



## Live Webinar Q&A Sheet:

### Real-Time MALS: A Breakthrough in Process Analytical Technology for Nanoparticles, Biopharmaceuticals and Polymers

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

#### RT-MALS general

*Q: What is the greatest advantage of using the [ultraDAWN™](#) and [real-time MALS](#)?*

A: The primary benefit of RT-MALS is the ability, as a PAT tool, to measure critical quality attributes during process development and in process.

*Q: What are the limitations of real-time MALS?*

A: The current implementation of RT-MALS is subject to two main limitations: flow rate and the requirement for constant concentration and buffer during molar mass monitoring (though not for size monitoring). Both of those can be extended or overcome with additional development.

*Q: Can we obtain the analyte concentration if we connect an Optilab refractive index detector in line with RT-MALS?*

A: Even if the concentration is measured along with MALS, for example using a dRI or UV detector, there are complications in the analysis related to concentration-dependent and buffer-dependent self-interaction effects ( $A_2$ ,  $A_3$ , etc.) and changing  $dn/dc$ . The current release of OBSERVER does not support this analysis, but it is not ruled out for future releases.

*Q: What is the resolution and recovery of the system?*

A: Repeatability: 2%; accuracy: 5%. In the in-line configuration, product is not lost and the recovery is 100%. In the on-line configuration, product is usually directed to waste, though except for dilution, the measurement is non-destructive and in a non-GMP situation the material could be recovered. The total amount directed to waste is usually quite minimal, and is set by the flow rate out of the reactor.



Q: *Can we see multimodality in  $M_w$  or  $R_g$ ?*

A: Since we are not doing a separation, RT-MALS measures the weight-averaged molecular weight and z-averaged radius. So, multimodality cannot be seen.

Q: *How is the ultraDAWN validated across its detection range?*

A: We validate the system with samples covering a range of molecular weights and sizes. Moreover, the optical architecture and data acquisition interface of ultraDAWN are very similar to those of the DAWN<sup>®</sup>, Wyatt's flagship MALS instrument.

## Applications

Q: *Has this been tested with a gene therapy vector to determine its size or aggregation at upstream stages in a bioreactor, or at BDS/DP stage?*

A: The ultraDAWN is a new product and at the time of the webinar has not yet been used for gene vector production, but this certainly would be a relevant application for RT-MALS. Since light scattering sees everything in the solution, clarification of the solution would be required for upstream monitoring.

Q: *Do you have any particular example for protein manufacturing?*

Q: *Can your technology be used online with process chromatography? I am particularly interested in flow-through aggregate removal of monoclonal antibodies.*

A: RT-MALS can, potentially, be used for certain types of process chromatography. A proof-of-principle for monitoring bind-and-elute purification, performed in collaboration with Merck, was published in the journal *mAbs*. Please see <https://doi.org/10.1080/19420862.2018.1505178>.

In general, this is not a plug-and-play installation; a preliminary evaluation is required to understand the limitations and capabilities.

Q: *Does a MALS detector degrade proteins?*

A: No. MALS is a passive technology that does not interact with or degrade the analyte. The illumination wavelength, 660 nm, is harmless.

Q: *Can we monitor the size of particles in the micrometer range, for example cells?*

A: No, the ultraDAWN only covers a size range of 10 to 250 nm.

Q: *Is it possible to measure the size of non-water-soluble nanoparticles?*

A: Yes, ultraDAWN can be used with both aqueous and organic solvents.



*Q: Is  $dn/dc$  constant during the growth of nanoparticle?*

A: That was our assumption, since the growth involved accumulation of the same type of material.

*Q: Do you think that MALS is a useful technique to monitor the growth of magnetic nanoparticles?*

A: No, dynamic light scattering is more appropriate for most magnetic nanoparticles which are metallic or semi-metallic and poor candidates for MALS. Standard DLS instruments such as the [DynaPro® NanoStar®](#) maybe be used for cuvette-scale measurements. In a large-scale production environment, a flow-through DLS detector such as the [Mobius™](#) can be set up for on-line monitoring.

### **RT-MALS measurement conditions**

*Q: You mentioned that the maximum flow rate is only 5 mL/min. Can higher flow rates be achieved?*

A: This is the maximum flow rate for a 1 cP solution; more viscous solutions are limited to proportionally lower flow rates—the product of flow rate and viscosity is limited to 5 cP · mL/min.

The flow rate of the system will be limited primarily by the back pressure experienced by the ultraDAWN, which in turn depends on the viscosity of the solution as well as flow rate. We are exploring the ramifications on system performance of relieving backpressure by increasing the tubing diameter.

*Q: Our polysaccharide starts out being very viscous. Are there any limits to the viscosity of the sample?*

*Q: What is the upper limit for solution viscosity and temperature before dilution?*

A: When configured for on-line operation, the maximum viscosity is 30 cP. This is a consequence of the requirement for high-efficiency in-line dilution, and accuracy of the dilution ratio (which affects accuracy of the molar mass result, but not of the size result).

Viscous polymer solutions pulled from a reactor or homogenizer are diluted on the way to the ultraDAWN by means of a quaternary HPLC pump. This both reduces viscosity and the self-interactions that must be understood and quantified as virial coefficients in order to report accurate molar mass.

The solution temperature entering the ultraDAWN should not deviate by more than a few degrees from the temperature at which the instrument was calibrated, otherwise loss of accuracy will result.



Q: *How important is keeping the concentration constant across the run?*

Q: *Which parameters need to be constant or stable to get reliable readouts of the MALS?*

A: The workflow for monitoring molar mass in the current version of OBSERVER assumes constant concentration as well as constant solvent or buffer conditions. Since molecular weight is inversely proportional to the concentration, 1% variation to the concentration entered in OBSERVER results in 1% error in molecular weight. Changes in ionic strength or pH will affect higher-order concentration-dependent corrections such as  $A_2$ . Changes to the solvent may also result in changes to  $dn/dc$ , which impact the reported  $M_w$  as well.

Note that this requirement does not apply to size monitoring, since the analysis of  $R_g$  by light scattering is only sensitive to concentration to higher order.

Maintaining a constant flow rate is also important, though secondary in nature, because excess backpressure fluctuations may impact instrument performance.

Q: *What is the typical delay time of the measurement?*

A: Delay time is defined as the time it takes for the solution to travel from the reactor or process chamber to the ultraDAWN flow cell. Delay time depends on the tubing length and diameter, and on flow rate. It can be as little as few seconds when the ultraDAWN is configured in-line, 30 seconds to a couple of minutes if it is configured on-line without dilution, and up to several minutes if the on-line configuration involves dilution by the quaternary pump.

Q: *Is there a limitation of turbidity of the product to be tested? Does it need to be a clear liquid?*

A: Yes, solution needs to be clear in the detection volume in the ultraDAWN. If dilution alleviates the product's turbidity, it can be diluted with the online pump before entering the ultraDAWN.

Q: *Is there a filter between the reactor and the MALS?*

A: Since concentrations during production are usually pretty high, a filter is not always needed, but in some applications an inline filter upstream of the ultraDAWN is required to mitigate the spikes in the light scattering data from the particulate matter in the reactor. The answer really depends on the specifics of the system.

Q: *If connected to field-flow fractionation, what impact will a change in flow-rate change (e.g. using a cross-flow gradient) have on RT-MALS?*

A: FFF is generally an analytical technique and not a production technology, though it can be used for semi-preparative work. In order to identify the fractions collected in semi-prep mode, our recommendation is to use standard FFF-MALS with post-processing rather than real-time MALS.



## OBSERVER software

*Q: Can OBSERVER™ software be used to determine the 2<sup>nd</sup> and 3<sup>rd</sup> virial coefficients?*

A: No, those must be determined offline using one of the analytical software packages. ASTRA® can determine 2<sup>nd</sup> virial coefficient, and CALYPSO® can determine both 2<sup>nd</sup> and 3<sup>rd</sup> virial coefficients. In order for OBSERVER to measure molecular weight in real time, the interaction terms such as 2<sup>nd</sup> virial coefficient must be entered as input parameters.

*Q: Does OBSERVER software come with a 21CFR11 compliance option?*

A: No, but 21CFR11 compliance can be achieved by integrating the system with standard 21CFR11-compliant production software like Delta V or SIPAT. We provide IQ, OQ and PQ for the OBSERVER software.

*Q: Does the vertical green line in the software indicate complete depolymerization or just initiation?*

A: The green vertical line indicates a trigger event, which is configured by the user as desired to represent passing a threshold in molar mass or size. Usually it means the end or completion of the process in a workflow.