GPC/SEC eBook Series

GPC/SEC Detection

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.

The topics of these eBooks will cover:

- GPC/SEC theory and background
- GPC/SEC columns
- GPC/SEC detection
- GPC/SEC troubleshooting
- GPC/SEC applications

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 detection

Every GPC/SEC system requires at least one concentration detector. Modern GPC/SEC instruments are often equipped with several detectors, as the combination of these detectors lends value to GPC/SEC. Multidetection turns GPC/SEC into a powerful technique for the comprehensive characterization of macromolecules. The first two sections of this eBook deal with detector types, peculiarities, and hyphenation.

In addition, this eBook provides more information on the most common GPC/SEC detectors:

- Refractive index detector (RI), a universal detector for all samples (even without chromophores) is discussed in section 1.3.
- Online light scattering detectors (LALS, RALS, or MALS), the most common molar mass sensitive detectors, are explained in section 1.6.
- Online viscometers (the most important detectors for structural analysis) are described in section 1.5.
- Evaporative light scattering detector (ELSD), the alternative concentration detector, is covered in section 1.4.

The last section of this eBook is about hyphenation of GPC/SEC with mass spectrometry, a technique that offers new opportunities for the analysis of low molar mass macromolecules.

1.1. Understanding GPC/SEC detector signals

What are the differences between detectors and their signals?

The signal intensity, SI, of all detectors depends on injected mass (concentration and injection volume), a sample related constant k_{Sample} (i.e., response factor), and a detector constant $K_{Detector}$.

Additionally, some detectors generate signals that are dependent on the molar mass of a sample.

Therefore, all detector signals follow one simple approach:

$$SI = K_{Detector} \cdot k_{Sample} \cdot c \cdot M^{x}$$

SI = signal intensity

$K_{Detector}$ = detector constant

k_{Sample} = sample constant or response factor

c = concentration

M = molar mass

The exponent x divides detectors into two categories:

- Concentration detector, x = 0
- Molar mass sensitive detectors, x ≠ 0

Table 1. Concentration and molar mass-sensitive detectors.

x	Type	Comment	Examples
0	Concentration	$M^0 = 1$, $SI = K_{Detector} \cdot k_{Sample} \cdot c \cdot 1$	Refractive index, RI UV/Vis Evaporative light scattering, ELS
1	Molar mass sensitive		Multi/right/low angle laser light scattering, MALS/RALS/LALS
α	Molar mass sensitive	Mark-Houwink exponent	Viscometer

Since constants and the influence of molar mass differ for concentration and molar mass-sensitive detectors, each detector yields a different signal output for a given sample. This difference is more pronounced for broad distribution samples.

Figure 1 depicts an RI trace, as well as two different molar mass-sensitive detector signals of a broad distribution sample. This omnipresent and apparent signal shift derives from different detection principles.

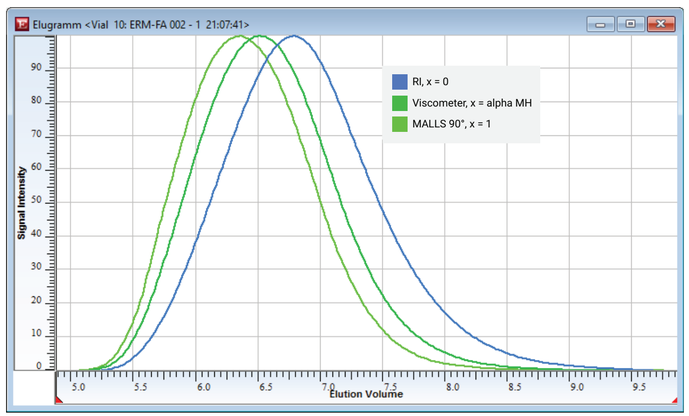

Figure 1. Comparison of detector signals: refractive index, viscometer, and light scattering detector of a broad distribution sample.

Are negative detector signals possible? How can they be avoided?

A) Negative peaks generated by sample properties

A positive value of k_{Sample} yields positive detector signals, while a negative value of k_{Sample} produces a negative detector signal. Based on the sample's related constant or response factor, it can also be determined if detection is possible. If k_{Sample} is 0 or close to 0, detection is impossible or difficult to achieve.

For RI and LS detectors, k_{Sample} is the refractive index increment, dn/dc. For UV/VIS detectors, k_{Sample} is the extinction coefficient, ε.

The value of dn/dc can be positive or negative. Light scattering detectors will always yield positive detector signals, since their signal intensity depends on $(dn/dc)^2$. Even samples with negative dn/dc in a given solvent yield positive signals.

One of the rare examples where dn/dc = 0 is polydimethylsiloxane (PDMS) in THF. This sample-solvent combination is isorefractive, so RI and light scattering detection is not possible. Therefore, PDMS needs to either be characterized in toluene (where dn/dc <0), or an ELS detector must be used instead. Figure 2 depicts RI signals for a PDMS mixture of three different PDMS standards in toluene. Since the sample-related constant is <0, negative signals are recorded.

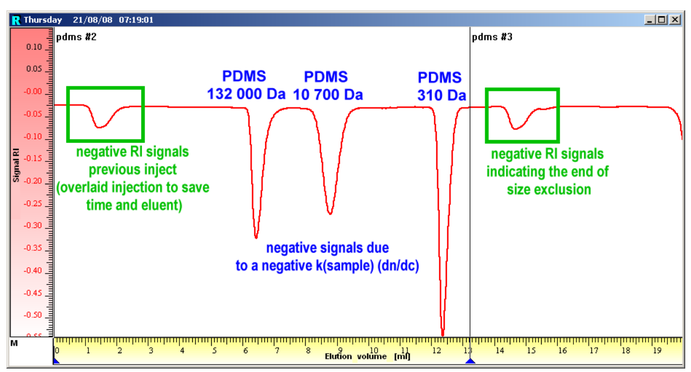

Figure 2. PDMS in toluene has a negative dn/dc; refractive index signals are negative.

Evaluation of negative detector signals is possible if an appropriate software is used. If the software cannot process negative signals, some detectors allow a polarity change of the signal output so that positive sample signals are recorded. However, formerly positive signals (e.g,. the internal flow rate marker) are acquired as negative peaks.

B) Negative peaks generated by detectors

Due to the detector design, some detectors generate negative signals that are not sample-related. It is important for correct data processing to identify these signals by injecting a blank sample.

All RI detectors show system peaks at the end of the chromatogram, and one or more of them can be negative (as shown in Figure 2). These signals indicate the end of the size exclusion separation. Even when pure solvent (a blank) is injected, these signals will be omnipresent.

Viscometers with delay columns also generate negative signals. If delay or hold-up columns are used, the sample in the solvent path will elute after the sample, and system peaks will elute as negative peaks. In Figure 3, a viscometer with delay columns (Figure 3A) is compared with a viscometer using a reservoir (Figure 3B).

The more delay columns are used, the more the negative peak is retarded. This also increases the total analysis time.

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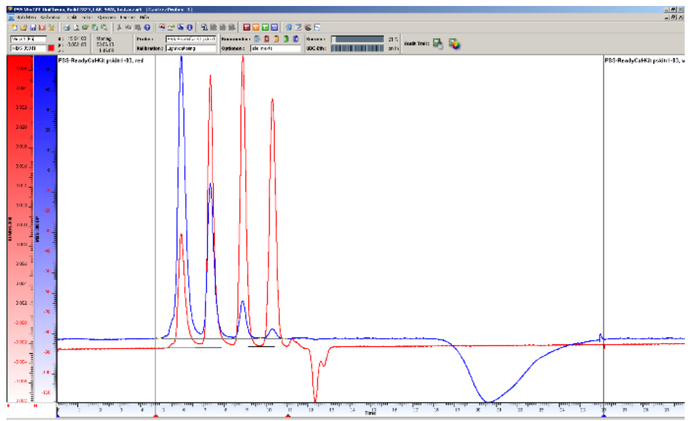
A



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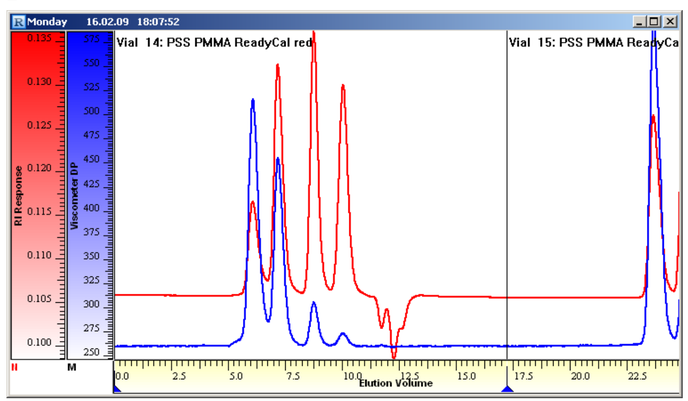


Figure 3. Comparison of a polystyrene mixture analyzed using a refractive index, a viscometer with delay columns (negative peak), and with a reservoir. (A) Mixture of four different polystyrene standards analyzed by RI (red trace) and viscometer with delay columns (DP, blue trace). The detector design yields negative signals at double elution volume. (B) The same mixture of four different polystyrene standards analyzed by RI (red trace) and viscometer containing a reservoir (DP, blue trace). The next sample can be injected earlier.

1.2. Detector combinations and detector sequence suggestions

Generally, GPC/SEC analytical methods use multiple detectors not only to generate traditional molar mass information (averages and distribution), but to learn more about the molecular structure of macromolecules. The use of traditional concentration-sensitive detectors (RI, UV, ELSD) combined with molar mass-sensitive detectors (light scattering and viscometer) has become quite common. There is also an increasing interest in adding information-rich detectors, such as FTIR, MS, or NMR to GPC/SEC instrumentation.[1] Table 1 summarizes common detector combinations and lists typical applications.

There is often some confusion and misunderstanding about how to combine multiple detectors in the best way to avoid errors in data generation.

There are different approaches to how detectors can be used effectively.[2] One approach is to split the effluent flow from the column with a flow splitting device, and to run the detectors in parallel. A drawback to this approach is that by splitting the effluent, the mass of analyte within a chromatographic slice is reduced by the split ratio. Since the signal intensity of a detector is related to the injected mass, this approach may not be feasible for many detectors.

A second approach is to daisy-chain the detectors, and install them in series. This approach also has limitations. It cannot be applied to all detector combinations, as band-broadening can become a problem.

Band-broadening can occur when the sample eluting from a column passes through several detector cells.[3] More detectors and longer-connecting tubings present a higher risk of generating significant peak-broadening, which will generate data with poor analytical quality.

Table 1. Typical detector combinations and their applications.

Detector Combination	Applicable for:
UV/VIS and UV/VIS	– Copolymer characterization: copolymer composition distribution, copolymer molar mass
UV/VIS and RI	– Copolymer characterization: copolymer composition distribution, copolymer molar mass – Heparin analysis according to Pharmeuropa: analysis of low molar mass heparins – End-group analysis
RALS/LALS/MALS and RI	– Homopolymer/protein characterization: absolute molar masses and molar mass distribution – MALS only: Radius of gyration, averages and size distribution, architecture, branching
Viscometer and RI	– Specific and intrinsic viscosity, molar masses, and molar mass distribution based on Benoit's Universal Calibration – Hydrodynamic radius, branching, architecture, and size distribution
RALS, viscometer, and RI (triple detection)	– Homopolymer characterization: absolute molar masses and molar mass distribution – Specific and intrinsic viscosity, molar masses and molar mass distribution based on Benoit's universal calibration – Hydrodynamic radius, branching, polymer architecture and size distribution
MALS, viscometer, and RI	– Homopolymer characterization: absolute molar masses and molar mass distribution – Radius of gyration averages and distribution, polymer architecture, branching – Specific and intrinsic viscosity, molar masses, and molar mass distribution based on Benoit's Universal Calibration – Hydrodynamic radius, branching, polymer architecture, and size distribution
UV/RI and FTIR/MALDI interface (offline FTIR/MALDI)	– Molar masses and molar mass distribution – Additives identification and quantification – Tacticity, copolymer composition

Figure 1 illustrates a comparison of two detectors in series: a UV detector with a 5 µL flow cell (red trace) and a test RI detector with a 50 µL detector cell* (green trace), which was placed directly behind the UV (as is standard in GPC/SEC setups).

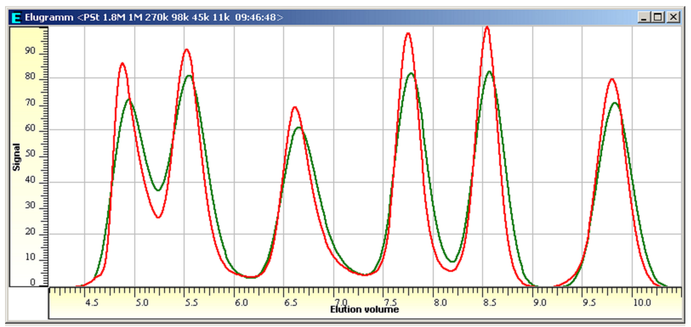


Figure 1. Test refractive index and UV 254 nm signal for a polystyrene mixture of different molar masses. The red trace is a UV detector with a 5 µL flow cell, and the green trace is a test RI detector with a 50 µL detector cell. In theory, the two signals should be comparable. The broader RI signal is a result of band-broadening.

* **Standard RIs:** 8 µL cell volume (e.g., Agilent RID), µRIs (e.g., µRI) 2.5 µL cell volume.

Another issue with the use of multiple detectors in line is that each detector sees the same sample slice at a different time, and this requires a detector-delay volume correction. This delay can be determined by injecting a monodisperse reference material and measuring the difference in peak elution volumes. Often, the delay is fine-tuned by supplementary measurements of a broad distribution reference standard. For GPC/SEC-viscometry, the interdetector delay is slightly adjusted until the Mark-Houwink plot yields the correct slope (the Mark-Houwink coefficient α).[3]

A correctly determined interdetector delay permits use of just one calibration curve for several concentration detectors. Evaluation of light scattering/viscometry/triple detection data can only be performed correctly with an accurate interdetector delay. A wrong interdetector delay yields wrong molar mass and architectural results.[4] The same holds true for composition analysis of copolymers.

How should detectors be positioned in a detector train?

As detectors rely on unique detection properties having distinctive detector cell types and specifications, several aspects must be considered when placing these in a detector chain.[2,5,6]

General detector rules:

- Detectors with the smallest cell volume should be first in the sequence; these are generally UV, DAD, and FLD detectors.
- Detectors with physically robust cells should be placed in front of detectors with pressure-sensitive cells.
- Detectors with cells that cannot handle increased backpressures should be put at the end of the train; these are RI and IR, whose flow cells are very weak.
- Detectors that yield pressure-sensitive signals should be placed last in line; these are RI and viscometer.
- Detectors that eliminate mobile phase should also be placed at the end of a detector sequence; these include ELSD, FTIR spray detectors, and MS detectors.

– Avoid daisy-chaining more than three detectors; otherwise the risk of cumulative band-broadening will be increased.

With cases of more than three detectors, or more than one detector requiring placement last in line, a flow split must be installed. By adding a T-piece, the pump flow is split into two pathways. The flow in each leg will depend on the backpressure of each leg.

Always consult the user manual or vendor for specific detector specifications, such as cell volumes, or pressure stability for optimizing the detector sequence.

Examples useful for system installations

1. GPC/SEC system consisting of UV, MALS, and RI detector:

Detector specification	UV	5 µL cell volume, 40 bar pressure stability
	RI	8 µL cell volume, pressure-dependent signal
	MALS	15 µL cell volume, 7 bar pressure stability

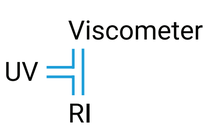
Best detector configuration:

UV = MALS = RI

2. GPC/SEC system consisting of UV, viscometer, and RI detector:

Detector specification	UV	5 µL cell volume, 40 bar pressure stability
	RI	8 µL cell volume, pressure-dependent signal
	Viscometer	8 µL cell volume, pressure-dependent signal

Best detector configuration:



3. GPC/SEC system consisting of UV, MALS, viscometer, and RI detector:

Detector specification	UV	5 µL cell volume, 40 bar pressure stability
	RI	8 µL cell volume, pressure-dependent signal
	MALS	15 µL cell volume, 7 bar pressure stability
	Viscometer	8 µL cell volume, pressure-dependent signal

Best detector configuration:

4. GPC/SEC system consisting of UV, FTIR, and ELS detector:

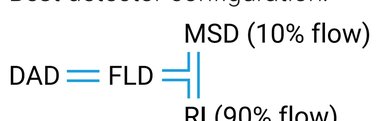
Detector specification	UV	5 µL cell volume, 40 bar pressure stability
	FTIR	10 µL cell volume, 20 bar pressure stability
	ELSD	5 bar pressure stability, evaporative

Best detector configuration:

5. GPC/SEC system consisting of DAD, FLD, RI and MS detector:

Detector specification	DAD	5 µL cell volume, 40 bar pressure stability
	RI	8 µL cell volume, pressure-dependent signal
	FLD	7 µL cell volume, 20 bar pressure stability
	MSD	Evaporative

Best detector configuration:



References

1. Kilz, P., Pasch, H. Coupled Liquid Chromatographic Techniques in Molecular Characterization; John Wiley & Sons Ltd, **2000**.
2. Striegel, A. *et al*. Modern Size-Exclusion Liquid Chromatography, 2nd ed.; Chapter 3, pp 49; John Wiley & Sons Ltd , New York, **2009**.
3. Held, D.; Radke, W. Inter-Detector Delay, LC/GC *The Column* **2017**, *(8)*.
4. Jackson, C.; Barth, H.G. Concerns Regarding the Practice of Multiple Detector Size-Exclusion Chromatography; Advances in Chemistry, Vol. 247, ACS, Washington, **1995**.
5. Netopilik, M. Influence of Peak-Broadening and Interdetector Volume Error on Size-Exclusive Chromatographic Analysis with Dual Viscometric-Concentration Detection Using the Universal Calibration Method. *J. Chromatogr. A* **2001**, *(915)*, 15.
6. Jackson, C.; Yau, W.W Computer Simulation Study of Size-Exclusion Chromatography with Simultaneous Viscometry and Light Scattering Measurements. *J.Chromatogr. A* **1993**, *(645)*, 209.

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1.3. Understanding your RI detector

The refractive index detector, RI, is the most common concentration detector used in GPC/SEC. The advantage of this "universal" detector is that it detects virtually everything, but the disadvantage is that it detects virtually everything.

This section offers some useful practical advice when working with RI detectors, as well as providing comparisons to other concentration detectors.

Principles of RI detection

RI detectors respond in general to a change of the refractive index, n. Most RIs are differential refractometers. They measure deflection of a light beam due to the difference in refractive index between pure solvent and solvent with sample. This difference is referred to as Δn and is expressed in refractive index units (RIU).

Figure 1 illustrates the detection principle, showing a quartz detector cell, divided diagonally into two parts. The cell comprises a sample side, continuously filled with flowing effluent from the column, which is compared to pure mobile phase in the reference side (normally nonflowing). If both sides contain the same mobile phase, the detector is in optical balance. When sample fractions elute from the column, the refractive index of the sample side content will change, and the light beam will be deflected. The change in intensity (measured as a voltage) is proportional to the concentration of the sample that passes through the cell after fractionation by size in the column.

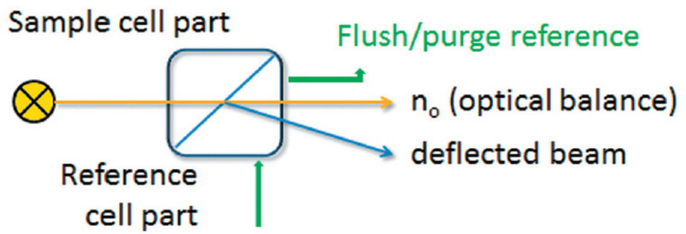

Figure 1. Refractive index detector cell design (divided cell with reference and sample part).

Advantages of RI detectors

Compared to UV detectors, samples do not require chromophores. Many types of samples (polysaccharides, starches, polyesters, etc.) can be detected.

Although ELSDs do not require chromophores, the RI detector output is much more linear with respect to response versus concentration than the ELSD response. Solvent evaporation is also not needed. Hence, no solvent restrictions apply to RIs (other than the fact that the sample and mobile phase cannot have the same refractive index, i.e., isorefractive). Additionally, RIs can detect low molecular species that are usually lost during evaporation when using an ELSD.

Disadvantages of RI detectors

Refractive index signals are generally linear in relation to changes of the refractive index. Thus, RI detectors can only be applied in isocratic mode; solvent gradients are not possible.

Changes in refractive index are also affected by:

- Temperature fluctuations
- Traces of contaminants
- Insufficient degassing
- Solvent composition

Consequently, when experimental conditions are altered, the RI will respond with a change of the signal, and a variation of the baseline. It may appear as if RIs are unstable or generate unstable baselines, but in fact, RIs are simply reflecting instrumental and environmental fluctuations.

RIs are less sensitive than UV detectors or ELSDs, so RIs require relatively high concentrations.

The cell design and detection principle can be detrimental for practical work. In a typical multidetector setup in GPC/SEC, the RI must often be put last in line.[1,2]

To achieve a stable baseline, it is required that the solvent in the reference cell and the mobile phase are of the same batch. Therefore, the reference cell needs to be flushed/purged regularly, or at least when the mobile phase in the reservoir is exchanged. The best time to purge/flush the RI is directly prior to analysis, after the system and columns have been thoroughly flushed and have had time to stabilize. If a purge/flush is performed during system (or column) equilibration, the solvent composition in the reference cell will be different than in the sample cell. Thus, no optical balance can be achieved before a run, and low signal quality may pose a problem during the run.

Troubleshooting

Negative peaks/system peaks

Figure 2 displays a common RI trace of a mixture of four different polymethyl methacrylate (PMMA) standards of different molar masses. The system peaks at the end of a chromatogram are very common. These peaks are usually negative, but a combination of negative and positive peaks can also occur.

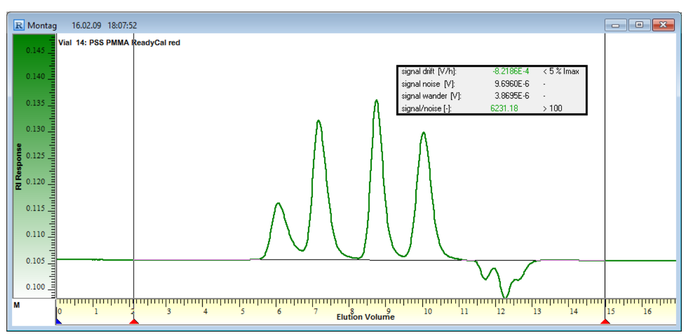

Figure 2. Refractive index chromatogram of a PMMA standard mixture with typical system peaks. The insert shows drift, noise, wander, and S/N (ISO13885-1).

It is good practice to prepare samples in solvent taken from the mobile phase reservoir to minimize the size of system peaks. Their intensity can be reduced further by:

- Decreasing the injection volume
- Cleaning the autosampler syringe and needle with the eluent used for analysis (if applicable)

The larger the compositional difference between the solvent used for sample preparation and the mobile phase, the more pronounced the system peaks will be. System peaks are identified by running a neat mobile phase, and overlaying the blank run with a sample run. Preparation of the mobile phase blank and the samples must be identical, including all filtration steps.

Problems occurring with RI detectors are comparable to those of other detectors in GPC/SEC. These include:

- Baseline drift (steady baseline shift up or down)
- Baseline wander (detector variation with frequencies between 6 and 60 cycles/hour)
- Detector noise (detector signal variation with frequencies above 1 cycle/second)[3]

Baseline drift

This type of drift is denoted by a continuous and steady change in the gradient slope (positive or negative). There are three major sources for baseline drift:

- Poor mobile phase quality
- Temperature differences between columns and flow cell
- Incomplete system equilibration

Using a fresh batch of high-quality solvent, and a sufficiently high sample equilibration time for columns, detector cells, and system should eliminate this issue. Baseline drift can be easily corrected, especially by using the recommended two-step evaluation procedure.[4]

Baseline wander

Detector wander is more difficult than drift, as it cannot be corrected as easily.

Figure 3 depicts an example of detector wander. In this example, the signal fluctuations were caused by an incompatibility between the degasser and mobile phase used. After removing the degasser from the setup, signal stability was restored.

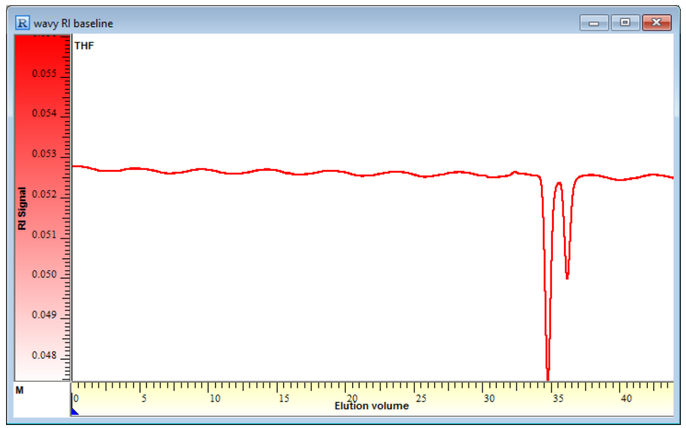

Figure 3. Refractive index detector wander example. Reasons for such fluctuations can be a malfunctioning degasser or column oven (heating cycle), or labs with poor air conditioning.

Similar fluctuations are generated by a malfunctioning vacuum pump in a degasser, or by a faulty column heater thermostat (heating cycles). In both cases, removal of the degasser and/or column heater yields better baseline stability than working with faulty components.

Air conditioning in a climate-controlled laboratory can also cause detector wander due to the heating/cooling cycling of the A/C unit.

Signal-to-noise ratio (S/N)

When discussing S/N ratio, it is important to understand the magnitude of the RI detector signal response of a sample. RI signal intensity depends on the concentration and the refractive index increment, dn/dc.

Two factors need to be evaluated when attempting to maximize S/N:

- The signal area increases with increasing concentration. In Figure 2, all PMMAs have the same dn/dc, so the same concentrations should result in identical peak areas. This holds true for this example, as the highest molar mass (eluting around 6 mL) has only half of the concentration compared to the other three samples, resulting in a peak area half the size.[5]
- The signal area increases with higher dn/dc. If, for example, a polystyrene (PS) mixture with the same molar masses and concentrations is run (THF at 35 °C), the PS peaks will be larger than the PMMA peaks, due to higher dn/dc of PS compared to PMMA in THF. For isorefractive samples with a dn/dc of zero, no signals (or only small signals resulting from end groups) will be obtained with an RI.

Other problems

An intriguing troubleshooting example is illustrated in Figure 4. In this figure, the internal standard signal starts off negative, and at peak minimum value, the polarity increases to a positive value. The reason for this behavior turned out to be a defective purge valve in the detector. Thus, the reference side was not completely shut during analysis and the sample was passing through both sides of the cell, yielding a positive and a negative signal. Gradual valve failure results in a steadily increasing negative dip before the peak. Replacement of the purge valve resolved this problem.

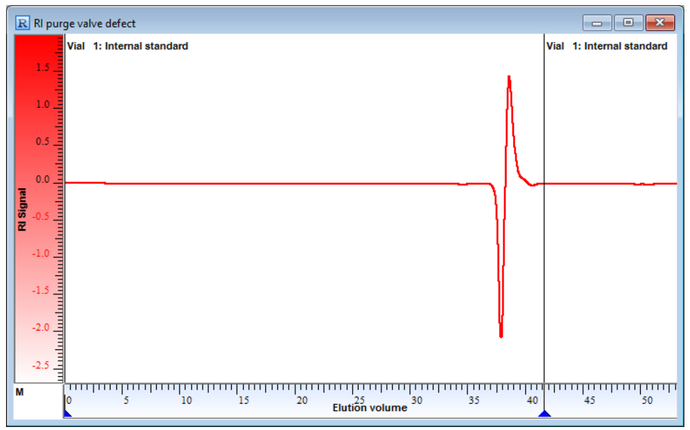

Figure 4. Refractive index signal obtained from a refractive index with defective purge valve. The negative to positive signal shift is characteristic of this problem.

Conclusion

- With linear response (signal response versus concentration) RI detectors are deemed to be universal detectors, and are suitable for many different types of macromolecules.
- Samples do not require chromophores, as the signal responds to a refractive index change.
- In GPC/SEC, RIs are commonly used in combination with light scattering detectors to estimate dn/dc.
- Disadvantages of an RI are the relatively low sensitivity (compared to UV and ELSD), the impact of laboratory environmental conditions, and the presence of a reference cell.
- RI detectors require high-quality solvents for stable baselines.
- Purging/flushing of the RI reference cell should be conducted as the last action before the analysis on a fully equilibrated system.

References

1. Held, D.; Kilz, P. What is the Best Detector Sequence? *The Column* **2012**.
2. Held, D.; Radke, W. Inter-Detector Delay. *The Column* **2017**.
3. Held, D. How to Get a Stable Baseline. *The Column* **2012**.
4. Held, D. GPC/SEC Do's and Don'ts for Data Analysis. *The Column* **2013**.
5. Held, D. The Art of Analyzing High Molar Mass Samples. *The Column* **2014**.

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1.4. Evaporative light scattering detection

In the 1980s, the evaporative light scattering detector (ELSD) was introduced as a sensitive and semi-universal detector, which detected any nonvolatile sample components.

ELSDs measure scattered light of solid analyte particles after mobile phase evaporation. This contrasts with light scattering detectors that work with polymers in solution. ELSDs are concentration-sensitive detectors (e.g., RI detectors). Most important is that their signal intensity is not influenced by molar mass. Hence, ELSDs cannot determine (absolute) molar masses.

So, using an ELSD in GPC/SEC still requires column calibration with standards of known molar mass.

Detection principle and influence of analytical parameters

ELSDs detect scattered light from particulate analytes in a gas phase. The operation principle consists of three steps:

- **Nebulization:** After entering the detector, the column effluent is nebulized using an inert carrier gas (N_2, air, He) to create an aerosol of effluent droplets.
- **Evaporation:** After aerosol formation, the carrier gas transports the aerosol through a heated evaporation tube. Here, the solvent is flash-vaporized, generating particles of pure analyte that will scatter light when crossing the light beam in the detector. Evaporation temperature should be high enough to evaporate the solvent, but should not evaporate volatile components of the analyte or cause thermal degradation.
- **Detection:** When a stream of particles passes through the detector's light path, they will scatter light. The amount of light detected by a photo diode is proportional to the analyte concentration and particle size distribution.

The effluent droplet size and uniformity are important parameters that impact sensitivity and reproducibility. The size of the aerosol droplet depends on many parameters, such as solvent viscosity, density, and surface tension, as well as gas and liquid stream velocities.[1]

Large effluent droplet size is desired to generate increased signal intensity. Increased gas flow rates will form smaller droplets. Too low a flow rate will cause baseline noise due to inefficient nebulization. In addition, care must be taken that oligomers are not removed during nebulization.

Advantages of ELS detectors

ELS detectors are (as RI detectors) universal detectors. UV active, chromophoric groups are not required. ELSD detects any nonvolatile sample compound.

Compared to RI detectors, ELSDs have a significantly lower limit of detection (LoD). They are approximately 10 times more sensitive.

System peaks resulting from solvent or low molar mass modifiers will most probably be eliminated during evaporation. In addition, ELSDs can also be used in interaction chromatography (IPC, Polymer-HPLC) when solvent gradient elution is applied. These advantages make ELS detectors a good choice in the second dimension of 2D-IPC-GPC/SEC.[2]

Disadvantages of ELS detectors

ELSD is a destructive detector, as it vaporizes the mobile phase and removes the analyte to waste. Therefore, it must go last in line in a multidetector chain.

An issue with ELS detectors is variable quantification of components due to the nonlinear detector response[3] (log-linear relationship). The signal intensity depends on many parameters such as molar mass, the chemical composition of the sample, mobile phase composition, and droplet size.

The measured peak area, A, changes with injected mass, m, and two sample-dependent coefficients, b and x:

$$A = b \cdot m^x$$

Thus, ELS detectors must be carefully calibrated before they can be used for quantification.

Practical advice for ELSD use

ELSD requires a particulate-free gas supply of several liters per minute (less for newer instruments).

Analysis of samples in aqueous mobile phases or polar organic solvents (DMF or DMAc) can present a problem due to their higher boiling points and vapor pressure.

If modifiers such as salts or other additives are required to suppress unwanted interactions, they must be volatile.

Examples of commonly used volatile modifiers are trifluoroacetic acid or ammonium acetate. Mineral acids, bromides, and nonvolatile buffers such as potassium phosphate cannot be used. Table 1 reports a variety of volatile additives and suggested concentrations. For ELS detection, it is recommended to use the lowest possible modifier concentration.

Table 1. Suitable volatile modifiers and recommended concentrations.

Additive	Concentration
Formic acid	0.1%
Acetic acid	0.1%
Trifluoroacetic acid	0.1%
Ammonium acetate	up to 10 mM
Ammonium carbonate	up to 10 mM
Ammonium formate	up to 10 mM

Conclusion

- ELSDs are superior to RI detectors for the following: limit of detection, applicability for gradient elution, and baseline stability for mixed solvents. Therefore, they are ideal as 2D-GPC/SEC second-dimension detectors.
- Potential loss of low molecular components, the use of volatile modifiers, and quantification difficulties due to nonlinear response are major concerns for method development and validation.

References

1. Schulz, R.; Engelhardt, H. The Application of an Evaporative Light Scattering Detector in Polymer Analysis. *Chromatographia* **1990**, *(29)*, 517–522.
2. Pasch, H., Trathnigg, B. Two-Dimensional Liquid Chromatography in Multidimensional HPLC of Polymers; Springer, **2013**.
3. Guiochon, G.; Moysan, A.; Holley, C. Influence of Various Parameters on the Response Factors of the Evaporative Light Scattering Detector for a Number of Non-Volatile Compounds. *J. Liq. Chromatogr.* **1988**, *11(12)*, 2547–2570.

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1.5. Viscometry – A versatile tool for structure determination and more

In comparison to other GPC/SEC detectors such as light scattering or RI detectors, viscometers require more care. Nevertheless, a viscometer is worth the effort, as it provides unique information that cannot be generated by other detectors.

How does an online viscometer work?

Although there are still some single and dual capillary viscometers in the field, most detectors offered today are based on the four-capillary bridge design originally developed by Max Haney[1] (see Figure 1). All four capillary viscometers are based on a similar concept. The sample solution is split into two parts upon entering the bridge; either 50:50 for symmetrical bridges (e.g., Malvern (formerly Viscotek) models) or 80:20 in asymmetrical bridges (e.g., ETA20xx/DVD1260 models).

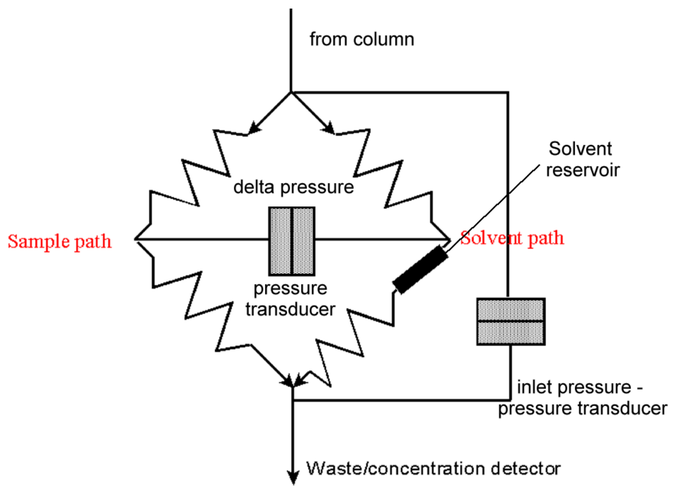

Figure 1. Four-capillary bridge design of a viscometer with sample and solvent path, and a reservoir to dilute to zero concentration.

One part of the sample flows through the sample path. The other part flows through the reference (or solvent) path. Here, either hold-up columns or a solvent reservoir are installed to dilute to zero concentration.

One pressure transducer continuously measures the pressure difference (delta pressure, (DP)) between sample and solvent paths. Additionally, most detectors also measure the inlet pressure (IP) before the sample solution is split.

If the sample portion will be delayed in the solvent path by hold-up columns, the solvent path content will elute after the sample and system peaks as negative peaks. If the sample portion is diluted to approximately zero concentration in a reservoir filled with pure solvent, these viscometers do not generate a negative breakthrough peak. These viscometers require flushing the reservoir (comparable to RI reference cells).

Figure 2 displays the raw data of a mixture of four narrow distribution polystyrene standards with different molar masses and the same concentration. With increasing molar mass, the viscosity increases, along with the signal intensity of the viscometer.

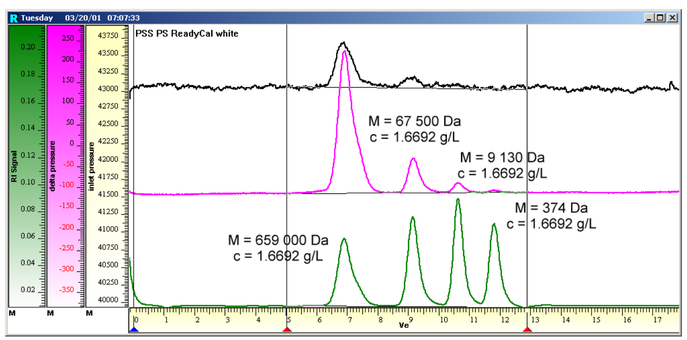

Figure 2. Delta (purple) and inlet (black) pressure signals of a mixture of four different polystyrene standards analyzed by a viscometer and a refractive index detector.

What kind of results are obtained by a viscometer?

A viscometer determines the specific viscosity of every elution volume slice. Depending on manufacturer and design, an equation is applied to calculate specific viscosity from inlet pressure and delta pressure.

For comprehensive viscosity data evaluation, concentration data from an RI or UV detector is required.

The concentration detector provides the concentration of every elution volume slice. Division of specific viscosity by concentration yields intrinsic viscosity, η, of every elution volume slice, as well as bulk intrinsic viscosity of the sample. The calculation assumes the concentration is so low, that extrapolation to zero concentration is negligible.

Slice intrinsic viscosity can then be used to obtain slice molar mass from a universal calibration curve. Benoit[2] and colleagues showed that all samples, independent of chemistry or structure, fall on one calibration curve if the logarithm of molar mass multiplied by intrinsic viscosity is plotted against elution volume (and not solely the logarithm of molar mass (see Figure 3)).

If a calibration curve is constructed using the concentration and viscosity data of reference materials, it is possible to determine true molar masses of samples that differ chemically and/or structurally from the calibrants.

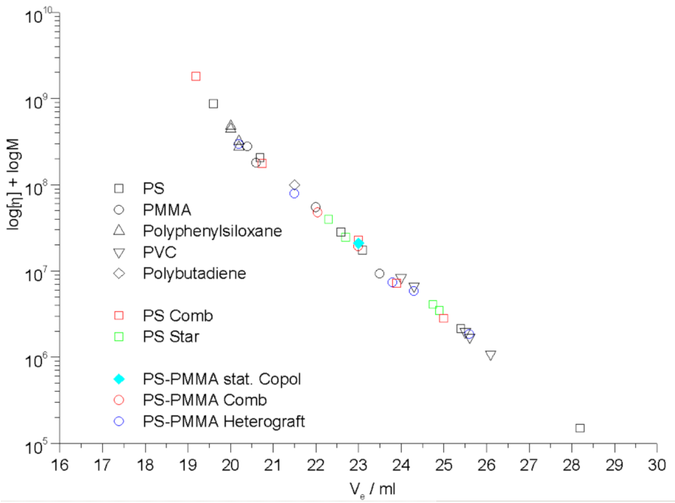

Figure 3. Universal calibration curve measured by Benoit *et al.* The Y-axis shows the logarithm of molar mass multiplied by intrinsic viscosity.

Other results can be derived from a Mark-Houwink plot. The Mark-Houwink equation relates molar mass and intrinsic viscosity:

$$[\eta] = K \cdot M^{\alpha}$$

M = Molar mass
η = Intrinsic viscosity
α = Slope
K = Y-axis intercept

In Figure 4, the logarithm of intrinsic viscosity (measured online) is plotted against the logarithm of molar mass. A linear fitted curve yields the slope and the axis intercept. Mark-Houwink α is a measure of the solution structure of a polymer chain.

Table 1 lists some examples.

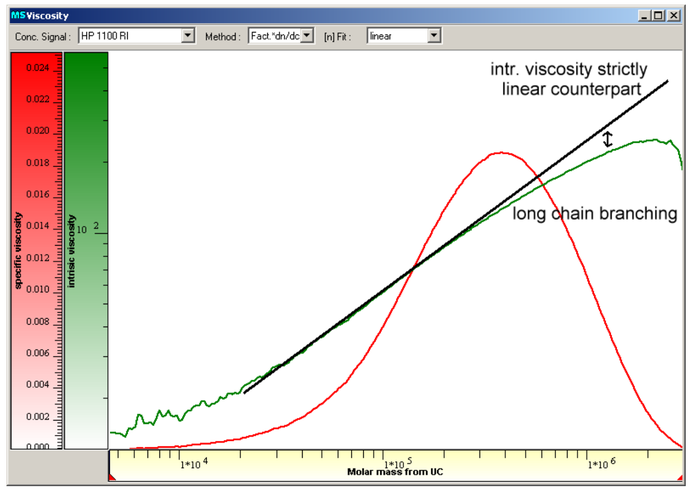

Figure 4. Mark-Houwink plot showing specific (red) and intrinsic viscosity (green) compared to intrinsic viscosity of a linear sample (black). The deviation shows where long chain branching starts.

Table 1. Solution structures for selected Mark-Houwink α values.

Mark-Houwink α	Proposed Structure
0	Compact sphere
0.5	Flexible coil (theta conditions)
0.5 ≥ α ≤ 0.8	Flexible coil (good solvent)
≥ 0.8	Semiflexible chains
2	Rigid rods

How are the data interpreted?

Intrinsic viscosity can be regarded as inverse density. This means that the higher the viscosity and signal intensity, the lower the density of the corresponding polymer chain. Thus, architectural information can readily be obtained from raw data.

The combination of a viscometer and a light scattering detector is ideal for yielding additional information, by comparing the signal intensity and order of appearance of both detectors. While a light scattering detector yields increased signal intensities for high molar masses, the viscometer generates high signal intensities for low densities. Both raw signals can determine if the molar mass is high and/or if the molecule is densely packed.

The Mark-Houwink plot is a good resource to learn more about sample characteristics like branching. If the structure of samples does not change with molar mass, there is a linear dependency of intrinsic viscosity on molar mass. When working with branched samples, it is beneficial to overlay sample plots, comparing intrinsic viscosities in the same molar mass range. If the intrinsic viscosity at the same molar mass is lower, the degree of branching is higher. A slope change in a sample plot indicates a structural (and density) change, such as long chain branching (see Figure 4) or, for example, the addition of more arms to a star polymer.

If a sample yields multiple peaks, it is best to rerun it on columns with better resolution to get the peaks baseline separated. If a proper separation of different species does not occur, an average intrinsic viscosity for the elution range with co-elution will be determined. As such, it is not recommended to interpret data between peaks that are not baseline separated, since interpretation will not reveal the true value.

Practical advice when working with viscometers

Since viscometers measure small pressure differences, it is important to work with a well-maintained, pulsation-free pump. Make sure that the system backpressure is high enough for the pump to work properly. If backpressure is too low, increase it by adding, for example, a restriction capillary. If noise of the DP signal increases, this could be an indication that the pump requires service. A Fourier analysis of the signal can show if there is, for example, an additional high frequency disturbance from the pump. If this is the case, a Fourier synthesis is a good tool to smooth data without changing peak position and shape (see Figure 5).

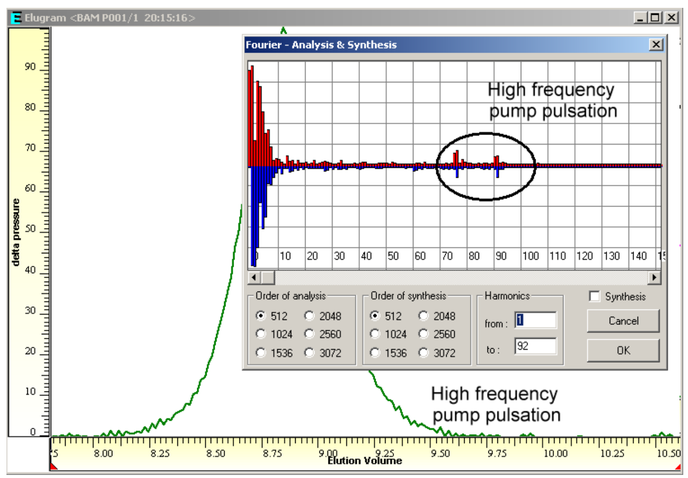

Figure 5. Fourier analysis of a delta pressure signal of a system with a poorly maintained pump leading to a high frequent disturbance.

Monitor the inlet pressure (IP) regularly to detect blockages, especially when working with buffers. IP should not increase during operation.

For viscometers with a reservoir: if a solvent exchange is required (e.g., from water to THF), do it manually and let the system stabilize overnight.

For viscometers with hold-up columns, the number of analytical columns and delay columns must match, so that the negative or breakthrough peak does not overlap with sample peaks. Subsequent samples can only be injected after the negative peak has eluted.

When measuring Mark-Houwink coefficients, make sure that the molar mass distribution of the investigated sample is broad enough. If Mark-Houwink coefficients are determined from a single sample, its polydispersity index (PDI) should be at least 1.3. A higher PDI is favorable, since it allows a linear fit with a much higher precision due to the increased number of data points. If the PDI is too small, precision can be increased by determining coefficients from several samples of different molar masses.

When measuring Mark-Houwink coefficients, it is important to accurately determine the interdetector delay between the viscometer and concentration detectors. The Mark-Houwink exponent α is most sensitive to the interdetector delay, and is actually used to fine-tune it. Use a linear, broad distribution sample (broad reference material such as polystyrene or special pullulan) to verify the interdetector delay between the concentration detector and viscometer.[3]

References

1. Haney, M.A. The Differential Viscometer. II. On-Line Viscosity Detector for Size-Exclusion Chromatography. *J. Appl. Polym. Sci*. **1985**, *(30)*, 3037–3049.
2. Benoit, H. *et al*. Étude par Chromatographie en Phase Liquide de Polystyrènes Linéaires et Ramifiés de Structures Connues. *J. Chim. Phys.* **1966**, *(63)*, 1507–1514.
3. Held, D; Radke, W. Inter-Detector Delay, LC/GC. *The Column* **2017**, *(8)*.

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1.6. How to choose a static light scattering technique

Light scattering is one of the few techniques capable of generating absolute molar mass data for characterization of macromolecules. Online light scattering detectors are used in GPC/SEC systems, in conjunction with a concentration detector to measure molar masses, radius of gyration, and for identification of high molar mass content at low concentrations.

Light scattering detection is a favorable method for characterization of high molar mass homopolymers, proteins, and other biopolymers, eliminating the need for conventional (column) calibration.

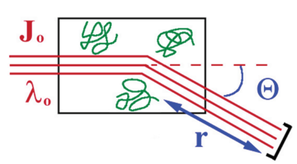
Signal intensity depends directly on molar mass, injected mass, and refractive index increment, $(dn/dc)^2$. Without knowledge of either of these, it is not possible to obtain true molar masses. There are several detectors available with different operating principles. By knowing and understanding the basic principles, users can choose the proper technology most suitable for a specific application.

The principle of static light scattering (SLS) of polymer solutions

When monochromatic light from a laser light source of known wavelength passes through a dilute polymer solution, light is scattered. Scattered light can be detected either using static or dynamic light scattering detectors (SLS versus DLS).

The term "static" does not denote the mode of operation of a light scattering experiment. It indicates that time-averaged scattering intensity is measured. Dynamic light scattering experiments involve measuring light intensity fluctuations. Both static and dynamic detection can be performed online (GPC/SEC, flow mode) or in batch-mode (stand-alone technique).

The intensity of the scattered light is measured at one or more fixed detector angles (Figure 1).



J_0 Intensity of primary beam
λ_0 Wavelength of primary beam and scattered light (elastic scattering)
r Distance of scattering center to detector
θ Scattering angle

Figure 1. Schematic of light scattering of polymers in solution.

Depending on cell design and geometry, detector angles can (but do not have to) be the true scattering angles:

- If intensity is directly measured in a cylindrical cell, the scattering angle and detector angle are always the same (Figure 2A).
- If common glass cells are used, the interface solution/glass is responsible for an offset of all detector angles (except for the 90° angle, which requires a correction for the true scattering angle (Figure 2B)).

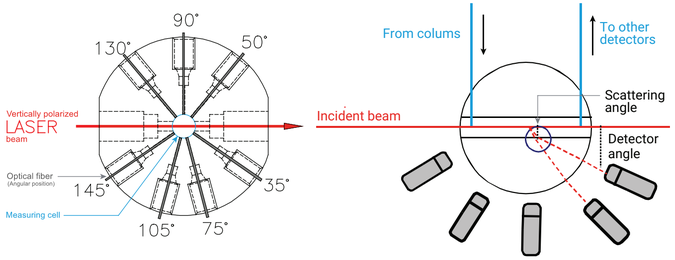


Figure 2. Cell designs, detector angle versus scattering angle.

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

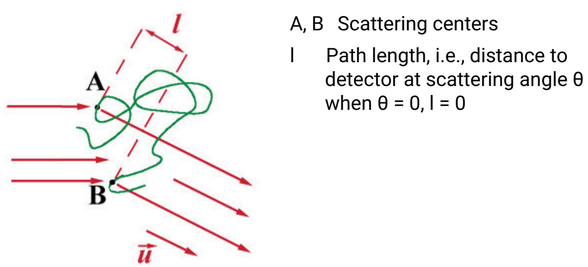
If dimensions of an analyte are small compared to the wavelength of the incident light beam, the analyte is regarded as a point scatterer. This occurs if the maximum distance between two scattering points of an analyte is smaller than ~λ/20 of the laser wavelength, λ. In this case, molar mass data generated at a single angle is accurate.

In general, results from one angle will 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
- Branched polymers over 200,000 Da (depending on branching density)[3]

With a simple experimental setup and very good signal-to-noise ratio, the 90° angle is often preferred for these applications.

If high molar mass species in solution have dimensions of up to a few hundred nanometers, the scattered light of different scattering points of the analyte is coherent and capable of interference (Figure 3).



A, B Scattering centers

l Path length, i.e., distance to detector at scattering angle θ when θ = 0, l = 0

Figure 3. Light scattering of large analytes.

The interference results in a reduction of scattering intensity for large analytes, compared to scattering intensity from all individual mass points. This makes scattering intensity angular-dependent, so that measurements of large analytes solely at 90° yield insufficient results.[1]

To obtain accurate results of unknown samples, it is recommended to not rely on 90° light scattering (LS) results alone.

Figure 4 depicts RI and light scattering signals of a large analyte. The LS signals were obtained at scattering angles 35°, 90°, and 145°. Because RI is a concentration-sensitive detector, and MALS is a molar mass-sensitive detector, there is an apparent shift in RI and MALS signals.[2]

The apparent signal shift of the three different LS signals derives from the angular dependence, emphasizing the importance of using several detector angles for characterization of large analytes.

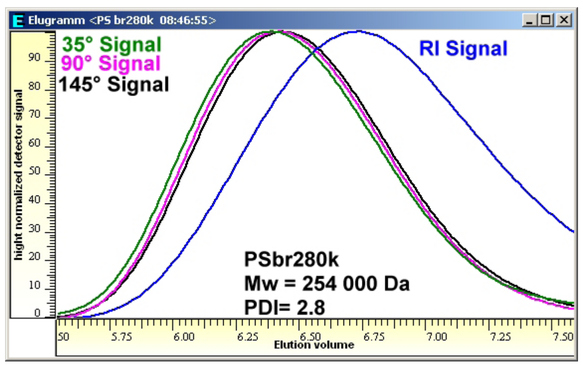


Figure 4. Refractive Index and three light scattering signals at 35°, 90°, and 145° for broad distribution polystyrene.

LALS, RALS, and MALS, explained

These abbreviations stand for low angle, right angle, and multi-angle laser light scattering techniques and detectors. The difference between these detectors derives from the number and position of the detection angles.

LALS and RALS instruments measure at just one angle, either at a low angle (e.g. 6 to 7°) or at right angle (90°), whereas MALS detectors measure simultaneously at several angles.

Right angle light scattering - RALS

RALS, 90° light scattering, has the advantage of having the most accurate and useful signal. Advantages include low stray light issues, and less interference from scattering due to dust particles that might be present in solution, deteriorating the quality of the signal. However, the range of accurately measured molar mass is limited to macromolecules of low to medium molar mass.

Low angle light scattering - LALS

LALS has the advantage that observed scattering intensities are very close to the theoretical intensity values expected at the 0° scattering angle. In theory, all light scattering measurements should be performed at the 0° angle, as they are not affected by some of the external interferences that affect RALS and MALS measurements. The range of molar mass is much wider than for other LS techniques, and molar mass values can be directly determined.

Unfortunately, LALS has the most experimental difficulties. Complex optics and an exceptionally clean system are required to measure the weak scattering signals with high precision and accuracy. This technique requires a lot of signal processing in the detector or software to generate useful data, but sometimes it is impossible to see true raw data. In some chromatographic systems (especially aqueous GPC/SEC systems), clean system requirements can almost never be met.

Multi-angle light scattering - MALS

For practical purposes, and to obtain accurate molar mass values over a very wide range, MALS instruments offer the best solution. The slope of a plot of scattering intensity versus detection angle can be extrapolated to the 0° scattering angle. Additionally, radius of gyration (R_g) averages can be calculated from the data.

One major disadvantage of MALS detectors is their high cost, which is due to the complexity of engineering.

Triple detection

In recent years, triple detection GPC/SEC has become a very popular technique. The original term "triple detection" was only used for a system composed of a viscosity, a 90° single angle light scattering, and a concentration detector. Data processing is conducted with a special algorithm that makes several assumptions.[5] It assumes that the sample is run under theta conditions (not GPC/SEC conditions), and that the sample is linear and can be represented by a chain model. Thus, the use of triple detection for unknown samples might yield faulty results if caution is not exercized.[6]

Today, the term is also used for all techniques combining a traditional refractive index detector with both light scattering (LALS, RALS, or MALS) and viscometer detectors. The technique is applicable to both natural molecules and synthetic polymers. The resulting molecular weight and structure information provides an invaluable tool for analysts.

In addition to absolute molar mass data, such triple detection systems can generate:

- Mark-Houwink-Sakurada parameters
- Mark-Houwink conformation plots
- Contraction factors and branching ratio information
- Universal calibration plots
- R_g and R_h information

However, triple detection GPC/SEC approaches are more complicated with respect to instrument configuration and data collection management/handling, and may not be applicable in all situations.

In combination with one low angle, higher molar masses can be determined. Figure 5 shows that the experimental error is highest for low angles, and hence the molar mass result accuracy can be improved when more than one angle is used.

Light scattering detection for the analysis of copolymers

When analyzing copolymers, light scattering detection (if used) will give incorrect molar mass data, because the dn/dc of the copolymer varies with composition and chain length. Differences in dn/dc, as well as low molecular compounds, yield less precise light scattering data due to low signal intensity.[1]

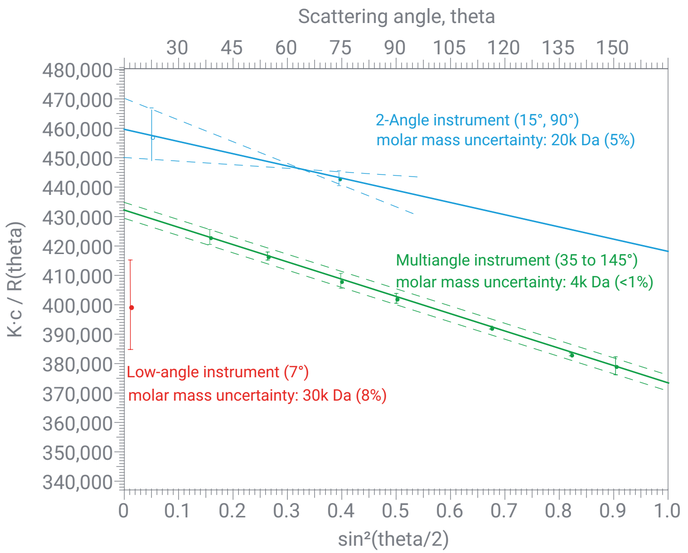


Figure 5. Error comparison of different scattering angles and of molar mass determination when using single or multiangle detection.

References

1. Kratochvil, P. Classical Light Scattering from Polymer Solutions; Elsevier, **1987**.
2. Held, D. Understanding positive and negative detector signals. *The Column* **2008**, *(12)*.
3. Mori, S., Barth, H. Size Exclusion Chromatography; Springer, **1999**.
4. Held, D.; Reinhold, G. A look at the importance of molar mass averages. *The Column* **2007**, *(10)*.
5. Haney, M.A.; Jackson, C.; Yau, W.W. SEC-Viscometry-Right Angle Light Scattering (SEC-VISC-RALS). *International GPC Symposium Proceedings* **1991**.
6. Radke, W. Chromatography of Polymers; in Macromolecular Engineering: Precise Synthesis, Materials Properties, Applications; Vol.3, K. Matyjaszewski, Y. Gnanou, L. Leibler Eds., Wiley-VCH Verlag GmbH & Co. KGa, **2007**.
7. Berry, G.C.; Cotts, P.M. Static and dynamic light scattering. Experimental methods in polymer characterization; Wiley & Sons Ltd., **1998**.
8. T. Chang *et al. Macromolecules* **1998**, *31*, 4114–411.
9. T. Macko, *Adv. Polym. Sci*. **2003**, *163*, 61–136.

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1.7. Information-rich detection with online mass spectrometry

GPC/SEC runs generally provide molar masses and distribution information that derive from a calibration curve established by reference standards. This easy-to-use technique generates reproducible results, and is easily applicable for sample comparison and robust quality control methods.

The resulting molar masses of chemically or structurally different samples and reference materials will yield apparent molar masses that differ significantly from true molar masses. This limitation can be overcome by applying different methods and techniques based on the molar mass range.

The latest addition to online detection solutions in GPC/SEC is hyphenation with mass spectrometry (MS). This online detection technique closes a gap in macromolecular characterization, since it is ideal for low molar mass samples, which other molar mass-sensitive detectors (LS and viscometer) struggle with. The most-applied and best described mass spectrometry method for hyphenation with GPC/SEC is electrospray ionization (ESI).[1] One of the major advantages of the ESI-MS approach is that a mass spectrometer can be easily connected to an LC system. It offers the possibility of multiple charged states, allowing analysis of higher molar masses.

For quantification, GPC/SEC-ESI-MS requires a concentration detector (RI or UV). Other than the LC components, a simple T-connector that splits the flow into appropriately portioned flows (e.g., 9:1) for the concentration detector and the mass spectrometer is the only requirement.

This type of GPC/SEC-MS coupling has been applied to a wide variety of natural and synthetic macromolecules such as polyacrylates, polyesters, polyethers, polyamides, resins, polycarbonates, proteins, and polystyrene. As a general rule, macromolecules must have at least one ionizable functional group per monomer unit in solution.

Advantages and disadvantages

- Easy-to-interpret spectra of fractions with a narrow molar mass distribution are transferred into the ESI-MS after separation is obtained.
- Sensitivity of the mass spectrometric analysis is significantly improved. The molar mass range after online hyphenation can be extended to 10 to 20 thousand Da (depending on the resolution of the mass spectrometer).

A disadvantage is that some GPC/SEC methods require salts or additives to minimize interactions during the separation. If these salts or additives are not volatile, they can compromise MS detection. So, as in the case of an ELS detector, the use of volatile salts is a prerequisite for hyphenation with MS. Ideal solvents are ones that are easy to evaporate, including THF, chloroform, methylene chloride, and toluene.

This powerful technique yields true molar mass distributions as a result. For every elution volume, a mass spectrum is available.

Figure 1 depicts an example evaluation. The upper part of the schematic displays detector signals (RI signal (blue trace); MS signal; total ion count (TIC), red trace); the lower part shows the mass spectrum at the elution volume of the green fraction marker. It provides automatic assignment of charged state, degree of polymerization, and the molar mass of the end group. By selecting a specific structure, or the base peak (most abundant signal in the spectrum), it is possible to display the distribution of this structure as a chromatogram. This yields the extracted ion chromatogram (EIC), sometimes also referred to as a single oligomer profile (SOP). Each of these structures generates its own matching calibration, because MS allows absolute determination of molar mass. With this calibration curve, and quantitative information from the concentration detector, true molar mass determination can be accomplished.

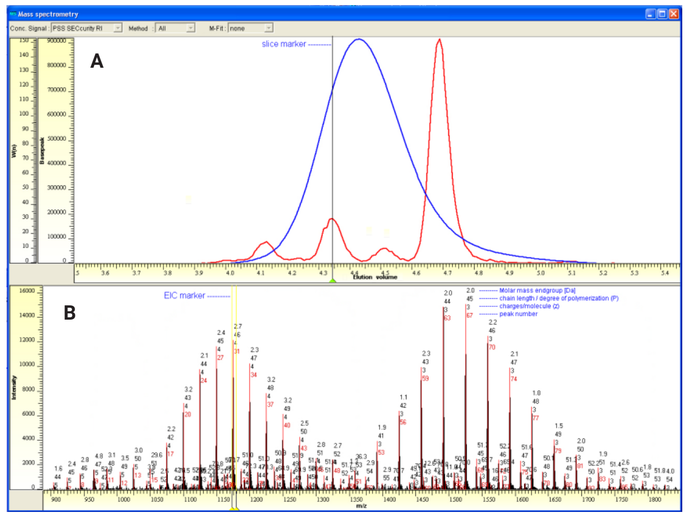

Figure 1. GPC/SEC-ESI-MS data.

Figure 1A shows the detector signals (RI signal, blue trace) and the MS signal (TIC), (red trace); Figure 1B shows the mass spectrum at the elution volume of the green fraction marker. Charged states, degree of polymerizations, and molar mass of the end groups are assigned automatically.

Reference

1. Gruendling, T.; Guilhaus, M.; Barner-Kowollik, C. Quantitative LC–MS of Polymers: Determining Accurate Molecular Weight Distributions by Combined Size Exclusion Chromatography and Electrospray Mass Spectrometry with Maximum Entropy Data Processing. *Anal. Chem.* **2008**, *(80)*, 6915 .

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Glossary

Da	Dalton (g/mol)
DAD	Diode array detector
DMac	Dimethylacetamide
DMF	Dimethylformamide
dn/dc	Refractive index increment
DP	Differential pressure
ELSD	Evaporative light scattering detector
EIC	Extracted ion chromatogram
ESI	Electron spray ionization
FLD	Fluorescent light detector
FTIR	Fourier transformed infrared
GPC	Gel permeation chromatography
H_2O	Water
HPLC	High performance liquid chromatography
IP	Inlet pressure
LALS	Low angle laser light scattering
LC	Liquid chromatography
LS	Light scattering
M_w	Weight-average molar mass
MALS	Multi-angle laser light scattering
Mobile phase	Liquid phase used in a chromatography system
MMD	Molar mass distribution
MS	Mass spectrometry
n	Refractive index
PDI	Polydispersity index ($D = M_w/M_n$)
PDMS	Polydimethylsiloxane
PMMA	Polymethyl methacrylate
PS	Polystyrene
R^2	Regression coefficient
RALS	Right angle light scattering
RI	Refractive index (detection/detector)
RIU	Refractive index units
SEC	Size exclusion chromatography
Solvent	Liquid in which a solute is dissolved to create a solution
THF	Tetrahydrofuran
TIC	Total ion count
UV	Ultraviolet (detection/detector)

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