APPLICATION NOTE

AN5007: Characterization of AAV-based viral vectors by DynaPro DLS/SLS instruments

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Summary

When developing adeno-associated virus (AAV) vectors as drug products, multiple quality attributes (QAs) must be monitored to ensure a safe and efficacious final product. Three such QAs are total AAV particle concentration, aggregate content, and thermal stability. The DynaPro NanoStar and DynaPro Plate Reader enable fast, easy measurements of these QAs in batch mode with combined dynamic light scattering (DLS) and static light scattering (SLS).

Introduction

Adeno-associated virus (AAV) is a class of virus, belonging to the family *Parvoviridae*, consisting of a non-enveloped protein capsid with diameter ~25 nm, packed with single-stranded DNA. With a history of over fifty years of study, AAV has become one of the most popular and well-characterized gene-delivery vectors for clinical applications.[1,2]

To provide safe and effective gene-therapy products, several quality attributes must be quantified throughout the manufacturing process, including in-process and final drug products. The most common QAs relate to stability, purity, and potency of the AAV product.[3]

A variety of analytical tools are used to monitor QAs of AAV vectors, including analytical ultracentrifugation (AUC) and real-time PCR. However, these techniques can be labor-intensive, costly, and destructive to the sample, making them unsuitable for early-stage, high-throughput screening. The use of SEC-MALS with a DAWN® multi-angle light scattering detector to characterize AAV QAs including size, aggregation, concentration and empty:full ratio is discussed in application note AN1617: AAV critical quality attribute analysis by SEC-MALS. SEC-MALS is non-destructive and easier than the aforementioned techniques; it provides detailed analysis, but requires approximately 30 minutes per sample and may not be appropriate for screening of processes and formulations.

In contrast, batch light-scattering techniques provide quick, easy, and high-throughput characterization of AAV solutions. Here, batch static and dynamic light scattering (SLS and DLS) were used to quantify three AAV quality attributes (QAs):

1. Aggregate content
2. Thermal stability
3. Total viral particle concentration

This application note highlights all three measurements in the DynaPro® Plate Reader and DynaPro® NanoStar® DLS/SLS instruments.

Materials and Methods

AAV9 samples were kindly provided by Virovek, Inc. (https://www.virovek.com/), specializing in large-scale AAV production (Table 1). Samples S1 and S2 represented purified 'empty' (no DNA payload) and 'full' (containing full-length, single stranded DNA) AAV samples, respectively. Samples S3 through S6 represent investigations of various buffer conditions on the stability and aggregate content of the AAVs.

Batch DLS and SLS measurements were performed with the DynaPro NanoStar and DynaPro Plate Reader as described below. Data acquisition and analysis were performed with DYNAMICS® software.

Table 1: AAV sample description

Sample ID	AAV	Buffer	Note
S1	AAV9	A	Purified, empty
S2	AAV9	A	Purified, full
S3	AAV9	B	Formulation testing
S4	AAV9	C	Formulation testing
S5	AAV9	D	Formulation testing
S6	AAV9	E	Formulation testing

DynaPro NanoStar

Low-volume DLS and SLS measurements were performed with DynaPro NanoStar to assess the size and size distribution for aggregation. For samples S1 and S2, 1.25 µL of neat and diluted AAV solutions were loaded into the quartz cuvette. Each measurement consisted of five, 5-second acquisitions. DLS and SLS data were collected to determine hydrodynamic radius (R_h), aggregate content, molar mass, and viral particle concentration.

DynaPro Plate Reader

High-throughput measurements were made using the DynaPro Plate Reader. All measurements were performed in a 384-well microtiter plate (Aurora), and each well was loaded with 30 µL solution. The plate was centrifuged at 400 *g* for 1 minute prior to loading into the plate reader.

AAV samples S1 and S2 were diluted 1:10 in buffer. In addition, three mixtures of S1 and S2 were created with ratios of full:empty AAV corresponding to 1:1, 1:10, and 1:50 (v/v). Each AAV solution was loaded into the microtiter plate in triplicate, and each well was capped with 1-2 drops of silicone oil to prevent evaporation. DLS and SLS data were collected to determine R_h, aggregate content, and viral particle concentration at 25 °C. For thermal stability measurements, the temperature was ramped continuously from 25 °C to 85 °C at a rate of 0.1 °C/min, and R_h was measured throughout the temperature ramp.

AAV samples S3 through S6 were loaded into single wells without dilution. The wells were sealed with tape (Nunc) to prevent evaporation. The plate was incubated at 37 °C for two hours, and the R_h distribution was measured over time to determine the effect of the formulation buffer.

Results and Discussion

Size and size distribution

Measuring the size and size distribution with batch DLS provides a quick approach to assess the gross amount of aggregation in AAV solutions. Among the six AAV samples tested, DLS provided clear differences in particle size and aggregate content. Purified AAVs (S1 and S2), exhibited autocorrelation functions (ACF) with smooth, fast decays, characteristic of monodisperse samples of the expected size (Figure 1, top). In contrast, the autocorrelation functions for other AAVs (S3 through S6) decay more slowly, indicating larger aggregates present in the solution. Fitting the ACFs with a regularization algorithm provides the size distribution plots in the lower plot of Figure 1.

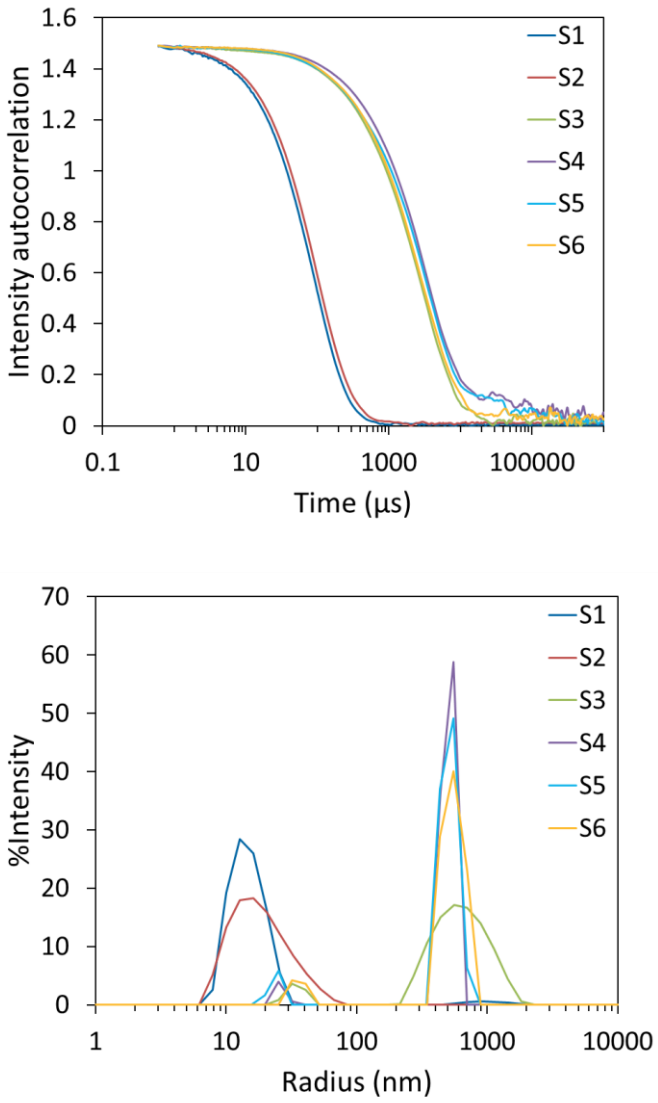


Figure 1. Autocorrelation functions (top) and size distribution via regularization (bottom) of AAVs for formulation screening.

Purified AAV samples S1 and S2 appear to be uniform solutions with R_h = 14.7 ± 0.7 nm and 15.6 ± 0.1 nm, respectively. Furthermore, the weight-average molar mass (M_w) determined by SLS from NanoStar are 3.67 ± 0.01 MDa for S1 and 6.78 ± 0.03 MDa for S2. The increase in molar mass is consistent with the incorporation of the DNA into the viral capsid and agrees well with molar mass measurements by SEC-MALS.[4]

Samples S3 through S6 appear to be highly aggregated (Figure 1), and the dominant species has an average R_h of 30 nm. Despite the presence of aggregate, a secondary peak with radius ~550 nm is apparent in the size distribution. This likely corresponds to AAV monomer or a mixture of monomer, dimer, and small oligomers. The various buffer conditions were thought to mitigate or promote different aggregate content. However, in this study, those differences were not apparent at 25 °C. They were only noticeable upon incubation at 37 °C (see "Thermal stability").

Thermal stability

Two measures of thermal stability were performed in this study. The hydrodynamic radii of samples S1, S2, and their mixtures were measured throughout a continuous temperature ramp to quantify aggregation temperature. S3 through S6 were incubated at a constant temperature of 37 °C to observe changes in size and size distribution caused by elevated temperature.

Both full and empty AAVs appeared to have the same aggregation temperature, suggesting the DNA payload does not change the thermal stability. Figure 2 shows R_h as a function of temperature for empty AAV (S1), full AAV (S2), and three different mixtures. The size remains constant for all the samples until the temperature is increased beyond 60 °C. A sharp increase in R_h was then observed, growing from 14 nm to 300 nm, indicating the formation of aggregates. Onset analysis in DYNAMICS was used to determine the onset temperature and radius for each sample. The onset temperature was relevantly constant at 62.5 ± 0.5 °C, and its corresponding onset R_h was 18.3 ± 1.1 nm.

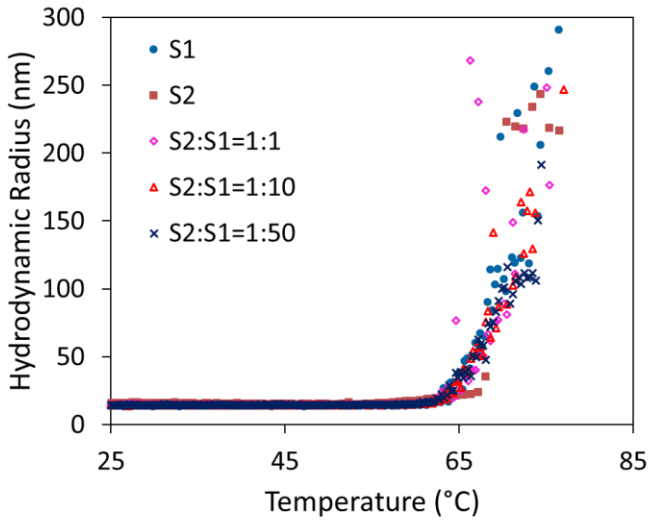
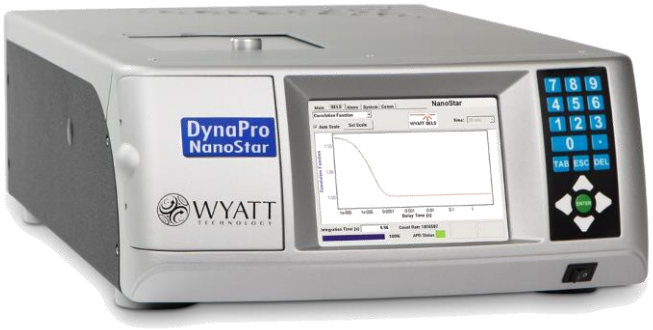


Figure 2. Aggregation screening of AAVs upon thermal ramping.

The response of aggregated AAV samples (S3 through S6) to thermal conditions was examined over time at a fixed incubation temperature of 37 °C. As shown in Figure 3, all four AAV solutions exhibit a decrease in R_h as a function of incubation time. At t = 0, the mean size of all four samples ranged from 450 nm to 530 nm, with S4 being the largest and S3 being the smallest. The rate of dissolution of the aggregates varied for each sample, depending on the additives in the formulation buffer.



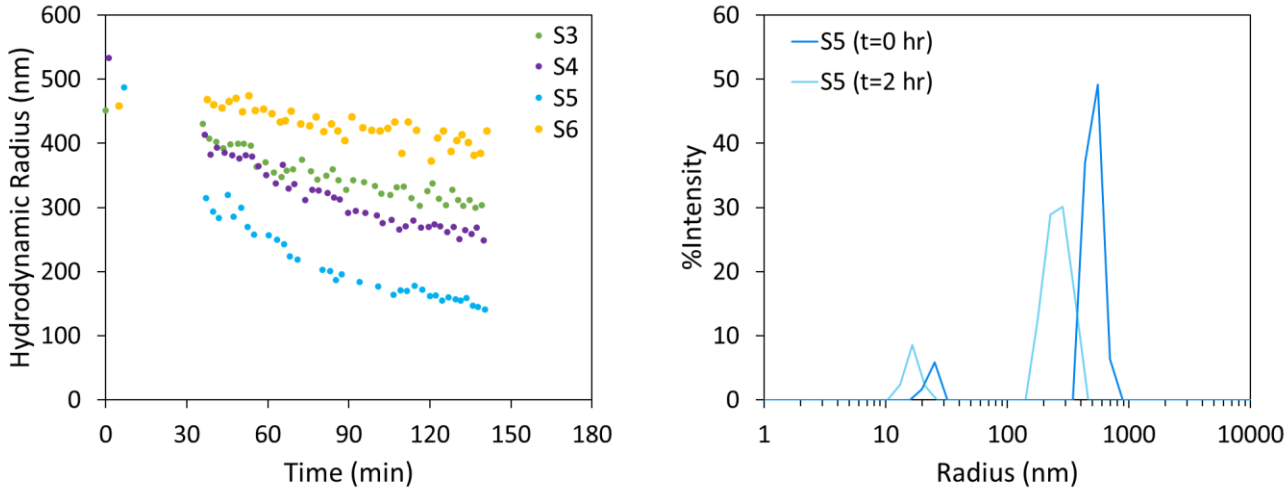


Figure 3. Hydrodynamic radius as a function of time of AAVs upon thermal incubation at 37 °C (left). An example of distribution difference between incubation at t = 0 and 2 hours (right).

R_h decreased the most for S5, which was formulated with the highest concentration of a particular buffer ingredient. It was hypothesized that this component should prevents AAVs aggregation, and it may also enable aggregates to be recovered as individual particles.

Particle concentration

Batch SLS and DLS measurements with the NanoStar and Plate Reader enable rapid quantitation of the particle concentration. Determining particle concentration requires knowledge of the particle shape—in this case a sphere. In addition, the refractive index (RI) of the particles and buffer must be specified. DYNAMICS comes preloaded with a library of RI values for common materials, and users may specify custom RI values for complex materials, like AAV. With these inputs, the R_h measured by DLS and the static light scattering intensity are used together to provide particle concentration. For typical AAV samples, the concentration measurable by this technique ranges from ~6x10^{10} mL^{-1} to ~10^{15} mL^{-1}.

The total AAV concentration was measured for samples S1 and S2. For the analysis, RI values of 1.43 and 1.48 were assigned to S1 and S2 AAV, respectively. Figure 4 shows the particle concentrations for S1 and S2 AAVs measured by batch DLS/SLS and calculated by DYNAMICS, compared to high-resolution measurements made by SEC-MALS. High-throughput measurements performed in microtiter plates agree well with SLS measurements made in the NanoStar quartz cuvette, demonstrating similar data quality.

DYNAMICS reports average particle concentrations of (8.2 ± 0.6) × 10^{13} mL^{-1} for S1 and (2.7 ± 0.1) × 10^{13} mL^{-1} for S2, respectively. These samples had been previously quantified by SEC-MALS to determine the molar mass, dimer and aggregate content, and the concentration of each species.[4] Batch measurements with the NanoStar and DynaPro Plate Reader provide comparable values, within 22% of the SEC-MALS high-accuracy measurement. This excellent level of agreement means that batch measurements can be used as fast, high-throughput screening for particle concentration without requiring labor-intensive, lengthy experiments.

Two main limitations are encountered for batch concentration measurements. First, while SEC-MALS enables separation of the monomer and aggregates and quantification of these particles separately, in batch mode the average measured R_h contains contributions from all the species in the solution. The average R_h by DLS is typically larger than the monomer radius measured by SEC-MALS, leading to an underestimation in the particle concentration in batch. Second, the choice of refractive index contributes significantly to the calculated concentration. In these experiments, RI values of 1.43 and 1.48 were empirically determined for empty and full AAV, respectively, and agree with more rigorous characterization by SEC-MALS using protein conjugate analysis and a model of a coated sphere.[4] Where the DNA loading is unknown, an average RI of 1.46 may be chosen, consistent with literature.[5] However, this ~2% difference in refractive index can lead to a 1.5× difference in calculated concentration.

For screening, this discrepancy is typically negligible, and further quantitation with high-resolution techniques like SEC-MALS can be used to refine the value.

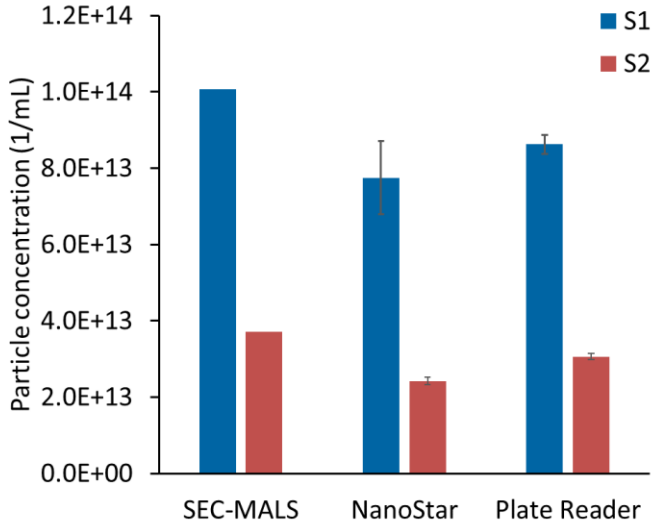


Figure 4. Comparison of viral particle concentration determined in DYNAMICS with those determined by number density in ASTRA.

Conclusions

Batch static and dynamic light scattering measurements provide low volume, quick screening tools for quantifying AAV quality attributes. Both the DynaPro NanoStar and DynaPro Plate Reader enable characterization of particle size and size distribution, thermal and colloidal stability, and total particle concentration. These methods are non-destructive and require no method development, making them ideal for incorporation into multiple areas of AAV drug development.

Acknowledgements

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