Trends in Biotechnology

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REVIEWS

Review

The Current Scientific and Regulatory Landscape in Advancing Integrated Continuous Biopharmaceutical Manufacturing

Adam C. Fisher,[1,3] Mark-Henry Kamga,[2,3,@] Cyrus Agarabi,[1] Kurt Brorson,[1,*] Sau L. Lee,[1] and Seongkyu Yoon[2,4,*,@]

There is a trend across the pharmaceutical sector toward process intensification and continuous manufacturing to produce small-molecule drugs or biotechnology products. For biotechnology products, advancing the manufacturing technology behind upstream and downstream processes has the potential to reduce product shortages and variability, allow for production flexibility, simplify scale-up procedures, improve product quality, reduce facility footprints, increase productivity, and reduce production costs. On the upstream side of biotechnology manufacturing, continuous perfusion cell cultures are fairly well established. However, truly integrated continuous biomanufacturing requires the uninterrupted connection of continuous unit operations (upstream and downstream) with no isolated intermediate or hold steps occurring between them. This work examines the current scientific and regulatory landscape surrounding the implementation of integrated continuous biomanufacturing.

Highlights

There is a trend across the pharmaceutical sector toward process intensification and continuous manufacturing to produce small-molecule drugs or biotechnology products.

For biotechnology products, advancing the manufacturing technology behind upstream and downstream processes has the potential to reduce product shortages and variability, allow for production flexibility, simplify scale-up procedures, improve product quality, reduce facility footprints, increase productivity, and reduce production costs.

Nevertheless, some scientific and regulatory challenges still exist in implementing integrated continuous biomanufacturing.

Advancing Integrated Continuous Biopharmaceutical Manufacturing: The Biologics Landscape

The application of recombinant DNA technology rapidly and dramatically altered the global pharmaceutical landscape. The era of commercial pharmaceutical biotechnology began on October 28, 1982, with the U.S. **FDA** (see Glossary) approval of Eli Lilly's recombinant human insulin [1]. Today, the majority of the top 10 best-selling drugs in the world are derived from recombinant bioprocesses [2] and monoclonal antibody products are one of the fastest growing drug product classes on the market [3]. Biotechnology medicines are regulated in the U.S. under the Food Drug and Cosmetic Act and certain provisions of the Public Health Service (PHS) Act. As such, their clinical development is regulated under **Investigational New Drug Applications (INDs)**, while the actual product marketing application is submitted as a **Biologics License Application**, including those submitted under section 351(k) of the PHS Act (i.e., **biosimilars**). In the U.S., the era of biosimilars has dawned with the approvals of the first biosimilar products: two filgrastim products, pegfilgrastim, three infliximab products, etanercept, two adalimumab products, trastuzumab, and bevacizumab [4–8]. Prior to this era, the major market drivers of pharmaceutical biotechnology have been minimizing development time and maximizing cost control. This naturally led to a focus on developing innovative products rather than processes, especially postmarketing. The advent of biosimilars has the

[1]Food and Drug Administration, Center for Drug Evaluation and Research, Office of Pharmaceutical Quality, Silver Spring, MD 20993, USA
[2]Department of Chemical Engineering, University of Massachusetts Lowell, Lowell, MA 01850, USA
[3]These authors have contributed equally to this article
[4]https://seongkyu-yoon.wiki.uml.edu
[@]Twitters: @UMLEngineering, @UMLEngineering

*Correspondence: kurt.brorson@fda.hhs.gov (K. Brorson) and seongkyu_yoon@uml.edu (S. Yoon).

potential to change market drivers. Now bioprocesses that deliver quality product more efficiently can potentially realize cost advantages.

Manufacturing and quality issues have been shown to cause nearly two-thirds of all biologics drug shortages [9]. The manufacturing and quality issues result in undesirable levels of variability that may expose patients to unnecessary risk of poor-quality products. To address manufacturing and quality issues, there is a major trend across the pharmaceutical industry toward process intensification and continuous manufacturing for both small-molecule drugs and biotechnology products [10]. For biotechnology products, advancing manufacturing technology – both upstream and downstream – has the potential to reduce shortages and variability, allow for production flexibility, simplify scale-up procedures, reduce facility footprints and capital costs, increase productivity, and reduce production costs [11,12].

On the upstream side, two common modes of cell culture operation are batch and fed-batch. An alternative approach is continuous processing often accomplished with a perfusion culture (Figure 1). In a perfusion culture, media and extracellular material containing the desired drug compound are continuously removed from the bioreactor. Cell bleeds remove cells from the bioreactor during the process to maintain a specified operational range to avoid critically high cell densities where process control cannot be maintained. As compared to a fed-batch process, a perfusion process can potentially achieve higher cell densities, specific productivity, and volumetric productivity [13]. Following the upstream operation, a fully integrated continuous biomanufacturing process harbors all continuous unit operations (upstream and downstream) with no isolated intermediate occurring between them. On the downstream side of biotechnology, fully continuous processing can decrease chromatography column residence times and eliminate intermediate hold steps to minimize the impact on sensitive molecules,

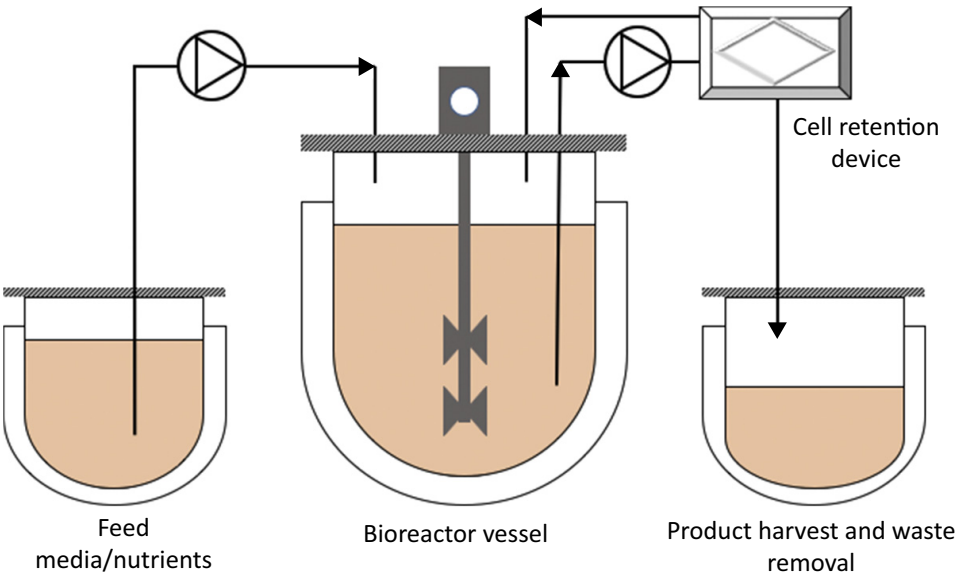

Figure 1. Perfusion Setup. In perfusion bioreactor processes, a pump adds media containing nutrients into the bioreactor while another pump flows a mixture of cells and spent media from the bioreactor through a cell retention device. The retention device separates the cells from spent media. The spent media are harvested while the cells are returned to the bioreactor.

Glossary

Alternating tangential flow (ATF): a system of moving a bioreactor mixture of media, cells, and cellular products tangential to a filtration membrane using an alternating pump and vacuum across a polymer diaphragm.

Biologics License Application: a request by a legal person or entity, for permission to introduce a biologic product into interstate commerce (21 CFR 601.2).

Biosimilar: a biological product that has been found to be structurally and functionally similar to an FDA-approved biological product and shows no clinically meaningful differences from the reference product in terms of safety, purity, and potency (i.e., safety and effectiveness).

Center for Drug Evaluation and Research: a center at the FDA that regulates over-the-counter, prescription, biological therapeutic, and generic drugs, thereby ensuring that only safe and effective drugs are available to persons in the United States.

Critical quality attribute (CQA): a physical, chemical, biological, or microbiological property that, to ensure the desired product quality of a pharmaceutical or biopharmaceutical product, must fall within a defined limit, range, or distribution.

Design of experiments (DOE): a systematic approach used to explain the output of a process by determining the relationship between the process parameters and the process output.

Emerging Technology Team (ETT): a small, cross-functional team at the FDA that has been created to keep pace with technical advancements.

European Medicines Agency: a decentralized agency in the EU charged with the scientific evaluation, supervision, and safety monitoring of medicines in the EU.

FDA: agency of the US Department of Health and Human Services that is responsible for protecting public health by regulating the manufacturing, marketing, and distribution of drugs, biological products, food, cosmetics, and radiation-emitting products.

while also minimizing manual operations and human decision making. A fully integrated continuous process has potential to improve quality, cost, speed, and flexibility [14].

With the rapidly increasing global sales in biologic drugs and the constant pressure from biosimilars, there is a significant drive for advanced technologies and improved efficiency to meet these needs. In addition, four top trends in biopharmaceutical manufacturing showed increased focus on continuous bioprocessing and manufacturing cost reductions from 2014 to 2017 [15]. Integrated continuous biomanufacturing can help meet these two important targets. Finally, the FDA's approval of a change in manufacturing of PREZISTA (darunavir) from batch to continuous (Box 1) gave a clear signal for manufacturing advancement, elucidated in its *Advancement of Emerging Technologies Applications to Modernize the Pharmaceutical Manufacturing Base* guidance. These factors make the current review very timely for biopharmaceutical manufacturers seeking to initiate or accelerate efforts in integrated bioprocessing and equally for academia focused on research to support this endeavor.

A Brief History of Scientific and Regulatory Challenges in Continuous Bioprocessing

While continuous upstream bioprocessing is reasonably well established, the integration of continuous downstream processing is still a developing field. In a typical process, an upstream bioreactor is followed by successive batch unit operations including clarification, hold steps, capture, and polishing (Figure 2). The use of a cell separation devices such as an **alternating tangential flow (ATF)** cell separation device typically addresses the clarification aspect of a continuous bioprocess. For continuous capture and polishing chromatography, the two main options are **periodic countercurrent chromatography (PCC)** and **simulated moving bed (SMB)** chromatography. A chromatographic column unit operation typically contains the steps of load, wash, elution, and regeneration. In both PCC and SMB chromatography, multiple columns in series run these steps in a cyclic manner (Figure 3). In a truly integrated continuous operation, the residence time in a column needs to exceed the time needed for successive column steps (i.e., equilibration, wash, elution, and regeneration), otherwise process synchronization will be difficult to achieve.

Although regulatory challenges are often cited as a concern in adopting continuous bioprocessing, the FDA approved the first biopharmaceutical product manufactured via continuous perfusion in 1993 [16] and today approximately 20 marketed biologic products, from multiple companies, use perfusion or other elements of continuous bioprocessing [17].

Box 1. What Is a 'Batch'?

A common misconception is that the concept of continuous biomanufacturing is not compatible with the paradigm of 'batches' and 'lots' [27]. In fact, regulations describe a 'batch' as a specific quantity of drug intended to have uniform character and quality within specified limits produced according to a single manufacturing order during the same cycle of manufacture [28]. A 'lot' is either synonymous with a 'batch' or is a specific identified portion of a batch. In both cases the definitions are based on a quantity of material and not a method of manufacture. In continuous processes, material traceability should be closely linked to the definition of a batch. A batch can be based on a set amount of production time, a specified quantity of product, equipment capacity limits, or the introduction of new materials into a process (i.e., different raw materials lots). In each case, the batch definition should be connected to the control strategy for the process, which ensures product with uniform character and quality backed by a representative sampling strategy. As continuous processes fit into the current regulatory paradigm, other regulatory expectations surrounding, for example, established conditions [29], process validation [30], and quality management systems [31–34] are no different for continuous and batch processes.

High-performance liquid chromatography (HPLC): a method used to separate, identify, and quantify two or more soluble products in a solution.
High throughput: a method of testing in which a high number of samples (dozens to thousands) can be simultaneously tested under different conditions.
Investigational New Drug Application (IND): a method through which a legal person or entity seeks exemption from the FDA to ship an investigational drug across state lines for the purpose of clinical investigation before the drug receives market approval.
IR: electromagnetic radiation with wavelengths between 700 and 1 000 000 nm.
Near infrared (NIR): electromagnetic radiations with wavelengths between 750 and 1400 nm.
PCR: an *in vitro* method for making many copies of a DNA region.
Periodic countercurrent chromatography (PCC): a method of purifying antibodies where two or more affinity columns are used. The first column is loaded to full capacity and the breakthrough from the first column is directly loaded onto the second. This ensures minimal material lost and higher purification yield.
Process analytical technology (PAT): the process of ensuring that final product quality meets specifications by designing, analyzing, and controlling manufacturing though periodic and/or continuous measurement of critical quality and performance attributes.
Quality by design (QbD): a systematic approach to development that applies sound science, process and product understanding, process control, and quality risk management to ensure the predefined product and process objectives are met.
Raman: a spectroscopic methodology used to identify molecules by taking advantage of their unique molecular fingerprint formed by specific rotational, vibrational, and low-frequency modes.
Simulated moving bed (SMB): a continuous method for separating a

For well over a decade, the FDA has advocated for **quality by design (QbD)** in pharmaceutical manufacturing processes, including those in biomanufacturing [18]. The QbD concept requires understanding drug product performance to identify **critical quality attributes (CQAs)** of the product. Equipped with this knowledge, the process and product formulation can be designed specifically to generate those attributes. This requires understanding the impact of materials and process parameters and adequately controlling sources of variability. In many ways, **process analytical technology (PAT)** is an enabling concept for the QbD initiative in that 'quality cannot be tested into products; it should be built-in or by design' [19]. A PAT framework allows for the design and development of a manufacturing process that delivers a consistent, defined quality material [20]. In addition to potentially reducing both risks to product quality, PAT and QbD can also deliver improved process efficiency. The assessment of material attributes can directly inform feedback or feedforward process decisions. Critical product attributes can be measured instantaneously (on-line, in-line, at-line) or before a decision point (near at-line). Importantly, PAT innovation needs to occur in biomanufacturing upstream, downstream, and in process development/validation to advance the level of process control available for integrated continuous processes [21–26].

mixture of substances in solution that is very useful when the substances in the mixture have similar affinity, such as enantiomers and diastereoisomers.

Building on the PAT and QbD initiatives, the FDA recently introduced new initiatives to help accelerate the adoption and implementation of new pharmaceutical manufacturing technologies. The **Center for Drug Evaluation and Research** actively encourages and supports the development and adoption of emerging technologies via the **Emerging Technology Team (ETT)** [35]. The ETT is a small, cross-functional team with representation from relevant assessment, inspection, and policy programs that is needed to keep pace with technical advancements in the field. The ETT facilitates the implementation of emerging technologies, including PAT and continuous manufacturing, by providing early engagement, supporting the quality assessment team in the review of submissions (including biologic license applications),

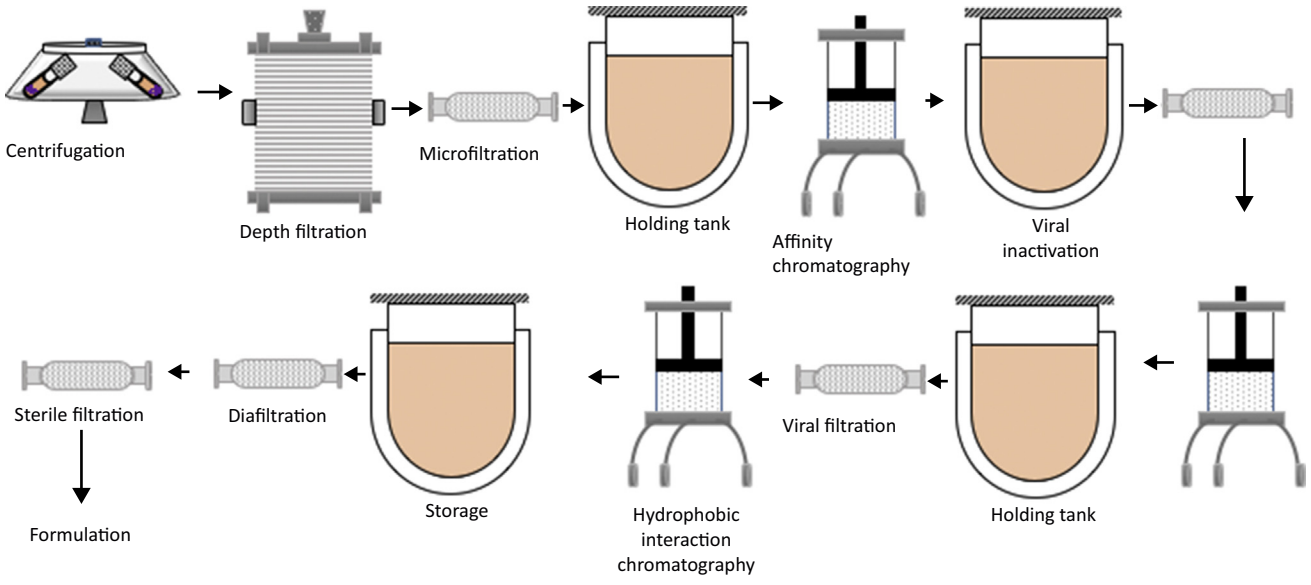

Figure 2. Downstream Processing Steps. Traditional downstream biomanufacturing processes include a combination of filtration, chromatography, and eventually formulation. The bulk of cells is usually removed by an initial centrifugation. Depending on the product, two or three chromatography processes might be required before a final viral filtration/sterile filtration step to obtain the product of interest.

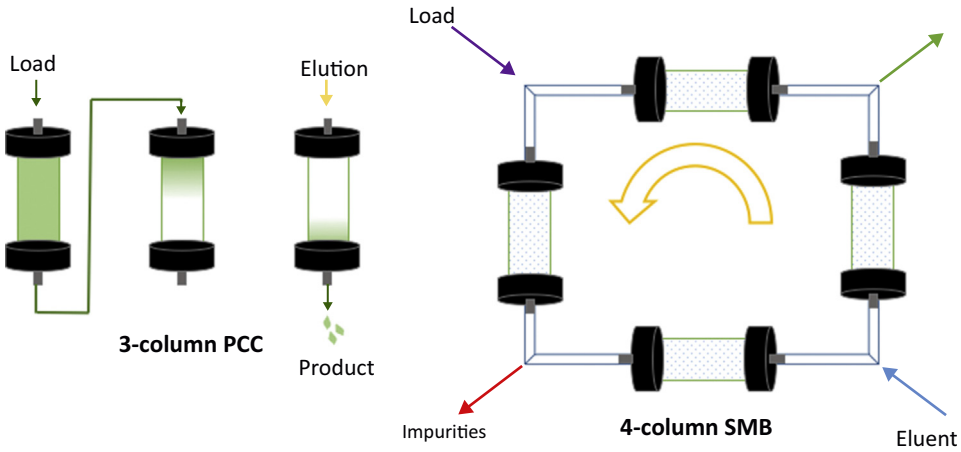

Figure 3. PCC and SMB Chromatography. Two important continuous chromatography processes gaining steam are the three-column PCC and SMB. In PCC, flow-through from a column is reloaded into subsequent columns to improve process yield. However, each column still undergoes the load–wash–elute–equilibrate cycle. In SMB, the inlet to each column is periodically 'moved', permitting continuous loading, elution, and harvesting, and resulting in lower buffer and resin use. PCC, periodic countercurrent chromatography; SMB, simulated moving bed.

and guiding the resolution of scientific and policy issues. A similar program was initiated in Europe in 2015: The EU Innovation Network. This network included the **European Medicines Agency** Innovation Task Force and innovation offices in each EU member state. This network has scientific, legal, and regulatory competencies and seeks to encourage early dialogue on innovative aspects of product development and encompasses applicants, academics, and researchers. Despite these programs, clearly, there is a need to keep regulators across the globe abreast of new technology developments to help assess applications using complex technologies. This is a joint challenge for regulators, academia, and industry (Boxes 2 and 3).

Box 2. Some Considerations for Implementing a Continuous Manufacturing Process in Different Phases of Development

From a regulatory standpoint, continuous manufacturing (CM) may be implemented prior to an IND, during development, and after marketing. These are all acceptable for CM development. The simplest point to implement continuous processing from a scientific and regulatory standpoint is likely prior to an IND. In this way, a continuous processing program can start at a small scale and scale up (potentially very rapidly using the same operation used for pilot plant development or early clinical supply) as development proceeds. The ETT can be engaged during development.

If a technology is introduced during development (i.e., during clinical studies), from a logistical standpoint it may make sense to transition to a continuous process at the same time that a significant scale up is required of the process. In this case, comprehensive comparability studies can address possible physicochemical or other changes to the drug product (e.g., post-translational modifications, degradation, impurities). The issue obviously becomes dramatically more complicated if significant analytical differences are observed in these studies.

Finally, a change could come after marketing approval. Introduction of a new technology postmarketing may be driven by process economics or a clear safety/quality imperative. Regardless of the point of implementation in the drug product life cycle, further developments in science and technology improve and enable the implementation of a continuous bioprocess.

Box 3. Is There a Business Case for Continuous Bioprocessing?

There is much written about the technical potential for continuous bioprocessing to improve quality, speed, and flexibility. However, continuous bioprocessing will never be widely adopted if the technology does not deliver clear economic advantages. Currently, the traditional economic drivers of biomanufacturing are in a state of flux. The new era of biosimilars introduces the potential for price competition. Whereas innovator drug development prioritizes speed to market and recovery of R&D cost, biosimilar development emphasizes minimizing the cost of goods per unit of product. Such process improvement has traditionally been driven by increasing cell culture titers. However, cell culture titers of some monoclonal antibodies are now approaching the practical limits of solubility and batch-based manufacturability [36], which limits the potential for significant titer improvement moving forward [37,38]. Further, the age of many existing biomanufacturing facilities and a drive toward more localized manufacturing incentivizes simplified facility design and lowered capital investments.

Recent work in the economic modeling of an integrated continuous biomanufacturing platform showed that over a 10-year period, the continuous platform could reduce average cost by 55% compared with conventional batch processing [12]. This model considered both capital and operating expenses. Further, the model predicted that savings could further increase by as much as 25% in situations when product demand exceeded projections. Other recent work examined the economic feasibility of an integrated continuous bioprocessing approach for monoclonal antibody manufacture across a product's life cycle from preclinical to commercial manufacture [39]. This analysis showed advantages on a direct cost per gram basis for a fully continuous strategy as compared to a batch strategy for steps earlier in the development process (e.g., preclinical, clinical) prior to commercial manufacturing. Thus, business drivers and technological capabilities seem to be coming together to make integrated continuous biomanufacturing economically viable and advantageous.

Upstream Technical Advancements

Bioreactors

The use of a perfusion system is not limited to the production bioreactor. High-density cell banks have been proposed as a potential alternative to the classical expansion seed train [40–42]. One method to generate the necessary densities and volumes for a high-density cell bank is to use a perfusion bioreactor coupled with a cell retention device. In these cases, cell densities can reach as high as 1–2 $\times$ 10^8 cell/ml [43,44]. Once cells reach the production bioreactor, the basic operating principle of continuous cell culture relies on the ability to simultaneously remove extracellular material (e.g., waste, product), replenish nutrient-rich media, and retain cells in the culture vessel [45]. In addition, a cell bleed can help remove debris and maintain a target cell concentration for engineering control of the cell culture. There are multiple options for cell retention devices common in continuous cell culture [46–49]. Most methods take advantage of filtration, centrifugation, or gravity. The earliest method reported used an internal spin filter within the culture vessel [50]. Variations of these spin filter modules are commercially available today at the laboratory and pilot scale. External centrifuges have equally been used as cell retention devices [51]. Here a mixture of cells and culture fluids are transported into an external filter with multiple settling zones and after centrifugation, fresh media are used to flush cells back into bioreactor. Gravity settlers have equally been developed [52]. Gravity settlers do not require any agitation, provide low stress on cells, and are not susceptible to clogging issues. However, as cell densities increase, high sparge might result in lower productivities. Moreover, an additional separation step is required to harvest material from gravity settlers. The most widely used method for cell retention recently has been ATF. At the laboratory and commercial scale, considerations for the cell retention device include ease of installation, maintenance, and operation; reliability over long-term culture; permeability toward cell debris, macromolecules, waste, and culture product; and adverse effects on cell growth and productivity.

Upstream PAT

PAT facilitates the consistent generation of products with predetermined quality attributes via real-time monitoring and process control. CQAs are properties that ensure the desired product

quality by meeting defined criteria. Understanding the impact of process variables on CQAs is required to design a control strategy. Process parameters whose variability has an impact on a CQA are called critical process parameters and need to be monitored or controlled to ensure the process produces a product of the desired quality. Counter to the principles of continuous processing, certain current testing procedures require lengthy offline protocols (e.g., bioburden). This increases cycle times, delays timely go/no-go decisions, and impairs any possibility for real-time release testing. To more closely link measurement and process control, real-time data can inform correlation models that provide predictive information on CQAs in real time. For example, in the bioreactor, PAT approaches could be based on the impact of metal ion concentrations [53–55], media additives [56,57], osmolality [58], and pH [59] on CQAs related to glycosylation [60]. Implementing PAT in batch and fed-batch processes has been contemplated, although product quality attributes are often not directly measured and controlled [23,61]. Classical process sensors can provide information on process variables such as temperature, pH, dissolved gases, and foam levels. For the advancement of continuous cultures, implementation of enabling PAT will require important improvements in sensor technology, configuration, and robustness [25,26,62,63]. Robust PAT may allow confidence within a design space, with flexibility to move within that design space during commercial manufacturing, using a PAT-driven control strategy.

Recently, novel on-line, at-line, and in-line sampling techniques, especially using spectrometric sensors, have been implemented for process monitoring in biomanufacturing [25,64]. Of particular interest is the rapidly evolving field of spectroscopic techniques, such as **near infrared (NIR)**, fluorescence, **IR**, and **Raman**. NIR spectroscopy has been most extensively studied to determine the concentration of individual components in cell culture broth [65–67]. Raman scattering differs from NIR or IR absorption and often can provide complementary information about chemical composition and molecular structure [68]. Raman spectroscopy in cell culture processes has been used to analyze broth component profiles [69–72] and complex cell culture media solutions [73]. A very attractive feature of Raman spectroscopy in the area of biopharmaceuticals is the ability to monitor structural/chemical changes of proteins [74–76]. Some examples of direct on-line measurement of quality attributes include glycoform patterns [77] such as sialylation [78]. The challenge moving forward will be to improve sensor design for easier incorporation in continuous bioprocesses, including implementing fiber optic technology [79,80], noninvasive process monitoring [81], and incorporating advanced sensors into automated process control strategies. New sensors may need to be amenable to redundancy and in-process recalibration [82,83] to address sensor failures and/or signal drift and the need for in-process replacement.

Downstream Technical Advancements

Continuous Purification

Continuous purification within the biopharmaceutical industry has evolved from SMB with six columns [84], through sequential multicolumn chromatography with four columns [85], to PCC with three columns [86], to twin-column chromatography with two columns [87]. Morbidelli and colleagues evaluated the twin-column setup relative to traditional batch methods [88]. Recently, a proof-of-concept cyclic one-column system was used to process perfusate from a perfusion bioreactor [14]. These different methods each have various advantages and drawbacks. Essentially, a balance between yield, capacity utilization, and productivity must be found, and the optimal process will depend on a range of factors, including operating conditions, and will likely be product specific.

As bioreactor titers improve, the eventual end point will be continuous purification schemes. At low titers, the cyclic batch method of one-column continuous purification [14] tends to have better productivities. However, as titers increase over 2 mg/ml, multicolumn continuous chromatography techniques become more advantageous in terms of productivity and buffer use [89].

Purification and Impurity/Viral Clearance

Compared to upstream processes in biomanufacturing, continuous downstream processing is not as established in manufacturing of marketed biopharmaceuticals. Typically, purification of therapeutic proteins after cell culture follows a three-step batch mode chromatography process: affinity (capture) and two ion-exchange steps. In addition to these three steps, multiple concentration/buffer-exchange steps and viral filtration can be incorporated. Various continuous capture techniques using more than one chromatography columns have been developed to allow continuous loading up to the point of column saturation without product loss. Examples include the three-column PCC [86] and the twin-column system [87]. In addition to these classical approaches, other continuous methods such as aqueous two-phase extraction [90], continuous precipitation [91], continuous countercurrent tangential chromatography [92], and high-performance tangential flow filtration [93] have been proposed.

These approaches all have the potential for reducing cost, offering flexibility, and reducing development time, which are important considerations for industry, especially in early development. However, while these novel approaches offer advantages and additional options, they introduce potential challenges in terms of validation and product quality. In multicolumn systems, elution streams from individual columns are typically pooled. To ensure that poor-quality eluent material from a faulty column is not pooled with material from a properly functioning column, real-time monitoring of individual columns with feedback control to divert material from defective column might be useful. Moreover, having multiple columns multiplies the odds of failure modes and the probability of process interruption since integration is typically achieved by very precise synchronization between upstream and downstream processing. For example, in the PCC method [86] flow through from an initial column is loaded onto a second column. This may introduce a risk of cross-contamination between columns. A robust monitoring and feedback control strategy might detect contamination and divert the effluent away from the second column. Without redundant systems, there is the risk of a loss of synchronization and a purification bottleneck.

Downstream PAT

The implementation of downstream PAT in biomanufacturing has been somewhat limited (reviewed in [94]). The limited use may be due, in part, to a perceived dearth of sensor options in downstream operations and equipment. For example, systems that only offer pH, conductivity, absorbance, temperature, and pressure sensors do not actually measure quality attributes of the biomolecule. With many sensors, information on actual CQAs to inform product pooling/diversion decisions cannot be directly obtained, including product properties (concentration, purity, etc.), biological impurities (host cell protein, DNA, endotoxins, etc.), variants (misfolding, etc.), and process-related impurities (leached protein A from affinity resin) [22]. Thus, PAT implementation can be a driver for process improvement in downstream operations for integrated continuous systems.

One such approach involves real-time data from chromatography systems. Multiwave UV spectra have been shown to be effective in determining the concentration of individual components in a protein mixture [24]. This approach was used to accurately predict the

concentration of aggregates relative to the protein-of-interest in an eluent stream. Column integrity values have also been used to determine the performance of columns under repeated cycles [95]. Expansion of sensor options by chromatography can be a driver for more effective process monitoring and control. A second approach is the use of at-line systems in eluent streams. **High-performance liquid chromatography (HPLC)**, fluorescence, and circular dichroism have been used to monitor in-process CQAs in chromatography unit operations [96–98]. By accelerating feedback from the monitoring systems, these established technologies can provide information well within the decision time [94]. Improved communication between on-line, at-line and in-line monitoring systems is needed before these types of automated control strategies can be realized.

Rapid upstream testing of bioreactor samples has potential downstream advantages, especially concerning bulk harvest disposition. Decisions made prior to transfer to downstream processing would allow contaminated cultures to be confined to the bioreactor, sparing clean-up of tanks and chromatography units. Rapid mycoplasma tests based on ribosomal **PCR** have been described in the literature [99–101], with a few allowing half-day turnaround times consistent with harvest dispositioning. Advanced methods such as next-generation sequencing could conceivably be applied toward virus screening [102], but such technologies would require more confidence in these methods in terms of qualification and rigorous approaches toward data analysis. Currently, an industry/regulatory consortium is working toward this goal [60,103]. Beyond testing, there have been proposals to modify existing viral clearance/inactivation validation approaches to accommodate modified unit operations in continuous bioprocessing [102]. These efforts and conversations are ongoing, but will likely involve updating assumptions and philosophies about what is critical to assure viral safety for biotech products.

Integration of Upstream and Downstream Technologies

Integrating Cell Culture with Capture

There have been several examples of integrating a continuous upstream process with immediate capture [86,104,105]. The major challenge with integrating these two processes is synchronizing the upstream perfusion flow rate with the downstream purification flow rate. If these processes are not synchronized, holding tanks or regular pooling may be required, rendering the process noncontinuous. This sometimes requires the use of multiple columns [14,86] or membrane chromatography [104]. Process synchronization can easily result in disruptions if problems such as clogged filters occur during manufacturing. To mitigate this, redundancies such as a surge tank and back-up columns can be incorporated in the process.

Upstream processes operate under sterile conditions. However, many types of downstream equipment do not yet feature sterile lines. For an integrated process, a sterile barrier needs to be installed between upstream and downstream systems. This can be achieved by installing filters on the surge tank inlet and outlet. However, since continuous processes may run for weeks to months, nonsterile operations may justify additional monitoring to ensure the stream is free of contaminants. In addition, upstream and downstream systems have historically been developed independently. Thus, synchronized control systems are lacking. This means a deviation upstream is not typically detected by downstream systems (i.e., feedforward control) or vice versa (i.e., feedback control). There have been some attempts to implement feedback control. For example, an at-line HPLC was installed to provide titer data on bioreactor harvest that was used to modulate downstream operations [105]. Such systems need to be developed because many upstream parameters (e.g., host cell protein) impact downstream operations (e.g., purification) [106].

Full End-to-End Integration

A full end-to-end integrated system might include upstream continuous production and clarification through a cell retention device, followed by continuous capture and polishing to produce a drug substance followed by concentration, viral inactivation, viral filtration, sterile filtration, and formulation to produce the drug product. To our knowledge, complete end-to-end integration at scale has still not been reported in the peer-reviewed literature. The most pressing challenge is developing a global monitoring and control strategy for the entire train. The monitoring and control would entail continuous measurements at all inlet and outlet streams into unit operations with a realistic feedback and feedforward control strategy to ensure that final product is within CQA specifications. In addition, this would require that data generated by one unit be interpretable by other units within the train. With these expectations, it is understandable that such a system has not been developed without broader standardization. One system combined a perfusion bioreactor and two PCC units for initial capture and successive ion-exchange steps [107]. Though not fully end-to-end, this system is clearly the foundation for a full end-to-end system that demonstrated the obvious advantages of continuous bioprocessing (e.g., elimination of redundant operations, smaller footprint, implementation of single-use systems).

End-to-End Closed Systems

End-to-end closed systems require a completely closed system in which the equipment is operated such that the process fluids are never exposed to the manufacturing environment. This is distinguished from a functionally closed system that may be opened periodically and closed with strict sanitization procedures to make process adjustments before returning the system to a closed state. To achieve end-to-end closed systems, cells must be frozen in cell bags with weldable tubing through which they are introduced directly into a closed bioreactor containing closed cap assemblies. From the bioreactor, the process fluid travels through closed systems right up to packaging of drug product. Implementing a truly closed system will require the extensive adoption of single-use systems with standardized inlet and outlets to facilitate sterile connections, probably through sterile welding, of one process unit to another. Implementing end-to-end closed systems would require the generation of a working cell bank in closed containers (such as weldable bags) and a single-use flow path incorporating weldable inlets and outlets for chromatography systems and filtration units. Implementation of such systems, even though challenging and expensive, will reduce risks of contamination and reduce cleaning and validation times. Such single-use systems may also introduce new challenges in terms of robust process monitoring tools and the control of leachables and extractables [108].

Process Scaling

Scale-Down Models and Process Optimization

Process optimization of cell culture can be expensive and time consuming. High-throughput process optimization for batch and fed-batch processes has been improved by the application of automated miniaturized reactor systems [109–111]. However, no complementary system has been reported to facilitate process optimization for perfusion cell culture. Current perfusion systems have high minimum volume requirements such that purchasing, installing, and operating these kinds of units in **design of experiments (DOE)** studies may be cost prohibitive and time consuming. Equipment manufacturers therefore have an incentive to develop comparable **high-throughput** perfusion microbioreactors for perfusion process optimization studies. By contrast, end users must be careful not to draw too many conclusions from a single DOE perfusion run. Results will have to be replicated (possible in shuffled order)

and validated in a final run. This would be important to ensure that the cellular memory under a previous condition does not impact the observation in the subsequent condition(s).

In addition, implementing response surface modeling (RSM) and executing Stage 1 process validation (PV) are of critical importance. Once the RSM model is available and validated, significant time and resources can be saved for process optimization. Both upstream and downstream processes need to be well integrated. The corresponding RSM models should be combined for integrated process validation and assessment. Because some unit operations are not perfectly continuous, one still has to address whether either batch or continuous model is used.

On the microscale, a high-throughput system of bioreactors may have a maximum working volume of ~15 ml. In a theoretical perfusion mode operating at one vessel volume per day, this requires a turnover of ~10 μL/min. To develop and implement such a system, microfluidics will play an important role. There has been some recent progress to this end. For example, miniature bioreactors have been equipped with peristaltic pumps fabricated by 3D printing [112]. Further, a perfusion microbioreactor with a 1-ml working volume operating at a high relative perfusion rate of 1 ml/h was recently developed [113]. The high cell counts observed in larger bioreactors (up to 100×10^6 viable cells/ml) might be challenging to reach and control in these microsystems. Therefore, the significance of results obtained in scale-down models to production systems will have to be investigated.

Downstream process optimization has equally faced many challenges. Historically, downstream operations have been the bottleneck in implementing a truly integrated continuous system [114]. An attempt to remedy this has been attempted by using a twin-column system to develop a predictive controller based on a multiparametric model followed by a closed-looped validation step [115]. Attempts like this have the potential to minimize the current bottleneck in downstream purification and facilitate the implementation of a truly integrated system.

Manufacturing Facilities

An important operational advantage of integrated continuous biomanufacturing is the ability to significantly reduce facility sizes while maintaining flexibility. To take full advantage of the footprint reduction, a continuous bioreactor operation can be coupled with a continuous media production line and continuous downstream purification. Otherwise, the use of large vessels for media compounding and/or harvest storage cannot be avoided. In a theoretical facility containing a 500-l bioreactor operating under perfusion at one vessel volume per day, continuous media production and downstream purification should operate at the rates of at least 500 l/day.

The ballroom-like facility is gaining increasing importance because it allows all unit operations to be flexible and enclosed in one production room. This includes manufacturing components such as media and buffer preparation, fermentation, and downstream processing [116]. These ballroom-like facilities coupled with single-use systems and fully closed end-to-end operation offer flexibility and permit multiproduct manufacturing capabilities while minimizing the risk of cross-contamination [110,116]. Recently, a dance floor approach to facility design was introduced, wherein the open ballroom is partitioned into smaller compartments by temporary walls that permit transfer of material [117].

Concluding Remarks

Continuous approaches for manufacturing both small-molecule drugs and biotechnology products are here to stay. There have been impressive developments in integrating continuous biomanufacturing both upstream and downstream and in single-use systems. However, to advance the field, there are still several elements requiring additional development (see Outstanding Questions). One is the integration of hardware and software, such that unit operations with components from different suppliers work together and communicate in a plug-and-play fashion. Unfortunately, the off-the-shelf continuous unit operations developed for small-molecule drugs and the chemical industry are not directly applicable to biotechnology processes [118,119]. Although multicolumn cycling is currently a popular approach in continuous processing, it is probably most accurately classified as a semicontinuous operation. Truly continuous separation technologies, with sufficient resolution, will be helpful in advancing the integrated downstream elements of biomanufacturing (e.g., continuous capture, polishing). Viral inactivation is mainly semicontinuous, as well, and could benefit from a truly continuous unit operation. A foundational technology for QbD [23], and especially for continuous processes, is PAT. Much of the PAT presently available to deploy during the manufacturing process is focused on indirect measurements of product quality or direct measurements performed off-line. This can limit the level of control of a process and often requires an intricate understanding of the relationship between critical process parameters and CQAs. Clearly there is an opportunity for advanced PAT to address issues including the direct and real-time measurement of drug CQAs during the process and at release, perhaps even to enable real-time release testing. As some of these advancements are being developed, the scientific approaches for assuring the quality of biotechnology products are evolving. A fully continuous, commercial, end-to-end bioprocess is not yet feasible due to some of these needs, though one may be realized in the next 5–10 years based on a review of potentially enabling technologies in the patent literature. Well-designed and intensified processes that deliver quality drugs are the future of the industry: a manufacturing paradigm mutually beneficial to both industry and patients.

Outstanding Questions

What is the current scientific status in integrated continuous biopharmaceutical manufacturing?

What is the current regulatory status in integrated continuous biopharmaceutical manufacturing?

In which direction is the community going to achieve regulatory and scientific satisfaction with continuous biopharmaceutical manufacturing platform?

Will the continuous unit operations developed for small-molecule drugs be directly applicable for biotechnology drugs?

Can the biotechnology drug be produced in completely integrated continuous operation?

Will a fully continuous, commercial, end-to-end bioprocess be feasible in the next 5–10 years?

How will well-designed and intensified processes deliver quality drugs as the future of the industry?

References

1. FDA (1982) Human insulin receives FDA approval. *FDA Drug Bull.* 12, 18–19
2. Philippidis, A. (2015) The top 25 best-selling drugs of 2014. *Genet. Eng. Biotechnol. News* 23, https://www.genengnews.com/the-lists/the-top-25-best-selling-drugs-of-2014/77900383
3. Ecker, D.M. *et al.* (2015) The therapeutic monoclonal antibody market. *MAbs* 7, 9–14
4. Holzmann, J. *et al.* (2016) Totality of the evidence at work: the first US biosimilar. *Expert Opin. Biol. Ther.* 16, 137–142
5. von Schaper, E. (2016) Celltrion's infliximab copy shows path to biosimilars in US. *Nat. Biotechnol.* 34, 454–455
6. FDA (2016) *FDA approves Erelzi, a biosimilar to Enbrel.*
7. FDA (2016) *FDA approves Amjevita, a biosimilar to Humira.*
8. FDA (2017) *FDA approves first biosimilar for the treatment of cancer.*
9. FDA (2013) *Strategic plan for preventing and mitigating drug shortages.*
10. O', T.F. and Connor *et al.* (2016) Emerging technology: a key enabler for modernizing pharmaceutical manufacturing and advancing product quality. *Int. J. Pharm.* 509, 492–498
11. Konstantinov, K.B. and Cooney, C.L. (2015) White paper on continuous bioprocessing. May 20-21, 2014 Continuous Manufacturing Symposium. *J. Pharm. Sci.* 104, 813–820
12. Walther, J. *et al.* (2015) The business impact of an integrated continuous biomanufacturing platform for recombinant protein production. *J. Biotechnol.* 213, 3–12
13. Xu, S. *et al.* (2017) Bioreactor productivity and media cost comparison for different intensified cell culture processes. *Biotechnol. Prog.* 33, 867–878
14. Kamga, M.-H. *et al.* (2018) Integrated continuous biomanufacturing platform with ATF perfusion and one column chromatography operation for optimum resin utilization and productivity. *Prep. Biochem. Biotechnol.* 48, 383–390
15. Langer, E.R. and Ronald, A. (2017) Top trends in biopharmaceutical manufacturing. *Pharm. Technol.* 41, 58–60
16. Langer, E.S. and Rader, R.A. (2014) Continuous bioprocessing and perfusion: wider adoption coming as bioprocessing matures. *BioProcess. J.* 13, 43–49
17. Hernandez, R. (2015) Continuous manufacturing: a changing processing paradigm. *BioPharm Int.* 28, In: http://www.biopharminternational.com/continuous-manufacturing-changing-processing-paradigm
18. Rathore, A.S. and Winkle, H. (2009) Quality by design for biopharmaceuticals. *Nat. Biotechnol.* 27, 26–34
19. FDA (2004) *Pharmaceutical CGMPs for the 21st century—a risk-based approach,* DHHS
20. FDA (2004) *Guidance for Industry. PAT—a framework for innovative pharmaceutical development, manufacturing, and quality assurance,* DHHS
21. Gottschalk, U. *et al.* (2012) The need for innovation in biomanufacturing. *Nat. Biotechnol.* 30, 489–492
22. Behere, K. *et al.* (2018) Protein a resin lifetime study: evaluation of protein a resin performance with a model-based approach in continuous capture. *Prep. Biochem. Biotechnol.* 48, 242–256

23. Bhatia, H. *et al.* (2016) A design space exploration for control of critical quality attributes of mAb. *Int. J. Pharm.* 512, 242–252
24. Kamga, M.H. *et al.* (2013) Quantification of protein mixture in chromatographic separation using multi-wavelength UV spectra. *Biotechnol. Prog.* 29, 664–671
25. Lee, H.W. *et al.* (2015) Dielectric spectroscopy-based estimation of VCD in CHO cell culture. *J. Chem. Technol. Biotechnol.* 90, 273–282
26. Lee, H.W. *et al.* (2012) Estimation of raw material performance in mammalian cell culture using near infrared spectra combined with chemometrics approaches. *Biotechnol. Prog.* 28, 824–832
27. Lee, S.L. *et al.* (2015) Modernizing pharmaceutical manufacturing: from batch to continuous production. *J. Pharm. Innov.* 10, 191–199
28. US Federal Government (2009) Current good manufacturing practice in manufacturing, processing, packing, or holding of drugs; general. *The Code of Federal Regulations 21 CFR210.3*
29. FDA (2015) *Draft guidance for industry: established conditions: reportable CMC changes for approved drug and biologic products.*
30. FDA (2011) *Guidance for industry. Process validation: general principles and practices.*
31. FDA (2006) *Guidance for industry. Q9 Quality risk management.*
32. FDA (2009) *Guidance for industry. Q8(R2) Pharmaceutical development.*
33. FDA (2009) *Guidance for industry. Q10 Pharmaceutical quality systems.*
34. FDA (2012) *Guidance for industry. Q11 Development and manufacture of drug substance.*
35. FDA (2015) *Draft guidance for industry. Advancement of emerging technology applications to modernize the pharmaceutical manufacturing base.*
36. Kunert, R. and Reinhart, D. (2016) Advances in recombinant antibody manufacturing. *Appl. Microbiol. Biotechnol.* 100, 3451–3461
37. Yang, Y. *et al.* (2017) Multi-criteria manufacturability indices for ranking high-concentration monoclonal antibody formulations. *Biotechnol. Bioeng.* 114, 2043–2056
38. Paul, A.J. *et al.* (2014) Direct analysis of mAb aggregates in mammalian cell culture supernatant. *BMC Biotechnol.* 14, 99
39. Pollock, J. *et al.* (2017) Integrated continuous bioprocessing: economic, operational, and environmental feasibility for clinical and commercial antibody manufacture. *Biotechnol. Prog.* 33, 854–866
40. Heidemann, R. *et al.* (2002) A new seed-train expansion method for recombinant mammalian cell lines. *Cytotechnology* 38, 99–108
41. Seth, G. (2012) Freezing mammalian cells for production of biopharmaceuticals. *Methods* 56, 424–431
42. Tao, Y. *et al.* (2011) Development and implementation of a perfusion-based high cell density cell banking process. *Biotechnol. Prog.* 27, 824–829
43. Wright, B. *et al.* (2015) A novel seed-train process. *Bioprocess Int.* 13, 16–25
44. Jin, C.B.X. (2016) Ultra-high density cell banking methods, US Patent WO2016044670A1
45. Velugula-Yellela, S.R. *et al.* (2018) Impact of media and antifoam selection on monoclonal antibody production and quality using a high throughput micro-bioreactor system. *Biotechnol. Prog.* 34, 262–270
46. Tokashiki, H.T.M. (1993) Perfusion culture apparatus for suspended mammalian cells. *Cytotechnology* 13, 149–159
47. Woodside, S.M. *et al.* (1998) Mammalian cell retention devices for stirred perfusion bioreactors. *Cytotechnology* 28, 163–175
48. Castilho, L.R. and Medronho, R.A. *et al.* (2002) Cell retention devices for suspended-cell perfusion cultures. In *Tools and Applications of Biochemical Engineering Science* (Schügerl, K., ed.), pp. 129–169, Springer
49. Voisard, D. *et al.* (2003) Potential of cell retention techniques for large-scale high-density perfusion culture of suspended mammalian cells. *Biotechnol. Bioeng.* 82, 751–765
50. Himmelfarb, P. *et al.* (1969) Spin filter culture: the propagation of mammalian cells in suspension. *Science* 164, 555–557
51. Takamatsu, H. *et al.* (1996) Large-scale perfusion culture process for suspended mammalian cells that uses a centrifuge with multiple settling zones. *Appl. Microbiol. Biotechnol.* 45, 454–457
52. Wen, Z.-Y. *et al.* (2000) A novel perfusion system for animal cell cultures by two step sequential sedimentation. *J. Biotechnol.* 79, 1–11
53. Crowell, C.K. *et al.* (2007) Amino acid and manganese supplementation modulates the glycosylation state of erythropoietin in a CHO culture system. *Biotechnol. Bioeng.* 96, 538–549
54. St Amand, M.M. *et al.* (2014) Identification of manipulated variables for a glycosylation control strategy. *Biotechnol. Bioeng.* 111, 1957–1970
55. Gramer, M.J. *et al.* (2011) Modulation of antibody galactosylation through feeding of uridine, manganese chloride, and galactose. *Biotechnol. Bioeng.* 108, 1591–1602
56. Fan, Y. *et al.* (2015) Amino acid and glucose metabolism in fed-batch CHO cell culture affects antibody production and glycosylation. *Biotechnol. Bioeng.* 112, 521–535
57. Kildegaard, H.F. *et al.* (2016) Glycoprofiling effects of media additives on IgG produced by CHO cells in fed-batch bioreactors. *Biotechnol. Bioeng.* 113, 359–366
58. Pacis, E. *et al.* (2011) Effects of cell culture conditions on antibody N-linked glycosylation – what affects high mannose 5 glycoform. *Biotechnol. Bioeng.* 108, 2348–2358
59. Ivarsson, M. *et al.* (2014) Evaluating the impact of cell culture process parameters on monoclonal antibody N-glycosylation. *J. Biotechnol.* 188, 88–96
60. Sha, S. *et al.* (2016) N-glycosylation design and control of therapeutic monoclonal antibodies. *Trends Biotechnol.* 34, 835–846
61. Read, E.K. *et al.* (2010) Process analytical technology (PAT) for biopharmaceutical products: part II. Concepts and applications. *Biotechnol. Bioeng.* 105, 285–295
62. Mora, A. *et al.* (2018) Sustaining an efficient and effective CHO cell line development platform by incorporation of 24-deep well plate screening and multivariate analysis. *Biotechnol. Prog.* 34, 175–186
63. Park, S.Y. *et al.* (2018) Metabolic responses and pathway changes of mammalian cells under different culture conditions with media supplementations. *Biotechnol. Prog.* 34, 793–805
64. Zhao, L. *et al.* (2015) Advances in process monitoring tools for cell culture bioprocesses. *Eng. Life Sci.* 15, 459–468
65. Navratil, M. *et al.* (2005) On-line multi-analyzer monitoring of biomass, glucose and acetate for growth rate control of a *Vibrio cholerae* fed-batch cultivation. *J. Biotechnol.* 115, 67–79
66. Triadaphillou, S. *et al.* (2007) Fermentation process tracking through enhanced spectral calibration modeling. *Biotechnol. Bioeng.* 97, 554–567
67. Arnold, S.A. *et al.* (2002) Use of at-line and *in-situ* near-infrared spectroscopy to monitor biomass in an industrial fed-batch *Escherichia coli* process. *Biotechnol. Bioeng.* 80, 405–413
68. Mungikar, A. and Kamat, M. (2010) Raman: use of in-line Raman spectroscopy as a non-destructive and rapid analytical technique to monitor aggregation of a therapeutic protein. *Am. Pharm. Rev.* 13, 78
69. Sivakesava, S. *et al.* (2001) Monitoring a bioprocess for ethanol production using FT-MIR and FT-Raman spectroscopy. *J. Ind. Microbiol. Biotechnol.* 26, 185–190
70. Shaw, A.D. *et al.* (1999) Noninvasive, on-line monitoring of the biotransformation by yeast of glucose to ethanol using dispersive Raman spectroscopy and chemometrics. *Appl. Spectrosc.* 53, 1419–1428

71. Bhatia, H. *et al.* (2018) In-line monitoring of amino acids in mammalian cell cultures using Raman spectroscopy and multivariate chemometrics models. *Eng. Life Sci.* 18, 55–61
72. Trunfio, N. *et al.* (2017) Characterization of mammalian cell culture raw materials by combining spectroscopy and chemometrics. *Biotechnol. Prog.* 33, 1127–1138
73. Li, B. *et al.* (2010) Rapid characterization and quality control of complex cell culture media solutions using Raman spectroscopy and chemometrics. *Biotechnol. Bioeng.* 107, 290–301
74. Gryniewicz, C.M. and Kauffman, J.F. (2008) Multivariate calibration of covalent aggregate fraction to the Raman spectrum of regular human insulin. *J. Pharm. Sci.* 97, 3727–3734
75. Hedoux, A. *et al.* (2006) Analysis of sugar bioprotective mechanisms on the thermal denaturation of lysozyme from Raman scattering and differential scanning calorimetry investigations. *J. Phys. Chem. B* 110, 22886–22893
76. Sane, S.U. *et al.* (2004) Raman spectroscopic characterization of drying-induced structural changes in a therapeutic antibody: correlating structural changes with long-term stability. *J. Pharm. Sci.* 93, 1005–1018
77. Kuribayashi, R. *et al.* (2012) Rapid evaluation for heterogeneities in monoclonal antibodies by liquid chromatography/mass spectrometry with a column-switching system. *J. Pharm. Biomed. Anal.* 67–68, 1–9
78. Markely, L.R. *et al.* (2010) A high-throughput method for quantification of glycoprotein sialylation. *Anal. Biochem.* 407, 128–133
79. Zibaii, M.I. *et al.* (2010) Measuring bacterial growth by refractive index tapered fiber optic biosensor. *J. Photochem. Photobiol. B* 101, 313–320
80. Pattison, R.N. *et al.* (2000) Measuring and control of dissolved carbon dioxide in mammalian cell culture processes using an *in situ* fiber optic chemical sensor. *Biotechnol. Prog.* 16, 769–774
81. Ge, X. *et al.* (2003) High-stability non-invasive autoclavable naked optical CO_2 sensor. *Biosens. Bioelectron.* 18, 857–865
82. Bychkovskiy, V. *et al.* (2003) A collaborative approach to in-place sensor calibration. In *Information Processing in Sensor Networks: Second International Workshop, IPSN 2003, Palo Alto, CA, USA, April 22–23, 2003 Proceedings* (Zhao, F. and Guibas, L., ed.), pp. 301–316, Springer
83. Zhang, Y. *et al.* System and method for sensor recalibration, US Patent 7384397
84. Keßler, L.C. *et al.* (2007) Step gradients in 3-zone simulated moving bed chromatography. Application to the purification of antibodies and bone morphogenetic protein-2. *J. Chromatogr. A* 1176, 69–78
85. Girard, V. *et al.* (2015) Large-scale monoclonal antibody purification by continuous chromatography, from process design to scale-up. *J. Biotechnol.* 213, 65–73
86. Warikoo, V. *et al.* (2012) Integrated continuous production of recombinant therapeutic proteins. *Biotechnol. Bioeng.* 109, 3018–3029
87. Angarita, M. *et al.* (2015) Twin-column CaptureSMB: a novel cyclic process for protein A affinity chromatography. *J. Chromatogr. A* 1389, 85–95
88. Baur, D. *et al.* (2016) Optimal model-based design of the twin-column CaptureSMB process improves capacity utilization and productivity in protein A affinity capture. *Biotechnol. J.* 11, 135–145
89. Gottschalk, U. (2017) *Process Scale Purification of Antibodies*, Wiley
90. Rosa, P.A.J. *et al.* (2013) Continuous purification of antibodies from cell culture supernatant with aqueous two-phase systems: from concept to process. *Biotechnol. J.* 8, 352–362
91. Hammerschmidt, N. *et al.* (2016) Continuous polyethylene glycol precipitation of recombinant antibodies: sequential precipitation and resolubilization. *Process Biochem.* 51, 325–332
92. Dutta, A.K. *et al.* (2016) Performance optimization of continuous countercurrent tangential chromatography for antibody capture. *Biotechnol. Prog.* 32, 430–439
93. van Reis, R. *et al.* (1997) High performance tangential flow filtration. *Biotechnol. Bioeng.* 56, 71–82
94. Rathore, A.S. and Kapoor, G. (2015) Application of process analytical technology for downstream purification of biotherapeutics. *J. Chem. Technol. Biotechnol.* 90, 228–236
95. Larson, T.M. *et al.* (2003) Use of process data to assess chromatographic performance in production-scale protein purification columns. *Biotechnol. Prog.* 19, 485–492
96. Rathore, A.S. *et al.* (2010) Large scale demonstration of a process analytical technology application in bioprocessing: use of on-line high performance liquid chromatography for making real time pooling decisions for process chromatography. *Biotechnol. Prog.* 26, 448–457
97. Rathore, A.S. *et al.* (2008) Case study and application of process analytical technology (PAT) towards bioprocessing: use of on-line high-performance liquid chromatography (HPLC) for making real-time pooling decisions for process chromatography. *Biotechnol. Bioeng.* 100, 306–316
98. Joshi, V.S. (2014) A rapid HPLC method for enabling PAT application for processing of GCSF. *LCGC N. Am.* 31, 948–953
99. Eldering, J.A. *et al.* (2004) Development of a PCR method for mycoplasma testing of Chinese hamster ovary cell cultures used in the manufacture of recombinant therapeutic proteins. *Biologicals* 32, 183–193
100. Asarnow, D. *et al.* (2010) Validation and international regulatory experience for a mycoplasma touchdown PCR assay. *Biologicals* 38, 224–231
101. Deutschmann, S.M. *et al.* (2010) Validation of a NAT-based *Mycoplasma* assay according European Pharmacopoeia. *Biologicals* 38, 238–248
102. Johnson, S.A. *et al.* (2017) Adapting viral safety assurance strategies to continuous processing of biological products. *Biotechnol. Bioeng.* 114, 21–32
103. Khan, A.S. *et al.* (2016) Advanced Virus Detection Technologies Interest Group (AVDTIG): efforts for high throughput sequencing (HTS) for virus detection. *PDA J. Pharm. Sci. Technol.* 70, 591–595
104. Vogel, J.H. *et al.* (2012) A new large-scale manufacturing platform for complex biopharmaceuticals. *Biotechnol. Bioeng.* 109, 3049–3058
105. Karst, D.J. *et al.* (2017) Process performance and product quality in an integrated continuous antibody production process. *Biotechnol. Bioeng.* 114, 298–307
106. Hogwood, C.E.M. *et al.* (2013) The dynamics of the CHO host cell protein profile during clarification and protein A capture in a platform antibody purification process. *Biotechnol. Bioeng.* 110, 240–251
107. Godawat, R. *et al.* (2015) End-to-end integrated fully continuous production of recombinant monoclonal antibodies. *J. Biotechnol.* 213, 13–19
108. Diehl, B. *et al.* (2015) A biopharmaceutical industry perspective on single-use sensors for biological process applications. *BioPharm Int.* 28, 28–31 53. http://www.biopharminternational.com/biopharmaceutical-industry-perspective-single-use-sensors-biological-process-applications
109. Moses, S. *et al.* (2012) Assessment of AMBRTM as a model for high-throughput cell culture process development strategy. *Adv. Biosci. Biotechnol.* 03, 918–927
110. Hsu, W.T. *et al.* (2012) Advanced microscale bioreactor system: a representative scale-down model for bench-top bioreactors. *Cytotechnology* 64, 667–678
111. Rameez, S. *et al.* (2014) High-throughput miniaturized bioreactors for cell culture process development: reproducibility, scalability, and control. *Biotechnol. Prog.* 30, 718–727
112. Balakrishnan, S. *et al.* (2015) A scalable perfusion culture system with miniature peristaltic pumps for live-cell imaging assays with

provision for microfabricated scaffolds. *BioRes. Open Access* 4, 343–357

113. Mozdzierz, N.J. *et al.* (2015) A perfusion-capable microfluidic bioreactor for assessing microbial heterologous protein production. *Lab Chip* 15, 2918–2922
114. Gronemeyer, P. *et al.* (2014) Trends in upstream and downstream process development for antibody manufacturing. *Bioengineering* 1, 188
115. Papathanasiou, M.M. *et al.* (2017) Intelligent, model-based control towards the intensification of downstream processes. *Comput. Chem. Eng.* 105, 173–184
116. Klutz, S. *et al.* (2015) Developing the biofacility of the future based on continuous processing and single-use technology. *J. Biotechnol.* 213, 120–130
117. Wolton, D.A. and Rayner, A. (2014) Lessons learned in the ballroom. *Pharm. Eng.* 34, 1–5
118. Johnson, B. *et al.* (2008) Use of continuous plate reactor technology – reshaping reactions in pharmaceuticals as well as fine and specialty chemicals. *Genet. Eng. Biotechnol. News* 28, 50–51
119. Short, P.L. (2008) Microreactors hit the major leagues. *Chem. Eng. News* 86, 37–38