Paving the Way for Real Time Process Monitoring in Biomanufacturing

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Introduction

The typical manufacturing process for biopharmaceuticals includes a cell culture process that generates the molecule of interest (upstream processing), a process to purify the molecule by removing process and product-related impurities (downstream processing), followed by formulation or lyophilization into the final drug product.[1] Despite the complexity of biomanufacturing processes, a significant amount of research and development has been invested into real time process monitoring to facilitate continuous manufacturing of biologics[2] and real time release (RTR) initiatives.[3] A systematic approach is essential for successful development and implementation of technology infrastructure for real time process monitoring.[4] A typical framework for implementation involves identification of critical process parameters (CPPs) that affect the critical quality attributes (CQAs) followed by deployment of appropriate analytical tools at critical control points (CCPs) of the unit operations involved in the manufacturing of the product.[3,5] Analytical sensors capable of acquiring real time information from the process and cyber-physical systems for automated data piping, processing and/or visualization are key components of any monitoring platform.[6,7]

True real-time data collection is enabled by integration of analytical tools in an in-line fashion, where the sensors and probes are placed within bioprocess streams and data acquisition is performed without removing samples from the unit operation. Vibrational spectroscopy such as Raman and Fourier Transform Mid Infrared (Mid-IR), UV-Visible such as Variable Path length Slope (VPE) spectroscopy, capacitance and Multi Angle Light Scattering (MALS) are common in-line analytical techniques for monitoring of bioprocesses.[4] Owing to the advancements in these technologies with bioprocess compatible probes, flow cells, integration scaffolds and improved analytical capabilities (such as superior sensitivities and response times), their utility in biomanufacturing for real time monitoring has gained significant momentum in recent years.[2] Even though these physical sensors enable real time acquisition of process information related to CPPs and CQAs, it is also vital to establish a data management infrastructure for automated piping, analysis, and visualization

of results.[8] The combination of process analytical sensors with an integrated data management platform (Figure 1) allows operators and scientists to monitor the results from the process in real time and make rapid process decisions enabling more robust control during manufacturing. Automated feedback and/or feedforward mechanisms can be used in some applications to control CPPs and achieve a target product profile.

Here, we review some of the most commonly deployed in-line process analytical technology (PAT) tools for real time monitoring of CPPs and CQAs in biomanufacturing processes and provide our perspective for their use in clinical and commercial manufacturing of biopharmaceuticals. Several case studies are discussed to emphasize the aforementioned key aspects of a typical real time monitoring platform.

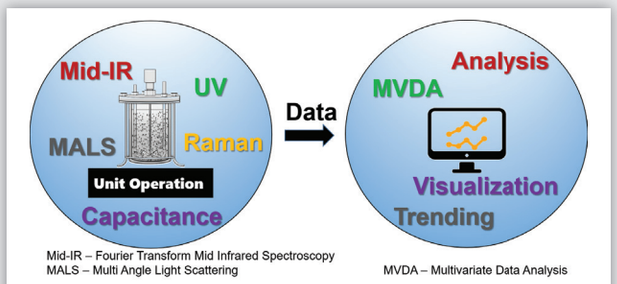

Figure 1. Technological Framework for Real Time Monitoring of Bioprocesses

In-line Vibrational Spectroscopy

Raman

Raman spectroscopy has grown in popularity since the publication of a seminal report by Abu-Absi et al. describing the use of Raman to monitor multiple upstream process parameters, such as glucose, lactate, and viable cell density, in an in-line fashion.[9] Modern Raman spectrometers for bioprocess applications offer sterilizable probes or non-contact optics and often integrated multivariate data analysis (MVDA) systems to streamline integration into cell culture bioprocess systems. Typical Raman sample collection parameters yield a new measurement every 10 to 15 minutes, which is an appropriate timescale to capture cell culture process dynamics and is far more frequent than traditional offline sampling, which is typically performed once or twice per day.

The most common use for Raman spectroscopy in bioprocessing is to monitor glucose and lactate in the cell culture bioreactor. In a typical application, the Raman probe is sterilized and placed directly in contact with cell culture (Figure 2). Spectra from the Raman system are analyzed by multivariate techniques, such as partial least squares, resulting in a prediction of nutrient or metabolite

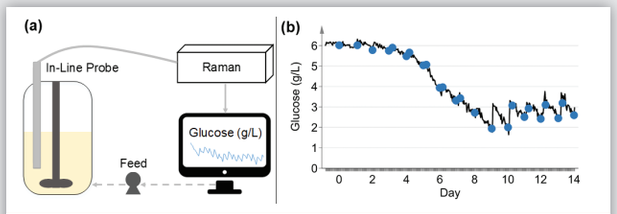

Figure 2. Raman for real-time monitoring of glucose in a cell culture bioreactor. (a) Schematic representation, where the Raman probe is positioned in-line in contract with the cell culture matrix and data are sent to a system with multivariate data analysis software to enable real-time visualization. Optionally, feedback control loops can be included to regulate bioreactor nutrient content. (b) Sample data comparing traditional offline sampling (blue dots) to Raman-based monitoring (black line). Real-time data collection and analysis enable more frequent measurements, allowing users to collect more information about the bioprocess.

concentration, which can be plotted and visualized in real time. In some applications, model predictions are linked with control systems to enable their use in regulating feed rates and, as a result, bioreactor nutrient concentrations.[10] In addition to glucose, lactate, and cell density, recent studies have reported the use of Raman to predict amino acid concentration,[11] protein titer,[12] glycosylation site occupancy,[13] and culture pH[14] in real-time. Thus, a notable advantage of Raman is the ability to use Raman spectra to predict multiple upstream variables with a single technique. However, since the prediction of these compounds from Raman spectra requires a model, care must be taken to characterize the model to understand its limitations and avoid its potential failure points.

Strategies to manage spectroscopic data are critical to a successful implementation of Raman technology. Traditionally, models have been developed on a product-by-product basis and managed using fit-for-purpose PAT data management software. A recent trend in the literature has been a shift from cell line- and product-specific models to generic models that can be applied across multiple cell lines or multiple products.[15,16] For example, Mehdizadeh et al., pooled calibration set data from multiple cell lines (seven total cell lines) and multiple scales of cultivation (1-L, 3-L, and 500-L) to generate PLS models for prediction of glucose, lactate, and viable cell density.[16] The authors' models predicted glucose, lactate, and viable cell density accurately for a new cell line not included in the calibration set. A generic model streamlines integration of Raman into multi-product facilities, but it can be challenging to build comprehensive calibration data sets to encompass potential sources of variation without compromising the accuracy of the generic model. Tulsyan et al. recently proposed an alternate approach using just-in-time learning as a generic framework for building models across different modalities, cell lines, media types, and process conditions.[17,18] The just-in-time approach stores diverse spectral data in a library and uses a machine learning algorithm to select the most relevant calibration data for any individual spectrum. Further advances in data processing and analysis should continue to provide improved accuracy and flexibility of Raman systems.

Mid-IR

The application of Mid-IR spectroscopy in bioprocess monitoring is not as well-established as Raman, likely due to the spectral interference from water present in the matrix and lack of instrumental configuration for easy integration into unit operations. However, modern Mid-IR spectrometers are capable of automatically subtracting water absorbance as part of background correction during spectral acquisition, and a variety of fiber optic probes, flow cells and attenuated total reflectance sensors are currently available to facilitate their in-line signal acquisition.[19] Mid-IR techniques are capable of capturing a single spectrum as quickly as ten seconds. This makes Mid-IR highly attractive for unit operations where quality attributes change rapidly during the process, such as ultrafiltration/diafiltration (UF/DF) and Protein A purification steps.[20,21] The application of MVDA at the fingerprint regions corresponding to multiple analytes of interest in the process enables real time monitoring of multiple CPPs and CQAs simultaneously.[22] For example, Mid-IR spectroscopic sensors were used to monitor multiple excipients and protein concentration variations during the UF/DF unit operation of biomanufacturing.[20] In brief, the technology platform featured integration of Mid-IR probe sensors into a UF/DF process by direct in-line insertion and through custom-made flow cells and acquired spectral signals were then shuttled automatically into a process monitoring software platform with pre-loaded MVDA models for real time monitoring of excipients and protein concentrations (Figure 3). This technology demonstrates the key features of a typical real time monitoring platform where results are generated almost instantly during the process (i.e., a measurement frequency of every 40 seconds), and in-built visualization capabilities enable rapid process decision making (Figure 3).[20]

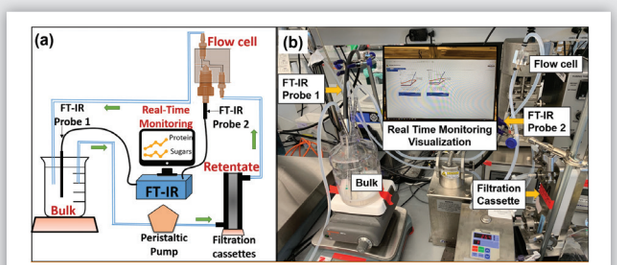

Figure 3. Mid-IR Sensor Platform for Real Time Monitoring of Protein and Excipient Concentrations at UF/DF Unit Operation. (a) Schematic representation, where sensor probes are positioned at the bulk solution and retentate line through a flow cell respectively for real time acquisition of spectra. The spectra are then automatically fed into the process monitoring platform for real time visualization of results; (b) Photographic illustration of the set up.

In-Line UV-Vis Spectroscopy

UV-Vis is one of the most well-established methods for determining drug product concentration during downstream bioprocessing, typically by measuring absorbance at 280 nm.[23] In-line UV-Vis flow cells have been a part of bioprocesses for decades. However, their dynamic range is limited by their use as single path length detectors, or if multiple path lengths are used, it is required to manually switch between them.[24] Variable Pathlength Slope (VPE) instruments, which measure absorbance at multiple path lengths automatically at a fixed wavelength to determine the concentration, have been an important breakthrough for UV-Vis analytical methods.[25] These instruments have a dynamic range that is orders of magnitude greater than their traditional fixed-path length counterparts; thus, protein samples from less than 1 mg/mL to over 200 mg/mL can be tested rapidly and accurately without dilution.[26] To ensure accuracy of the measurements in VPE technologies, specific algorithms are built into the software to scan the path lengths and search for a starting path length at midpoint optical density (OD) where Beer's law shows the best linearity. A significant advantage of determining concentration by slope, as opposed to using a single or a few path lengths, is that this approach eliminates unwanted background effects. Thus, VPE is highly amenable to platforming, with minimal development required for individual biopharmaceuticals to achieve high accuracy in late-stage downstream processing.

VPE technology in conjunction with a flow cell (In-line VPE) allows in-line integration to unit operations of the bioprocess and hence real time acquisition of UV signals. For example, in-line VPE tools enable real time monitoring of protein concentration in UF/DF unit operations. As shown in Figure 3, the integration of an In-line VPE tool at the retentate line during an UF/DF operation allows protein concentration measurements in real time. Protein concentration is part of the control strategy of a typical biomanufacturing process; e.g., diafiltration and final ultrafiltration during UF/DF step are performed at pre-determined concentrations. Thus, a PAT platform such as In-line VPE makes a significant contribution towards complete automation of this unit operation by providing real time protein concentration variations during the process while allowing feedback or feedforward control.

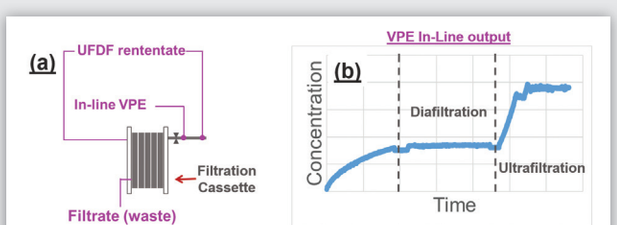

Figure 4. Real time protein concentration monitoring using VPE technology in UF/DF unit operation. (a) A schematic representation of the setup, where VPE instrument is positioned in-line; (b) Real time variation of protein concentration during the process.)

In-line VPE technology is not limited to quantification of the drug product during downstream purification unit operations. For example, Brestrich and coworkers recently demonstrated the utility of in-line VPE technology and MVDA for real time monitoring and quantitation of selective proteins with a broad dynamic range of concentrations during downstream unit operations.[27] The authors claim monitoring

of downstream chromatography runs with highly loaded columns where product and product-related impurity peak concentrations varied between 30 g/L -80 g/L, and 4 g/L to 20 g/L respectively.[27] In summary, VPE technology with sufficient precision and dynamic range can now be used in many downstream operations with real time decision making capability.[3] It is highly likely this technology will be a critical component of the control strategy for bioprocessing in the years to come.

Future Perspective

Intensified and continuous biomanufacturing platforms[1,28] with real time process monitoring capabilities are attractive developments to enhance productivity, reduce cost of goods and support a growing pipeline of therapeutic modalities. While there are well-established PAT tools for real time measurements in bioprocesses, several unmet needs for certain parameters and quality attributes such as host cell proteins, bioburden, and residual DNA still exist. This could be due to the lack of in-line or on-line technologies, and inherent analytical assay challenges such as sample pretreatment needs. On-line PAT tools, where a sample is taken out from the process stream in an automated fashion for analysis, can be employed for the types of analysis which involve significant sample preparation and pretreatment before analysis. In addition to applications in real time process monitoring, as adoption of PAT technologies becomes more widespread in the biopharmaceutical industry, they are likely to be increasingly used in adaptive process control using automated feedback or feedforward loops to improve process robustness. Finally, real time analytics not only enables precise monitoring and control of the process but also leads to collection of enormous amounts of data that can then be used for more holistic understanding of the manufacturing process using advanced data interrogation techniques.[29]

References

1. Zydney AL. Perspectives on integrated continuous bioprocessing - opportunities and challenges. Curr Opin Chem Eng. 2015;10:8-13. doi:10.1016/j.coche.2015.07.005.
2. Holzberg TR, Watson V, Brown S, et al. Sensors for biomanufacturing process development: facilitating the shift from batch to continuous manufacturing. Curr Opin Chem Eng. 2018;22(October):115-127. doi:10.1016/j.coche.2018.09.008.
3. Jiang M, Severson KA, Love JC, et al. Opportunities and challenges of real-time release testing in biopharmaceutical manufacturing. Biotechnol Bioeng. 2017;114(11):2445-2456. doi:10.1002/bit.26383.
4. Wasalathanthri DP, Rehmann, MS, Song, Y, Gu Y, Luo M, Shao, C, Chemmalil L, Lee, J, Ghose S, Borys MC, Ding J, Li ZJ. Technology Outlook for Real Time Quality Attribute and Process Parameter Monitoring in Biopharmaceutical Development – A Review. Biotechnol Bioeng. 2020; 1-17. doi:10.1002/bit.27461
5. ICH. (2009). Pharmaceutical development Q8(R2). Retrieved from https://database.ich.org/sites/default/files/Q8_R2_Guideline.pdf.
6. Steinwandter V, Borchert D, Herwig C. Data science tools and applications on the way to Pharma 4.0. Drug Discov Today. 2019;24(9):1795-1805. doi:10.1016/j.drudis.2019.06.005.
7. Wasalathanthri DP, Ding J, Li ZJ. Real Time Process Monitoring in Biologics Development. Am Pharm Rev. 2020;23(April):72-75.
8. Cao H, Mushnoori S, Higgins B, et al. A systematic framework for data management and integration in a continuous pharmaceutical manufacturing processing line. Processes. 2018;6(5):1-21. doi:10.3390/pr6050053.
9. Abu-Absi NR, Kenty BM, Cuellar ME, et al. Real time monitoring of multiple parameters in mammalian cell culture bioreactors using an in-line Raman spectroscopy probe. Biotechnol Bioeng. 2011;108(5):1215-1221. doi:10.1002/bit.23023.
10. Berry BN, Dobrowsky TM, Timson RC, Kshirsagar R, Ryll T, Wiltberger K. Quick generation of Raman spectroscopy based in-process glucose control to influence biopharmaceutical protein product quality during mammalian cell culture. Biotechnol Prog. 2016;32(1):224-234. doi:10.1002/btpr.2205.
11. Bhatia H, Mehdizadeh H, Drapeau D, Yoon S. In-line monitoring of amino acids in mammalian cell cultures using raman spectroscopy and multivariate chemometrics models. Eng Life Sci. 2018;18(1):55-61. doi:10.1002/elsc.201700084.
12. André S, Cristau L Saint, Gaillard S, Devos O, Calvosa É, Duponchel L. In-line and real-time prediction of recombinant antibody titer by in situ Raman spectroscopy. Anal Chim Acta. 2015;892:148-152. doi:10.1016/j.aca.2015.08.050.
13. Li MY, Ebel B, Paris C, Chauchard F, Guedon E, Marc A. Real-time monitoring of antibody glycosylation site occupancy by in situ Raman spectroscopy during bioreactor CHO cell cultures. Biotechnol Prog. 2018;34(2):486-493. doi:10.1002/btpr.2604.
14. Rafferty C, O'Mahony J, Burgoyne B, Rea R, Balss KM, Latshaw DC. Raman spectroscopy as a method to replace off-line pH during mammalian cell culture processes. Biotechnol Bioeng. 2020;117(1):146-156. doi:10.1002/bit.27197.
15. Webster TA, Hadley BC, Hilliard W, Jaques C, Mason C. Development of generic raman models for a GS-KOTM CHO platform process. Biotechnol Prog. 2018;34(3):730-737. doi:10.1002/btpr.2633.
16. Mehdizadeh H, Lauri D, Karry KM, Moshgbar M, Procopio-Melino R, Drapeau D. Generic Raman-based calibration models enabling real-time monitoring of cell culture bioreactors. Biotechnol Prog. 2015;31(4):1004-1013. doi:10.1002/btpr.2079.
17. Tulsyan A, Wang T, Schorner G, Khodabandehlou H, Coufal M, Undey C. Automatic real-time calibration, assessment, and maintenance of generic Raman models for online monitoring of cell culture processes. Biotechnol Bioeng. 2019. doi:10.1002/bit.27205.
18. Tulsyan A, Schorner G, Khodabandehlou H, Wang T, Coufal M, Undey C. A machine-learning approach to calibrate generic Raman models for real-time monitoring of cell culture processes. Biotechnol Bioeng. 2019;116(10):2575-2586. doi:10.1002/bit.27100.
19. (a) Bruker Optics. FT-IR / FT-NIR Process Spectrometers. Available at: https://www.bruker.com/fileadmin/user_upload/8-PDF-Docs/OpticalSpectrospcopy/FT-NIR/MATRIX-F/Brochures/MATRIX-F_Brochure_EN.pdf. Accessed on June 20, 2020. (b) Mettler Toledo. ReactIR In Situ Reaction Analysis Available at: https://www.mt.com/us/en/home/products/L1_AutochemProducts/ReactIR.html. Accessed on June 20, 2020.
20. Wasalathanthri DP, Feroz H, Puri N, Hung J, Lane G, Holstein M, Chemmalil L, Both D, Ghose S, Ding J, Li ZJ. Real Time Monitoring of Quality Attributes by In-line Fourier Transform Infrared Spectroscopic Sensors at Ultrafiltration and Diafiltration of Bioprocess. Biotechnol Bioeng. 2020; Accepted.
21. Großhans S, Rüdt M, Sanden A, et al. In-line Fourier-transform infrared spectroscopy as a versatile process analytical technology for preparative protein chromatography. J Chromatogr A. 2018;1547:37-44. doi:10.1016/j.chroma.2018.03.005
22. Wasalathanthri DP, Tewari JC, Kang X, Hincapie M, Barrett SL, Pollock JS. Multivariate spectral analysis and monitoring for biomanufacturing. 2019; Patent No. US 2019/0272894 A1. United States. Available at: https://patents.google.com/patent/US20190272894A1/en.
23. LONZA. Technical Reference Guide- Determination of Protein Concentration. Available at: https://knowledge.lonza.com/downloadasset.ashx?assetId=31460. Accessed July 1, 2020.
24. Flowers PA, Callender SA. Variable path length transmittance cell for ultraviolet, visible, and infrared spectroscopy and spectroelectrochemistry. Anal Chem. 1996;68(1):199-202. doi:10.1021/ac950580w.
25. Shih, T, Salerno M. Validating Slope Spectroscopy Methods: A Formula for Robust Measurements. C-Technologies White Paper 2008; Available at: https://cdn.technologynetworks.com/TN/Resources/PDF/spwp2.pdf. Accessed July 1, 2020.

26. McKechnie WS, Tugcu N, Kandula S. Accurate and Rapid Protein Concentration Measurement of In-Process, High Concentration Protein Pools. Biotechnol Prog. 2018;34(5):1234-1241. doi:10.1002/btpr.2695

27. Brestich N, Rüdt M, Büchler D, Hubbuch J. Selective protein quantification for preparative chromatography using variable pathlength UV/Vis spectroscopy and partial least squares regression. Chem Eng Sci. 2018;176:157-164. doi:10.1016/j.ces.2017.10.030.

28. Xu J, Xu X, Huang C, et al. Biomanufacturing evolution from conventional to intensified processes for productivity improvement: a case study. MAbs. 2020;0(0). doi:10.1080/19420862.2020.1770669

29. Hong MS, Severson KA, Jiang M, Lu AE, Love JC, Braatz RD. Challenges and opportunities in biopharmaceutical manufacturing control. Comput Chem Eng. 2018;110:106-114. doi:10.1016/j.compchemeng.2017.12.007.

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