AAV Virus Titer and Aggregation Characterization

Characterization of Gold Labelled AAV and Other Small Viruses by Nanoparticle Tracking Analysis (NTA)

PARTICLE CONCENTRATION

PARTICLE SIZE

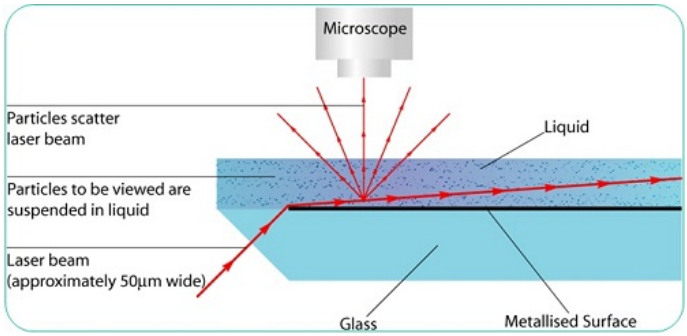
Introduction

In this application note, we describe a novel method to rapidly measure the size and concentration of Adeno-Associated Virus (AAV) that utilizes the Nanoparticle Tracking Analysis (NTA) technique and a gold labeling technique. By labelling AAV capsids with small gold nanoparticles, we were able to increase the amount of light scattered by each capsid, enabling rapid NTA characterization to determine the virus concentration and aggregation state. This method should be equally applicable to other virus types presuming teh surface chemistry is similar. NTA characterization of AAV was previously impossible without gold labeling.

This new technique extends the benefits of simultaneous size and concentration measurements, both as an alternative or supplement to traditional titer assays and as a check of aggregation state. The differing stability of formulations may have a significant effect on final product performance but may not be adequately measured with traditional techniques.

Overview of NTA

Nanoparticle Tracking Analysis (NTA) utilizes the properties of both light scattering and Brownian motion in order to obtain the particle size distribution of samples in liquid suspension. A laser beam is passed through the sample chamber, and the particles in suspension in the path of this beam scatter light in such a manner that they can easily be visualized via a 20x magnification microscope onto which is mounted a camera. The camera, which operates at approximately 30 frames per second (fps), captures a video file of the particles moving under Brownian motion within the field of view of approximately 100 µm x 80 µm x 10 µm (Figure 1).



$$Dt = \frac{TK_B}{3\pi\eta d}$$

NTA is not an ensemble technique interrogating a very large number of particles, but rather each particle is sized individually, irrespective of the others. An example of the size distribution profile generated by NTA is shown in Figures 2 and 3.

In addition, the particles' movement is measured within a fixed field of view (approximately 100 µm by 80 µm) illuminated by a beam approximately 10 µm in depth. These figures allow a scattering volume of the sample to be estimated; by measuring concentration of the particles within this field of view and extrapolating to a larger volume it is possible to achieve a concentration estimation in terms of particles per mL for any given size class or an overall total.

The lower sizing limit for NTA is related to the refractive index of the particle compared to the surrounding media. For high scattering materials like gold and silver nanoparticles, the lower sizing limit is 10 nm. However for low scattering materials such as biological particles the lower sizing limit is 30-40 nm.

Adeno Associated Virus (AAV)

AAV is small virus with a diameter of approximately 25 nm [1]. It is commonly used by gene therapy researchers as a viral vector due to its many attractive properties that include its low pathogenicity [2], ability to infect non-dividing cells [3], ability to integrate into a specific location on the human chromosome 19 [4], and its tunable tropism towards different tissue types [5]. To date there have been over 130 clinical trials worldwide that utilize AAV as a viral vector [6].

AAV is a well characterized virus with a known crystal structure[7]. The AAV capsid is composed of three types of capsid proteins (VP1, VP2, VP3) in a 1:1:10 ratio. These three proteins form 5 subunits, for a total of 60 capsid proteins, resulting in an icosahedral symmetry.

Despite the well-known structure of AAV, gene therapy researchers are still limited in their ability to rapidly measure AAV particle concentration. The plaque assay, which is commonly used today in academic labs, was first developed in the 1950s to measure virus titer[8]. However, the plaque assay is time intensive,

and can require days or even weeks to complete. Industry has adopted modern techniques like quantitative polymerase chain reaction (qPCR) [9] and colorimetric assays such as enzyme-linked immunosorbent assay (ELISA) [10] to measure genome counts and capsid proteins to estimate AAV titer. Despite the advantages of these techniques over the plaque assay, these modern techniques still require hours to days to complete. As a result, a large demand exists for a method that can rapidly and directly measure AAV particle concentration.

Gold Labeled AAV NTA Results

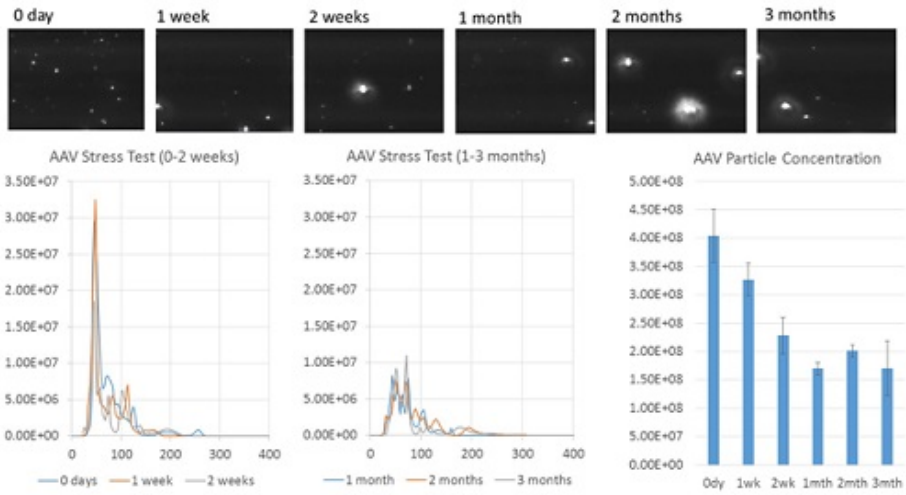
The unlabeled AAV virus is too small and does not scatter enough light to be characterized by NTA. However, a recently developed method to label the virus surface with gold nanoparticles has enabled the NTA characterization of AAV. This approach utilizes the electrostatic attraction between a highly scattering material like gold nanoparticles and capsid. The resulting gold labelled virus particles scatter enough light to be visualized and tracked by the optical system, enabling the use of NTA to measure the size and concentration of AAV.

Using this approach, a series of AAV samples were measured. Firstly, a highly-purified gold-labeled sample was selected to compare the NTA measured concentration to the expected titer. The gold labeled AAV produced a size distribution with a primary peak and a particle concentration of $3.55x10^{8}$ particles/mL, representing only a 22% difference from the expected concentration of $4.5x10^{8}$ particles/mL based on a 100,000-fold dilution from the neat AAV titer of $4.5x10^{13}$ determined by qPCR (Figure 2A).

A control experiment without gold but otherwise under identical conditions was performed. In the resulting size distribution the primary peak was not detected, and the measured concentration was much lower (Figure 2B). The small number of particle tracks that were tracked in the unlabeled AAV sample were likely a small number of virus aggregates. Additional control experiments confirmed that the primary peak was not due to the presence of gold nanoparticle aggregates or background particles from the AAV vehicle buffer (data not shown).

The results show an increase in polydispersity and a decrease in particle concentration over time. A primary peak representing monodisperse AAV is clearly visible for up to 2 weeks, but within 1 month the peak begins to diminish. These results show that AAV is mostly stable at 37 ºC for up to 1 week, but then show significant signs of aggregation after 2 weeks. However, the size distribution and concentration measurements show little change between 1 month and 3 months, suggesting that AAV does not continue to form larger aggregates over that time period. These results highlight the usefulness of this NTA gold labelling characterization technique not only to measure AAV

concentration, but also to measure aggregation state and formulation stability as well.



Conclusion

By utilizing the electrostatic attraction between gold nanoparticles and AAV, we have enabled the NTA characterization of AAV. For the first time, it is now possible to measure AAV particle concentration in minutes instead of hours or days. Additionally, this approach is a direct measure of particle size and concentration, and does not require any assumptions regarding AAV genome loading efficiency, infection efficiency, capsid protein integrity, or aggregation state.

References

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