Waters™ | WYATT TECHNOLOGY

Live Webinar Q&A Sheet:

Advances in automated dynamic light scattering characterization and zeta potential of macromolecules and nanoparticles

The recorded webinar may be viewed from the ELS 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

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

A: The biggest problems 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 of the DynaPro™ ZetaStar™ compare to the DynaPro™ NanoStar™ and DynaPro™ 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 the DynaPro ZetaStar 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 set-up 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 any overcome any issues with dilution.

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

A: For uniform standards, like our mobility standard, we typically measure 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: 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: *To measure virus particle concentration/aggregation, what´s the limit of concentration?*

A: The limit of quantitation depends on the particle size. For AAVs, which have a radius ~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 ~10^{8} particles/mL. For any size, we can measure the concentration across several orders of magnitude.

Q: *What software controls the autosampler?*

A: To control the ZetaStar with integrated automation, our DYNAMICS and HPLC CONNECT software are required. The Experiment Designer in DYNAMICS, allows for easy vial selection and parameter specification while the HPLC CONNECT software is used for directly controlling the autosampler pump.

Q: *Can you quantify lipids in the particles?*

A: The ZetaStar cannot be used to quantify the amount of lipid. However, we do offer a suite of LNP analyses using SEC or FFF combined with MALS for quantifying size, payload, encapsulation, and size-based payload distribution. You can find more information here.

Q: *How is temperature monitored and controlled?*

A: Temperature of the sample cell is monitored with a high precision RTD. Each instrument goes through a multipoint temperature calibration using NIST traceable sensors to achieve an accuracy of within 0.5 °C across the temperature range. DLS and SLS measurements can be made from -10 °C to +120 °C, and for ELS measurements can be made from +4 °C to +70 °C.

Q: *For AAV applications, can the DynaPro ZetaStar distinguish different sizes of the DNA payload?*

A: In general, we have not found charge to be a good indicator of AAV payload. In some specific cases, such as validating IEX buffer conditions, you may be able to quantify a difference in charge between empty and full AAVs, but this is not always the case. For payload analysis, we recommend using SEC-MALS combined with our Viral Vector Analysis for simultaneous quantitation of total concentration (Cp), genome titer (Vg), capsid content (Vg/Cp), and DNA payload molar mass. You can find more information here.

Q: *Legacy Mobius dip cells have an upper R_h limit of 200 nm. Any changes to those limits in the DynaPro ZetaStar? What is the upper size limit between the disposable ELS-cuvette vs. dip cell vs. the automated flow cell?*

A: For DLS measurements, we did improve the upper size limits that can be measured across all the cuvettes. Both the quartz cuvettes and 4 µL disposable cuvettes can be used to measure sizes from 0.2 nm to 1 µm. You can find a full list of specifications on our website.

Q: *Is it possible to measure the ELS of LNP in a solution with a high concentration of sucrose (example 10%)?*

A: Yes, both DLS and ELS measurements are possible in the presence of high concentrations of sucrose.

Q: *Is there any contribution of the electrophoretic mobility on the particle size/diffusion during the DLS measurement? Or is that corrected within the software?*

A: The motion of the particles in response to the electric field generally provides a constant velocity that does not impact the relative motion due to diffusion. However, the software does allow you to take a DLS measurement prior to each DLS+ELS measurement. This has the added benefit of allowing you to assess your particle's stability and other impacts of the electric field.

Q: *During the measurements in 1X PBS, can you perform multiple measurements of the same sample without damaging the sample (especially fragile proteins)?*

A: In general, yes, we can perform replicate measurements under physiological conditions to provide robust, reliable data. In the webinar, we showed data for LNPs measured in 150 mM salt (PBS). We showed the standard deviation of 5 measurements taken at a time for each injection. For more fragile samples, it may only be possible to take a few measurements. Users can also customize the measurement parameters to limit degradation, such as reducing the applied current or measuring at lower temperatures. Importantly, users can observe degradation, if it happens, in the raw DLS and ELS data and can easily filter or exclude inappropriate results.

Q: *Can the temperature ramp be programmed for DLS measurements?*

A: Yes, temperature ramp experiments can be performed like the DynaPro NanoStar workflows. The temperature range for DLS is -10 °C to +120 °C.

Q: *Could you use the DynaPro ZetaStar to measure and quantify the efficiency of oligo conjugation to antibodies?*

A: Although the conjugation of oligonucleotides would be expected to change the net charge of the antibody-oligonucleotide conjugate (AOC), this may not be the best way to quantify the efficiency or the degree of conjugation. Rather, we suggest using SEC-MALS with protein conjugate analysis to measure the molar mass of the protein and oligonucleotide. This will allow you to quantify the payload and payload distribution for the AOC. You can find more information here.

Q: *What is the typical applied voltage?*

A: Typical voltage for most experiments is 2-3 V using the flow cell and dip cell, which have been optimized to have very small electrode gaps. For the disposable cell, since it has longer path length, it requires in the order of 20 V for most experiments. The ZetaStar is also capable of applying electric fields of hundreds of V/cm to extract robust signals in the presence of poorly conducting samples.

Q: *How do you clean the reusable cuvette after using it for an upstream sample?*

A: Depending on the sample of interest, we recommend a few different flushing procedures. For most typical proteins, we'd recommend a cuvette washing procedure of detergent and water, then drying with filtered dry air. The flow cell can be cleaned in situ with a similar procedure or disassembled for deep cleaning.

Q: *Since the DynaPro ZetaStar has a different base theory of FIDELIS compared to PALS, is there a way to validate/compare results with that from an instrument that uses PALS?*

A: All our validation protocols have included side-by-side comparisons and found the ZetaStar provides equivalent results as the Mobius which uses PALS technology. Due to the inherent nature of fiber optic interferometer incorporated in FEDELIS technology, stray light rejection is greatly improved which further improves the accuracy of the results, especially at the lower end of the sensitivity limits.

Q: *If the original buffer is 1X PBS, what happens if you measure zeta potential after diluting it in water?*

A: For most samples, we'd expect the zeta potential to increase in magnitude after diluting in water. In high salt conditions, we expect a higher shielding effect that reduces the measured zeta potential.

Q: *Can the DynaPro ZetaStar be used in line with older SEC-MALS systems from Wyatt like the DAWN Heleos?*

A: The ZetaStar optical fiber can be connected to any supported Wyatt MALS detector for online DLS measured inside the MALS flow cell. Please check in with Wyatt Support for configuration compatibility.