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Live Webinar Q&A Sheet:

Exploring Enhanced Macromolecular and Nanoparticle Analysis through the Integration of Dynamic, Electrophoretic, and Static Light Scattering (DLS/ELS/SLS) with the ZetaStar™ Instrument

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

Q: *How difficult is it to swap the DLS fiber from the ZetaStar to the DAWN for inline DLS?*

A: Unlike the previous generations of instruments (Mobius and NanoStar I), there is no need to move the optical fiber between instruments when switching from inline DLS mode in the DAWN or miniDAWN to batch DLS mode in the ZetaStar or NanoStar II. The optical fiber is connected once during installation, and all the switching is software-controlled by DYNAMICS or ASTRA. You can read more about this configuration here: On-line MALS and DLS in a shared flow cell.

Q: *What's the limit of concentration to measure virus particle concentration?*

A: The limit of quantitation depends on the particle size. For AAVs, which have a radius of ~13 nm, we can measure particle concentration down to ~$6x10^{10}$ particles/mL. For larger viruses, the limit of quantitation decreases. In the LNP examples that we showed, where the radius was ~50 nm, we could easily measure concentrations of ~10^8 particles/mL. For any size, we can measure the concentration across several orders of magnitude.

Q: *How fast does a typical measurement take?*

A: For zeta potential and charge, a measurement can take anywhere between 15 and 60 seconds. The instrument software utilizes our adaptive mode to optimize both the duration and strength of the applied field for best results.

Q: *How do we measure in high salt? What have you measured?*

A: The biggest challenges with measuring in high salt or formulation buffer are the issues of electrolysis (bubble formation), sample degradation, and electrode degradation. By pressurizing the flow cell, any bubbles that are formed from electrolysis are squeezed down so they're virtually invisible during the measurement. With the optimized flow cell design, we're able to apply very low voltages for these same measurements, typically around 2-3 V. To avoid electrode degradation, we use platinum electrodes that are extremely resilient.

In the lab, we've successfully measured proteins like BSA in 150 mM and 300 mM salt conditions. We've also successfully measured lipid nanoparticles, liposomes, and virus-like particles in PBS (150 mM salt).

Q: *How does the DLS sensitivity compare to the NanoStar and Plate Reader?*

A: The DynaPro ZetaStar maintains the excellent sensitivity of the DynaPro NanoStar. You can see a side-by-side comparison of the specifications on our DLS products page.

Q: *What HPLC pump and autosampler is it compatible with? What are the typical volumes?*

A: The ZetaStar is compatible with the Arc HPLC Pump and Autosampler from Waters. This setup does require a few specific parts for proper integration like a 500 µL syringe and 1 mL loop assembly. The automation package can be purchased directly from Wyatt to ensure the proper setup is configured for the ZetaStar. We'd recommend injection volumes of at least 500 µL to ensure the sample is thoroughly flushed through the cell during measurement. While the actual volume inside the cell is 170 µL, the 500 µL volume is necessary to saturate the cell and overcome any issues with dilution.

Q: *What's the typical zeta potential reproducibility for LNP?*

A: For reference materials, like our mobility standard, we typically measure a relative standard deviation (RSD) of 5%. LNPs can be more complicated since they are heterogeneous, containing a wide range of sizes and charge states. The data in the webinar showed typical RSD of 5-10% for these samples.

Q: *Can you do simultaneous ELS & DLS with a disposable cell?*

A: Yes. Any time an ELS measurement is made in the ZetaStar, a simultaneous DLS measurement is made as well, regardless of the cell or cuvette used. The dip-cell, flow cell, and zeta disposable cell will all do simultaneous ELS & DLS measurements.

Q: *When analyzing the size distribution of LNPs with a size (diameter) of approximately 100 nm and a concentration of ~2x10^{13} particles/mL using Zetastar, is sample dilution necessary?*

A: In general, to measure size and particle concentration in the ZetaStar, the solution must be optically clear (not turbid). The maximum measurable concentration will depend on the sample size distribution and composition. The LNPs in the webinar were provided at concentrations up to ~2x10^{12} particles/mL and were typically diluted 10x - 100x prior to measurement.

Q: *This is perhaps the lowest volume measurement of turbidity that I have seen. Does Waters/Wyatt have any white paper applications for measuring turbidity similar to USP method (comparing sample to turbidity of formalizing standard)?*

A: The method of using static light scattering intensity measured at 90° in a low-volume cuvette has been compared to other traditional turbidity measurements in this paper: Characterization of Opalescence in low Volume Monoclonal Antibody Solutions Enabled by Microscale Nephelometry - ScienceDirect. You can find more information about performing low-volume turbidity measurements with the ZetaStar or NanoStar, II along with additional references on our website: Measuring Turbidity/Opalescence - Waters | Wyatt Technology.

Q: *Based on my understanding, SLS is not compatible with flow cell. If needing high throughput, is the DynaPro Plate Reader better for DLS/SLS?*

A: The SLS detector is physically blocked by the electrodes during the zeta potential and size measurement. We're able to get around this with the dip cell by inserting the electrodes after the SLS & DLS measurements have already been completed.

While we can achieve automated measurements by pairing the ZetaStar with an Arc HPLC, the measurements are limited to only DLS & ELS. They can take several minutes per measurement since each sample has to be thoroughly flushed out between measurements. For high-throughput DLS/SLS measurements, the Plate Reader is the ideal instrument with access to 96, 384, and 1536-well plates.

Q: *Can the instrument measure the particle size distribution of highly polydisperse particles?*

A: The ZetaStar uses dynamic light scattering to measure the z-average hydrodynamic size of a polydisperse population. For samples composed of multiple populations with sizes differing by ~3-5x or more, the ZetaStar can report an average size for each population. However, a separation technique, like FFF, coupled to multi-angle light scattering (MALS) is required for detailed particle size distribution of polydisperse samples. You can read more about how light scattering measures size and size distribution on our website here: Nanoparticle and macromolecular size by light scattering (wyatt.com).

Q: *What is the accuracy of estimated particle concentration of highly polydisperse particles (PDI>10)?*

A: Since we assume a single average particle size, the estimated particle concentration for polydisperse samples can carry some error. Typically, for monodisperse samples, the accuracy of the particle concentration is ~30%. For polydisperse samples, the error in the estimate can increase to a factor of 2-3x. Accurate concentration requires measuring and counting each individual size in the distribution, which FFF-MALS can accomplish.

Q: *How do you get weight average MW with single angle SLS? What is the accuracy of this estimation? Can a batch SLS + DLS measurement give molar mass of each component of polydisperse samples?*

A: The static light scattering (SLS) detector in the ZetaStar provides an absolute weight-average molar mass of molecules and particles in solution. Performing this measurement requires knowing the weight/volume concentration of the analyte. The total light scattering intensity measured by SLS combined with the hydrodynamic radius, measured by dynamic light scattering (DLS), allow the ZetaStar to determine molar mass from as low as 300 Da to as high as 10 MDa, depending on the conformation of the molecule with typical accuracy of 5%. The reported molar mass will be a weight-average of the entire solution. Separation, such as SEC or FFF, combined with multi-angle light scattering (MALS) is required to determine the molar mass distribution of a polydisperse sample.

The combination of DLS and SLS can also be used to measure particle concentration. In the case of a multimodal sample (two or more discrete populations with sizes at least 3-5x different), the ZetaStar can report the concentration of individual populations.

Q: *What type of laser does the instrument use? At what wavelength does it work and how stable is it? Is there a substantial warm up time for the laser source?*

A: The ZetaStar uses a 785 nm laser, allowing for easy measurements of fluorescent samples. On the first "cold start," the instrument requires up to 30 minutes to properly warm-up and reach thermal stability. Once warmed-

up, the instrument can be used immediately for any follow-up measurements. Users can turn the laser on and off independently of the instrument, and little-to-no warmup time is required.

Q: *What is the size range for DLS and ELS measurements?*

A: For DLS measurements, the size range is limited to 0.2 nm – 1 µm (radius). For ELS measurements, the instrument range is from 1 nm to 50 µm. A full set of specifications can be found here: The DynaPro ZetaStar: Dynamic Light Scattering Instrument - Waters | Wyatt Technology.

Q: *Can we use the Zeta cell for the neutral samples? What is the expected data distribution and does the software consider data quality for the report? Based on the recorded data, do you have any suggestions? Can the Zeta cell be used for the neutral samples? How well can we expect the Zeta distribution? Does the software allow for quality of the data to be considered for the report? What can we expect based on the recorded data?*

A: The Zetastar can measure neutral samples with any one of the ELS-compatible cells. The expected data distribution or measurement-to-measurement repeatability will rely on the samples' overall size, concentration, and applied field strength. Our adaptive software will optimize the applied field based on the conductivity of the sample for best results, but further refinement can be done manually if needed.

For each measurement, the DYNAMICS software will provide data quality indicators to help you assess the quality of the measurement taken. Additional details are provided to assess the quality for each measurement. More information can be found here: DYNAMICS Software - Waters | Wyatt Technology.

Q: *Is measurement temperature of ZetaStar controlled? Can one make DLS measurement with a temperature ramp?*

A: *The ZetaStar has temperature control from -10C to 120C with ramp rates up to 15 ˚C/min and an accuracy of up to 0.01 ˚C. The instrument can perform a wide variety of temperature-based experiments that include collecting data continuously over a temperature ramp or holding at specific temperature while the instrument collects data.*

Q: *What is the recommended maximum conductivity for zeta measurements with ZetaStar?*

A: With the ATLAS pressurization accessory, the maximum recommended conductivity is up to 100 mS/cm. Without it, the maximum is about 7 mS/cm where you'll begin to notice the effects of electrolysis and bubble formation negatively impacting the measurement.

Q: *Have you done an independent confirmation of particle concentration with another technique like NTA?*

A: Static light scattering has long been used to measure particle concentration. Classically, this measurement involves fractionation, like SEC or FFF, combined with multi-angle light scattering (MALS), as in this reference: Quantitation of influenza virus using field flow fractionation and multi-angle light scattering for quantifying influenza A particles - ScienceDirect. The batch SLS and DLS measurements in the ZetaStar are based on the same principle. However, since the samples are unfractionated, the result has lower resolution and reduced accuracy compared to SEC-MALS or FFF-MALS. We have compared our batch results with the more rigorous FFF-MALS results as well as with controls of known particle concentration. You can find more information and additional references on our website: Particle Concentration - Waters | Wyatt Technology.