Chapter

Advanced Process Control and Automation with Special Focus on Emerging Continuous Bioprocessing

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Abstract

Legacy batch processing carried out in pharmaceutical and biopharmaceutical sectors is undergoing transformation to adopt the next generation continuous processing to produce safe and effective drugs with better efficiency and consistency at a reduced cost. To facilitate innovative continuous processing, enabled by an end-to-end process with a single uninterrupted production scenario, it is essential to generate real-time or near-real-time data using process analytical technology (PAT), which has been defined by the FDA as a system for designing, analyzing, and controlling manufacturing through timely measurements to ensure final product quality. Based on quality by design (QbD) principles, PAT-enabled data monitoring is essential for the timely control of critical process parameters (CPPs) and critical quality attributes (CQAs) to keep the process in a desired state of control to achieve a predefined product quality. Based on QbD philosophy, quality cannot be tested into products; it should be built-in or should be by design. Deployment of PAT tools for real-time monitoring is integral to align with the guiding principles of QbD-enabled workflow to enhance process and product understanding to administer a control strategy to keep the process within the design space. Aim of this chapter is to highlight the recent advancements in PAT tool-development to monitor and control CPPs and CQAs.

Keywords: cell culture, continuous bioprocessing, process analytical technology, quality by design, critical quality attribute, critical process parameters

1. Introduction

Pharmaceutical and biopharmaceutical medicines are diverse entities of therapeutics with shared common mission of advancing scientific knowledge into the development of breakthrough treatment modalities for complex medical conditions. While pharmaceutical small molecule drugs are leveraging traditional chemical synthesis, the large biopharmaceutical drugs are being produced in living organisms, employing recombinant DNA (rDNA) technology leveraging the deeper

understanding of biochemical pathways and human physiology. Widely known rDNA technology, often referred to as genetic engineering, is the method of recombining two or more genetic DNA sequence-fragments from different species to create a novel gene with a unique function. Such genetically engineered expression vector, carrying the gene of interest, is then integrated into a host cell to express a desired protein through gene expression during the cell culture process. Animal cell culture process leveraging rDNA technology is an indispensable tool to produce protein therapeutics such as monoclonal antibodies (mAbs), lymphokines, interleukins, hormones and other drugs for various disease indications including oncology, cardiovascular diseases, and inflammatory diseases [1]. Applications of rDNA technology is spanning over various fields including healthcare, agricultural sector, environmental segment, and others. The contribution of rDNA technology is significant in the healthcare arena for developing vaccines, biopharmaceutical drugs, diagnostics, etc. [2]. In the environmental sector, rDNA technology is applied to generate biological pesticides and microbes to maintain environmental sustainability [3]. In the agricultural sector, genetic tools are employed to introduce foreign gene to achieve higher yield with desired qualities such as disease resistance and nutritional enhancement [4].

For biopharmaceutical drug development, the commonly used host cell is Chinese hamster ovary (CHO) cells over bacterial, fungal and yeast cells due to the specific need of eukaryotic expression systems. Prokaryotic expression systems are not suitable for achieving eukaryotic post-translational modifications such as desired glycosylation [5]. Upstream bioprocess development as well as pilot and production scale manufacturing are continuing to evolve to produce high quality drugs at reduced cost. Although small molecule medicinal chemistry drugs can be synthesized with utmost reproducibility, biopharmaceutical drugs produced in living cells face inherent variability. Therefore, effective control strategy is critical to keep the process in a steady state with the guidance to operate strictly within the well-established boundaries of the design space. One of the ways in which the cost-effective manufacturing of biopharmaceutical drugs with reduced variability can be achieved through the adaptation of QbD enabled integrated continuous bioprocessing. Fully automated connected end-to-end process eliminates human intervention and hence reduces risk and improves drug safety. Despite the significant progress made in the field of PAT-enabled continuous bioprocessing, exemplified by the successful research conducted at Novartis-MIT Center for Continuous Manufacturing, the end-to-end integration is still not advancing to the forefront [1]. With the emergence of digital transformation, the opportunity for a connected continuous bioprocessing with the adaptation of intensification and data integration is not far from reality.

Mammalian cell culture with engineered cell line is a prerequisite for the modern-day biopharmaceutical processing to produce biological therapeutics such as mAbs, fusion proteins, hormones, interferons, tissue plasminogen activator, EPO, colony stimulating factors, clotting factors, enzymes, vaccines, and others. In addition to cell culture, remarkable growth is on the horizon for the tissue culture, leveraging 3D cell culture model, mimicking the physiological microenvironment, to form a fully developed organs, resembling the structure and function of their natural counterparts. The complex 3D cell culture is leveraging multidisciplinary technologies including bioengineering, mechanics, material science and chemistry [6]. Irrespective of the type of cell culture being considered, it is essential to supplement continuous stream of growth medium containing nutrients and growth factors to the culture. Monitoring and controlling of essential media components as well as minimizing the levels of certain metabolites such as lactate is critical to the steady growth and

proliferation of cells. Maintenance and controlling of cell's surrounding environmental factors such as pH, temperature, osmolarity, oxygen and carbon dioxide are equally significant. Advent of rDNA technology in conjunction with the adaptation of animal cell culture have landmarked various breakthroughs. Recombinant proteins therapeutics, recombinant vaccines, CAR-T Cell therapy and gene therapy are some of the examples of milestones achieved throughout the bioengineering journey. The birth of biotech began to accelerate subsequently to the 1982 approval of rDNA-based insulin produced in bacteria. 60–70% of recombinant therapeutics produced since then have been in mammalian cells [7], including the first recombinant therapeutic drug tissue plasminogen approved in 1986. Approval of recombinant vaccines against hepatitis A & B also was resulted from the application of rDNA technology [8, 9].

Despite the tremendous progress made in the biopharmaceutical drug development in conjunction with the enhanced understanding of human biology, the surging drug development cost and increased failure rates are major industry-wide concerns. Drug development trajectory is directly opposite of Moore's' law, predicted by Intel CEO Gordon Moore stating that the computing power of a microprocessor would double every two years while the cost would reduce by half. In the pharmaceutical sector, to reduce the overall failure rate requires a transformational shift from the current platform of clinical trial to embrace relatively risk-free approaches such as the utilization of organ-on-a-chips (OoC) platform to screen out non-potential candidates early in the program, without investing too much time and resources into the lengthy and expensive clinical trials. OoC is an emerging technology in which the microfluidic technology is coupled with biology to model human physiology ex-vivo. OoC, leveraging tissue engineering, was awarded as one of the top emerging technologies by the World Economic Forum in 2016 [10]. The ex-vivo model generated using OoC provides an in depth understanding of the drug safety and efficacy of potential drug candidates even before it enters the clinical trial.

From the process perspective, modernization of cell culture process and downstream purification steps can improve process efficiency to cut down the overall cost of manufacturing. QbD driven continuous bioprocessing is the most effective strategy for agile manufacturing to achieve targeted product quality. Integration of intensification of processes along with the establishment of design spaces is critical to the success in concert with continuously monitoring and controlling of the upstream cell culture process to maintain the cell genotype and phenotype. To improve upstream bioprocessing efficiency, selection, and optimization of the specialty media specific for the cell type is critical. Establishing the correlation between process parameters and desired product attributes is being leveraged to establish a design space using DoE. To enable the faster delivery of high-quality biopharmaceuticals at maximum efficiency and enhanced flexibility, modernization of the process is required with a transition from the legacy batch processing to the next generation continuous bioprocessing along with the integration of PAT. Bioinformatics plays an integral role in the effective deployment of integrated continuous bioprocessing. Adaptation of mechanistic modeling to create digital twin to facilitate data driven operation is critical to establish an agile and cost-effective bioprocessing with reduced cost and product variability.

2. Continuous processing

For the last several decades, pharmaceutical and biopharmaceutical companies have been adhering to the traditional batch processing to manufacture therapeutic drugs. Recent technological advancements have encouraged drug manufacturers to

divert from batch processing to embrace more efficient continuous processing. The FDA is taking proactive steps to encourage drug makers to implement emerging technologies, including continuous operation to improve product quality [11]. Although continuous operations are widely embraced and routinely practiced in the chemical engineering field, their implementation in the biopharmaceutical and pharmaceutical sectors is requiring additional assessments [12]. Recently published white paper series from the MIT-Strathclyde symposium on continuous manufacturing is highlighting the current state of thinking on the modernization of pharmaceutical and biopharmaceutical processing landscape [12]. Based on the recent shift in paradigm to adopt the guiding principles of engineering concepts and product design, drug companies are embracing risk-based approaches to build quality into the product with an informed process understanding. With the increased emphasis on safety and quality of drugs with simultaneous reduction in cost, there is an increased acceptance of science-based approach for structured process development. To implement a well-established chemical engineering knowledge in the drug manufacturing landscape, FDA and ICH have drafted guidance for PAT and QbD, respectively [13, 14]. While several industries have undergone a manufacturing evolution using continuous technologies, the transition is still in the early stage for the pharmaceutical industry. The setback is even greater for biopharmaceutical sector due to the lack of innovative technologies to modernize such complex operations. The biopharmaceutical platform is getting more dynamic with the introduction of new modalities such as bi & tri-specific molecules, antibody-drug conjugates, cell therapy, mRNA technology, gene therapy, aptamer technology, aptamer-drug conjugate, and other continuously evolving drug development platforms. The recent development in continuous bioprocessing with the incorporation of PAT tools in conjunction with digital technology is leading the way to build a continuous manufacturing with digital transformation to support the current paradigm shift.

3. Process analytical technology (PAT)

The scientific and risk-based framework of PAT is not only intended to modernize pharmaceutical and biopharmaceutical processes through innovation, but also facilitate the enhancement of process and product understanding [13]. FDA is encouraging manufacturers to use the PAT framework to develop and deploy efficient innovative approaches in pharmaceutical development and operation. With an essentially important role in health care, drug manufactures need to employ innovation and apply cutting edge scientific and engineering technologies [13]. Current practice of generating offline analytical data is not only inefficient, but also can exhibit various logistical challenges. Because of longer analytical turnaround time associated with the offline testing, timely process control is not achievable. Also, due to the prolonged sample storage and multiple sample transfers associated with offline testing, issues such as sample stability and data integrity are legitimate concerns. With real-time or near-real-time testing, PAT-tools provide the opportunity for timely control of the process to build desired product qualities into the product. This type of control strategy is critical for supporting continuous processing in which there is no option to send samples for offline testing and waiting for results. Therefore, PAT is referred to as the analytics of future to support the next generation of continuous processing.

PAT has been designed to analyze and control CPPs, CQAs and performance attributes of raw materials and in-process samples with the goal of ensuring desired final product quality. With the utilization of PAT as an enabling technology for assessing the

quality of intermediates during in-process steps, the need for final product testing can be eliminated, resulting in a significant reduction in lead time. Various PAT technologies including 1D- & 2D- LC, Raman, Mid-IR, Near-IR, Flow VPX, RI sensors, sequential injection analysis, Mass spec-based on-line multi-attribute methods and other emerging technologies can be deployed for real-time or near-real-time monitoring and control of product titer, product quality, glycan profiling, peptide mapping, metabolite profiling and others. Implementation of PAT tools in bioprocessing can support the supply chain to achieve cost reduction via enabling Real-Time-Release Testing (RTRT) of therapeutic products, which is aligned with the framework of innovative drug development guiding principles. Therapeutic drug development continues to evolve with the increased emphasis on science and engineering principles to improve the efficiencies of both manufacturing and regulatory landscapes. The goal of PAT is to enhance the process and product understanding as well as to control the manufacturing process to build quality into the products through innovation. The emphasis of building quality into the product requires increased understanding of the process and product knowledge as well as the multi-factorial relationships between materials, process variables, environmental factors, and their effects on product quality.

4. QbD-driven process development

QbD provides a robust framework for the design and deployment of science-based processes to achieve a pre-defined product quality. Data driven risk analysis is carried out to understand how the process design affects quality target product profile (QTPP) that identifies the CQAs and critical material attributes (CMAs). Implementation of risk management strategy, formalized design of experiments (DoE), and advanced data analysis techniques as well as process modeling and control are critical to maintain a desired product quality. Testing products at the end of the manufacturing process limits the options to employ control strategies to remediate the process. By combining PAT with QbD, companies can move away from traditional quality approaches, and employ data-driven strategies to deliver high quality drugs at a reduced cost. Regulatory agencies are the active proponents of QbD implementation, inspired by its power to produce high quality drugs with reduced variability. ICH has provided guidance to define terms such as CQA, CPP, QTTP and design space [14]. PAT- enabled QbD requires upfront investment in money, time, and resources. However, the return on investment is significant in terms of reduced product variability, risk minimization, cost reduction and improved regulatory compliance. Once the design space is established, adjustment within the design space is free of new registration with the regulatory agencies. QbD approaches also facilitates RTRT, which enables the process engineers to make informed decisions to provide timely intervention. QbD offers a better understanding of the manufacturing process, making the process scale-up relatively easier. As continuous process-performance verification is done in real-time with QbD, formal process validation is not a requirement. Drug manufacturers are enthusiastic about the PAT/QbD approach, attributed to the ability to manufacture high-quality drugs at a reduced cost. Finally, the patients are benefited from receiving safe and efficacious drugs.

QbD enabled-knowledge gained from raw materials and in-process samples along with additional insights gained from extended characterization performed using biophysical, biochemical, and microbiological techniques offers opportunity to implement better control strategy. To establish a statistically enabled design space for the safe operation, real-time or near-real-time monitoring of process parameters and product

quality attributes are better served than relying upon the offline data. The flexibility to operate within the established design space helps to build desired quality into the products. Implementation of PAT systems in pharmaceutical industries have been resulted from the regulatory authorities' initiative to improve and modernize the pharmaceutical industry to enhance product quality with the adaptation of QbD/PAT concepts. The common theme of QbD philosophy is to build quality into the products instead of testing the product to ensure quality. QbD/PAT enabled control strategies ensure the development of robust and efficient processes to deliver high quality drugs with desired product quality at reduced variability. QbD/PAT principles will be the norm rather than an exception for the QbD driven next generation continuous bioprocessing.

5. QbD enabled continuous bioprocessing

To facilitate next-generation continuous bioprocessing, deployment of PAT tools for real-time or near-real-time monitoring is integral for process understanding and timely process control to keep all CPPs within the boundaries of the design space to align with the guiding principles of QbD to achieve predefined CQAs. While conventional offline testing is done in labs outside of the process operation area, PAT enabled inline, at line and online analyses are carried out within the immediate vicinity of the process operations. To reduce the cost while improving product quality, continuous operation is a highly recommended platform for pharma and biopharma companies to improve efficiency and reduce cost. There is an alignment between the major stakeholders to provide a strong foundation for building and facilitating the implementation of continuous processing. There are four pillars for providing foundational stability to continuous processing, which is based on the interplay between the government, regulatory agencies, the vendors and the drug companies. Government is in support of continuous processing, attributed to the benefit of reducing the cost of drugs. Regulatory agencies are in concert with the strategy, owing to the benefit of high quality drugs with improved safety and regulatory compliance. Vendors are excited about the opportunity to design and develop new enabling technologies to support the new platform. Pharma and biopharm companies are in full alignment due to the results of various economical benefits including, increased speed, enhanced flexibility, reduced manufacturing footprint, improved robustness, minimized variability, enhanced product quality, diminished product failure, etc. The next generation continuous bioprocessing resides on four pillars consisting of process intensification, single-use technology, real-time data monitoring and RTRT as elucidated in the following sections. Connectivity between these four pillars is essential to develop and deploy a fully integrated continues bioprocessing.

5.1 Process intensification

The paradigm shift in the adoption of next generation bioprocessing is impacting all areas of biopharmaceutical operations. The process intensifications (PI) of upstream and downstream processes require adaptation of perfusion technology and the deployment of multi-column chromatography, respectively. PI involves design, development and deployment of efficient technologies and novel devices to bring dramatic changes to the process operation with spectacular improvements to the process plants, without sacrificing product safety and product quality. Design of single unit facility can lead to substantial reduction in footprint, significant decrease in equipment size, attrition in energy consumption and minimization of waste generation. Ramshaw, one of the

pioneers in the field, defined process intensification as a strategy for making significant reductions in the footprint of chemical plant, either by minimizing the size of individual equipment or reducing the number of unit operations by consolidating multiple operational units into fewer units [15]. PI applies to the common scientific areas of chemical process engineering, mathematics, physics, quantum chemical approaches, classical molecular simulations, thermodynamics, classical mechanics, transport phenomena, numerical mathematics, electrodynamics, chemical kinetics, etc. [16]. PI allows biomanufacturers to produce more product using less raw materials and smaller equipment in a reduced space. With the innovative principles applied in processes and equipment design, PI brings significant benefits in terms of process efficiency at a lower capital and operating expenses to produce higher quality products. While the implementation of PI improves productivity and product quality, the single use scale-down technologies help to reduce the size of the facility to bring the overall cost down. Without having appropriate PAT tools to generate real-time or near-real-time analytical data, the upstream and downstream continuous bioprocessing is inconceivable.

5.2 Single use systems (SUS)

Single use bioprocessing systems are being utilized predominantly for pre-clinical and clinical manufacturing than in commercial setting. SUS provides tremendous economic benefits for small scale manufacturing, attributable to the significant reduction in facility footprint and other operational simplicity. For large scale commercial manufacturing, stainless-steel bioreactors are preferred as it is economically more beneficial. In addition to the miniaturization of facility footprint, the disposable single use bioreactors do not have to undergo cleaning, sterilization, and validation as they come in ready-to-use plug-and-play format. Adoption of upstream process intensification strategies has added pressure to the downstream to adopt similar intensification processes to handle higher titers evolved from the upstream intensification process. Adaptation of disposable chromatographic columns had faced resistance as chromatographic resins are quite expensive and they are intended to be re-used for several cycles without having major issues. Multi-column continuous chromatography systems are now getting traction as this technique eliminates the inherent limitations of traditional large columns to be utilized fully to its maximum capacity during the capture affinity step. In multi-column chromatography, the large column is split into several smaller columns with flexible valve configuration allowing the breakthrough of the product stream from the 1st column to enter the 2nd column, which enables the utilization of the full capacity of the column resin through overloading. Modular platforms such as BioSC from Sartorius could connect and orchestrate multiple downstream modules with a single platform and unified software to increase efficiency in operation of the connected platform.

5.3 Tools for real-time data monitoring

Measurement tools such as sensors and probes that are immerced in sample sources such as a bioreactors or directly into the sampling interfaces are labeled as in-situ in which the sample is not removed from the process stream and can be invasive or noninvasive. Inline measurement is continuous without removing the probe or samples from the process. Online measurement is very similar to inline with a key difference in which the sample is diverted from the manufacturing process and may be returned to the process stream. Online analysis usually involves diverting a portion of the product from the main process line to perform measurements on the diverted portion of the product

through a sampling loop, flow cell or sampling interface. The diverted sample can be either re-introduced into the process stream or to the waste, depending on the application. Both inline and online measurements offer the ability to continuously carryout measurements. At-line and offline measurements usually is requiring manual collection of samples to perform analysis separately from the process. For at-line measurements, the sample is being removed and analyzed in close-proximity to the process stream, while offline analysis is carried out apart from the process. When it comes to real-time and near-real-time data monitoring, a fast response from an inline sensor (in-situ) is considered real-time. On the other hand, in-situ with sampling bypass and ex-situ through sampling modules are considered as near-real-time. For example, data generated using in-situ spectroscopic measurements in which probes are immersed in the source is real-time. Same measurements taken ex-situ using a loop or flow-cell is categorized as near-real-time. UPLC systems interfaced with a sampling interface utilized for the measurements of titer, nutrients, metabolites and CQAs from upstream and downstream unit operations also falls in the category of near-real-time. Other near-real-time analyses include μSIA-based glycan analyis, amino acid analysis, peptide mapping, etc.

5.4 Real-time release testing (RTRT)

RTRT recognizes that an appropriate combination of CPP with pre-defined material attributes may provide greater assurance of product quality than end-product testing [17]. RTRT is based on monitoring and controlling of the process using PAT tools to ensure desired product quality of in-process samples and final end-products. The PAT component of RTRT includes a valid combination of measured material attributes and process controls [17]. In-process testing of process parameters and attributes to enhance process understanding along with the deployment of control strategy can be used to justify the replacement of routine end-product testing. RTRT is a system for product release that gives assurance that the product meets the desired quality, based on the process understanding and product knowledge acquired during the process steps. RTRT recognizes that an appropriate combination of process control and pre-defined material attributes to achieve a desired CQA may provide greater assurance of product quality than traditionally performed end-product testing. Release of a product can be a combination of RTRT approach for certain critical quality attributes (CQAs) and a more conventional evaluation for certain other quality attributes. The application of RTRT may offer advantages from a manufacturer's perspective as well as from a regulatory point of view to gain enhanced knowledge throughout the process. Other potential benefits include real time monitoring and the opportunity to provide feed-back or feed-forward controls. RTRT strategy should be based on a thorough understanding of the process and of the relationship between process parameters, in-process material attributes and product attributes. RTRT comprises a combination of process controls utilizing PAT in combination with relevant process control and material attributes. PAT-based data monitoring along with timely process control could enable RTRT to replace traditional end-product testing.

6. Existing and emerging PAT tools

To support next-generation continuous bioprocessing to fulfill QbD-driven PAT initiatives, various emerging PAT technologies are now available for online monitoring and controlling of pharmaceutical and biopharmaceutical processing. Some of the PAT techniques unique to the biopharmaceutical sector consists of online monitoring of

product titer, amino acid analysis, peptide mapping, glycan profiling and CQA assessments using chromatography-based hybrid techniques. Other product attributes can be monitored and controlled using techniques such as NIR, FT-IR, UV/Vis, Flow VPX, Fluorescence spectroscopy, LC/MS, and other techniques. At-line fluorescence detection and chemometric modeling is an alternative to UPLC-PSM-based approach for purity based peak pooling. IR sensors and Solo-VPE are useful for the measurements of real-time protein concentration of downstream samples. FTIR, Raman and NIR are useful for product quality assessments based on chemometric modeling. LC/MS interfaced with online autosampler is suitable for online monitoring of multi-attributes simultaneously. Process and product understanding accomplished on real-time and near-real-time monitoring can provide opportunities to keep the process in a desired state via potential feedback control. Compared to offline or at-line testing, inline and online measurements can augment speed, sample integrity, and convenience. There are many tools available to facilitate process understanding, continuous improvement, and development of scientific risk-mitigation strategies [13]. Some prominent tools applicable for biopharmaceutical applications are presented in subsequent subsections.

6.1 HPLC/UPLC based PAT tool

For online monitoring of upstream titer and nutrients as well as assessing upstream and downstream product quality of biopharmaceutical drugs with the option to perform timely process control, UPLC system interfaced with a process sample manager (PSM) can be utilized. A 1D- & 2D-LC system interfaced with an appropriate online sampling device is useful for various applications including online titer measurements, online product quality assessments and online quantitation of amino acid contents during upstream cell culture process [18]. In all cases, samples are withdrawn automatically from bioreactors through a FISP probe or equivalent device with appropriate filter discs to remove all debris from the bioreactor culture samples before introducing it into the HPLC/UPLC. Since the upstream samples require purification prior to product quality assessment, the 1st dimension of 2D-LC is leveraged for protein purification while the 2nd dimension is utilized for product quality assessment [19]. A UPLC system with PSM autosampler is better suited for upstream titer measurement and downstream product quality measurements [20]. The same UPLC system with PSM autosampler can facilitate online purity assessment during peak pooling [21]. Near-real-time data generated using online testing using chromatographic, spectroscopic, and other techniques help to enhance process and product understanding and provide an opportunity to control the process to achieve a pre-defined final product quality. Also providing speed and efficiency to improve sample integrity with the elimination of protein degradation and undesired post translational modification associated with prolonged sample storage prior to the offline testing.

Figure 1 and **Table 1** exihibit acceptable comparability of a representative batch bioreactor titer results generated using online UPLC with PSM autosampler vs. at-line and offline results of the same sample set. Statistical analysis confirmed that there are no statistical differences between the three data sets. **Figure 2** illustrates acceptable comparability of online titer data generated using UPLC/PSM vs. at-line UPLC data of a perfusion bioreactor sample set. Online amino acid profile of a typical cell culture media overlaid with amino acid standard is shown in **Figure 3**. As shown in **Figure 4**, the spike and recovery of online OPA method is equivalent to the offline AccQ-Tag method. Tabulated recovery data is shown in **Table 2. Figure 5** is illustrating the power of 2D-LC interfaced with a sampling device such as segFlow for online measurement of titer and product quality of bioreactor samples from a single analysis work stream.

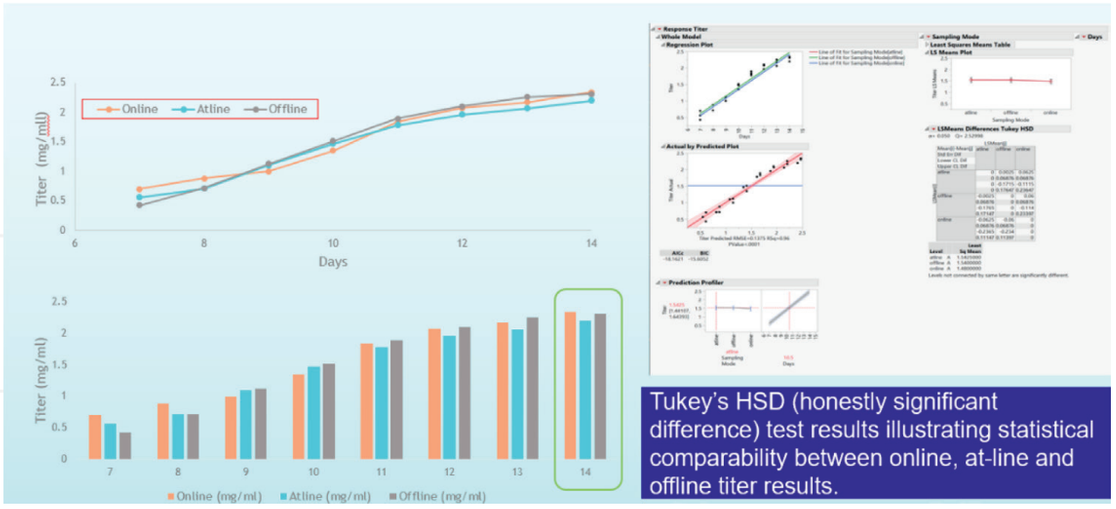

Figure 1.
Online at-line and offline mAb titer data from batch bioreactor using1D-LC with PSM.

Days	At-line (g/L)	Online (g/L)	Offline (g/L)
7	0.7	0.56	0.426
8	0.88	0.71	0.713
9	1	1.1	1.122
10	1.35	1.47	1.511
11	1.83	1.78	1.891
12	2.07	1.96	2.099
13	2.17	2.06	2.253
14	2.34	2.2	2.305

Table 1.
Titer data for mAb-1 in three sampling modes (At-line, Online, and Offline).

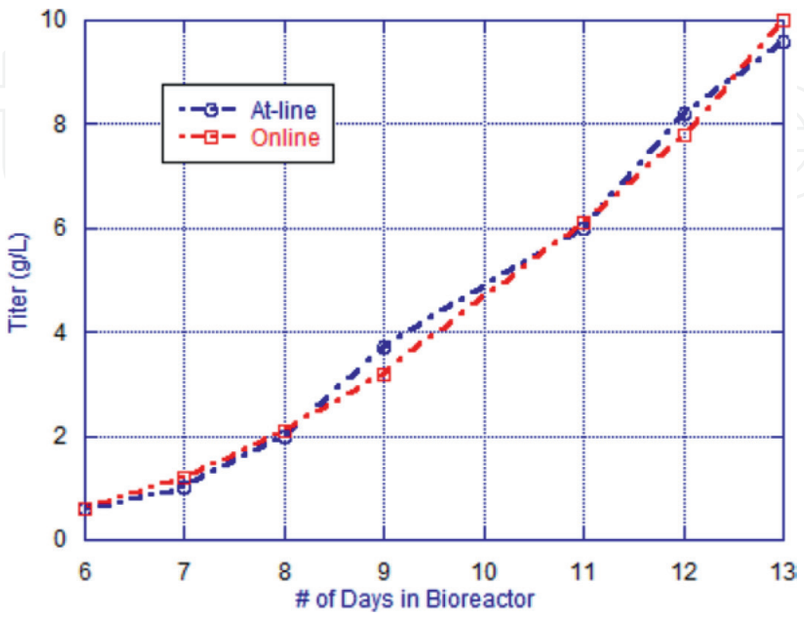

Figure 2.
Online and at-line mAb titer data from an ATF bioreactor using 1D-LC with PSM.

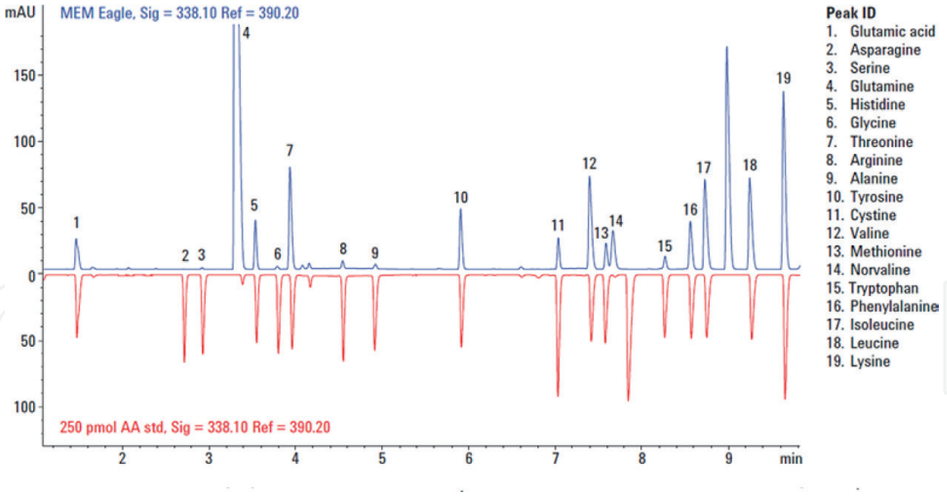

Figure 3.
Representative online amino acid profile of MEM media (blue) mirrored against amino acid standard (red) generated using 1D-LC with OPA derivatization.

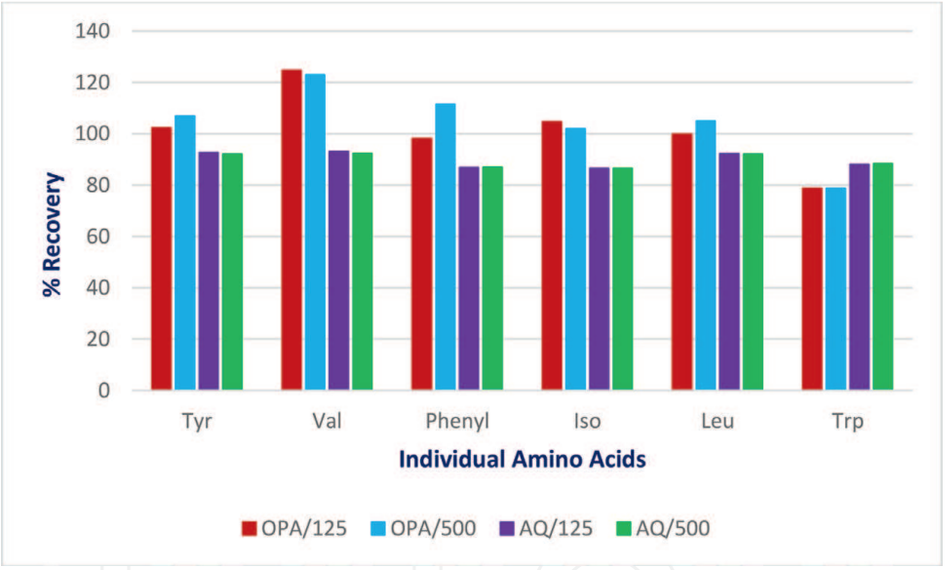

Figure 4.
Spike & Recovery study results of online OPA method vs. conventional offline AccQ-Tag method demonstrating acceptable recovery (80–120%) of 6 essential amino acids for both methods.

Amino acid	AccQ-Tag method (500 mM spike) (%)	OPA method (125 mM spike) (%)	AccQ-Tag method (500 mM spike) (%)	OPA method (125 mM spike) (%)
Tyrosine	95.7	98.2	102.6	114.3
Valine	101.9	102.5	108.2	118.2
Isoleucine	95.3	101.2	101.0	116.6
Leucine	101.3	109.2	107.7	125.5
Phenylalanine	90.3	93.4	97.4	109.4
Tryptophan	90.7	92.8	96.6	107.6

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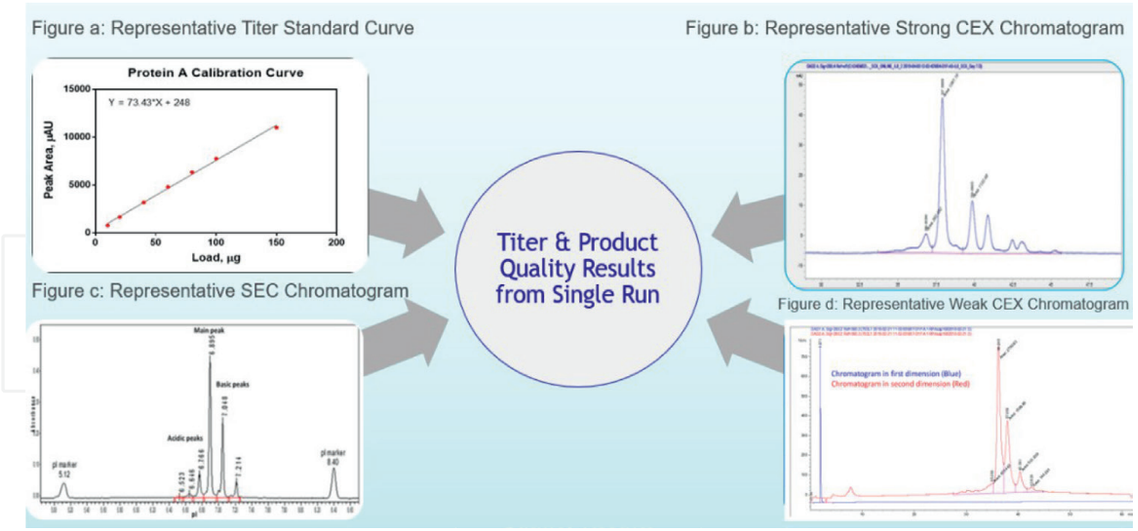

Figure 5.
Online chromatograms of 2D-LC system-3 with [1]D Pro-A and [2]D product quality.

6.2 Sequential injection analysis (µSIA system)

For complex online analytical techniques requiring laborious sample preparation workflow, a contemporary approach such as µSIA would be ideal. This novel system featuring a fully automated sample preparation modules can be programmed to execute various commands within the workflow using python scripting. The system can be interfaced with online sampling devices such as SegFlow or FIA lab's new built-in architecture (proSIAmpler) to withdraw samples from bioreactors. For N-linked glycan analysis, the script can be written to withdraw samples followed by protein-A based affinity purification within the system's architecture. The purified protein is then be subjected to PNGase-F digestion to release the glycan from the protein. The released glycan subsequently can undergo derivatization and clean-up step before transferring to an integrated online HPLC/UPLC equipped with a fluorescence detector for N-linked glycan mapping. Similarly, for online peptide mapping, the samples withdrawn from the bioreactors are subjected to protein-A purification followed by denaturation, reduction, alkylation, and a subsequent clean-up step to remove the excess reagents. Then desired protease such as trypsin is added to digest the protein to generate assorted peptides. The peptides are then analyzed on an integrated online HPLC/UPLC interfaced with Mass Spectrometry for peak identification and characterization. This architecture provides an ideal platform for the online Multi-Attribute Method (MAM) to monitor multiple critical quality attributes (CQAs) simultaneously. In addition, this platform is suitable for online amino acid analysis using AccQ-Tag, complementary to the online OPA derivatization presented above using the Agilent system.

6.3 LC/MS/MS for online metabolite analysis

LC/MS with integrated inline samplers such as SegFlow is suitable for online metabolite analysis of samples directly from bioreactors. The identification and quantification of the metabolites during cell culture process provides an insight into the homeostasis of the growing cells as well as to gain understanding of how the levels of metabolites influence the phenotypic nature of the living cells to

enhance insight into the mechanisms of cellular functionality [22]. In contrast to proteomic analysis data, the metabolite analysis data is more dynamic and challenging [23]. It was identified that molecules related to carbohydrate and amino acid metabolic processes provide insight regarding the dynamic changes happening during the cell culture process [24]. The complexity of metabolome; consisting of varying concentrations of carbohydrates, ketones, organic acids, amino acids, lipids, and other assorted natural products along with their shorter half-life makes it difficult to analyze the entire metabolome. Various statistical and mathematical modeling as well as computational modeling and simulations have been developed for metabolome analysis. The most powerful experimental approaches are LC/MS and NMR-based techniques for structural elucidations. In addition to the multivariate analysis and chemometric modeling of the data generated from LC/MS and NMR, the data can be applied to conventional statistical and AI-based machine-learning techniques to gain understanding of the fate of metabolites through simulation. It has been shown that this deep learning algorithm is reliant on large numbers of training data to significantly enhance the accuracy of the model [25]. Additionally, mass spectral libraries like the Golm metabolome database, linking mass spectrum and chromatographic retention time to specific compounds, have been developed [26]. Various machine-learning and deep learning software packages for different tasks in metabolomics analysis are available. Ion-pair chromatography coupled with mass spectrometry is gaining popularity in virtue of its power to analyze both hydrophobic and hydrophilic compounds in a single analysis in contrast to performing RP-HPLC and HILIC runs separately for hydrophobic and hydrophilic compounds [27].

6.4 Spectroscopic techniques with chemometric modeling

Inline spectroscopic measurements utilizing Raman, FT-IR and NIR provide the platform for real-time monitoring of various nutrients, metabolites, and excipients to keep the process in a state of control. Multivariate analyses are often necessary to extract critical process knowledge for real-time control and quality assurance [13]. Real-time and near-real-time testing of critical product quality provides timely control of process parameters to achieve a desired product quality. With the convenience of integrating these PAT tools to upstream and downstream unit operations through fiber optics provides tremendous flexibility to interface with different unit operations for QbD-driven continuous processing. The process performance can be evaluated rapidly with the utilization of multivariate analysis, chemometric modeling and design of experiments using real-time PAT data. A PLS model can be built using the spectroscopic data for the real-time monitoring. AI-based machine learning techniques can transform the spectroscopic data into structural prediction of therapeutics. Inline monitoring features of spectroscopic techniques in conjunction with chemometric modeling provides an excellent platform for testing certain attributes throughout the product life cycle including in-process measurements. An alternative to the use of sophisticated predictive modeling techniques using FTIR with expensive ATR crystals, single use FT-IR is evolving with the use of silicon-based inexpensive ATR in combination with proprietary algorithms. These innovative systems can be connected directly to the bioreactors for continuous monitoring and control of nutrients and metabolites like glucose and lactate using a single-point standard calibration, reducing the effort of time-consuming chemometric modeling.

6.5 Online multi-attribute method (MAM)

Recent advances in process analyzers are making real time monitoring and control of multiple CQAs using a single analytical work stream during manufacturing is becoming a reality. Online MAM, targeted for measuring multiple product quality attributes simultaneously using a single workflow has received tremendous traction due to the benefit of saving time and resources. Various attributes including protein truncation, amino acid sequence coverage, post translational modifications, glycan profiling, glycation measurement, host cell protein monitoring, charge distribution, product related impurity assessment and more can be accomplished from a single MAM analysis. Simultaneous monitoring of multiple CQAs has significant advantage for QbD driven drug development paradigm in which the goal is to build desired quality into the product to achieve a quality target product profile (QTPP), revolutionizing the traditional approach of analytical testing. With the acquisition of multiple CQAs near-real time, prompt control of process parameters is feasible. Post translational modifications including asparagine and glutamine deamidation, succinimide and pyroglutamate formation, asp-isomerization, methionine oxidation, tryptophan oxidation, glycosylation, glycation, etc. can be monitored near-real time to provide feed-back or feed-forward control to modulate the process promptly to produce therapeutics with a desired product quality attributes. As FDA has been encouraging drug companies to adapt PAT as an enabling platform to modernize the manufacturing via QbD driven continuous processing, adaptation of MAM in an online setting to measure multiple product qualities straight from the bioreactors and other in-process steps is a revolutionary pathway. This approach will be in perfect alignment with the proposed paradigm shift from end-product testing to in-process monitoring and control. With the deployment of MAM in an online setting, the information-rich product quality data acquired at near-real-time can help to guide timely process control to establish a fully integrated automated online PAT tool.

MAM can overcome the challenges of obscured measurements of CQAs in conventional methods due to the co-elution of multiple components in each peak [28]. Furthermore, conventional analytical techniques are not capable of monitoring site-specific CQAs. In addition to providing detailed characterization of complex proteins, MAM can be implemented as a tool for process characterization (PC) during QbD-driven bioprocessing. Model fitting and simulations could be performed to evaluate the impact of process parameters on measured product quality attributes. A central composite design can be formulated to model the effects of these process parameters on product quality. Through model fitting and simulation, acceptance criteria of product quality attributes can be established and monitored during PC. Statistical models can be established based on the correlation between the process parameters and product quality attributes. Once the reduced model exhibits statistically acceptable correlation between the process parameters and quality attributes, a tolerance interval can be established through simulation at 95% confidence interval to predict the product quality in reference to the variation of process parameters [28]. In addition to providing comprehensive characterization of therapeutic drugs using QbD principles, MAM helps to reduce the number of assays required in a traditional release testing panel to a single workstream. The online MAM streamline the process further to generate CQAs at near-real-time to support QbD driven continuous bioprocessing. MAM consists of multiple modular components including automated sampling device, sample preparation platform such as μSIA, sampling device such as SegFlow, HPLC/UPLC and high-resolution mass spectrometry with MS/MS capability.

7. Timely process control

Control strategy is derived from product and process understanding that ensures optimal process performance and desired product quality [14]. A control strategy is designed to ensure that CPPs remain in a constant state of control to achieve a desired product quality during manufacturing [29]. The control ranges are determined based on statistical models in which the limits of the input parameters are controlled such that the response variables meet predefined specifications. With the establishment of design space and the operations within the boundaries of the established design space enable to attain pre-defined product quality. For the timely control of the process as well as the synchronization of the operation between the process equipment and PAT analyzers, two-way data communication across systems is essential. The legacy systems mandate the conversion of analog to digital data as well as 4–20 mA conversion of analog data before sending the signal to the distributed control system (DCS). For transmitting analog-based process information, the 4–20 mA loop is the dominant industry standard. The sensor measures a process variable, the transmitter translates the measurements into current signal, the signal travels through a wire loop to a receiver, and the receiver displays or performs an action with the received signal. Continued evolution of new technologies in conjunction with digital transformation is revolutionizing the way the data being transitioned across and between different types of instruments to build smart factories. For example, analytical process interface (API) serves as a juncture promoting communication between two different software packages to virtually connect with each other and serves as an intermediary between the two applications. Established enterprise scientific platform (ESP) workflow with bi-directional capability can push data between process systems and PAT instruments via data connectivity through a unified platform. Digitalization dealing with information processing can be used to improve workflows through the automation of existing processes to serve as the path towards digital transformation.

8. Building lab of the future through AI, ML & digitalization

In addition to the implementation of PAT tools to generate real-time or near-real-time data, an automatic control strategy is essential to maintain the process in a steady state. FDA's Process Validation Guidance [30] states that process knowledge and understanding are dynamic, and it is critical to establish a process control strategy for each unit operation as well as for the entire process. FDA's 2019 guidance on continuous manufacturing recommends input material control, process monitoring and control, RTRT deployment as well as system integration and data management [31]. Drug companies have been investing in the integration of PAT and data systems to assimilate data for controlling the process effectively and providing real-time predictions of the product quality [32]. Establishment of bi-directional feed-loop control for the synchronization between the process systems and PAT devices is inevitable. To this end, establishing a unified digitalized platform orchestrating the data flow back and forth from a centralized data depository is a way to overcome the challenge of data connectivity between incompatible systems. Harnessing the emerging technologies is essential for companies to stay competitive to foster innovation. Pharmaceutical and biopharmaceutical companies, engaged in leading-edge technological endeavors, are interdependent on un-related technologies such as informatics, statistical modeling, multi-variate analysis, artificial intelligence (AI), machine learning (ML)

and other emerging technologies to promote continuous growth to stay ahead of the curve through innovation and modernization. Convergence of the enterprise's core technologies with enabling technologies such as informatics, IT and automations is essential to establish a successfully integrated architecture. To facilitate bidirectional data synchronization between the process equipment and analytical instruments, closed loop communication via feedback control is a prerequisite.

For monitoring and controlling of the processes to achieve a desired product quality, orchestrated digitalization of PAT data is the right course of action. Digital technologies are revolutionizing the biopharmaceutical industry, eliminating data communication silos to create more proficient processes through enhanced communication between various data systems. As part of the enterprise modernization and infrastructure connectivity, digital transformation is leading the way to build a smart factory with enhanced digital communication. High-tech companies such as telecommunication and aerospace industries have successfully implemented digital plants, embracing artificial intelligence and digitalization. In response to their success, drug companies are following the suit to join the digital revolution to modernize their manufacturing process. Such digital transformation is an example of a technology convergence in which unrelated technologies are intersecting at a juncture, resulting in a powerful technological transformation. With the utilization of intelligent systems such as DCS (distributed control systems) or HMI/SCADA, real-time monitoring, visualization, and control of both process parameters and product quality attributes is becoming a reality. Informed insight through data integration and feedback communication as well as timely control of inter and intra lab systems have made enormous progress through digital transformation. With digitalization and centralized visualization, informed decision can be made in real-time such that timely control strategies can be facilitated to achieve better plant performance and improved product quality. Legacy systems lacking opensource software are difficult to institute communication with other systems and require custom approaches to establish connectivity through digitalization. With the development of data analytics and visualization tools, monitoring the status of plants across the globe can be achieved virtually from anywhere in the world.

9. Building digital twin

The initial scientific conceptualization of digital twins was originated from NASA as a computerized simulation model to improve physical-model simulation of spacecraft [33], and now being extended to the pharmaceutical and biopharmaceutical companies. Digital twin, the digital equivalent of the physical system/process serves as the digital counterpart for process monitoring and computer modeling to predict the fate of a product through simulation. Building a virtual replica of a given process, leveraging information and communication technologies in the form of digitalization, enables cross-functional communication and synchronization of multiple activities [34]. Number of technologies such as machine learning, artificial intelligence and advanced robotics can influence the way the digital twins can be crafted and deployed. Digital twin technology is based on the connectivity between analytical instruments integrated with the process equipment. The data is transmitted and communicated to the digital twin through digitalization and various integration technologies to synchronize between the virtual and real system. The concept of digital twin, generating the best model using the input and output data enables the prediction of performance of the process. In addition to predict end-product quality, process bottlenecks can be

identified and eliminated through simulation and timely process intervention. Using Big Data analytics, it is possible to access the data for a rapid decision making. With the use of algorithms based on artificial intelligence, digital twin acts as an efficient and intelligent alternative to test, predict, and solve problems virtually.

10. Summary

Pharmaceutical and biopharmaceutical companies have imprinted their signatures on successful development of diverse drug modalities for various disease indications. Despite having many successes, the drug companies are facing enormous challenges on producing cost effective drugs with highest product quality and adequate reproducibility. While the synthetic version of pharmaceutical drugs can be produced with minimal variability, the biopharmaceutical drugs produced in living cells tend to suffer significant challenges of inconsistency. Therefore, control strategy plays an integral role in manufacturing of high-quality biopharmaceutical drugs with minimal variability. Contrary to the pharmaceutical operations, the biopharmaceutical landscape is intensely more complex with convoluted multi-factorial processes as well as its inherent variability associated with its production in living organism. In addition to the complexity of in-process matrix components, the larger size of the biopharmaceutical drugs contributes additional challenges to perform reliable product characterizations to ensure safety and efficacy. As a result, many of the spectroscopic, chromatographic, and mass spectrometric techniques developed and deployed in the pharmaceutical sector for real-time data monitoring cannot be extended to the biopharma application without making significant modifications to their architectural design. Various novel and emerging techniques specific for biopharmaceutical applications are in the process of being developed and deployed.

With the ongoing efforts to make changes to biopharmaceutical landscape to transition from batch to continuous bioprocessing, there is an enhanced emphasis on PAT implementation. While the legacy batch process provides the flexibility to wait for offline data for decision making before advancing to the subsequent unit operation, continuous bioprocessing requires real-time or near-real time data to make timely decision to execute the control strategy. In terms of choosing the most appropriate PAT techniques for upstream and downstream testing, the ideal scenario would be to use an existing offline method either in an inline, at-line or online mode. With the emergence of contemporary sampling devices that can be integrated into the existing and emerging analytical tools, the scope of the PAT implementation and timely control of the processes are steadily advancing. UPLC-PSM with modular technology tailored to draw samples from bioreactors and downstream unit operations can be deployed as an ideal PAT platform for analyzing and controlling upstream titer and downstream product quality. 1&2-dimensional chromatography interfaced with an appropriate sampling device is suitable for titer measurement, amino acid analysis, and product quality assessments of upstream samples. Fully automated μSIA system is suitable for complex analysis with complicated workflow such as peptide mapping, amino acid analysis and glycan profiling. Additionally, the adaptation of emerging information technology infrastructure can facilitate timely process control.

Integrations of PAT tools to the process equipment is involved with interconnectivity between various parts of the integrated system with a flow of information channeling through the network with close alignment between systems. Sharing data between different platforms is not always straight forward with off-the-shelf data sharing tools, and

often requires customized solutions, leveraging innovative technologies. Harmonized data communication platforms can be harnessed for systems with open-source software architecture. However, instruments with restricted access requires special licensing to customize the network architecture with a closed-loop communication feedback. For example, the synchronization of the operation between the process systems and PAT analyzers requires conversion of voltage to current followed by analog to digital conversion using the converter located in the analog input module of PLC or DCS systems. Alternatively, an ESP data automation workflow with standard data connectors can be implemented to establish an integrated online PAT solution for two-way data communication between the process systems and PAT analyzers. Tremendous amount of fundamental scientific, engineering and informatics knowledge as well as the technical tools are available for developing and implementing fully integrated innovative engineering principles into an integrated continuous bioprocessing architecture. A more collaborative approach between scientists, engineers and software teams is required at the interface of these multidisciplinary juncture to successfully integrate PAT tools and bioinformatics to deploy effective engineering controls. A continued effort in integrating multiple skillsets including chemistry, engineering and informatics is required to bridge the existing gaps and establish a fully integrated automatic analytical solution such as PAT to support fully integrated continuous bioprocessing.

11. Conclusion

Recombinant DNA (rDNA) technology and animal cell culture processes are the foundational pillars for the rapidly growing life science sector with potential applications including biopharmaceutical drug development, cell therapy, gene therapy, organ development, and other exploratory scientific approaches targeted for treating life-threatening diseases. Considering that the growth and maintenance of engineered cells in a bioreactor is heavily dependent upon its surrounding environment, it is essential to monitor, and control environmental factors and process parameters. Real-time or near-real-time monitoring and timely control of nutrients, metabolites, pH, CO_2 level, and temperature, impacting product titer and product quality attributes, are critical to achieve a desired target product profile (TPP). Quality by design philosophy along with PAT-enabled control strategy can help to achieve a TPP with the adaptation of integrated continuous manufacturing (ICM). ICM provides various benefits including the alleviation of variability encountered in the widely practiced batch processes, facilitating improved safety and enhanced product quality. Compared to Quality by testing (QbT) approach, QbD pathway provides the flexibility to produce high-quality drugs faster at a reduced cost with improved robustness and efficiency. To establish a state-of-the-art fully integrated end-to-end process platform, it is essential to merge various technological frontiers including process operation units, PAT tools and bioinformatics techniques. Fully automated ICM, enabled by the QbD-driven PAT initiatives, provides enhanced process and product understanding and facilitate a closed loop feedback control to keep the process in a desired state of control.

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Conflict of interest

The authors declare no conflict of interest.

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Abbreviations

AI	Artificial Intelligence
API	Analytical Process Interface
ATR	Attenuated Total Reflectance
CHO	Chinese Hamster Ovaries
CMAs	Critical Material Attributes
CPP	Critical Process Parameters
CQA	Critical Quality Attributes
DCS	Distributed Control System
DoE	Design of Experiments
FDA	Food and Drug Administration
ICH	International Council for Harmonization
mAb	Monoclonal Antibody
MAM	Multi Attribute Method
ML	Machine Learning
OoC	Organ on a Chip
PAT	Process Analytical Technology
PC	Process Characterization
PI	Process Intensification
QbD	Quality by Design
QbT	Quality by Testing
QTPP	Quality Target Product Profile
rDNA	Recombinant DNA Technology
RTRT	Real Time Release Testing
SUS	Single Use System

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