



## Live Webinar Q&A Sheet:

### Characterizing Polymers and Polymer Aggregates Using SEC-MALS and Batch-Mode MALS

The recorded webinar may be viewed from the [SEC-MALS](#) webinars page. These questions were submitted by live viewers. Additional information on SEC-MALS, DLS, CG-MALS, and FFF may be found in the Wyatt web library under [Webinars](#), [Application Notes](#), 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.

#### Batch MALS

*Q: Which instrument is used for batch static light scattering? How is this typically done?*

A: The MALS detector is used for batch static light scattering (SLS) experiments. This is achieved by flowing different concentrations of a sample through the MALS instrument with either a syringe pump or HPLC pump. Another method for batch SLS is to use microcuvettes in conjunction with the MALS microcuvette accessory.

*Q: Does batch mode determine the size of the polymer or aggregates? How do you know this is from polymer or its aggregates?*

A: Batch mode will tell you the MW of anything in the solution that scatters, provided that you know the  $dn/dc$  value and concentration. If there are large aggregates, they will scatter much more light than individual polymers, so you will effectively see only the signal from the aggregates.

*Q: Do you have any experience with batch MALS measurements in mixed solvents?*

A: No. That becomes difficult because you would need to determine the solvent mixture parameters yourself. Parameters for most single solvents are already embedded in ASTRA<sup>®</sup>.

*Q: What kind of software did you use to analyze the batch mode data?*

A: Everything was analyzed in ASTRA.

*Q: Can batch mode and SEC-MALS be done at the same time in a run?*

A: No, these are two different types of MALS measurements. You need to take the detector offline to do batch-mode measurements.



Q: *Did you perform batch measurements in the first experiment with a microcuvette or by injecting into the detector?*

A: We performed batch mode experiments using the adapter from Wyatt, in 20 mL scintillation vials.

## SEC-MALS

Q: *What is the advantage of using the MALS detector in SEC experiments rather than just an RI detector and calibration standards?*

A: A MALS detector provides absolute  $M_w$  values, where calibration standards rely on comparison of retention time and are only accurate if the sample being measured is the same type of polymer as the calibration standards. Additionally, MALS is key for block copolymers, where there are no useful standards because different blocks can have different enthalpic interactions with the stationary phase in the columns. The BCPs on slide 22 show overlapping traces, but the molecular weight curves show that there was near-quantitative chain extension. With RI only, one would not be sure that complete chain extension had occurred because the retention times are similar between the homopolymer (5 kg/mol) and the BCP (10 kg/mol).

Q: *For the polymer projects, why not do batch mode experiments to get MWs instead of SEC?*

A: For those projects it was important for us to prepare micelles with narrow size distributions. We know that polymer dispersity translates well to micelle size dispersity, so it was imperative for us to know we had low dispersity and monomodal polymers. While batch mode SLS would have gotten us  $M_w$  values for our polymers, SEC-MALS lets us calculate  $M_w$  and  $M_n$ , and therefore the dispersity  $D = M_w/M_n$ , so we could evaluate polymer dispersity.

Q: *How do you know if certain detectors should be disabled to avoid skewing the result?*

A: If certain detectors appear to be well off the fit line consistently, meaning at several data points in a curve, then it may make sense to disable them.

Q: *Is there a way to view a few light scattering angles instead of monitoring all 17 angles in MALS?*

A: You can deselect detectors from the display and you can also deselect them from the analysis after the measurement. Alternatively, you can set up a method with specific detectors not selected and use it for all experiments. However, if you constantly need to remove detectors, there could be an issue like a dirty flow cell.



*Q: During SEC-MALS analyses are there ways to tell if the polymer distribution is a normal non-aggregated distribution compared to one that is aggregated?*

A: The molar mass profile can distinguish between these two examples. For a polydisperse sample, the molar mass will be a smooth continuous sloped line. The light scattering peak itself will also typically be monomodal. The molar mass profile of an aggregated sample will show a noticeable change in slope where the aggregate is. There are usually shoulders or multimodal peaks present due to these aggregates.

*Q: Can you comment on angle dependence of the MALS measurements of your aggregates? Is there an advantage to having more angles vs just 1 or 2?*

A: It's about robustly fitting the angular data at each slice, where the slope of the fitted line gives you  $R_g$  and the y-intercept gives you molar mass. If you only have two data points, any error will be magnified versus the quality of fitting 17 data points. You can get away with the three-angle mini-DAWN in some cases, but there will be larger error in the numbers than with an instrument with 17 detectors.

*Q: Are  $M_w$  and  $M_n$  absolute measurements?*

A: Using SEC-MALS, one can determine an absolute  $M_w$ . The  $M_n$  value, and  $\bar{D}$ , which is  $M_w/M_n$ , can also be determined. However, the accuracy of the  $M_n$  value will depend on how well your columns separate your sample. Bad separation will give an  $M_n$  value that is higher than the true value, and thus a lower  $\bar{D}$  value than the true value.

*Q: When working on new polymers, what can be done without knowing  $dn/dc$ ?*

A: It's often critical to know or at least estimate  $dn/dc$ . If you don't know  $dn/dc$ , you can measure it using a differential refractometer, or estimate it based on similar polymers in the same solvent. You cannot just leave  $dn/dc$  out of an equation though.

*Q: What is the right  $dn/dc$  value when we have two different molecules like protein and polysaccharide in a dispersion sample?*

A: The  $dn/dc$  value of a conjugate is the mass average of the  $dn/dc$  values of the components. If the mass fraction of component A is  $x$  and of component B is  $1-x$ , the overall  $dn/dc = x * (dn/dc_A) + (1-x) * (dn/dc_B)$ . Don't forget that the  $dn/dc$  value of any analyte depends on the solvent.

*Q: Did you measure  $dn/dc$  for MALS or use the literature values?*

A: It depends. Often, we use literature values for homopolymers. Sometimes we take the time to measure  $dn/dc$  ourselves on the refractometer, but this requires a fair bit of sample. If needed, we use the 100% mass recovery assumption that can be done in ASTRA.



## Application-specific

*Q: How do you know that you were measuring scattering from nanocrescent morphologies rather than single peptides or aggregates of nanocrescents during the SLS experiments on the peptides?*

A: Anything that scatters light would show up in this experiment with a strong dependence on size. Small peptides therefore would not give a significant signal, but nanocrescents and any aggregates would. We can't be sure from the SLS experiments alone that there is no aggregation occurring, but given that the aggregation number calculated from SLS is consistent with the estimate based on volume calculations for nanocrescents, we are confident that nanocrescent morphology was present during the SLS experiments.

*Q: Why was it useful to calculate an aggregation number for the nanocrescent forming peptides?*

A: There are very few examples of peptide-derived nanocrescent morphologies in the literature, and so we sought to thoroughly analyze this relatively new structure. Also, aggregation number lets us approximate the H<sub>2</sub>S-donating ability of each discrete nanocrescent, which could be useful in assessing their bioactivity in future works.

*Q: Does the aggregation number equal the degree of polymerization?*

A: The degree of polymerization is not the same as aggregation number here. I used the term 'aggregation number' of the block copolymers to describe the number of polymers that make up a micelle, while 'degree of polymerization' refers to individual polymer chains.

*Q: How does CMC differ from aggregation number, or is there a difference?*

A: Aggregation number is the number of polymers, on average, in each aggregate. CMC is the concentration above which aggregation occurs.

*Q: What are the different techniques you used to characterize your materials?*

A: We used many different light scattering, spectroscopic, and imaging techniques, including SEC-MALS, batch-mode MALS, transmission electron microscopy, NMR spectroscopy.

*Q: How efficient are post polymer modifications?*

A: The ones I discussed were quite efficient, near quantitative under our conditions.