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Live Webinar Q&A Sheet:

Shining Light on AAVs: Using Light Scattering Technologies to Solve Challenges in Characterization

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

Q: *Have you tested samples that have the same number of particles but a different empty and full ratio?*

A: We've successfully measured a few different controls where we keep the AAV titer consistent but varied the full-to-total ratio (Vg/Cp) from 3% up to 97% full. With the SEC-MALS method we can detect changes in Vg/Cp as little as 3% and quantify differences as low as 5%.

Q: *How long did it take for you to develop your FFF method?*

A: The answer here isn't straight forward. If someone is already familiar with the technique, it can take anywhere from 2-6 months for optimization. However, it can take several months, up to 1 year to become an expert. Leveraging what has already been done by Wyatt can keep these times on the lower end. Timelines differ significantly based on expertise and overall scope, 3-12 months is a typical timeline depending on assay needs e.g. process development vs. QC qualification/validation.

Q: *When you get a novel AAV how much time does it take to tailor it?*

A: MALS is a platform method, which means that it works for any serotype or novel engineered AAVs with no or very little optimization. The only parameter that changes is the molar extinction coefficient of the AAV (genome and capsid).

Q: *What is the calibrant that is used for MALS?*

A: We actually don't need to calibrate; MALS is an absolute technique. All the detectors are calibrated absolutely with toluene. We do recommend a suitability standard to check the system's health; otherwise calibration standards aren't required.

Q: *What was the benefit to adding FFF-MALS in addition to SEC-MALS?*

A: FFF-MALS was able to uncover aggregated species that we didn't initially detect in the SEC-MALS. It's possible that these large aggregates were either sheared in the SEC column or removed by the column frit.

Q: *Do you see agreement between ddPCR and SEC-MALS when aggregation is high?*

A: We haven't seen a difference, but it could depend on the amount of aggregation. Our products are anywhere 80-90% monomers and it is possible that at this high level of purity we wouldn't be able to see this difference.

Q: *What are the upper limits for FFF-MALS?*

A: We can measure up to 1 µm, independent of the mode of separation. Separation is usually where the limitation is located, where the column's stationary phase can shear the sample or remove larger species, like aggregates. Most SEC columns work best when the molecule's radius is ~85 nm or less. With FFF-MALS we can fractionate and measure the full range of sizes.

Q: *What is the SEC-MALS column that you used?*

A: We used the newly released XBridge™ Premier™ GTx BEH SEC Column, 450 Å and there is also a legacy, both available at our column store.

Q: *Have you tested any upstream samples?*

A: We have tried this, but the issue is the titer is close to the limit of quantification and the high level of impurity tends to complicate the measurement. The combination of the sample complexity and low titer makes this measurement difficult and is less qualitative.

Q: *What version of ASTRA is needed for the AAV workflow option?*

A: The Viral Vector Analysis used for AAVs was introduced with ASTRA 8 and is not available in earlier versions. The latest version of ASTRA 8 includes our most up-to-date AAV SOP Guidance Manual and data collection capabilities for successful AAV quantitation.

Q: *How transferrable is SECM-MALS and FFF_MALS to QC?*

A: There is a clear path to QC with the compliant ready software. But one major challenge in implementation is having well trained QC analysts who can reliability execute the assay, perform data analysis, and troubleshoot when needed.

Q: *How do you optimize your FFF recovery?*

A: We pre-condition the membrane with a few injections of the serotype we are characterizing.

Q: *AAVs are notoriously sticky, how do you mitigate this problem?*

A: Formulation is really important, we add Pluronic and salt to help reduce the "stickiness" and reduce aggregation. Also, some serotypes like AAV-9 are less prone to sticking and aggregation like other AAVs but mobile phase optimization can help and we have found one mobile phase that works well for all of the serotypes and downstream samples we run.

Q: *Does the mobile phase impact results?*

A: Definitely. Certain serotypes may behave differently from others. When we did our initial mobile phase screening using dynamic light scattering (DLS), we saw that when salt wasn't high enough, certain serotypes would aggregate. We also saw significant column interactions or retention on the column with sub-optimal mobile phase. For example, there is a definite need for sufficient ionic strength, enough Pluronic, and serotypes like AAV-2 and AAV-6 that aggregate more readily may require additional optimization. However, we have developed a platform method and mobile phase in our released AAV SOP Guidance Manual, and this mobile phase has worked for all serotypes tested, including engineered serotypes.

Q: *Have you compared the A260/A280 from the LC optical detector to your MALS results?*

A: Although this comparison wasn't shared in the presentation the following reference does include this type of comparison: Werle, A. K. et al. Comparison of analytical techniques to quantitate the capsid content of adeno-associated viral vectors. Molecular Therapy - Methods & Clinical Development 23, 254–262 (2021).

Q: *After you run an AAV do you see high background noise in the MALS?*

A: No, we have protocol for maintaining the health of LC and column and the noise stays low. With these best practices, we are able to achieve high throughput and are not spending significant time troubleshooting or re-collecting data.