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Application Note

An Innovative Approach to Streamline Raman Implementation for Cell Culture Processes: One-batch Calibration

Abstract

Standard model building methods for Raman spectroscopy involve time-consuming and resource-intensive processes, leading to limitations in efficiency and potentially creating a roadblock for the general adoption of the technology. One-batch calibration, the innovative approach detailed here, greatly simplifies the Raman calibration phase while providing comparable levels of accuracy for real-time monitoring compared to standard modeling methods involving multiple calibration batches.

The monitoring of cell cultures is crucial for gaining a deeper understanding of the process and ensuring the production of safe and high-quality products. Raman spectroscopy can monitor several cell culture parameters in-line and in real time. However, prior to implementing Raman for process monitoring, calibration models that correlate Raman spectra with the off-line reference measurements of target parameters need to be built.

By utilizing one-batch calibration, the time, effort, consumables, and human intervention required to generate data for Raman implementation can be significantly reduced four-fold. This approach eliminates the need for multiple calibration batches, allowing for a more streamlined and efficient workflow. Moreover, the one-batch calibration approach not only achieves a comparable level of accuracy compared to traditional methods, but also enhances the robustness of Raman modeling and facilitates the technology transfer to other sites for efficient implementation.

Highlights

- **Streamlined workflow:** The one-batch calibration method simplifies the Raman implementation process, saving valuable time and resources.
- **Unmatched flexibility:** This innovative approach enables seamless adaptation to various cell lines, clones, media, scale, feed conditions, and instruments as well as facilitating site-to-site technology transfer.
- **Enhanced robustness:** By leveraging advanced algorithms and statistical techniques, the approach improves robustness and reliability of Raman measurements.

Introduction

Prior to implementing real-time monitoring, it is necessary to gather a large amount of spectral data to develop robust and reliable calibration models. Depending on the specific process, this model building phase can take several months and result in significant costs. The Raman spectral fingerprints captured throughout the duration of cell cultures, combined with the biochemical complexity of these processes, may affect the accuracy and specificity of the models built using traditional methods. Consequently, the adoption of the Raman technology in the industry may be hindered.

This novel modeling approach offers significant time, cost, and resource savings compared to standard approaches. The methodology leverages a proprietary model library and advanced algorithms to streamline the Raman calibration phase. By using this methodology, coupled with spectral preprocessing methods and chemometrics, models are constructed to quantify key compounds in multiple runs of a fed-batch process of Chinese Hamster Ovary (CHO) cell culture producing monoclonal antibodies (mAbs).

The benefits of adopting this innovative approach are three-fold. First, it saves valuable time and resources, enabling Raman users to focus on their core work rather than spending excessive efforts on Raman calibration. Second, the improved robustness of the model enhances the reliability and reproducibility of Raman spectroscopy results.

Third, the method offers unparalleled flexibility in process development as it seamlessly integrates with any CHO cell line, clone, media, and feed conditions, providing a versatile and customizable solution. We explore here the details of this innovative approach and compares its performances with standard modeling techniques.

Table 1: Summary comparison between both approaches

	Standard calibration method	One-batch calibration method
		
Number of batches	Run **three to six batches** under typical process conditions to generate sufficient data to build the model	Run **one batch** under typical process conditions and leverage advanced statistical methods
Sampling cost	Samples taken twice a day across several batches	Samples taken twice a day from one single batch
Labor cost	Presence of a bioprocess expert during several batch runs	Reduced labor cost to one single batch
Reference analytical costs	Reference analytical measurements required for samples across several batches	Reduced reference analytical costs required for samples from one single batch
Time to implement	Three to six months or more	Two weeks to one month (depending on the process)
Sensitivity to process scale changes	Sensitive to process scale changes	Agnostic to process scale changes
Sensitivity to media or feed changes	Sensitive to media or feed changes	One-batch adaptation to media or feed changes
Sensitivity to cell line or clone changes	Sensitive to cell line or clone changes	One-batch adaptation to cell line or clone changes
Sensitivity to instrumental changes	Sensitive to instrumental changes	One-batch adaptation to instrumental changes
Site to site technology transfer	Sensitive to technology transfer between sites	One-batch adaptation for technology transfer between sites
Accuracy	Equivalent monitoring accuracy for both methodologies	
Raman expert support offering	Our Raman experts can help you create and implement advanced monitoring capabilities regardless of your preferred methodology	

Experimental setup

Fed-batch cell cultures (n = 5) were performed on an Ambr® 250 mL Modular bioreactor with a CHOZN® GS -/- backbone cell line (SAFC®) producing an IgG1 using EX-CELL® Advanced CHO fed-batch medium (SAFC®). The process parameter setpoints for the cell culture were as follows: 37 °C, pH 7, 40% dissolved oxygen and 300 rpm agitation speed.

During the batch runs, samples were taken twice a day and measured with a BioProfile® FLEX2 off-line analyzer (Nova Biomedical) to determine the concentrations of glucose, lactate, and viable cell density (VCD). The samples were also analyzed with two-dimensional liquid chromatography coupled to tandem-mass spectrometry (2D-LCMS) for mAb titer measurements.

The samples collected from the process bioreactor were also analyzed by Raman spectroscopy using the ProCellics™ Raman Analyzer (Millipore®) with its specific measurement cell enabling low volume at-line measurements. All the data acquisitions were performed with Bio4C® PAT Raman Software (Millipore®) and the data analysis was done with Bio4C® PAT Chemometric Expert (Millipore®).

Study plan and model calibration performances

Standard and one-batch calibration methods were compared for accuracy. The following approach was tested:

a. One-batch calibration: data collected from the first batch and the one-batch calibration method is used.

b. Standard calibration: data collected from the first three batches and the standard calibration method is used.

In order to evaluate the model calibration performance, the coefficient R^2, the cross-validated correlation coefficient Q^2 and the Root Mean Square Error of Cross-Validation (RMSECV) were determined (**Table 2**). Then, both models were used to monitor two new batches and compare the performance and accuracy.

Table 2: Calibration performance table

	Standard calibration method				One-batch calibration method			
	Glucose ($g.L^{-1}$)	Lactate ($g.L^{-1}$)	VCD ($Mcells.mL^{-1}$)	Titer ($g.L^{-1}$)	Glucose ($g.L^{-1}$)	Lactate ($g.L^{-1}$)	VCD ($Mcells.mL^{-1}$)	Titer ($g.L^{-1}$)
N. batches	Three batches				One batch			
N. samples	40 samples				13 samples			
R^2	0.84	0.89	0.95	0.96	0.99	0.99	0.97	0.94
Q^2	0.80	0.82	0.93	0.95	0.97	0.99	0.94	0.84
RMSECV	0.66	0.28	1.28	0.13	0.40	0.19	1.12	0.14

Results and Discussions

The resulting performance table, performance summary bar graph, and Raman measurements compared to off-line measurements plots are respectively shown in **Table 3**, **Figure 2** and **Figure 1**. The accuracy of the measurements was assessed through the evaluation of Root Mean Square Errors of Prediction (RMSEP) and the relative error. The relative error is defined as the ratio between RMSEP and the maximum value of the range in the validation batches for each parameter, ensuring comparability across different units. **Figure 1** shows that the one-batch calibration method can track the process kinetics with remarkable accuracy. These results confirm the robustness and precision of this method.

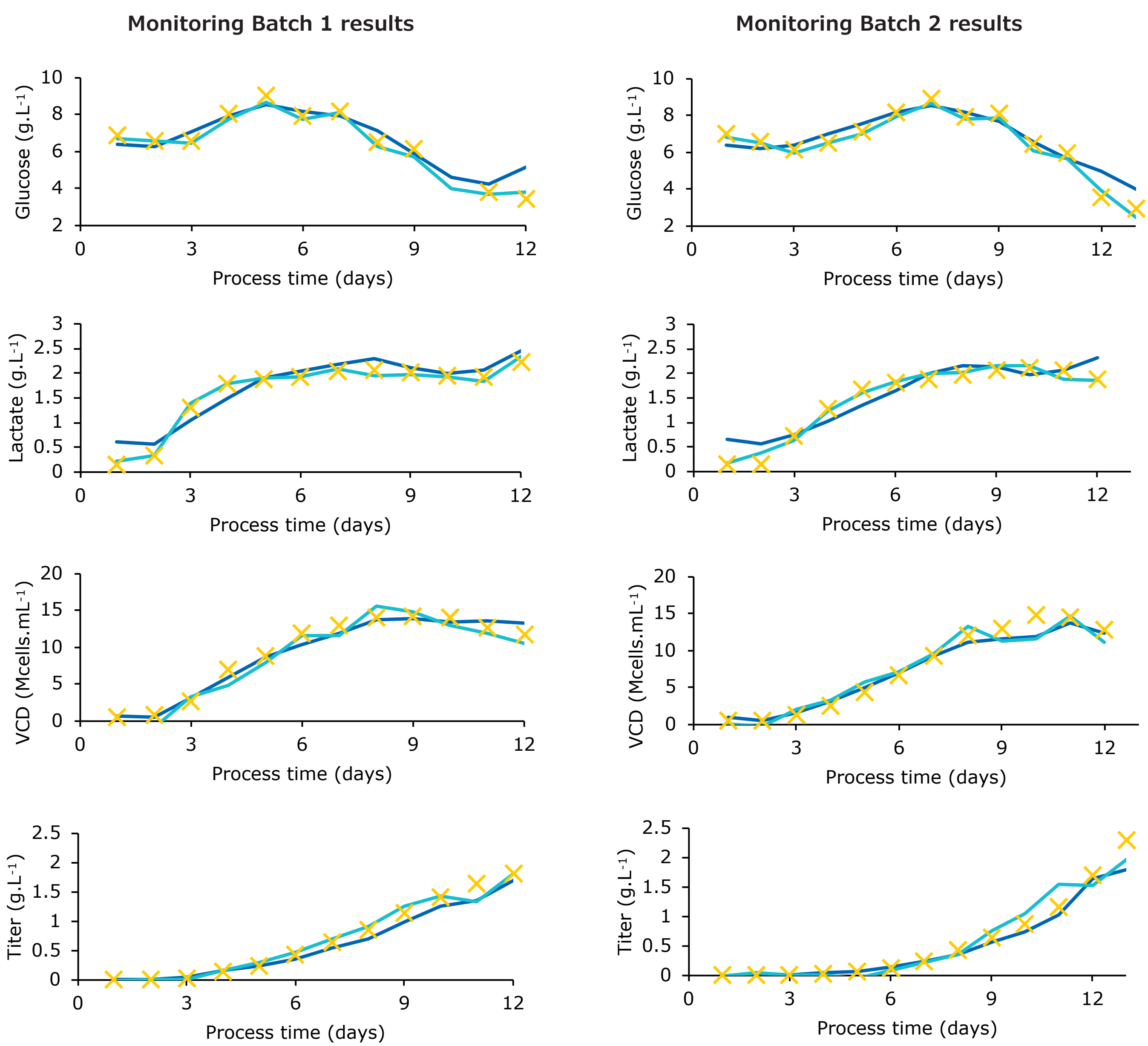

Figure 1: Plot of Raman predictions of the one-batch calibration method (light blue -), standard calibration method (dark blue -) and reference measurements (yellow crosses ×) for the two monitoring batches.

Table 3: Monitoring performance table

		Standard calibration method				One-batch calibration method			
		Glucose ($g.L^{-1}$)	Lactate ($g.L^{-1}$)	VCD ($Mcells.mL^{-1}$)	Titer ($g.L^{-1}$)	Glucose ($g.L^{-1}$)	Lactate ($g.L^{-1}$)	VCD ($Mcells.mL^{-1}$)	Titer ($g.L^{-1}$)
First monitoring batch	**RMSEP**	0.66	0.22	0.90	0.12	0.25	0.07	1.23	0.09
	Relative Error (%)	7%	10%	6%	7%	3%	3%	9%	5%
Second monitoring batch	**RMSEP**	0.62	0.27	1.05	0.16	0.27	0.10	1.32	0.17
	Relative Error (%)	7%	13%	7%	7%	3%	5%	9%	8%

The results in **Table 3** underscore the high reliability of the one-batch calibration method, which notably enhances the Relative Error (%) by over two-fold for both glucose and lactate parameters.

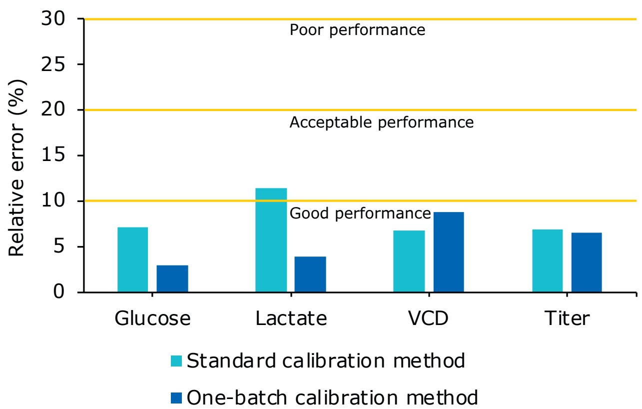

Figure 2: Average relative errors for both monitoring batches to compare the two methods

As observed in **Figure 2**, the standard calibration method, which requires running three batches, and the one-batch calibration method, which streamlines the calibration process, obtain satisfactory results in terms of accuracy. Moreover, the method presented here offers significant benefits beyond accuracy. By simplifying the calibration process and eliminating the need for multiple batches, it reduces the time and effort required for Raman implementation, while maintaining confidence in the results.

Conclusion

These study results show how the one-batch calibration method addresses the limitations of traditional model building methods, and provides an alternative approach that is more efficient, accurate, and flexible.

By leveraging innovative algorithms and statistical techniques, coupled with a proprietary model library, the accuracy and reliability of Raman modeling are enhanced, enabling users to make confident decisions backed by robust data. This way, the usual laborious and complex processes associated with Raman calibration can be replaced by a streamlined workflow that saves time, effort, and valuable resources.

Ultimately, this groundbreaking approach to streamline Raman implementation through one-batch calibration offers a compelling solution to the limitations of traditional model building methods and enables a seamless adaptation of the technology to various cell-lines, clones, media, bioreactor scale, feed conditions, instruments as well as facilitating site-to-site technology transfer.

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