enter the pores. Region 2 starts at the exclusion limit. This is where efficient size separation begins as the molecules can enter some pores but are excluded from others. Here the molar mass distribution can be accurately determined. Region 2 ends at the limit of total penetration. At the total penetration limit, the molecules in solution are so small that all of them can penetrate all pores. Region 3 begins after the total penetration limit. It is this region that is the most interesting for learning more about low molar mass fractions or additives.

In theory, if the driving force for separation is pure interaction-free size-exclusion, it should not be possible to separate molecules such as toluene from butylated hydroxytoluene (BHT) or acetone. However, there is often a separation.



Figure 1: A typical GPC/SEC calibration curve constructed using molar mass standards with a narrow molar mass distribution (each green dot is one standard) illustrates the three regions. At the exclusion limit and the total penetration limit separation starts and ends, respectively.

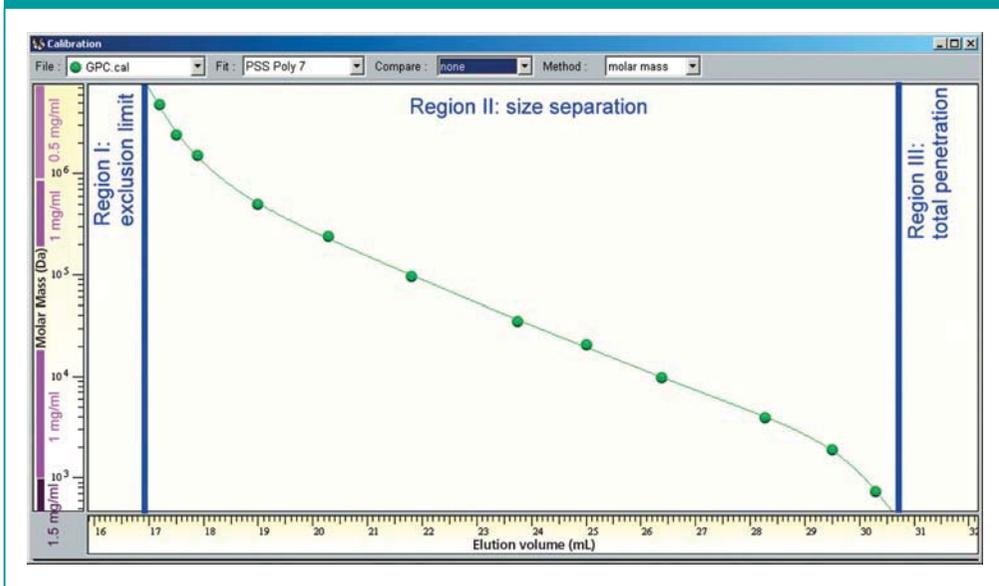


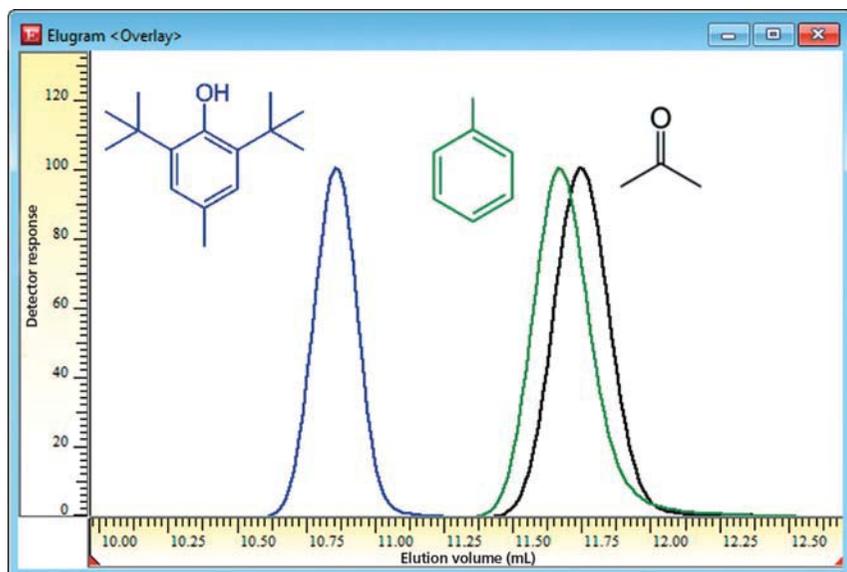
Figure 2 shows the overlay of chromatograms of BHT, toluene, and acetone obtained using a column with a medium molar mass separation range. There is a (baseline) separation, which cannot be explained by the size difference of the molecules alone. Therefore, the separation must result from enthalpic interactions between the analyte and the stationary phase, indicating that a mixed mode mechanism

is operating. This means that both molecular size and interaction of the molecules with the stationary phase contribute to the elution behaviour.

Although interactions should be strongly avoided when determining molar masses of polymers by GPC/SEC, this mixed separation can be an advantage because it allows the identification and even quantification of low molar mass substances.



Figure 2: Overlay of BHT (blue), toluene (green), and acetone (black) injected in three individual runs onto the same GPC/SEC column with a medium molar mass separation range. Although the size of the molecules in solution is so small that they all fit into pores, a separation is observed.



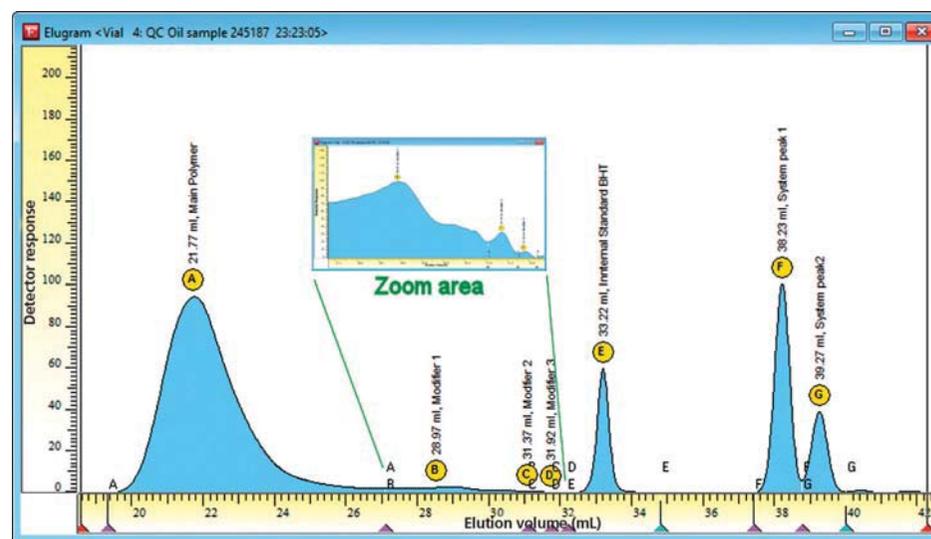
Identification

Figure 3 shows a chromatogram of a QC sample with a broad polymer peak and different peaks eluting at the low molar mass end (high elution volumes) and close to the total penetration limit. The very first step is to distinguish between peaks belonging to the sample and typical system peaks. This can be done by measuring a blank sample — pure mobile

phase (ideally solvent taken from the mobile phase bottle) — which has been treated in the same way as the sample.

An overlay of the blank injection with the sample can show which peaks are system peaks. In Figure 3 the two peaks F and G have been identified as system peaks (1). Peak E also does not belong to the sample and results from the added internal flow marker, BHT (2). The sample

Figure 3: Chromatogram of an oil sample. Overlay with a blank injection allowed peaks F and G to be identified as system peaks. Peak E is the internal flow marker BHT. The figure inset shows a zoom of the low molar mass fractions, where peaks C and D should be identified and quantified.



itself contains a polymer part (Peak A) with a long tailing. In the tailing area three peaks (B, C, and D) should be further characterized.

Several strategies can now be applied to identify the peaks. If there is already a suspect (for example, educts from the production process, residual monomer, or a known solvent, initiator, or additive) and the pure substances are available, they can be prepared and injected as separate

samples. Assigning the peaks can be done by overlaying the chromatograms or comparing the elution volumes. It is also possible to spike the original sample with the suspected substance and to monitor an increase in the respective peak area.

However, relying only on the peak position might not be sufficient. Verification can be performed by advanced detection techniques or fraction collection followed by an off-line

characterization. This is also recommended when there is no information at all about potential additives or contaminants.

An easy and cost-effective approach is the use of additional ultraviolet (UV) detection. Often GPC/SEC systems already comprise two different types of concentration detectors: the more universal refractive index detector (RI), and the more specific UV detector (or diode array detector/photodiode array [DAD/PDA]). If the substance is UV-active, the UV wavelength can be chosen to specifically detect the substance. If a DAD/PDA is available, the spectra can also be used for identification.

It requires more effort to hyphenate GPC/SEC with on-line mass spectrometry (MS), nuclear magnetic resonance (NMR), or (for a limited number of solvents) with Fourier-transform infrared spectrometry (FTIR) detection (3).

A very practical approach is fractionation followed by an off-line analysis. As GPC/SEC is a non-destructive technique, it is possible to collect fractions and to apply spectroscopic or spectrometric techniques off-line to characterize the fractions. If the amount of collected fraction is not sufficient, repeated fractionations can be performed. Analytical GPC/SEC systems can easily

be upgraded to semi-preparative systems by replacing the analytical column with a preparative column, so that higher amounts can be injected. Automation is possible by adding a fraction collector.

An elegant way to hyphenate GPC/SEC with FTIR connection is the use of an on-line collection module. This FTIR interface is connected as the last device in the system. A heated nozzle evaporates the solvent as the effluent leaves the column and the nonvolatile components are deposited on a rotating Germanium disk, resulting in a special separation of the different components. After the run is finished, the trace on the Germanium disk is analyzed spot by spot using an FTIR spectrometer. A similar approach is possible for matrix-assisted laser desorption/ionization (MALDI) instruments.

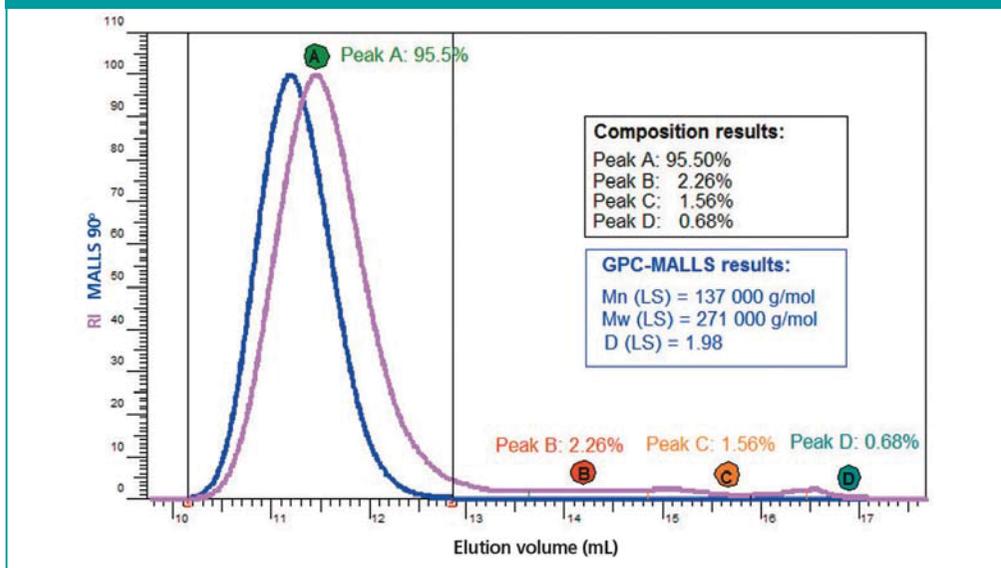
Problematic for both approaches, fraction collection and FTIR/MALDI hyphenation, are nonvolatile additives such as salts, which are sometimes required to ensure proper GPC/SEC separation. Replacement by volatile salts is therefore needed (4).

Quantification

Figure 4 shows detailed example results for the high molar mass peak and the



Figure 4: Chromatogram of another oil sample with the results displayed. The absolute molar masses for peak A are given (GPC/SEC–light scattering) as well as the relative amount of the four identified peaks in this sample.



three low molar mass peaks. Here the absolute molar mass distribution of the polymer peak is determined using GPC/SEC–light scattering. Simultaneously the RI detector signal is used to determine the relative peak areas (composition results). Therefore, within just one run the sample is fully characterized.

Quantification in such a setup is possible. Just as in high performance liquid chromatography (HPLC) analysis, the area percentage of each peak is

easily determined. However, absolute concentrations can also be determined. Once the peaks are identified the pure substances can be injected at known concentrations to determine the RI detector response factors for every single peak.

Once the response factors are known they can be used to determine the concentration of each individual peak.

Please note that this detector calibration differs from the typical GPC/SEC

calibration. While the detector calibration requires a pure substance with a known concentration to be injected, GPC/SEC calibration requires the injection of several standards with known molar masses to yield a calibration curve similar to the one shown in Figure 1 (5).

Summary

- GPC/SEC is a non-destructive fractionation technique.
- Two different types of information can be obtained simultaneously, the percentage peak area and the molar mass distribution.
- Overlays can help to identify peaks if there are hints from production or processing. Special detection (UV [spectra], FTIR, MS) can be applied to identify peaks either on-line or off-line after fractionation.
- Detector response factors can be determined analogous to typical HPLC analysis. These factors allow the

concentration determination using the measured peak area to be found.

References

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