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Application Note

In-Line Monitoring of CPPs and Capsid Titer in Upstream AAV Process with ProCellics™ Raman Analyzer

Abstract

In recent years, adeno-associated virus (AAV) production has become a key focus in gene therapy, driven by growing demand for safe and effective treatments. As AAV manufacturing evolves, the need for precise and efficient monitoring methods throughout upstream processing (USP) becomes paramount.

This application note explores the innovative use of the ProCellics™ Raman Analyzer for in-line monitoring of critical process parameters (CPPs) during the growth and production phase as well as post-lysis capsid titer in the bioreactor of an AAV production process that utilizes Human Embryonic Kidney (HEK) cell culture and transient transfection.

The ProCellics™ Raman Analyzer is a robust, non-destructive, in-line analytical tool that enables real-time monitoring of CPPs such as viable cell density (VCD), glucose, and capsid titer in AAV processes. By integrating this Raman analytical tool into the production workflow, manufacturers can achieve enhanced process control, improving process knowledge, and batch consistency, ultimately facilitating the delivery of high-quality AAVs for therapeutic applications.

All experiments shown in this application note were performed in **collaboration with Ascend Advanced Therapies** at the Ascend Munich facilities using ProCellics™ Raman Analyzer in their AAV production processes. Ascend Advanced Therapies is a CDMO for high-quality, cost-effective gene and advanced therapy development and manufacturing, from clinic to commercialization.

Highlights

- **Accurate Real-time Monitoring:** The ProCellics™ Raman Analyzer achieved excellent accuracy in monitoring process parameters (glucose, lactate, ammonium, glutamine, viable cell density (VCD), and capsid titer) with monitoring errors ranging on average from 4% to 11%.
- **Streamlining AAV Production:** Measuring capsid titer in-line in the bioreactor post lysis allows for a direct transition to downstream processing (DSP), eliminating the need for in-process sampling and standard at-line or off-line analytical assays. This results in significant time savings.

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Material and Methods

Experimental plan

The study was conducted using HEK293 cell cultures producing AAV with proprietary media utilizing Ascend's EpyQ® platform, efficient split-2 plasmids designed to drive high yield and quality. A total of eight cultures were performed, comprised of four runs for model building and four runs for real-time monitoring. In two of the model-building runs, glucose feeds were intentionally added to introduce process variability into the data. Each run has an initial cell growth phase, a transfection phase, a chemical lysis phase, and an endonuclease treatment.

The parameters of interest were glucose, ammonium, glutamine, lactate, VCD, and capsid titer. VCD was measured with a Vi-CELL BLU (Beckman Coulter, Inc., Brea, CA, USA), capsid titer was measured with Gyrolab® immunoassay (Gyros Protein Technologies AB, Uppsala, Sweden) and the remaining metabolic parameters were measured with a BioProfile® FLEX2 analyzer (Nova Biomedical Corp., Waltham, MA, USA).

The ProCellics™ Raman Analyzer was used in a multi-channel configuration enabling the measurement of four parallel bioreactors. Two bioreactors were at a 2 L scale and two others at 5 L scale. Each batch had a total duration of five days with three off-line samples taken each day, totaling approximately 15 samples per batch.

The Design of Experiment (DoE) enabled the integration of a slight bioreactor scale variability, process variability via the glucose feed as described above, and also AAV serotype variability.

Raman acquisitions

The acquisition of Raman spectra and real-time monitoring were performed using ProCellics™ Raman Analyzer with Bio4C© PAT Raman Software. For this study, the Raman acquisition settings included an integration time of 45 seconds per spectrum with 10 spectra collected, resulting in a total measurement time of approximately 11 minutes per measurement. The Bio4C® PAT Raman Software proprietary straylight noise reduction feature was activated to ensure spectral data quality.

The raw data from the first four batches were imported into the Bio4C® PAT Chemometric Expert module to enable spectral preprocessing, exploratory qualitative analysis using Principal Component Analysis (PCA) and quantitative analysis using Partial Least Squares (PLS) regression modelling.

For spectral preprocessing, a proprietary water normalization was first used, followed by a Savitzky-Golay 1st order derivative, 2nd order polynomial, five points smoothing window and focusing on different spectral ranges of variables depending on the target parameter and its Raman spectral signatures. The preprocessing step enhances the signal, removes potential signal noise, and emphasizes Raman bands linked to the molecules of interest.

Table 1.

Summary of experiments.

Batch	Bioreactor scale	Data type	Probe	AAV serotype
Batch 01	5 L	Model building	Probe 01	AAV2
Batch 02	5 L	Model building	Probe 02	AAV2
Batch 03	2 L	Model building	Probe 03	AAV2
Batch 04	2 L	Model building	Probe 04	AAV2
Batch 05	5 L	Monitoring	Probe 01	AAV2
Batch 06	5 L	Monitoring	Probe 02	AAV9
Batch 07	2 L	Monitoring	Probe 03	AAV2
Batch 08	2 L	Monitoring	Probe 04	AAV9

Results and Discussions

Exploratory analysis

Exploratory PCA was performed on the preprocessed spectral data. PCA enables a first glance at the data, facilitating the visualization of the samples to extract trends and patterns.

PCA exploratory analysis of the data showed minimal batch-to-batch variations across all eight bioreactor runs as shown in **Figure 1 (A)**. Additionally, no impact from the differences in bioreactor scale was observed in the Raman data **(B)**. Likewise, the analysis reveals no noticeable hardware variability between probes **(C)** of the ProCellics™ Raman Analyzer multi-channel unit in the first two principal components, which account for 80.6% of the spectral variability. While not depicted here, higher principal component dimensions were examined, and neither scale nor probe variabilities were observed.

These results suggests that the Raman signals collected amongst different batches, scales, and probes were consistent, highlighting the quality and reliability of the dataset generated.

Model building strategy and performance

Table 2 summarizes the calibration model details for each parameter, including statistical criteria to evaluate the ProCellics™ Raman Analyzer performance.

The results of the PLS regression models demonstrate the robust performance of the ProCellics™ Raman Analyzer in modeling critical process parameters during the AAV USP process.

Each parameter exhibits high predictive capability, with R^2 values ranging from 0.95 to 0.99, indicating excellent models fit. The Q^2 values, which assess the models predictive abilities in cross-validation, also show good results with values ranging from 0.87 to 0.99. The low Root Mean Square Error of calibration (RMSEc) and Root Mean Square Error of cross-validation (RMSEcv) values further reinforce the reliability of the models.

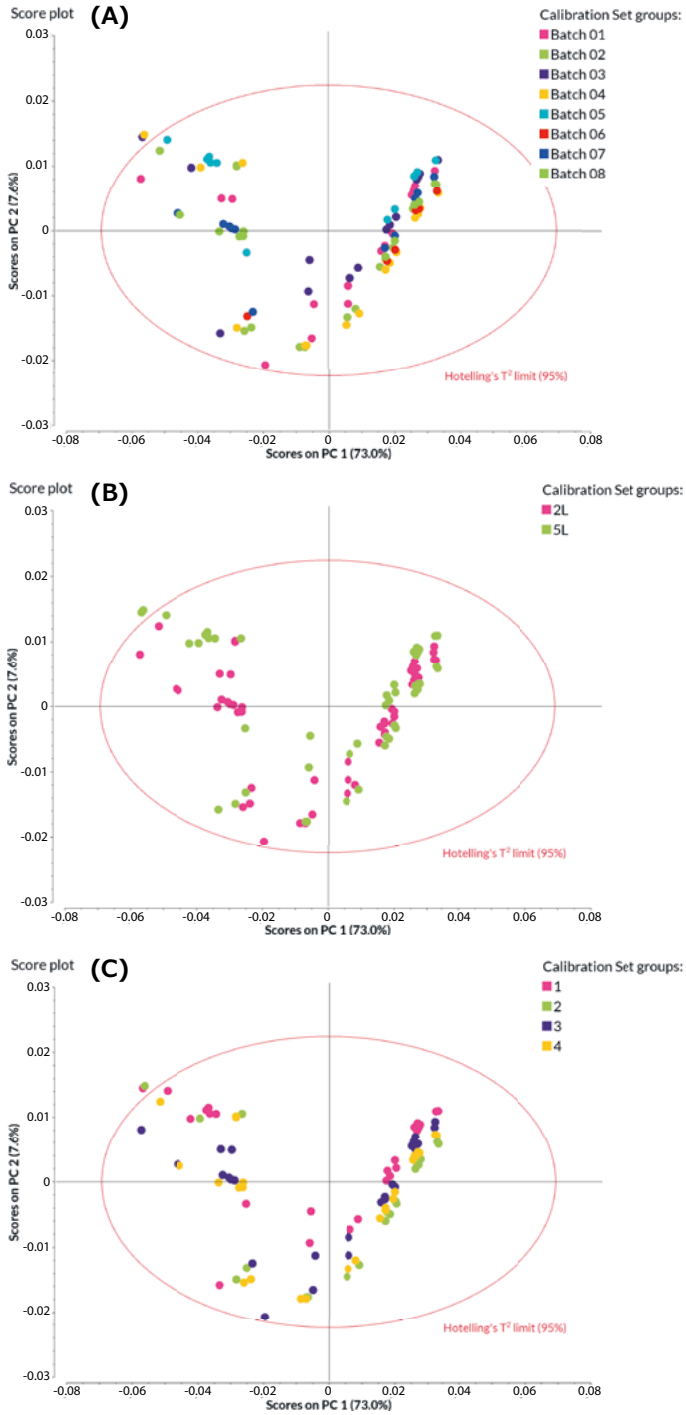

Figure 1.

PCA score plots colored according to **(A)** batch, **(B)** bioreactor scale and **(C)** probe number.

Table 2.

Summary of model calibrations based on AAV2 serotype.

Parameter (Unit)	N. samples	Range	LVs	R^2cum.	Q^2cum.	RMSEc	RMSEcv
Glucose (g/L)	54	0–6.2	4	0.99	0.97	0.25	0.30
Lactate (g/L)	55	0–7.9	3	0.99	0.99	0.17	0.20
VCD (×10^6 cells/mL)	49	0–1.9	4	0.97	0.95	0.10	0.13
Glutamine (mM)	55	0.5–4.3	2	0.97	0.96	0.24	0.25
NH_4 (mM)	55	0.6–3.3	4	0.95	0.87	0.17	0.30
Capsid titer (×10^{11} cap/mL)	22	0.3–7.2	2	0.95	0.93	0.65	0.73

Monitoring results

The models were used for real-time monitoring on new batches, and the Raman estimated values were compared to the off-line references analysis to estimate accuracy. The statistical performance metrics calculated to judge monitoring accuracy were the Root Mean Square Error of prediction (RMSEp) and the Relative Error (%) corresponding to the RMSEp divided by the maximum value of the validation range.

As there were four monitoring batches and six parameters each, the resulting 24 monitoring plots were summarized to judge the monitoring results. To do so, the real-time monitoring plots of one batch were shown in detail, complemented by a summary of errors plot averaged across all four monitoring batches. All detailed performance metrics for each batch and each parameter are displayed in **Table 3**. Due to the very low number of total samples for capsid titer compared to other parameters, one independent batch was used for model validation (as opposed to the strategy mentioned above) to provide the most amount of data for model building. The models built were uploaded into the Bio4C® PAT Raman Software to enable real-time monitoring.

Figure 2 shows the plots of values across batch duration for one monitoring run. The study for glucose, lactate, glutamine, VCD, and ammonium was focused on the performance before chemical lysis of the cells, whereas, the study for capsid titer measurements was focused after the lysis step. Especially for capsid titer, this distinction was done to ensure all the genetic material was released freely into the medium and well detected by the Raman sensor. The red crosses (×) represent off-line reference analytics, which are closely aligned with the blue continuous line (–) representing Raman monitored values, reaffirming the reliability of the Raman model.

These results demonstrate the effectiveness of using the ProCellics™ Raman Analyzer to monitor AAV process parameters and to ensure the critical quantification of capsid titer yield. Moreover, all four model-building runs used an AAV2 template. For the monitoring runs, however, two runs used AAV2 datasets while the other two used an AAV9 template. Overall, these findings highlight the effectiveness of the system in providing precise and consistent measurements, essential for understanding and optimizing AAV production processes.

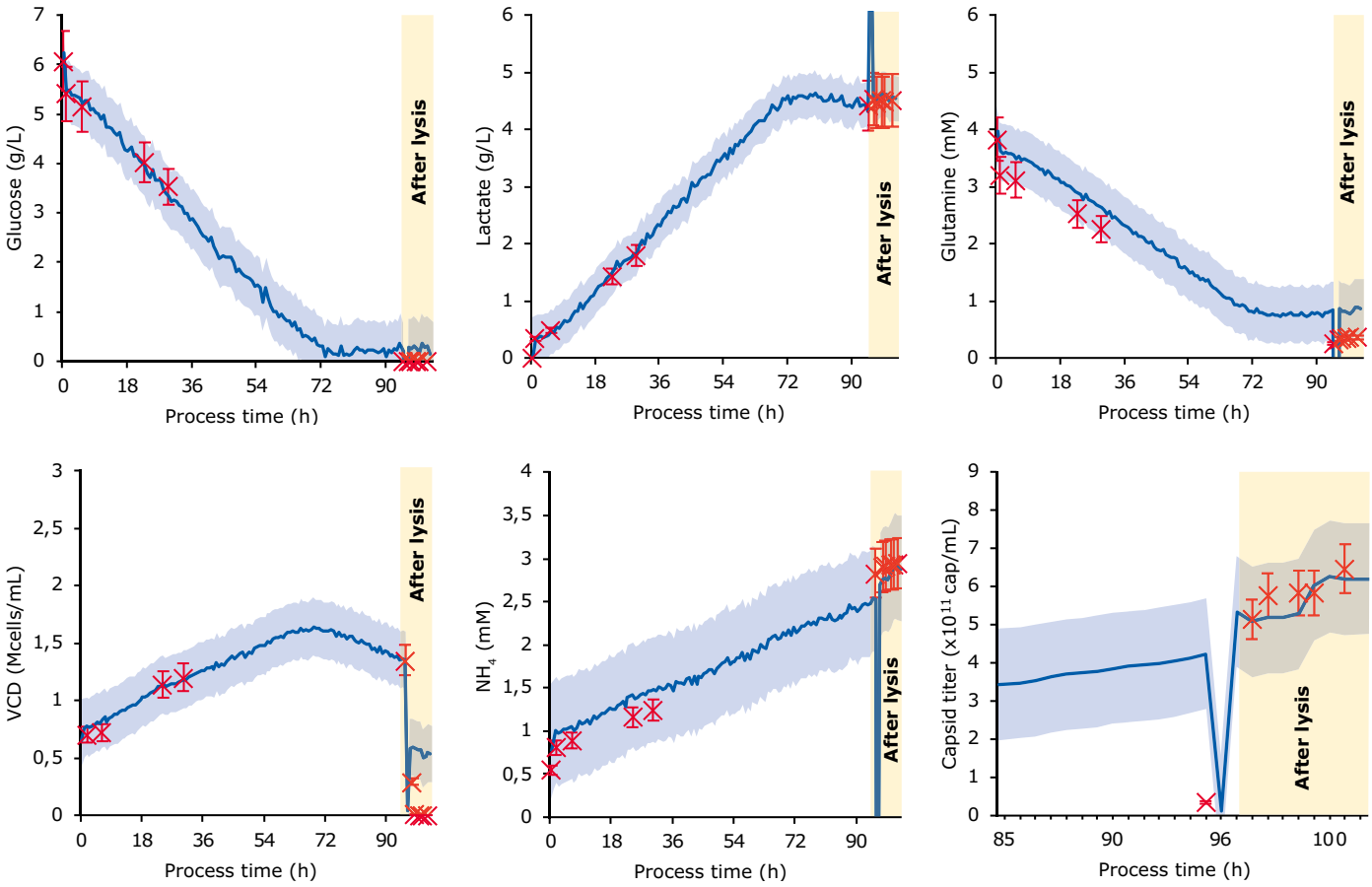

Figure 2.

Real-time monitoring plots for one batch, featuring red crosses (×) to indicate off-line reference analytics and their corresponding reference errors. The blue continuous line (–) represents the Raman monitored values, while the light blue cloud illustrates the 95% confidence interval of the Raman model. The yellow rectangle representing the process time after lysis.

Table 3.

Detailed table of performance for all four monitoring runs.

Parameter (Unit)	Monitoring	Range	RMSEp	RE	Averaged RE
Glucose (g/L)	Batch 05 (AAV2)	0–5.9	0.3	5%	4%
	Batch 06 (AAV9)	0–6.0	0.2	3%	
	Batch 07 (AAV2)	0–6.1	0.3	5%	
	Batch 08 (AAV9)	0–6.1	0.1	2%	
Lactate (g/L)	Batch 05 (AAV2)	0–4.3	0.2	4%	4%
	Batch 06 (AAV9)	0–4.2	0.1	3%	
	Batch 07 (AAV2)	0–4.5	0.1	7%	
	Batch 08 (AAV9)	0–4.4	0.1	2%	
VCD ($\times 10^6$ cells/mL)	Batch 05 (AAV2)	0.7–1.4	0.1	4%	7%
	Batch 06 (AAV9)	0.7–1.4	0.1	8%	
	Batch 07 (AAV2)	0.7–1.4	0.1	9%	
	Batch 08 (AAV9)	0.7–1.7	0.1	8%	
Glutamine (mM)	Batch 05 (AAV2)	0.2–3.7	0.6	15%	11%
	Batch 06 (AAV9)	0.2–3.8	0.4	10%	
	Batch 07 (AAV2)	0.2–3.8	0.4	10%	
	Batch 08 (AAV9)	0.3–3.8	0.4	11%	
NH_4 (mM)	Batch 05 (AAV2)	0.6-2.8	0.2	8%	10%
	Batch 06 (AAV9)	0.5–2.8	0.3	11%	
	Batch 07 (AAV2)	0.5–2.7	0.2	13%	
	Batch 08 (AAV9)	0.5–2.7	0.2	9%	
Capsid titer ($\times 10^{11}$ cap/mL)	Batch 05 (AAV2)	5.1–6.4	0.39	6%	5% (AAV2)
	Batch 06 (AAV9)	ABSENCE OF POST-LYSIS DATA			
	Batch 07 (AAV2)	3.9–6.2	0.16	3%	
	Batch 08 (AAV9)	5.3–9.5	5.8	61%	

Figure 3 shows a summary of errors averaged across all four monitoring batches.

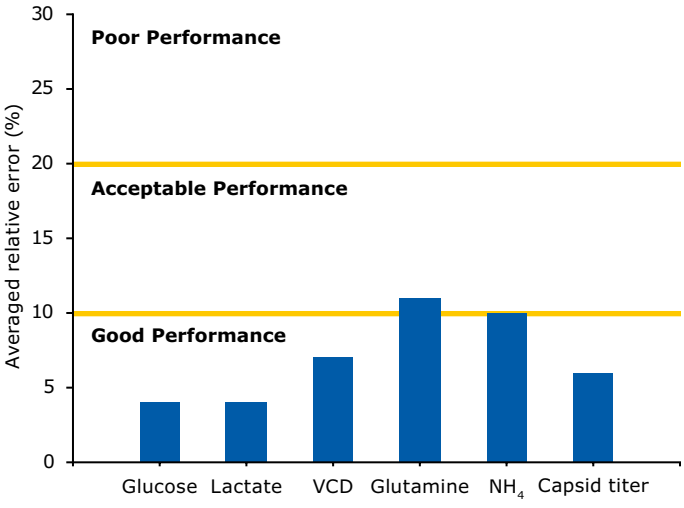

Figure 3.

Averaged errors across the four monitoring runs.

With an average RE of only 4% for glucose and low RMSEp values, the system delivers reliable and precise measurements. Lactate monitoring showcases its adaptability, consistently performing well even in fluctuating conditions. VCD measurements achieve a commendable average RE of 7%, while glutamine and ammonium levels yield close to 10% RE. The capsid titer results further substantiate the system's effectiveness as validated on batches 05 and 07 (AAV2), with an average RE of 5%.

Finally, **Table 3** demonstrates that ProCellics™ Raman Analyzer provides good real-time monitoring accuracy across all tested parameters which includes glucose, lactate, VCD, glutamine, ammonium, and capsid titer.

Batch 06 and batch 08 had a different AAV serotype than the one used for generating the model. It did not impact the monitoring accuracy for the classical parameters, but it did impact the capsid titer monitoring which could be attributed to higher productivity of the AAV9 serotype and absence of AAV9 serotype data in the initial model building phase.

These monitoring results were obtained using a four-batch model-building strategy based only on AAV2 serotype. Incorporating additional batches for model refinement and adopting improved strategies, such as serotype-dependent models for capsid titer, will highlight areas for further training – ultimately improving the system's robustness and accuracy.

Conclusions

This study demonstrates the ProCellics™ Raman Analyzer's effectiveness in real-time monitoring of critical parameters during AAV USP. The system consistently monitored all parameters with an accuracy close to or below 10% across all four monitoring runs, underscoring its reliability and precision in a dynamic production environment. The current study confirms an excellent alignment between the capsid titer measured post lysis with the Raman and offline analytics.

While it would be highly beneficial to measure capsid titers in real-time during the production run before the lysis step, it is important to note that capsids for most serotypes are not released until the cells are lysed. Utilizing Raman spectroscopy for in-line real-time process monitoring helps to maximize process efficiency and reliability. Measuring capsid titer in-line in the bioreactor post lysis offers the possibility to move straight to the DSP process steps eliminating the need for time- and resource-intensive analytical assays and resulting in substantial cost and time savings.

Looking ahead, future research will focus on developing a capsid titer model specifically tailored to various AAV serotypes, across a wide range of concentrations. By addressing the challenge of variability in the experimental set up, this investigation will aim to provide a comprehensive assessment of monitoring precision and versatility across various serotypes with ProCellics™ Raman Analyzer.

The successful collaboration between MilliporeSigma and Ascend Advanced Therapies has been instrumental in integrating this innovative technology into AAV production workflows, setting a benchmark for future collaborations in the gene therapy field. By enhancing monitoring precision and process control through in-line, real-time monitoring and a targeted modeling strategy, we are contributing to establishing more robust and successful AAV manufacturing processes. This proactive approach aligns with the evolving needs of biopharmaceutical manufacturers by improving manufacturing efficiency and performance, and paving the way for more effective therapeutic applications.

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