



Live Webinar Q&A Sheet:

Structural Characterization of Biopolymers by Analytical Separation Techniques with Advanced Detectors

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

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

SEC-MALS general

Q: How does the MALS detector give the molar mass of molecules? How do you compare that to mass spectrometry?

A: The molar mass from a multi-angle light scattering detector such as a [DAWN[®]](#) or [miniDAWN[®]](#) is calculated from the ratio of intensity of scattered light at zero angle, to the concentration. While the accuracy of light scattering analysis is only about $\pm 5\%$, it compares well to mass spectroscopy results. The advantage of light scattering is a much larger molar mass range and simpler analysis. I have measured some oligomers and compared molar mass distributions by [SEC-MALS](#) with MS spectra; the agreement was very good.

Q: How can dn/dc be measured?

A: It can be measured off-line by injecting series of solutions of known concentration, e.g., 0.2 mg/mL to 5 mg/mL, directly into the flow cell of RI detector ... or online from the injected mass and RI peak area. Both calculations are quite comparable and supported by Wyatt's [ASTRA[®]](#) software.

Q: How easy is it to quantify polymers in matrices using SEC or AF4 in absence of standards? I understand that the molar mass may be identical but the chain length distribution may vary.

A: Quantification of polymer in matrices can be performed using a three-detector method combining MALS with two concentration detectors. The ratio of polymer to matrix is obtained by comparing two different concentration signals, most commonly from UV and RI detectors. In the case of complex polymer systems such as blends or copolymers, the analysis is easier when one component is UV absorbing whereas the other is not. Finally, the molar mass of each component is calculated by adding the information from the MALS detector.



SEC-MALS-IV general

- Q: What are the shear rates experienced in the viscosity detector? Are there any concerns with shear thinning or degradation in the viscometry detector independent of the SEC column?*
- A: The shear rates depend on the flow rate and viscosity. For water flowing at 1 mL/min, the shear rate in Wyatt's **ViscoStar**[®] differential viscometer is 5000 s⁻¹. Hence, at typical flow rates of 0.5 – 1 mL/min, the results are not affected by shear.
- Q: Can you utilize viscometry to characterize the difference of density in similar biopolymers?*
- A: Intrinsic viscosity measured by an online differential viscometer is closely related to the ratio between hydrodynamic volume and molar mass, and therefore is indicative of molecular density or specific volume.

AF4 general

- Q: What are possible solvents for AF4?*
- A: Wyatt's **Eclipse**[®] AF4 modules support aqueous buffers and various organic solvents such as THF, chloroform, toluene, methylene chloride.
- Q: What is the molar mass range for AF4?*
- A: It starts around 5000 g/mol and goes to hundreds of million g/mol.
- Q: For polymers that are not soluble and highly cross-linked, would the AF4 technique be suitable for the determination of molecular weight?*
- A: In the case of highly crosslinked species containing more than a single molecule, strictly spoken we do not measure molecular weight, but the mass of some supermolecular structures (we can call them nanogels). Such species can be characterized quite well by AF4-MALS. In the case of macro-gels, the material cannot be characterized by AF4.
- Q: Is it possible to vary the length of the AF4 cell to increase resolution?*
- A: Yes, it is possible, and Wyatt does offer two AF4 channel lengths. However, optimization of the cross-flow profile is a more effective way to increase resolution. Wyatt's **SCOUT DPS**[™] software simulates AF4 separations to perform virtual optimization, eliminating a lot of the trial-and-error traditionally involved in method optimization.



Conformation and branching

Q: What is the difference between conformation estimation by using M-H plot vs the conformation plot (RMS radius against molar mass)? *Is there any advantage of viscometry for the characterization of branching?*

A: The SEC-MALS-IV method is somewhat more sensitive than SEC-MALS alone. The exponent of Mark-Houwink equation is in the range of 0 for compact spheres versus ≈ 0.7 for linear random coils in thermodynamically good solvents, whereas the slope of a conformation plot (radius of gyration versus molar mass) ranges from 0.33 to ≈ 0.6 . In addition, the intrinsic viscosity does not lose sensitivity at low sizes (R_g by MALS is limited to > 10 nm) and thus the Mark-Houwink plot can be measured down to ≈ 1000 g/mol.

Both plots allow detection and quantification of branching if information is available for the equivalent linear polymer of the same chemical composition. Specifically, one must be able to specify the intrinsic viscosity or rms radius of the equivalent linear polymer as a function of molar mass, whether previously measured or taken from the literature. In case one does not have data for the linear polymer, it is possible to at least differentiate the conformation and relative branching ratios of different samples.

Q: *Can Mark-Houwink plots also be used to make a statement about structural changes or aggregation of proteins?*

A: The hydrodynamic radii of proteins and aggregates can be calculated from molar mass (MALS) and intrinsic viscosity (differential viscometry) with higher precision than their determination by dynamic light scattering, and therefore MALS + IV offers a more sensitive indication of conformational changes than does MALS + DLS. In addition, plotting intrinsic viscosity against molar mass of monomeric and aggregated proteins yields a Mark-Houwink plot similar to that of polymers; the slope can be analyzed to learn if the aggregates are amorphous or fibrillar.

Specific applications

Q: *How would you recommend to monitor reactions such as polymerization or depolymerization?*

A: If the reaction is relatively slow, on the time scale of many hours or longer, I would periodically measure the entire molar mass distribution by SEC-MALS, or alternatively by AF4-MALS, and calculate moments including M_n , M_w , M_z and polydispersity M_w/M_n . If the reaction is relatively fast, on the time scale of minutes to a few hours, and the polymer system is compatible with UHPLC, then the same properties can be monitored in fast separations of 1-2 minutes each.

If the reaction is fast but the system is not amenable to UHPLC, the progress of the weight-average molar mass and size can be monitored in real time by flowing the solution through MALS and dRI detectors without separation.



Q: Would you recommend analyzing cellulose in native form or after derivatization?

A: If the sample can be dissolved, then I would recommend native form. However, the derivatization for some samples may be the only way.

Q: How do you measure the molecular weight of Xanthan? In aqueous mobile phase it aggregates.

A: I would try disaggregation using longer dissolution time (even several days) and/or elevated temperature.

Q: Why is RI used as the concentration detector for lignin given that UV absorbance at 280 nm generally gives a much stronger signal? Is there a benefit to using RI over UV in this case?

A: UV can be used for lignin, yet RI is more general concentration detector. In case of UV detector, the extinction coefficient for each lignin type would have to be known in order to calculate the concentration from UV signal whereas the dn/dc value is more or less uniform.

Q: How does one determine shell surface of a nanoparticle, if any? Also, with known nanoparticle, how does one determine PEGylation?

A: If the nanoparticle is spherical, the refractive indices of the core and shell materials are known and the core radius is known, it is possible to determine the shell thickness using multi-angle light scattering with the Particle module in Wyatt's ASTRA software. This method may also be useful in assessing PEGylation of a nanoparticle. Additionally, if the nanoparticle is relatively small ($\sim < 50$ nm in radius) and optically not too different from the solvent (e.g. lipidic or proteinaceous) then PEGylation can be calculated using ASTRA's protein conjugate analysis method using MALS + UV + RI.