Current Trends in Raman Spectroscopy for Upstream Bioprocess Monitoring and Control: Capability and Efficiency

Introduction

More than ten years have passed since the creation of the Food and Drug Administration’s (FDA) Process Analytical Technology (PAT) framework [1], during which the bioprocess has continuously evolved resulting in new challenges that have been addressed with a multitude of innovative measurement and control solutions.

The FDA has identified a number of hurdles that will need to be addressed if these innovations are to scale-up from research, into the process development suite, and ultimately the GMP manufacturing suite [2,3]. However, as evidenced by the growing number of success stories being shared by the industry, this is well underway [4-17].

This application note covers some new trends in bioprocess monitoring and control. It focuses on the increasing use of Kaiser Optical Systems Inc. (Kaiser) Raman spectrometers as multi-measurement bioprocess sensors to enable quality by design, process optimization, process control and continuous processing. Links to mature manufacturing methodologies such as Six Sigma and Lean Manufacturing are also made throughout this note to highlight the value of Raman-based solutions for improvement of capability and efficiency.

Bioprocess Monitoring Using Raman Spectroscopy

Bioreactor monitoring and control are particularly challenging given the complex nature of biological matrices. Furthermore, in the absence of a design space, conservative nutrient and by-product control ranges are usually established during development in order to de-risk the process and achieve a consistent process capability. In order to keep the process working within tight ranges, the process needs frequent cumbersome in-process testing with multiple analytical techniques, many of them not suited for in situ and/or real-time measurements. These factors can add significant non-value-added cost and activities (inefficiencies) to the manufacturing process which goes against advocated lean practices.

In order to improve upstream bioprocess probing and to facilitate control, Kaiser has developed a series of solutions to measure multiple components at once in an in situ and real-time manner, Figure 1. These solutions enable frequent spectral measurements from biological matrices easily and safely without the typical water interference encountered when using other vibrational spectroscopy analyzers such as NIR. Raman measurements can be used to make process adjustments in real time when evolving away from optimal operation conditions, guaranteeing a consistent and reliable process. This approach is well aligned with Right First Time and Zero Defect initiatives.
Mammalian Cell Culture Monitoring

One of the most popular Kaiser Raman bioprocess applications is the monitoring of mammalian cell cultures. Kaiser Raman spectrometers have been successfully used during mammalian cell cultures to monitor multiple critical constituents and metabolic indicators such as glucose, glutamine, glutamate, lactate, ammonium, osmolality, viability, viable cell density (VCD), and total cell density (TCD) [7-15]. Naturally, glucose has been the most critical monitored constituent due to its role as the primary source of nutrients for the cells.

Figure 2 (a) shows clear systematic spectral variation of glucose in typical mammalian cell culture media, whereas Figure 2 (b) shows off-line reference measurements (red circles) superimposed to in-line Raman glucose predictions (solid line) for a mammalian cell culture process.

Figure 3 shows a comparison between off-line reference results and in-line Raman predictions for additional critical nutrients such as glutamate, by-products such as ammonia and lactate, as well as VCD, TCD, and osmolality. In addition, Figure 3 shows the application & scalability of Raman to three different process scales from PD, Pilot, and GMP Manufacturing. Figure 4 shows a comparison for critical culture parameters such as VCD and titer. Comparisons depicted in Figures 2 to 4 were obtained from different media types and cell lines, demonstrating Raman’s versatility throughout the bioprocess pipeline. In addition, Raman-based methods that are applicable across different cell lines have been successfully developed using spectral data pooled from multiple media types and multiple cell culture stages [12, 13].
Microbial Fermentations

Microbial Fermentation is a very rapid process, progressing in days not weeks, and as such has proven challenging to most analytical instruments but not Raman. This is attributed in no small part to Ramans real time in situ measurement of the fermentation process [9-11]. Figure 5 (a) shows the in-line–obtained Raman data of a simultaneous saccharification and fermentation of corn mash. Figure 5 (b) shows good agreement between Raman ethanol predictions and High Performance Liquid Chromatography (HPLC) off-line method results.

Continuous Bioprocessing

Continuous manufacturing is a stated goal of the FDA for the purpose of improving process efficiencies and reducing costs. One of the requirements of continuous processes is the use of robust process measurement techniques. These are needed in order to ensure stable operations as well as to support the high degree of automation needed to enable continuous processing. Kaiser bioprocess monitoring solutions enable monitoring multiple critical-to-quality parameters in real time for extended periods without degradation of the measurement source. Robust bioprocess analyzers such as Raman, that enable truly continuous bioprocessing open a new realm of opportunities for process developers, and enable a wide array of novel solutions to traditional bioprocessing challenges and emerging challenges alike.

Bio-Process Control

Improved process control, achieved through a better understanding of what is occurring in the process, is the ultimate goal of the PAT initiative. With Ramans proven ability to output its multitude of measurements to any bioprocess control platform the resulting control strategies can be as simple or complex as is required. Figure 6 shows an example of a model predictive controller for a bioprocess application [18].
Conclusion

Process Raman can play a fundamental role in biopharmaceutical production and bioprocess development due to its real-time, in situ capabilities and low water sensitivity. During bioprocess development, Raman spectroscopy can be pivotal when adopting QbD principles aiming to define manufacturing design spaces and demonstrate holistic process and quality understanding. During production, a single Raman sensor can be used to monitor and enable control of multiple critical parameters. This can help to improve bioprocess reliability and promote leaner manufacturing by reducing the cost and risk associated with contamination consumables, equipment maintenance, and operator overburden. Also, a bioprocess control strategy which utilizes process Raman as a real-time PAT method is well suited for continuous processes, 60, 90, 120 days or more, thereby maximizing output while reducing costs. The breadth and depth of insight into the bioprocess facilitated by process Raman, and the associated control strategies made possible, give those utilizing this technology a competitive advantage at the process development, pilot and GMP manufacturing scale.

References

1. PAT — A Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance
3. Sharmista Chatterjee, Ph.D., FDA Perspective on Continuous Manufacturing, IFPAC, Annual Meeting, 2012
4. Ronald a Rader and Eric Lander, 30 Years of Upstream Productivity Improvements, Bioprocess International

Figure 6. Schematic diagram of a model predictive controller system and illustration of its incorporation into a bioprocess.