BioTech

CHAUVIN ARNOUX
INDATECH

Raman spectroscopy: transforming the control of monoclonal antibody bioprocesses with PAT tools

Introduction

Biotherapies have profoundly transformed the medical landscape by offering more targeted and customized treatments than conventional therapies based on small chemical molecules. Now accounting for more than 50% of new drugs, advances in bioprocess engineering and automation are essential to improve the efficiency of biological systems and thus reduce the costs of bioproduction.

In the context of a French economic recovery plan encourageing the national bioproduction sector, the CLIMBIN project aims to provide an innovative solution for process analytical technology using molecular optical spectrometry tools.

Objectives

The main objective of the project is the production and qualification of a process analytical solution for monitoring the critical process and quality parameters for bioproduction, as well as the quantification and qualification of the various elements of interest (metabolites, proteins and therapeutic biomedicines).

This solution should enable real-time, *in situ* monitoring of bioproduction without sampling, thus improving on conventional bioprocess analysis practices. Coupled with an embedded chemometric data analysis function, which must be adapted to a variety of bioreactors and production scales in order to supervise the entire bioproduction process. The approach has been demonstrated on a CHO cell line for therapeutic antibody manufacturing.

Equipment & methods

Unique on the market, VISERION allows up to 4 simultaneous Raman measurements using 4 probes and 4 lasers. Lasers with different emission wavelengths of 785 or 830 nm can be combined, each with adjustable power. A very low temperature cooling system allows high quality spectroscopic analysis with very low noise levels, thus facilitating the use of long integration times, which can be critical for bioprocesses.

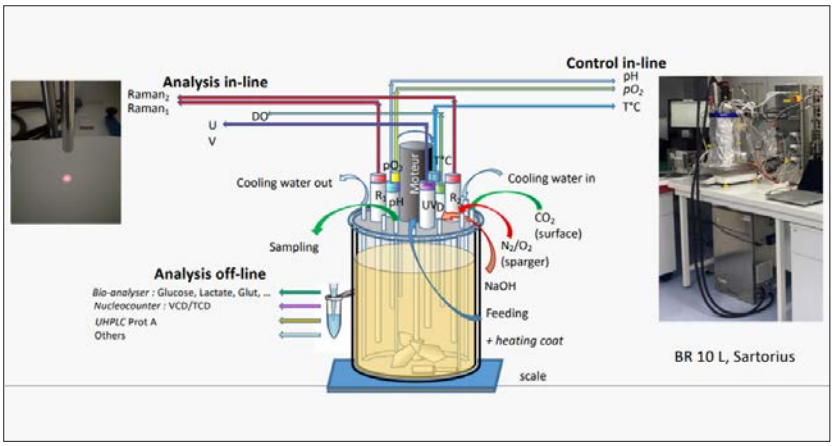
VISERION has an industrial PC integrated into the stainless steel casing to make it as robust as possible. Several interlocks guarantee laser safety. The 21 CFR part 11 compliant software allows not only the recording of measurements and modulation of their acquisition parameters, but also the integration of chemometric models, enabling critical process parameters to be monitored. The system is able to communicate with other PLC software using the OPC-UA communication protocol to fully automate production monitoring.

Indatech has developed an entire ecosystem around the Raman optical probe, offering great flexibility for Raman measurements thanks to an immersion probe coupled with a set of configurable end-fittings that can be adapted to different measurement contexts and applications. The user can easily switch between transflection probe tips or probe tips with variable working distances (WD).

The principle of the transflection probe involves the use of a reflective surface. This doubles the optical path, increasing sensitivity to low-concentration samples. In the case of Raman measurement, INDATECH has developed a transflection fitting that also considerably reduces the parasitic light inherent in the long integration times of Raman measurement in complex media such as cell cultures. This fitting is very effective in eliminating ambient light during production, but makes it all the more sensitive to increases in the density/turbidity of the medium. A non-transflection probe simply measures the light after a single pass through the sample, making it more suitable for more concentrated or opaque samples. Another advantage of the non-transflection fitting is that the working distance can be adjusted, allowing for further adaptation to the characteristics of the medium being measured. The range of probe tips offered by Indatech can therefore be adapted to any type of medium with a variety of physical and chemical properties and optical behaviors.

The configuration of the VISERION device selected for CLIMBIN after several experimental measurement campaigns consists of two measurement channels at 785 nm, each equipped with non-transflection probe tips, guaranteeing the best sensitivity under the physical and chemical conditions of the bioprocess being monitored. This involves the cultivation of CHO cells, which are widely used in the pharmaceutical and biotechnology industries for the production of therapeutic biomedicines, particularly antibodies.

The database consists of 5 batches of cell cultures, fed in 3 cycles, in a BioBlu 10C (10 L) bioreactor (Figure 1). Monitoring is carried out continuously in-line with two Raman probes (one probe per bioreactor) by taking a measurement every 30 minutes, and in parallel sampling before and after feeding for reference measurements (metabolites, nutrients, cell density). The entire data analysis was carried out using PLS_Toolbox (Eigenvector Research) running in the MATLAB environment (The Mathworks).



Run	Bio-reactors	Number of samples	
		Raman spectra	References
1	1	758	31
2	2	1 140	31
	3	1 140	31
3	4	768	20
	5	629	20
	TOTAL	**4 435**	**133**

Figure 1: Presentation of the measurement configuration and the acquired data sets

Results & discussions

An initial exploratory analysis was performed on the first batch of cell culture using principal component analysis (PCA), presented in Figure 2.

The first graphic representation of PC1 vs PC2 shows the evolution of the culture as a function of cell density, which can be observed via the evolution of the baseline on the Raman spectra. Components 3, 4 and 5, on the other hand, cover the chemical information, confirmed by the appearance of well-defined peaks in the loadings.

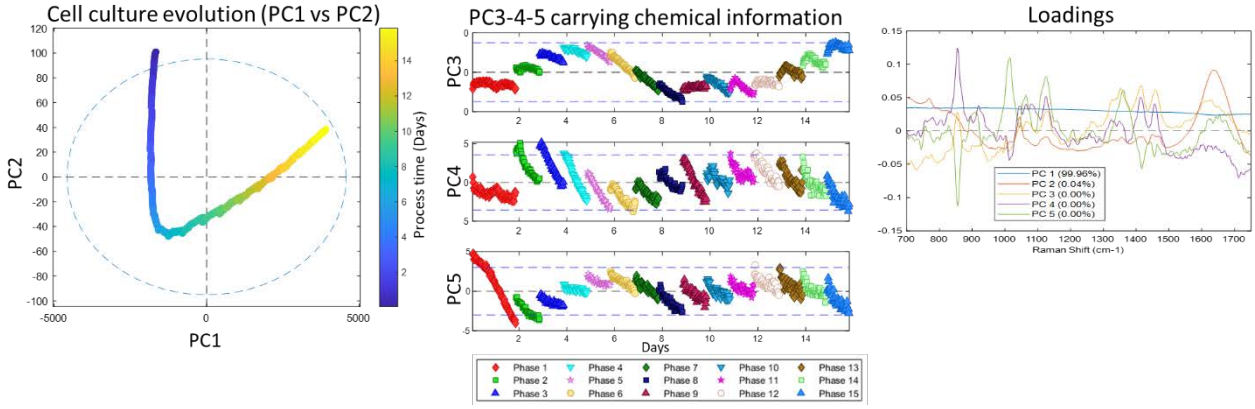


Figure 2: Exploratory analysis of the first bioreactor

By carrying out this same exploratory analysis on all 5 batches, the observations made on a single batch are confirmed. PC1 does indeed appear to be associated with the evolution of cell density and the diffusion effect induced by the latter, while PC2 is representative of the probe effect, i.e. the observable differences between the two Raman probes. Chemical changes during the processes can be observed in components 3, 4 and 5.

Following an exploratory analysis, several prediction models were created, including PLS (Partial Least Squares) models on the same database consisting of 5 batches for a total of 4435 Raman spectra based on 133 reference measurements. These prediction models seek to predict critical process parameters (CPP) and critical quality attributes (CQA) such as cell density and antibody quantification, as well as a maximum of production metabolites (glucose, lactate, glutamine, etc.). Cross-validation was performed on 5 blocks, per batch, i.e. using the 1-batch-out method.

Figure 3 shows all the prediction models with viable modeling characteristics, suitable for use for validation in new bioreactors. The correlation factors start at 0.86-0.87 for lactate and glutamine and go up to 0.98 for IgG antibodies.

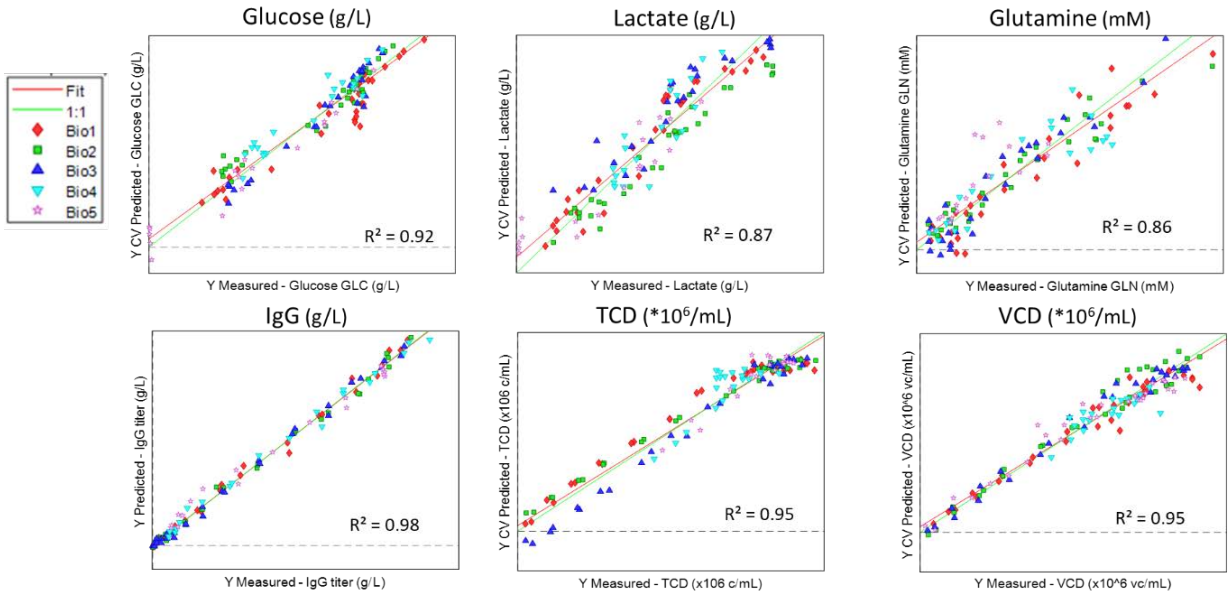


Figure 3: Calibration models for 6 critical production parameters

Conclusions & perspectives

Coupled with machine learning tools, in-line Raman spectroscopy enables non-destructive analysis and automated quality control of bioprocesses, such as the production of monoclonal antibodies (mAbs) in CHO cell cultures.

Thanks to real-time monitoring of CPPs and CQAs, batch reproducibility and yield can be significantly improved.

The reliability of the tool and its application will depend on the optimized configuration of the measurement probe, the acquisition of numerous datasets (both «golden» and non-conform) and spectral data of satisfying quality. Combined with appropriate chemometric or machine learning methods, the device will thus enable precise calibration of the online spectroscopic method in relation to offline reference methods.

The same methodology can be applied to a wider range of biopharmaceutical applications in the quest for process automation and integrated control.

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FRANCE
Indatech
1900, Boulevard de la Lironde
34980 Montferrier-sur-Lez
Tél : +33 4 80 78 01 40
Fax : +33 4 80 78 01 42
sales@indatech.eu
www.indatech.eu