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Preparation, Separation,
Filtration & Monitoring Products

Application Note

Cell-free modeling approach for efficient cell culture monitoring using Raman spectroscopy

Abstract

Monitoring cell cultures is crucial for gaining a deeper understanding of processes and ensuring the production of safe and high-quality products. Raman spectroscopy offers the capability to monitor in-line and in real time several important parameters simultaneously such as the concentration of glucose or lactate.

However, prior to using Raman to monitor a specific process, a calibration phase is required to build models that correlate Raman spectra with the target parameters. It is mandatory to conduct this phase with multiple batches to build robust models that account for biological variability. The user needs to run at least three to six batches in real process conditions with the collection of around 15 to 20 samples per batch. Then, each sample must be analyzed with off-line equipment to provide reference measurements, correlate the parameters' variations to the spectral changes, and build the Raman calibration method. This model building phase can be time-consuming, take several months and require significant resources from the analytical team. The industry is actively seeking solutions to simplify and expedite this step without compromising accuracy. Moreover, the current model building methodology has limitations when it comes to changing cell culture medium, cell line, or process scale.

The novel synthetic model approach provides a significative gain of time and resources for the calibration phase which is reduced to just a few days. The methodology involves using cell-free samples of cell culture media that are spiked with various compounds of interest. This methodology was evaluated for its ability to monitor glucose and lactate concentrations in real time across several runs of fed-batch processes. The synthetic model approach was compared to the standard methodology to assess its effectiveness and reliability.

Highlights

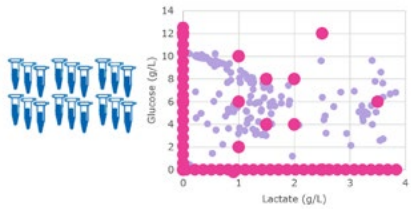
- Successful application of a novel synthetic model approach allows significant reduction in time and resources to enable in-line and real-time monitoring of bioprocesses.
- The synthetic model approach enables a dramatic acceleration of the Raman implementation process without compromising accuracy compared to the standard approach. Two weeks are sufficient to generate the nutrient model calibration.
- The combination of ProCellics™ Raman Analyzer with Bio4C® PAT Raman Software provides a convenient and reliable solution for implementing Raman technology using the cell-free modeling approach.

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 process, this model building phase can take several months and result in significant costs. The Raman spectral fingerprints captured throughout the duration of the cell cultures, combined with the biochemical complexity of these processes, affect the reliability and specificity of the models built using traditional, time-consuming, and expensive methods. Consequently, the adoption of Raman technology in the industry may be hindered.

This application note introduces a novel modeling method that offers great advantages compared to traditional approaches. The methodology utilizes cell-free cell culture media (CCM) spiked with varying concentrations of the compounds of interest for real-time measurement. By employing chemometric methods, models are constructed to measure these compounds in multiple runs of a fed-batch Chinese Hamster Ovary (CHO) mAb-producing cell culture process.

Table 1: Comparison between standard and synthetic model methodologies

	Standard methodology	Synthetic model methodology
		
Method overview	Perform three to six cell culture runs in order to generate adequate data to build a robust model	Spiking compounds of interest in the cell-free CCM In this example, the DoE was built by analyzing 13 samples of glucose/lactate mixture in triplicate in addition with a linear range for each component.
Sampling frequency	Two samples per day across all the batches	Total of 13 samples in triplicate for DoE and two samples per component for automated addition with pump
Labor requirement	Frequent presence of a bioprocess expert across the batch durations	Reduced labor cost due to streamlined time to implementation
Time to implement	Three to six months	One to two weeks
Sensitivity to Process Scale Changes	Sensitive to process scale changes	Agnostic to process scale changes
Accuracy	Equivalent monitoring accuracy for both methodologies	
Raman expert support offering	Our Raman experts can help you to create and implement advanced monitoring capabilities regardless of your preferred methodology	

Materials and Methods

Experimental setup

a. Cell-free samples

The samples were prepared in centrifuge tubes and consist of CCM in which a solution containing glucose and lactate is added in various concentrations. A Design of Experiments (DoE) was constructed to determine the composition of each mixture within the desired range. The specific range for the synthetic sample mix used in the study was carefully determined based on the concentration ranges of the target cell culture process. To ensure accuracy, the synthetic sample mix used covered a broader range of analytes that what is typically encountered in the actual process.

Additionally, a sterile bioreactor vessel is filled with a cell culture medium that does not contain glucose or lactate. The Raman probe is then inserted into the vessel to collect the spectral data. The medium within the bioreactor vessel is subjected to agitation, aeration, and temperature control, simulating the conditions typically maintained during cell culture. Subsequently, a linear ramp up of either glucose or lactate is gradually added to the vessel. During this process, the Raman spectra are automatically collected at regular intervals.

The concentrations of glucose and lactate observed in a cell culture process are depicted in **Figure 1**. The composition of the synthetic sample mix was selected to align with the concentration mix represented on the graph.

b. Cell culture experiments

The cell culture batches were run using a CHOZN® cell line and EX-CELL® Advanced medium (Merck KGaA, Darmstadt, Germany). The cells were cultivated in a 3 L glass vessel with the process parameters specified in **Table 2**.

During each run, samples were taken twice a day in triplicate and measured with an analytical off-line analyzer to determine the concentration of various components including glucose and lactate.

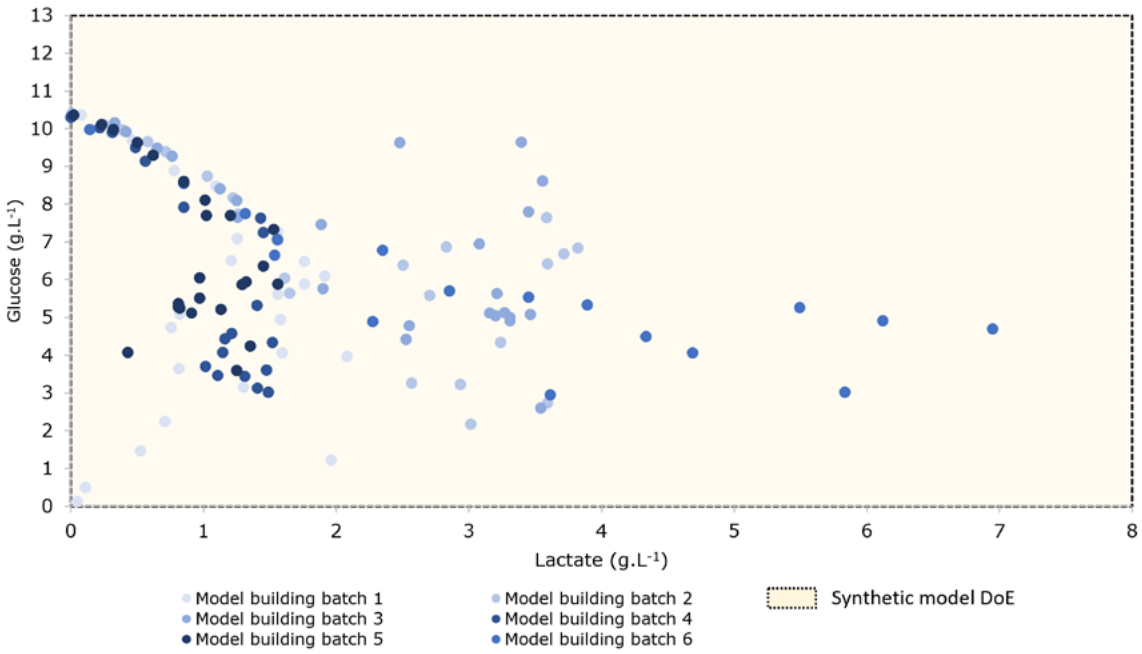


Figure 1: Graphical representation of the DoE approach showing the distribution of values comparatively between cell culture runs and cell-free samples.

Table 2: Process parameters and control mechanisms for cell culture batches

Process Parameter	Setpoint	Control
Temperature (°C)	37	Water jacket
pH	7.0 (dead band 0.1)	0.5N NaOH or sparging CO_2
Dissolved oxygen (%)	40	Mix of gas (air, O_2)
Agitation speed (rpm)	100	Marine impeller, magnetic drive

Raman spectroscopy

The ProCellics™ Raman Analyzer (Merck KGaA, Darmstadt, Germany) was used in single channel mode. The instrument has a 785 nm laser with laser power around 350 mW at the probe-tube output. The probe was immersed into the samples contained in centrifuge tubes or into a bioreactor using PG13.5 cable gland adaptors. To isolate the samples from external straylight and avoid any measurement disturbance, aluminum foil or a lightproof cover was used depending on the setup. Laser excitation and spectral data collection were controlled by Bio4C® PAT Raman Software using an Ethernet connection between the ProCellics™ Raman Analyzer and the computer. Each Raman measurement was the result of an integration time of 30 seconds and an average of 30 spectra (15 minutes of acquisition per measurement in total).

Model building

Off-line data were associated to their corresponding Raman spectra in Bio4C® PAT Raman Software to generate a consistent dataset. The spectral data were preprocessed with the standard preprocessing pipeline available in the software. The dataset containing the off-line measurements and the preprocessed spectral data was generated and used with Bio4C® PAT Chemometric Expert Software to perform data analysis and Partial Least Squares (PLS) model building.

The number of Latent Variables (LVs) was selected based on minimizing the Root Mean Square Error of Calibration (RMSEC) and the Root Mean Square Error of Cross-Validation (RMSECV) while maximizing the cumulative explained variance (R^2Y) and the cumulative predicted variance (Q^2Y) without overfitting. The models created were then imported into Bio4C® PAT Raman Software to enable real-time monitoring of cell cultures. The predictive performance of the models was evaluated by calculating a Root Mean Square Error of Prediction (RMSEP) and a relative error (%) based on the maximum value of the concentration range of the monitoring batches.

The modeling steps were performed with both the standard and synthetic model approaches. The performance of the calibrations are displayed in **Table 3** below.

Table 3: Performance of synthetic and standard models on glucose and lactate measurement

Method	Parameter	Range ($g.L^{-1}$)	Number of samples	LVs	R^2Y	Q^2Y	RMSEC	RMSECV
Synthetic Models	Glucose	0 – 12.9	150	3	0.99	0.99	0.16	0.16
	Lactate	0 – 11.2	200	5	0.99	0.99	0.05	0.06
Standard Models	Glucose	0.1 – 10.4	129	6	0.99	0.99	0.21	0.24
	Lactate	0 - 6.9	128	6	0.99	0.99	0.13	0.15

Results and Discussions

To evaluate the effectiveness of each methodology, fed-batch processes were carried out using three different CHOZN® cell culture runs as validation sets.

The resulting performance of each methodology and Raman measurements compared to off-line measurement plots are shown in **Table 4**, **Figure 2** and **Figure 3**.

Table 4: Performance of synthetic and standard models for glucose and lactate monitoring

			Synthetic Models		Standard Models	
Parameter	Monitoring Batch	Range	RMSEP	Relative error	RMSEP	Relative error
Glucose ($g.L^{-1}$)	Batch 1	2.4 – 9.5	0.39	4%	0.44	5%
	Batch 2	0.2 – 10.4	0.79	8%	0.45	4%
	Batch 3	3.1 – 10.4	0.56	5%	0.47	5%
Lactate ($g.L^{-1}$)	Batch 1	0.1 – 5.1	0.46	6%	0.20	4%
	Batch 2	0 – 9.1	0.90	10%	0.77	8%
	Batch 3	0.1 – 8.2	0.65	8%	0.90	11%

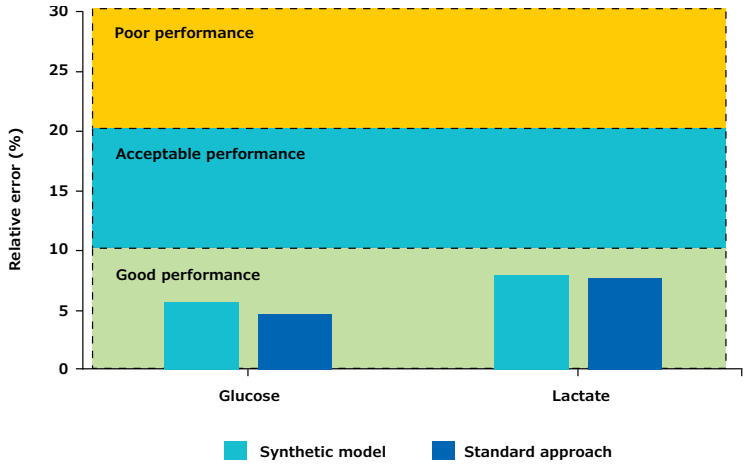
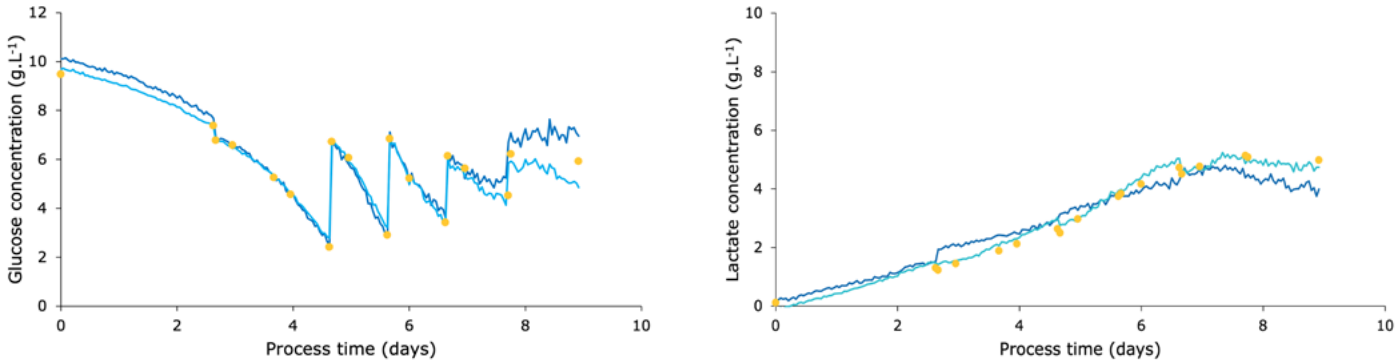
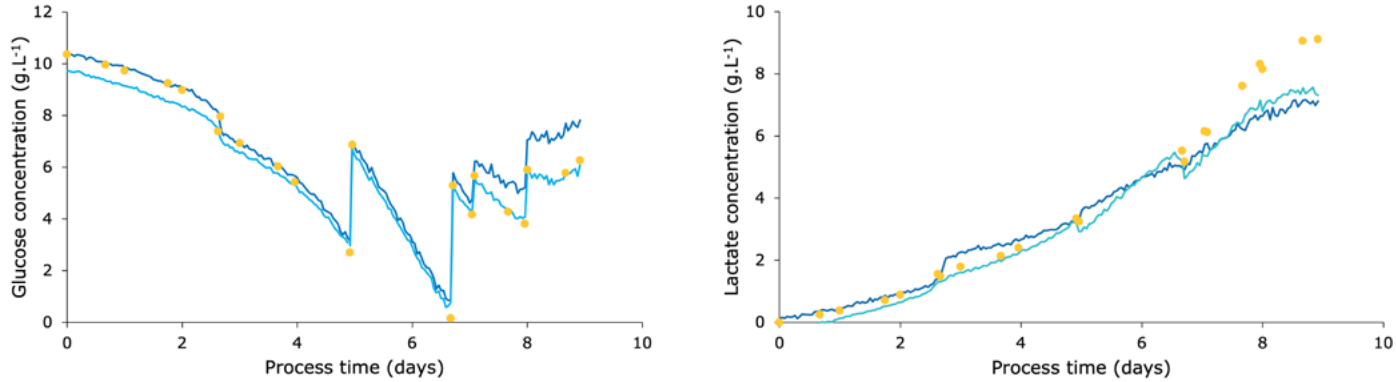


Figure 2: Comparison of average relative errors obtained from three monitoring batches using synthetic and standard methodologies for glucose and lactate parameters.

A. Batch 1



B. Batch 2



C. Batch 3

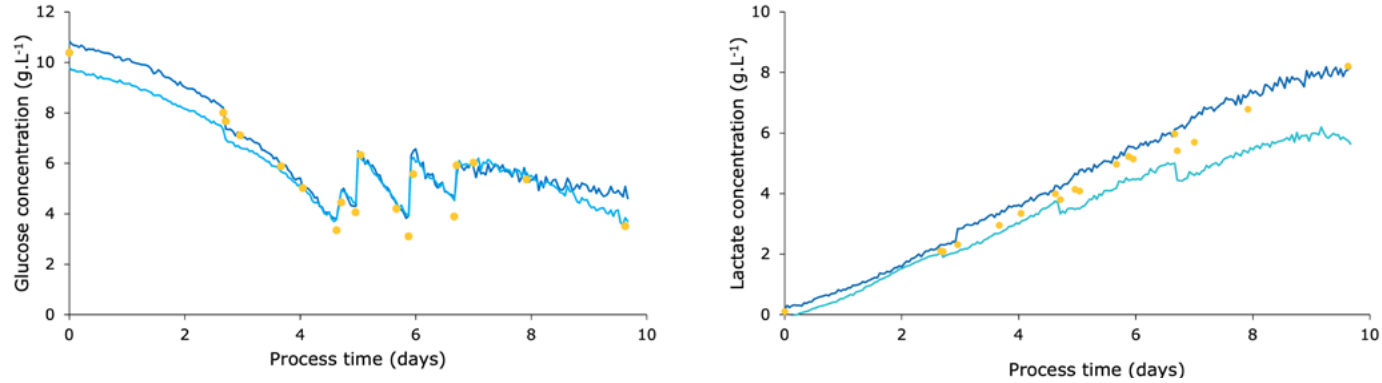


Figure 3: In-line real-time monitoring comparison of the standard methodology (light blue line –) vs the synthetic models methodology (dark blue –) with regards to off-line reference measurement (yellow dots ●)

The synthetic models and the standard models were both able to accurately predict the glucose and lactate and also follow the process trend variations and concentrations for the three monitoring batches. Each time a glucose feed was performed during the process, the increase was accurately measured by the Raman analyzer in alignment with the off-line reference measurements (**Figure 3**). Both the standard models and the synthetic models have low RMSEP values and relative errors close to or below 10% (**Table 4**).

While the accuracy is close for both methodologies, the innovation of the synthetic modeling resides in the fact that it does not require any cell culture runs to calibrate the models before moving into real-time monitoring. Such leading-edge improvement in comparison to the standard method allows users wishing to use Raman technology to monitor their bioprocess to reduce the time (from three to six months for the standard approach compared to one to two weeks for the synthetic modeling approach), resources and cost needed to implement the technology.

Conclusion

Raman spectroscopy is becoming widely accepted as a Process Analytical Technology (PAT) tool in the bioprocessing industry. One drawback often limiting its usage is the lengthy implementation process. This is no longer the case as this application note presents a novel modeling approach using cell-free samples to monitor a cell culture process. The results indicate that the synthetic models approach enables accurate measurement of glucose and lactate parameters. In addition, the accuracy was comparable to a standard modeling methodology while enabling a significative reduction in time-to-implementation, cost, and resources.

Further research is on-going to demonstrate the capabilities of synthetic models to monitor other type of processes, cell lines, scales, and cell culture media.

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