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Live Webinar Q&A Sheet:

Determination of critical production parameters of proteins from research to production

The recorded webinar may be viewed from the Biotherapeutics webinars page. These questions were submitted by live viewers. Additional information on SEC-MALS, DLS, FFF, CG-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.

Questions & Answers

MALS QUESTIONS

Q: *Can you use any GPC for a GPC-MALS study?*

A: The Wyatt MALS detector is compatible with a variety of different GPC systems. We recommend a Waters GPC for the tightest integration where one software can control the HPLC system and the MALS. Alternative configurations would use an analogue connection and would require two software packages. One to run the HPLC and the other for data analysis and MALS control.

Q: *How complicated is it to implement SEC-MALS?*

A: It depends on your reference point. SEC-MALS is not complicated compared to classical SEC with an optical detector. You can think of the instrument as an advanced detector. The analysis is straightforward for most samples. You would set the baseline and some sample specific parameters and after that the software provides all the MALS information like molecular weight and size/radius of gyration (R_g) if applicable.

Q: *Can I use an RI detector for AEX-MALS?*

A: In principle you can. However, RI detectors are sensitive to salt gradients and the RI signal will fluctuate, so we suggest against it. For those users that insist upon it we recommend a baseline subtraction to try to correct for the effects of the gradient.

Q: *Can I use organic solvents in MALS?*

A: Yes, you can use a variety of organic solvents for MALS.

Q: *For SEC-MALS applications, usually there is a column peak which corresponds to the pressure change within the column due to the physical injection, sometimes this column peak interferes with the peak of the molecules of*

interest. How do you minimize this peak and maximise the signal to noise ratio to ensure accurate calculation of molecular weight in this case?

A: In a normal SEC-MALS run you start by setting the flow to a desired rate before starting the injections. This minimizes overall pressure changes during the run. Pressure changes occur normally during injection which does not interfere with the sample peak or if you have large contaminants that stick to column and lead to increased pressure over time. Under normal circumstances UV and light scattering detectors are not affected by these pressure changes. The shape of the peak might change but this does not affect separation quality that much if a column suitable for the respective application is used. MALS analysis is anyway independent of elution times and peak shapes as molar mass is determined absolute at every timepoint in the chromatogram. The RI detector on the other hand is more successive to changes in pressure. Pressure fluctuation typically leads to more background noise but will not influence results much if you use reasonable amounts of your protein for the analysis.

Q: Is there an upper limit for Protein concentration in RT-MALS?

A: This is complicated, when the protein concentration is higher, there will likely be an interaction phenomenon. The second virial coefficient (A2) can be used to correct for this additional event. This is important because during RT-MALS for bioprocess samples the concentrations are typically high, in the 10s of mg/mL and the concentration changes over the course of the collection. You must correct for these interactions which will also be different in different elution buffers. You can't just use a single value; you might need multiple values at the different conditions or focus on a specific part of your purification process. We recommend using DLS for screening for the A_2 values.

FFF QUESTIONS

Q: How often do you need to change the FFF membrane?

A: This is sample dependent, for most samples the membrane will last for 50-100 injections, but this depends on "stickiness' of the sample.

Q: What type of sample processing required prior to FFF-MALS

A: In principle it is the same as for SEC-MALS. For an unknown sample, we usually do a first check in DLS and based on that information and the average molecular weight you can create a method for FFF and then optimize.

DLS QUESTIONS

Q: What is the typical analysis time for the DLS plate reader instrument? And what is the cost per sample?

A: The measurement time per well is ~30sec on average. The 96 well plate can be completed in ~1hr and the 384 well plate would take 2-3hrs. The well-plates compatible with our system are vendor agnostic and are usually 5-20 Euros per plate.

Q: *What are the differences for T_m using DLS against DSC? Which one is more recommended to determine the protein's stability in the long run?*

A: There are many ways to measure T_m, even outside of DLS or DSC, for example, you could also try CD. The important thing to understand is what you are directly measuring with each technique and what other parameters you get with them. DLS delivers two critical readouts: size and molar mass. With this the aggregation is easily measured. Determining the Tm is trickier because you are looking at smaller difference in the sizes. But I would challenge the value of solely relying on Tm for stability, it is a part of a larger number of stability indicators. The colloidal stability parameters which are the second viral coefficient A_2 and the diffusion interaction parameter K_d are in general much more reliable when it comes to long term stability of a protein formulation.

Q: *Why were some columns excluded in 96-well plate?*

A: No reason to exclude any columns of a well-plate. You can work with the plates how you want.

Q: *For samples where we have multiple aggregation sizes, like in the process of amyloid fibers aggregation, would DLS be able to distinguish or not between the different species?*

A: For a set of different aggregation sizes, DLS will struggle because DLS measures on unseparated sample. If the size difference is small for the aggregates, it will look like one broad aggregate peak. Normally a size difference of at least 3-5 times is needed in DLS to distinguish different species. Nonetheless, DLS can be helpful for comparing different samples as you will get a landscape that can be used for comparison, or you use it an initial screening tool before following up with FFF-MALS for in-depth characterization. An example where this has been fully executed is for prions.

Q: *Can DLS detector measure K_d of protein-protein interactions?*

A: First, we should define what K_d is. There is K_d, the dissociation constant, and k_d, the diffusion interaction parameter. DLS can't distinguish slight changes in the radius due to protein-protein interaction at different concentrations – it potentially could be used to screen for binding partners, but this requires a strong change in average radius between the single components and the complex. For association reactions where you want to report a quantitative value we recommend composition gradient-MALS (CG-MALS), CALYPSO, that injects different concentrations of ligand/protein into a MALS detector. CG-MALS doesn't use separation and can measure molar masses to see if a complex is formed and then use that to determine the K_D.