Millipore®
Preparation, Separation,
Filtration & Monitoring Products

Application Note

Seamless Integration of Glucose Control Using Raman technology in CHO Cell Culture

Abstract

Process analytical technology (PAT) and quality by design (QbD) are used in the biopharmaceutical industry to ensure quality is designed into a process and to achieve innovative quality improvements. In this study, ProCellics™ Raman Analyzer with Bio4C™ PAT Raman Software (Raman PAT platform) was used to implement a feedback control loop in a CHO cell culture process to monitor glucose concentration and enable maintenance of stable glucose levels without the need for human intervention. The ability to monitor and maintain the desired glucose concentration leads to improved process quality and supports proper glycosylation of the drug product.

The feedback control loop was based on a direct Open Platform Communications United Architecture (OPC-UA) connectivity between the Raman PAT platform and the bioreactor control system. The culture was fed with medium containing glucose. With use of the feedback control loop, the glucose concentration was steadily maintained for three days and process performances were similar to those of regular fed-batch cultures. The process was completely automated for glucose concentration management and did not require any human intervention. In addition, a noteworthy decrease of 35% in lactate production was observed.

Introduction

PAT and QbD guidelines, published by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA), reflect the concept that quality cannot be tested into a product but must be deployed throughout process development.

Seamless integration of monitoring into a bioprocess and the application of analytical data are crucial for understanding the process and proactively addressing manufacturing challenges. One of the biggest challenges is in-line monitoring of critical quality attributes (CQA) such as glycosylation that affects the stability, immunogenicity, safety and potency of the biomolecule. Maintaining the glucose concentration at a steady level in the bioreactor is essential to control and optimize the process yield and quality, including glycosylation[1,2]. Manual sampling and feeding of the bioreactor are costly and time consuming and increase the risk of contamination each time the sterile boundary is penetrated.

This application note describes the use of ProCellics™ Raman Analyzer with Bio4C™ PAT Raman Software to monitor glucose levels in a bioreactor and trigger automated addition of feed to maintain the desired concentration. Communication between Bio4C™ PAT Raman Software and Eppendorf DASware® control software was enabled by an OPC-UA[3] connectivity.

The combination of accurate measurements by the Raman analyzer and programming of complex feedback loops as functions of different parameters resulted in stable glucose concentration without the need of human intervention.

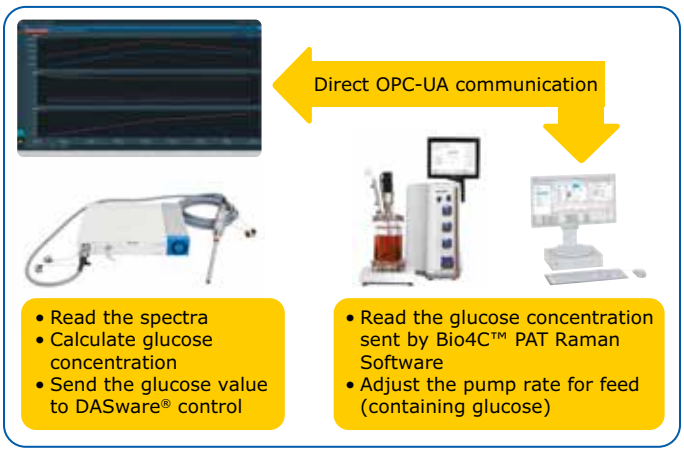

Figure 1.

Bio4C™ PAT Raman Software communicated directly with the bioreactor-controlling software to send the glucose concentration measured in the bioreactor.

Material and Methods

Media and Cell Line

FreeStyle™ CHO-S (Gibco®) cells were cultivated in CD-CHO medium (Gibco®) with 8 mM glutamine, 1‰ anti-clumping agent (Gibco®) and 0.5% penicillin/streptomycin.

Bioreactor Control System and Process Parameters

Process monitoring and control were performed using a BioFlo® 320 bioprocess controller with a water jacketed 3 L glass bioreactor. The bioreactor was equipped with a ring sparger and a pitched-blade impeller. The DASware® control 5.4.1 software was used to control the bioreactor. The bioreactor was inoculated with cells at a density of 0.4×10^6 cells/mL in a starting volume of 2 L. The bioreactor was shielded with a lightproof cover to ensure the Raman measurements were not affected by external light. Bioreactor settings are provided in Table 1.

Parameter	Setpoint	Control
Temperature	37 °C	Water jacket
pH	7.0 (deadband 0.1)	Sparging CO_2 or 0.5 N NaOH
pO_2	40%	Mix of air and O_2 sparging (flow rate max 0.1 vvm)
Stirring	80 rpm	

Table 1.

Process parameters and cultivation conditions

Feeding Strategy

Monitored batch: the culture was fed with a 15% v/v CHO CD EfficientFeed™ B (Gibco®) feed solution on day zero. Glutamine was added when the concentration dropped below 4 mM; constant glutamine feeding began on day three. For glucose feeding, a control loop was programmed based on the desired glucose concentration. The pump rate of the complex feed solution was controlled by a normal law (on DASware® control 5) based on the glucose concentration read by the ProCellics™ Raman Analyzer probe to maintain a glucose concentration of 5 g/L. The communication was integrated via OPC-UA and the function was defined as:

$$\text{Pump rate} = 2{,}000e^{\left(\frac{-[\text{Glucose}]^2}{5}\right)}$$

Control conditions: the cultures were fed using CHO CD EfficientFeed™ B (Gibco®) with 15% v/v on day 0 and 10% v/v on day 3, 6 and 9. When the glucose concentration dropped below 4 g/L, a highly concentrated glucose solution was added. Glutamine was also added when the concentration dropped below 4 mM in addition to a constant feed of glutamine starting on day 3.

Model Building for Raman Monitoring

Model building was used to correlate the reference values obtained by an offline analyzer (Nova Biomedical FLEX2) and ProCellics™ Raman Analyzer. The spectra were preprocessed on the Bio4C™ PAT Raman Software (SNV on the water region, Savitzky Golay derivative with 3 points (15 cm^{-1}, polynomial order 2nd and 1st derivative) and spectral selection (350–1,775 cm^{-1} + 2,800–3,000 cm^{-1})) to create a data set. The reference values were automatically linked to their corresponding spectra. The chemometric models for the monitoring were based on four standard fed-batch cultures (with a total of 103 points). A Partial Least Squares (PLS) model was developed for each monitored parameter using multivariate analytics modeling software. Models for viable cell density (VCD), glucose and lactate were created.

Raman Monitoring

The ProCellics™ Raman Analyzer with Bio4C™ PAT Raman Software acquired and preprocessed Raman spectra and calculated the process parameters including glucose concentration. Measurements were taken every 30 minutes and based on these measurements, the pump rate was adjusted.

Sensor Integration

Communication between Bio4C™ PAT Raman Software and DASware® control 5 software was enabled by an OPC-UA connectivity.

Results and Discussion

Glucose was consumed by the cells and stabilized around 5 g/L. As shown in Figure 2A, the glucose concentration was precisely maintained at 5 g/L for 3 days by the programmed feedback loop. As a control to compare the accuracy of the Raman analyzer measurements to traditional offline sampling methods, daily, offline samples were collected to measure glucose concentration.

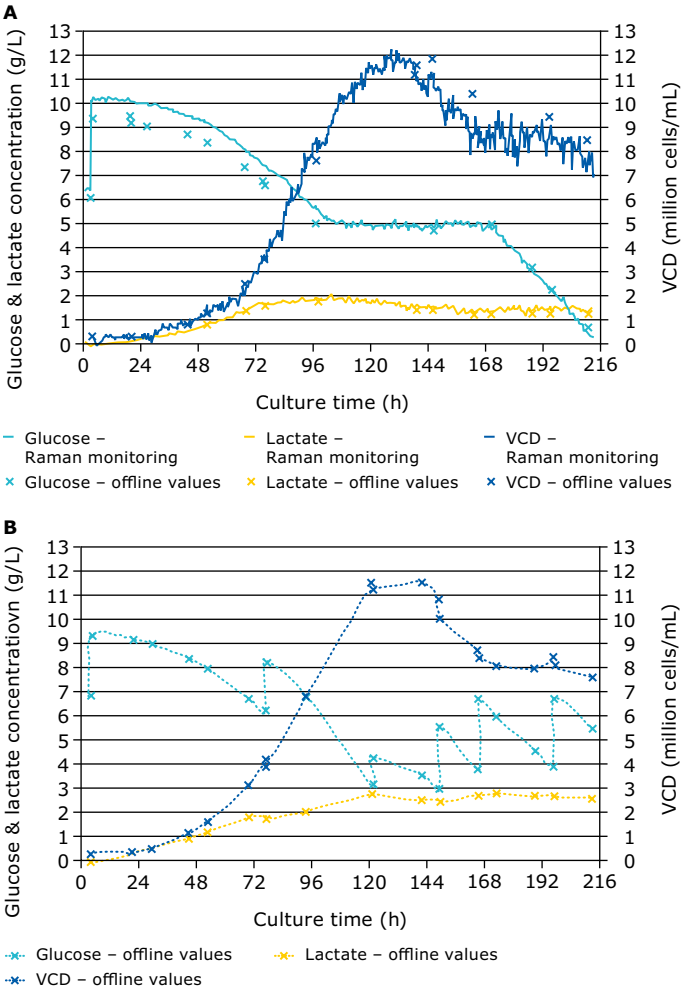

Figure 2.

Real-time monitoring of glucose, lactate and VCD for a fed-batch culture with a glucose feedback control loop strategy (A) and for a fed-batch culture with a manual glucose addition (B).

Feeding was stopped when the maximum vessel volume was reached; the process was stopped when the glucose concentration in the vessel dropped below 1 g/L. As a control, a classical fed-batch process with manual glucose addition was performed (Figure 2B). Cell growth kinetics and the maximum cell density in the feedback-controlled run were comparable with the classical fed-batch run. However, the lactate concentration in the feedback-controlled run was lower (1.8 g/L) in comparison to the control run (2.8 g/L). This is a noteworthy result, since high lactate concentration can be toxic for cells.

Figure 3 shows the culture parameters as displayed by Bio4C™ PAT Raman Software.

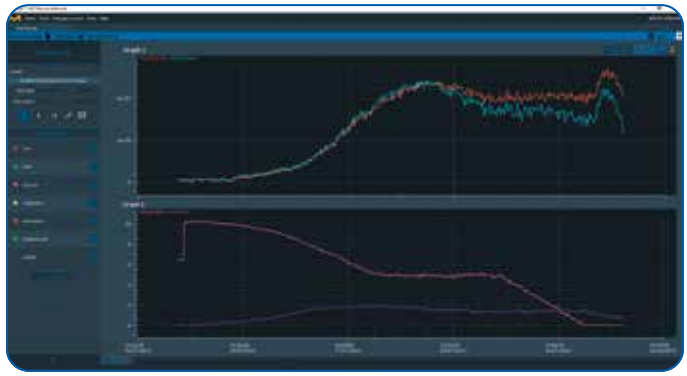

Figure 3.

Cell culture parameters (glucose, lactate, TCD and VCD) measured over the duration of the bioreactor culture as displayed in real-time on Bio4C™ PAT Raman Software.

Conclusion

Data monitored by Bio4C™ PAT Raman Software were efficiently and easily communicated to the DASware® control 5 software via an OPC-UA protocol. Following setup, automation of the feedback control loop was complete and reliable. An extremely stable glucose concentration was achieved along with accurate measurements by the Raman analyzer. This automated approach minimizes the number of human interventions needed to sample the bioreactor and maintain the desired glucose concentration, reducing the risk of contamination. Additionally, it reduces the risk of batch failures due to a lack of glucose resulting from gaps in manual monitoring such as during the night or on weekends.

Literature

1. Berry, BN et al., Quick Generation of Raman Spectroscopy Based In-Process Glucose Control to Influence Biopharmaceutical Protein Product Quality during Mammalian Cell Culture. Biotechnology Progress 32(1):224–34 (2016). https://doi.org/10.1002/btpr.2205
2. Yuk, IH et al., Controlling Glycation of Recombinant Antibody in Fed-Batch Cell Cultures. Biotechnology and Bioengineering 108(11):2600–2610 (2011). https://doi.org/10.1002/bit.23218
3. Lange, J et al., Frank Iwanitz, and Thomas Burke, OPC From Data Access to Unified Architecture, 4th rev. Ed., OPC Foundation – Softing (VDE Verlag GMBH, 2010)

Merck KGaA
Frankfurter Strasse 250
64293 Darmstadt
Germany

For additional information, please visit **www.MerckMillipore.com**
To place an order or receive technical assistance, please visit **www.MerckMillipore.com/contactPS**

Lit. No. MK_AN7251EN Ver. 0.1
04/2021