



## Live Webinar Q&A Sheet:

### Quantifying Viral Vector Attributes with 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, CG-MALS, and FFF 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](mailto:info@wyatt.com) with any additional questions.

#### AAV analysis by SEC-MALS

*Q: In the case of AAV quantification, how does the presence of partial AAVs affect the results?*

A: The presence of partial will not affect the total AAV concentration. In other words, the SEC-MALS method measures the total AAV particle concentration (or physical titer) directly, whether the AAV is empty, full, partial, or mixture of them. However, the SEC-MALS method cannot tease out the percentage of partially filled AAVs from a mixture of empty and full. It considers a population of partials as a fraction of empty AAVs and a fraction of full AAVs.

*Q: What is the variability of AAV concentration by SEC-MALS?*

A: If the AAV concentration is relatively high, say  $10^{12}$  AAV/mL, the repeatability is 2-3% or better.

*Q: Up to what degree of error(s) can be observed between sample to sample (or reproducibility) for measuring cp/vg ratio using SEC-MALS?*

A: The reproducibility is roughly 1 : 20 if you consider cp/vg, or  $\pm 0.05$  in terms of the fraction full AAV/total AAV (which varies between zero to one).

*Q: Have you done a comparative analysis of SEC-MALS and qPCR methods to obtain viral genome copy numbers? Are these techniques comparable in terms of data similarity and percentage errors?*

A: Many of our customers have compared SEC-MALS results with qPCR (or ddPCR), ELISA, and AUC. Generally they report that the correlation is excellent. The SEC-MALS method measures the AAV concentration directly, and its accuracy is very high. Anecdotally, one customer shared that they trust the value from SEC-MALS more than from the other techniques.



*Q: Does the software support analysis of AAV concentration and empty:full ratio using two UV wavelengths, 260 nm and 280 nm, instead of the more common analysis that uses dRI and one UV wavelength?*

*Q: We have a MALS instrument but not a dRI detector. How does this limit the parameters we can estimate? Is there a workaround?*

*A:* Our approach allows us to use any two of the following concentration detectors, dRI, UV at 260 and UV at 280 nm, so it support the use of UV260/UV280. However, I would recommend include the dRI detector in the system especially during method development, verification, and validation. Once you understand your system and AAV samples well, you may consider using only the UV signals for QC.

In order to use the data from two UV wavelengths, a UV detector with two analog outputs is required. In the future we expect to be able to acquire the data from certain instruments digitally, without the need for analog connections.

*Q: Can a fluorescent detector be used instead of UV detection with the SEC-MALS system?*

*A:* Yes. Fluorescent detectors provide superior sensitivity compared to UV detection; however, the response factor relating the fluorescence signal and sample concentration varies from serotype to serotype. The UV response factors (i.e., the extinction coefficients for capsid proteins and DNA) remain the same for all the serotypes we have tested, so analysis with UV is easier to use and more reliable than fluorescence.

*Q: Are there 3rd party CROs that run the SEC-MALS system to QC AAVs?*

*A:* While the AAV method is new at the time of this webinar, there are multiple CROs that run Wyatt SEC-MALS instruments and can quickly adopt the method as needed. Please see [www.wyatt.com/ContractLabs](http://www.wyatt.com/ContractLabs) for a list of CROs that offer SEC-MALS services.

#### **AAV analysis conditions**

*Q: Which column was used for SEC-MALS for such good resolution?*

*A:* Wyatt offers an AAV-specific column. In order to implement the entire AAV characterization method, we now offer a comprehensive one-day, on-site AAV Training & Method Transfer service. This service includes our well-tested AAV method, with details on the column and mobile phase conditions.

*Q: In which solvents do you recommend to measure the size of viral particles?*

*A:* Generally, our customers as well as our lab test the viral particles in common buffers like PBS or formulation buffer.



*Q: What is your injection volume of AAVs for SEC-MALS?*

A: It depends on the AAV concentration. For a concentration of  $10^{13}$  AAV/mL, less than 10  $\mu$ L of injection volume is used.

### AAV analysis by IEX-MALS

*Q: How will the salt gradient in ion-exchange chromatography affect the light scattering measurement?*

A: The LS baseline signal increases slightly (and linearly) with salt concentration, so the effect of the salt gradient on light scattering is automatically canceled in the usual way, by setting a baseline in [ASTRA® software](#). However, the dRI baseline is affected quite significantly. ASTRA offers a *Baseline subtraction* method: you run a blank gradient (no sample injected) with exactly the same timing, and the method subtracts the blank dRI signal from the sample dRI signal, leaving just the dRI sample peak.

Most chromatography RI detectors cannot handle typical ion-exchange salt gradients, since the change in RI is greater than their measurement range, and they reach saturation. Wyatt's [Optilab® differential refractometer](#) has a very large range so it can cover common salt gradients, up to about 300 mM NaCl (or equivalent with another ion). If the salt gradient to be run is greater than this, we recommend the Optilab HC high-concentration model which can cover the equivalent of 2 M NaCl.

### DLS analysis of AAVs

*Q: Batch DLS is not a method of choice for aggregate determination for mAbs—there has to be a certain size difference between aggregates and monomers in order for them to be resolved. So, how is it justified for AAVs?*

A: Just like with mAbs, batch DLS cannot resolve small oligomers from a main monomer population. It is useful for analyzing aggregation in two ways: 1) DLS can detect the presence of oligomers via the polydispersity index and a shift in average size; and 2) it is great tool to rapidly detect the presence of large aggregates (larger than monomers by at least 3-5x in radius). The new particle concentration feature in [DYNAMICS® software](#) also allows a quick estimation of AAV physical titer.

Unlike batch DLS, online DLS coupled to a separation technique such as SEC, IEX or FFF can readily resolve and characterize small oligomers. Wyatt offers the [WyattQELS™ embedded DLS module](#) for our online MALS instruments for this purpose.



*Q: How reliable is the concentration measurement from DLS? It is commonly known that DLS is not quantitative.*

A: It is true that, traditionally, batch DLS has not been considered a quantitative method in that the size distributions it provides are relative (actual concentration is not determined), and depend on various assumptions about particle and aggregate conformation. Recent advances combine static light scattering (SLS) with DLS to provide estimates of particle concentration, which compare well with other particle-counting or particle concentration measurement techniques. The analysis works best for monomodal (especially monodisperse) populations, and requires some basic a priori information, including particle shape and refractive index. Particle concentration can also be determined for multimodal samples, but the accuracy is lower. The analysis is fully implemented in DYNAMICS beginning with version 7.10.

There are many benefits to using DLS/SLS for particle concentration including convenience, speed, throughput, no sample preparation and no clogging of nanopores or sieves.

*Q: How long does a thermal ramp take to find a  $T_{onset}$  for AAV?*

A: For a single sample, using the cuvette-based [DynaPro® NanoStar®](#), a typical ramp rate is 5 °C/min, so a run from 25 to 85 °C takes 12 minutes (ramping back down can be the same, or faster if measurements are not needed). However, depending on the thermal kinetics of the sample, this may be too fast and will overestimate  $T_{onset}$ , so a slower rate may be desirable.

For characterizing multiple samples, such as different serotypes or formulations, the [DynaPro Plate Reader](#) is preferable. The specific ramp rate depends on the number of samples to be measured; there is a calculation tool in DYNAMICS to help set this up. In a typical example, measuring 24 samples every 1 °C between 25 to 75 °C takes 2.5 hours.

*Q: What is the detection limit for AAVs and aggregates in batch DLS?*

A: For monomers,  $6 \times 10^{10}$  AAV/mL. For aggregates, it depends on size... small aggregates such as dimers and trimers cannot be resolved from monomers, but a 50-mer (about 4x the radius of a monomer) could be resolved and detected at  $\sim 2.5 \times 10^7$  mL<sup>-1</sup>. The minimum detectable concentration is inversely proportional to the sixth power of the radius.

#### FFF-MALS analysis of non-AAV vectors

*Q: Adenovirus is still the most widely-used viral vector in vaccines/oncolytics, will these techniques still apply to adenovirus considering it is a bit larger size?*

A: Absolutely, though SEC separation must be replaced with FFF by adding an Eclipse controller and separation channel to the HPLC system. FFF-MALS can detect small amount of aggregates in



adenovirus samples, measure DNA content and particle concentration, help select formulation buffers, and study sample stability.

*Q: Does the measure of protein/nucleotides could be done in Lentivirus which is enveloped?*

A: Yes, it is possible to measure protein and nucleotides in a lentivirus that is enveloped, in a manner very similar to AAVs. As of the date of the webinar, the work is in progress and the method and results will be shared in the near future.

*Q: Can you expand on what kind of setup we would need to carryout FFF?*

A: An FFF system consists of HPLC degasser, pump, and autosampler; Eclipse FFF controller and separation channel; and downstream detectors including a DAWN MALS instrument, Optilab dRI detector and the HPLC UV detector. If you do not have an available HPLC system from Agilent, Shimadzu or Thermo, Wyatt can provide a complete turn-key setup.

Since the FFF system utilizes standard HPLC components and SEC-MALS detectors, an FFF system can be configured to alternate quite readily between FFF-MALS and SEC-MALS.

*Q: What is the best technique for bacteriophage analysis, SEC-MALS or FFF-MALS?*

A: It depends on the size of bacteriophage. Generally, when the longest dimension of the nanoparticle is more than 50 nm (true for most bacteriophages), we recommend using FFF-MALS.

### General light scattering

*Q: What can the  $R_g/R_h$  ratio tell us?*

A:  $R_g$  is the mass-averaged radius, measured from angular dependence of the MALS detector;  $R_h$  is the hydrodynamic radius measured by the DLS detector, defined as the radius of an imaginary sphere that has the same diffusivity as the sample. The ratio of  $R_g$  to  $R_h$  gives us information on shape or structure. The  $R_g/R_h$  ratio is often used to characterize the cargo or payload content of nominally hollow bioNPs such as liposomes, since it distinguishes between uniform spheres and spherical shells. It can also distinguish between spherical monomers and their aggregates such as dimers.

*Q: Can we apply the same principle that was described for analyzing proteins and DNA in AAVs to characterize polysaccharide-protein conjugates?*

A: Yes, ASTRA software's *Protein Conjugate* method utilizes data from MALS, dRI and UV to determine the molar masses of the protein and polysaccharide in the conjugate at each elution volume. It provides the protein fraction in the conjugate as well as the total molar mass. The method is subject to limitations at high molar masses, in the range of tens of millions and above, due to excess UV extinction resulting from scattering.