



Live Webinar Q&A Sheet:

Real-time process analytics and control for vaccine nanoparticles and macromolecules

The recorded webinar may be viewed from the [RT-MALS](#) webinars page. These questions were submitted by live viewers. Additional information on SEC-MALS, DLS, CG-MALS, RT-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 with any additional questions.

Viruses and other nanoparticles

Q: I would be interested in using RT-MALS to measure virion concentration, but I don't know the refractive index of the virion particle. How can it be measured or calculated?

A: There are values in the literature for refractive index of influenza virus and others, roughly $n_{\text{virion}} = 1.50$. If you know the relative content of proteins, nucleic acid and lipids you could even calculate the virion refractive index, since it is just the weight-average of the refractive indices of those components, each of which is well known. Alternatively, you could use a well-purified sample, calibrate the concentration using an offline technique such as electron microscopy, nanoparticle tracking analysis or TRPS, and adjust the refractive index to get the same value, then use that value going forward.

Q: Are some of the methods that we will be discussing today also applicable for Adeno Associated Vector (AAV) manufacturing for gene therapies?

A: Yes, though a solid application might require some exploratory work as to conditions. For MALS, AAVs are at the border between macromolecules and nanoparticles, so depending on conditions it might be appropriate to choose the Macromolecules workflow (molar mass and size) or Nanoparticles (size and particle concentration).

Q: Should we also measure B_{22} offline for nanoparticles systems to correct the MW?

A: For nanoparticles, RT-MALS does not usually measure molecular weight – it measures size. Even if one would wish to measure MW, the concentrations of nanoparticles that can be measured generally are much lower than the concentrations where virial coefficients are needed (above about 1-2 mg/mL). An exception might be highly concentrated, small particles with radii in the range of 10-15 nm where it might be possible to reach concentrations of a few mg/mL or higher without reaching turbid conditions.



Q: What is limits of detection for viral load?

A: This depends on size. For typical ~ 100 nm diameter viruses, the lower limit is $10^6 - 10^7$ particles/mL and the upper limit $10^{10} - 10^{11}$ particles /mL. For smaller viruses such as AAVs, the lower limit is about 10^{11} particles/mL and the upper limit 10^{15} particles/mL.

Q: If one is working with polydisperse populations with particles with a mean size in the range of 150-200 nm but has some particles > 250 nm, will this cause problems with function and/or interpretation of UltraDAWN data?

A: It certainly will not cause malfunction of the ultraDAWN, but the degree of misinterpretation depends on how many and how big... The average size reported by MALS is the 'z-average', which means it is weighted by the square of the molar mass, in other words the 6th power of the radius. So if you have 99.9% 150 nm particles and 0.1% 300 nm particles, the z-average is 159 nm and the result is in error by 6%. On the other hand, if the main population is 99.9% 200 nm particles then the z-average is 201 nm, off by just 0.5%.

Q: In case of measuring virus titer in an upstream process, which kind of smart at line purification methods do you suggest / are you investigating?

A: We are certainly not experts in purification methods and don't have much experience in upstream process monitoring (so far we have only seen downstream applications, which are a better fit for ultraDAWN). However, I would recommend looking into various available methods available such as small mixed-mode columns that are specific to viruses, or 2D-LC. I would be less concerned about removing small molecules such as metabolites and small proteins, but the main thing is to remove large lysate chunks (e.g. by coarse filtration) and the larger proteins and nucleic acids.

Proteins and polymers

Q: Can RT-MALS determine the degree of aggregation in a monoclonal antibody purification process?

A: Roughly yes, under certain conditions: constant concentration and constant buffer, which are typical of certain flow-through chromatographic purification processes. Since RT-MALS determines a weight-average molar mass you would then assume that the value obtained corresponds to monomers and dimers, and figure out the fraction of dimers that gives this value. The assumption of monomers and dimers is a worst case, in that if higher oligomers are present, their concentration would be lower than the calculated dimer concentration.

In the future we expect to be incorporate additional algorithms that will allow for relaxation of the constant buffer and constant concentration constraints.



Q: How does the ultraDAWN-OBSERVER system know the concentration for molar mass determination? Can it connect to a UV or refractive index concentration detector?

A: At this time, it does not use an additional concentration detector, you simply type in the concentration which must be constant throughout the run. In the future we expect to be able to incorporate online concentration measurements with an appropriate detector, e.g. UV for proteins or dRI for polymers.

RT-MALS general

Q: Why does the buffer have to be constant for molar mass measurements?

A: Production processes typically take place at concentrations of several mg/mL and above, where intermolecular interactions affect molar mass measurements. Those interactions depend on concentration and on the buffer.

The effect of interactions on light scattering as a function of concentration is typically quantified in terms of the virial expansion, using the so-called virial coefficients. Currently OBSERVER software lets you enter virial coefficients (which are usually measured offline) for only one buffer. Stay tuned, we expect to enhance the algorithm to allow for continuously changing buffer as well.

Q: Does molar mass measurement depend on the shape or conformation of the molecule?

A: Not when measured by MALS. Unlike dynamic light scattering, which measures hydrodynamic size and must assume a shape or conformation in order to estimate molar mass, multi-angle light scattering measures molar mass independently of the shape or conformation.

Q: Can you explain how the flow rates affect DLS measurements? What is the flow range for the RT-MALS? Are there plans to incorporate QELS or other DLS approaches into UltraDAWN?

A: Dynamic light scattering measures particle motion over a finite time period, so adding a flow component – especially at the high flow rates typical of production processes - certainly will skew the results. On the other hand, multi-angle light scattering, which is a form of static light scattering, is essentially an instantaneous measurement, so RT-MALS does not care about flow rate except to the extent that the flow can physically pass through the instruments without excess back pressure.

The current formal specification for the ultraDAWN is 5 mL/min, but actually this can be increased with some minor modifications to the instrument, up to perhaps 50 mL/min. We are working on models that will allow for higher flow rates as well. Because it is not as suitable for systems with high flow rates (i.e., high linear velocity) we do not have plans at this time to incorporate DLS into ultraDAWN.



Q: Can you comment on how to obtain virial coefficients for macromolecules in solution?

A: This is done by measuring the scattered light intensities for a series of known concentrations, and fitting the result to a simple equation. The analysis is taken care of by our software, and you can even use the ultraDAWN to do this.

UltraDAWN

Q: How can the ultraDAWN be sterilized? Does it have a single-use option?

A: The current ultraDAWN does not have a single-use option. It can be sterilized using 10% nitric acid, organic solvents or hot water (the system can accommodate incoming fluid temperatures up to 150 °C). While the current system is most appropriate for process development as well as lab and pilot scale, we are planning a single-use option for a production-scale unit.

Q: How does the ultraDAWN communicate with an AKTA FPLC to begin and end fraction collection?

A: Using the AKTA I/O Box, which has analog and digital inputs that are read by Unicorn, the AKTA software. We connect the trigger output from the ultraDAWN to a corresponding input line on the I/O Box and program Unicorn to read the trigger and act accordingly.

Q: Is the dynamic range of the LS signal the same as a DAWN MALS instrument?

A: Yes: 3,300,000:1, and if you include the possibility of decreasing the laser power to 10%, 33,000,000:1.

Q: For molar mass determination, how is the dn/dc quantity is measured by the equipment?

A: UltraDAWN does not measure dn/dc , for that you need a separate instrument, the Optilab differential refractometer. You run a series of known analyte concentrations through the Optilab, and ASTRA software fits the data to a linear regression to calculate dn/dc .

Q: What is the particle size resolution?

A: UltraDAWN measures an average size, so it is somewhat incorrect to specify a resolution. However, the precision and repeatability are in the range of 1-2%, so if the sample quality is good you should be able to see changes in size of that amount.