



## Live Webinar Q&A Sheet:

### Latest Advancements in Absolute Characterization of Proteins, Polymers and Nanoparticles

The recorded webinar may be viewed from the [SEC-MALS](#) webinars page. These questions were submitted by live viewers. Additional information on FFF-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](mailto:info@wyatt.com) with any additional questions.

#### SEC-MALS basics

*Q: What is the minimal MW SEC-MALS can reliably detect?*

A: All Wyatt [MALS detectors](#) can reliably measure [molecular weights](#) as low as 200 g/mol, and even less. This is not a typical application for MALS, but does come up in the context of small monomers/oligomers of polymeric building blocks and small peptides. More commonly, SEC-MALS is used in the low MW range for peptides, [proteins](#) and [polymers](#) from about 1000 g/mol and up to tens and even hundreds of millions of g/mol.

*Q: Can SEC-MALS be used with a DAWN HELEOS II detector to characterize proteins?*

A: Certainly! Before the latest DAWN model was released in March 2019, the DAWN HELEOS II was the premier detector for SEC-MALS.

*Q: Is SEC-MALS compatible with Agilent HPLC?*

A: Yes, Wyatt's SEC-MALS detectors are compatible with Agilent and most other HPLC and FPLC systems. What's more, Wyatt's software can control Agilent HPLC modules for seamless operation.

*Q: Can we use RI as concentration determination in gradient runs for MALS analysis?*

A: The [Optilab](#)<sup>®</sup> has an extraordinary range of dRI and an even more extraordinary dynamic range. It can cover, with high sensitivity, a significant fraction of mild gradients such as are encountered in ion-exchange chromatography or hydrophilic interaction chromatography, from 0 and up to about 500 mM NaCl. For larger gradients, there is a high-concentration version of the Optilab (Optilab HC) which covers up to 2 M NaCl, more than enough for almost all such gradients as well as for acetonitrile-water reverse-phase gradients.



In order to use RI for concentration determination, ASTRA offers a 'Baseline subtraction' method. You measure the gradient without sample, and subtract the signals from the measurements acquired with the sample. Typically the sensitivity limit is determined by pump pulsation, which causes imperfect gradient mixing, and shows up in the RI signal as noise. Therefore, MALS analysis under gradient conditions are not as sensitive as under isocratic elution, and you may need to inject more sample.

*Q: Can the DAWN or miniDAWN be used with FPLC instrumentation, for example AKTA pure?*

A. Yes, a large fraction of our customers use their Wyatt MALS instrumentation with FPLC where it can be operated in the standard analytical mode or inline with purification techniques such as ion-exchange chromatography (see <http://www.wyatt.com/files/literature/app-notes/iex-mals/AN8004-Coupling-MALS-with-pIEX-for-structural-biology.pdf>).

The primary challenge in using MALS with FPLC is maintaining system cleanliness for good analytical results when the FPLC might be exposed regularly to crude solutions. This is definitely possible, though might entail some adaptations on the part of the FPLC user.

*Q: The new interface is attractive and packed with helpful information, but in the end, will it be easy or complicated to perform SEC-MALS measurements?*

A: SEC-MALS measurements are not complicated! The detectors are simply added downstream of the SEC system, and operation is similar to SEC. The main challenge for new practitioners is learning how to identify and maintain a clean, particle-free chromatography system, and the new System Ready and Health Indicators take a lot of the guesswork out of that learning process, making it even easier to make good quality measurements every time.

### **SEC-MALS experimental details**

*Q: How much sample must be injected for good signal-to-noise and accuracy?*

A: While this depends on the molar mass of the sample, our usual recommendation is 100 µg, which allows for good sensitivity to very heterogeneous polymers that are extensively dispersed by the SEC column(s), as well as to small (but narrow) impurity peaks in proteins and monoclonal antibody samples. Smaller molecules in the range of a couple of kilodalton or less will require larger quantities, while larger molecules in the range of megadaltons and more require less.

If you are measuring narrow peaks such as proteins, and are interested in characterizing the main species rather than impurities, you can get away with much less material, e.g. 10 µg.



*Q: Which SEC columns are required for SEC-MALS?*

A: Basically, any column that does a good job of separating the macromolecules in solution is OK. However, some columns are better than others in terms of resistance to shocks and preventing the shedding of fine particles that interfere with MALS measurements. Wyatt offers protein separation SEC columns that have minimal shedding and have been QC-tested to ensure low particle content. Our application specialists can help you identify an optimal column for your specific samples.

*Q: Is there a solvent/mobile phase dependency in SEC-MALS, specifically aqueous conditions?*

A: The light scattering measurement is generally not very sensitive to different types of aqueous mobile phase, because the  $dn/dc$  value of the sample changes by an insignificant amount.

However,  $dn/dc$  may change appreciably if the mobile phase differs significantly in refractive index from water or PBS, e.g. if it contains high levels of glycerin or arginine. If you are working with proteins under such conditions, it is quite straightforward to determine the correct  $dn/dc$  value by measuring a known protein such as BSA, and adjusting the value of  $dn/dc$  until the calculations yields the correct molar mass. Since the  $dn/dc$  of nearly all proteins are the same under given conditions, you can use the modified value for the sample of interest.

*Q: What is a good approach to address peak tailing in SEC-MALS?*

A: Peak tailing is usually due to chromatographic conditions – inappropriate column chemistry or mobile phase; however, it can arise from other reasons such as dynamic equilibrium or column overloading. SEC-MALS can help understand if the tail you see has the same molar mass as the peak region (hence is true tailing or column overloading) or a different molar mass (and therefore is actually impurities or a dissociation of the complex eluting in the main peak). Armed with that knowledge, you can proceed to optimize the chromatography and/or identify the impurities.

*Q: Can you explain how to address column shedding (ghost peaks) in MALS data?*

A: Ghost peaks can arise from shocking the column or dirty injectors.

- A dirty injector can be identified and cleaned by injecting blanks (and, if necessary, detergent or some other cleaning solution) without a column in the flow path.
- If the problem is not a dirty injector but rather from shocking the column, it is important to 1) always ramp up the flow gradually instead of going directly to the final flow rate; 2) perform overnight equilibration at the measurement flow rate; and 3) maintain continuous flow at a constant rate. In addition, some column packing materials are less sensitive to mechanical shocks,



and some injector valves (“make-before-break”) eliminate pressure shocks that cause ghost peaks.

*Q: With regard to using in-line DLS (QELS), does the flow impact the accuracy of hydrodynamic radius measurement?*

*A:* It does, depending on particle size and flow rate, but not over the typical size and flow ranges of SEC-MALS which is appropriate for hydrodynamic radii up to 50 nm. If you need to measure larger particles, for example with separation by FFF, Wyatt offers an optional flow cell that extends the  $R_h$  range to 300 nm.

*Q: How does extinction coefficient determination work? Are there any related application notes? How good is the repeatability of the method?*

*A:* We do have a technical note available for customers who would like to measure extinction coefficients. It describes a simple ASTRA method that entails injecting an aliquot, which passes through the UV and dRI detectors. The MALS detector is not actually part of this method. Assuming  $dn/dc$  is known (commonly the case for proteins in aqueous buffers), the extinction coefficient can be calculated.

#### SEC- and FFF-MALS

*Q: What is the size limit for using SEC for size characterization? At what average size one should switch to FFF?*

*A:* SEC size ranges depend on the specific columns, and usually do not go above millions (linear polymers) or tens of millions (proteins) of g/mol, or ~ 50 nm in size. FFF is essential above that range. However, FFF can also be the separation technique of choice for smaller molecules, if SEC presents difficulties such as column interactions or shear.

*Q: Is there a high temperature option for the FFF Eclipse system?*

*A:* No, the Eclipse® field-flow fractionation system operates at ambient temperature. In principle it is possible to place the separation channel in an oven that maintains temperature up to 90°C.

#### SEC-MALS applications

*Q: Can SEC-MALS analyze DNA, RNA or protein-DNA complexes?*

*A:* Absolutely! SEC-MALS is appropriate for characterizing most soluble macromolecules as long as they can be separated nicely on SEC columns, including nucleic acids and complexes with proteins. In particular, this technique can determine their molar mass, size and conformation, as well as the absolute stoichiometry (i.e., how many molecules of each are present in the complex)



of oligomers and heterocomplexes of proteins and nucleic acids. While SEC-MALS cannot usually determine binding affinity,  $K_d$  can be measured—without labeling or immobilization—by a related technique, [CG-MALS](#).

Some good examples may be found in these application notes:

[https://www.wyatt.com/files/literature/app-notes/cg-mals/WP3001\\_SEC-MALS-and-CG-MALS-characterize-protein-DNA-interactions.pdf](https://www.wyatt.com/files/literature/app-notes/cg-mals/WP3001_SEC-MALS-and-CG-MALS-characterize-protein-DNA-interactions.pdf)

<https://www.wyatt.com/files/literature/app-notes/sec-mals-biopolymers/AN1616-SEC-MALS-method-for-characterizing-mRNA.pdf>

*Q: Is there any specific way to distinguish between aggregates and large polymers by SEC-MALS?*

A: Since light scattering essentially provides a structural determination – molar mass, size, conformation – in order to differentiate, there would need to be some difference in the overall structures of the aggregate and large polymer in order to make that distinction.

*Q: Do you have more examples of your instruments characterizing nanoparticles? I'm interested in the 500-10,000nm range.*

A: MALS is appropriate up to a radius of about 500 nm, or diameter of 1000 nm. The field-flow fractionation system can separate larger sizes, but without online size determination. However, one could collect size fractions and measure them offline, e.g. using dynamic light scattering.

*Q: Do you have examples/application notes for polysaccharide degradation by enzymatic reaction?*

A: No, but we do have application notes and examples of monitoring the degradation of polysaccharides and other polymers by other means, such as radiation, oxidation, mechano-chemical, aging or shear: see

<https://www.wyatt.com/blogs/highlights-recent-publications-in-the-field-of-natural-polymers.html> and

<https://www.wyatt.com/library/application-notes/an1305-irradiation-induced-degradation-of-sodium-alginate-by-sec-mals.html>

Examples specifically related to SEC-MALS monitoring of polysaccharide degradation by enzymatic reactions may be found by searching the peer-reviewed literature at [www.wyatt.com/Bibliography](http://www.wyatt.com/Bibliography), e.g. <https://doi.org/10.1016/j.ijbiomac.2013.06.025> or <https://doi.org/10.1016/j.carbpol.2016.03.087>.