

SEC Analysis of Polymers with Light Scattering Detection

A comparative Overview of Measuring Principles

For the chromatographic analysis of macromolecules in solutions light scattering (LS) has been used for about the past 20 years. The selection of a light-scattering instrument can be time-consuming due to the number of suppliers and measuring principles available in the market. A comparison of measuring principles of instrumentation is included to provide the reader with an experienced overview of methods, hopefully to ease the selection of light scattering detectors. Customer demands and laboratory requirements, specifically to meet the needs of biopolymer applications, calls for instrumentation, that provides high performance, reliable software, compact dimensions and of course attractive price.

Size exclusion chromatography (SEC, also called GPC or GFC) is the most important test method for the determination of the molecular properties of synthetic and natural macromolecules in solution. Different from traditional methods which only establish average property values, SEC allows the simple and fast determination of property distributions of macromolecules, e.g. molecular weight distribution, structure distribution, end group distribution or branching distribution [1].

SEC is often calibrated with reference materials in order to obtain molecular weight distributions and to validate instruments and analytical methods. However, to obtain more extensive structure information (branching, density, etc) or to abstain from a calibration, the use of light scattering detectors for SEC is necessary [2]. Thus, the molecular weight can be measured continuously during

elution. Depending on the type of LS instrument used, various qualitative and quantitative properties can be determined:

- Radius of gyration (Rg)
- Degree of branching (long chain branching)
- Molecular structure changes
- Aggregation/agglomeration behaviour
- Aging/storage processes.

A comparison of the results between conventional SEC and light scattering measurements can show differences, especially when the standards used in conventional SEC and the samples differ. An example is shown in table 1, caused by the unequal hydrodynamic volume of gluten and pullulan molecules. Here the results from conventional SEC with narrow standards can be used only for the comparison of different samples. This is not the case, when using light scattering measurements, where absolute M-values are obtained.

Table 1: Comparison of Gluten Results with Conventional SEC and SEC-Light Scattering

| SEC-Method | LS Detection (SLD7000) | Pullulan Calibration |
|------------|------------------------|----------------------|
| M_n [D] | 10,800 | 18,600 |
| M_w [D] | 14,600 | 41,400 |
| M_w/M_n | 1.36 | 2.22 |
| M_p [D] | 8,530 | 55,700 |

Basic Measuring Principles

A number of light scattering instruments have been used for about 20 years in SEC and have – depending on the manufacturer – distinctive advantages and disadvantages which can be quite important. PSS has kept a close eye on customer demands and the requirements of the laboratory environment and developed a light scattering solution combining innovative ideas with established methods. Table 2 summarises the different principles and instruments for light scattering measurements.

Low Angle Laser Light Scattering (LALLS)

Due to its construction the low angle laser light scattering (LALLS) has the most complicated optical set-up [3]. This often results in signal interference impulses (“spikes”) when small air bubbles or par-

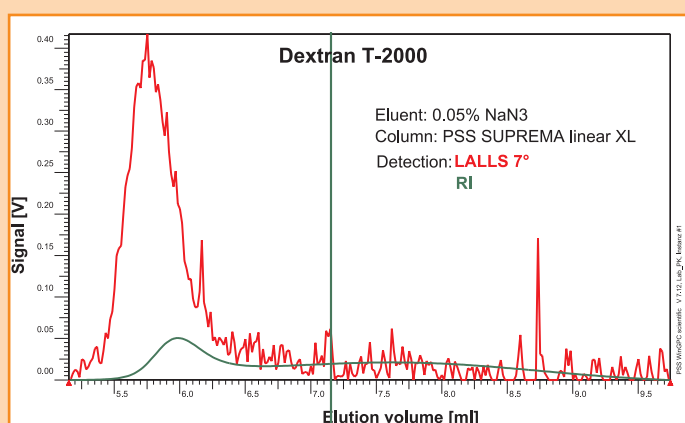


Fig. 1: SEC detection of a high molecular weight dextran in aqueous solution shows the interference of the 7° LALLS signals (red) and the excellent signal/noise ratio of the RI signal (green).

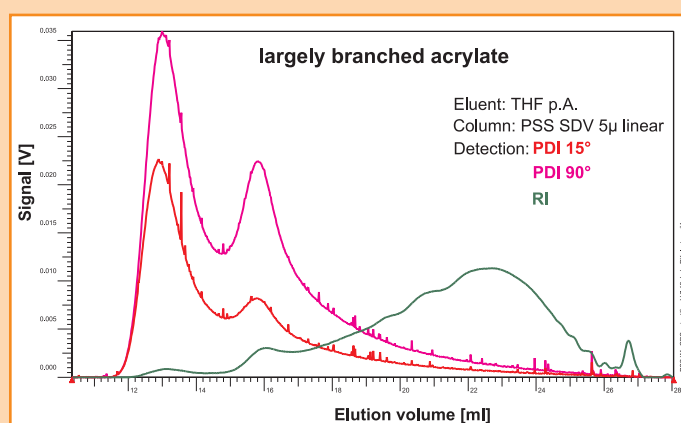


Fig. 2: Raw data view of a two-angle light scattering instrument shows less signal interferences in THF (with a largely branched PMMA). The different structures can be differentiated in terms of quality, but not easily quantified with the TALLS procedure.

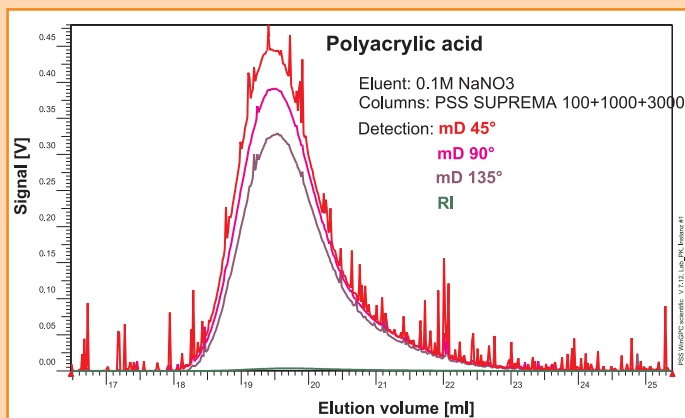


Fig. 3: Light scattering and RI signal of a high molecular weight polyacrylic acid measured with a three-angle LS instrument. Here the susceptibility to interferences of the LS-signals at small angles is shown in comparison with the wide-angle scattering (RI signal not very well visible in this graph).

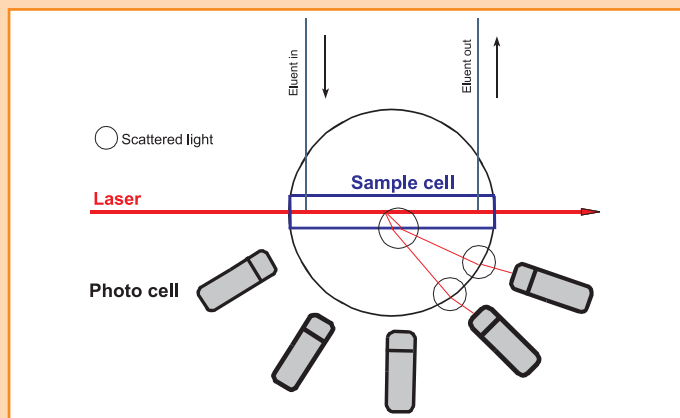


Fig. 4: Schematic set-up of a commercial MALLS light scattering cell. The light path (red) shows the refraction effects. Therefore the angle position of the photo cell deviates from the true scattering angle and needs to be corrected.

ticles get into the measuring cell (Fig. 1). The cell needs to be cleaned frequently since even the smallest disposal affects the optical behaviour and contribute to considerable stray light scattering. While this type of instrument provides molar masses only, it does not deliver any other information. For example, the determination of branching degree is not possible, because this is an angular-dependent measurement. The advantage of this concept is the exact measurement of high molecular weights (> 10 Mio. g/mol) without the principle-related errors which often occur with extrapolations [4].

Right Angle Laser Light Scattering (RALLS)

The construction of the light scattering with only one 90° scattering signal (RALLS = Right Angle Laser Light Scattering) is very simple and sturdy. Interferences of the light scattering signal are

usually not very strong. However, this type of light scattering produces correct results only for relatively small molecular weights (about 200 kg/mol or molecule sizes smaller than 10 nm) and due to its measuring principle it does not deliver any structural information about the sample. In case of higher molecular weights the light scattering signal has to be corrected with a viscosity detector [5]. For this a number of assumptions are necessary which in case of normally unknown substances cannot easily be verified.

Two/Tree-Angle Laser Light Scattering (TALLS)

Use of two- or three-angle light scattering instruments does not considerably improve the situation mentioned above. The two-angle light scattering operating at 15° and 90° often combine the disadvantages of LALLS and RALLS instruments [6]. This means you have to work on the

basis of assumption and/or the signal of the small angle shows interferences (Fig. 2). The range of angles offered by the available three-angle instruments is too small (realistically about 50–120°) to be able to deliver independent information about the structure over an extensive molecular weight range. These instruments usually deliver a reasonable quality of the signals even if there might be interferences especially in case of aqueous applications (Fig. 3). For small molecules (isotropic scattering) both types of instruments can be used; however, the much less expensive RALLS detector would be also sufficient for such applications.

Multi Angle Laser Light Scattering (MALLS)

The available multi angle laser light scattering instruments (MALLS) overcome the above mentioned limitations and make the best use of the possibilities of

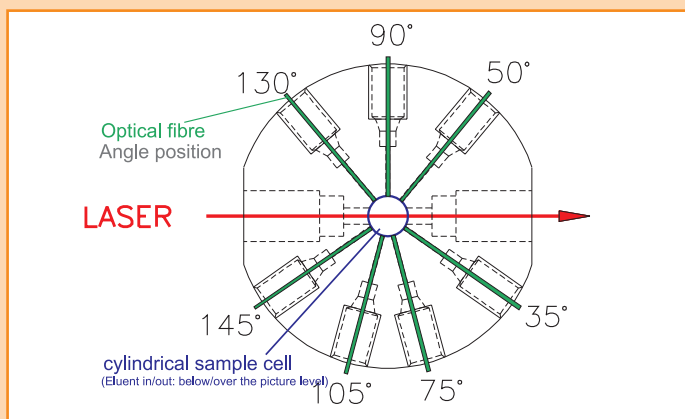


Fig. 5: Set-up of the cylindrical light scattering cell [8b] of the light scattering instrument SLD 7000 MALLS detector. The optical fibre (green) leads directly into the measuring cell and there is only a single 90° phase transition leading to a significantly better signal quality (Fig. 6).

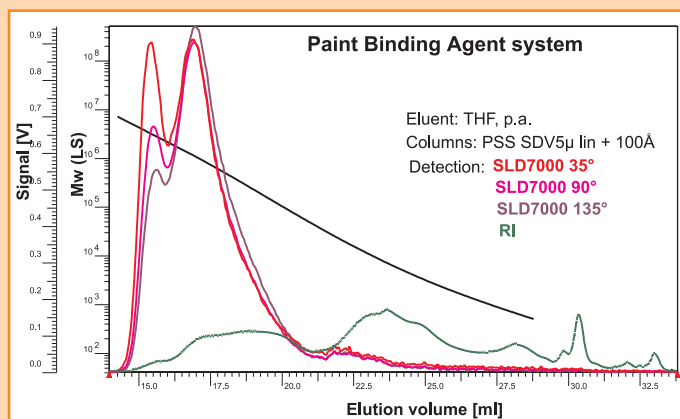


Fig. 6: SEC analysis of a paint binding agent with SLD 7000 in THF. The multi-modal substance covers a very wide molecular weight range (black curve).

Table 2: Comparative Overview about Light Scattering Procedures and Instruments

| Type | Method | Application | Limitation | Conditions* |
|--|--|---|---|---|
| LALLS | | | | |
| Low angle laser light scattering | - Molar mass measurement without extrapolation | - MWD - high molecular weight samples | - frequent "spikes" - high maintenance - no Rg-measurement | - extreme clean system (without particles, dust) |
| RALLS | | | | |
| Right angle laser light scattering | - Molar mass measurement without angular correction | - only MWD of low molecular weight samples - comparative analysis | - no angular correction - Rg only in connection with viscometer | - $M < 200000$ D - sample properties must be known |
| TALLS | | | | |
| Two/Three angle laser light scattering | - Molar mass measurement with 2(3)-point extrapolation | - MWD - high and low molecular weight samples | - "spikes" at 15° - limited angular correction - Rg inaccurate | - coil statistics should be known |
| MALLS | | | | |
| Multi angle laser light scattering (e.g. PSS SLD 7000) | - M- and Rg- measurement with zero angle extrapolation | - accurate MWD - reliable Rg - branching - structure information | | |

*) general condition for all SEC light scattering methods:

- The value of the refraction index increment (dn/dc) has to be known very accurately [9a] because its square value is used for the determination of the molecular weight. It is important, that dn/dc corresponds to the measuring conditions (solution, wavelength, etc.) [9b].
- Use of good SEC columns without particle shedding in order to avoid "spikes"
- flexible and simple software for an extensive analysis of the data, calculation and graph of the results so that the number of different data systems in a lab can be reduced [10].

light scattering [7]. The range of angles actually available (typically $30\text{--}150^\circ$) is even more important than the number of angles. For the precise description of most scattering functions seven angles are completely sufficient, further angles lead to redundancy and unnecessarily increase the cost of purchasing and operation. Independent of the number of angles the quality and stability of the signals are important quality criteria. They highly depend on the construction of the measuring cell. A MALLS detector frequently used in SEC uses a glass block with a longitudinal bore (Fig. 4). Here, the scattered light is refracted at several spots (e.g. phase transition solution-glass). Especially in case of small angles this can lead to signal interferences or even to completely useless measuring signals for individual angle positions.

Improvement of the MALLS Technique

A recently developed light scattering system (PSS SLD 7000 with WinGPC LS software) is based on an improved MALLS technique. PSS actively participated in the development of the light scattering system in close cooperation with Brookhaven Instruments. The combined expertise produced an instrument with unparalleled features. The instrument has traditional cylindrical-cell geometry, with the advantage that optical fibres

directly measure in the cell (Fig. 5). This prevents light refraction effects and minimises the flare of external light [8], leading to high signal/noise ratios and excellent signal forms. This self purging, cylindrical cell has small dead volume and high pressure stability.

Summary of the PSS SLD7000 highlights:

- practical relevance: simultaneous measurement of seven angles between 35 and 145° .
- reliability: self purging, cylindrical measuring cell with index matching, small dead volume and high pressure stability.
- modern optical set-up: optical fibre technique with high sensitivity and low noise.
- modern electronic components: ultra-sensitive CCD detector, USB data transfer.
- high signal quality: small cell volume ($100\ \mu\text{l}$) preventing band broadening and other artefacts.
- exact results: extremely small scattering volume ($20\ \text{nl}$) for high precision
- high reliability: optimised flow paths and separated optical/electronic compartments
- simple handling: plug&play operation, seamless WinGPC integration also in connection with additional SEC equipment and further detectors (e.g. on-line viscometers)

- compact design: optimised instrument design taking advantage of all known miniaturisation concepts

Fig. 6 shows the raw signals at 35° , 90° and 145° as well as the concentration signal in direct comparison. The paint binding agent is multi-modal and covers a wide molecular weight range (10 million up to $1000\ \text{g/mol}$). The black curve shows the molecular weight measured with the SLD 7000 which even for relatively small concentrations and molecular weights can be determined very reliably. In the high molecular weight range the quality of the raw signals is very good, below about $10\ \text{kg/mol}$ a slight noise can be detected. This is due to the used measuring principle since the strength of the light scattering signal is proportional to the concentration and the molecular weight. When both factors are small their product is also very small and therefore the signal/noise ratio will naturally decrease.

Summary

Light scattering detection in SEC can lead to considerably more information. The selection of a light scattering instrument for a special application can be rather time-consuming in view of the number of suppliers and measuring principles. The new SLD 7000 is an universal instrument which combines high performance and compact dimensions with an

attractive price. In connection with an efficient and flexibly expandable software solution like the PSS WinGPC the SEC light scattering combination smoothly fits in the existing laboratory environment and delivers fast and sound answers on current questions about natural, synthetic and (bio-)polymers.

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