



## Live Webinar Q&A Sheet

### Vaccines, Illuminated: Biophysical Characterization, PAT, and Quality Control via Light Scattering Techniques

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

#### Specific vaccines

*Q: Can MALS be used with UHP-SEC of antigen proteins and whole viruses?*

A: MALS measurements with the [microDAWN®](#) and UHP-SEC are used routinely for a whole host of proteins, including antigen proteins and small viruses. You can find more information in the 2019 eBook, [Advancing HPLC/UHPLC Analysis with Multi-Angle Light Scattering](#).

The pore size for typical SEC and UHP-SEC columns does not provide appropriate separation for larger viruses. For molecules and virus particles with radius >50 nm, we recommend asymmetric field flow fractionation (AF4) combined with detection with the [DAWN multi-angle light scattering detector](#). You can read more about AF4 on [www.wyatt.com/Theory/FFF](http://www.wyatt.com/Theory/FFF) and [www.wyatt.com/FFF-MALS](http://www.wyatt.com/FFF-MALS).

*Q: Can FFF-MALS be used to purify viruses or lipid nanoparticles?*

A: The [Eclipse AF4 field-flow fractionation system](#) provides the option for a semi-preparative channel that can be used for small-scale purification of viruses and lipid nanoparticles. Combining AF4 with a MALS instrument allows for on-line characterization of the size, molar mass, and number of particles in the collected fractions.

*Q: Can FFF-MALS analyze the size distribution and concentration of enveloped viruses?*

A: Yes, FFF-MALS can be used to separate and quantify the size and concentration of enveloped and non-enveloped viruses, lipid nanoparticles, and other bionanoparticles. In the webinar, we showed examples of FFF-MALS characterization of influenza virus, which is an enveloped virus. We referenced validation of this method performed by the CDC in [Bousse et al., J. Virological Methods \(2013\)](#).



*Q: Do you have DLS or MALS data with an mRNA vaccine in liposome or LNP formulation?*

A: Examples of lipid nanoparticle characterization with MALS and DLS can be found in this [white paper](#).

*Q: Are there any data on lentivirus aggregation or characterization that you could share with us?*

A: Examples of lentiviral characterization with FFF-MALS can be found in this on-demand webinar, [Quantifying Viral Vector Attributes with Light Scattering](#).

*Q: Any difference in this approach to DNA vs. RNA viruses?*

A: Measuring molar mass, size, and concentration with light scattering techniques is the same process whether considering DNA or RNA viruses.

### **Regulatory aspects**

*Q: Are results obtained with Wyatt's MALS and DLS instruments validated and recognized for regulatory filings?*

A: Wyatt's MALS and DLS instruments have been submitted as part of regulatory filings. For information about method validation and other specifications, please contact [support@wyatt.com](mailto:support@wyatt.com).

### **Specific analyses**

*Q: How do you measure number-based particle concentration? Which technique you apply? How do you calculate number of particles using DLS or MALS? Can we quantify empty capsids and capsids containing nucleic acid?*

A: In the webinar, we used static light scattering to determine the particle number concentration via the ratio between the measured scattered intensity and the intensity scattered by a single particle. The single-particle scattering intensity is proportional to the square of its volume, which can be determined either by MALS or DLS. With MALS, we can determine the volume of the particle based on the angular dependence of the scattered light. With DLS, we can determine the hydrodynamic volume of the particle. When combined with the static light scattering intensity and the appropriate shape model, these data provide particle concentration directly, without the need for a UV or dRI concentration detector.

For small viruses, such as adeno-associated virus, we may also quantify the capsid content and particle concentration by SEC-MALS combined with UV and dRI detection. You can find out more information in application note [AN1617: Quantifying quality attributes of AAV gene therapy](#) and the webinar, available on demand, [Quantifying Viral Vector Attributes with Light Scattering](#).



*Q: Is Protein Conjugate Analysis required to estimate glycan molar mass? How do you measure the glycan mass from the spike trimer protein? Can you share again the reference of the publication with SARS protein?*

*A:* For glycosylated proteins, ASTRA's [Protein Conjugate Analysis](#) is an ideal way to determine the protein and glycan molar masses of an eluting species. The method measures MALS along with UV absorbance *and* dRI detection. The user must enter the extinction coefficient and  $dn/dc$  values of each component. ASTRA then determines the amount of each component and the total molar mass of the molecule. This technique was utilized to determine the oligomeric state and glycan content for SARS and MERS spike proteins in the publication [Walls et al. Cell \(2019\)](#).

*Q: Please comment on the need for determining different  $dn/dc$  values for different forms of vaccine molecules.*

*A:* The refractive index increment ( $dn/dc$ ) for a vaccine molecule in a particular solvent depends on the chemical composition of the molecule. This value is used both to determine the concentration by differential refractive index (dRI) and as part of the light scattering equation. Inaccurate  $dn/dc$  values, therefore, result in inaccurate molar mass determination by MALS.

For a single component (e.g., pure protein, pure mRNA, or pure polysaccharide), the choice of  $dn/dc$  is relatively straightforward. Two-component systems, such as protein-polysaccharide conjugates or protein viruses filled with DNA, are best characterized by protein conjugate analysis. This means that the user needs to know only the  $dn/dc$  value of each component, and the conjugate composition and overall  $dn/dc$  will be automatically determined based on the UV and dRI signals in a SEC-UV-MALS-RI measurement. For other complex molecules,  $dn/dc$  can be measured by injecting a series of known concentrations into the [Optilab® differential refractive index detector](#).

*Q: Where can one find an overview of the protein conjugate analysis method? Any technical notes you would recommend?*

*A:* The Wyatt website provides several references with additional information, including this [overview](#) and this "[Ask the Expert](#)" article. Wyatt customers can login to the [Support Center](#) to view [TN1006 Performing Protein Conjugate Analysis in ASTRA](#), describing step-by-step analysis with ASTRA.

### Other applications

*Q: Is it possible to analyze lipid nanoparticles (LNP) with SEC-MALS instead of FFF-MALS?*

*A:* Although some LNPs may be separable by SEC, typically, we find that LNP can interact with or be broken up by the SEC column packing. Since FFF does not use a stationary phase to perform the separation, the LNP is less likely to be sheared or degrade.



*Q: Is it possible to determine concentration-dependent oligomerization with MALS?*

A: Yes, composition-gradient multi-angle light scattering (CG-MALS) measures the weight-average molar mass of a solution as a function of concentration or composition. In the webinar, we showed an example of CG-MALS for measuring the reversible association of an antibody and antigen. The same technique can be used to measure the self-association of proteins, such as [insulin](#) and [antibodies](#). For additional information, visit [www.wyatt.com/CG-MALS](http://www.wyatt.com/CG-MALS).

### Process monitoring

*Q: Can process monitoring by RT-MALS be used for the fermentation process? How does turbidity or heterogeneity affect the measurement?*

A: Process monitoring with [real-time MALS](#) may be used to monitor changes in molar mass or size in different processes. The solutions must be clear to avoid multiple scattering effects and provide accurate results. For any process, RT-MALS measures the weight-average molar mass of all the molecules and particles in the solution. Therefore, unreacted monomer, host cell proteins, metabolites and other species present in the solution will impact the apparent molar mass of the product, making this less attractive for upstream processes than for downstream processes. For more information, please contact [info@wyatt.com](mailto:info@wyatt.com).

*Q: During at-line or on-line monitoring, how do you adjust right concentration of samples to be delivered to ultraDAWN detector?*

A: On-line monitoring with real-time MALS makes use of a quaternary HPLC pump to draw product from the process stream and deliver it continuously to the [ultraDAWN™](#) for determination of molar mass, size and particle concentration. The same pump may be programmed to dilute the solution by a pre-determined ratio. Usually this is done to reduce intermolecular interactions, which affect the apparent molecular weight by MALS; to reduce viscosity, for lower backpressure; or in the case of nanoparticles, to reduce turbidity so as not to saturate the detector.

UltraDAWN is not used in at-line monitoring. However, Wyatt's [microDAWN® MALS detector for UHPLC](#) may be coupled to the process via a 3<sup>rd</sup>-party automated sampling system and UHP-SEC such as the Waters PATrol. In that case, the concentration is adjusted by the sampling system or by the quantity injected onto the SEC column.

*Q: In the example of ion-exchange purification of viruses, why is the light scattering signal for viruses so much larger than for proteins and DNA, even though the UV signal is much smaller?*

A: UV absorbance is proportional to the mass concentration of material in the solution. However, light scattering intensity is proportional to the product of molar mass x concentration of molecules and particles. Although the concentration of the virus may be lower, the virus particle size is significantly larger than the proteins, resulting in a higher light scattering intensity.



## SEC-MALS

*Q: What SEC column(s) was used in the vaccine work?*

A: The choice of SEC columns depends on the type of vaccine molecule and its expected molar mass. For protein vaccine products, including small viruses and VLPs, we presented data using [Wyatt SEC columns](#). Separation and characterization of larger viruses, lipid nanoparticles, and other particles may require [field-flow fractionation](#) (FFF) in place of SEC.

*Q: Have the MALS results been compared to results from techniques such as native mass spec?*

A: Like mass spectrometry, MALS provides absolute molar mass, and both can be used in combination with size exclusion chromatography. Where MS can provide molar mass with accuracy <1 g/mol, molar mass measured by MALS carries  $\pm 5\%$  accuracy and  $\pm 2\%$  precision. However, MALS carries several advantages over MS, which can be critical at different stages of development.

- The upper molar mass limit by MALS is  $\sim 10^9$  g/mol, making it suitable for viruses, lipid nanoparticles, and other bionanoparticles, which is outside the range of MS.
- MALS measurements are performed in solution and do not require “MS-friendly” buffers or similar considerations. As such light scattering can be used to study molecules and particles in their formulation buffer.
- MALS can be used to measure reversible and irreversible associations which can be destroyed during MS measurements.
- MALS can also determine the rms radius of a polymer chain.

*Q: Some polysaccharide interacts with SEC column matrix and gives two populations for the same polysaccharide. What can be done in such case to determine the polydispersity?*

A: Polysaccharides, especially branched polysaccharides, can exhibit delayed elution due to pore anchoring and other column interactions. In these cases, we recommend separation with field-flow fractionation, rather than SEC. This allows the entire size and molar mass distribution to be measured with no stationary phase. More information can be found in this [white paper](#).

*Q: What could cause rms radius moments to not be calculated in an ASTRA report?*

A: The report structure in ASTRA is quite customizable; you can include all of the file, configuration, sample, method, and data processing information as well as all results including graphs and moments, per your preference. If the rms radius was calculated in the procedures but the moments do not appear in the report, simply click on the Report Designer button and select the appropriate moments from the Results checklist.



*Q: Which controls (positives) are used for nanoparticles such as VLPs which are smaller than 20 nm?*

A: MALS is an absolute measurement of molar mass and size, and does not require any molecular weight or size standards. The method for determining particle concentration also does not require any virus or VLP standards. Typical SEC-MALS standards, such as BSA, may be run as system suitability standards.

#### FFF-MALS

*Q: How can you be sure that during FFF measurements, particles do not escape through the membrane?*

A: Typical FFF membranes have a molecular weight cutoff between 5 kDa and 30 kDa. This is much smaller than most vaccine particles. In addition, we can recommend several controls and method development tricks to ensure the particles do not interact with the membrane.

#### DLS

*Q: Can DLS measure aggregation inhibition in the presence of surfactants or surfactant like excipients?*

A: Yes, DLS can be used to measure changes in size and size distribution in the presence of many excipients, including surfactants. An example showing the effect of surfactant on drug nanosuspensions can be found in the application note [AN5002: High-Throughput Tools for Optimizing Drug Nanosuspensions](#). Additional examples can be found at [www.wyatt.com/Bibliography](http://www.wyatt.com/Bibliography), such as studies on gold nanoparticle aggregation at <https://doi.org/10.1166/jnn.2020.18121> and for monoclonal antibodies at <https://doi.org/10.1002/jps.23405>.

*Q: For DLS do you recommend looking at %intensity or %number? What is the best way to report the readout?*

A: The regularization analysis of DLS data directly calculates the %intensity from each species and does not require any assumptions of particle conformation or molar mass. Thus, this distribution is typically most reliable. The %number distribution requires knowledge of the molecular conformation and involves multiple assumptions. It can be helpful for determining the most abundant species but is typically not recommended and may contain large errors, especially for particles >200 nm.

*Q: Is there any parameter called Aggregation Index in DLS measurement? We have heard Polydispersity Index but would like to know about aggregation index.*

A: As far as I know, there is no defined parameter called "Aggregation index." However, different aspects of the DLS measurement can inform on the level of aggregation. These include the



average radius (by cumulants), polydispersity index, and regularization size distributions. In addition, dynamic light scattering can be used to measure the propensity to aggregate or reversible self-interactions via the diffusion interaction parameter ( $k_D$ ) and also colloidal stability via aggregation or unfolding temperature.