Live Webinar Q&A Sheet:

Multi-Attribute Quantification of Viral Vectors Using Light Scattering

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, 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 results from SEC-MALS AAV titer quantification compare to results from analytical ultracentrifugation (AUC) or capsid titer ELISA?*

A: AAV capsid and genome titers measured by SEC-MALS multi-attribute quantification are typically in excellent agreement with results from orthogonal methods such as AUC, ddPCR, and capsid titer ELISA. Please see e.g., Troxell, B et al. (2023) *Hum Gene Ther*. 34(7-8):325 or Werle, AK et al. (2021) *Mol Ther Methods Clin Dev.* 1:23:254 for peer-reviewed comparisons of the different techniques. SEC-MALS multi-attribute quantitation also provides the added benefit of being a 21 CFR part 11 compliant platform method that delivers multiple quality attributes, including genome titer, capsid titer, and Vg/Cp from a single experiment.

Q: *What kind of purification did the AAV sample go through (Slide 8)?*

A: This example used a typical AAV purification protocol combining filtration steps and column chromatography. For example, ion exchange chromatography (IEX) and tangential flow filtration (TFF) steps were used to improve the purity of the downstream purification (DSP) samples compared to the upstream purification (USP) sample.

Q: *How are you defining USP filtrate? Post-depth filtration?*

A: The USP filtrate is the sample obtained from the final upstream purification step, immediately prior to the first downstream purification step.

Q: *Can you explain what is causing the USP samples to have a slightly lower peak radius than the DSP samples (Slide 8)?*

A: The USP sample is less pure and contains additional components compared to the DSP samples. The slightly lower peak radius for the USP sample is due to the presence of contaminants in the USP sample (e.g., proteins smaller than AAV capsids), that are removed by the downstream purification steps.

Q: *For IEX/AEX-MALS can you explain the separation of full & empty AAV and why there is a higher light scattering signal of the full AAV?*

A: Empty capsids elute at lower salt concentration than full capsids, as the empty capsids bind less tightly to the AEX column. Given that the empty and full capsids are similar in size, the greater peak area of the full peak compared to the empty peak indicates that the sample contains more full capsids than empty capsids.

Q: *How do you separate out debris often found in the sample peak of monomers, dimers and trimers?*

A: Both filed flow fractionation (FFF) and SEC separate particles by size. Debris typically has a different size and molar mass than monomers, dimers and trimers, and is thus separated out by these size-based separation techniques.

Q: *How does absorbance and refractive index data obtained from FFF-MALS and SEC-MALS comprehensively differentiate between miss-folded or inactive AAV serotypes from the functionally active monomers peak obtained?*

A: Please see our application note AN1617: Quantifying quality attributes of AAV gene therapy vectors by SEC-UV-MALS-dRI for an overview of the principle behind the analysis. In addition, misfolded or aggregated AAV typically differ in size compared to the functionally active AAV monomers and may thus be separated from the monomers by either FFF or SEC, as both methods provide size-based separation. Please see e.g., our recent application note AN2004: Why and how to quantify AAV aggregates by FFF-MALS, for additional information.

Q: *What instrument provides particle measurement? in #/ml (Slide 3)*

A: The DynaPro™ Plate Reader, NanoStar™, and ZetaStar™ all offer particle concentration measurements. The particle concentration is provided in units of mL^{-1}, e.g., capsids/mL.

Q: *Is DLS sensitive to pick out the effect of filtration (Slide 6)?*

A: Yes, batch DLS is an excellent tool for measuring how filtration impacts a sample.

Q: *Why can DLS have a larger error to quantify virus concentration?*

A: Particle concentrations measured with Wyatt's MALS or DLS methods have high precision and accuracy and are generally in good agreement with results obtained with orthogonal methods. The precision and sensitivity depend on the specific method. For example, the AAV concentration by SEC-MALS, which uses MALS with UV and RI concentration detectors, is precise to 1 – 2% and accurate to ~ 5%, and even precision in Vg/Cp of AAVs is 3% with a minimum quantifiable change of 5% for SEC-MALS multi-detector analysis. Even a relatively complex technique, the SEC/FFF-MALS multi-detector LNP payload analysis, has been validated to within a couple of percent by size-based fractionation of various LNPs with subsequent offline analysis of the fractions (Jia, X et al., (2021) *J Chromatography B*. 1186:123015). Particle concentration measurements by MALS or DLS that rely on determining the radius are somewhat less robust, since a 5% deviation in radius can result in a 30% deviation in concentration, and for 50 nm radius polystyrene we have measured a 17% uncertainty. Likewise, an error of 1% in the specified refractive index results in 20-30% error in concentration. For viruses or LNPs, where sample composition can be more complex, I would expect the method to yield reproducibility within 30%. The ASTRA and DYNAMICS software provide multiple utilities for rapid evaluation of data quality, including estimates of statistical precision and data quality indicators.