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Live Webinar Q&A Sheet:

In-Line and On-Line Monitoring of CQAs for Biologics, Vaccines and Gene Vector

The recorded webinar may be viewed from the RT-MALS webinars page. These questions were submitted by live viewers. Additional information on SEC-MALS and RT-MALS 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.

Specific applications

Q: *Is MALS suitable for adenovirus (AdV) titer determination?*

A: Absolutely. Examples of AdV size and titer determination using real-time multi-angle light scattering (RT-MALS), both in ion-exchange chromatography and in UF/DF, were shared in the presentation and the measurements were shown to agree well with offline measurements.

Q: *Can RT-MALS be used to monitor polymerization of PLGA or other polymers?*

A: Generally, yes, we would use the auxiliary pump to pull the solution at a steady rate from the polymerization reactor and deliver it to the ultraDAWN™. If the polymer concentration is high, we would recommend using the pump's dilution capability for reliable measurements. If the concentration isn't high, you can both measure and recirculate the material because the technique is non-destructive.

Q: *If you wanted to monitor in real time a reaction in non-aqueous solvent (and assuming LS intensity is high from solvent), is there a way to do a background subtraction for RT-MALS analysis?*

A: Background, or baseline, subtraction is something that is always done in RT-MALS analysis. Since it's actually measured prior to the real-time process measurements, it's important that the background scattering is constant – the solvent scattering properties should not be changing during the process run.

Q: *We used a DAWN MALS instrument to monitor VLPs, with a quad pump recirculating the sample at 10 mL/min flow rate. Even at 10% laser power, the first 9 detectors were saturated. My question is, can we reduce the gain any further or should we switch to an ultraDAWN?*

A: It is not recommended to reduce the DAWN™ or ultraDAWN laser power below 10% because the laser might become unstable. An ultraDAWN would overcome the saturation issue since it accepts 100x attenuators in front

of the photodiodes. With these attenuators it is literally impossible to saturate the detectors, so it would provide the measurements you need.

Q: *Can you give an example of how to perform a size calculation for the radius of a lipid nanoparticle?*

A: The only parameter needed by OBSERVER™ RT-MALS software for calculating the size of a lipid nanoparticle or similar is the refractive index of the buffer, which you can usually find in the literature for different levels of excipients like sucrose or salts. The operation itself is straightforward: the particles flow into the instrument, their scattering properties are measured and OBSERVER software immediately produces a size. With the addition of the refractive index of the particle material (roughly 1.46 for LNP) you also get the particle concentration. For heterogeneous particles the result is an average size and concentration.

Upstream application questions

Q: *Can MALS be used upstream to determine transfection efficiency for AAVs?*

A: If you can clean up and remove the lysate you could feed that into an analytical system like SEC-MALS to see the transfection efficiency. Please note that MALS does not calculate payload (full capsid ratio) for larger viruses like lentivirus or, for that matter, for cells, only for smaller viruses like AAV.

Most upstream processes occur over long time scales of hours to days, so it's better to go with at-line analytics that provide more detailed information than with RT-MALS. RT-MALS provides fast responses of seconds to tens of seconds, albeit with far less detail.

Q: *Could RT-MALS be used for in-line monitoring of a fermentation process expressing a recombinant protein to identify bacterial contaminants?*

A: MALS provides a rather non-specific type of measurement, which here would be the particle size. Obviously bacteria are much larger than proteins and MALS would discriminate between them, but would also pick up fermentation cells if they were to enter the instrument.

Q: *For mAb purification, is it possible to incorporate MALS to measure titer of cell harvest supernatant?*

A: MALS is not an appropriate means for measuring protein titer, and definitely not suitable for any type of analysis of cell harvest supernatant due to the presence of large lysates.

RT-MALS capabilities

Q: *What are the limits for particle size and concentration in RT-MALS?*

A: The nominal size measurement range for the ultraDAWN is 10 – 250 nm in radius, though actually we see good measurements of smaller sizes when the concentration is not too low, and can also measure larger sizes. The concentration range depends on particle size and type. For example, AAV can be analyzed from the 10^{10} Vp/mL to the 10^{16} Vp/mL range; the lower limit is actually dependent on the sensitivity of the UV detector, not the ultraDAWN, so if your UV detector sees the AAV, so will the ultraDAWN. For 100 nm LNPs it would be in the vicinity of 10^{7} – 10^{13} LNP/mL.

Since turbidity is a limitation, there is a rule of thumb for estimating whether or not the ultraDAWN can handle the turbidity level: aliquot some of your solution in a 1-inch vial, and if it's clear or translucent, it can be measured without dilution. If it's opaque, you will need to dilute it - the online configuration supports up to 20x continuous dilution.

Q: *Can we integrate this PAT tool for DSP with any other chromatography system than Cytiva? What about other types of unit operations?*

A: In principle you can use systems other than Cytiva (chromatography or otherwise), they just need to provide the same type of analog and digital pulse inputs and outputs in order to achieve the same level of integration:

- Digital pulse output to the ultraDAWN to synchronize data collection. If this is not available then synchronization between the RT-MALS and chromatography systems would have to be done manually.
- Analog outputs of the UV signal for calculating protein or nucleic acid MW (one UV wavelength) or AAV payload etc. (two UV wavelengths). UV is not needed for nanoparticle analysis.
- Analog inputs to receive a trigger signal and a signal proportional to the measured value back into the chromatography system. This is very convenient and nice to have, especially for automated process control, but not necessary for process monitoring.

The other option is digital integration, where OBSERVER is controlled by an external PAT or system control application via OPC-UA communications. In this case control and data transfer are all done by an OPC-UA client that users prepare for their specific needs (we provide all support required for interacting with OBSERVER).

Q: *If dilution is required, how is the dilution performed and controlled?*

A: Dilution may be desirable when the solution is highly turbid (high particle concentration) or when macromolecules are present at high concentration and/or in a low ionic strength solution, leading to strong intermolecular interactions that affect light scattering properties. Dilution is available in the online PAT configuration, where a binary pump is used to both draw the product slipstream and to dilute prior to detection. The pump is controlled from within the OBSERVER RT-MALS application.

Q: *Can we connect RT-MALS with single use flow kits?*

A: ultraDAWN does not support single-use flow cells, but we are thinking about it for future RT-MALS products. In terms of connecting to other single-use instruments, you would just need an appropriate intermediate adaptor:

- Transition to and from 10-32 cone fittings for inline operation
- A tee or Y-connector splitting off to 1/16” capillary tubing for online configuration

Q: *How can we differentiate impurities from biological molecules differentiated with MALS*?

A: Generally impurities are differentiated from the molecules of interest by differing biophysical properties such as molar mass or size. MALS is particularly adept at identifying aggregates or foreign particulates. Note that co-presence of such impurities with product is found by the change in solution molecular weight.

Q: *Biological macromolecules are usually viscous and frothy, how does we overcome this using incline MALS*

A: Since RT-MALS is primarily for downstream processes, this is usually not a problem. In fill-finish operations that involve reformulating or blending drug substance, the solution might initially be frothy but it will settle down, so it’s best to begin drawing solution after bubbles are no longer present. If necessary a degasser can be incorporated for online RT-MALS.

General

Q: *Can we connect ultraDAWN to typical UPLC/HPLC equipment? If so, are the scan rates compatible with UPLC flow rates?*

A: UltraDAWN is used with process equipment so it isn't the ideal instrument for analytical UPLC or HPLC. The DAWN™ and microDAWN™ provide similar MALS measurements (except for real-time response) and are appropriate for analytical HPLC and UPLC, respectively. However, if need be, ultraDAWN can do double duty to work with standard HPLC (but not UPLC) equipment.

Q: *When would you need a standard curve for RT-MALS?*

A: For most applications you don't need a standard curve. The calculations are based on first principles equations that make use of fundamental material parameters such as the refractive indices of solvent and particle, which you can obtain from the literature or measure using common lab equipment.

With that said, there are some instances where you won't have sufficient information for a full absolute calculation, like protein-polysaccharide conjugation processes where there will be an indeterminate mixture of free and conjugated molecules with different degrees of conjugation. For such cases you might want a standard curve to relate the apparent molecular weight (determined by MALS) to the desired end condition where you would quench the conjugation process, or more simply just determine the appropriate apparent MW value that corresponds to the endpoint by comparison with offline testing

Q: *How do you prevent the RT-MALS flow cell from becoming 'dirty' or clogged? Is there a recommended purification step before which RT-MALS should not be used and how is the instrument cleaned and sterilized?*

A: MALS flow cells become dirty when particulates like lysates adhere to the glass. The optimal utilization of ultraDAWN is for downstream processes, post clarification, or possibly following an affinity step, where this is not usually a concern. Typically sterilization is performed along with your system – for example if it's an FPLC, the sodium hydroxide (NaOH) solution would clean the ultraDAWN as well. For inline operation, the ultraDAWN is generally set up with tubing and flow cell bore diameter similar to or larger than that of the process tubing, so if the process does not clog then neither will the ultraDAWN.

If working with the online configuration, you have alternative options for cleaning and sterilization solution, since the binary or quaternary pump used can provide organic solvents, detergent or nitric acid as well as NaOH. You also have the option of adding an inline filter to remove particulates and so prevent flow-cell contamination. The filter pore size can be selected to minimize filter clogging and prevent flow cell clogging, but the membrane may have to be changed periodically depending on the particle load. It's possible to set up two parallel filters with a selection valve between them, so you can continue measuring the process with one filter while changing the membrane in the other.

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