DLS Sizing of Adeno-Associated Viruses (AAVs)

1 Introduction

The adeno-associated virus (AAV) is a prominent vector used in gene therapy, thanks to its low immune response and wide host-cell range. Among the critical quality attributes (CQAs) necessary to control for proper manufacturing, aggregation has a particular influence on the stability and shelf-life of therapeutic formulations[1]. Analytical techniques such AUC, TEM, or SEC are used to characterize such formulations and measure possible aggregates. However, these techniques are time-consuming, destructive, and incur low throughput[2]. In this context, Dynamic Light Scattering (DLS), when carried out using advanced instrumentation, provides a straightforward and rapid way of evaluating the particle size distribution (PSD) in AAV formulations and detecting the presence of aggregates - responsible for product degradation - with high sensitivity and in-situ. In this application report, we present DLS sizing results performed on a popular AAV serotype, using a highly powerful DLS instrument.

2 Materials & Methods

An AAV9 sample containing no DNA payload and labeled "empty" was received from Virovek Inc (USA). No dilution or filtration was performed. The sample was measured on the goniometer-based variable multi-angle light scattering instrument (V-MALS) 3D LS Spectrometer™ from LS Instruments equipped with a 100 mW laser with a 660 nm wavelength. The measurement angle was set to 90°, and the measurement was carried out with activated modulated 3D technology to remove any possible artifacts from multiple scattering[4]. 10 repetitions of 60 seconds were carried out. The results were obtained by means of the CORENN algorithm[3].

3 Results & Discussion

Fig1 shows a representative PSD measured for the AAV9 "empty" sample.

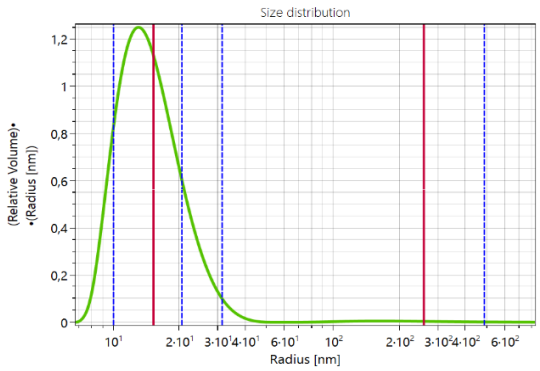

Fig. 1: Volume-weighted particle size distribution of the AAV9 "empty" sample.

A main particle population with an average (red vertical bar) diameter of 30 nm is identified. We also notice the presence of a small amount of aggregates with an average size of 310 nm. The averaged results are summarized in the following table:

Pop.	Amount [%]	Radius [nm]	PDI
1	99	14.5	0.81
2	1	310	0.49

4 Conclusion

Using a popular AAV serotype, we show that such systems can be characterized in a fast and straightforward manner using advanced DLS. The sensitivity provided by novel fitting algorithms and high laser power enables proper detection of low amounts of aggregates.

5 Authors

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6 References

[1] Wright, J. F. et al., Gene Therapy, **2008,** 15, 840–848.

[2] Gimpel, A. et al., *Mol. Ther. - Methods Clin. Dev.*, **2021**, *20*, 740–754.

[3] https://lsinstruments.ch/en/theory/dynamic-light-scattering-dls/dls-data-analysis-the-corenn-method.

[4] https://lsinstruments.ch/en/theory/dynamic-light-scattering-dls/modulated-3d-cross-correlation.