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Application Note

In-Line Real-Time Monitoring of CHO Cell Culture Process Parameters using Raman Spectroscopy

Introduction

Cell culture processes are complex and highly variable and yet only a handful of key parameters such as temperature, pH, and dissolved oxygen (DO) are typically controlled in real time. While measurement and control of these parameters are essential for a robust process, they do not provide a direct indication of the culture content itself; instead, they provide only broad assumptions on the culture's true state and offer limited insights into the process and cell growth.

In contrast, critical process parameters (CPP) such as glucose, lactate, or ammonium and key performance indicators (KPI) such as total cell density (TCD) and viable cell density (VCD) provide direct indication of the culture's content and state. These measurements are typically measured offline, however, and do not provide real-time information or effective process control. Another challenge of monitoring cultures is the requirement for daily, manual sampling which increases the risk of batch failures due to the increased risk of contamination.

This application note describes use of the ProCellics™ Raman Analyzer with Bio4C™ PAT Raman Software (also known as Raman PAT Platform) to perform in-line and real-time measurement of TCD, VCD and the concentration of glucose, lactate and ammonium in a bench-scale bioreactor.

Material and Methods

Cell Culture

FreeStyle™ CHO-S (Gibco®) cells were inoculated at $0.4x10^6$ cells/mL in CD-CHO medium (Gibco®) with 8 mM glutamine (Gibco®), 1‰ of anticlumping agent (Gibco®) and 0.5% of penicillin/streptomycin in a water jacketed 3 L glass bioreactor. The cultivation conditions were set at 37 °C, pH 7.0 and DO 40% regulated by sparging CO_2 and 0.5 N NaOH for pH and a mix of air and O_2 for DO.

The cells were agitated with a pitched blade impeller at 80 rpm. The bioreactor was shielded with a lightproof cover to ensure that the Raman measurements were not affected by external light.

Feeding Strategy

The cultures were fed with CHO CD EfficientFeed™ B (Gibco®) feed solution with 15% v/v on day zero and 10% v/v on day three, six and nine. When glucose concentration dropped below 4 g/L, a highly concentrated glucose solution was added. Glutamine was added when the concentration dropped below 4 mM, and a constant glutamine feeding began on day three.

Sample Collection and Offline Analysis

Samples were collected twice a day when no feed was performed. For each feed, a sample was taken before and after the feed in addition to the daily sample. All samples were taken in triplicate and each triplicate was analyzed with an automated analyzer (Bioprofile® FLEX2™ - Nova Biomedical) for quantitation of TCD, VCD, glucose, lactate and ammonium.

Raman Measurements

Raman measurements were performed using the ProCellics™ Raman Analyzer with Bio4C™ PAT Raman Software. The optical probe of ProCellics™ Raman Analyzer was directly immersed into the bioreactor using a PG13.5 cable gland adaptor as shown in **Figure 1**. Laser excitation and spectral data collection were controlled by Bio4C™ PAT Raman Software using an Ethernet connection between the instrument and the computer. Each Raman measurement was the result of an average of 20 spectra of 45 seconds (15 minutes of acquisition in total) and measurements were scheduled every 30 minutes.

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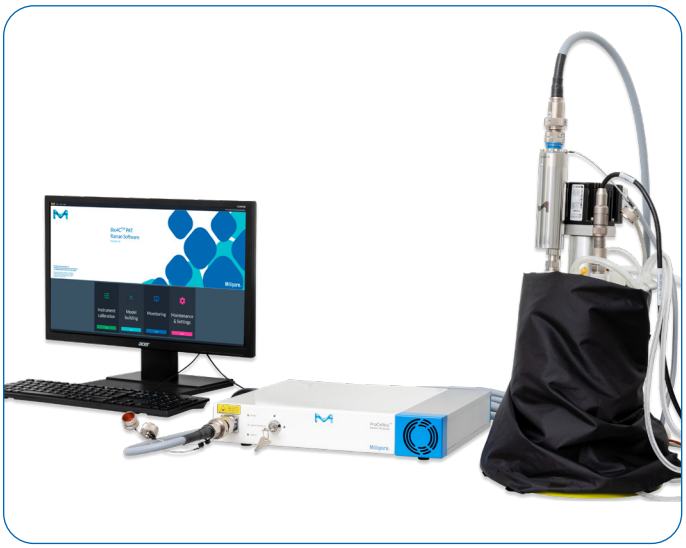

Figure 1: ProCellics™ Raman Analyzer probe immersed into the bioreactor, protected from external straylight with a lightproof fabric. The instrument is controlled by Bio4C™ PAT Raman Software.

Model Building

Offline data from three cell culture runs (a total of 76 points for all parameters and 73 for glucose) were automatically linked to their corresponding Raman spectra to generate a consistent dataset in Bio4C™ PAT Raman Software. The spectra were preprocessed in Bio4C™ PAT Raman Software using Standard Normal Variate on the water region, Savitzky-Golay derivative with 5 points (15 cm^{-1}, polynomial order 2 and a 1st derivative) and spectral selection (350 - 1,775 cm^{-1} + 2,800 - 3,000 cm^{-1}) to create a calibration dataset. A partial least squares (PLS) model was built for each process parameter (TCD, VCD, glucose, lactate and ammonium) using a multivariate analytics modeling software. Chemometric models were uploaded in the Bio4C™ PAT Raman Software and used to perform in-line and real-time monitoring of process parameters.

Results and Discussion

The use of Raman spectroscopy to monitor process parameters first requires chemometric model building with a calibration dataset. In this study, Raman spectra from three independent culture runs were collected using the ProCellics™ Raman Analyzer, preprocessed by Bio4C™ PAT Raman Software and used to build chemometric models for TCD, VCD, concentration of glucose, lactate and ammonium. These models were used by the Bio4C™ PAT Raman Software for real-time monitoring of process parameters for a new cell culture run from the acquired Raman spectra **(Figure 2)**.

Figure 2: Real-time monitoring of a CHO cell culture (red: TCD, light blue: VCD, orange: ammonium, pink: glucose, purple: lactate).

To assess the accuracy of Raman measurements compared with traditional offline sampling methods, samples were collected daily and analyzed. The root mean square error of prediction (RMSEP) and its percentage error were calculated for each parameter **(Table 1)**.

Table 1. PLS chemometric model validation results

Parameter	Range	RMSEP	Max value % error
TCD (10^6 cells/mL)	0.38 – 12.58	0.63	5%
VCD (10^6 cells/mL)	0.38 – 12.50	1.28	10%
Glucose (g/L)	2.51 – 9.54	0.35	4%
Lactate (g/L)	0.00 – 2.13	0.17	8%
Ammonium (mM)	0.77 – 21.85	1.50	7%

For cell densities, real-time monitoring and offline values presented the same cell growth kinetics trend **(Figure 3A)**. On day 6, the addition of a large volume of feed led to a cell dilution that was successfully monitored by the ProCellics™ Raman Analyzer with Bio4C™ PAT Raman Software. At the end of the culture, the total and viable cell densities were readily distinguished by Raman measurements and the analyzer enabled real-time tracking of the decrease of the cell viability. In this study, the total and viable cell densities were predicted in real time with an error of 5% and 10%, respectively **(Table 1)**.

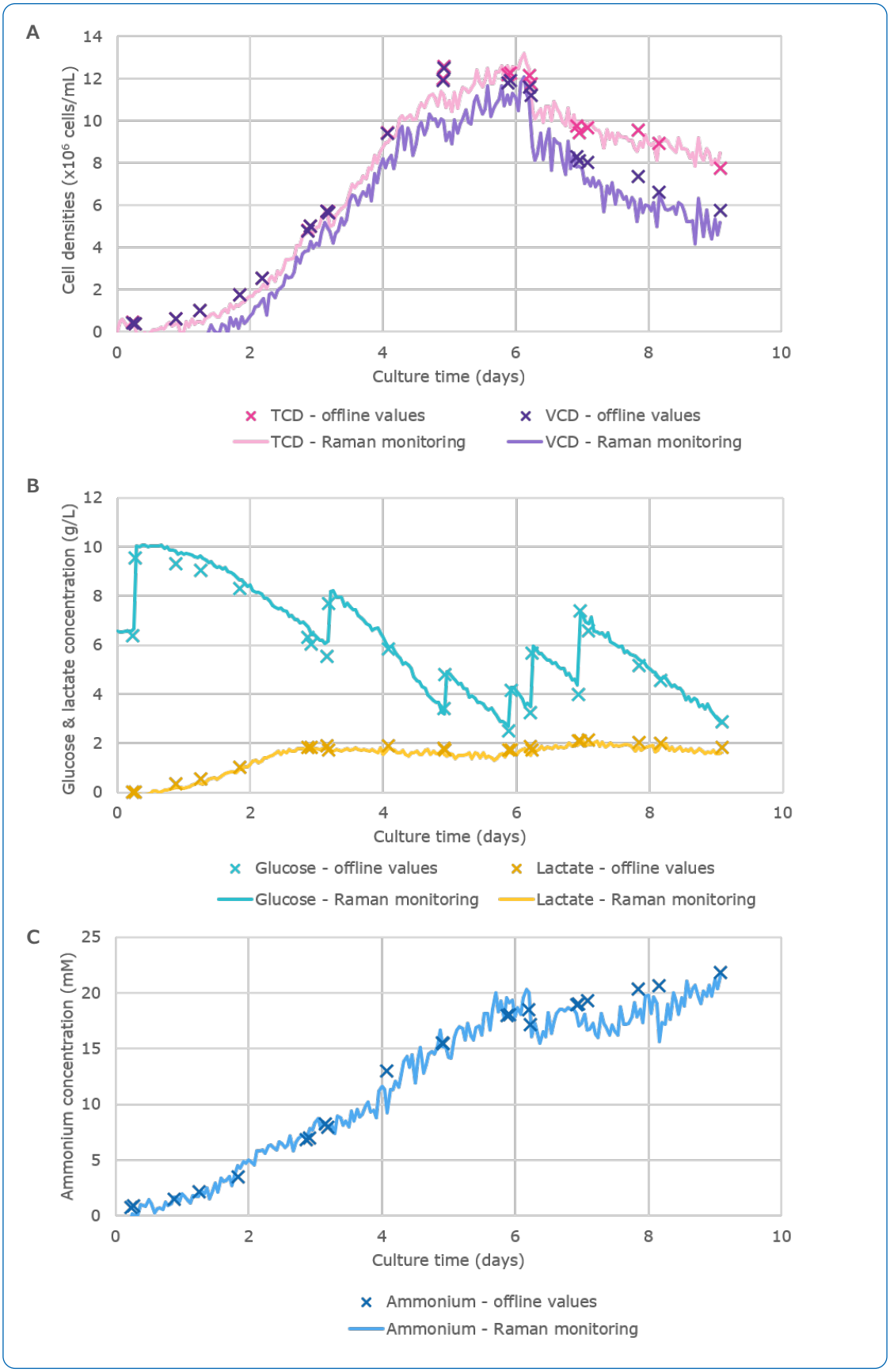

Figure 3: Comparison of offline measurements and real-time monitoring performed by ProCellics™ Raman Analyzer with Bio4C™ PAT Raman Software of TCD and VCD (A), glucose and lactate (B) and ammonium (C).

Real-time measurement errors are 4% for glucose, 8% for lactate and 7% for ammonium **(Table 1)**. As shown in **Figure 3B and 3C**, real-time measurements are consistent with offline data throughout the duration of the culture. All increases of glucose level following the feeds were accurately monitored by Bio4C™ PAT Raman Software.

Conclusion

ProCellics™ Raman Analyzer with Bio4C™ PAT Raman Software enabled in-line and real-time measurement of the critical process parameters glucose, lactate and ammonium, and the key performance indicators total and viable cell densities, in a bench-scale bioreactor.

By providing much more data than what is possible with offline sampling, the Raman PAT Platform helps to better understand processes and to collect digital data of processes. With real-time analytical data of the culture's content and status, this PAT tool helps increase process control and reproducibility and may lead to yield and quality improvement.

It could also decrease the risk of contamination and minimize the risk of batch failure. Finally it can also enable the implementation of an automated nutrient control loop strategy to reduce offline sampling and manual feeding, a first step toward process automation. Please refer to the Seamless Integration of Glucose Control using Raman Spectroscopy in CHO Cell Culture application note,

SigmaAldrich.com/deepweb/assets/sigmaaldrich/product/documents/309/390/seamless-integration-an7251en-mk.pdf

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