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Live Webinar Q&A Sheet:

Successful SEC-MALS Analysis of Proteins and AAVs
Dr. Leo Liu

The recorded webinar may be viewed from the Biotherapeutics webinars page. These questions were submitted by live viewers. Additional information on SEC-MALS, DLS, FFF, CG-MALS and RT-MALS 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 Solutions page of our web site.

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

Questions & Answers

MALS QUESTIONS

Q: *How long does method development and optimization take?*

A: It should be minimal. The beauty of SEC-MALS is having absolute molar mass determination without the reliance on column calibration. That being said, better separation is always nice, and XBridge™ columns offer broad platform compatibility and help reduce LC method developments. You can find additional information on our recent White Paper: WP1618: Enhanced SEC-MALS analysis of biotherapeutics with XBridge columns (amazonaws.com)

Q: *Is there a mass limit for SEC-MALS to accurately detect large aggregates?*

A: The larger the size, the more complex its light scattering angular dependence is. Hence, we need more angles to capture the anisotropic light scattering accurately. The upper limit for the 19-angle DAWN is around 1 GDa or about 1 µm in radius. You can find more specification-related information here: DAWN Line of Multi-Angle Light Scattering (MALS) Detectors (wyatt.com)

Q: *What is the size cut-off to change from an SEC-MALS with a column to the FFF system??*

A: SEC has about a 30nm upper limit. For larger particles, finding a column with a sufficient pore size is difficult. FFF can cover a higher upper limit and is ideal for larger particles like lipid nanoparticles and large aggregates. The other benefit or use of FFF is to check for any possible column interactions that could be skewing SEC-MALS results, like sample or column interactions. FFF has no stationary phase, making it an ideal orthogonal separation technique.

Q: *Is it possible to get reliable analysis results without determining the dn/dc of the molecules (e.g., membrane proteins in detergents)?*

A: It is essential to incorporate the *dn/dc* in light scattering analysis. It is used in the equation for calculating molecular weight and size. Estimating *dn/dc* is possible with 100% mass recovery or utilizing the UV extinction coefficient. In the case of proteins in aqueous solutions, a commonly used value is 0.185, as reported in the literature. See our Tech Note for more details: TN4001 Online *dn/dc* Determination Rev A (zendesk.com). Additionally, you can use our proprietary conjugate analysis to discern the percentage contribution from proteins and micelles. Many *dn/dc* values can be found in relevant literature sources. We provide an online database on some *dn/dc* values. Database of *dn/dc* Values – Wyatt Support Center Home (Zendesk.com)

Q: *How is MALS able to resolve two co-eluting species during an LC separation?*

A: We can resolve two components in our conjugate analysis with two concentration sources. Most commonly, it is UV and RI or dual-wavelength UV. In this case, by measuring the molar mass contribution from two concentration sources, we can resolve the conjugation ratio of the two co-eluting species, such as antibody-drug ratio, empty vs full AAV, and such. More details on performing conjugate analysis: TN1006E – Performing a Protein Conjugate Analysis in ASTRA

Q: *Can materials be separated based on size (even if they are constituents of different compositions)? For example, synthetic polymers and naturally occurring proteins?*

A: The most important parameter is identifying a solvent or mobile phase that will work for both species. SEC separates based on hydrodynamic volume, and if these two materials have different volumes, then in theory, they should be candidates for separation.

Q: *What is the limit of detection for AAVs, and what is the typical concentration that you run?*

A: For best results, we recommend a concentration above 10^13 particles/mL. The Limit of Quantitation (LOQ) is about $5x10^{10}$ particles/mL.

Q: *Is the Wyatt MALS compatible with AKTA™ Avant specifically??*

A: Yes, the ultraDAWN™ is compatible with the AKTA Avant FPLC system. This system is different from the MALS shared today and is operated with different software. For more information, please visit: https://www.wyatt.com/products/instruments/ultradawn.html

COLUMN QUESTIONS

Q: *Can these two columns be used to characterize ferritin-antigen nanoparticles (The proteins co-expressed with ferritin_480 KDa plus rec Protein 100-500 KDa)?*

A: In total, you are looking in the 1 MDa range. The GTx column should be in the correct size range, and I would recommend starting with the 450 Å pore size column.

Q: *Does the pore size filter out higher-degree aggregates?*

A: The pore size usually doesn't filter out aggregation. You could have other unwanted elution behaviors, like column anchoring, which can be seen in the MALS data.

Q: *Are XBridge™ Premier™ Protein SEC 250™ 250Å 2.5µm and XBridge Premier GTx BEH SEC 450Å 2.5 µm columns suitable for both microDAWN™/µdRI and HELEOS II™)/ Optilab™TrEX™ system??*

A: They are compatible, but for optimal instrument performance, like a faster run time, you will want to use a UPLC-specific column. The UPLC instrument is compatible with HPLC columns and the DAWN detector, and we have many customers that run this configuration.

Q: *Do the Xbridge™ Premier™ columns work for peptides with a molecular weight of 4000 Da??*

A: A 125 Å pore size column would be better than the 250 Å. Please explore our or the Waters website for more columns and pore sizes.

Q: *Do you have any experience with these new columns when biopolymers are being looked at? (e.g., Lignans, Starch, or degraded PFAs)?*

A: I have tried this application and suggest checking our column selection tools online (https://store.wyatt.com/sec-columns/) or from Waters™ (https://www.waters.com/nextgen/us/en/products/columns/sec-columns-category.html) to help with your specific application.