Raman-based advanced process control in upstream bioprocessing for parameters beyond glucose

Benefits at a glance

- Raman spectroscopy has become a first-choice analysis technology for bioprocessing
- Raman instrumentation continues to become more amenable for non-specialist use, facilitating new uses in process development and clinical manufacturing
- Newer reports demonstrate Raman-based monitoring and control of glucose and additional parameters including lactate, amino acids, and cell attributes

Introduction

Raman spectroscopy in bioprocessing is an important real-time monitoring tool for chemical species of interest in upstream or downstream bioprocessing operations. The value of Raman comes from its ability to non-destructively quantify multiple parameters with a single probe. Raman-based information provides a round-the-clock time course and greater process characterization capabilities. Technology developments have enabled new applications in cGMP, cross-scale and cross-platform model transfer and bioprocess control.

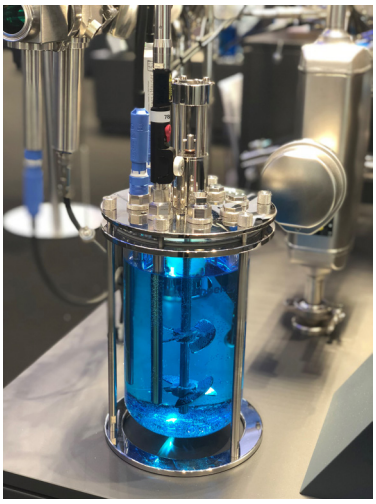
Recently, there have been a wealth of publications focused on data analytics, advanced process control, and new Raman applications in high throughput and automated modeling, single use, perfusion, cell and gene therapies, downstream and more. New studies of Raman in upstream bioprocessing highlight the use of Raman to control glucose, metabolites, and additional parameters such as amino acids and cell quality attributes.

Materials and methods

A series of three papers by Rafferty et al in 2020 researched avenues to expand the functionality of in-line Raman and predictive models.[1–3] The studies were performed on CHO cells producing a monoclonal antibody using a Raman Rxn2 analyzer, powered by Kaiser Raman technology, operating at 785 nm, equipped with a Rxn-10 probe and bIO-Optic for installation in a laboratory-scale bioreactor (1-5 L) or a manufacturing scale bioreactor (2000-15,000 L). Depending on the study, Raman data were compared against in-line and/or off-line reference measurements. Predictive models were developed in SIMCA® (Sartorius).

Results and discussion

In one study, in-line Raman was compared to in-line pH, off-line pH, off-line lactate and carbon dioxide partial pressure (pCO_2) to determine if Raman spectroscopy could provide secondary pH measurements in two CHO cell lines.[1] pH is a critical process parameter, but the drift of in-line pH meters requires daily off-line confirmation and the daily sampling poses a contamination risk. The authors hypothesized that changes to pH-influencing molecules are implicit in the Raman spectra, and Raman-predicted lactate and pCO_2 values could be used to predict off-line pH. Raman spectra were first compared to off-line pH values across the entire 17-day culture, but they found that full-scale pH was too complex to model. To reduce model complexity of the full-length data set, the authors segmented the culture data into an early stage and a late stage to generate an early and a late model. This approach slightly improved the model predictions, but prediction errors were not consistent between the two cell lines. For example, there



The Rxn-10 and bIO-Optic used in advanced bioprocess control

were model switching errors in cell line A that were not observed in cell line B, and there were underpredictions in cell line B on days 9-11 that were not observed in cell line A. In another approach, the authors generated two off-line pH models from lactate and pCO_2 values. The first model was based on off-line lactate and pCO_2 values and the second model was based on in-line Raman-based lactate and pCO_2 values. The model complexity was reduced by incorporating off-line parameter data. These first results support further model development for an overall goal of sampling-free bioprocesses.

The second study by Rafferty et al explored Raman-based feeding strategies based on cell health, as measured off-line by capacitance.[2] Capacitance measures important cell parameters including viable cell density, viability, and viable cell diameter. However, the technique is affected by physiological conditions and signal interference from non-viable cells. The study was performed in eight production-scale cultures of CHO cells producing monoclonal antibodies. In-line capacitance and Raman were collected throughout the 13-day culture. A combined approach using both capacitance and Raman reduced the dependence on one measurement type for complex feed strategies which could support other applications such as inoculation processes.

Finally, Rafferty et al assessed the performance of Support Vector Machines, Random Forests, and Cubist to predict glucose, lactate, and ammonium and compared those results from a PLS model.[3] Data were collected from three bioreactor scales (1L, 2L, and 2000L) from two CHO cell lines. For glucose, lactate, and ammonium, the Cubist model slightly outperformed the PLS model, indicating that non-linear tree-based models could be applied to bioprocesses.

Conclusions

Recent work in upstream bioprocessing further expands the use of Raman for measuring major biochemical process parameters such as amino acids, pH, cell viability, and cell volume. This newer body of work shows an increasingly sophisticated use of Raman spectroscopy for advanced applications such as non-linear predictive models, feedback-based process control, automation, integration of sensors and mechanistic knowledge, and efficient model optimization. These recent successes in upstream monitoring applications and a deeper knowledge of Raman's capabilities support rapid process development and technology transfer into clinical manufacturing.

References

1. Rafferty, C.; O'Mahony, J.; Burgoyne, B.; Rea, R.; Balss, K. M.; Latshaw, D. C. Raman Spectroscopy as a Method to Replace Off-Line pH during Mammalian Cell Culture Processes. *Biotechnol. Bioeng.* **2020**, *117* (1), 146–156. https://doi.org/10.1002/bit.27197.
2. Rafferty, C.; O'Mahony, J.; Rea, R.; Burgoyne, B.; Balss, K. M.; Lyngberg, O.; O'Mahony-Hartnett, C.; Hill, D.; Schaefer, E. Raman Spectroscopic Based Chemometric Models to Support a Dynamic Capacitance Based Cell Culture Feeding Strategy. *Bioprocess Biosyst. Eng.* **2020**, *43* (8), 1415–1429. https://doi.org/10.1007/s00449-020-02336-2.
3. Rafferty, C.; Johnson, K.; O'Mahony, J.; Burgoyne, B.; Rea, R.; Balss, K. M. Analysis of Chemometric Models Applied to Raman Spectroscopy for Monitoring Key Metabolites of Cell Culture. *Biotechnol. Prog.* **2020**, *36* (4), e2977. https://doi.org/10.1002/btpr.2977.