



WYATT
TECHNOLOGY

6300 Hollister Avenue
Santa Barbara, CA 93117
Tel: +1 (805) 681-9009
Fax: +1 (805) 681-0123
Web: www.wyatt.com

Live Webinar Q&A Sheet:

Pulp Non-Fiction: Absolute Macromolecular and Nanoparticle Characterization of Lignocellulosic Materials

The recorded webinar may be viewed from the [Polymer webinars page](#). These questions were submitted by live viewers. Additional information on MALS, DLS, and FFF may be found in the Wyatt [Library](#) under Webinars, Application Notes, Featured Publications and Bibliography, as well as on the corresponding [Product](#) and [Theory](#) pages of our website.

Please contact info@wyatt.com with any additional questions.

Applications

Q: *Why would someone need to know the amount of aggregates in a SEC solvent?*

A: If lignin samples contain significant amounts of aggregation prior to injection onto a SEC-MALS system, it may be impossible to achieve baseline resolution of the soluble polymer peak from the aggregate peak. Because of this, elimination of large aggregates from the sample prior to injection is essential when the goal of the SEC-MALS experiment is to measure parameters for soluble lignin polymer. Batch DLS can be used as a quick screen to identify if a sample prep contains very large lignin aggregates which are clearly differentiated from the majority of the soluble polymer. In those situations, the scientist can rework the sample prep in an attempt to reduce or eliminate the lignin aggregates prior to injection onto the SEC-MALS system.

Q: *Why do you think 89 kDa is high and why do you expect them [i.e., the samples] to be in the region of 27 kDa?*

A: It is true that 89 kDa is not an unreasonable expectation for lignin molar mass. The goal of the example shown during the webinar was to demonstrate the impact of fluorescence on the calculation of molar mass and to illustrate how reducing the impact of fluorescence via hardware modification can greatly alter the molar mass result.

It is known that fluorescence adversely impacts molar mass calculations by over-reporting the true intensity of scattered light, and hence the calculated molar mass. The photodiode detectors cannot differentiate between fluoresced light and scattered light, so the reported intensity of scattered light will be artificially high according to the LS relationship, $I_{scattered} \propto M \cdot c \cdot \left(\frac{dn}{dc}\right)^2$. Reducing the amount of fluoresced light by using an IR laser and interference filters cuts down on the fluorescence and the fraction of fluoresced light reaching the detector, making the reported intensity of scattered light more accurate and hence the calculated molar mass more accurate as well.

Since the fluorescent behavior of lignins is well established, the best course of action for analysis is to perform the measurements with a system that effectively reduces the impact of fluorescence. This is achieved in the DAWN MALS detector by an IR laser and fluorescence-blocking filters.

Q: *As far as I know, these techniques require the polymer to be fully dissolved to be accurate. What about solubility issues for highly crosslinked or branched lignin or hemicellulose? Can you comment on this issue?*

A: Polymers that are either in solution or stable suspension can be measured accurately by light scattering. For highly crosslinked samples, molecular weights are typically not the goal of the analysis but size is. In these situations, MALS can still be used to calculate R_g , and DLS or intrinsic viscosity can be used to calculate R_h . Heavily branched polymers wherein all of the chains might not be fully solvated can still have accurate molar masses calculated.

Q: *Can you determine the weight average molecular weight of nanocrystalline cellulose nanoparticle?*

A: Yes. I just opted not to display that information for the sake of time.

Q: *What is dn/dc for cellulose and cellulose nanoparticles you have obtained?*

A: The dn/dc for the cellulose polymer in DMAc was provided to me by a customer and not verified here in our labs. The value was 0.136. The dn/dc for the cellulose nanocrystals was not measured and did not enter the analysis since it is only required for molar mass determination; only the R_g or rod length of the cellulose nanoparticles was analyzed, and this does not make use of dn/dc.

Q: *In AF4 experiment, the molecular mass was quite stable across the whole elution profile (pink in color). I would expect aggregates having much higher molecular mass.*

A: The scaling on the Y-axis was logarithmic. I should have altered the axis scaling to better show the polydispersity across the elution profile. The leading edge of the soluble polymer peak had a reported molar mass of ~30 kDa, and the tailing edge had a reported molar mass of ~120 kDa. The aggregate peak had a reported molar mass of >1.0 MDa. You are correct in that the aggregate molar mass for AF4 is lower than the reported molar mass for the SEC column. Likely the SEC conditions are causing additional aggregation in the system, driving the molar mass higher in the SEC analysis but not the AF4 analysis.

Q: *Can batch DLS effectively deconvolve size distributions of samples with polydisperse aggregates?*

A: Batch DLS can deconvolve sizes of polydisperse aggregates, but the resolution is nothing like that achieved with SEC separation upstream. In order to get baseline resolution between two different species, their R_h values must be

4x – 5x larger/smaller than each other – implying > 100x difference in molar mass. If this 4x – 5x size variation is not met, then the reported R_h distribution will not reach baseline between the two samples. Instead, you would simply see a broadening of a single peak relative to the peak width of a monodisperse sample.

Q: *How to accurately measure organosolv lignins with M_w s of approx. 1000? Does light scattering give any information apart from aggregation?*

A: The lowest molecular weight we have measured is 200 Da. Samples with M_w of ~1 kDa are therefore not far from the lower detection threshold of MALS and differential viscometry, so the feasibility of analysis depends on their concentration and dn/dc values being sufficiently high. I would recommend running proof-of-concept analyses to determine whether MALS + dVIS can work for your samples.

Instrumentation

Q: *In the DLS example, are there any mobile phase limitations?*

A: Batch DLS is typically performed either in a quartz cuvette, plastic cuvette, or plastic multi-well plate. The plastic cuvette and multi-well plate have certain solvent restrictions particularly when it comes to using organic solvents. The quartz cuvette is resistant to most organic solvents, but has pH limitations since alkaline solutions can etch the quartz. Please contact Wyatt Technology for more details regarding the specs of the different sample cells.

Q: *Is it possible to analyze huge cellulose with A4F technique using DMAc/LiBr solvent (risk of membrane dissolution)?*

A: If an appropriate AF4 channel membrane can be found, there is no reason AF4 cannot be used to analyze very large cellulose polymers. Currently, Wyatt Technology offers PES membranes and Regenerated Cellulose membranes. Unfortunately, neither of these are compatible with DMAc + LiBr. However, if the

user can identify an appropriate membrane, we can provide a template for the user to cut his/her own channel membranes to fit the AF4 system. Some AF4 users have definitely taken advantage if this option for other solvent conditions, but I'm not familiar with anybody using this for DMAc.

Q: *Can you comment on drawbacks associated with a 2 angle LS detector instead of a MALS detector?*

A: Your samples can in principle have sizes that range from a few nm up to tens or hundreds of nm. However, detectors with only two angles cannot determine masses or sizes for macromolecules larger than ~ 20-30 nm (R_g), since beyond that the light scattering variation with angle is nonlinear and more than two angles are required to fit the curve. An instrument with only two angles also cannot tell you if there is nonlinear angular dependence in order to know that 2 angles do not suffice... Three or more angles provide a far more reliable fit to a linear angular dependence, provide a fit to nonlinear angular dependence when the R_g is larger than 20-30 nm, and also indicates curvature pointing to larger R_g values which cannot be fit with just two angles. With two angles you may think you are measuring light scattering but are actually left in the dark!

In addition, most two-angle light scattering instruments place one detector at 90° and one at ~ 15°. The low angle signal tends to be very noisy since particles shed from the column preferentially scatter a lot of light into low angles, and so determination of sample mass and size (which depend on only two points, one at 90° that is relatively stable and one at 15° which is noisy and so skews the determination of slope) is erratic. With three or more angles, if the low angle signal is noisy it can be ignored, and both mass *and* size calculated from two *stable* signals. Wyatt's 3-angle detector design places the lowest angle at ~ 40° in order to improve signal-to-noise which usually makes *all three* detectors useful.

Q: *Is there any drawback to using the 786 nm laser for non-fluorescing analytes?*

A: The only drawbacks are, the 786 nm laser is slightly less powerful than the 665 nm laser, and the molecular scattering efficiency at longer wavelengths is lower, so the overall intensity of scattered light will be less, impacting the ultimate

system sensitivity (when column shedding is not the real limitation to sensitivity). One other possible drawback is that the lower R_g detection limit will increase very slightly since that parameter is wavelength dependent. It may go up from 10 nm to ~12 or 13 nm.

Q: *As a crystal does not scatter light according to theory why is it possible to measure cellulose nano-crystals?*

A: Actually, only an infinite and perfect crystal does not scatter light, and that is only true if the wavelength of light is much longer than the atomic spacing. X-rays have a wavelength comparable to or smaller than the interatomic spacing and therefore do scatter; however, a very large slab of perfect crystal will scatter almost no visible light.

Crystals of finite size in solution, whether nanocrystals or macroscopic, scatter visible light primarily from the solid-solvent interface, because the crystal is not infinite in extent and the 3D interface can be considered an “imperfection”. So long as the crystal nanoparticle size is appreciably smaller than the wavelength of incident light, in this case 660 nm for the cellulose crystals, the Rayleigh-Gans scattering approximation holds and may be used to determine the size. Larger particles with sizes up to ~ 1000 nm can be analyzed with shape-specific light scattering models to determine the ellipticity.

Separation parameters

Q: *What types of SEC columns were used to separate the lignin samples?*

A: We used MZ-Gel SDplus LS columns. But please be aware that just because those columns worked for the lignin samples that we analyzed, they might not work for your samples. Different source woods, pulping methods, etc. can produce significantly different lignin polymers that may or may not have different column interaction behaviors.

Q: *What are the settings for the AF4 analysis of Lignosulfonate (Cross flow?)*

A: I used a Wyatt short channel with a 5 kDa cutoff PES membrane, 0.5 mL/min channel flow, and 3 mL/min crossflow to achieve the baseline resolution between polymer and aggregate.

Q: *Could you please elaborate on the SEC conditions used to separate the lignin samples? Most conditions in literature involve harsh caustic conditions which can be problematic to the system so are there any methods which use less harsh conditions?*

A: Most of the sample were run at relatively benign conditions either in neat THF or DMF with 5% LiBr for the organic soluble samples, or 50 mM sodium nitrate buffer pH 7 for the aqueous soluble samples. Wyatt detectors have a broad range of solvent compatibilities. Acceptable pH range is typically from 1 – 10, the detectors can handle acids very well, but not bases as it will etch the flow cell or quartz cuvette. Please contact Wyatt Technology for detailed information regarding solvent compatibility and wetted materials for all of our detectors.

Samples and solvents

Q: *How important is filtration of mobile phase for SEC-MALS analyses? What cutoff would you propose?*

A: Mobile phase filtration is very important to reduce the light scattering noise from the system. I usually recommend a 0.2 µm pore size membrane to filter all mobile phases prior to use.

Q: *In the DLS example, what type of lignin was measured and what is the mobile phase?*

A: A softwood lignosulfonate was analyzed. Sample 1 was dissolved in pure water, Sample 2 was dissolved in 50 mM sodium nitrate buffer pH 7, Sample 3 was

dissolved in 50 mM sodium nitrate buffer pH 10. I do not know what the pulping method or sulfonation method used for these samples was.

All lignins are not created equal. Your samples might have different aggregation behavior in these conditions.

Q: *What is the eluant used for nanocrystalline cellulose analysis in A4F?*

A: 50 mM sodium nitrate buffer, pH 7.