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A new approach for high-resolution full and empty AAV capsid analysis utilizing a high-throughput method for comprehensive AAV evaluation on a single CE platform

アデノ随伴ウイルス(AAV)の高分離 Full、Empty カプシド割合解析、キャピラリー電気泳動(CE)装置による包括的ハイスループットメソッド

Key Words : 遺伝子治療、ウイルスベクター、AAV、カプシド、純度評価、SDS ゲル分子重量解析キット、RNA 9000 純度・完全性評価キット、BioPhase 8800

遺伝子/細胞治療用ベクターのアデノ随伴ウイルス (AAV) は、その蛋白質カプシド内に遺伝子として一本鎖 DNA を含みますが、製造においては DNA 欠損体 (Empty カプシド) や部分断片 DNA を含む Partial カプシドも生成されます。これらは治療効果や安全性に関連するため、完全な DNA を含む Full カプシドに対して不純物となり、品質管理上その割合評価は重要な CQA の一つです (Table 1)。

SCIEX の CE 分離では、カプシド蛋白質構成割合及びプロファイリングの解析条件 (Fig. 3)、及び 1 本鎖 DNA 遺伝子完全性評価の解析条件 (Fig. 6、7) が標準化されており、これらは幅広い AAV セロタイプに対応します (Fig. 4 for カプシド、Fig. 8 for 1 本鎖 DNA)。これらの解析条件でそれぞれの CQA を評価するとともに、さらにカプシドにおいては VP3、遺伝子においては完全長 DNA をそれぞれ定量し、その存在割合から Full カプシド割合を純度評価として算出する方法を提案しています (Fig. 1)。

VP3 及び完全長 DNA の検量線は、AAV 8 サンプルの希釈系列から作成され、それぞれ 3 桁以上の直線域が得られました (Fig. 5 for VP3、Fig. 9 for 完全長 DNA)。これに AAV 8 Test Sample の結果をプロットしてそれぞれの力価 (GC/mL) を算出し、その割合から Full カプシド割合を算出 (Fig. 10) した結果は、透過型電子顕微鏡や超遠心分析法による結果とよく一致しました (Fig. 11)。

双方を別々に定量して解析する方法は他の分析技術でも提案されています (Table 2) が、SCIEX の高効率生物製剤解 8 本キャピラリー電気泳動システム BioPhase 8800 では、Full/Empty 解析のそれぞれの検量線作成とサンプル測定だけでなく、複数の CQA 解析が同一装置上で効率的・迅速に行え、全体の所要時間においても有利です (Fig. 12)。8 本キャピラリーの効率性が、特に検量線作成において発揮されます。

A new approach for high-resolution full and empty AAV capsid analysis utilizing a high-throughput method for comprehensive AAV evaluation on a single CE platform

Featuring the BioPhase 8800 system

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To ensure the safety and efficacy of gene therapies delivered by adeno-associated virus (AAV) vectors, multiple critical quality attributes (CQAs) must be well characterized before regulatory submission.¹ Different analytical platforms are currently being used to characterize multiple CQAs. However, these methods have limitations or cannot be used to analyze all serotypes without significant optimization. In this technical note, we present an approach for high-throughput multiple CQA analysis of AAVs using a single platform: the multi-capillary BioPhase 8800 system. High-accuracy analytical data were collected in a single day for multiple CQAs, including nucleic acid loading and titer, capsid protein profile and ratio, and capsid titer from the same samples.

A new approach was used here to obtain empty, partial, and full capsid ratios by utilizing data for other CQAs (Figure 1). The workflow is serotype-independent and amenable for development and QC. Using a single, multi-capillary platform for multiple AAV CQA analyses saves time and cost and reduces laboratory footprint compared to using different platforms.

Key features of the comprehensive AAV characterization workflow

- High-definition full, partial, and empty analysis is accomplished with a new methodology
- High-throughput, comprehensive analysis of multiple CQAs for the same AAV sample is performed on a single platform within a single day
- High-resolution, serotype-independent capsid profile, ratio, and impurity analyses, and titer determination are achieved with a CE-SDS-LIF workflow
- High-resolution, serotype-independent genome integrity analysis and titer determination are achieved with a CGE-LIF workflow
- Multi-capillary capability enables fast result generation for all methods
- Time and cost saving, and laboratory footprint reduction are attained by using a single tabletop platform for the analysis of multiple CQAs

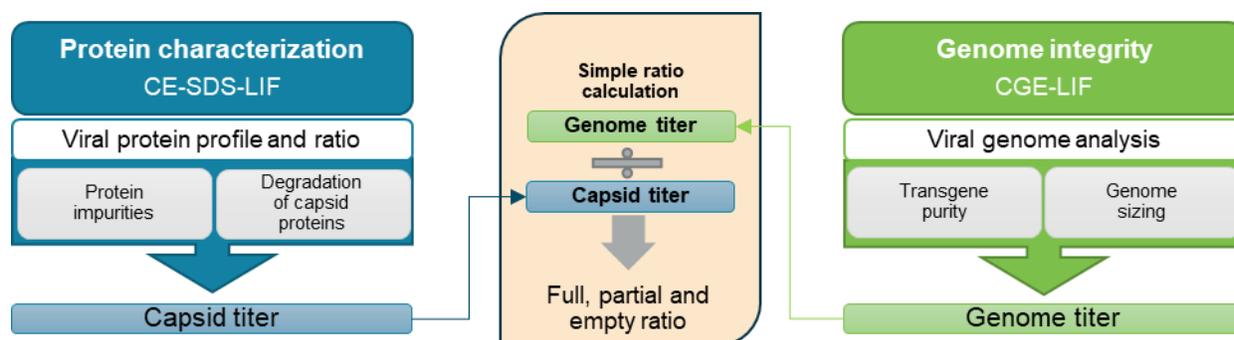


Figure 1. A new approach for high-resolution full and empty AAV capsid analysis as part of a high-throughput, comprehensive analysis suite of AAV CQAs on the BioPhase 8800 system. Each sample is analyzed with 2 workflows: the CE-SDS-LIF for protein characterization including the capsid titer determination; the CGE-LIF for genome integrity analysis including genome titer determination. The percentage of the full capsids is calculated by dividing the genome titer by the capsid titer.

Materials and methods

Materials: The BioPhase CE-SDS Protein Analysis kit (PN C30085), the BioPhase BFS Capillary Cartridge (PN 5080121), the BioPhase Sample and Reagent Plates (PN 5080311), the RNA 9000 Purity & Integrity kit (PN C48231) and Sample Loading Solution (SLS) (PN 608082) were from SCIEX (Framingham, MA). The Chromeo P503 dye (PN 15106) was from ACTIVE MOTIF (Carlsbad, CA). Dithiothreitol (DTT, PN V3151) was from Promega (Fitchburg, WI). QIAquick PCR purification kit (PN 28104) was from Qiagen (Germantown, MD). Single-stranded RNA ladder 0.5-9 kb (PN N0362S) was from New England BioLabs (NEB) (Ipswich, MA). Nuclease-free water (PN AM9932), 10x Phosphate Buffered Saline (PBS) (PN AM9624), and RNA 6000 Ladder (PN AM7152) were from Thermo Fisher Scientific (Waltham, MA).

Samples: The packaged AAV8 of pAV-CMV-GFP ($\sim 2.62 \times 10^{13}$ GC/mL), packaged AAV8 of pAV-CMV-BuB1, and AAV8-CMV-GFP reference standard with full capsids (PN RS-AAV8-FL) were from Charles River (Rockville, MD). The AAV1-CMV-GFP (PN SL100803), AAV2-CMV-GFP (PN SL100812), AAV2-CMV-LacZ (PN SL100854), AAV5-CMV-LacZ (PN SL100857) and AAV9-CMV-LacZ (PN SL100868) were from SignaGen Laboratories (Rockville, MD).

Instrument and software: A BioPhase 8800 system (PN 5083590) equipped with LIF detection was from SCIEX. The excitation wavelength was 488 nm, and the emission wavelength was 520 nm for AAV genome integrity analysis. A 600 nm bandpass emission filter (PN 5085177) from SCIEX was used for CE-LIF analysis of AAV capsid proteins. Data acquisition and analysis were performed using BioPhase software, version 1.0 (SCIEX).

Sample preparation for AAV capsid protein analysis by CE-SDS-LIF on the BioPhase 8800 system: A 20 μ L sample of AAV was diluted to 1×10^{11} GC/mL with 1x PBS. The diluted AAV sample was then mixed with 20 μ L of sample buffer from the BioPhase CE-SDS Protein Analysis kit and 4 μ L of 1M DTT and incubated at 70°C for 10 minutes. After samples were cooled to room temperature, 2 μ L of 1 mg/mL Chromeo P503 dye was added, and the mixture was incubated at 70°C for another 10 minutes. After the samples were again cooled to room temperature, 154 μ L of water was added to the mixture. AAV1, AAV2, and AAV8 samples were all prepared following the same sample preparation procedure. A 100 μ L sample of the diluted, prepared solution was transferred into a sample well on the sample plate. Analysis was then performed on the BioPhase 8800 system using separation conditions described previously.²

Sample preparation for AAV capsid titer determination by CE-SDS-LIF on the BioPhase 8800 system: The packaged AAV8 of pAV-CMV-GFP (2.62×10^{13} GC/mL) was used as a standard and serially diluted 2-fold with 1x PBS to generate results to build a standard curve. A total of 15 serial dilutions were made with AAV concentration in a range of 2.62×10^{13} GC/mL to 1.60×10^9 GC/mL. From each diluted standard solution, 15 μ L was removed and added to 15 μ L of sample buffer from the BioPhase CE-SDS Protein Analysis kit and 3 μ L of 1M DTT and incubated at 70°C for 10 minutes. After the mixture was cooled to room temperature, 1.5 μ L of 1 mg/mL Chromeo P503 dye was added, and the mixture was incubated at 70°C for another 10 minutes. After the samples were cooled to room temperature, 115.5 μ L of nuclease-free water was added to the mixture to quench the reaction. An AAV8-CMV-GFP reference standard with full capsids from Charles River was used as the test sample, and 15 μ L of it was labeled with Chromeo p503 dye as described above. Then, 90 μ L of the diluted, prepared sample solution was transferred into a sample well of the sample plate for analysis on the BioPhase 8800 system. After CE separation, results were analyzed using the BioPhase 8800 software. The corrected peak area of the VP3 peak for each dilution point of the standard was plotted against the AAV titer in GC/mL to generate the standard curve. A linear equation was obtained from the standard curve. The capsid titer of the AAV test sample was determined using the corrected peak area of the VP3 peak of the test sample and the linear equation.

Sample preparation for AAV genome integrity analysis by CGE-LIF on the BioPhase 8800 system: The AAV samples were prepared following a simplified workflow for genome release and purification. First, 20 μ L of each AAV sample was mixed thoroughly with the binding buffer from the QIAquick PCR purification kit. The manufacturer's instructions were followed except that the column was washed twice, and the AAV genome sample was eluted from the column using 50 μ L of 10x diluted elution buffer (Buffer EB from the kit diluted with nuclease-free water). Then, 10 μ L of the eluted AAV genome solution was mixed with 40 μ L of nuclease-free water and 50 μ L of SLS, heated at 70°C for 10 minutes, and immediately cooled on ice for 5 minutes. The final 100 μ L mixture was transferred into a sample well of the sample plate. CE separation conditions were the same as described previously.³

Sample preparation for AAV genome titer determination by CGE-LIF on the BioPhase 8800 system: Packaged AAV8 of pAV-CMV-GFP (2.62×10^{13} GC/mL) was used as a standard and serially diluted 2-fold with 1x PBS to generate results for building a standard curve. A total of 14 serial dilutions were made with AAV concentration in a range of 2.62×10^{13} GC/mL to 3.20×10^9 GC/mL. From each diluted standard solution, 110 μ L

was used for genome extraction using the QIAquick PCR purification kit, following the manufacturer's instructions, except the column was washed twice, and the AAV genome sample was eluted from the column using 50 μ L of 10x diluted elution buffer. Before loading onto the instrument for analysis, 10 μ L of the eluted AAV genome solution was prepared as described above. As a test sample, an AAV8-CMV-GFP reference standard with full capsids from Charles River was used for genome extraction using the QIAquick PCR purification kit, following the same process as for each standard dilution. After CE separation, results were analyzed using the BioPhase 8800 software. The corrected peak area of the intact genome peak for each dilution point of the standard was plotted against the AAV titer in GC/mL to generate the standard curve. The genome titer of the AAV test sample was determined using the corrected peak area of the intact genome peak in the test sample and the linear equation.

Calculation of the percentage of AAV full capsids: The percentage of full capsids in the test sample was determined by dividing the genome titer by the capsid titer from the same test sample and multiplying it by 100.

Analytical challenges for AAV analysis

AAVs are safe and effective gene delivery vehicles to drive persistent transgene expression in gene therapy. Over 100 unique clinical trials with recombinant AAV (rAAV) vectors have been approved by US and European regulatory agencies.¹ Rapid growth in this gene therapy space created a need to manufacture rAAVs at a large scale and with high quality. Table 1 shows the reported CQAs and their potential impact on the quality of rAAVs. These CQAs must be characterized and monitored throughout the manufacturing process to ensure product efficacy and safety. The full-to-empty capsid ratio is especially important because full capsids deliver intact transgenes into target cells and directly impact the potency of AAV therapeutics. Nonfunctional empty capsids reduce transduction efficiency and might exacerbate vector-related immunotoxicity. Furthermore, partially filled capsids containing truncated genomes or fragments of host cell nucleic acids or vectors could cause low efficacy, genotoxicity, and immunotoxicity. Therefore, it is essential to use a high-resolution sizing methodology to resolve the intact genome from the partial genome and other impurities. Monitoring the full and empty capsid ratio is critical for process improvements in removing empty and partial capsids for better efficacy and safety of AAV-based gene therapy. Currently, biopharma scientists often use

Table 1. Quality considerations for rAAV.

CQA	Vector Potency	Immunogenicity	Transduction efficiency	Packaging efficiency
<i>Viral protein profile and ratio</i>	●	●	●	●
<i>Genome integrity</i>	●	●	●	
<i>Titer</i>	●	●	●	
<i>Full and empty capsid ratio</i>	●	●	●	
<i>Protein impurities</i>	●	●		
<i>Nucleic acid impurities</i>	●	●		

different platforms to analyze different CQAs. Table 2 compares current technologies to determine multiple AAV CQAs, their limitation, and the cost of each platform. The information in Table 2 indicates that comprehensive AAV analysis of multiple CQAs on different platforms is time-consuming and costly. Additionally, different laboratories are required to house different platforms and specially trained scientists are needed to run different instruments and perform data analysis using additional software. In this technical note, a single multi-capillary electrophoresis system, the BioPhase 8800 system, was utilized to provide a complete set of gene therapy product quality analysis for multiple AAV CQAs (Figure 2). Two main workflows were used. The CE-SDS-LIF workflow was used to analyze viral protein profile and ratio, protein impurities, and capsid titer. The CGE-LIF workflow was used for high-resolution analysis of genome integrity and genome titer. A new approach was employed to determine the percentage of full capsids by a calculation, as shown in Figure 1. Results in this technical note demonstrate that the BioPhase 8800 system provides a fast, high-throughput, comprehensive analysis of multiple AAV CQAs.

Table 2. Comparison of current technologies to determine multiple AAV CQAs. Abbreviations: ELISA: enzyme-linked immunosorbent assay; SDS-PAGE: Sodium Dodecyl Sulfate-polyacrylamide gel electrophoresis; IEX: ion exchange chromatography; SEC: size exclusion chromatography; MALS: multi-angle light scattering.

Technology	Limitation	Cost of platform
Real-time PCR (qPCR) Digital PCR (dPCR)	qPCR: Low accuracy and precision dPCR: False positive, limited sizing	\$~20,000 to \$~120,000
ELISA/SDS-PAGE	Low accuracy and precision	\$~20,000
Spectrophotometry	Impurity interferences; low accuracy	\$~18,000
Chromatography (IEX and SEC)	Needs a large sample amount; cannot distinguish partial from full capsids	\$~80,000
SEC-MALS	Cannot distinguish partial from full capsids Assumes 100% mass recovery and needs extinction coefficients	\$~100,000
Electron microscopy (EM)	Low throughput; time-consuming, Cannot distinguish partial from full capsids; expensive	\$~100,000 to \$~7 million
Analytical ultracentrifugation (AUC)	Low throughput; needs a large sample amount Expensive; difficult to implement as a QC release assay	\$~100,000 to \$~400,000
Charge detection mass spectrometry (CDMS)	Low throughput; expensive; difficult to validate as a QC assay	>\$750,000



BioPhase 8800 system

- ✓ 8-capillary sample analysis
- ✓ High sensitivity with LIF
- ✓ Compliant-ready software
- ✓ Low sample consumption



Complete gene therapy product quality analysis

Viral vector protein characterization

- Viral protein profile and ratio
- Protein impurity screening
- Capsid titer

Capsid and transgene analysis

- Transgene purity and size analysis
- Genome titer
- Full to empty capsid ratio

Residual nucleic acid analysis

- Host cell DNA/RNA screening

Figure 2. Comprehensive AAV analysis on the BioPhase 8800 system for key AAV CQAs. High-throughput, high-resolution sample analysis is enabled using the pre-assembled cartridge with 8 capillaries. High sensitivity is achieved with laser induced fluorescence detection or LIF. The system comes with compliant-ready software. A small sample volume is sufficient for analysis. This system offers flexible solutions designed for all phases of the drug development process including discovery, development and QC.

High-resolution AAV capsid characterization

The CE-SDS-LIF workflow shown in Figure 3 was used for high-throughput, high-resolution AAV capsid characterization. AAV samples were denatured in the presence of DTT and the sample buffer from the BioPhase CE-SDS protein analysis kit, then labeled with Chromeo p503 dye, and further separated on the BioPhase 8800 system using a BioPhase BFS capillary

cartridge. As shown in Figure 4, baseline resolution was achieved in separating VP1, VP2, and VP3 peaks for AAV samples of 3 different serotypes: AAV1, AAV2, and AAV8. These peaks were also well-separated from impurities. Furthermore, baseline resolution was achieved in separating the VP3' (VP3 variant) peak from the VP3 peak for all 3 serotypes. These results indicate that the CE-SDS-LIF workflow works for all 3

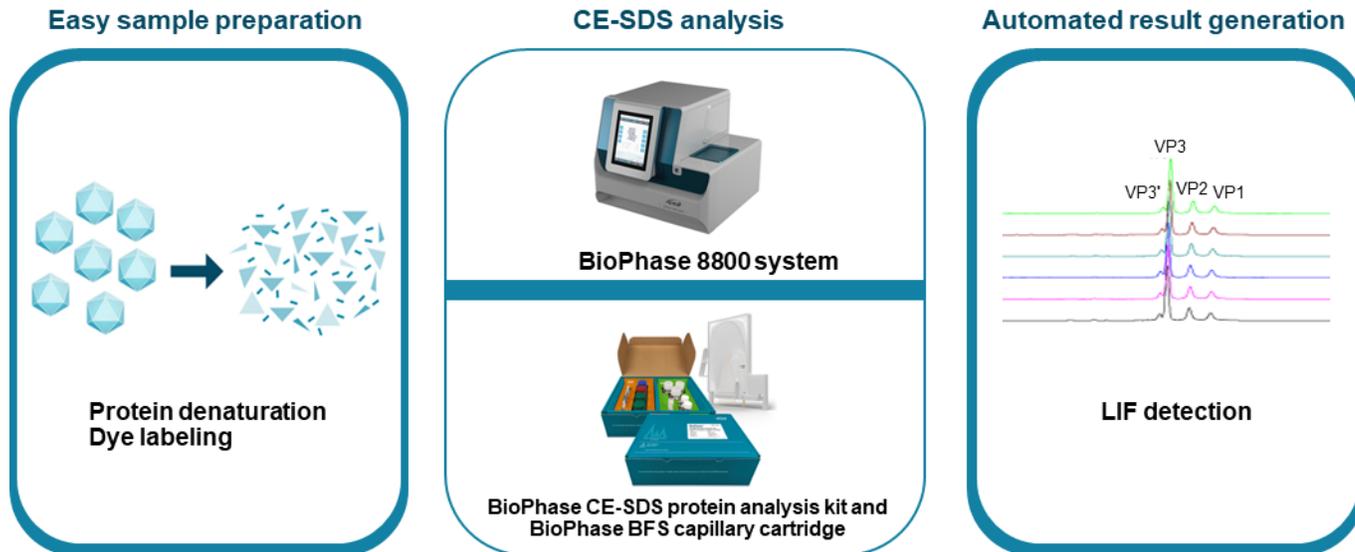


Figure 3. High-throughput, high-resolution CE-SDS-LIF workflow for AAV capsid characterization on the BioPhase 8800 system. Samples were denatured in the presence of SDS, labeled with a fluorescent dye, and separated by CE with LIF detection to achieve high sensitivity. Data were collected and analyzed automatically. Three viral capsid proteins and the VP3 variant peaks were well-separated for easy assessment of their ratio.

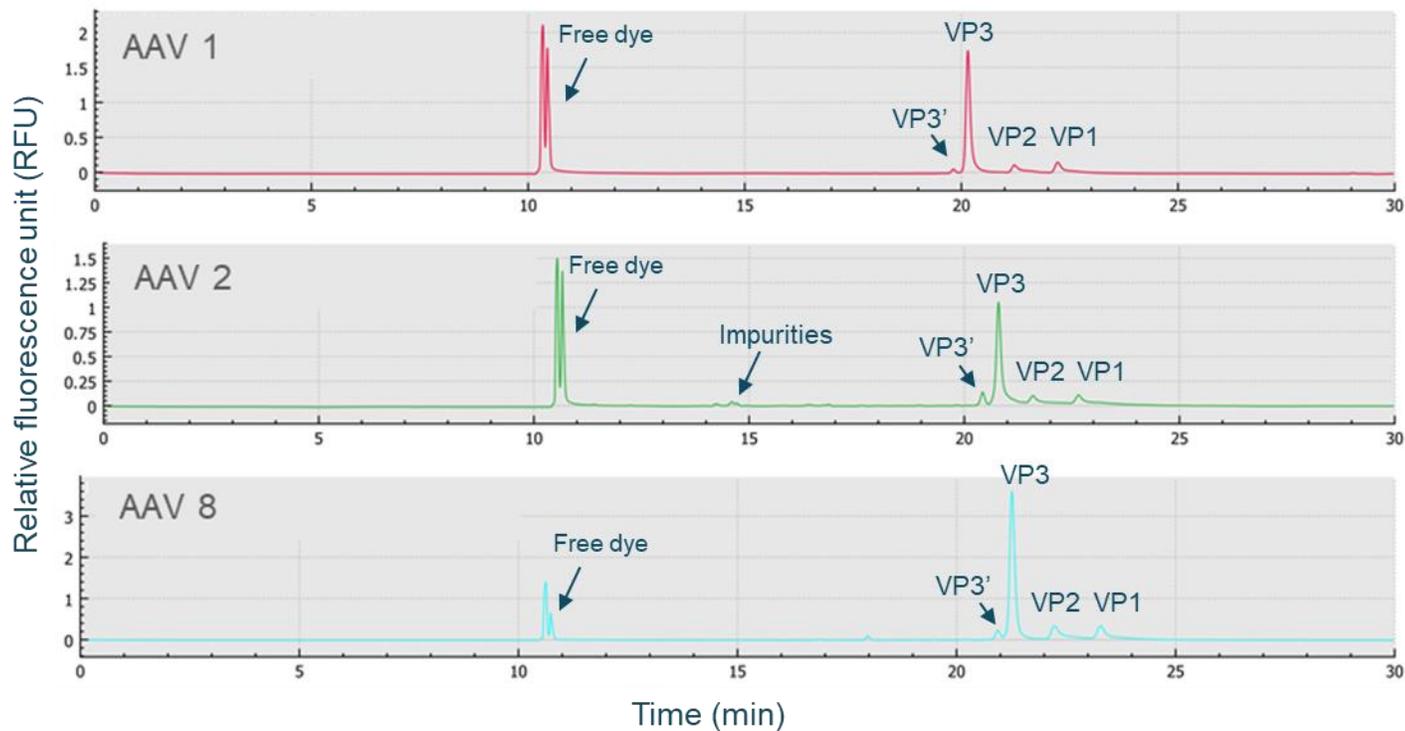


Figure 4. Analysis of capsid proteins from AAV samples of different serotypes using the same CE-SDS-LIF workflow. Samples of 3 different serotypes, AAV1, AAV2, and AAV8 were analyzed on the BioPhase 8800 system. Baseline resolution of VP1, VP2, VP3 and VP3' was achieved for all 3 serotypes. The presence of impurities were detected as well. These results demonstrate that this workflow is serotype-independent.

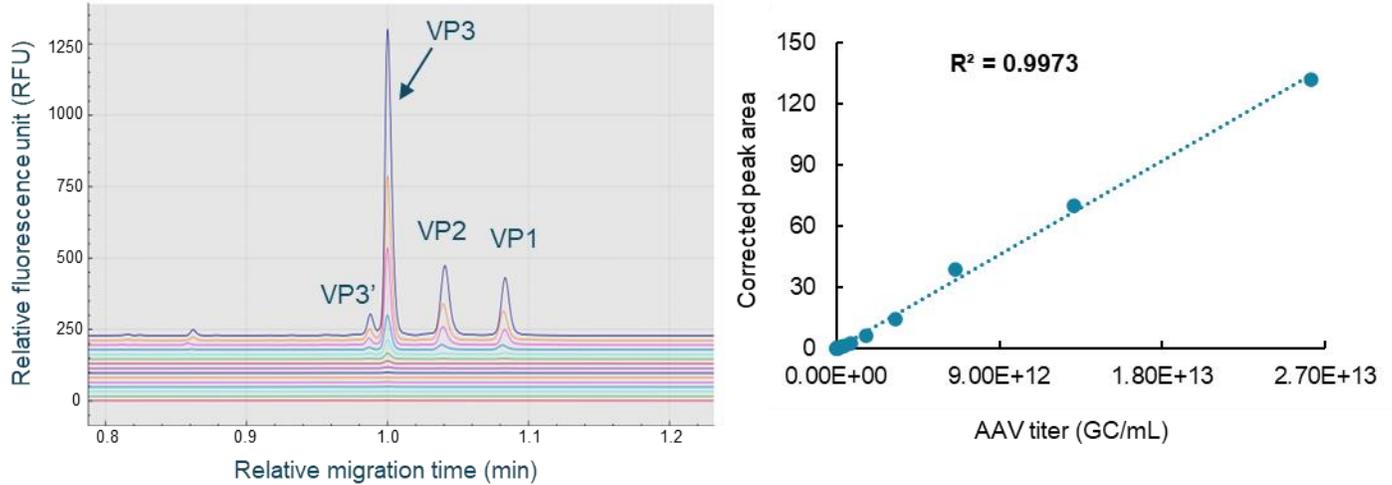


Figure 5. Fast, high-throughput AAV capsid titer determination using the CE-SDS-LIF workflow on the BioPhase 8800 system. The panel on the left is the overlay of electropherograms obtained with samples from different dilution points. The panel on the right is the plot of the corrected peak area of VP3 against the AAV titer in GC/mL with excellent linearity. The LOD was 1.60×10^9 GC/mL. The LOQ was 6.40×10^9 GC/mL. The linear detection range was 6.40×10^9 GC/mL to 2.62×10^{13} GC/mL.

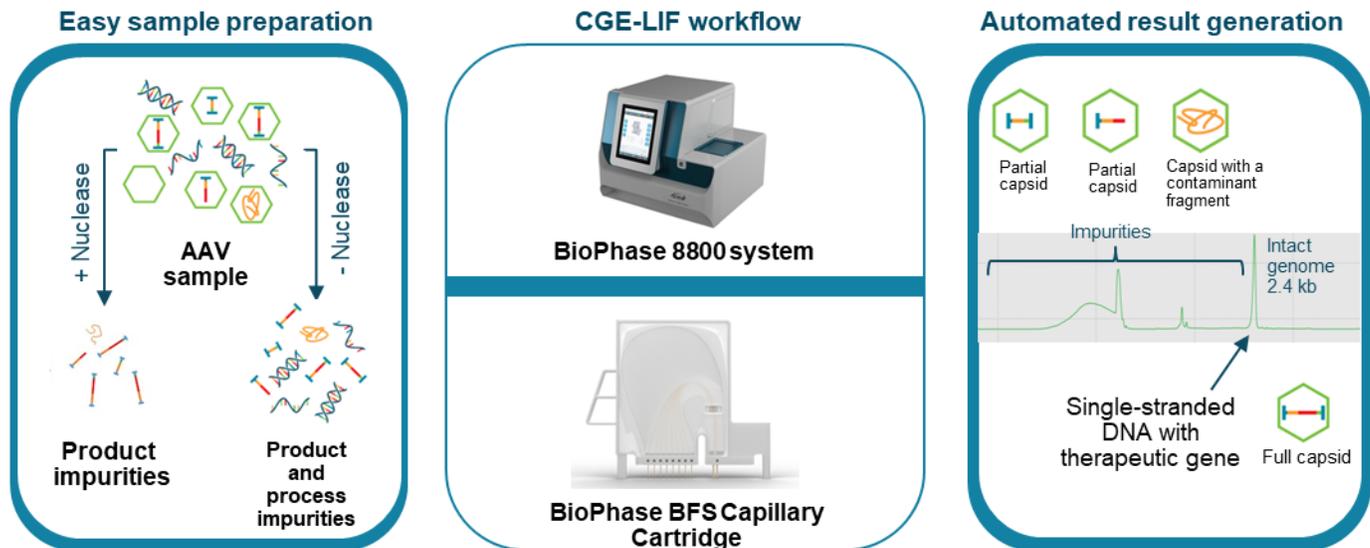


Figure 6. High-throughput, high-resolution CGE-LIF workflow for AAV genome integrity analysis on the BioPhase 8800 system. Two sample preparation options can be used. AAV samples can be treated with nuclease prior to nucleic acid extraction for analysis of product-related impurities. Alternatively, nucleic acids can be released and purified directly from the AAV samples without nuclease treatment for analysis of both product and process related impurities. Extracted nucleic acids were analyzed by CGE-LIF with the use of a fluorescent dye, SYBR™ Green II Gel Stain*, for high sensitivity analysis. The intact genome was well separated from partial genome and other impurities.

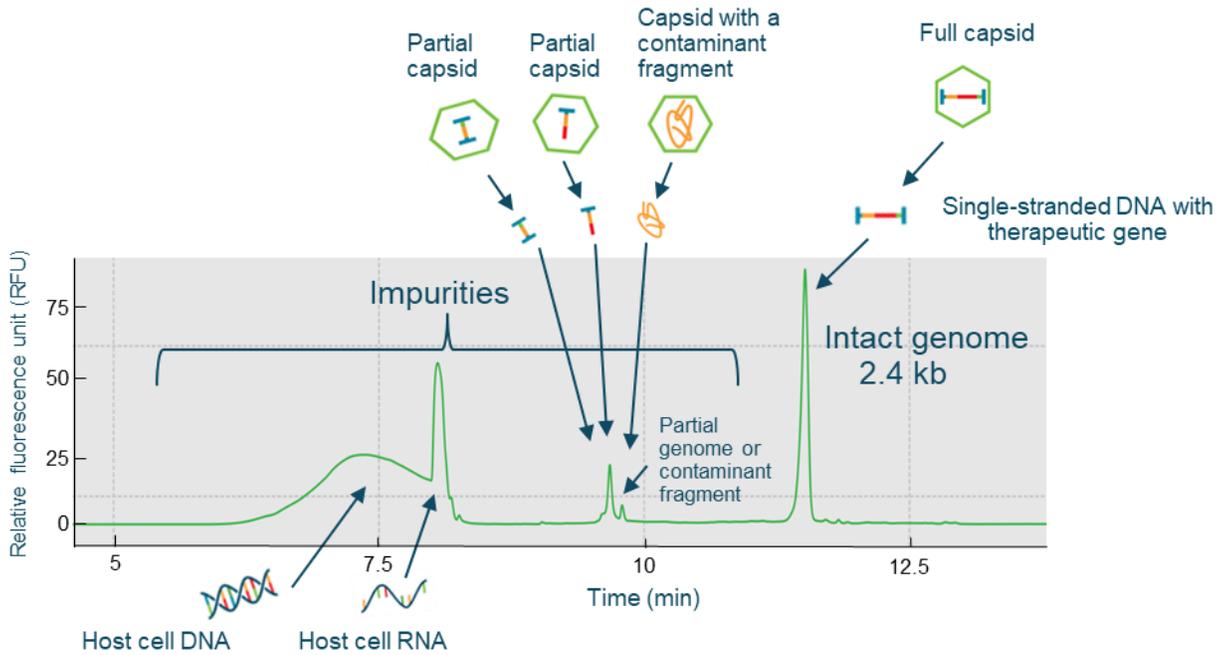


Figure 7. High-resolution genome integrity analysis on the BioPhase 8800 system. The intact genome of 2.4 kb was well separated from potential partial genomes, contaminant fragments, small-sized host cell RNA, and host cell DNA impurities.

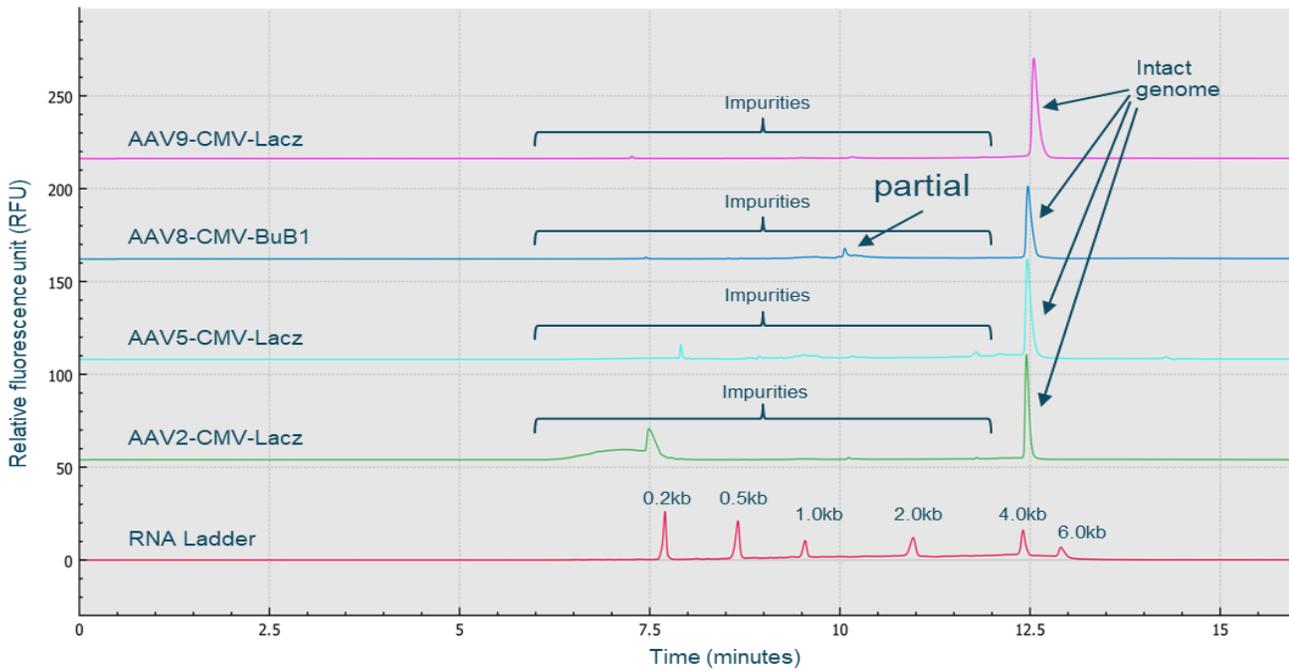


Figure 8. High-throughput, high-resolution genome integrity analysis of multiple AAV serotypes using the CGE-LIF workflow on the BioPhase 8800 system. Nucleic acids extracted from 4 different serotypes, AAV2, AAV5, AAV8, and AAV9 were analyzed. High-resolution separation of the intact genome from impurities was achieved for all 4 serotypes, demonstrating that this workflow can be implemented independent of serotype.

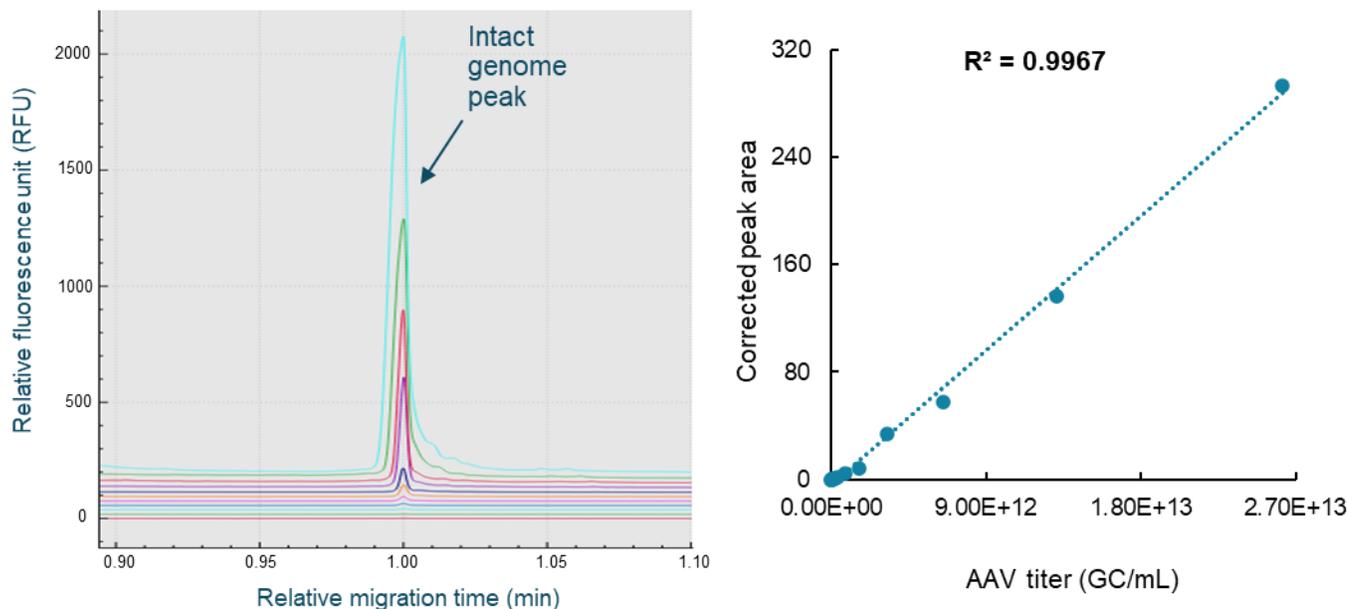


Figure 9. Fast, high-throughput AAV genome titer determination using the CGE-LIF workflow on the BioPhase 8800 system. The panel on the left shows the overlay of electropherograms obtained with samples from different dilution points. The panel on the right shows the linear plot of the corrected peak area of the intact genome peak against the AAV titer in GC/mL. The LOD was 1.28×10^{10} GC/mL. The LOQ was 2.56×10^{10} GC/mL. The linear detection range was 2.56×10^{10} GC/mL to 2.62×10^{13} GC/mL.

serotypes and is a serotype-independent workflow. The VP3' and VP3 peaks differ by 8 amino acids due to their difference in translation start site, as reported previously.⁴ The baseline resolution between VP3 and VP3' peaks demonstrates the outstanding resolving power of CE on the BioPhase 8800 system.

Fast, high-throughput AAV capsid titer determination

An AAV8 sample with a known titer was used as the standard. It was serially diluted 2-fold for 15 dilutions with 1x PBS buffer. Sample from each dilution point was denatured in the presence of DTT and the sample buffer, labeled with the Chromeo p503 dye, and separated on the BioPhase 8800 system. As shown in the left panel of Figure 5, baseline resolution was obtained for VP1, VP2, VP3, and VP3' peaks. The Optimizer feature on the "Integration" tab in the BioPhase 8800 software was enabled to select a best-fit analysis automatically. After minor adjustments to peak integration parameters, results were exported for simple processing in Microsoft Excel. The corrected peak area for the VP3 peak was plotted against the AAV titer in GC/mL. As shown in the right panel of Figure 5, excellent linear curve fitting was achieved, evidenced by an R^2 value of 0.9973. The limit of detection (LOD)

was 1.60×10^9 GC/mL. The limit of quantification (LOQ) was 6.40×10^9 GC/mL with a linear range of about 3 logs. A linear equation was obtained from the linear curve fitting. An AAV8 test sample was processed in the same experiment, using the same workflow as the AAV standard. The corrected peak area of the VP3 peak from this test sample was used to calculate its AAV capsid titer using the equation from this linear curve. The capsid titer for this test sample was 2.65×10^{12} GC/mL.

High-resolution AAV genome integrity analysis

The CGE-LIF workflow shown in Figure 6 was used for high-throughput AAV genome integrity analysis. Two sample preparation options can be used. A fast method was used for the analysis of both product- and process-related impurities with direct extraction of the single-stranded DNA genome from the AAV sample using the Qiagen PCR purification kit. An optional benzonase treatment step can be used for the analysis of product-related impurities in the AAV sample. The extracted AAV genome sample was analyzed by CGE-LIF on the BioPhase 8800 system. Figure 7 shows the high-resolution results obtained with an AAV2-CMV-LacZ sample. The 2.4 kb intact genome peak was well-separated from 2 distinct peaks with sizes around 1 kb and small-sized impurities of around 0.2 kb.

The 2 distinct peaks with sizes around 1 kb could be potential partial genome peaks or contaminant fragments from the host cell genome or plasmid vectors. There are 2 groups of small-sized impurities: the one with a sharp peak shape could be host cell RNA impurities, while the broad peak could be small-sized host cell DNA fragments. Since the BioPhase BFS capillary cartridge has 8 capillaries, the same high-resolution genome integrity analysis can simultaneously be performed for up to 8 AAV samples. Figure 8 shows the results obtained with 4 different AAV samples: AAV2-CMV-LacZ, AAV5-CMV-LacZ, AAV9-CMV-LacZ and AAV8-CMV-BuB1, and the RNA ladder. High-resolution of intact genome peaks and partial and small size impurities was achieved for all 4 AAV samples. This result demonstrates that the CGE-LIF workflow on the BioPhase 8800 system is a serotype-independent workflow.

Fast, high-throughput AAV genome titer determination

The same AAV8 sample with known titer used in the aforementioned AAV capsid titer determination was also used as the standard for building the standard curve for AAV genome titer determination. The AAV8 sample was serially diluted 2-fold for 11 dilutions with 1x PBS buffer. The sample from each dilution point was subjected to nucleic acid extraction using the Qiagen PCR purification kit. The extracted nucleic acid samples were heat-treated and analyzed on the BioPhase 8800 system using the multi-capillary BioPhase BFS capillary cartridge. Samples from the 14 dilution points and a test sample were completed in 2 60-minute runs. After CE separation, results were analyzed with the BioPhase 8800 software. Results were exported. Data processing was performed in Microsoft Excel. The corrected peak area of the intact genome peak was plotted against the AAV titer of the standard (Figure 9). Excellent linearity was demonstrated by the R^2 value of 0.9967. The LOD was 1.28×10^{10} GC/mL and the LOQ was 2.56×10^{10} GC/mL with a linear range of about 3 logs. The AAV genome titer of the AAV8 test sample was 2.21×10^{12} GC/mL.

A new approach for AAV full, partial and empty capsid analysis

Since the capsid titer and the genome titer for the same AAV8 test sample were determined in the previous experiments, the percentage of the full capsids in this test sample was obtained by dividing the genome titer of 2.21×10^{12} GC/mL (value A) by the capsid titer of 2.65×10^{12} GC/mL (value B) and multiplying by 100, as shown in Figure 10. The percent of full capsids in this AAV8 sample was 83.4%. No additional experiments were needed. As shown in Figure 11, the percentage of full capsids obtained by CE was consistent with the values determined by

two orthogonal technologies: transmission electron microscopy (TEM) and AUC.

Benefits of comprehensive AAV analysis of multiple CQAs on a single, multi-capillary CE system

As listed in Table 1, multiple CQAs must be characterized and monitored throughout the AAV manufacturing process to ensure the efficacy and safety of AAV-based gene therapy drugs. Currently, biopharma scientists often use multiple, different platforms for the analysis of all key AAV CQAs. As shown in

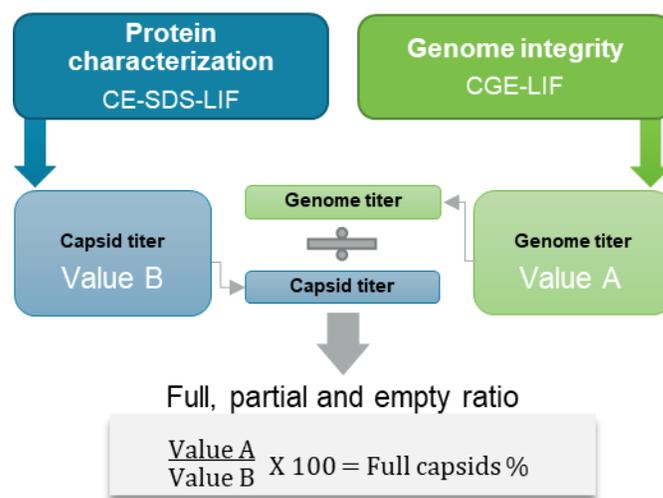


Figure 10. A simple calculation for determining the percentage of full capsids in an AAV sample. The percentage of the full capsids is calculated by dividing the genome titer (value A) by the capsid titer (value B), and multiplying by 100.

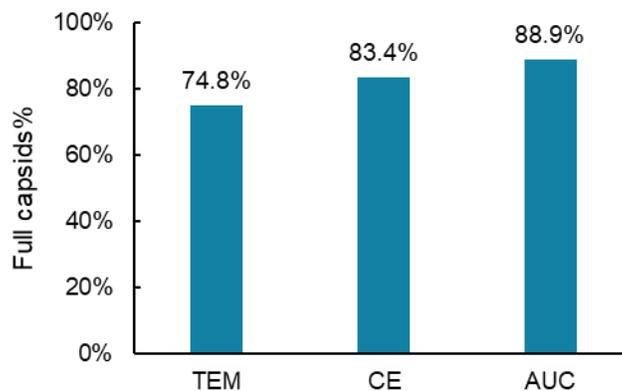


Figure 11. Comparison of the percentage of full capsids determined by three different technologies: TEM, CE, and AUC. The TEM and AUC results were obtained from the vendor of the AAV test samples.

A combination of different technologies



A single CE platform: BioPhase 8800 system



Figure 12. Comparison of the timeline for analysis of all key AAV CQAs per sample between different methods. Time for sample preparation is included. When a combination of 4 different technologies is used, the total amount of time needed for all analyses is 13 to 22 hours.⁵⁻⁶ However, the same number of CQAs can be analyzed on the BioPhase 8800 system in 8 hours. Therefore, using the BioPhase 8800 system saves significant time compared to an approach using a combination of different platforms.

Figure 12, when other platforms are used, the total time for comprehensive analysis of all key AAV CQAs for an AAV sample would be 13-22 hours including sample preparation time. In addition, multiple laboratories might be needed to house instruments for different platforms. Analysts with different skill sets are also required to perform tests on different instruments. In contrast, a comprehensive analysis of all key CQAs can be done on a single platform, the BioPhase 8800 system, within 8 hours. With its tabletop design, the BioPhase 8800 system would not only save cost in equipment purchasing but also save laboratory space, enabling a streamlined quality test process with high efficiency.

Conclusions

- It is essential to characterize and monitor multiple CQAs to ensure the safety and efficacy of AAV drug products
- High-throughput, serotype-independent, comprehensive analysis of all key CQAs for the same AAV sample was achieved on a single CE platform within a single day
- Fast result generation for all methods was accomplished using the multi-capillary cartridge, saving time and costs
- High-resolution full, partial, and empty capsid analysis as part of a comprehensive set of analytical methods for AAV was achieved on the BioPhase 8800 system
- High-resolution, high-throughput gene therapy platform method development from discovery through quality control (QC) can all be carried out on the BioPhase 8800 system

- Platform methods on the BioPhase 8800 system with fast process times can shorten development timelines, save cost, streamline the QC process and accelerate time to market

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Patent pending.

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