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Live Webinar Q&A Sheet:

To SEC-MALS and Beyond: The Road to Biotherapeutic Development Illuminated by Light Scattering

The recorded webinar may be viewed [here](#). These questions were submitted by live viewers. Additional information on SEC-MALS and Wyatt instrumentation may be found on 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.

Q: What is the resolution of the detection for an antibody with a 2 kDa conjugate? Can we differentiate 150 kDa and 152 kDa using the TREOS?

A: We specify the accuracy of the molar mass to within 5% and the precision within 2%. This means the addition of a 2-kDa conjugate (~1.3% change in molar mass) may still be within the run-to-run variation for a given SEC-MALS system. However, you should be able to distinguish an antibody with >3-kDa conjugate from the bare antibody. This could mean a single polymer chain (such as PEG) conjugated to a single protein or multiple smaller modifiers, such as the 6-10 drugs conjugated to the antibody in our ADC example.

Q: Is the number of angles important?

A: The number of angles determines the maximum size and molar mass that can be measured by MALS. With 3 angles in the TREOS, we can measure root mean square radius of 10-50 nm for arbitrary geometries or up to 150 nm for spheres. With the TREOS, we can measure molar masses of 300 g/mol up to $\sim 10^6$ g/mol for arbitrary geometry or up to a few million g/mol for compact structures, like proteins. With 18 angles in the HELEOS, we can measure root mean square radius up to 500 nm and molar mass up to $\sim 10^9$ g/mol.

Q: What's the MW accuracy spec range determined by SEC-MALS?

For a routine analysis, what would be the typical variance in measurement of molecular weight?

A: We specify the accuracy of the molar mass to within 5% and the precision (variance among measured molar mass on the same SEC-MALS hardware) within 2%. This takes into account the accuracy in the concentration measurement, dn/dc , and other parameters.

Q: Do we expect to have the same diffusion coefficient for a sphere or rod shaped molecule?

A: Two molecules with the *same* molar mass but different shape would not be expected to have the same diffusion coefficient. This is one reason that analytical SEC may fail. The measured molecule has a different conformation as compared to the molar mass standards and, thus, elutes at a different rate.

Two molecules of *arbitrary* mass with different shapes could have the same diffusion coefficient and, thus, the same hydrodynamic radius (r_h). However, the molar mass, radius of gyration (r_g), and geometric dimensions of each molecule would be different. For example, r_h is equal to the radius of a sphere, and for a rod, $r_h = L/6$.

Q: So the MALS detector has a DLS detector incorporated in it?

If you want to use DLS detection in the HELEOS system, do you need adjustments to the equipment and/or software, or is it readily available in current settings?

A: Both our HELEOS and TREOS have the option of an embedded dynamic light scattering (DLS) detector. This allows you to measure both multi-angle static light scattering (MALS) and DLS simultaneously in the same measurement volume. For more information, see www.wyatt.com/SEC-MALS.

Once the dynamic light scattering module is installed in the HELEOS, using it is simple and straightforward with our ASTRA software. No additional adjustments are required by the user prior to a given experiment.

Q: When we say MALS, it's always static light scattering?

A: Yes, MALS always means multi-angle (static) light scattering. Static light scattering intensity varies as a function of angle according to the root-mean-square radius of the molecule being measured. Thus, it is critical to collect static light scattering data at multiple angles to calculate molar mass accurately. Dynamic light scattering (DLS) is only measured at a single angle to quantify the hydrodynamic radius of a molecule in solution.

Q: Using the Protein Conjugate analysis, how can you calculate the fraction of protein and modifier with SEC-MALS?

A: With the Protein Conjugate analysis, three detectors are required—UV, MALS, and refractive index (RI). The protein and conjugate each have different UV extinction coefficients and differential refractive indices (dn/dc). From the UV detector, we get the concentration of protein; from the RI detector, we get total concentration; and from the MALS detector, we get total molar mass. With that information, we have all the data necessary to calculate the mass of protein and the mass of polysaccharide.

Q: Can you please go over the calculation of the drug-antibody ratio (DAR) using SEC-MALS in detail?

A: With the Protein Conjugate analysis, the total mass of the ADC, protein mass, and drug mass can be individually calculated (see previous question). In the example that we showed in the webinar, the mass of a single drug molecule was known by the manufacturers to be ~1.2 kDa. The DAR was calculated by dividing the total mass of drug conjugated to each antibody by the mass of each drug. For example, one of the ADC's we presented had a total mass of 167.8 kDa. The antibody mass was 155.2 kDa, and the drug mass was 12.6 kDa. The drug-antibody ratio is calculated as follows:

$$(12.6 \text{ kDa drug per antibody}) / (1.2 \text{ kDa per drug}) = 10.1 \text{ drug molecules per antibody}$$

Q: You showed that LS detected the higher aggregates whereas no UV absorbance was visible. Can this LS signal be quantified somehow?

A: The light scattering signal can be used to determine the root-mean-square radius of the aggregate, provided that it is >10 nm. We can also report the relative light scattering intensity for the aggregates as compared to the “main” peak. For example, you might say that 5% of the scattering intensity is due to aggregates and 95% due to the monomer. For general particulate samples where concentration data do not exist, we can perform Particle Analysis to determine the radius and number density of particles from light scattering data.

Q: Can MALS be used only with high performance liquid chromatography (HPLC), or can low pressure chromatography be used?

Can MALS be used with different separation modes, e.g., affinity chromatography, reverse-phase chromatograph (RP), or ion-exchange chromatography (IEX)?

A: Multi-angle light scattering can be used to measure molar mass and radius, regardless of if the sample is fractionated or not. Macromolecules may be separated by size using low pressure chromatography (e.g., FPLC), high pressure chromatography (HPLC), or field-flow fractionation (FFF) before flowing through the light scattering detector for

measurement of absolute molar mass. For other forms of chromatography, such as IEX, baseline subtraction must be included to account for the changing refractive index of the mobile phase over the course of the experiment.

Q: Can light scattering measure the K_D of the protein-ligand directly?

A: Composition-gradient multi-angle light scattering (CG-MALS) is a powerful tool for measuring both the affinity (K_D) and absolute stoichiometry of a macromolecular interaction. This technique requires the molar mass of the protein increase by ~10% or more upon binding. Thus, it is well-suited for protein-protein, protein-DNA, and protein-polymer interactions but not for interactions between a protein and small molecule. You can find more information at www.wyatt.com/CG-MALS.

Q: Did you show any CG-MALS data illustrating protein function, i.e., a hetero-interaction?

A: CG-MALS data for hetero-associations was outside the scope of this webinar, but you can learn more in our archived webinars and in our CG-MALS page (www.wyatt.com/CG-MALS). Here, you'll find links to our application notes and other references.