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WHITE PAPER

WP2303: Lignin and lignosulfonate characterization with SEC-MALS and FFF-MALS

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Introduction

Lignin is the second most abundant natural polymer and a major constituent of green plants. In contrast to cellulose, it has no industrial/technical use except incineration for energy production. In view of the push for sustainability, efforts to find new ways for utilization of lignin have been increasing and more research is devoted to finding productive applications.

The most fundamental property of biopolymers is their molar mass distribution. Originally, lignin was assumed to be a very large macromolecule that provides support for cellulose in cell walls; then there seemed to be evidence that it is merely an oligomer consisting of a few monomer units. A significant breakthrough in the molar mass characterization of lignin and its derivative lignosulfonate was achieved by coupling the DAWN® online multi-angle light scattering (MALS) instrument to size-exclusion chromatography (SEC) or the Eclipse™ field-flow fractionation (FFF) device[1,2].

Lignin and lignosulfonate analysis are among the most demanding applications of SEC-MALS and FFF-MALS. This work highlights how the advanced technical features of the DAWN and the Eclipse contribute to successful characterization.

Common characterization methods for lignin, and their challenges

State-of-the-art routine methods for molar mass determination do not work well for lignin:

- Mass spectrometry techniques, both MALDI-MS and ESI-MS, only work for narrow fractions of lignin, because of its heterogeneous chemical nature. It is not possible to achieve effective ionization of an unfractionated sample
- Analytical SEC combined with UV or RI detection has been widely applied. But because lignin standards are not available, and lignin can be highly branched, accurate column calibration is not feasible.
- Universal Calibration (UC) using SEC with a differential viscometer is an improvement over standard analytical SEC, but it does not provide reliable molar mass information. This is because of 1) branching (UC is only defined for linear polymers) and 2) non-ideal interactions of this chemically heterogeneous molecule with the SEC column matrix (retention is not based purely on diffusion).
- SEC-MALS is generally preferable for polymer analysis since it is an absolute method that does not rely on calibration standards or assumptions about conformation and ideal column interactions. SEC-MALS performed with the standard red laser (λ = 660 nm) is, unfortunately, not a good solution for most lignin samples, which contain a broad range of fluorescing moieties that are excited at this wavelength. Absorption by the fluorophores reduces the incident light intensity in the cell, while fluorescence creates excess light intensity at the detector. Both effects interfere with accurate MALS analysis.

A common difficulty in SEC analysis of lignosulfonates is the tedious sample preparation required – crude samples must be extensively purified to remove salts and sugars which otherwise degrade chromatography and may even damage the column.

Advanced solutions for characterizing lignin and lignosulfonates

As an absolute method, SEC-MALS is reliable and accurate—if the challenges of absorption and fluorescence can be overcome. As we will see below, this is feasible with the right technology. In addition, FFF-MALS provides advanced characterization capabilities that greatly simplify the analysis of these materials.

Unique features of the DAWN for lignin characterization

A combination of three technical features of the DAWN are crucial for the characterization of lignin, as well as other strongly fluorescent samples.

- The DAWN's Forward Monitor measures the intensity of light transmitted through the flow cell. If absorption by the sample occurs, the reading of the Forward Monitor decreases relative to pure solvent, and the actual light intensity at the scattering volume can be calculated.
- A 785 nm near-infrared laser option is available which reduces absorption and fluorescence compared to the standard wavelength
- Fluorescence-blocking filters can be added to prevent fluorescent photons from reaching the photodetectors that quantify scattering.

As will be discussed below, all these features are critical to achieve reliable molar-mass determination for lignin.

A DAWN MALS detector integrates with standard HPLC systems.

Learn about FFF-MALS-DLS

An FFF-MALS-DLS system comprises two main subsystems:

1. **Separation**: an Eclipse™ FFF device plus standard HPLC components such as pump, degasser and autosampler from leading HPLC vendors.
2. **Characterization:** a DAWN® online MALS instrument incorporating a WyattQELS™ online DLS module and additional HPLC UV or fluorescence modules. MALS determines the rms radius R_g (radius of gyration) while DLS determines the hydrodynamic radius R_h. In addition, R_h can be estimated from the FFF retention time using any of the detectors to detect the peak.

An introduction to asymmetric-flow FFF (AF4) and Wyatt's Eclipse can be found in www.wyatt.com/Theory/FFF. An introduction to multi-angle light scattering and dynamic light scattering may be found in www.wyatt.com/Theory.

The Eclipse FFF system includes the flow controller device and several options for the separation channel, two standard analytical channels, the dispersion inlet channel for aggregation-prone samples and the semi-preparative channel.

Molar mass determination for lignin

For SEC analysis lignins are dissolved in DMSO, with LiBr salt added to minimize interaction with the column matrix. There are only few choices for column calibration standards in DMSO; sodium polystyrene sulfonate (Na-PSS) is considered the best choice for lignin. But, as Figure 1 demonstrates, Na-PSS elutes quite differently compared to lignin of the same molar mass: the model oligomers do not fall on the calibration curve.

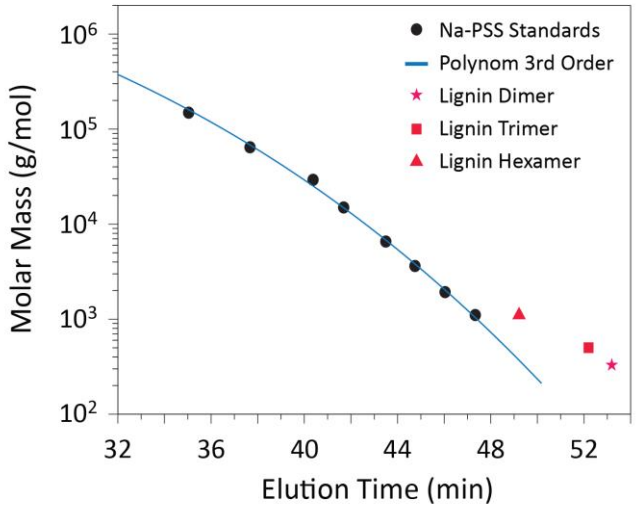

Figure 1. Comparison of lignin oligomer elution time (red symbols) against sodium polystyrene sulfonate standards (blue line). Separation was done in DMSO on a set of three SEC columns. The lignin oligomers deviate from the Na-PSS calibration curve. (Figure adapted from Ref. 1).

Lignin represents a case where SEC calibration with standards clearly fails, but the use of MALS for absolute measurement of molar masses is not straightforward. The first reason is strong absorption of the incident light by the lignin sample, and the second is fluorescence.

Dealing with absorption

In standard SEC-MALS analysis, the laser intensity is stabilized and monitored through the Laser Monitor which records laser intensity prior to entering the flow cell. This is the most accurate method when the sample does not absorb, but is insufficient when absorption occurs.

Figure 2 shows the dip in forward monitor intensity when a lignin sample at relatively low concentration passes through the MALS flow cell. A comparison of the red and infrared laser wavelengths demonstrates that absorption is minimized in the IR. Since molar mass is calculated from the ratio between incident and scattered light, any change in the intensity reaching the sample in the detection region of the cell will adversely impact the analysis.

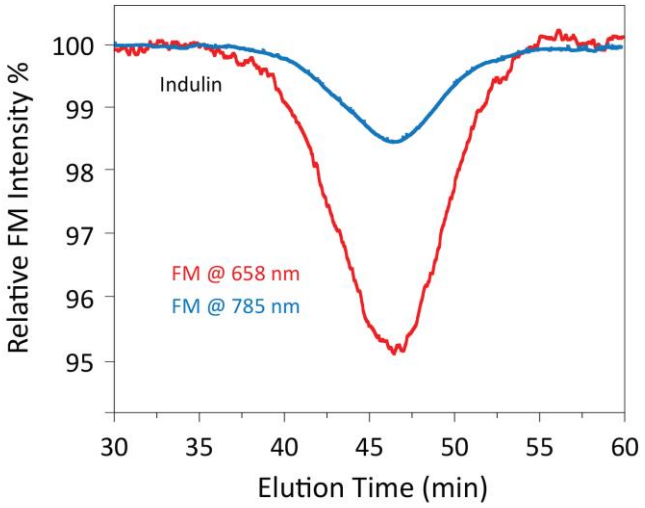

Figure 2. Intensity variation of the forward laser monitor during passage of the sample. The decrease comes from absorption of the incident light by the sample. Absorption is reduced from almost 5% at 658 nm to less than 2% at 785 nm. The sample is a kraft lignin sample (indulin). (Figure adapted from Ref. 1)

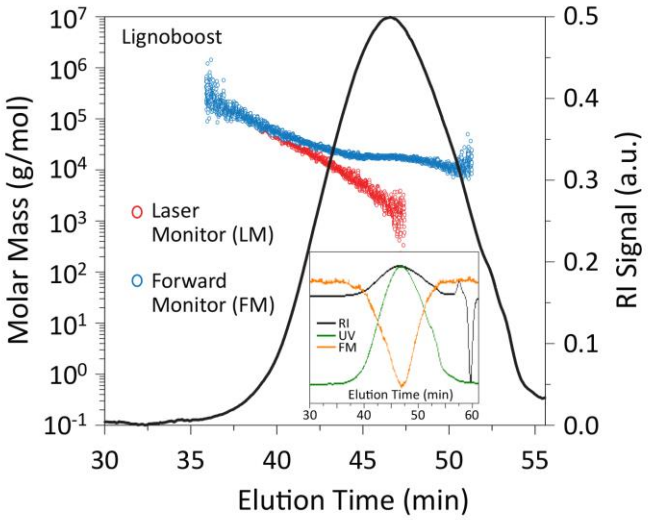

Figure 3. Effect of using the laser monitor (LM) or the forward monitor (FM) to calculate the molar mass of a strongly absorbing lignin sample. Using the LM which is the uncorrected laser intensity leads to an underestimation of the molar mass. The insert shows the forward monitor reading in an overlay with the RI trace. (Figure adapted from Ref. 1)

Using the Forward Monitor signal, the loss of intensity can be corrected—if it is not too severe. For moderate absorption, up to ~ 40%, the correction is feasible and reliable, as Figure 3 illustrates for a strongly absorbing lignin illuminated at 660 nm.

Dealing with fluorescence

Once the correct laser intensity is applied, the effect of fluorescence becomes evident. This is shown in Figure 4 for several combinations of laser wavelength and fluorescence-blocking filters. Reduction in the apparent molar mass across the peak is a result of suppressing fluorescence, with filters and a longer laser wavelength. It is evident that the lower the calculated molar mass, the closer it will be to the real value. So even with a 785 nm laser, the filters are indispensable.

But is the lowest plot now the real molar mass of the lignin sample? The fact that the molar mass does not decrease across the peak is suspicious. Lignin is a polydisperse material and the column should separate the material with elution going from higher to lower molar masses. The fact that such a decrease is not seen means there is still residual fluorescence present which the filters and IR laser cannot fully suppress.

The scattering intensity is proportional to the product of molar mass and concentration, while fluorescence is proportional to concentration alone. Hence the impact of fluorescence is higher with decreasing molar mass; the apparent molar mass is too high in the tailing flank of the peak.

Absolute molar mass determination for this lignin sample only works if the molar mass is high enough so that the fluorescence light contribution can be neglected. It is possible to extrapolate the molar mass versus elution time to evaluate the lower part of the distribution. as shown in Figure 5. This method can also be applied in cases where the light scattering intensity becomes too noisy at low molar masses.

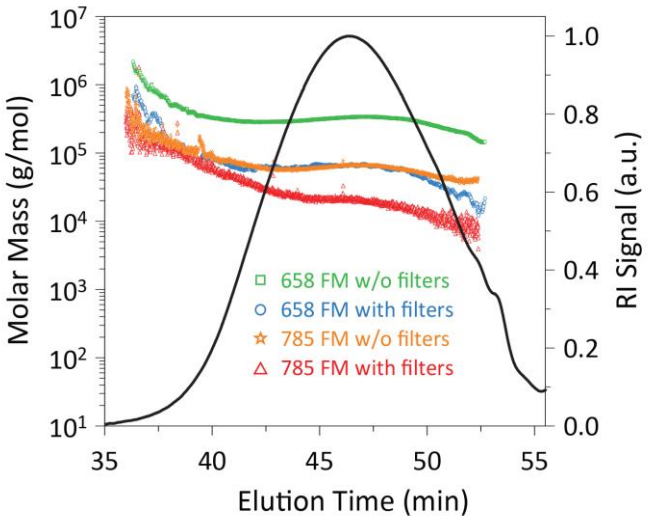

Figure 4. Comparison of calculated molar mass using different configurations of the MALS instrument. Two different laser wavelengths with and without fluorescence-blocking filters have been applied. At the infrared wavelength of 785 nm fluorescence is reduced compared to the 685 (red) wavelength. But even then, filters are necessary to minimize the effect of light not coming from scattering. (Figure adapted from Ref. 1)

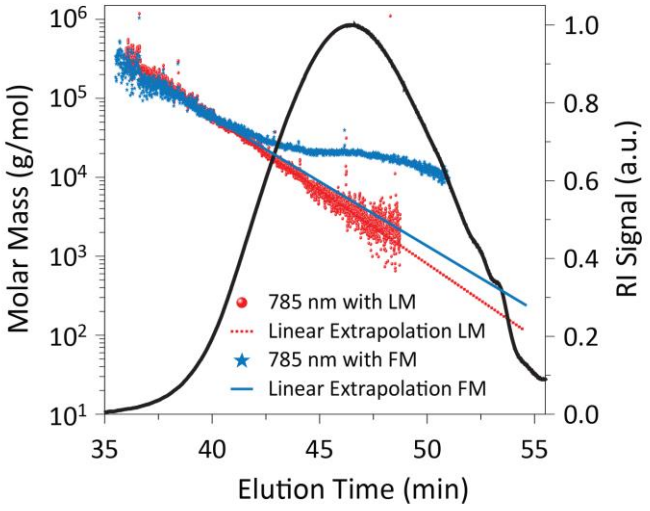

Figure 5. Calculated molar masses using the uncorrected laser intensity (LM) and the forward monitor (FM) which takes absorption into account. Values based on the FM are too high as the filters are not 100% effective to exclude the fluorescence light. A method to approach the true molar mass is an extrapolation from early elution times. It is important to base the extrapolation on the FM derived vales, otherwise the result will be too low, as seen from the red line (LM extrapolation) versus blue line (FM extrapolation). (Figure adapted from Ref. 1)

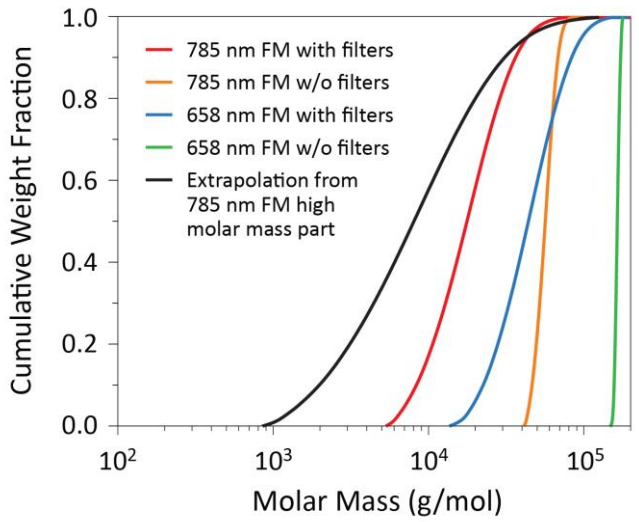

Figure 6. Comparison of the cumulative molar mass distribution from different instrument configurations and the use of extrapolation. Influence of fluorescence light leads to an overestimation of the low end of the distribution even with filters installed. (Figure adapted from Ref. 1)

With this combination of IR laser, fluorescence-blocking filters and the use of the FM monitor, the correct molar mass distribution can be evaluated by extrapolating to the low molar mass region. The misjudgment of the molar mass distribution in the other instrument configurations are evident from Fig. 6.

If the necessary instrumentation is available, the task of lignin molar mass determination becomes less challenging and a routine application for industrial lignin samples is made feasible.

Characterization of lignosulfonates by AF4-MALS

Lignosulfonates (LS) are derivatives of lignin and a byproduct of the sulfite pulp process, produced in kiloton quantities. They have several industrial uses with more are in development. This water-soluble derivative of lignin can be characterized by SEC, but only after tedious preparation of samples drawn from raw pulp. Therefore, it is of high interest to develop a characterization method which can deal with a direct injection of raw pulp. AF4-MALS is a suitable method, as described in a recent publication[2].

Advantages of AF4 for lignosulfonate characterization

AF4 separation is achieved in a channel composed of an upper plate and an ultrafiltration membrane forming the bottom, or accumulation wall (Figure 7). The sample is concentrated against the membrane during the focusing step prior to elution and separation. Separation is achieved purely based on diffusion of the sample components up into faster streamlines of the eluent flowing towards the channel outlet.

The pores of the membrane must be small enough to prevent the sample from crossing the membrane, but will allow low-molar-mass impurities to pass. Those will permeate during the focusing step with the cross-flow waste, realizing sample purification “on the fly”. This is the major advantage of the AF4 technique for raw pulp samples.

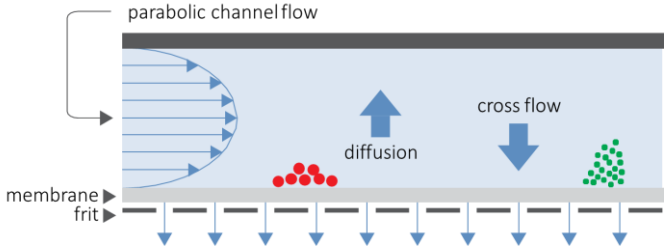

Figure 7. AF4 separation mechanism.

Careful choice of the membrane and cutoff is important for successful characterization. Two types of membrane material, regenerated cellulose (RC) and polyethersulfone (PES), were screened in different cutoffs (pore sizes). RC membranes have less electrostatic interaction with the sample but, at the smaller pore sizes needed for good recovery, generate more backpressure. Using an RC 3 kDa MWCO membrane, the channel can be calibrated with polystyrene sulfonate standards and a relative molar mass distribution for lignin generated. As Figure 8 shows, for fluorescent samples like Kraft lignin, this can be an alternative when MALS at 785 nm is not available.

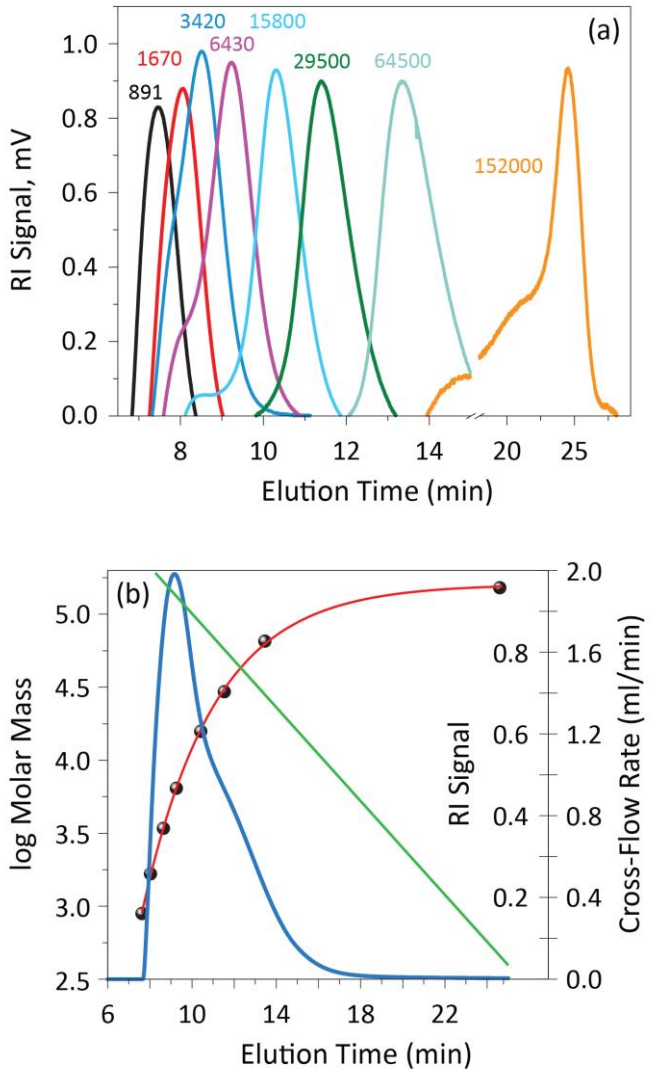

Figure 8. Separation of polystyrene sulfonate standards using a 3 kDa RC membrane (a). From the known peak molar mass values, a calibration curve is generated which can be used to estimate the molar mass distribution of a fluorescent Kraft lignin sample (b). The RI trace is shown in blue, the cross-flow rate in green and the molar mass calibration curve in red. (Figure adapted from Ref. 2).

However, MALS is the gold standard for molar mass determinations to generate absolute results. The added advantage is that electrostatic interactions which influence the retention time do not compromise the results. Therefore, PES membranes can be used with lower cut-off as they produce less back-pressure. MALS at 785 nm with fluorescence-blocking filters gives accurate results, because the lignosulfonates have much less fluorescence compared to lignin. Figure 9 shows the measurement of a sample with the calculated molar mass as a function of retention time.

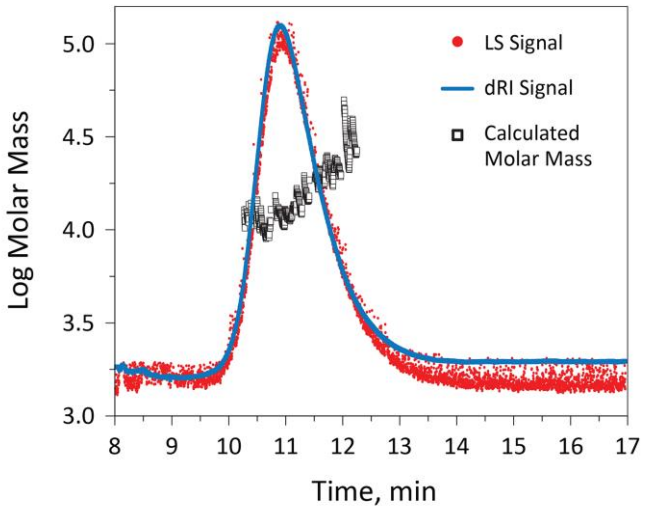

Fig. 9 AF4 separation of a lignosulfonate sample using a polyether sulfonate membrane. Molar masses are determined using a MALS detector at 785 nm with fluorescence-blocking filters. As this sample has less fluorescence compared to lignin, correct values can be calculated.

Summary

Molar mass characterization of lignin is one of the most challenging applications, because of strong absorbance and fluorescence of this biopolymer. MALS can be applied, but accurate values depend on correcting for absorption using the Forward Monitor and minimizing the influence of fluorescence light with a 785 nm laser plus filters in front of the scattering detectors.

The molar mass on the low end of the distribution may still be overestimated so an extrapolation of molar mass to longer elution times should be applied. In the end, reliable molar mass distribution values for lignin are achieved. This represents significant progress, since even the order of magnitude of the molar mass was not well-established before publication of Ref. 1.

In a second study the characterization of lignosulfonates using AF4-MALS was described. Here the advantage of FFF is that crude pulp samples can be readily analyzed without the tedious sample preparation normally required for SEC. AF4-MALS analysis provides a fast and reliable tool to determine the molar mass distribution which will facilitate research for new industrial applications and optimization of processes for lignosulfonate products.

References

1. Zinovyev, G. et al. Getting Closer to Absolute Molar Masses of Technical Lignins. *ChemSusChem* **11**(18), 3259-3268 (2018).
2. Sulaeva, I. et al. Molar Mass Characterization of Crude Lignosulfonates by Asymmetric Flow Field-Flow Fractionation. *ACS Sustainable Chemistry & Engineering* **7**(1), 216-223 (2018).

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