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Application Note

Implementation of Raman Spectroscopy for in-line monitoring of critical process parameters of CHO cell perfusion cultures

Abstract

This application note introduces a case study for the implementation of a Raman spectroscopy soft-sensor for in-line and real-time monitoring of critical process parameters (CPP) in mammalian perfusion cell cultures reaching 160 x 10^6 cells.mL^{-1}. Partial Least Squares (PLS) was applied for multivariate regression analysis between the respective changes of spectra and bioprocess parameters. The prediction accuracy of the Raman analyzer was estimated to be on average 3% for all the CPPs except for Ammonium (15%), being comparable with the accuracy of current off-line and at-line analyzers and hence demonstrates its utility for in-line monitoring and subsequent real-time control.

Highlights

- Development and implementation of a Raman soft sensor for in-line and real-time monitoring of critical process parameters-such as titre, cell densities and nutrient concentrations-in mAb producing, high cell density perfusion CHO cell culture.
- Use of ProCellics™ Raman Analyzer with Bio4C® PAT Raman Software, easy-to-use and 21 CFR Part 11 compliant, for the implementation of Raman spectroscopy capabilities in bioprocessing.
- Use of Bio4C® PAT Chemometric Expert Software as an easy-to-use platform for unsupervised and supervised multivariate data analysis.

Introduction

Process Analytical Technology (PAT) is a regulatory framework initiated by the United States' Food and Drug Administration (FDA) that encourages innovation in pharmaceutical development, manufacturing, and quality assurance. The goal of PAT is to build quality into biopharmaceutical production processes by monitoring and controlling the process in real time. Through the identification and monitoring of critical process parameters (CPP) and critical quality attributes (CQA) within a specified design space, Raman spectroscopy enables understanding of cell culture processes and improvement of product quality by real-time monitoring. Rather than measuring the quality by testing, we are incorporating quality into the design of the process, making it more robust and transparent.

This application note describes the development and implementation of Raman spectroscopy-based soft sensors for process monitoring of CPPs in bench scale, CHO cell, intensified seed trains (N-1). The framework presented herein combines the use of ProCellics™ Raman Analyzer with Bio4C® PAT Raman Software for spectral acquisition, data management and process monitoring as well as the use of Bio4C® PAT Chemometric Expert Software for multivariate model development. The introduced workflow for data pre-processing and processing stages demonstrated a potential to employ soft sensors to provide effectively continuous monitoring capabilities for multiple key cell culture process parameters in intensified upstream processing.

Materials and Methods

Intensified cell culture processing

Cell line maintenance and cell culture media

A CHOZN® GS (MilliporeSigma) cell line producing IgG1 was sub cultivated in polycarbonate baffled shake flasks with vent caps and with 20% working volume. Expansion cell cultures were seeded at low cell density ($3x10^{-5}$ cells.mL^{-1}) and maintained at 37.0 ºC in a humidified (80%) and controlled atmosphere of 6% (v/v) CO2 at a constant rotational speed of 125 rpm. An animal-component free and chemically defined perfusion medium platform (EX-CELL® Advanced HD Perfusion medium, MilliporeSigma) was used for cell cultivation.

Perfusion process setup and operation

Intensified seed train cell cultures (N-1) (n=6 runs) were performed in 3L glass stirred tank bioreactor systems with a working volume of 2 L and a seeding density of $5x10^{-5}$ cells.mL^{-1}. Process operation parameters for all vessels (Finesse Trubio) are summarized in **Table 1.** To enable cell retention in bioreactor and perfusion operation, the Cellicon™ Filter (Durapore® hydrophilic PVDF with 5.0 µm microfiltration membrane, MilliporeSigma) was connected on-line to the process stream in conjunction to the Cellicon™ Controller (MilliporeSigma) for pressure monitoring of the feed, retentate and permeate streams (**Figure 1**). Perfusion operation was controlled gravimetrically on the bioreactor system by preserving a null net weight change from inlet fresh medium and outlet of permeate. Perfusion rate was adjusted daily based on a desired constant cell specific perfusion rate of 20.0 pL.$cell^{-1}$.day^{-1} and the predicted increase of cell density.

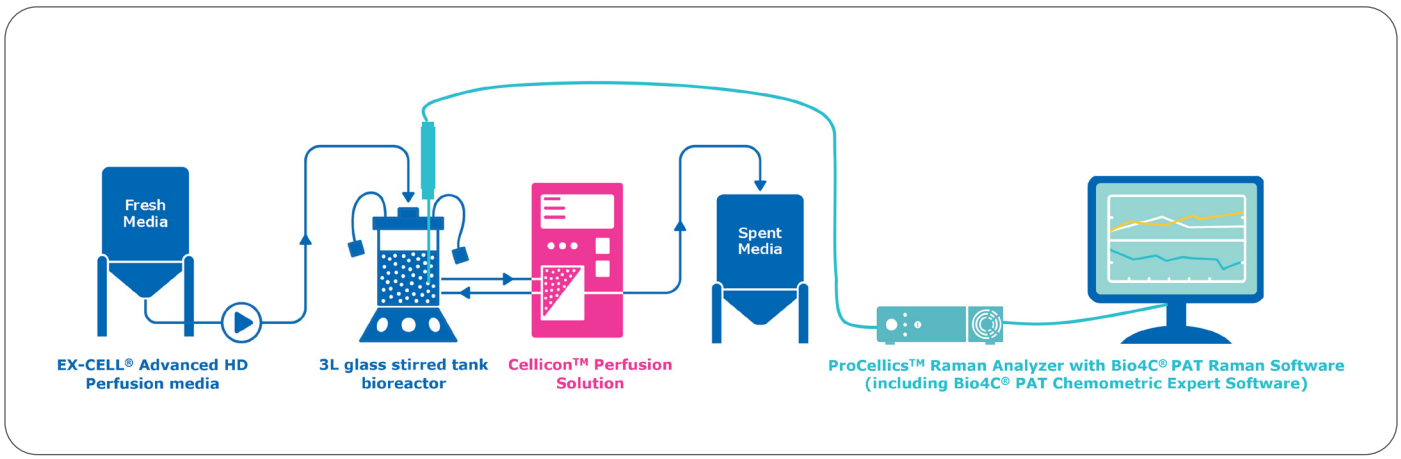

Figure 1: Scheme of perfusion process configuration (bench-scale stirred tank bioreactor and Cellicon™ Perfusion Solution) and integration of an in-line Raman sensor (ProCellics™ Raman Analyzer with Bio4C® PAT Raman Software).

Table 1. Process parameters and control for intensified cell culture processes

Process Parameter	Setpoint	Control
Temperature (T)	36.5±0.5 °C	Electrical jacket
pH	7.0±0.05	Inlet of carbon dioxide or 1M sodium carbonate
Dissolved Oxygen (DO)	50.0±2.0%	Inlet of gas mixture (Air-Oxygen)
Headspace	50.0 $mL.min^{-1}$	Constant inlet of Air
Agitation speed (N)	300 rpm	Mechanical stirring

Standard analytical methods

Multiple daily off-line samples were acquired during bioreactor runs and analyzed for reference measurements. These reference values were associated with the corresponding Raman spectra and were used for building the PLS calibration models.

Measurements of key metabolites and osmolality were performed using an automatic metabolic profiler (Flex2 Bioprofile Analyzer, Nova Biomedical Ltd.). Live and dead cell concentration, viability, and mean cell diameter were determined using an automated cell staining and counting analyzer system (Vi-Cell XR, Beckman Coulter). Secreted antibody in the culture broth was quantified using a High-Performance Liquid Chromatography system (Agilent 1100, Agilent Technologies) with affinity-based separation using a bind-elute cycle in a small-scale protein A column (0.1 mL).

Raman spectroscopy analysis

ProCellics™ Raman Analyzer with Bio4C® PAT Raman Software (MilliporeSigma) was used to achieve real-time, in-line monitoring of selected CPPs. The Raman probe was autoclaved before each batch/ experiment and connected in-line to the process (**Figure 1**). Spectral acquisition, reference data association, data management and monitoring were conducted using Bio4C® PAT Raman Software. Spectral acquisition parameters are detailed below:

Table 2. Spectral Acquisition Parameters

Spectral Acquisition Parameters	Value
Laser wavelength	785 nm
Laser power	400 mW maximum at probe tip
Spectral range	+150 to +4,000 cm^{-1}
Exposure time	30 s
Number of spectra per acquisition	30 spectra
Total measurement time	15 min

Spectral Pre-processing

Spectral pre-processing was done using Bio4C® PAT Raman Software. Standard normal variate (SNV) was applied to the whole spectra based on the water signature region (3,100 to 3,700cm^{-1}) to achieve the batch normalization followed by Savitzky-Golay 1^{st} order derivative, 2^{nd} order polynomial, and 5 points window smoothing (15 cm^{-1}). Finally, spectral selection (350-1,775 cm^{-1} and 2,800-3,000 cm^{-1}) was applied to select fingerprint regions for the process parameters and truncated in other regions.

Calibration and Monitoring of parameters

Pre-processed spectra were uploaded to the Bio4C® PAT Chemometric Expert Software and used to build a Partial Least Squares (PLS) model for each parameter. PLS calibration models were built using 5 batches in order to get a sufficient number of samples for calibration and ensure the robustness of the models. Glucose, lactate, Ammonium, osmolality, viable cell density (VCD), total cell density (TCD), and monoclonal antibody titer were calibrated **(Table 3).**

PLS calibration models were imported into Bio4C® PAT Raman Software for direct integration, visualization, and automated pre-processing of incoming real-time spectral data for monitoring of the process parameters. An independent intensified seed train cell culture (n=1 batch, 21 process samples) was conducted for model validation **(Table 4)**.

Results and Discussion

Seven independent PLS calibration models were built for the correlation of Raman spectra and the observed concentration of monoclonal antibody, glucose, lactate, ammonium osmolality and cell densities (VCD, TCD) in N-1 perfusion CHO cell cultures. Models were calibrated using off-line analytical data collected from 5 independent bench scale N-1 perfusion cultures (n=5) with a duration of 7-8 days, which translated into a total number of observations ranging from 86-101 observations across process parameters of interest **(Table 3)**. The PLS calibration model performance was evaluated by considering the determination coefficient R^2 (percentage of variance in the calibration dataset reflecting the goodness of fit), the cross-validated correlation coefficient Q^2 (percentage of variance explained by the cross-validation reflecting the predictive ability of the model) and the Root Mean Square Error of Cross Validation (RMSEcv). **(Table 3)**

Table 3. Experimental calibration dataset and PLS model

Parameter	Units	Sample Size	Range Value Min -Max	Latent Variables	R^2	Q^2	RMSEcv
VCD	10^6 cells.mL^{-1}	89	0 - 143	5	0.997	0.994	3.5
TCD	10^6 cells.mL^{-1}	89	0 - 148	6	0.999	0.997	3.6
Antibody	mg.L^{-1}.day^{-1}	86	0 - 1357	2	0.948	0.937	70
Glucose	g.L^{-1}	101	0 - 13.4	6	0.994	0.984	0.48
Lactate	g.L^{-1}	101	0 - 3.4	5	0.959	0.936	0.23
Ammonium	mM	101	0 - 5.5	5	0.915	0.873	0.37
Osmolality	mOsm.kg^{-1}	101	290 - 359	6	0.949	0.889	5.5

In general, the seven PLS models showed a good coefficient of correlation (R^2>0.91) during calibration and showed satisfactory performance during cross-validation (Q^2>0.87).

An independent batch was used as test validation set for model evaluation and real-time monitoring for each of the seven parameters. Model validation has been performed using the same process setup as calibration experiments and conducted over a period of 10 days. 21 observations for each parameter have been collected for offline reference values. The accuracy of the measurements was evaluated by considering the Root Mean Square Errors of prediction (RMSEp). The relative error (%) is also presented in **Table 4** as the ratio between the RMSEp and the maximum value in the prediction set for each parameter.

Table 4. **Process parameter prediction using PLS models**

Parameter	Units	Sample Size	Range Value Min -Max	RMSEp	Relative error (%)
VCD	106 cells.mL^{-1}	21	0.4 – 163	1.7	1.0%
TCD	106 cells.mL^{-1}	21	0.5 – 169	2.6	1.5%
Antibody	mg.L^{-1}.day^{-1}	20	37 - 1275	64	5.0%
Glucose	g.L^{-1}	21	0 - 10.5	0.33	3.1%
Lactate	g.L^{-1}	21	0.2 - 3.6	0.20	5.6%
Ammonium	mM	18	0.7 – 3	0.45	15%
Osmolality	mOsm.kg^{-1}	20	328 - 352	6.3	1.8%

For these attributes, a similarity in order of magnitude between RMSE values and a low relative error percentage, calculated based on RMSEp, was observed indicating an overall good model fit during prediction and therefore confirming their utility for deployment for bioprocess monitoring. **Figure 2** illustrates the use of these multivariate regression models as soft sensors for in-line and real-time monitoring of glucose, lactate, ammonium, osmolality, VCD, TCD and antibody volumetric productivity. On each graph the calibration ranges are highlighted in pink.

Based on **Figure 2**, ProCellics™ Raman Analyzer showed robust monitoring of CPPs with a maximum relative error of 15% for ammonium, among all the 7 CPPs that were selected for this study. For VCD and TCD, the model performed well even outside the calibration space with relative errors of 1.0% and 1.5% respectively. One of the many advantages of real-time monitoring is its ability to continuously measure the process parameters thereby filling the gaps in our understanding of the process, specifically, at the time points when manual samples are not available. In **Figure 2 B** and **2 D**, between day 9 and 10 there was a spike in lactate concentration and osmolality followed by a sharp drop. Around the same time there was a drop in ammonium concentration (in **Figure 2 C**) followed by a significant increase. These trends, which are difficult to capture by manual off-line samples, could be very valuable to scientists and engineers in improving their understanding of the cell culture process while reducing the risk of contamination associated with manual sampling. Thus, real-time monitoring through ProCellics™ Raman Analyzer with Bio4C® PAT Raman Software can help expand knowledge of the process and minimize process related deviations by being able to capture them ahead of time.

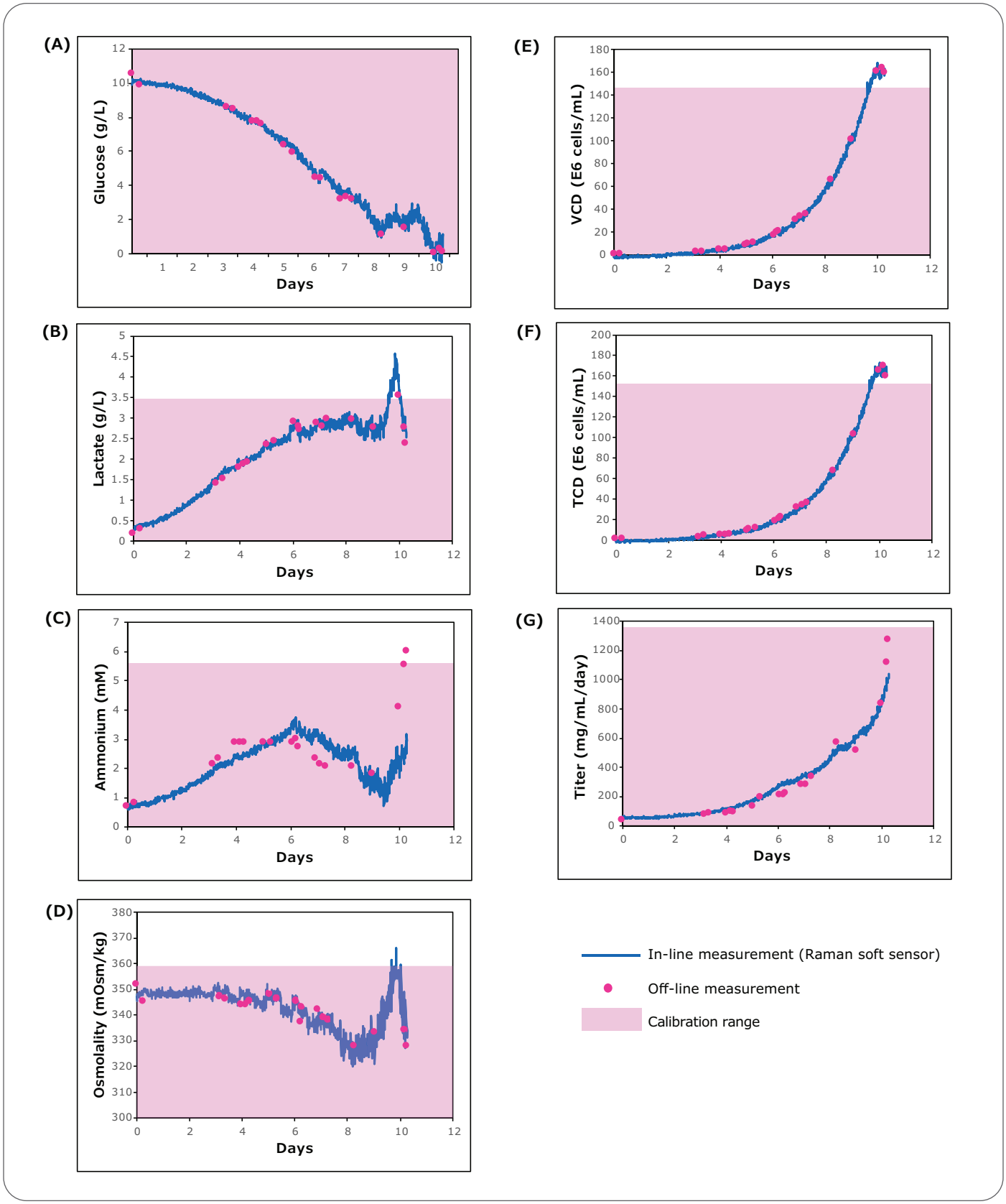

Figure 2: Deployment of Raman soft sensors for the in-line and real-time monitoring of critical process parameters in a N-1 perfusion CHO cell culture. The time series profile of: (A) Glucose (g L^{-1}), (B) Lactate (g L^{-1}), (C) Ammonium (mM), (D) Osmolality (mOsm.kg^{-1}), (E) Viable Cell Density (VCD, 10^6 cells mL^{-1}), (F) Total Cell Density (TCD, 10^6 cells mL^{-1}) and (G) Antibody volumetric productivity (mg/mL/day). In-line soft sensor predictions are displayed with a blue line, off-line reference measurements are displayed with dark pink dots, and the calibration range for each parameter is highlighted with a pink background.

Conclusion

This study highlights an experimental and multivariate modelling approach for the implementation of Raman spectroscopy for in-line and real-time monitoring of high cell density perfusion mammalian cell cultures. By using ProCellics™ Raman Analyzer with Bio4C® PAT Raman Software, we were able to successfully monitor multiple key cell culture variables based on in-line and real-time Raman spectra acquisition in a high cell density mAb producing perfusion CHO cell culture. Additionally, the Raman analyzer can greatly improve our understanding of the CHO cell perfusion process as the key cell culture variables are measured every 15 minutes in a non-destructive manner. Consequently, the implementation of combined advanced sensor technology, automated platforms and process data analytics tools can enable process monitoring, and subsequent control, to improve process yields and meet product quality requirements in the next generation of intensified upstream platforms.

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