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APPLICATION NOTE No. 415

Seamless Integration of Glucose Control using Raman Spectroscopy in CHO Cell Culture

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Abstract

In the context of Process Analytical Technologies (PAT) implementation in the biopharmaceutical industry, Quality by Design (QbD) is being developed and widely implemented and used. In upstream processes, one compound of great interest to monitor is glucose, and specifically, being able to control its concentration during the process. Such a monitoring leads to process quality improvement, including glycosylation of the product of interest. In this study, a Raman analyzer has been successfully used to implement a feedback control loop in a CHO cell culture based on glucose concentration. The feedback control loop implied a direct OPC UA connectivity between the analyzer and the bioreactor control system The culture was fed with a complex feed containing glucose. As a result, glucose concentration was maintained steady for three days. The process performance remained similar to the ones of regular fed batch cultures and a noteworthy decrease in lactate production was observed. The process was completely automated for glucose concentration management and did not require any human intervention throughout the process.

Introduction

The Process Analytical Technology (PAT) and Quality by Design (QbD) guidelines, promoted by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) aims to support the idea that quality cannot be tested only into a product but must instead be deployed throughout design.

Seamless integration of monitoring and control of analytical data into a bioprocess is crucial to understand a process and to overcome manufacturing challenges.

One of the biggest challenges is the monitoring of quality attributes such as glycosylation. Important characteristics like stability and immunogenicity are affected by glycosylation. In order to receive regulatory approval, glycosylation is a Critical Quality Attribute (CQA) ensuring the safety and potency of biopharmaceutical products.

Maintaining the glucose concentration steady is key for the control and optimization of processes' yields and quality [1, 2]. Manual bioreactor sampling and feeding can be a costly endeavor, both in terms of labor costs as well as increased risk for contamination each time the sterile boundary is penetrated.

In this application note, researchers from Merck KGaA, Darmstadt, Germany integrated the ProCellics™ Raman Analyzer via OPC UA (Open Platform Communications United Architecture) connectivity into DASware® control software to optimize their bioprocess, controlled by a BioFlo® 320 bioreactor control system. DASware control allows the easy integration of third-party devices. OPC UA allows the independent implementation into a process while being safer, more stable, and more flexible than older OPC versions (such as OPC DA – Data Access) [3]. The programming of complex feedback loops as functions of different parameters, and the accurate measurements of the Raman analyzer, resulted in stable glucose concentrations without the need of human interaction.

Merck KGaA, Darmstadt, Germany *develops and commercializes a Raman analyzer for the biopharmaceutical industry, dedicated to in-situ monitoring of bioprocesses.*

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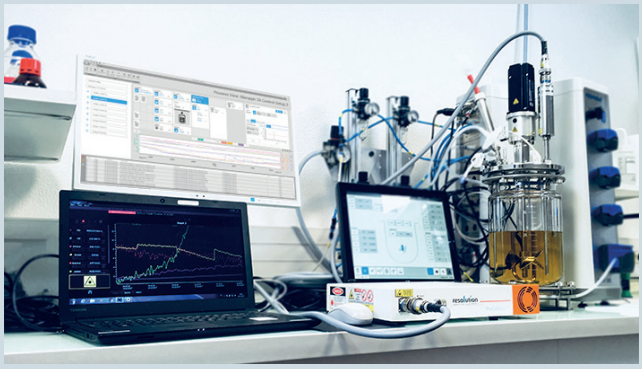

Fig. 1: Illustration of the complete system setup with the ProCellics probe inside the bioreactor controlled by DASware control 5.

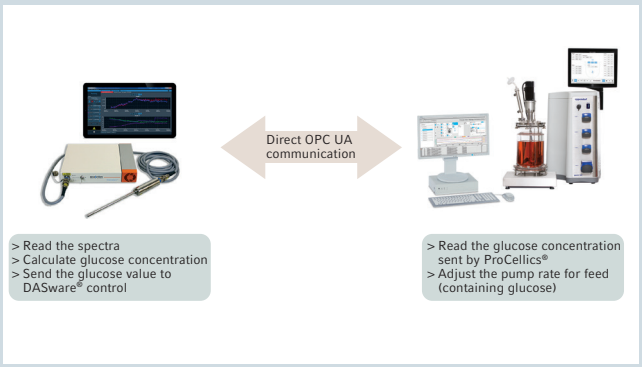

Fig. 2: Experimental setup: The analyzer communicates directly with the biocontroller to send the glucose concentration read in the bioreactor.

Material and Methods

Media

We used FreeStyle™ CHO-S (Gibco®) cells cultivated in CD-CHO medium (Gibco) with 8 mM glutamine, 1‰ of Anti-Clumping Agent (Gibco) and 0.5 % of Penicillin/Streptomycin.

Bioreactor control system and process parameters

We performed the CHO cultivation, and process monitoring and control with 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 experiment. The bioreactor was inoculated with cells at a density of 0.4 x 10^6 cells/mL, with a starting volume of 2 L. Bioreactor settings to control the process are listed in Table 1.

Table 1: Process parameters and cultivation conditions.

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

The bioreactor was shielded against external light to make sure that the Raman measurements were not affected by external light.

Feeding strategy

The culture was fed with 15% v/v EfficientFeed™ B (Gibco) on day zero. Glutamine was added when the concentration dropped below 4 mM. On day three, we started with constant glutamine feeding. For glucose feeding, a control loop was programmed based on the glucose concentration: the pump rate of feed B (containing glucose) was controlled by a normal law (on DASware control 5) based on the glucose concentration read by the ProCellics Raman Analyzer to maintain a glucose concentration of 5 g/L. The communication was integrated via OPC UA. The used function was:

$$\text{Pump rate} = 2000e^{\left(\frac{-[\text{Glucose}]^2}{5}\right)}$$

Model building for Raman monitoring

To perform monitoring with Bio4C® PAT Raman Software, a step of model building is needed to correlate the reference values obtained by the BioProfile® FLEX2™ (Nova Biomedical®) and ProCellics Raman Analyzer (Merck KGaA, Darmstadt, Germany). 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 (350-1775cm^{-1} + 2800-300 cm^{-1}) to create a dataset. The reference values were automatically linked to their corresponding spectra. The chemometric models for the monitoring are based on four standard fed-batch cultures (total of 103 pt). A PLS model was computed for each monitored parameter using SIMCA® Software (SARTORIUS STEDIM BIOTECH®). Models for Viable Cell Density (VCD), Total Cell Density (TCD), glucose, glutamic acid, ammonium and lactate were performed.

Raman monitoring

The ProCellics Raman Analzyer acquired and preprocessed Raman spectra, and calculated the process parameters

including glucose concentration. Based on the scheduled frequency, measurements were carried out every 30 minutes. Following the previously described loop settings, the pump rate was adjusted according to the measurements every 30 minutes.

Third-party sensor integration

Connectivity between the ProCellics Raman Analyzer and the DASware control software was implemented with OPC UA.

Results and Discussion

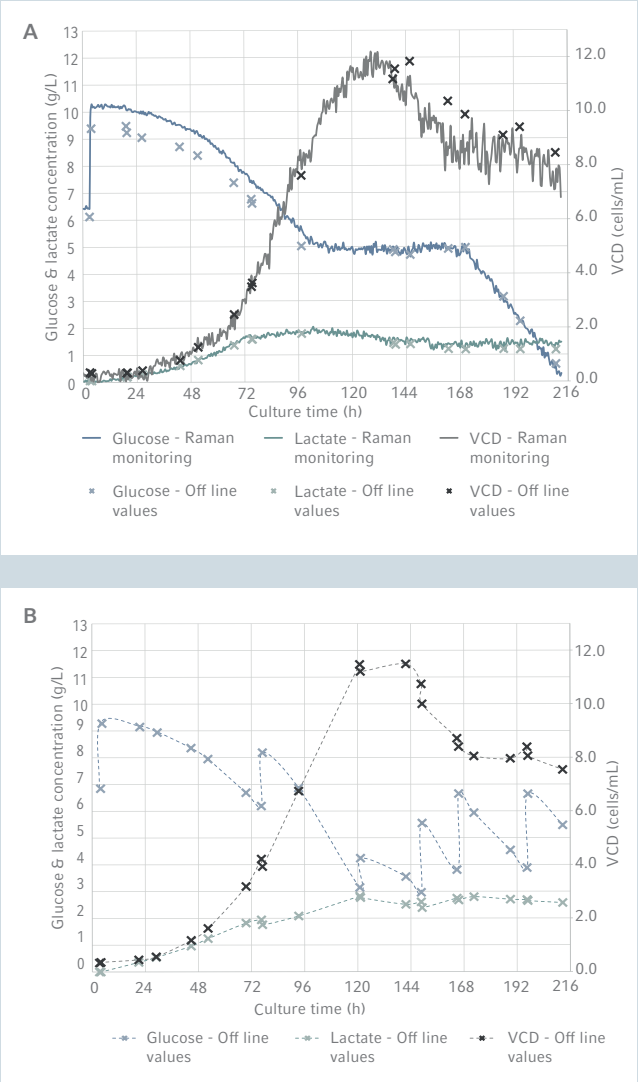

Fig. 3: Cell culture parameters evolution.
A: Glucose feedback control loop based on Raman measurement (full lines) in comparison with offline measurements for reference (cross)
B: Control: Classical fedbatch culture (offline measurements only)

Until day 4 glucose was consumed by the cells until the minimum set value of 5 g/L was reached. The glucose concentration was precisely maintained at 5 g/L for 3 days by the programmed feedback loop (Figure 3A). In parallel, glucose concentrations have been measured offline with FLEX2 in order to assess Raman monitoring. These measurements confirmed that the Raman analyzer accurately measured the glucose concentration.
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 run with manual glucose addition was performed (Figure 3B). The cell growth kinetics and the maximum cell density in the feedback-controlled run was comparable with the classical fed-batch control run. However, the lactate concentration in the feedback-controlled run was lower (2 g/L) in comparison to the control run (3 g/L). This is a noteworthy result, since to high lactate concentrations can be toxic for cells.

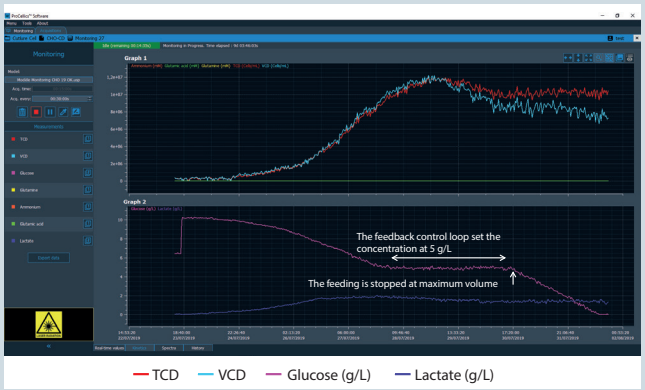

Fig. 4: Cell culture parameters evolution over the cultures, displayed on Bio4C PAT Raman Software.

Conclusion

DASware control 5 enabled the efficient and easy integration of the ProCellics Raman Analyzer via OPC UA protocol. OPC UA is more secure compared to OPC DA. With this setup, we were able to prove that ProCellics Raman Analyzer is fully ready for process automation. Once set up, the automation of the feedback control loop was complete and reliable.
A great level of confidence for the extremely stable glucose concentration, and accurately measured with the Raman analyzer was achieved. This allows to reduce the number of human interactions needed, thus reducing the risk of contamination due to repeated sampling. Less sampling is needed due to the automation, resulting in minimized manual work and reducing the risk of contaminations. Additionally, the risk of batch failures due to a lack of glucose during the night or at weekends is reduced.

Literature

[1] Brandon N. Berry 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, no. 1 (2016): 224–34, https://doi.org/10.1002/btpr.2205

[2] Inn H. Yuk et al., "Controlling Glycation of Recombinant Antibody in Fed-Batch Cell Cultures," Biotechnology and Bioengineering 108, no. 11 (2011): 2600–2610, https://doi.org/10.1002/bit.23218

[3] Jürgen Lange, Frank Iwanitz, and Thomas Burke, OPC From Data Access to Unified Architecture, 4th rev. Ed., OPC Foundation - Softing (VDE Verlag GMBH, 2010)

Ordering information

Description	Order no.
BioFlo® 320, overlay gas option, 1 TMFC (0.05 – 5 SLPM)	1379502111
Software License, DASware® control 5, for one culture vessel	78600166
Vessel Bundle, for BioFlo® 320, water jacket, magnetic drive, 3 L vessel	M1379-0311
Pitched-Blade Impeller Kit, magnetic drive, 3 L	M1379-5069
Harvest tube	M1287-9483

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