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APPLICATION NOTE No. 491

Implementing Raman Spectroscopy for Automated Monitoring of a Cell Culture Bioprocess

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Abstract

Optimizing upstream bioprocessing in the biopharmaceutical industry relies heavily on data on process parameters, cells, and products. The automation of data acquisition through inline measurements offers significant advantages by reducing manual labor and enabling real-time measurements, which can serve as basis for process control via automated feedback loops. As a Process Analytical Technology (PAT), Raman spectroscopy is a valuable method for the analysis of process parameters in real-time. The interpretation of Raman spectra needs chemometric models, whose development requires a substantial number of reference data points obtained through alternative analytical devices. This application note describes how bioprocess data for Raman model development were efficiently acquired by conducting four bioprocesses in parallel using SciVario® twin bioreactor control systems (Eppendorf SE) equipped with a ProCellics® Raman Analyzer (Merck KGaA, Darmstadt, Germany) in multi-channel configuration. Furthermore, we detail how we used Raman spectroscopy to monitor a CHO cell fed-batch process in real-time.

Introduction

Mammalian cells are the foundation of many biopharmaceutical production processes, like those of many therapeutics, vaccines, and diagnostics. The product types are diverse and include proteins, viral vectors or, in the case of cell therapies, the cells themselves. Data on the bioprocess, cells, and product are vital in ensuring efficient production of the desired product at consistently high quality.

Process parameters: The effective production of the desired product is dependent on the establishment of appropriate culture conditions. This encompasses the optimal values for process parameters, including pH, temperature, and dissolved oxygen (DO), in addition to the concentrations of essential nutrients. A carbohydrate source and essential amino acids must be provided during cell growth and protein production, as these are used during this process. Concurrently, cell metabolism generates by-products such as lactate or ammonia, which, at specific concentrations, can impede cell growth or even be toxic to the cells.

Glucose is a principal source of carbon used by the cell for growth and protein production. Therefore, monitoring glucose levels is essential for ensuring sufficient availability for cellular processes. Real-time measurement enables the user to ascertain the optimal timing and dosage of glucose supplementation to the culture medium. Lactate production

serves as an indicator of cell metabolism, and significant lactate production may indicate a deviation in the process. Furthermore, elevated lactate levels can exert a toxic effect on cells. Monitoring this parameter provides an early warning of potential issues in the bioproduction process.

Cell data: The monitoring of the viable cell density and viability is of paramount importance in the assessment of the status of the bioprocess and must be conducted throughout the process. The monitoring of cell quantity and health, particularly during the process development stage, enables the identification of parameters that can be modified to enhance yields. During the production phase, the monitoring of cell densities and viability helps assure that the process is operating in accordance with the specifications established during the process development stage.

Product data: The objective of bioprocess development is the efficient and high-quality production of the desired product. Data on product yield and critical quality attributes are essential to achieve this objective.

The collection of data necessitates the use of sensors. In principle, there are two methods for data collection in upstream bioprocessing. The first is to obtain a sample from the bioreactor and measure the parameters in that sample. The second is to measure the parameters without taking a sample but using integrated sensors. The latter is particularly advantageous because it allows parameters to be measured in real-time, and the data obtained can be used as a basis for process control via feedback loops, thus enabling process control to be automated.

Raman Spectroscopy

Raman spectroscopy is one of the technologies that enables the analysis of bioprocess parameters in real-time. The Raman spectrum can be used as a molecular fingerprint that provides information about various process parameters. The performance of this technology has been demonstrated in the literature and in industry for a number of parameters, including viable and total cell density, protein titer, glycoforms, metabolites, and nutrients such as glucose, lactate, ammonia, glutamine, glutamic acid, and other amino acids [1, 2].

Raman models are usually built from experimental data. For chemometric modelling, bioprocesses are conducted and process parameters of interest are measured at different times of the process using alternative analytical methods.

Principle of Raman spectroscopy

The Raman effect

The Raman effect is the exchange of energy between a photon and the vibrations of the molecule. In simple terms, it means that when light interacts with a chemical bond, the color of the light changes, resulting in a Raman spectrum. This color change is specific to the chemical bond in question: A Raman spectrum can therefore be considered as a molecular fingerprint.

Raman spectroscopy in bioprocessing

In bioprocessing, Raman spectroscopy can be used to analyze a range of process parameters. A Raman sensor is introduced into the culture medium, and a laser emits light from an analyzer via an optical cable to the end of the sensor. The Raman effect occurs in the culture medium, is collected by the sensor, and returned to the analyzer via the optical cable. The analyzer breaks down the different Raman signal contributions of each measurement into a Raman spectrum. Subsequently, the Raman spectra are sent to a software program where calculations are conducted using chemometric models, resulting in concentration values for process parameters such as nutrient concentrations and cell densities.

Raman model building

In bioprocessing, the culture media contain many compounds, so the resulting Raman spectra can be very complex to analyze. In order to extract concentrations from the spectra, a modelling step is essential in bioprocess applications. A first step for modelling is to collect Raman spectra along with reference measurements to model correlations between the concentrations of the specific compound and the Raman spectra, so that once the model is built, the values can be calculated directly from the spectra in real-time.
Raman spectroscopy can then measure any molecule-related parameters, provided there is a reference measurement available for modelling and it is in sufficient concentration, as with any analytical instrument.

Get support

As multivariate data analysis and chemometric modelling can be a different area of expertise from bioprocessing, Merck experts are available to build chemometric models for users. Users simply need to export the data set from the software and send it to the support team. The support team performs the data analysis and provides the user with a model file along with explanations. After importing the model file into the software, it can be used for bioprocess monitoring.

These data are then employed in the chemometric modelling process. It is essential to obtain a sufficient number of data points to develop a robust model. Parallel processing can facilitate the rapid acquisition of data.

This application note demonstrates the use of Raman spectroscopy to monitor a CHO fed-batch bioprocess. It describes how parallel bioprocessing using two SciVario twin bioreactor systems, each with two bioreactors, and a 4-fold ProCellics instrument enabled the development of a chemometric model after four parallel runs. It also shows how the quality of the Raman spectroscopy data was assessed and compares the effects of straylight on different types of Raman sensors. Finally, it demonstrates the use of Raman spectroscopy to monitor total and viable cell density and the concentrations of glucose, lactate, and glutamate in real-time.

Material and Methods

Bioreactor system
Two SciVario® twin bioreactor systems (Eppendorf SE) were used. Each system was equipped with two DR03C glass bioreactors with a working volume of 1 L (Figure 1).

Raman analyzer
The bioreactors were equipped with ProCellics Raman Analyzer with Bio4C® PAT Raman Software (Merck KGaA, Darmstadt, Germany) (Figure 1). Measurements were carried out in multi-channel configuration with immersion sensors running four cell cultures in parallel. A standard sensor and an advanced sensor for straylight management were compared. The sensor tube was placed on a Pg 13.5 (12 mm port) on the bioreactors' head plate and autoclaved with the vessel. Before autoclaving, the Raman sensor calibration was checked using immersion in isopropyl alcohol.

Offline analytics
A BioProfile® FLEX2 analyzer (Nova Biomedical) was used for reference measurements on samples to build the chemometric models.

Bioprocess
CHOZN® cells (Merck KGaA, Darmstadt, Germany) were precultured in a shake flask in a shaking incubator at 37 °C, 100 rpm, and 5 % CO_2. The preculture was used for bioreactor inoculation at a cell density of 0.5 x 10^6 cells/mL. The cells were cultured in the bioreactors in fed-batch mode for eight days. The process parameters and process control strategies are summarized in the Tables 1 and 2. Four cell culture runs were run simultaneously using the same process parameters and process conditions. pH, DO and temperature were measured using the sensors provided with the bioreactor. Glucose, lactate, glutamic acid, viable cell density (VCD) and total cell density (TCD) were analyzed using Raman spectroscopy. The models are built from data from a previous four parallel runs conducted in the same way.

Fig 1A: SciVario twin – ProCellics Raman Analyzer Multi-Channel Unit setup in a dark environment used for the experiment shown in Figures 2 and 3 with opaque protections on the four bioreactors.

Fig 1B: SciVario twin – ProCellics Raman Analyzer Multi-Channel Unit setup in a light environment used for the experiment shown in Figure 5 without opaque protections on the four bioreactors.

More runs in less time

The SciVario twin is capable of operating two bioreactors individually in any combination of compatible type and working volume.

For more technical information, please visit
www.eppendorf.group/sci-vario

Table 1: Process parameter setpoints and control

Process parameter	Setpoint	Control
pH	**7.0 (deadband 0.1)**	0.5 N NaOH or sparging CO_2
DO	**40 %**	Mix of gas (air, O_2)
Temperature	**37 °C**	Heating blanket
Agitation	**100 rpm**	Marine impeller, magnetic drive

Table 2: Cell culture conditions

Cell line	CHOZN®
Cell culture media	EX-CELL® Advanced Medium + 0.4 % of penicillin/streptomycin
Inoculation density	0.5 × 10^6 cell/mL
Feeding strategy	**Feed:** 50 %/50 % mix of EX-CELL® Advanced CHO Feed 4 and CellVento® 4Feed COMP; 5 % v/v on day 3 and 5, and 7.5 % v/v on day 7 **Glucose:** added as bolus when under 4 g/L and constant glucose addition when needed

Results

Measurement of process parameters using Raman spectroscopy

All four cell culture runs achieved approximately 20 million cells/mL in a fed-batch process after eight days of cultivation and were accurately monitored using Raman spectroscopy. Figure 2 shows a screenshot of the Bio4C PAT Raman software used to monitor four cell culture runs in real-time. The four runs using two SciVario twin bioreactor systems

Fig 2: Bio4C PAT Raman Software monitoring four cell culture runs in real-time.
Brown: Ammonium [mM]. Magenta: Glucose [g/L]. Blue: Glutamic acid [mM]. Red: Glutamine [mM]. Yellow: Lactate [g/L]
Green: Total cell density [× 10^6 cells/mL]. Purple: Viable cell density [× 10^6 cells/mL].

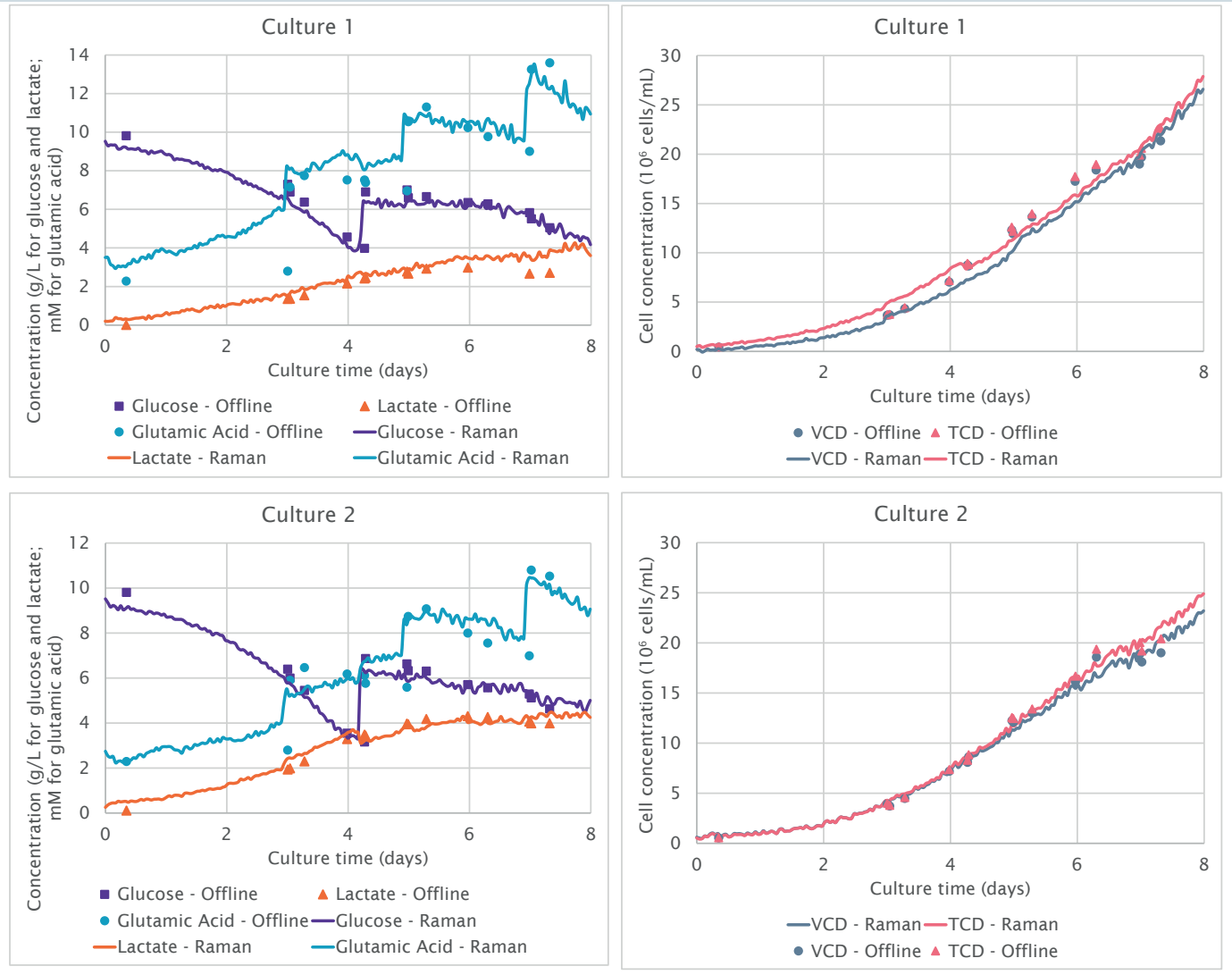

Fig 3: Comparison of cell culture monitoring using Raman spectroscopy or monitoring based on sampling for two batches.
Left: Concentrations of glucose [g/L], lactate [g/L], and glutamic acid [mM].
Right: Viable cell density (VCD) and total cell density (× 10^6 cells/mL).

in parallel performed similarly in terms of cell growth and metabolic evolution.

An example of data monitoring of two of these batches is shown in Figure 3. It compares the Raman spectroscopy-derived values with values obtained using an automated offline analyzer FLEX2 used a reference. For both batches, the viable cell density (VCD) and total cell density (TCD) values obtained by Raman spectroscopy overlap completely with the reference values, demonstrating the good monitoring quality of the cell densities during the exponential growth of the cells. Glucose was also well monitored in both cases, and the glucose concentration was successfully maintained above 4 g/L by constantly adding glucose via the bioprocess controller's pumps. For lactate and glutamic acid, while the accuracy of Raman measurements could be improved, the trends of evolution of

the concentration is overall similar to those observed in the offline samples.

The root mean square error of prediction (RMSEP) of two batches are summarized in Table 3, along with the relative errors of the Raman measurement compared to references measurement on samples. Except for lactate on the first batch, all the relative errors are below 10 %, which is typical for any analytical method, and demonstrate the ProCellics Raman Analyzer capabilities as an analytical instrument for cell culture monitoring (Figure 4).

Table 3: Root Mean Square Errors of Prediction (RMSEP) and relative errors (%) of the Raman measurement compared to references measurement on samples of the respective cell culture runs

	Culture 1		Culture 2	
Parameter	**RMSEP**	**Relative error (%)**	**RMSEP**	**Relative error (%)**
Glucose [g/L]	0.41	4	0.36	4
Lactate [g/L]	0.37	12	0.28	6
Glutamic acid [mM]	1.21	9	0.88	8
VCD [× 10^6 cells/mL]	1.55	7	0.86	5
TCD [× 10^6 cells/mL]	1.00	4	0.77	4

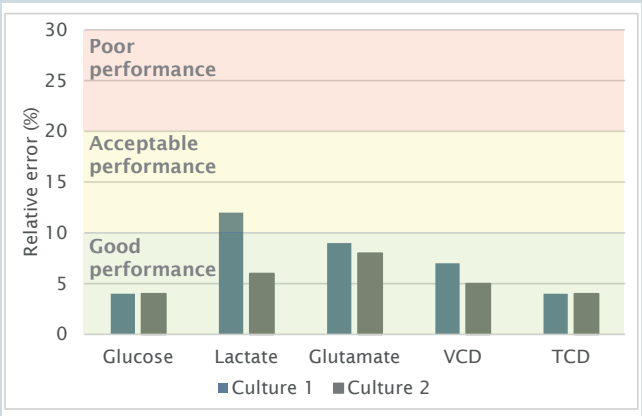

Fig. 4: Relative errors for Raman measurement compared to offline measurement.

Comparison of a standard Raman sensor and an advanced sensor for straylight management

Raman measurements can be affected by external light, leading to inconsistencies in the values of the cell culture parameters. Figure 5 shows an example of the same cell culture run monitored in a light environment (see set up in Figure 1B) using a standard Raman sensor as reference and the advanced solution for straylight management. This advanced solution is the combination of hardware (sensor with light-reducer cap) and software (noise reduction filter) features. As can be seen in the graph, with a standard sensor, the measurement of the cell culture parameters is strongly affected and irrelevant in the presence of daylight, whereas with the advanced solution with straylight management available with the Raman PAT Platform, the measurement of glucose and VCD remains stable days and nights.

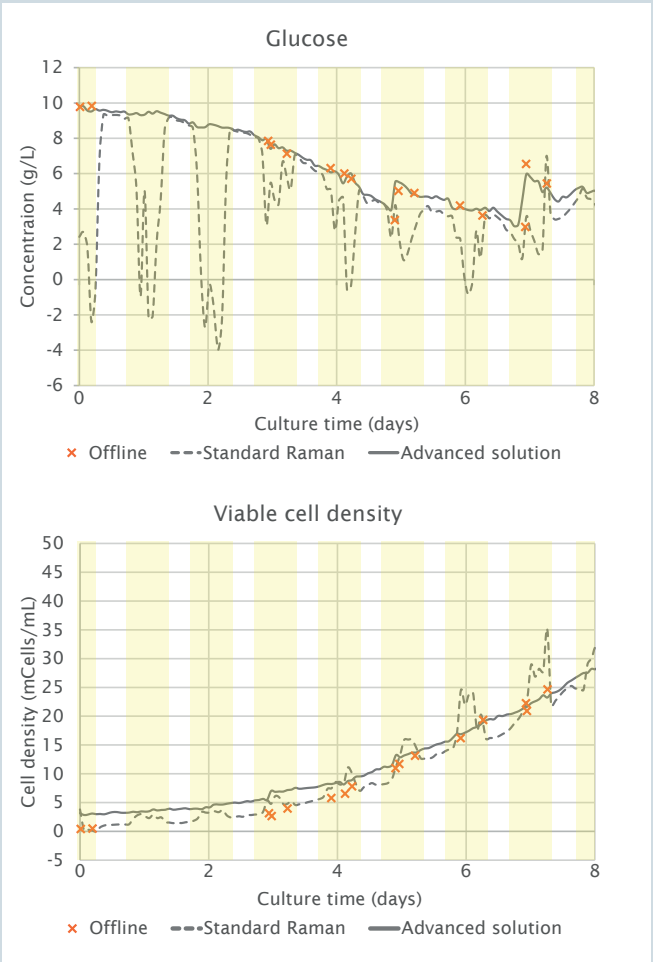

Fig. 5: Monitoring of glucose and VCD in a light environment using a standard Raman sensor or the advanced Raman sensor with straylight management feature of the Raman PAT Platform; in yellow, the time of the day with daylight in the lab.

Conclusions

Raman spectroscopy was implemented for real-time monitoring of a CHO fed-batch bioprocess. The combination of a multi-channel ProCellics Raman Analyzer with two SciVario twin parallel bioreactor systems resulted in significant four-fold time savings compared to standalone systems, as four cell culture runs processed in parallel delivered sufficient data for the creation of a chemometric model.

The availability of real-time data provided the basis for effective control of critical process parameters, including lactate production and glucose concentration. A further improvement in data utilization may be achieved by leveraging the Raman PAT Platform with the bioprocess control software DASware® control through OPC UA connectivity (as demonstrated in Application Note 415). This will enable the data gathered through Raman spectroscopy to be displayed and exploited concurrently with other parameters within the same software, facilitating direct utilization for automated process control.

References

[1] Graf A, Neubrand C, Baur D, Kadisch M. Comparison of Raman and MIR spectroscopy for bioprocess monitoring of mammalian cell cultures. Pharm. Ind. 83, 11, 1523-1529, 2021.

[2] Li M, Ebel B, Chauchard F, Guédon E, Marc A. Parallel comparison of in situ Raman and NIR spectroscopies to simultaneously measure multiple variables toward real-time monitoring of CHO cell bioreactor cultures. Biochemical Engineering Journal, Vol 137, 205-213, 2018.

Ordering information

Description	Order no.
SciVario® twin Fermenter/Bioreactor Control System, base unit, 100–240 V/50/60 Hz, for 2 vessels	7600100001
DASGIP® Vessel, DR03C, 750 mL–2.7 L, dip tube, overhead drive	76DR03C

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AA491-020-01-102024