SIMPLIFYING AAV PROTEIN ANALYTICS WITH MAURICE

Will McElroy and Chris Heger, Ph.D.

ProteinSimple, a Bio-Techne Brand, 3001 Orchard Parkway, San Jose, CA 95134, USA

INTRODUCTION

Adeno-associated viruses (AAV) are promising vectors for the delivery of genetic material in gene therapy. During the manufacture of AAV, critical quality attributes (CQAs) like charge heterogeneity, purity, and empty/full status must be carefully monitored because they can impact the product's safety and efficacy. Imaged capillary isoelectric focusing (icIEF) and capillary electrophoresis sodium dodecyl sulfate (CE-SDS) are two powerful methods to respectively characterize and quantitate charge heterogeneity and purity, but, traditionally, two separate platforms are required to perform these analyses. Here, we used a single Maurice platform to analyze AAVs by icIEF and CE-SDS methods to ensure product stability, identity, and purity. In addition, we show that the icIEF mode on Maurice, coupled with dual wavelength detection, affords insights into the empty/full status of AAVs. Taken together, the poster will show that Maurice is a powerful fully integrated analytical tool for gene therapy development.

MATERIALS AND METHODS

MATERIALS

AAV2, AAV9, Maurice CE-SDS PLUS Application Kit (PS-MAK03-S) Maurice cIEF Method Development Kit (PS-MDK01-C), Compass for ICE Software.

CE-SDS METHOD

AAV2 (1 x 10^{13} GC/mL) samples were concentrated, and buffer exchanged prior to analysis. In all samples, 20% BioRad SDS solution was added to Maurice CE-SDS PLUS Sample Buffer to a final concentration of 4% SDS. Samples were denatured in the presence of TCEP at 70 °C for 10 minutes, cooled to RT for 5 minutes, and mixed by vortex. Samples were injected for 15 seconds at 4600 V and separated for 30 minutes at 5750 V.

icIEF METHOD

AAV9 (2 x 10^{13} GC/mL) were diluted into a final mixture containing 0.35% MC, 3% Ampholyte 3-10 blend, 2.5mM Arginine, in a Formamide/Urea blend with pI markers pI 4.05, 5.2, 8.4, 9.5. Samples were focused for 1min at 1.5kV, followed by 8min at 3kV. Absorbance and native fluorescence images were captured using the Compass for iCE software.

RESULTS

MAURICE CE-SDS MEASURES AAV PROTEIN PURITY AND CAPSID RATIO

Protein purity is a CQA that needs to be addressed for AAVs. These measurements are classically done with SDS-PAGE, but the method has significant disadvantages. In recent years, CE-SDS has become a strong tool for biopharmaceutical protein purity. Therefore, we reasoned that Maurice could be used for AAV protein purity. We analyzed an AAV2 sample on CE-SDS for specificity (Figure 1), repeatability (Table 1) and linearity (Figure 2).

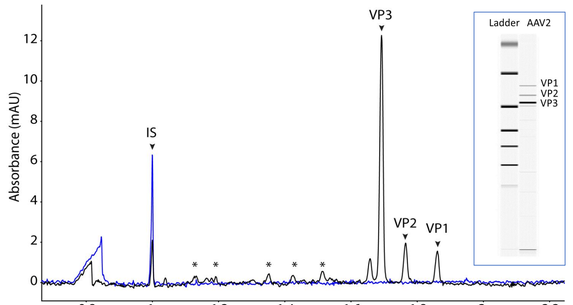

Figure 1. Maurice CE-SDS method detects and resolves AAV capsid proteins. The AAV2 sample is shown in the black trace and the blank sample is shown in the blue trace. The internal standard is labeled as IS and impurities are labeled with an asterisk. Inset = NEW! Lane view for CE-SDS in Compass for iCE software.

Table 1. CE-SDS AAV2 Repeatability. The AAV2 sample was injected as five replicates and the percent peak area for each capsid protein and their respective ratios were calculated. The percent relative standard deviations (%RSDs) of VP3, VP2 and VP1 were 0.5%, 4.0%, and 4.3%, respectively. The average capsid ratios were 7.6:1.3:1 for VP3:VP2:VP1.

	% CORRECTED PEAK AREA			CAPSID PEAK RATIO		
Injection Name	VP3	VP2	VP1	VP3	VP2	VP1
Injection 1	71.6	12.4	8.9	8.0	1.4	1.0
Injection 2	71.0	12.8	9.7	7.3	1.3	1.0
Injection 3	71.3	12.5	9.0	7.9	1.4	1.0
Injection 4	70.8	11.7	9.9	7.2	1.2	1.0
Injection 5	70.6	13.2	9.1	7.8	1.5	1.0
Average	71.1	12.5	9.3	7.6	1.3	1.0
%RSD	0.5	4.0	4.3	4.5	6.9	0.0

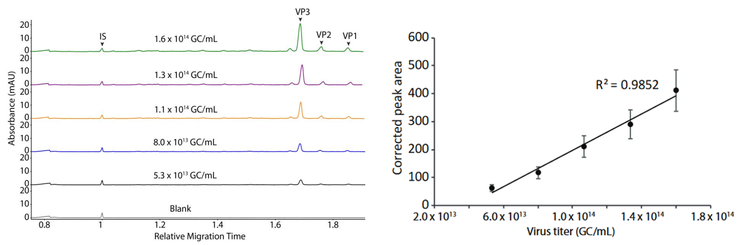

Figure 2. Representative CE-SDS electropherograms from AAV titration. AAV2 was diluted from 1.6 x 10^{14} GC/mL (green trace) to 5.3 x 10^{13} GC/mL (black trace) prior to analysis on Maurice. The bottom (gray) trace is the sample blank.

LEVERAGING DUAL WAVELENGTH DETECTION FOR INTACT AAV EMPTY/FULL ANALYSIS

AAVs are utilized as gene therapy delivery vehicles because they can hold up to 5kb of DNA and have low immunogenicity. During manufacturing, AAV capsids lacking DNA (empty capsids) are formed. These empty capsids can reduce efficacy and need to not only be removed, but ideally minimized during the manufacturing process. Current methods to characterize empty/full capsids are long and challenging to implement in quality control. We examined the capability of Maurice to help analyze these samples. Because Maurice detects absorbance (280nm) and native fluorescence (~320-450nm), it is possible that contributions from DNA will be observed only in absorbance (where DNA still absorbs light) and contributions from protein are observed in both absorbance and native fluorescence (DNA is not detected with NF). We separated two AAV9 samples with different % Full associated (determined by the manufacturer) and compared the absorbance and NF for either a nearly full AAV9 (91%) or an empty (7% Full) AAV9 (**Figure 4**). The NF signals (black traces) are overlaid with the absorbance (shaded green). Native fluorescence from the two samples was quantified on an individual peak basis, as well as the total NF for each sample (total NF area of all peaks). This comparison shows both samples have nearly the same NF signal (Figure 4B). Using the slight difference in NF between the samples to normalize the absorbance, we observed a significant difference in the absorbance of the empty sample (Figure 4C), confirming that Maurice can be used to characterize empty/full AAVs.

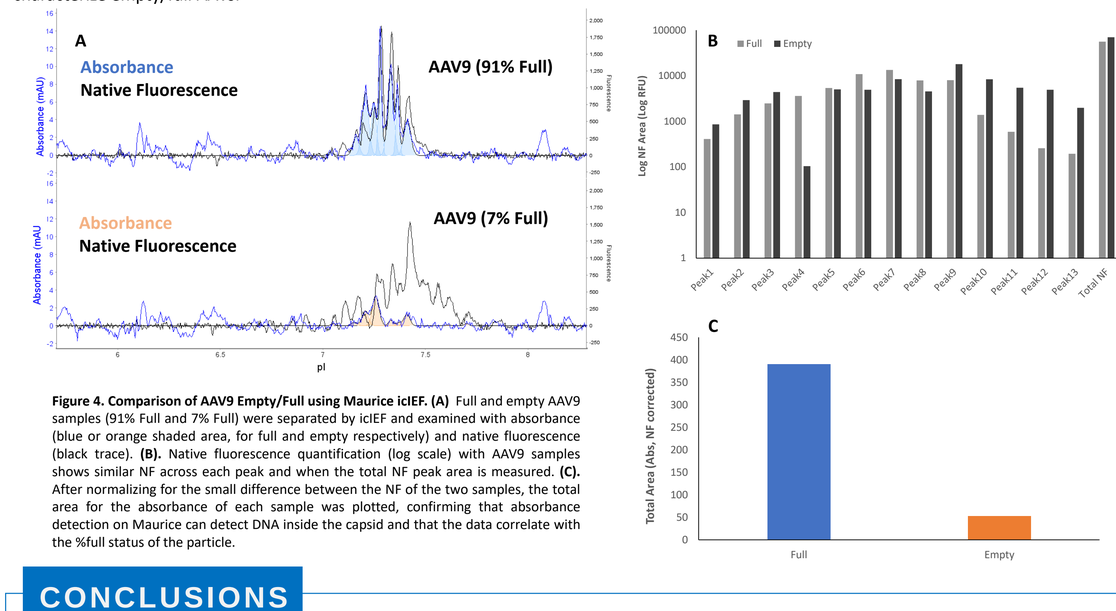

Figure 4. Comparison of AAV9 Empty/Full using Maurice icIEF. (A) Full and empty AAV9 samples (91% Full and 7% Full) were separated by icIEF and examined with absorbance (blue or orange shaded area, for full and empty respectively) and native fluorescence (black trace). **(B).** Native fluorescence quantification (log scale) with AAV9 samples shows similar NF across each peak and when the total NF peak area is measured. **(C).** After normalizing for the small difference between the NF of the two samples, the total area for the absorbance of each sample was plotted, confirming that absorbance detection on Maurice can detect DNA inside the capsid and that the data correlate with the %full status of the particle.

CONCLUSIONS

- Maurice is a two-in-one analytical platform, capable of running icIEF and CE-SDS.
- AAV CQAs require characterization of protein purity and identity but are evolving. This evolution will likely require additional characterization of AAV proteins and could extend to charge heterogeneity.
- Using the dual wavelength icIEF detection, Maurice can make important observations about the DNA content of intact AAV particles.

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CONTACT: chris.heger@bio-techne.com

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