Live Webinar Q&A Sheet:

Stability Characterization of Vaccines, Gene Vectors and Therapeutic Antibodies with Light Scattering

The recorded webinar may be viewed from the RT-MALS webinars page. Additional information on SEC-MALS, DLS, CG-MALS, and FFF may be found in the Wyatt website Resources section under Webinars, Application Notes, and Bibliography, as well as on the corresponding Product page and Solutions page of our website.

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

RT-MALS Applications

Q: *What are the applications you see for direct bioreactor monitoring with RT-MALS?*

A: Typically, real-time multi-angle light scattering (RT-MALS) using an ultraDAWN™ will not be coupled directly to a bioreactor, because MALS is a species-agnostic measurement. Cell lysate, host cell proteins and other debris will be measured together with the product. For certain products, especially viral vectors, it might be feasible to do a quick purification in-line with the ultraDAWN.

Q: *Is it possible to directly measure unfractionated, protein-based or mAb-based samples, in-line? Are there further concentration limits for these kind of samples?*

A: Yes, it is possible to do so, either in post-process mode (with ASTRA® software) or in real-time mode (with OBSERVER™ software). MALS provides the weight-average molar mass, and for concentrations above a couple of mg/mL, it will usually be necessary to determine the A_2 and A_3 virial coefficients offline in order to obtain accurate values.

This procedure works well when the buffer composition is constant, for example in purification by size-exclusion chromatography or in flow-through ion-exchange or mixed-mode chromatography. It will be more challenging in bind-and-elute chromatography where changing buffer conditions cause the protein-protein interactions, and hence the virial coefficients, to change. We are currently involved in a proof of concept study with BioPhorum's Technology Roadmapping Phorum, Inline Monitoring/Real-time Release Workstream to evaluate a concept for overcoming this challenge in bind-and-elute cation exchange polishing.

Q: *Can RT-MALS accurately measure AAV CQAs (capsid concentration, full:total ratio) in a crude lysate, with concentration around 1x10^{11} capsids/mL, or will the background noise be too high?*

A: Most likely the background noise from cell debris will be too high, and co-elution of AAVs with host cell proteins and nucleic acids will render the results unreliable. RT-MALS is best

implemented downstream of a capture step in order to eliminate most of this background material. Post-capture it can measure AAV concentrations of $1x10^{11}$ capsids/mL. More details on Wyatt's suite of solutions for characterizing AAVs may be found on the AAV Services page.

Q: *We're monitoring a homogenization to graph sizing using this technology. Are dilutions performed by a quaternary pump accurate enough? Current practice is to dilute samples manually.*

A: So far, we have been using Agilent's quaternary pump, which mixes via a proportioning valve. If one solution is viscous and the other is not (a typical situation for polysaccharides or other polymers) then the dilution ratio may not be sufficiently accurate in such a device. It would be interesting to explore the use of a binary pump, which incorporates two independent pump heads rather than a proportioning valve, and therefore can be expected to provide more accurate mixing when the viscosities are disparate.

In addition to mixing ratio, mixing efficiency may not be good enough, and in that case, it would be beneficial to incorporate a small dynamic mixer downstream of the pump.

Q: *Can real-time monitoring of chromatographic elution differentiate between reversible and irreversible aggregation?*

A: In a single real-time chromatographic purification run it is generally impossible to differentiate reversible and irreversible aggregates, because you cannot tell if a change in apparent molar mass is due to changing admixtures of, say, monomer and dimer, or dissociation of dimers due to changing buffer conditions or sample concentration. The way to differentiate between reversible and irreversible aggregation is by running the same method at different starting concentrations.

With that said, you can sometimes get a general indication of dynamic equilibrium if you see a correlation between protein concentration and apparent molar mass across an eluting peak: on both sides of the peak, decreasing concentration leads to dissociation and a corresponding decrease in weight-average molar mass.

Q: *We've developed bind-and-elute chromatographic purification connected to SEC-MALS. It's on-line, but feasible, on an ÄKTA FPLC system.*

A: This is certainly feasible, but would not really be a real-time measurement since even UHP-SEC-MALS measurements take at least 5 minutes per run.

MALS General

Q: *What is the upper limit of protein concentration that MALS can handle without dilution?*

A: Wyatt's MALS instruments can "handle" any protein concentration, in the sense that measurements can almost always be made. Two general caveats are: 1) the solution viscosity

does not preclude appropriate fluidic behavior and 2) the laser intensity may need to be reduced to prevent detector saturation. Both of those behaviors are sample-specific.

However, correct interpretation of MALS data at high concentration may not be straightforward. Protein-protein interactions may lead to a non-linear relationship between scattered intensity, concentration, and molar mass. The concentration range at which those interactions begin to impact the analysis is dependent on the specific protein, but a general rule of thumb would be concentration in the flow cell of 1 – 2 mg/mL.

Q: *How good is MALS for measuring the particle distributions as compared to other techniques?*

A: MALS on its own cannot determine particle size distributions, since it just measures the average size of the particles present in the flow cell at each instant in time. That is why MALS is usually coupled with a separation method to determine size distributions. Size distributions by SEC-MALS or FFF-MALS generally provide more detail and better resolution than non-separated methods like dynamic light scattering (DLS) or nanoparticle tracking analysis (NTA), and since additional detectors like UV or fluorescence can be added online, they provide richer data regarding each particle size than batch (unfractionated) methods.

The answer is different for AAVs than for larger particles. While AAV concentration can be measured in the manner described above, because of its small size, it is better to determine concentration with a UV or dRI detector. These give the mass concentration (mg/mL) which is converted to particle concentration (particles/mL) via the molar mass of the AAV.

Q: *How does MALS distinguish empty virus particle from full virus particle?*

A: Analysis of viral gene vectors requires information from additional detection modes such as UV, dRI or fluorescence. Combining two concentration signals that have distinct responses from the empty virus and the payload gives the required data to discriminate empty and full particles.

Q: *How can one measure particle concentration by MALS or MALS/UV without a standard or a reference?*

A: The beauty of MALS is that it is an absolute method and does not require reference standards. Characterization of particle concentration derives from the basic physical relationship between particle size, refractive index, particle concentration and light scattering intensity which was shown in the presentation. Since MALS also measures particle size, all you need is the refractive indices of the solvent and the particle's constituent material, such as proteins or lipids. The only calibration in MALS is an absolute standard, toluene, which provides the relationship between photodiode voltage and Rayleigh ratio (essentially the ratio between incident and scattered light, with some other system parameters).

RT-MALS Technical

Q: *What kind of quaternary pump was used for online sample dilution? Can any pump be integrated or is it a complete solution sold by Wyatt?*

A: Wyatt's RT-MALS system utilizes an Agilent 1260 infinity II quaternary pump, which is directly controlled by OBSERVER software. Other pumps can be used, but they are not directly controlled by OBSERVER.

Q: *What are the minimum and maximum capillary tube sizes for RT-MALS?*

A: We provide four sets of internal capillary tubes for the ultraDAWN, with internal diameters (i.d.) of 0.010" (0.25 mm), 0.020" (0.5 mm), 0.030" (0.75 mm) and 0.040" (1.0 mm). The 0.010" i.d. tubing set is primarily for use with the Agilent pump in the on-line configuration, while the other sizes were selected to match typical tubing used in FPLC for in-line applications. However, these sizes do not limit the external tubing i.d., which may be larger than the internal tubing in order to reduce overall system backpressure.

Q: *How do you clean and validate cleanness of flow path inside the RT-MALS detector?*

A: The ultraDAWN can be cleaned with commonly used cleaning solutions such as 1 M NaOH, 10% HNO_3 or 30% ethanol. The only wetted materials are 316 stainless steel, fused silica and Kalrez. Wyatt does not provide a protocol for cleaning validation, but when the instrument is operated in-line (or on-line with measured solutions returned to the process) then it is an integral part of the system, and its cleanliness is validated with the entire process unit. If the ultraDAWN is operated on-line and the measured solution is discarded to waste, cleanliness validation is usually not so critical.

Q: *Are any hands-on adjustments necessary during real-time operating?*

A: No adjustments are needed, but user input may be required to start and stop data collection. For example, the workflows for on-line monitoring of molar mass or particle size assume that the user will open a valve in order to begin sampling solution with the auxiliary pump, and after doing so the user must click in the software to notify that the system is ready to begin sampling.

Q: *Can OBSERVER interact also with Agilent or Shimadzu HPLC?*

A: OBSERVER can control an Agilent quaternary pump. However, at this time it does not interact with other Agilent modules or with Shimadzu HPLC modules. In the future we will consider connecting to an Agilent UV detector in order to obtain digital data, as we do in ASTRA and VISION software.

Q: *What is the UV detection limit?*

A: Wyatt does not provide the UV detector. It acquires analog data from a 3rd-party UV detector such as an ÄKTA UV module or FlowVPE. The detection limit is specific to each detector and flow cell path length.