Acceleration of method optimization for AAV capsid purity analysis using multi-capillary electrophoresis platform

BioPhase 8800 system

Tingting Li and Sahana Mollah
Biopharma
SCIEX/Brea

Sample preparation conditions play a crucial role in the assessment of the capsid protein purity profile of adeno associated viruses (AAV's). Multiple factors such as incubation temperature and sample buffer concentrations affect the AAV capsid protein stability, inducing separation artifact in the final results. Additionally, the analysis time for optimization of multiple experimental parameters can be time-consuming. Here, we show how the optimization time can be significantly reduced from 48 hrs when using a single capillary electrophoresis (CE) system to 4 hrs using the multi-capillary BioPhase 8800 system. This 8 capillary system allows for faster processing of samples and overall increased throughput.

Adeno associated viruses (AAV) are a popular viral vector in gene therapy and are composed of capsid proteins which have critical implications in the efficacy of the viral vector.[1] A robust analytical method for assessing capsid purity that can provide reliable results in a timely fashion is desirable. CE-SDS (capillary electrophoresis sodium dodecyl sulfate) is a popular application technique for protein analysis, quantitation, and profiling in the biopharmaceutical industry because it offers high specificity, resolution, reproducibility and is automation-friendly.[2] More specifically to AAV analysis, CE-SDS assay results are consistent across serotypes, an important quality to consider when parameters, such as temperature and sample buffer conditions, affect the stability and assembly of the capsid proteins.[3,4] Method optimization for these studies was done using a single capillary format, which can limit throughput.

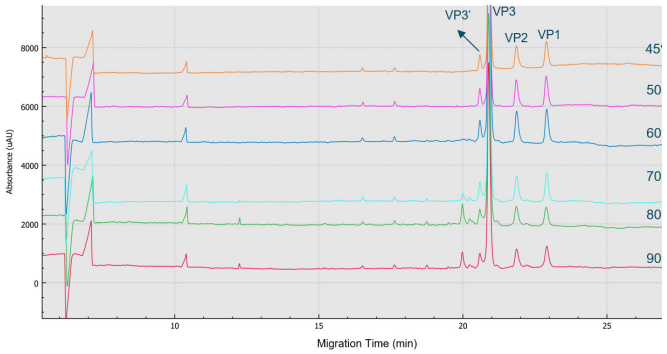


Figure 1. Electropherograms of the analysis at temperatures ranging from 45° C to 90° C to determine the optimized incubation temperature.

Figure 2. The BioPhase 8800 system equipped with LIF and UVdetectors and consumable/reagent kits.

This technical note highlights how a multi-parameter approach using CE-SDS with laser-induced fluorescence detection (LIF) on BioPhase 8800 system can significantly expedite methods development and optimization of AAV capsid purity analysis and thus improve throughput.

Key features

- Optimizing 3 separate parameters takes 4 hours to complete on the multi-capillary BioPhase 8800 system compared to 48 hours on the single capillary PA 800 Plus.
- Easy-to-use new processing software provides flexibility in viewing and reporting of data results.
- Optimized parameter settings obtained on the BioPhase 8800 system correlate well with values on the PA 800 Plus.
- Efficient labeling by Chromeo dye P503 and a simple sample preparation procedure completed in less than 1 hour.
- Analysis of 4 rows of samples were completed in 4 hours using the BioPhase 8000 system compared to 48 hours for single capillary platform.

Methods

Chemicals: The SDS-MW Analysis Assay kit (Part # 390953, SCIEX, Brea, CA) with the SDS-MW gel buffer and sample buffer were from SCIEX (Framingham, MA, U.S.A.). The Chromeo P503 dye (PN 15106) was from ACTIVE MOTIF (Carlsbad, CA, U.S.A.). Sodium dodecyl sulfate (PN L4390-100G), and 2-mercaptoethanol (PN M3148-100ML), and all other chemicals were from Sigma Aldrich (St. Louis, MO, U.S.A.).

Samples: Packaged AAV8 of pAV-CMV-GFP with titer at 1.00 X 10^{13} GC/mL (titer as supplied by vendor) was purchased from Vigene Biosciences (Rockville, MD, U.S.A.). An AAV1-CMV-GFP (Cat # SL100803, Lot # AAV62058) sample and an AAV2-CMV-GFP (Cat # SL100812, Lot # AAV62099) were purchased from SignaGen Laboratories. All the samples with different sample preparations were loaded onto a 96 well plate for analysis, as shown in figure 3.

Sample preparation for AAV CE-SDS-UV: The method is developed and optimized using AAV8 samples. For the optimization of the sample preparation procedure, 5 µL of AAV8 sample solution was mixed with 5 µL of incubation buffer and 1.5 µL of 2-mercaptoethanol in a 0.65 mL microcentrifuge tube at a constant temperature for 10min. Samples were then allowed to return to room temperature before 38.5 µL of DI water was added to the mixture. The diluted mixture was transferred into the appropriate well of the injection sample inlet plate for analysis on the BioPhase 8800 system. Different incubation buffers and incubation temperatures were evaluated to achieve optimal sensitivity and minimum sample preparation. 1X, 2X, 4X, 5X, 8X,10X, and 20X dilutions of sample buffer from the SDS-MW kit (100 mM Tris-HCl pH 9.0, 1% SDS) and SDS solutions at different concentrations from 0.25%-5% were compared in this technical note. The incubation temperature ranging from 40° C to 90° C was also evaluated for the optimal sample preparation conditions.

Sample preparation for AAV CE-SDS-LIF BioPhase: 20 µL of AAV sample, diluted to 1 X 1011 GC/mL in PBS, was mixed with 20 µL of Tris sample buffer and 4 µL of 1M DTT and incubated at 60° C for 10 min, followed by adding 2 µL of 1 mg/mL Chromeo P503 dye[5] and incubated at 60° C for another 10 min. After cooling the samples down to room temperature, 154 µL of DI water was added to the mixture. AAV1, AAV2, and AAV8 were prepared following the sample preparation procedure. 100 µL of the diluted, prepared sample solution was transferred into the well of the injection sample inlet plate for analysis on the BioPhase 8800 system. The leftover 100 µL of the diluted prepared sample solution was transferred to the sample vial for analysis on the PA 800 Plus.

Instrumentation: All single capillary electrophoresis analyses were carried out using a PA 800 Plus Pharmaceutical Analysis system configured with an LIF detector and solid-state laser with an excitation wavelength of 488 nm and a 600 nm bandpass emission filter from Edmund Optics (Barrington, NJ). CE-SDS separations were performed using the EZ-CE cartridge (Part # A55625) with a 20 cm effective length (30 cm total length). A 50 µm I.D. bare fused silica capillary was filled with the SDS-MW gel-buffer system.

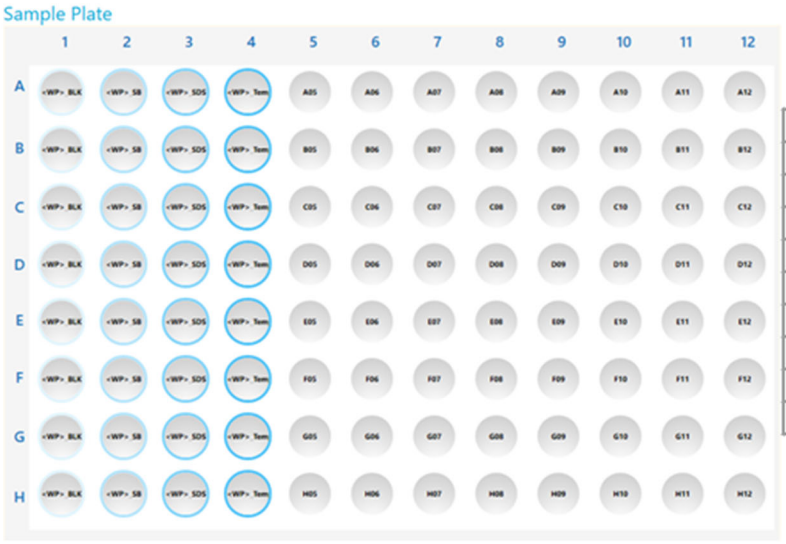
Capillary conditioning was: 0.1 M NaOH rinse for 3 minutes at 70 psi,0.1 M HCl rinse for 1 minute at 70 psi, HPLC grade water rinse for 1 minute at 70 psi and SDS-MW gel buffer rinse for 10 minutes at 80 psi before each run. The applied electric field strength was 500 V/m for all capillary electrophoresis analyses in reversed polarity mode (anode at the detection side). The samples were electrokinetically injected at 5 kV for 20 seconds. The 32 Karat software version 10.1 was used for data acquisition and processing.

The multiplexed separation utilized the BioPhase 8800 system. The gel-buffer system, capillary conditioning, injection, and separation conditions (Figure 4) were the same as those for the single capillary analyses. Separations were accomplished in the BioPhase BFS Capillary Cartridge – 8 x 30 cm. The BioPhase software version 1.0 was used for data acquisition and processing.

Results and discussion

Sample buffer optimization

Different sample preparations and buffers were evaluated to achieve optimal sensitivity and resolution of the capsid proteins for the AAV 8 serotype on the BioPhase 8800 system using CE-SDS-UV. One of the parameters optimized was the sample buffer. Sample buffer from the SDS-MW kit (100mM Tris-HCL, pH9.0, 1% SDS) was used at 1x-20x dilution range, as shown in Figure 5. All 7 dilution points were obtained from a 30 min single analysis on the multi-capillary. The analysis shows the best peak intensity was obtained using 1x sample buffer, which correlates well with previous studies on the PA 800 Plus.[3]



	Column #			
Capillary	1	2	3	4
A	Blank	1X SB	5% SDS	40° C
B	Blank	2X SB	4% SDS	45° C
C	Blank	4X SB	3% SDS	50° C
D	Blank	5X SB	2% SDS	60° C
E	Blank	8X SB	1.5% SDS	70° C
F	Blank	10X SB	1% SDS	80° C
G	Blank	20X SB	0.5% SDS	90° C
H	Blank	Blank	0.25% SDS	Blank

Figure 3. Configuration of the D.O.E. samples in the sample plate. Blankswere placed in row 1, column A to H. Blank samples were placed in row 1. Samples with various diluted sample buffer were placed in row 2, samples with different % SDS in row 3, and samples with different incubation temperatures in row 4

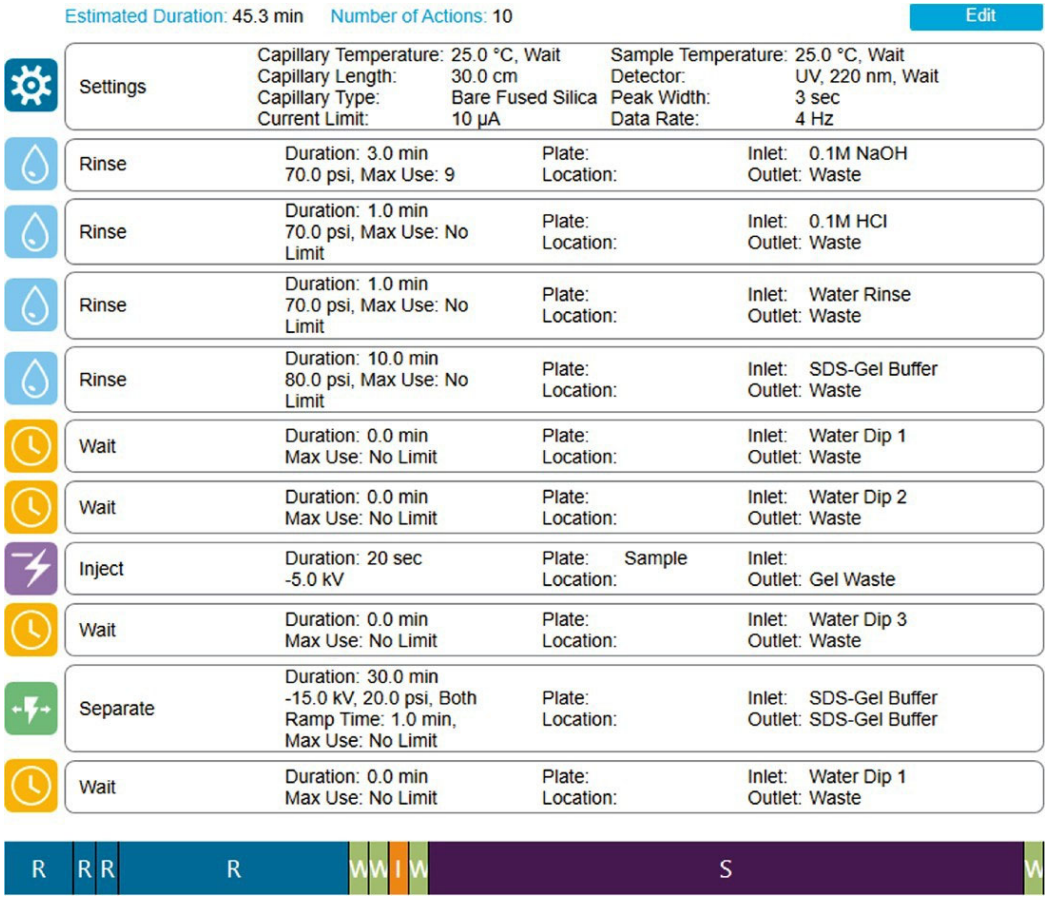


Figure 4. Separation method steps as shown using the BioPhase 8800 system

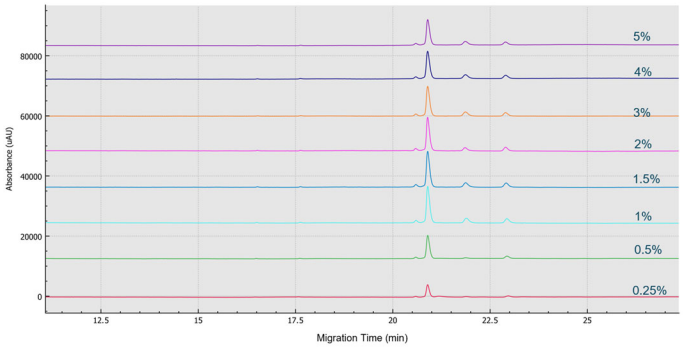


Figure 6. BioPhase 8800 electropherograms from CE-DS-UV sample analysis using various % SDS ranging from 0.25% to 5%. Optimized valueobtained was 1 - 1.5% SDS

Incubation temperature optimization

The optimization of the sample incubation temperature for the AAV8 serotype is shown in Figure 1. The peak intensity of all 3 capsids is optimized at 50° C. With increased incubation temperature greater than 50° C, heat-induced impurity peaks with increased intensity is observed while the VP3 protein peak intensity decreases. These smaller peaks are likely degradation products of the VP3 proteins. This method is optimized for the AAV 8 sample. The optimal incubation temperature can differ for different serotypes.

% SDS optimization

Figure 6 shows the optimization of the % SDS concentration used for the optimal method. 1% - 1.5% SDS provided the optimal peak shape and sensitivity. This provides sufficient amounts for protein binding and minimal residual salt concentration for the best efficiency of electrokinetic sample injection.

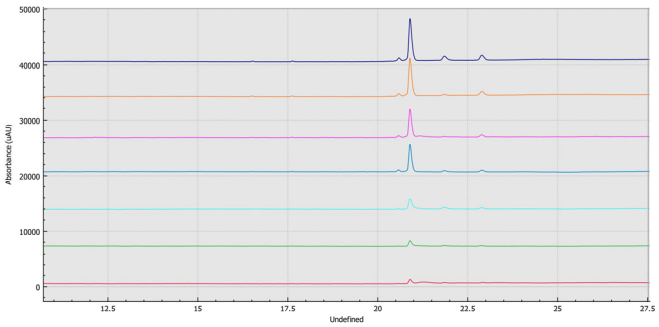


Figure 5. Sample buffer optimization with dilutions from 1x - 20x range using CE-SDS-UV on the BioPhase 8800 system. The best peak intensity for all 3 capsid proteins was obtained using 1x dilution.

The comparison between BioPhase 8800 and PA 800 Plus

To compare the data quality between the multi-capillary and the single capillary platforms, analysis of 3 different AAV serotypes, 1, 2, and 8, respectively, was performed on the PA800 Plus and BioPhase 8800 systems. The sensitivity and migration time of the 3 capsid proteins align well between the 2 systems (Figure 7). Additionally, as expected, the migration times obtained for each serotype also correlated well between the 2 systems.

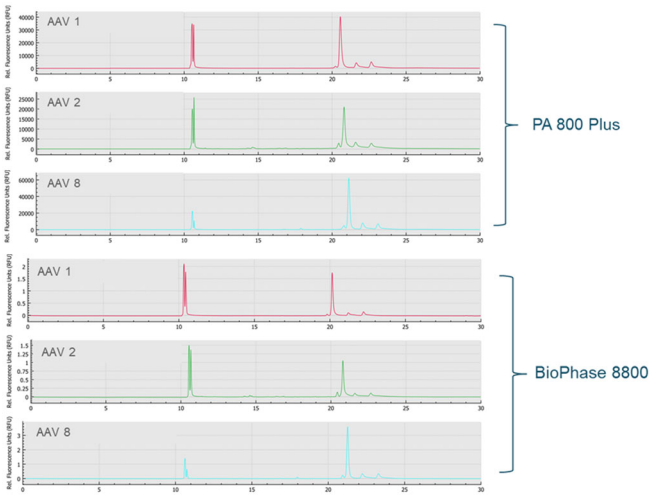


Figure 7. Comparison of CE-SDS-LIF analysis of AAV1, AAV2, and AAV8 on the single capillary PA 800 Plus and the multi-capillary BioPhase 8800 system using a LIF detector. Results obtained on both instruments correlate well. The analysis on the multi-capillary system was completed 3x faster than the single capillary system

Conclusions

- Multiplexing capability of the BioPhase 8800 allows for 12x faster analysis compared to the single capillary systems for the method optimization of AAV purity analysis by CE-SDS.
- The sample preparation with the Chromeo P503 dye labeling for LIF analysis was straightforward. It did not require any buffer exchange of sample cleanup, helping decrease the time for sample preparation and overall workflow completion time.
- Optimized conditions obtained on the multi-capillary system correlate well with the PA 800 Plus system values. Thus, providing a seamless and easy method transfer from the single to the multi-capillary platform.

References

1. Kewal, J.K.; Drug Delivery Systems,.2008; 437: 51–91

2. Ying, Shi.; Zen, Li.; Li, Jun; Anal. Methods, 2012,4, 1637-1642

3. Purity Analysis of Adeno- Associated Virus (AAV) Capsid Proteins using CE-SDS method, RUO-MKT-02-9761-A

4. Zhang, C.; Meagher, M. M. Anal. Chem. 2017,

5. Sensitive AAV capsid protein impurity analysis by CE using easy to label fluorescent Chromeo dye P503, RUO-MKT-02-*10600-A*

Headquarters
500 Old Connecticut Path | Framingham, MA 01701 USA
Phone 508-383-7700
sciex.com

International Sales
For our office locations please call the division headquarters or refer to our website at
sciex.com/offices