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SPOTLIGHT

INNOVATOR INSIGHT

A reproducible, high-throughput platform to quantitatively study AAVs

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The adeno-associated virus (AAV) has the potential for major therapeutic advances in the future due to its low immunogenic response in humans. Studying AAVs through sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) electrophoresis presents multiple challenges in user variability and time consumption. In order to resolve these challenges, a microfluidic platform, the LabChip® GXII Touch™ system, was used to characterize AAV serotype 8 (AAV8) particles. This reproducible technology can be used for high-throughput, quantitative characterization of AAV particles.

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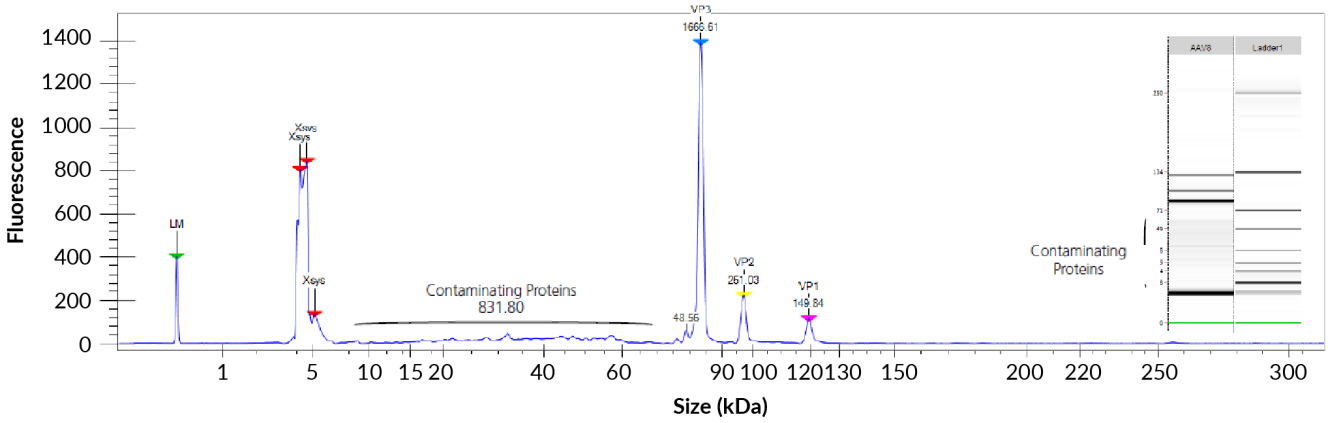
Adeno-associated virus may play a major role for gene therapy applications in the near future. This is due to their ability to infect dividing and quiescent cells while only causing a mild immune response in hu-mans. These small viruses, when engineered to carry specific genes, can be a powerful tool in fighting many diseases. In order to achieve this, AAVs must be well characterized and studied.

Typically, AAVs are studied using SDS-PAGE gels with silver stain. This highly sensitive method yields con-vincing qualitative results but is time consuming and user dependent. Studying AAVs through SDS-PAGE with silver stain in a high-throughput manner is a difficult task [1,2]. This article will specifically look at a microfluidic electrophoresis-SDS platform as a potential solution to simplify the quantitative study of AAVs.

µCE-SDS uses electric fields to create a migration difference based on the charge of the analytes. The ana-lytes are then exposed

FIGURE 1

The output of the LabChip® GXII Touch system is an electropherogram or a virtual in-silico staining gel (insert).



LM is the lower marker and Xsys are system peaks. VP3 (blue), VP2 (yellow) and VP1 (pink) represent the denatured VPs and their concentration (ng/µl). Each peak is annotated with the expected VP type and concentration (ng/µl). Identifying the presence of contaminating proteins is very useful to ensure the overall quality control process for AAV characterization and further analysis.

to fluorescent light to measure the amount of each in the sample. This process is highly reproducible and has comparable sensitivity to SDS-PAGE with silver stain [2,3]. µCE-SDS platforms, such as the LabChip® GXII Touch™ system, have the potential to quantitatively study AAVs in a consistent manner.

The LabChip® GXII Touch™ system is a reproducible quantitative instrument to characterize analytes. This system has a highly sensitive standardized analysis for the size, concentration and purity of proteins. Run-ning 150 nl samples in under 65 s, this automated system is 21 CFR 11 GMP compliant. In this article, we show how AAV8 was characterized using the LabChip® GXII Touch™ system.

AAV8 CHARACTERIZATION

AAV8 particles (catalog#A81000, Welgen Inc., Worcester MA) were generated from HEK-293 cells and contained at least 5×10^{12} genome copies (GC)/ml. This material was placed in a hardshell 96 well V-bottom SBS plate (catalog #6008870, PerkinElmer, Waltham MA) along with the ProteinEXact™ assay's nonreducing sample buffer. The plate was sealed and heated at 70°C for 10 minutes. Once cooled to room temperature, Milli-Q® water (Millipore, Bedford MA) was mixed into each well. The plate was then analyzed on the LabChip® GXII Touch™ system once the plate was spun at 1200 RCF to remove any bubbles.

The AAV capsid forms an outer shell with three viral proteins (VP) known as VP1, VP2, and VP3. These proteins not only protect the AAV genome, but also perform host cell binding. The denatured AAV8 cap-sid proteins were measured using the ProteinEXact™ Assay on the LabChip® GXII Touch™ system. The resulting electropherogram is shown in Figure 1. This assay classifies the three viral proteins as well as determining the presence of contaminating proteins. This is highly useful in the overall quality control process for AAVs manufacturing and study. LabChip® assays are designed to minimize cross contamination between sips.

The AAV8 VP ratios were determined through automatic calculation of the corrected area under the curve (AOC) by the LabChip® Reviewer software. To demonstrate the reproducibility in this determination, 12 samples were superimposed and compared (Figure 2).

This AAV capsid ratio profile can be further used to distinguish AAV serotypes. Purified AAV serotypes were run through the LabChip® GXII Touch™ system and the

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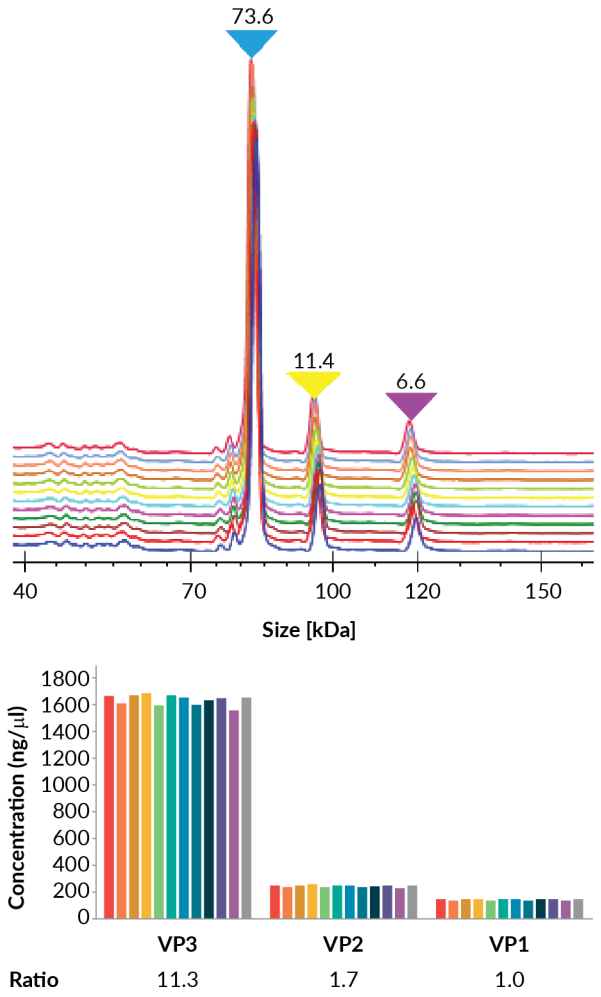
FIGURE 2

An electropherogram overlay (A) and calculated ratio (B).

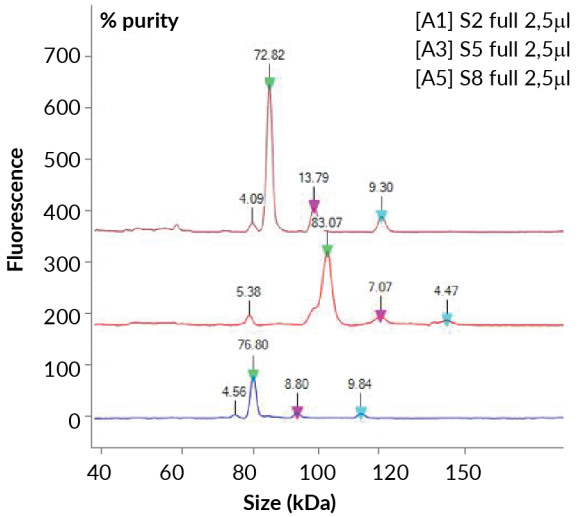


(A) Electropherogram overlay showing 12 runs of each denatured VP peak: VP3 (blue), VP2 (yellow) and VP1 (pink). (B) The calculated ratio was based on the average concentration of each peak.

peaks were determined (Figure 3). In order to properly validate serotype ratios in your

FIGURE 3

AAV capsid profiles of serotype 2 (blue line), serotype 5 (red line), and serotype 8 (brown line).



The peaks for VP3, VP2 and VP1 are shown in green, pink and turquois respectively. Serotypes were highly purified before they were characterized with the LabChip® GXII Touch™ system.

experiments, it is essential to confirm complete capsid breakage.

The LabChip® GXII Touch™ system is an efficient, accurate and reproducible µCE-SDS alternative to SDS-PAGE with silver stain for the quantitation of proteins. This system yields high-quality quantitative results with a faster analysis time and increased throughput than SDS-PAGE with silver stain4. The LabChip® GXII Touch™ system supports high-throughput AAV research by standardizing the analysis of the size, concentration and purity of proteins.

For research use only. Not for use in diagnostic procedures.

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APPLIED GENOMICS

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