BIOMANUFACTURING TECHNOLOGY ROADMAP

CONTINUOUS DOWNSTREAM PROCESSING FOR BIOMANUFACTURING

AN INDUSTRY REVIEW

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About BioPhorum

The BioPhorum Operations Group's (BioPhorum's) mission is to create environments where the global biopharmaceutical industry can collaborate and accelerate its rate of progress, for the benefit of all. Since its inception in 2004, BioPhorum has become the open and trusted environment where senior leaders of the biopharmaceutical industry come together to openly share and discuss the emerging trends and challenges facing their industry.

Growing from an end-user group in 2008, BioPhorum now comprises 53 manufacturers and suppliers deploying their top 2,800 leaders and subject matter experts to work in seven focused Phorums, articulating the industry's technology roadmap, defining the supply partner practices of the future, and developing and adopting best practices in drug substance, fill finish, process development, manufacturing IT, and Cell and Gene Therapy. In each of these Phorums, BioPhorum facilitators bring leaders together to create future visions, mobilize teams of experts on the opportunities, create partnerships that enable change and provide the quickest route to implementation, so that the industry shares, learns and builds the best solutions together.

BioPhorum Technology Roadmapping

BioPhorum Technology Roadmapping establishes a dynamic and evolving collaborative technology management process to accelerate innovation by engaging and aligning industry stakeholders to define future needs, difficult challenges and potential solutions. The Phorum involves biomanufacturers, supply partners, academia, regional innovation hubs and agencies, serving to communicate the roadmap broadly while monitoring industry progress.

For more information on the Technology Roadmapping mission and membership, go to https://biophorum.com/phorum/technology-roadmapping/

1.0

Executive summary

BioPhorum's First Edition Technology Roadmap[1] outlines a ten-year vision for therapeutic protein production in the biopharmaceutical industry. This paper builds on the first edition by using a model monoclonal antibody (mAb) process to look at technology and regulatory gaps in the area of continuous downstream drug substance processing of therapeutic proteins. Increasingly, continuous processing (CP) for the manufacture of biologics is being discussed as a feasible approach, with commercially available, production-scale equipment for the key unit operations now available.

The pharmaceutical industry recognises that CP offers advantages over batch-based processing by:

- **producing products with a more consistent quality attribute profile**
- **allowing greater flexibility to react to changes in market demands**
- **reducing up-front capital investment in facilities, due to process intensification**
- **optimizing cost of goods (COG) through process intensification.**

In the small-molecule manufacturing arena, continuous manufacturing has been used to make five FDA-approved products[2], but CP has not yet been adopted more widely for clinical or commercial production of therapeutic proteins. This is a timely opportunity to discuss the gaps, limitations and regulatory landscape across continuous downstream unit operations.

This paper performs an end-to-end gap analysis of a typical continuous mAb downstream process from primary capture to bulk drug substance (BDS), identifying current technology and regulatory gaps that are preventing the implementation of continuous biomanufacturing. The intention is to provide a focus to enable the industry and its suppliers to target effort and resources most effectively to generate solutions. This approach results in a catalog of challenges facing continuous biomanufacturing (see Table 1). The authors, members of both the biomanufacturing and supplier communities, believe this list can be used to address the root causes that limit adoption of CP to date.

The gaps identified are grouped into categories:

- unit operation technologies
- single-use (SU) technologies
- automation
- modeling
- regulatory

Common terminology has been defined to clarify meaning. It is hoped that closing the gaps identified in this paper will turn the promise of continuous bioprocessing into a reality, so that patients, biomanufacturers and suppliers may all benefit.

Table 1: Overview of gaps hindering the adoption of continuous biomanufacturing

Section	Gap summary	Opportunity
Process description	Alternative meanings used within the industry for key descriptors.	Standardization of terminology and identification of a baseline model process for benchmarking innovation and efficiency gains.
Technology gaps	Complex equipment design, controls (see *Automation* row in table) and maintenance that require robust operation for long durations compared to batch processes. Robust, accurate and precise sensors required for process monitoring and control, and with performance-checking strategies and real-time replacement protocols. Current bioburden control strategies are based on batch knowledge and their appropriateness to CP is currently unknown. Limited range of product portfolios across operating scales (small to large) for all continuous unit operations available. No universal sampling approach or proven method for obtaining representative samples across all stages of a continuous process.	Development of robust skid, sensor and implementation strategies for CP. Development of novel technologies and scale-down systems for process development and characterization. Development of new methodologies for bioburden detection and control. Development of wider and scalable product portfolios for continuous equipment.
SU vs stainless steel technology	Continuous processes require flow paths that are robust, durable, precise and accurate throughout a biomanufacturing campaign.	Development of 'continuous-use grade' disposable plastics to meet the requirements of prolonged manufacturing durations.
Automation	Although automation can be applied to batch processes, it is critical for CP. Current gaps in automation are limited to real-time monitoring and data analysis, no residence time distribution models for raw material tracking and deviation management, the absence of process-level systems for inter-skid communication and skid-to-enterprise inventory system communications. Most industrial automation systems require specialized expertise to develop, maintain and achieve compliance, and do not provide/use full plug-and-play capabilities.	Development of automation systems for equipment monitoring and coordination, process tracking, monitoring and control, and efficient utilization of facilities and resources to support a flexible manufacturing strategy.
Modeling	Continuous-modeling assumptions are largely based on batch-operated technologies and current working practices, which are temporarily overshadowed by the adoption costs of new technologies and implementation strategies.	Understanding the impact of future continuous technologies on operational staffing, facility design and layout, and quality control/real-time release (RTR) in building robust models for the economic evaluation of CP.
Regulatory	No approved viral inactivation and clearance protocols established for manufacturing or laboratory characterization scales. Novel control strategies for continuous biomanufacturing processes need verification for good manufacturing process (GMP) manufacture.	Development of unified approaches for qualification, validation and viral clearance packages. Gain regulatory acceptance of continuous process qualification, validation and control strategies.

2.0

Introduction

The biopharmaceutical industry has gained momentum over the last five years as shown by the number of new products on the market and is constantly increasing production capacity for therapeutic proteins [3, 4, 5].

As molecules progress through the clinical development cycle, the demand forecast can change dramatically up to and beyond product launch. This has led to the adoption of platform processes that can be operated in very large facilities (20,000L stainless-steel bioreactors) and can cost over $1bn to construct.[6] To enable manufacture at this scale and facilitate the considerable scale-up required for clinical campaigns, a lengthy technology transfer activity is typically required. In some cases, this scale-up can be associated with changes in cell culture performance and, ultimately, the product quality attribute profile.

Many biomanufacturers are investing in research programs and laboratory-scale demonstrations of continuous bioprocessing, but few have transitioned to clinical or manufacturing scale. To address the complexity of implementing continuous bioprocessing, a group of 26 BioPhorum member companies—from biomanufacturers, supply partners, equipment producers and engineering companies—have contributed to this white paper. This unity helps to synchronize the entire manufacturing chain from equipment and SU development to raw material suppliers and producers in identifying the real and perceived gaps in continuous downstream processing (DSP) for mAb BDS production.

Continuous bioprocessing in SU-enabled facilities has been proposed as a means to address some of the challenges where bioreactors can be designed to operate at similar scales for both clinical and commercial production. These flexible facilities may more easily react to market pressures by extending production times, while using a relatively inexpensive and flexible infrastructure (such as small, SU surge vessels vs large stainless-steel (SS) hold tanks), thereby lowering the total upfront capital investment in the facility due to its reduced footprint. Also, by operating at the same or similar bioreactor scales, comparable product quality attribute profiles are expected in the production facility, reducing the technology transfer burden from clinical to commercial operations. The continuous approach can be realized for an entire process or single process steps depending on the product and process.

The transition to continuous bioprocessing requires either adapted or entirely new design principles at the unit operation level. However, there is a broad agreement that the production process itself must maintain the same downstream unit operations as a batch process, but be transformed to run in a continuous manner. This approach can open the door to further flexibility by enabling a seamless transition between continuous and traditional batch processing for production.

The need for closed processing is amplified with continuous downstream bioprocessing, as the equipment train may be operated over a long period. Closed processing reduces the risk of contamination, and resulting batch loss. Thus, the control strategy for microbial contamination is critical in the design and operation of continuous bioprocesses to prevent the build-up of bioburden. This will draw heavily on established closed-processing concepts regarding established SU, aseptic connection technologies, together with established sterilization procedures. Although closed processing will not immediately negate the need to process in a classified environment, it is likely that the area dedicated to higher-grade clean rooms may be reduced in the future as learning is gained from operating with closed processes. The integration of the whole downstream process into a single flow path requires each unit operation to be synchronized by a central control system. While being challenging to implement, a central control system ultimately provides tighter control and monitoring of process parameters leading to lower variability in product quality profiles.

Also, reliance on automated control systems will reduce human intervention within the process, lowering the risk of operator error.

Although feed material can be derived from either fed-batch or perfusion-mode cell culture fermentations, the upstream aspects of CP are not covered in this paper because perfusion technology, and its integrated cell-retention capability, is already established in commercial biomanufacturing. For fed-batch bioreactor inputs into a continuous downstream process, harvesting will be considered part of the upstream operation

Furthermore, the integration of drug product generation is not considered in this document as it usually occurs independently of drug substance production. It is not the intention of the authors to present solutions but to highlight where the industry should target their energy to enable the implementation of continuous DSP. A further outcome of this white paper is to start the alignment of the bioprocessing industry with regulatory thinking and, vice versa, regarding the implementation of continuous biomanufacturing for clinical and commercial production.

This paper highlights the technology and regulatory gaps that are preventing the implementation of continuous biomanufacturing and focuses on the efforts of both the industry and its suppliers on generating solutions. The gaps have been classified into the following categories:

- technology
- SU equipment and consumables vs SS equipment
- automation challenges
- economic modeling
- regulatory landscape for continuous biomanufacturing.

Table 2 summarizes the key gaps and the opportunities they present to the biomanufacturing industry. Although this approach results in a catalog of the challenges, the team believes that this will inspire and help prioritize solutions that address the root causes that are limiting CP adoption. The paper performs an end-to-end gap analysis of a typical continuous mAb downstream process from primary capture to BDS. The gaps are grouped into a number of overarching categories to help focus attention and target solutions. The paper defines common terminology (see Table 3). We believe that narrowing the gaps identified in this paper will enable the community to turn the promise of CP into a reality, for the benefit of both the patients and biomanufacturers.

Table 2: Summary of gaps in continuous downstream processing, with related sections

Gap identified	Opportunity	Related sections in this paper
Limited continuous unit operations or existing hybrid skids, at appropriate scales of operation, with required technology readiness	Immature technologies and limited experience with robust continuous GMP systems provides an opportunity to develop and adopt new bioprocessing philosophies and approaches	4.2, 4.3, 4.4, 4.5, 4.7, 6.1, 6.2 and 6.3
Bioburden control strategy requirements concerning cleaning, sterility and closed systems not defined	Industry and regulators to refine standards and adopt novel strategies for cleaning and sterilization limits and detection methods in continuous downstream process platforms	4.1 and 8.2
No standardized virus inactivation and clearance validation strategies	Development of validation strategies for challenging CP to handle worst-case viral clearance scenarios	4.3, 4.4 and 8.2
Continuous buffer preparation, supply and on-line release approach undefined	Integration of continuous buffer preparation systems with various CP platforms. System configuration, documentation of on-line release and hold vessel staging needs adapting to CP	4.7
Limited or no robust, precise and accurate disposable sensors and probes for long-term operation and/or change-out strategies for on/in-line control and release	Opportunities to develop on-line sensors that meet the operational life (30 to 120 days), process conditions and required sensitivity ranges or change-out strategies that do not impact on continuous operations	4.8
Limited availability, automation and flexibility of existing process development and pilot-scale equipment for integration into continuous downstream processes	Opportunity for the development of smaller-scale continuous equipment or to retrofit legacy equipment into a continuous configuration	5.3, 6.1 and 6.2
Connector and tubing usability for sterile boundary maintenance, operating scale, standardization and long-term robustness unknown	Introduction of 'continuous-use grade' disposable components developed for CP at small to large scales to provide fit-for-purpose consumables across the whole downstream process	4.1 and 5
Need for hold/surge container design for inter-unit operations connections to receive continuous or 'packets' of liquid, plus monitor and control the process	Develop smart surge vessels/disposable bags with automated sampling, accurate sensors and appropriate inlets and outlets	4.2, 4.7 and 5.3
Need for engineering and automation definitions for plug-and-play networks and continuous control strategies	Establish new workflows in existing facilities and define future CP networks through robust integration of facility workflows into process development activities	6.4 and 6.5
Limited understanding of the business case for continuous downstream platforms	Opportunity to increase understanding of how continuous downstream processing, as opposed to end-to-end CP, impacts on process economics, realizing cost savings and expanding global market access	7
Need to develop real-time knowledge management	Develop tools to enable real-time data handling, analysis and reporting for process control and real-time product release	6.1, 6.2, 6.4, 6.6 and 8.2
No standardized material disposition and batching strategies	Define batch or lot assignments for continuous processes to support quality assurance trace windows of operation with proven productivity, raw material and consumable lots, and process deviation exclusions	6.4, 8.1 and 8.3

The predicted timelines for closing the technology gaps reflect the general opinion of the authors.

This white paper represents the views of industry members from biomanufacturers through to suppliers, and no recommendations are made about specific products. The paper aims to be agnostic to any supplier or specific technology.

"These flexible facilities may more easily react to market pressures by extending production times, while using a relatively inexpensive and flexible infrastructure..."

3.0

Process description

3.1 Introduction

The term 'continuous processing' has garnered a lot of discussion and debate because each end-user has a different interpretation of what the final solution will look like. While the desired end state for one company may be a continuous process where all unit operations are linked, at another company existing assets may require the linkage of only two or three unit operations together followed by batch operations. Further complications arise when considering the method of protein production in the bioreactor, whether fed-batch or n-stage perfusion. Also affected are the methods by which clarified cell-culture fluid is delivered to the downstream process for purification, such as batch harvest, pooling perfusate or direct perfusate connection. This section provides definitions of key terms and standardized process descriptions to gain industry alignment.

3.2 Definitions

As the field of continuous bioprocessing has matured, the associated terms and definitions have evolved. In today's environment, ambiguous language is a source of confusion and a lack of clarity. To illustrate this, definitions for key terms from a *White Paper on Continuous Bioprocessing*[7], definitions from the FDA and definitions from the BioPhorum end-user community are presented in Table 3. In this document, we will use the BioPhorum definitions.

Table 3: Definitions of key terms used in continuous bioprocessing

Term	White Paper on Continuous Bioprocessing (Konstantinov and Cooney, 2014)[7]	FDA Definition	BioPhorum Definition
Continuous step or unit operation	A continuous unit operation is capable of processing a continuous flow input for prolonged periods of time. A continuous unit operation has minimal internal hold volume. The output can be continuous or discretized in small packets produced in a cyclic manner.		A continuous unit operation has average inlet and outlet flows with fixed ratios, over a long operation time. The output can be continuous or discretized in small packets produced in a cyclic manner
Continuous process	A process is continuous if it is composed of integrated (physically connected) continuous unit operations with zero or minimal hold volumes in between. To emphasize that all the unit operations are continuous and integrated, such processes are also referred to as fully continuous or end-to-end continuous.	Material is simultaneously charged and discharged from the process	A process composed of all continuous unit operations running concurrently (physically connected) via direct connection or small-volume surge vessels
Integrated process			A process with a control system such that there is coordination and a physical connection between all unit operations
Semi-continuous process		Like continuous manufacturing, but for a discrete time period	A process consisting of both continuous and batch-fed operations
Hybrid	A process is hybrid if it is composed of both batch and continuous unit operations		A process made of both SS and SU components
Batch		A specific quantity of a drug or other material that is intended to have uniform character and quality, within specified limits, and is produced according to a single manufacturing order during the same cycle of manufacture. Batch refers to the quantity of material and does not specify the mode of manufacture	Same as FDA definition
Lot		A batch, or a specific identified portion of a batch, having uniform character and quality within specified limits; or, in the case of a drug product produced by a continuous process, it is a specific identified amount produced in a unit of time or quantity in a manner that assures uniform character and quality within specified limits	Same as FDA definition

3.3 Process linkages

Continuous bioprocessing, whether in a continuous or semi-continuous process, needs two or more unit operations to be linked to each other via a fluid flow. This linkage can be made in one of three classes of connection:

- Direct connection where the flow rate is conserved, and the stream composition is compatible with the subsequent unit operation (i.e. normal flow filtration)
- Direct connection with in-line conditioning where flow rates are compatible with the subsequent unit operation, but an adjustment must be made to the feed stream (i.e. pH or conductivity)
- Indirect connection through a surge vessel where flow rates are not compatible with the subsequent unit operation or decoupling unit operations is desirable to control minor process upsets.

In the last instance, surge vessels allow for simultaneous flow into and out of the vessel with good mixing characteristics. Surge vessels can also be used as a location for stream conditioning if in-line conditioning is challenging or not preferred.

3.4 Start-up and shutdown of continuous processes

Continuous processes are operated under constant conditions, but require time to reach this state. Due to lower operating flow rates and connected unit operations, the time to reach an equilibrium of continuous processes is expected to be significantly longer than batch processes. During this time, the generated material may not meet the critical quality attributes (CQAs) of the drug substance. Understanding the duration of this pre-production stage and the impact on quality is likely to be system-, process- and product-specific. Similarly, the shutdown phase of the continuous process, where the unit operations are transitioning from processing product to a washout phase, will see a transition that may have an impact on the product attribute profile. Data on the start-up and shutdown phases will be required to inform the start point of production campaigns and may support batch management within a continuous process. See Section 8.1 for a regulatory perspective on batch definition.

3.5 Reference process descriptions

This section will define the reference types of continuous and semi-continuous processes for several product mass flow scenarios. These reference processes are intended to illustrate the points being made throughout the remaining sections of the document. Protein production in the bioreactor and connection to the downstream process may be highly variable among the end-users; however, the paper will focus only on the downstream process and not where or how the purification feed stream originates from. As such, harvest unit operations are not considered, and it is assumed that feed is available at defined liquid and product mass flow rates into the system. To align with The BioPhorum First Edition Biomanufacturing Technology Roadmap, the reference downstream process descriptions provided refer to a standard mAb platform with the following steps:

- Protein A capture chromatography (bulk purification, bind- and elute-mode)
- VI (low pH hold)
- Anion exchange chromatography (virus and DNA removal, flow-through)
- Polishing chromatography (fine- and product-related purification, bind and elute, i.e. cation exchange, mixed mode, etc.)
- Viral filtration (VF) (nanofiltration, normal flow)
- Ultrafiltration (UF) and diafiltration (DF) (tangential flow).

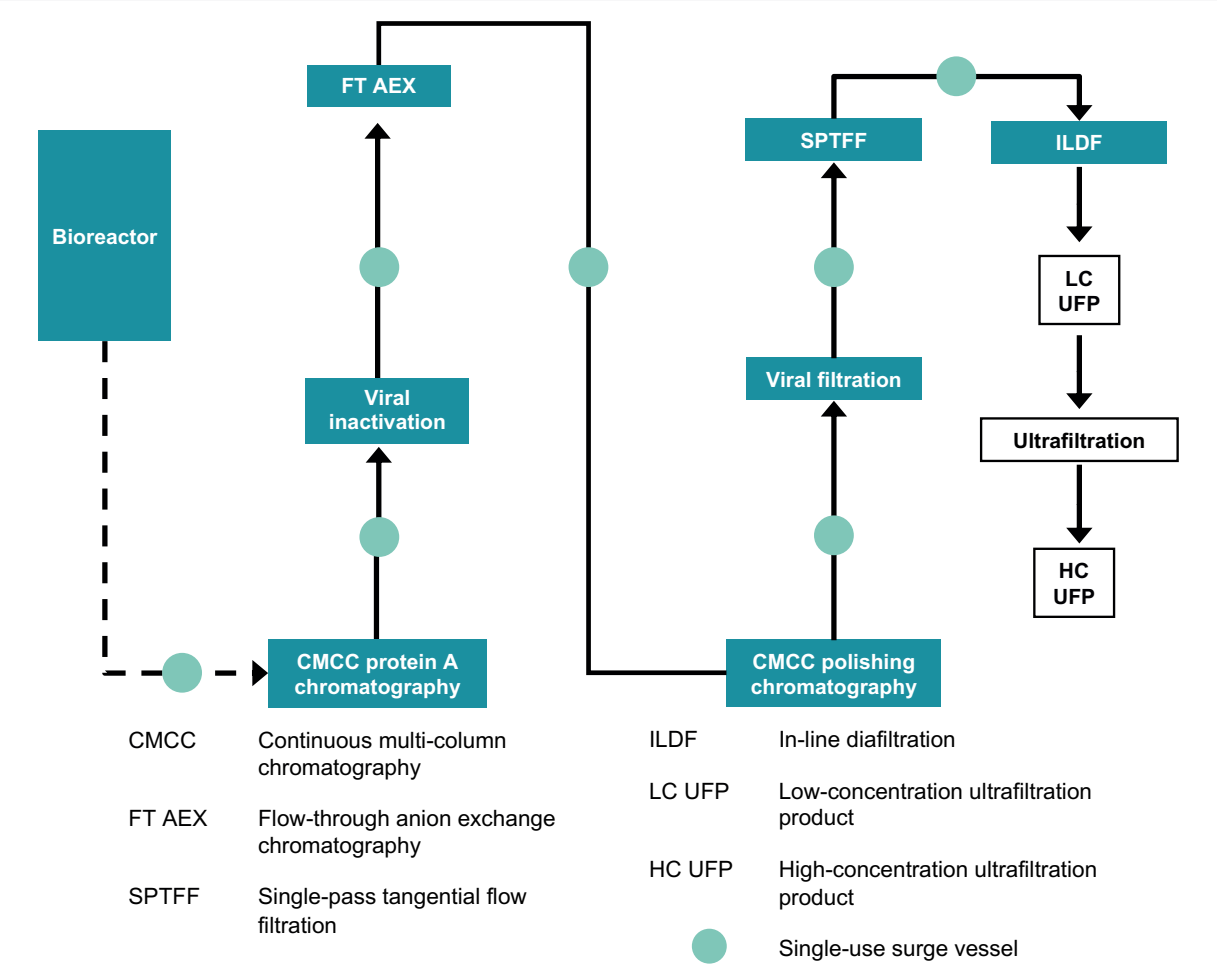
Although the trend towards higher modality diversity in the biopharmaceutical pipeline is growing at a rapid pace, the concepts presented here can be extended to other molecules on a unit operation basis.

3.5.1 Continuous process

A continuous process is shown in Figure 1, where each unit operation is separated by a surge vessel to decouple variable flows and stream composition from the neighboring unit operations. It is acknowledged that some platforms may directly connect unit operations without the use of surge vessels at one or more locations. Also, some platforms may run flow-through chromatography steps such as anion exchange chromatography (AEX) in either continuous multi-column chromatography (CMCC) or batch mode. It should further be noted that some platforms may rearrange the order or add/subtract to the number of processing steps; for example, placing the polishing step (cation exchange, mixed mode, etc.) before AEX.

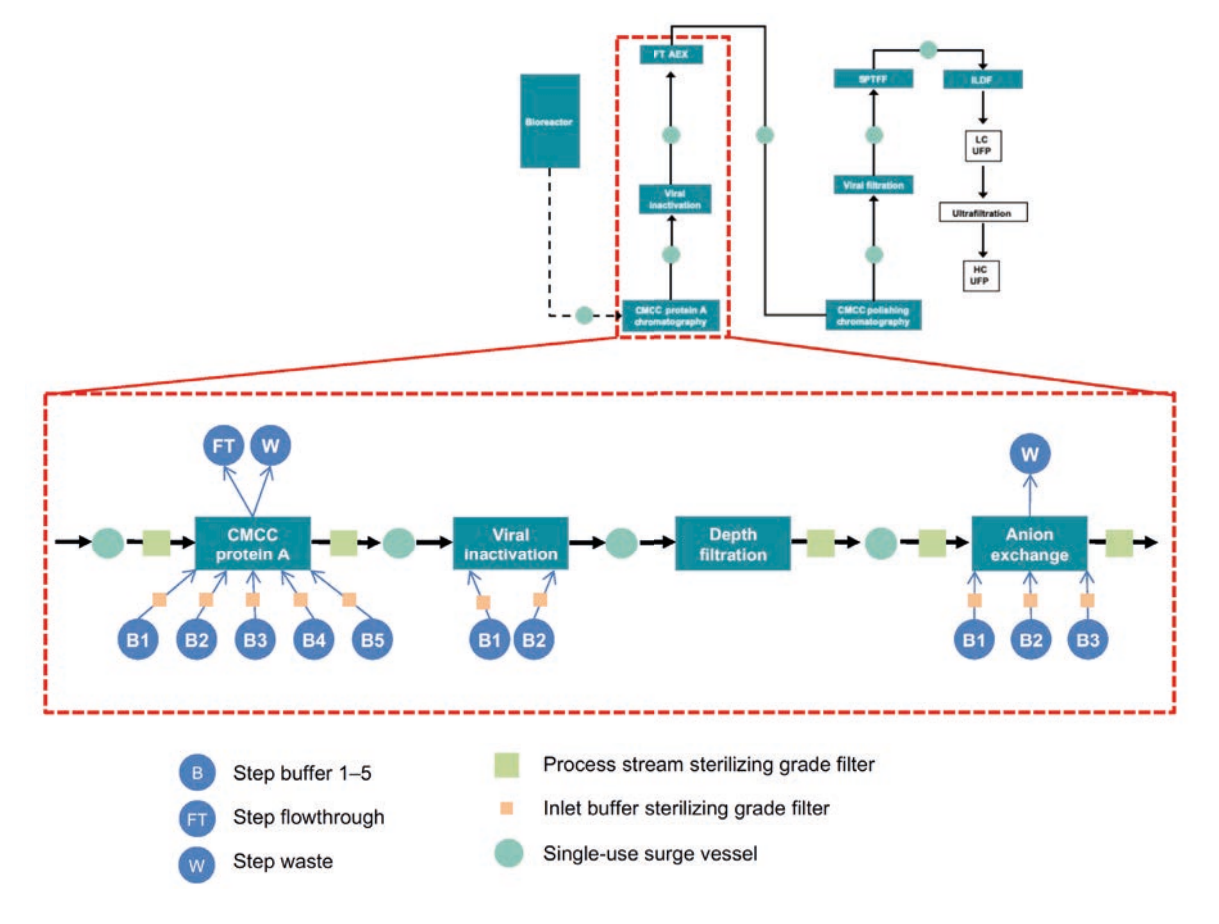
In Figure 1, all process steps from capture chromatography through to in-line diafiltration (ILDF) are carried out continuously. In this configuration, the process will be concentrated to an intermediate value and buffer exchanged through the ILDF module and is labeled low-concentration drug ultrafiltration product (LC UFP). The LC UFP may range in concentration from 20–80g/L depending on the efficiency of the single-pass tangential flow filtration (SPTFF) operation. The trend for mAb therapies is towards high-concentration formulations (>150g/L) to ease patient administration. In these cases, an additional off-line UF step may be required to reach the desired high-concentration ultrafiltration product (HC UFP). For clarity, many of the operational details are omitted from Figure 1, including filtration operations, external pumps, probes and automation.

Figure 1: High-level block flow diagram for a continuous process from a clarified cell culture fluid through to a typical monoclonal antibody platform process. Flow from one unit operation to the next is depicted through the use of surge vessels from the continuous multi-column chromatography capture step through to the low-concentration ultrafiltration product.



An expanded view of the first three unit operations is shown in Figure 2 and captures the sterilizing grade filters that may be required for operation with minimal risk of bioburden transmission within the process. Platforms may require bioburden reduction membrane filtrations around unit operations and buffer inlets for bioburden control and particulate reduction. Additionally, some platforms may require additional (depth) filtration steps. The system is more complex than shown in Figure 2, as there are no pumps, probes or automation shown.

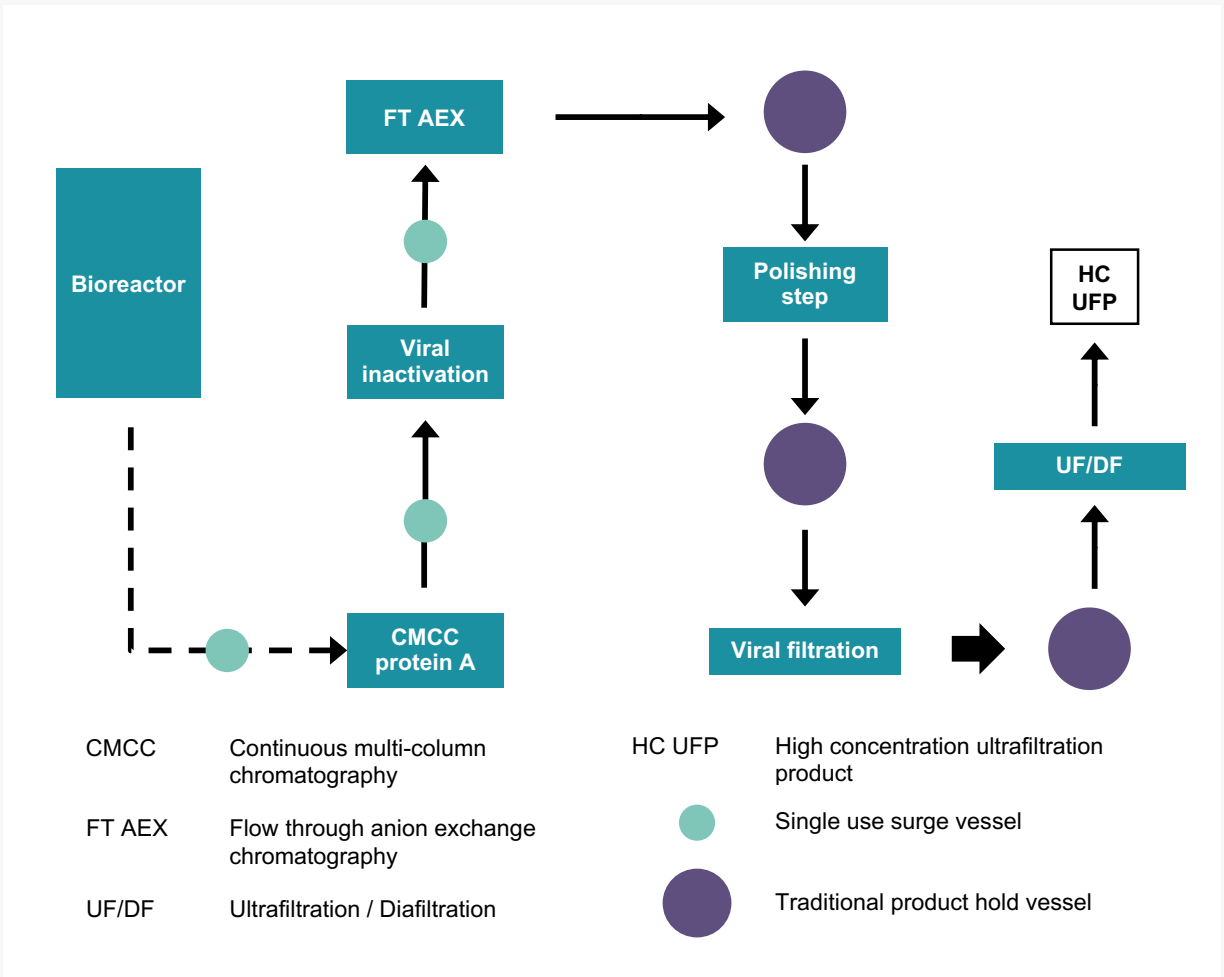
Figure 2: Detailed view of a partial continuous process showing potential buffer feeds and sterilizing grade filters on both the process stream and the incoming buffers. The system is even more complex, as there are no pumps, probes or automation shown.



3.5.2 Semi-continuous process

It is not possible to define a single semi-continuous process, as each end-user may have different solutions to the challenges faced by their network of facilities. End-users are using CMCC to increase the productivity of the capture step. As an example, the initial three unit operations are linked for discussion and initial modeling within this paper, see Figure 3. Protein A is combined with continuous VI and AEX with the use of surge vessels. After the linked unit operations, product is pooled into a traditional product hold vessel. From there, the next unit operation is performed in a batch manner up to the HC UFP. Note that some semi-continuous platforms may link to other unit operations in the process flow and may directly connect unit operations without the use of surge vessels at one or more locations in the process. Hybrid processes allow for a large number of process configurations.

Figure 3: High-level block flow diagrams for a semi-continuous process with the Protein A, viral inactivation and anion exchange steps linked. After the anion exchange step, the product stream is pooled before and between subsequent unit operations. The use of larger volume product hold vessels will be required to accommodate the increased volume of the stream.

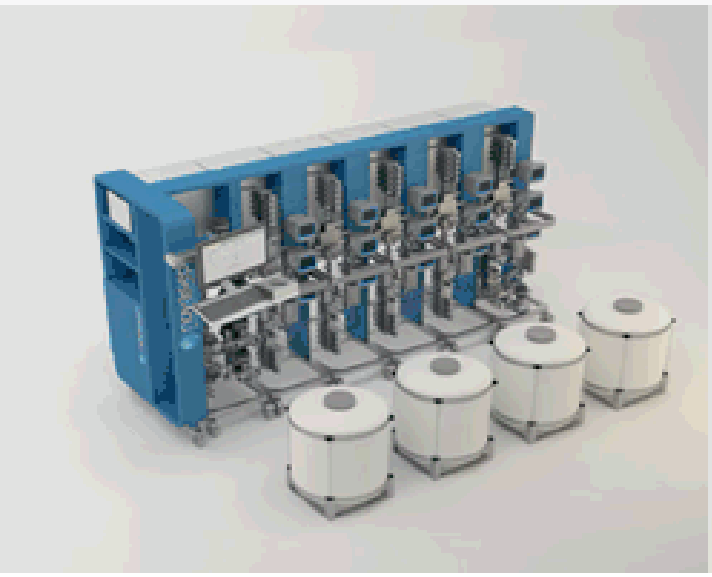


3.5.3 Detailed scenario definitions

The size and scale of continuous downstream unit operations are determined by liquid flow (i.e. determining pumping and column diameter requirements, etc.) and mass flow (i.e. determining resin volumes and cycle times, etc.) into the process or step. Several scenarios exist that will help to characterize continuous processes for both economic and operational feasibility. Most scenarios fall within an order of magnitude for liquid flow (0.4–4.3L/min) whereas the product mass flow rate into the system has a much broader range (0.7–65g/min). These scenarios are summarized in Table 4 and are a basis for the initial modeling work discussed in Section 7.

Table 4: Liquid and product feed flow scenarios to continuous and semi-continuous downstream bioprocesses for different bioreactor configurations. The number of bioreactors and length of culture are not considered and flow rates have been determined by assuming a harvest cadence

Bioreactor configuration	Titer/productivity	Liquid flow rate (L/min)	Product flow rate (g/min (kg/day))	Notes
2,000L SU intensified	10–20 **g/L**	0.46–0.69	4.6–13.9 (6.6–20.0)	Harvest cadence 2–3 days
12,500L SS	7.5–15 **g/L**	2.9–4.3	21.7–65.1 (31.2–93.7)	Harvest cadence 2–3 days
2,000L SU perfusion	2–4 **g/L/day**	1.4–2.8	2.8–11.1 (4.0–16.0)	1–2 vessel volumes per day exchange
500L SU perfusion	2–4 **g/L/day**	0.35–0.69	0.7–2.78 (1.0–4.0)	1–2 vessel volumes per day exchange



4.0

Technology gaps

The following gap analysis explores the areas where technology improvements are required to attain continuous and semi-continuous DSP. The review will start by assuming a continuous feed stream and product titer from perfusion or staggered batch cultures supplied from harvest.

We will not consider harvest technologies such as tangential flow filtration, alternating flow filtration, depth filtration, centrifugation, inclined settler, acoustic wave, spin filters or other techniques for cell separation. The predicted timelines for closing the technology gaps reflect the general opinion of the authors.

4.1 Bioburden control

Bioburden control is a requirement for all biomanufacturing processes. Control strategies are well established for traditional batch biomanufacturing using various approaches. These include sterilizing grade (0.2µm) filtration of process streams and buffers, low-temperature processing, steam-in-place (SIP) procedures, sterilization of assemblies and devices in an autoclave, and clean-in-place (CIP) using chemical sanitization with sodium hydroxide or other sanitizing fluids, such as ethanol and benzyl alcohol. A batch process downstream unit operation is typically operated for <24 hours followed by cleaning and sanitization for bioburden control. Due to their short nature of operation, most steps are considered 'low bioburden'. In continuous processes, operating times could be from a few days to weeks, giving a much greater time for any low-level of bioburden to proliferate. Additionally, the location of the sterile boundary around the upstream bioreactor in an integrated end-to-end process needs to be defined. Therefore, bioburden control strategies need to be considered for the specific risks and opportunities continuous DSP presents and one-size-fits-all solutions may not be feasible.

The risks and opportunities vary depending on the specific controls and exposures in each continuous system. Cleaning and sanitization processes that are adequate for batch processing may not be acceptable for continuous, as bioburden levels may exceed acceptable limits after processing for extended times. Basic environmental control is achieved by adopting closed-processing and sterile-wetted components in the system. Additional control can be achieved by designing flow paths with consideration of cleanability and reducing bioburden harboring areas by preventing uneven flow distribution or air pockets. Aseptic control of all feed streams into the continuous process will be required to mitigate the risk of further bioburden contamination. The use of bioburden reduction filters upstream of the unit operation, as shown in Figure 2, will provide aseptic control; however, prolonged operation of these filters could lead to blinding or growth through the filter. This adds complexity to the process as regular replacement or CIP requires interruption or diversion of the process and feed flows through surge tanks or duplicated unit operations. As the technology matures and robustness of closed, aseptic processes increases, the need for most process-intermediate filtrations for bioburden control is expected to reduce.

Discontinuous-mode unit operations within a continuous process, such as bind-elute chromatography column steps, easily lend themselves to the inclusion of a sterilizing CIP step to control bioburden within the unit operation. Continuous-mode unit operations, such as SPTFF and VF, will require their own risk-mitigating strategies. There is the additional need to consider product CQAs as well as bioburden control.

For example, long-term use of the same SPTFF membrane may lead to membrane fouling, which could compromise optimal process operation and support microbial growth. Having a robust strategy for allowing switch-out or cleaning of absolute filters and tangential flow filtration membranes without disrupting process operations and product CQAs will be key to supporting continuous biomanufacturing over extended periods. These strategies need to be developed as new processing technology is adopted into biomanufacturing processes.

The bioburden controls for continuous processes using SU technologies are no different from SS systems. However, the construction materials affecting their mechanical strength, chemical and thermal stability, and resistance to environmental factors become an additional consideration. These will impact on the process design concerning sanitization and preventing the ingress and accumulation of adventitious agents. Defining the cleaning regime, change-out frequency or bioburden control strategies to mitigate against the loss of sterility are areas that have yet to be clearly defined. The currently identified gaps are given in Table 5.

Table 5: Gaps in bioburden control

Gaps or perceived gaps	Importance for continuous processing	Expected implementation date			
		Current	2022	2025	2030
Absence of in-line, real-time bioburden monitoring for prolonged bioburden control	H				
Lack of connectors able to connect and disconnect aseptically or perform multiple connections/disconnections aseptically	M				
Need for sensors in closed flow paths that are pre-calibrated, can withstand gamma irradiation or ozone, shipping and storage before use	H				
No easy means to integrate stream sampling for bioburden contamination	H				

Key:
L = Enhancement of capability, M = Less efficient workarounds available, H = Must have for operation with high benefit

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The weak points in maintaining a very low bioburden in an integrated process are the junctions between unit operations, feed lines and surge vessels. In SS facilities, these connections have standardized on tri-clover sanitary fittings, where connections are made under septic conditions and then sanitized through SIP or CIP once they are functionally closed. For SU plastics, there is a plurality of vendor-specific sterile connector designs. This either restricts processing equipment to vendor-specific technologies or introduces complexity through the introduction of linking connectors. Current aseptic connecting devices have high validated sterile connection success rates when used correctly. However, some are better than others in their pressure rating and chemical compatibility.

Moreover, there are few, if any, sterile connectors on the market that can both connect and disconnect aseptically with the same device and few choices for aseptic disconnectors. Therefore, manifolds are required for repeated connections during a long-term continuous process, increasing consumable costs. A lack of standardization on aseptic or closed connection/disconnection technology still makes tube welding attractive. However, welding takes longer than using an aseptic connector and there is the potential for integrity loss or flow path blockages, additional capital investment needs and equipment limitations when welding tubing full of liquid. Standardization on a universal sterile connector technology that is multi-use, robust, simple to operate and scalable to service small-volume personalized and stratified medicine markets, as well as large multi-drug facilities, is required by the industry to support the adoption of CP.

4.2 Continuous multi-column chromatography

Continuous chromatography offers the promise of process intensification through increased resin use (per cycle and batch), reduced asset footprint demand, reduced buffer consumption (L buffer/g purified protein) by overloading the columns, and the ergonomic advantages of using smaller column sizes[7,8,9,10]. Continuous flow into and periodic flow out of the step (averaged over time) enables connectivity to neighboring unit operations for connected or CP. The continuous mode of operation can be applied across all chromatography steps in a process, either in a continuous or semi-continuous approach. As terminology differs across technology supply partners and biomanufacturers, this section assumes CMCC, simulated moving bed, periodic counter-current and twin-column chromatography are functionally equivalent.

4.2.1 Continuous chromatography systems

Process development continuous chromatography systems have been available for some time for bench-scale operations, though the more recent emergence of process-scale skids has promoted the concept of continuous chromatography as being plausible for GMP production. Such technologies are available and in terms of technology readiness, most suppliers are focused on delivering formats to service 2,000L SU bioreactors, whereas opportunities exist to provide formats designed to process 12,000L SS bioreactors. The currently identified gaps are given in Table 6.

Table 6: Gaps in continuous chromatography systems

Gaps or perceived gaps	Importance for continuous processing	Expected implementation date			
		Current	2022	2025	2030
In-line sensor recalibration during a run, and verification procedures for continuous operations, are not well established in GMP operations	M				
Non-standard validation packages for CMCC steps compared to batch processes (i.e. viral clearance)	H				
Need for development of scale-down models to demonstrate viral clearance	H				
Robustness of SU components for long-duration operation in SU skids is unknown (chemical and mechanical stability)	H				
Complexity of the equipment design compared to batch chromatography and system maintenance	L				
Additional training of operations staff for complex operations/equipment	M				
No standardized system cleaning and sanitization procedures for long duration operation	M				
Potential bioburden contamination from chromatographic media and no bioburden control strategy	M				
No methodology for accurately closing mass balances when product pools are not collected	M				
Impact of column-to-column variability in packing height and efficiency on separation performance when loading multiple columns is unknown	L				
No detection method of multi-column performance deterioration and real-time replacement strategy	L				
Strategies for handling non-steady states during start-up and shutdown periods not developed	M				
No SU DSP equipment available for 12,000L-scale operation	L				
Impact of feed variability on column performance (loading and separation) is unknown	M				
Need for streamlined integration into manufacturing execution systems with linkages to surge vessels and/or other unit operations	H				

Key:
L = Enhancement of capability, M = Less efficient workarounds available, H = Must have for operation with high benefit

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4.2.2 Continuous Protein A chromatography

A range of process economic analyses in the literature shows that the greatest impact on COG is in using CP in bind and elute unit operations[11, 12, 13]. For Protein A antibody capture, where the expectation of step performance is bulk purification of cell culture fluid through affinity interactions with the capture ligand, characteristics such as peak asymmetry and the number of theoretical plates in the column are less important to the separation performance. As a result, the change to CMCC is perceived as a lower risk for Protein A than some other chromatography modalities. Hence, many biomanufacturers have started investigating CMCC for the Protein A step with positive technical performance results[14]. With the emergence of GMP-scale CMCC systems, CMCC Protein A chromatography is expected to be in use before the connecting of Protein A chromatography to other unit operations for semi- or continuous bioprocesses, due to the reduced burden of systems and control integration. A specific gap associated with CMCC Protein A chromatography is the sensitivity of the proteinaceous ligand. If a cycle were interrupted, ligand lifetime could be affected through two mechanisms:

1. Increased contact time of a column to the caustic solution, increasing the rate of degradation of the ligand
2. Increased contact time with the feed solution and increased rate of column fouling.

Although similar failures may occur in batch systems, corrective action and method restart is more easily and rapidly applied to a single column rather than multiple columns in different steps of the process.

4.2.3 Continuous cation exchange chromatography (and other high-resolution bind and elute modalities)

Unlike Protein A, high-resolution chromatography steps depend on the column to provide fine separations of product-related variants (isoforms, aggregates, etc.). In this instance, column packing, buffer gradients and fine fractionation may be required to meet the demands of the step. Also, overloading the column may negatively affect the peak shape and ultimate separation performance of the step. For these reasons, the barrier to implementation of CMCC is increased. Specific gaps associated with fine separations of this type are listed in Table 7.

Table 7: Gaps in continuous cation exchange chromatography

Gaps or perceived gaps	Importance for continuous processing	Expected implementation date			
		Current	2022	2025	2030
Some manufacturing GMP-scale CMCC systems can perform gradient elution, others need development	M				
Peak cutting on some systems may be more difficult than batch systems as system hold-up/ dispersion may not be optimized post-column, as it is in traditional batch chromatography skids	M				
Variability in the feed stream may impact on separation performance affecting the quality and purity of the product	H				

Key:
L = Enhancement of capability, M = Less efficient workarounds available, H = Must have for operation with high benefit

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4.2.4 Continuous anion exchange chromatography (and other flow-through modalities)

Many flow-through steps in protein purification scavenge impurities from the feed stream and, in most cases, there is little benefit from loading columns in series. For these reasons, CMCC skids for flow-through steps may not be needed for continuous DSP. The emergence of membranes, absorbers and other monolithic supports with low residence times could enable rapid cycling on a standard chromatography skid. Hence, any potential switch-out of columns or membranes remains a challenge in a continuous process.

Vendors have created CMCC configurations to allow for running flow-through steps on the same skid as a preceding or subsequent bind and elute modality. However, these methods may become burdensome to execute based on limitations imposed by switch times, matching peaks and method complexity. The means of validating such an approach in GMP is unclear.

4.2.5 Continuous flow-through processing

An emerging field for continuous bioprocessing is the concept of continuous flow-through processing (CFTP), which shows great potential to simplify mAb purification, increase productivity and decrease costs. CFTP relies on capturing all impurities in flow-through mode while passing the mAb unbound. This approach approximates a more continuous product flow as the product never sees a bind and elute chromatography column. CFTP promises to reduce the process footprint, the number and volume of aqueous buffers and simplify controls, thereby lowering capital and operational costs while increasing productivity. Further advantages for host cell protein capture-based methods are accelerating biomanufacturing, reducing process footprint and operational expenses, reducing the cost of adsorbents and enabling SU and recyclable formats[15]. The CFTP concept is inherently compatible with other continuous, flow-through processing strategies, including the tubular reactors for VI and SPTFF discussed in Sections 4.3 and 4.5 respectively. A lack of commercially available peptide ligands or other resins for host cell protein removal is a current limitation to the realization of CFTP processes. Advances in this field are currently under development at the Biomanufacturing Training and Education Center (BTEC) at NC State University[15].

4.2.6 Continuous chromatography media

Most continuous chromatography that is documented in the literature refers to the use of chromatographic resin, though this paper considers other base matrices such as membranes, non-woven fibers, monoliths and other bead alternatives. The gap analysis can be considered in the context of all such media forms. Early continuous chromatography used resins made for batch processing, but new resins optimized for continuous chromatography are commercially available. Optimal media must demonstrate:

- Base matrix to enable high flow rates and low back pressure (critical for higher resolution needs in ion exchange polishing steps)
- Minimized sensitivity to column overloading
- CIP stability to support sanitization and high cycle numbers
- Sharp elution volumes despite increased bead loading
- High binding capacity.

4.3 Continuous or semi-continuous flow into a viral inactivation process

Virus inactivation is considered one of the two orthogonal steps required for virus safety in a biotherapeutic production process from mammalian cell culture. For the low-pH VI step in a batch process, the Protein A column is cycled two to four times and the eluates are pooled in a holding vessel. For inactivation, titrant is added to the pooled material through multiple manual acid additions to reach the inactivation pH set point. Each addition is pumped in and the system mixes to ensure homogenization. A sample is then taken and the pH is measured off-line using a calibrated probe. Some platforms transfer the product into a second hold vessel to ensure homogeneity and to mitigate the risk of hanging drops. Finally, the homogenized product is held in the vessel for a target time to ensure inactivation.

Adjusting this process to be automated and continuous poses several challenges and this has led to multiple approaches to performing continuous VI. These approaches can be loosely categorized as tubular reactor, column-based and stirred tanks. These fundamentally different approaches are discussed in sub-sections 4.3.1, 4.3.2 and 4.3.3, which include a gap analysis for each approach.

4.3.1 Continuous viral inactivation in a tubular reactor

The tubular, plug flow reactor approach for low-pH VI continuously processes eluates from the Protein A column and provides a solution close to the ideal of a continuous flow of product[16, 17, 18, 19]. Eluate-pH can be adjusted directly to the hold-pH via an in-line static mixer as it leaves the Protein A column. From here, the eluates are passed through a tubular reactor that ensures that the product has the target inactivation time. A plug flow profile from the tubular reactor is desired but, in practice, dispersion will always be present. The challenge is to design a tubular reactor that decreases axial mixing in the laminar regime and provides little pressure drop. Operating in the turbulent regime will result in an unmanageable length for the typical 60-minute hold time and in a high-pressure drop across the unit operation.

Several publications have compared the residence time distribution efficiency across multiple tubular reactor designs[16, 17]. After a thorough comparison for each design, the one that provided the most efficient performance and that can be robustly scalable is the 'jig in a box' design[16, 20]. The advantages of the 'jig in a box' are that it provides a rigid flow path, avoids a variation in incubation volume ensuring precise incubation times, and reduces run-to-run variability. The 'jig in a box' is modular, scalable and can be made from SU materials.

Using a tubular reactor has many advantages over alternating hold-bags, including operational simplicity, a smaller footprint, savings in time and effort for cycle-to-cycle cleaning, the ability to use a fully disposable incubation chamber, the ability to accommodate integrated processes and possibly truly continuous end-to-end manufacturing[16]. The effective inactivation time in a tubular reactor does not just depend on its incubation chamber volume and flow rate, but also on minimizing dispersion effects through efficient reactor design. If dispersion is present and not accounted for, then a fraction of the product stream will be underincubated, which may potentially impact on viral clearance, or over-incubated, which may impact on product quality[21, 22]. To address this, a 'minimum residence time' approach is employed[22] to give a target time that ensures >4 log reduction in virus[21]. This assumes a constant flow scenario, which can be achieved if the Protein A elution is pooled before VI and then pumped at a fixed flow rate. Any pause will result in an additional inactivation time. This will also allow the use of an extra pump to decouple Protein A elution from VI as a standard approach for emptying the tubular reactor, if required. Extreme variations in flow rate are not a big risk as a flow meter should be used to monitor and/or control flow rates. The currently identified gaps are given in Table 8.

Table 8: Gaps in continuous viral inactivation in a tubular reactor

Gaps or perceived gaps	Importance for continuous processing	Expected implementation date			
		Current	2022	2025	2030
No simple and robust method for obtaining representative samples from the process	H				
Dispersion in tubular reactors can lead to an under-inactivation time of the virus or over-exposure time for the protein	H				
Need for scale-down models for tubular reactors and potential to run inactivation studies at production scale	H				
Need for accurate (absolute value and fast response) pH monitoring and dose control to balance VI against product stability	H				
No standardized, validated VI strategy	H				
Spiking virus into a pool may inactivate the virus before introduction into the tubular reactor	H				
Need to determine worst-case conditions that may deviate from the process center point	H				
How to mitigate the impact of fluctuations in conductivity and protein concentration, coming from preceding Protein A column is unknown	H				
Requirement for commercially available GMP and scalable solutions for the tubular reactor	M				

Key:
L = Enhancement of capability, M = Less efficient workarounds available, H = Must have for operation with high benefit

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If the flow rates of the acid or product elution are perturbed, there is a risk that the desired pH target is not achieved. Mitigation for pump excursions should be considered in the design space. The variance of the curve, the average residence time, and the minimum residence time must be equivalent for multiple flow rates. For SU disposable devices, an understanding of leachables and extractables is required (see Section 5) and for closed systems the device must pass an integrity test.

To operate a continuous commercial GMP process, a validated viral clearance strategy is needed, either at scale or in a proven scale-down model[21, 23]. It should be noted that when the product feed is pooled and homogenized before entering the VI step, the complexity of viral clearance validation reduces, but this is offset by the increase in complexity of the integrated downstream process. Obtaining representative samples for monitoring VI could be done by adopting the split-stream approach.

Industry partners have developed this approach where a pump is automated to take a split stream that represents a virtual pool. However, this split stream will not capture variations in product quality over time and mitigating against pump excursions should be considered.

4.3.2 Column-based strategies

The main challenge of the plug-flow approach is ensuring that all the product stays within the reactor for the entire hold time. This has led to the evaluation of column chromatography approaches, including performing the low-pH hold while the product is bound to either a Protein A[24] or cation exchange column[25] or adding a size-exclusion chromatography (SEC) column to the process[8]. In the later SEC-based technique, dispersion in the column can be assessed through classical pulse-type injections to the column in question. Table 9 outlines the identified gaps.

Table 9: Gaps associated with column-based strategies

Gaps or perceived gaps	Importance for continuous processing	Expected implementation date			
		Current	2022	2025	2030
Bind and elute chromatography step productivity not optimized for on-column, low-pH hold	L				
Protein A resin not designed for on-column VI strategies	L				
No means to prevent particulates from fouling the column	M				
SEC media not developed to prevent deterioration in packing integrity or fouling when used as a VI step	M				
No simple approach for inclusion and scale-up of additional SEC column	L				
No standardized, validated VI strategy	H				

Key:
L = Enhancement of capability, M = Less efficient workarounds available, H = Must have for operation with high benefit

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"To operate a continuous commercial GMP process, a validated viral clearance strategy is needed, either at scale or in a proven scale-down model."

4.3.3 Virus inactivation using alternating biocontainers or continuous stirred-tank reactors

This approach could be considered to be the most direct transfer of the current batch VI operation into an integrated continuous process. In common with the batch process, a few Protein A elutions are pooled and processed together, but this approach increases complexity. More than one container is required and, for many scenarios, the intervals between column elutions may be shorter than the requisite low-pH hold time.

A two-tank, SU solution has been designed and commercialized using the failure modes and effects analysis approach. Each biocontainer has a single-use mixer (SUM), pH probe to monitor acid addition (inactivation) and then base addition (neutralization) and is connected through a control system that directs the fluid flow. Drift of the critical pH probe is mitigated through the initial selection of a robust probe and applying a periodic single point calibration. The two SUMs are used alternately and asynchronously. The complete VI process, including feed pooling, acidification, hold and neutralization takes place in that mixer. While the whole VI process is occurring in the first SUM, the second SUM receives the next tranche of eluates from the Protein A column until the first VI cycle is complete. Once the first SUM is ready to receive more Protein A eluate, the second SUM performs the whole VI process.

In this design, very little residual material is expected to be retained and the likelihood of product being retained beyond two cycles is very low. This is deemed acceptable as most products are expected to be stable over two cycles of VI. Liquid additions are made sub-surface to prevent splashing, and mixing is ensured using a zero-hold-up recirculation loop. Confirmation of the inactivation of adventitious agents by this approach was shown with a model organism, demonstrating that the system effectively inactivates viruses at low pH and no hanging drops or hold-ups re-infected the treated material. Finally, because the hold tanks have to be emptied as quickly as possible to continue processing incoming elutions, the next unit operation has to either operate with a high flow rate (and then pause) or have a surge tank to normalize the flow rate. Table 10 outlines the identified gaps.

Table 10: Gap analysis on virus inactivation using alternating hold-bags

Gaps or perceived gaps	Importance for continuous processing	Expected implementation date			
		Current	2022	2025	2030
Potential for hanging drops to contaminate subsequent bags	M				
Process automation requires a continually immersed pH probe, which must be robust, accurate and precise	H				
When acid and base are added sub-surface, the potential for back-mixing and pH deviation during the hold step may occur	M				
No capability for periodic sanitization	M				
J-tubes are not available for SUMs; liquid addition from the top of the biocontainer is likely to cause splashing and foaming	M				
SU system does not fully drain; potential to over-incubate protein	M				
Scalability of the system is not proven	M				
No standardized, validated VI strategy	H				

Key:
L = Enhancement of capability, M = Less efficient workarounds available, H = Must have for operation with high benefit

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Although the commercially available system is more complex than the current batch process it mimics, the route to viral clearance validation may be simpler as it replicates the current approach. Introduction of automation, once validated, should further help mitigate process control risks for this design approach. A further advantage is that the development of alternate operating scales can easily be achieved using the current approach for scaling batch processes. Furthermore, the discussed system demonstrates the current process understanding and the approaches that can be adapted for future continuous biomanufacturing. It is also amenable to further development, such as the inclusion of a flow path for periodic sanitization or a pre-VI sterilizing filter[8].

4.4 Continuous viral filtration

Virus filtration is a robust method for virus clearance in downstream applications and is amenable for use in a continuous process, as it is a flow-through process and can be run in constant-flow. To date, however, there is little data published on this subject[26]. Intensified processing has created additional motivation to further understand the technical and operational drivers in establishing a virus filtration step in a continuous mode and address any challenges towards manufacturing implementation. The translation of a continuous process with multiple linked unit operations into discrete unit operations for virus clearance testing poses a significant challenge[27].

Section 4.4 is organized according to the filter lifecycle and states the gaps, listed in Table 11, that need to be addressed for the use of virus filters in continuous DSP. The lifecycle covers the installation of the filter unit, the sterilization procedure if the unit cannot be sourced in a fully sterile and self-contained fashion, the pre-wetting and optional pre-use integrity testing, buffer preconditioning, processing and product recovery, post-use integrity testing and, finally, discarding the filter unit. This unit operation is usually after a chromatographic capture and at least one chromatographic polishing step, as shown by the figures in Section 3.

Table 11: Gaps in continuous viral filtration

Gaps or perceived gaps	Importance for continuous processing	Expected implementation date			
		Current	2022	2025	2030
Need for scale-down model validation equipment	H				
Virus stability impacting on long time duration loads in spiking studies	M				
Need for filter installation and pre-use integrity testing strategy for CP	M				
Impact of process variations on virus retention not understood in CP	H				
Impact of transmembrane pressure peaks on viral clearance due to feedstock variation not known in CP	H				
Limited information on continuous bioprocessing for defining worst-case conditions for testing	H				
Methodology for mimicking of total parvovirus load challenge in viral clearance study of CP not defined	H				
Need for post-use integrity testing strategy for CP	M				

Key:
L = Enhancement of capability, M = Less efficient workarounds available, H = Must have for operation with high benefit

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4.4.1 Filter installation

One mode for installing the new filters into a continuous process could be in parallel. One filter would be active and the other filter(s) placed on standby to allow immediate switching as each filter reaches capacity or is blinded. Either a bank of filters or a pair of filters with a surge vessel can be used for protecting the process when filter change-out is required. The filters should have a sufficient filtration area for use in the order of days rather than hours in batch mode. Filters in parallel will help minimize process interruptions but will require increased connectivity, modularity and sterility options. The availability of sterilized, connectable, SU functional filtration units could contribute to minimizing the downtime for sterilization or sanitization procedures. The filter preparation steps for pre-use integrity test (if required) and flushing will still be needed as in batch mode. A constant flow method with an appropriate pump is suggested for the whole operation.

4.4.2 Process variation

A thorough assessment is needed to determine the worst-case conditions for filtration in continuous mode. Not all of the batch-mode, worst-case criteria will translate to the continuous process scheme, but much can be learned from batch processing. Process perturbations without transmembrane pressure (TMP) on the virus filter need to be carefully considered if parallel filter lines are used. Historically, it has been shown that interruptions in the range of minutes to hours can decrease virus retention; however, this is now generally observed only in older types of small-scale virus filters[28, 29]. Robustness and resistance to repeated pressure releasing have been shown in current major virus retentive filters.

"Virus filtration is a robust method for virus clearance in downstream applications and is amenable for use in a continuous process, as it is a flow-through process and can be run in constant-flow."

The feed constitution will be different in a continuous process into the filter compared to a batch mode. Depending on the mode of operation, the feed may consist of a series of protein elution peaks from a previous chromatography step, which might be more or less dispersed by consecutive processing[13]. Therefore, appropriate control strategies are needed to deliver constant viral clearance. Conversely, fluctuations in TMP, especially through variations in protein and contaminant concentrations, may lead to peaks that may exceed a filter's specific TMP limits. This will either be due to the maximum operational protein concentration being reached or the total throughput of the filtration step being concentration-dependent. Mitigation is by using a mixing vessel between the chromatography step and virus filter that 'homogenizes' the material and delivers a more constant feed stream and hence TMP profile.

Small surge vessels before and after the filtration step can reduce many concerns around fluctuating feed stream compositions, serve as an intermediate storage container to bridge downtimes, and compensate for unexpected pressure spikes from upstream operations. It may also introduce the requirement to clean or change-out these surge vessels for bioburden control.

A lower volumetric flow, where the convectional transport of liquid in the filter is competing with diffusional motion, can also contribute to a reduction in viral clearance. The physicochemical parameters, such as low-pH and high salt conditions, also play a role in viral retention[28]. However, there is evidence that virus retention by virus removal filters is largely able to tolerate feedstock variations, such as protein concentration, salt, conductivity and pH fluctuations[30]. Therefore, understanding the properties and interactions of the individual products with the filters will de-risk VF, but potentially complicate platform small-scale model approaches to capture these identified fluctuations during continuous VF.

Prolonged exposure also poses a new challenge. Some publications show that a filter might become potentially overloaded with virus and the retention might decrease when a certain number of parvovirus particles per filter area is exceeded[31]. Typically, this is an artifact of viral clearance studies and most bioreactor contaminations would not overload the filter. To create a realistic assessment of the VF capacity, it is imperative to challenge it with realistic levels of (parvo) virus over the planned period of continuous operation in a viral clearance study.

4.4.3 Scale-down model validation

The small-scale model should be designed to account for process-specific issues. An additional challenge for the model used for virus clearance for continuous process validation is the virus spiking strategy and stability over the extended durations. Although viruses, such as minute virus of mice and other parvoviruses, may be sufficiently stable for several days, such study designs might not be suitable for a continuous spiking using a more suitable panel of adventitious agents. The heterogeneity of the studies might increase the effort for all of the stakeholders. The filtration and virus clearance validation might be additionally complex when adsorptive pre-filters are used in the process. In general, these filters allow for higher volumetric throughput, which is desirable, but all of the filter exchange, preparation and qualification aspects will need to be extended. Validation of switching the viral filter mid-process will be required where such strategies are used for extended continuous VF operations. Finally, the design of current viral clearance studies, focused on batch processes, uses in-line adventitious agent spiking, which captures protein fluctuations but does not simulate fluctuations in virus. Here again, having an intermediate mixing vessel between the column and VF step would simplify the validation of a viral clearance study on a continuous process. Therefore, full-scale continuous processes may need to respect the validation-scale challenges and/or include/develop sensors appropriate for use. Further regulatory discussion on VF is found in Section 8.2.

4.4.4 Post-use integrity testing

The last step in the filter lifecycle is the post-use integrity test. Two key challenges need to be overcome: removal of viral filters under aseptic conditions in preparation for the integrity test and, in the rare event of a test failure, the affected batch needs to be clearly defined and risk mitigation methods applied.

4.5 Continuous ultrafiltration/ diafiltration

Although SPTFF technology is one of the key enablers for the successful implementation of continuous bioprocessing platforms, it also presents important challenges for widespread adoption. The concentration aspect of this unit operation has been taken up well and has gained acceptance within the industry in the past several years. Implementation and utilization have varied immensely based on the multiple publications and case studies shared at conferences even though concentration factors of up to 20x have been reported with the technology[32].

Recent developments in continuous DF unit operations further complement the single-pass technology portfolio and fills a technology gap for this operation. As single-pass DF can be performed using alternative designs and operating modes (i.e. co-current and counter-current), its operational simplicity will be the main technology driver from proof-of-concept to implementation at a commercial scale. Continuous DF modules also present new opportunities such as combining concentration and DF within a single module. This would support integrating drug substance with drug product operations under the final formulation umbrella, enabling a fully integrated end-to-end biomanufacturing platform. Both unit operations also enable easy integration and/or coupling of in-line, on-line or at-line analytics and smart sensors to better monitor, understand and control key CQAs (see Section 4.8).

“This would support integrating drug substance with drug product operations under the final formulation umbrella, enabling a fully integrated end-to-end biomanufacturing platform.”

Much effort needs to be invested in well-designed engineering platforms and/or flexible infrastructures that can easily incorporate and/or welcome customization as these unit operations mature and develop for the target applications and intended processing scenarios. Table 12 identifies the gaps in this area.

Table 12: Gaps in technology for continuous ultrafiltration and continuous diafiltration

Gaps or perceived gaps	Importance for continuous processing	Expected implementation date			
		Current	2022	2025	2030
Lengthy cleaning and regeneration cycles do not seamlessly fit into a continuous paradigm without having multiple membrane modules	M	Development	Manufacturing	Manufacturing	Manufacturing
Limited product configurations: membranes are currently offered in discrete module sizes as dictated by the manufacturing supply partners that may not fit the application or scale of interest	M	Development	Development	Development	Manufacturing
Scale-down model validation is difficult with the modular membrane format	H	Development	Development	Manufacturing	Manufacturing
Lack of user-friendly skids, which can be easily integrated into process automation systems	M	Development	Manufacturing	Manufacturing	Manufacturing
Current continuous DF modules may require 1.5–3x as much buffer as a batch process to achieve similar buffer exchange	H	Research	Development	Manufacturing	Manufacturing
Start-up and shutdown sequences may lead to an out-of-specification product until a steady state has been reached	M	Development	Development	Manufacturing	Manufacturing
Viscosity variation of protein products at high concentrations may make continuous UF difficult to implement in platforms that require high final concentrations (>100g/L). In some cases, high-concentration images may need to be further concentrated off-line of the continuous equipment train	M	Research	Development	Development	Manufacturing
Limited membrane chemistries and molecular weight cut-off offerings in continuous filtration cassette modules forces the user into a standard offering, which may limit applicability to new protein modalities (e.g. fragments, enzymes, fusion proteins)	L	Research	Development	Manufacturing	Manufacturing

Key:
L = Enhancement of capability, M = Less efficient workarounds available, H = Must have for operation with high benefit

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4.6 Continuous formulation

Formulation of ultra-filtered material is critical to the final drug substance image. Product and excipient concentrations must be tightly controlled to meet release criteria. As discussed in the previous section, platforms that rely on DF into the final matrix can rely on continuous DF methods for formulation. Currently, there are two available approaches for performing DF in a continuous process. Firstly, where the new buffer replaces the existing buffer as it is removed from the product stream by repeated iterations of SPTFF and in-line dilution. Secondly, through a hybrid of SPTFF, for feed stream concentrating to a small volume then conventional re-circulating DF for buffer exchange followed by a final SPTFF concentration. Platforms that require specific excipient additions after DF will require a form of feedback control on the actual product concentration to mix the product and excipient streams. However, if a control strategy for concentration is executed on the continuous UF and DF steps, excipient additions may become simpler with continuous stream-blending as you could streamline in-process monitoring and control for determining their concentrations. Reliance on automation and feedback control are ubiquitous in continuous manufacturing and are no more onerous on the formulation step. Therefore, an opportunity exists to further develop continuous formulation to optimize the efficiency of the buffer exchange process, support excipient additions and provide automated systems for integration into a continuous biomanufacturing process.

The formulation step, either as part of DF or the blending of excipients, may become a primary focal point for process analytical technology (PAT) integration if the ultimate goal is RTR and product disposition. At the end of this process, sensors or assays can be inserted for targeted release parameters such as pH, conductivity, bioburden, activity, concentration and aggregation. This will ensure product quality attributes, such as purity, are met as the formulated product is being collected into drug substance bags to de-risk product disposition and move towards RTR. Table 13 lists the identified gaps for continuous formulation and a discussion of the current gaps that need to be addressed for RTR is in Section 4.8.

Table 13: Gaps in technology for continuous ultrafiltration and continuous diafiltration

Gaps or perceived gaps	Importance for continuous processing	Expected implementation date			
		Current	2022	2025	2030
Lack of monitoring/controlling of excipient concentrations, especially in light of any changes in process flow rates	H				
Inefficient mixing with higher viscosity solutions	H				
Need representative sampling as input to testing to RTR assays and/or appropriate in-line sensors for PAT	H				

Key:
L = Enhancement of capability, M = Less efficient workarounds available, H = Must have for operation with high benefit

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4.7 Continuous buffer supply considerations

Buffer preparation and hold is a well-established practice for traditional batch processing. Buffers are made batch-wise in a buffer preparation tank and then 0.2µm filtered into a buffer hold vessel, which is connected to the unit operation. Typically, a buffer is required for one process step that lasts around one day or less, and then the next lot is processed one or two days later. This gives one or two days of 'idle' time to clean the buffer hold tank and lines, prepare another batch of buffer and transfer it to the buffer hold tank. In CP, process steps can last for weeks or months and buffer may be required to be flowing into that process step continuously; there is no idle time to turnaround and refill a buffer tank as in batch processing. Therefore, the traditional discrete batch approach will not work, and new methods of buffer preparation and hold are required.

Two methods of buffer preparation can be considered. Batch dissolution of buffer to the 1x formulation and continuous production of buffer from buffer concentrates. Two methods to reach the 1x buffer formulation from concentrates are in-line dilution of concentrate and buffer stock blending, where concentrates of individual salts are blended to give the final buffer composition

There are three buffer hold delivery methods to fill the gap of CP buffer supply. These are twin hold vessels, buffer hold tank top-off and continuous buffer production on-line.

The first continuous, uninterrupted method for continuous buffer supply would be to provide twin hold vessels, or A/B tanks. Buffer would be flowing from one tank and the second tank would be 'idle' allowing for turnaround and refilling as in batch processing. The advantage of this approach is that buffer preparation, transfer and scheduling are similar to traditional batch processing. The disadvantage is that twice as many buffer hold vessels are required, requiring roughly twice the capital and twice the facility footprint. The use of SU bags, which are commonly used for buffer hold up to 3,500L volume, instead of SS tanks can greatly reduce the capital cost impact, but a significant increase in footprint is still required.

The second continuous, uninterrupted supply of buffer option is buffer tank top-off. In batch processing, buffer tanks are often drained between refills to give batch lot segregation (firewall). If a lot of buffer has a quality issue (such as a raw material used that was not properly released), the impact of deviation is limited to that lot of buffer. 'Topping off' the tank has the advantage of always having sufficient buffer in the tank and minimizing turnaround times, and it is well suited to continuous biomanufacturing. The disadvantage is the discrete firewall around buffer lots is lost and the impact on tracing buffer quality needs to be defined. Many companies have developed approaches to allow refilling buffer hold tanks before they are empty to address this risk. These include appropriate controls around buffer preparation so that the quality risk become acceptably low (which could be used for some buffers in CP).

The third continuous, uninterrupted supply of buffer option is continuous buffer production with a buffer dilution skid, or buffer stock blending skid. The amount of buffer required for a continuous process run is very large, too large for SU bags and perhaps even for SS tanks. If the buffer can be highly concentrated, and then continuously diluted with water for injection (WFI) by a buffer dilution skid on-line to the use point, one could realize a continuous buffer flow that could use smaller and lower-capital SU bags for buffer concentrate hold, reducing buffer preparation activities. One disadvantage is that the number of relatively expensive buffer dilution skids needs to be determined and optimized. A second is that the buffer dilution skid would need to release test the buffers via PAT and, if the buffer was not in specification, divert it to drain. The process would, therefore, require surge bags to allow for the uninterrupted flow of buffer to the process skid during these temporary diversions to drain, adding additional complexity and costs.

Another approach could be a combination of the above methods, using a buffer dilution skid to feed buffer bags, whether A/B bags or buffer tank top-off. While the buffer dilution skid will not provide true CP, the advantage is that the buffer preparation rate can be much higher than traditional buffer preparation tanks. Therefore, the buffer hold tanks can be refilled quickly, which is an advantage given the challenges of buffer management in CP. An advanced approach for buffer dilution is buffer stock blending, which is being explored by the BioPhorum Buffer Preparation Team[33].

The following key areas of system design are involved in properly equipping a continuous process train:

- Number of continuous buffer preparation units
- Number of process buffers and use schedule
- System, header, instruments and cleaning cycle
- Buffer supply surge vessel size
- Process upset duration and resolution
- In-line release of buffer.

One preparation that is not conducive to the continuous buffer preparation system is formulation. The challenges for a continuous process continuous formulation buffer supply is based on the ability to reliably measure the final product stream out of the UF/DF unit operation. This information would then be used to meter the flow of bulk formulation buffer into the final bulk aliquots on the bulk fill line. It is unlikely that the risks/benefits will ever drive one to attempt continuous feed of final formulation buffer to the final buffer formulation unit operation based on the volume required and value of the final product.

The benefits of using a continuous buffer preparation and delivery system can be realized for large-volume buffer consumption over long periods. A reduced buffer hold tank footprint, as well as a reduced buffer preparation area requirement, are envisioned. Two to three continuous buffer preparation systems are likely to be a minimum requirement for a base-case plant. With increased purification capacity, numbers of units would be optimized through buffer delivery systems and optimized scheduling. Refer to Table 14 for the identified gaps for continuous buffer supply.

Table 14: Gaps in continuous buffer supply

Gaps or perceived gaps	Importance for continuous processing	Expected implementation date			
		Current	2022	2025	2030
On-line instrumentation for on-line buffer release	M				
Need a method for establishing on-line buffer bioburden load for continuous buffer preparation and supply to unit operations. Determine if filtration is required	M				
Need for cost-effective continuous buffer preparation, i.e. the number of units required for manufacturing	M				
No fully automated buffer and media preparation systems integrated into continuous production planning	L				

Key:
L = Enhancement of capability, M = Less efficient workarounds available, H = Must have for operation with high benefit

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4.8 On-line monitoring and instrument probe challenges

A control strategy for a continuous process is required to mitigate transient disturbances, such as input material attributes, process conditions or environmental factors[34]. PAT plays a critical role in ensuring the state of control of the process. CP allows for an increased control capability that was previously unattainable in batch unit operations pending the implementation of robust, reliable on-line monitoring methods. While many options exist for measuring quality attributes, these gaps focus on passive detection methods and probes that interface with the process streams (in-line and on-line) and less so on rapid analytical techniques, where the process impact is limited to sterile in-line sampling (at-line).

The FDA definition of 'in-line monitoring' is "measurement where the sample is not removed from the process stream and can be invasive or non-invasive" and 'on-line monitoring' is specified as "measurement where the sample is diverted from the manufacturing process and may be returned to the process stream"[35]. The success of these monitoring techniques will not only be highly dependent on the speed and accuracy of the probe or detection technique, but equally on the integration ability of sensors that support other key facets of the continuous paradigm. Specifically, these sensors must be robust over the extended period of continuous operation, maintain the closed (or functionally closed) process boundary and be compatible with all potential process buffers and cleaning techniques employed. See Table 15 for identified gaps.

Furthermore, different weights are assigned to these gaps based on SS or SU integration and must be holistically considered when evaluating potential solutions. BioPhorum has a team working in this area and an extensive list to guide the development of new monitoring techniques and specific attribute testing can be found in the In-line Monitoring/Real-time Release section of the first edition BioPhorum Technology Roadmap[36]. This section aims to identify the practical implementation gaps in operating and maintaining CP.

4.8.1 Instrument calibration

While sensor installation and calibration are not unique to CP, the strategy around preparing a SU system compared to a SS system is significantly different and places a different set of requirements on the flow path and sensors. Many SS facilities perform out-of-place calibrations and checks, allowing the flexibility of off-line, open calibration techniques. This is anticipated to be the case for instrument use in SS for CP application as well. SU applications, on the other hand, require the installation of a new flow path that includes the SU sensors. The installation occurs at the start of each run, which must not only cover the physical attachment of the disposable flow path to any non-disposable hardware skid, but also sensor calibration and/or checks. Less ideal is a single-point spot check of the sensors after installation, such as an initial buffer flush on the wetted path to ensure the sensors are measuring accurately. Sensor calibration strategy is noted as a gap with a high criticality for CP, with very few solutions readily available.

4.8.2 Instrument performance life and recalibration

Lifetime requirements of sensors used in CP will also differ from current batch expectations. Current batch sensors experience discrete exposure to process extremes and off-line redundant measurements are often taken when key process parameters rely on accurately calibrated sensors. These sensors are often cleaned weekly or monthly and occasionally involve a neutral storage solution to prolong their lifetime. In continuous process design, certain sensors measuring key process parameters may be exposed to process extremes for the entirety of the planned continuous campaign. Sensor compatibility and stability with the intended process fluids is paramount. For example, a pH sensor in place to monitor low-pH for VI may be subject to a process stream of pH <3.5 for the duration of processing. Not only does this exposure risk deposit formation and advance sensor aging (resulting in drift) but a lack of redundancy or planned off-line measurement risk these effects going unobserved. In these instances, sensor check, recalibration, reconstitution and change-out must all be viable options to validate and maintain process monitoring control, and must be done without process interruption while adhering to a closed or functionally closed operation. These gaps, together with concerns around initial calibration, are highly important for CP operations.

4.8.3 Instrument cleaning vs single-use

While the sensor must be compatible with the in-process fluids, non-disposable probes must also be wholly compatible with any cleaning techniques used. In SS scenarios, sensors are subject to the validated cleaning techniques while within the final process flow path, typically using cleaning agents through CIP and/or SIP. The need to endure harsh environments means these sensors are likely to be more robust and potentially better suited to CP. These SS sensors are then often left in place through multiple runs and may not require recalibration for between six months to a year. Often, SU components are less resilient and cannot be removed for calibration and cleaning. These sensors and their associated flow path constituents need to be chemically compatible with all calibration, check standards, cleaning solutions and preferably supplied in a pre-calibrated format. Even when gamma irradiation is an option for flow kits and incorporated sensors, often additional cleaning steps may be employed. In these cases, chemical compatibility must be assured. Some SU items make compromises between robustness, dynamic range and dry storage to achieve the cost-benefit of a SU component, while others maintain their high performance, but compromise on delivering a sterile, dry sensor for integration into a closed flow path.

The advent of continuous DSP creates greater challenges for the SU polymer-based sensors because of the long duration of operation and change-out strategies must be taken into account.

Defining the end-user requirements of disposable sensors with respect to measurement accuracy, sensor lifetime and flow path lifecycle will help vendors deliver appropriate sensors that are economical for 'continuous-use grade' SU biomanufacturing. Current examples are sensors used for in-line buffer measurements or indirect measures of product quality/titer by spectroscopic methods. Whereas technology to determine in situ bioburden or direct product quantification has yet to be developed. Moving to RTR of continuous processes, several analytical challenges as well as the need to develop appropriate connectors and probes for in-line, real-time interaction between the analytical instruments and the disposable flow path. However, some of these may be mitigated against by greater use of 'quality by design' strategies[37] using enhanced/SU-compatible versions of current measuring systems. Predicted improvements in product quality control in continuous biomanufacturing processes are yet to be understood and may help streamline the types of analytics required. The gaps in this area are summarized in Table 15.

Table 15: Gaps in on-line monitoring

Gaps or perceived gaps	Importance for continuous processing	Expected implementation date			
		Current	2022	2025	2030
No sensor calibration or check while in-service and no change-out possibility during process operation with closed or functionally closed systems	H				
Sensor compatibility with calibration standards and process fluids is unknown	M				
Limited knowledge on sensor chemical/radiation stability for the duration of the process	H				
Limited sensors for product attribute measurement	M				

Key:
L = Enhancement of capability, M = Less efficient workarounds available, H = Must have for operation with high benefit

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5.0

Single-use technologies

There is an increased focus on SU systems for delivering biomanufacturing processes[62]. However, most current biomanufacturing facilities were designed for SS operations. In the context of this discussion, SS refers to re-used, non-disposable components predominantly made from SS.

Due to the higher productivity of continuous manufacturing over batch processing[1], see modeling in the The BioPhorum First Edition Technology Roadmap appendix[38], smaller scales of operation will generate the same quantity of drug substance in the same unit time. This allows both SS/'not disposable' and SU/'disposable' systems to be considered for continuous bioprocessing. Current examples of continuous downstream bioprocessing systems, such as Bayer's MoBiDik or PALL's Westborough laboratory, have been designed with SU in mind. This section will look at the merits of SU systems in continuous DSP, as applied to the example process models, and highlights the identified gaps, in Table 16, preventing adoption into GMP manufacture.

Table 16: Gaps in single-use technologies

Gaps or perceived gaps	Importance for continuous processing	Expected implementation date			
		Current	2022	2025	2030
Lack of validation data for process and SU hardware for continuous timescales (1–3 months)	H				
No integrated process automation to unify the process	H				
Effects of longer exposure times of continuous processes on SU technology, regarding for example leachables and extractables, is not understood	H				
Lack of small-scale SU devices as precursors for large-scale SU, preventing adoption	H				
Low-pressure rating of some SU components (e.g. bags) limits the ability to pressure integrity check the end-to-end flow path	M				
Absence of plug-and-play-style SU systems	M				
Absence of standardized SU connectors to connect all downstream process steps into a single automated production line	M				
CP flow rates can be lower than current SU or SS components or sensors are validated/ designed for	M				
Limited range of measurement technologies covered by current disposable SU sensors for process monitoring and control	M				
Lack of installation strategy for SU systems with physically linked continuous processes	M				
Definitions for 'single-use' and 'disposable' sensors are not consistent with the operating philosophy required for SU CP	L				
Disposable bags require additional identification of inlets, outlets, level sensing and instrumentation for a continuous process flex point (for process upsets)	L				

Key:
L = Enhancement of capability, M = Less efficient workarounds available, H = Must have for operation with high benefit

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5.1 Durability and robustness

Central to the concept of CP is the need to have a flow path that is in constant use, performs consistently for the duration of the process and does not materially or physically change. Consequently, flow path components need to meet these fundamental parameters for consideration in continuous processes, as the links and connections between each unit operation will remain in place for the duration of the process. Moreover, break tanks/bags that can buffer variable process flow rates or act as processing vessels will be required for continuous processes that can withstand wider extremes of operating conditions (e.g. pH). SS has historically been used because it can meet these requirements over the lifetime of a manufacturing facility. SU components were specifically developed to supply disposable flow paths for batch campaigns. Therefore, there has not been a driver to develop 'continuous-use-grade' SU components with usage lifetimes up to three months with a resistance to aggressive cleaning solutions, as well as mechanical and chemical robustness. The recent interest in transitioning processes to a continuous biomanufacturing platform has identified several questions around selecting the appropriate flow path.

Prolonged mechanical working of plastic/polymer tubing will lead to deformation or integrity failure. In batch processing, tubing fatigue is generally not a concern because the use is intermittent or of a short duration. An exception to this is mechanical deterioration in a peristaltic pump used for prolonged transfers. In a continuous process, considerations include the continuous exposure of tubing to solutions such as strong acids or caustic solutions for prolonged periods that may affect its strength and extractables. Therefore, tubing requirements must be matched to the process solutions being used at each step. Mechanical deterioration in peristaltic pumps is even more pronounced, driving the need for pumps that do not work by action on the tubing, or new tubing materials that can withstand prolonged exposure to peristaltic pumping (e.g. up to 90 days).

5.2 Leachables and extractables

The predicted reduction in the operating scale of a continuous process compared to a batch process of equivalent productivity has significant implications for assessing leachables and extractables. The increase in the surface-area-to-volume ratio can impact on the allowable contribution of leachables and extractables. Additionally, the SU components will remain in contact with the product stream for the duration of the biomanufacturing process. Although individual lots of product might only have a transient interaction with the flow path components, the effect of continuous exposure to the process solutions on the leachables and extractables profile regarding exposure durations up to 90 days[39] needs to be obtained. Mitigating strategies such as pre-use water/buffer flushes, leachable clearance through the biomanufacturing process and SU change-out during the continuous run can be built from the data developed.

5.3 Flow rate

Table 4 in Section 3 outlined potential scenarios encountered in continuous DSP with their expected average flow rates. At commercial scales, the flow rates differ significantly between a batch and a continuous process producing comparable product yields. Batch processes typically operate at 10–30L/minute, while continuous processes are closer to 0.1–2L/minute. At clinical scales, the flow rates may be even lower. These differences in flow rates will become more significant as processes intensify and smaller bioreactor volumes can be used. Miniaturization of the processes, which are advantageous from capital and operational perspectives, will challenge the current capabilities of equipment intended for GMP manufacturing and should drive the development of the next generation of downstream processing equipment. The range of pumping devices available to attain the flow rates considered in this paper is currently quite restrictive compared to the pump designs available at larger GMP manufacturing scales.

Additionally, detection of lost flow must be considered in a continuous process. The mass flow through a process and across each unit operation must balance; where the output is less than the input flow, a loss has occurred. Typically, this will be indicative of leaks or process failure. This may be determined using in-line flow meters or pressure sensors with signal outputs that can be attached to alarms within process control software. Manual means of detection are also available, such as ball indicators and rotating vanes, etc. However, these manual means must always be watched during a continuous process, which may be challenging. Sensors and alarms should, therefore, be used in a continuous process to determine any loss of flow. Continuous processes will require pumps that can operate at constant low flow rates with a high accuracy to allow for adjustments with a minimum process upset.

6.0

Automation

The industry's shift from large-scale, fixed-tank processing facilities to more flexible, SU technologies has demonstrated the desire to move towards flexibility in biomanufacturing.

This has created an equivalent need for more flexible automation for these facilities. The industry's aspiration to change from batch to semi-continuous to continuous production also creates a need for expanding the scope of automation to deliver the planned performance. This section will identify key needs for applying automation technology in continuous downstream production and references The BioPhorum First Edition Technology Roadmap (Automated Facility)[40].

6.1 Improved equipment operation

In the future, a paradigm shift will be required in the use and operation of processing equipment regarding the tolerance for equipment failures, deviations or excursions. In the past, with batch-wise operation, a problem in a single batch impacted only on that batch. In CP, we can no longer rely on the limits of that small batch as a problem early in a run could impact on a much larger volume of product. Ensuring continuous and proper performance of each piece of equipment in the process therefore becomes a key operational need.

Table 17 identifies a number of gaps to drive changes in equipment operation to:

- Eliminate the time lag between taking a sample and getting the test results from the laboratory, and then determine if changes to operational equipment are needed
- Ensure the equipment is running consistently
- Characterize the operation of a piece of equipment so that more consistent performance is achieved shift-to-shift.

Table 17: Gaps in equipment operation

Gaps or perceived gaps	Importance for continuous processing	Expected implementation date			
		Current	2022	2025	2030
Need to communicate the status of basic automation and control applications to confirm proper working conditions so that advanced control applications layered on top of them will perform	H				
Lack of analytical measurements in-line or at-line to provide closed-loop quality control	M				
Need for in-line/at-line sampling to verify analytical measurement performance	L				
Need for common integration of real-time analyzers for closed-loop quality control	H				
Require easy development of multi-variate data analysis models to provide a comprehensive understanding of continuous unit operations	M				
Need to ensure proper running of continuous processes with regulatory approvals e.g. using multi-variate statistical process control on-line in real-time	H				
Lack of comprehensive equipment effectiveness monitoring to confirm proper equipment processing	M				

Key:
L = Enhancement of capability, M = Less efficient workarounds available, H = Must have for operation with high benefit

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"In CP, we can no longer rely on the limits of that small batch, as a problem early in a run could impact on a much larger volume of product."

6.2 Overall process-optimization requirements

When the overall DSP process starts changing from batch to continuous, the need to coordinate between and across the individual unit operations grows. Downstream units need to be aware of any interruptions in upstream units. Adjustments may be needed in the surge tanks between units to ensure the next unit will operate effectively. The overall yield of the full production train needs to be accounted for to determine the run time to meet the overall production plan.

Table 18 identifies a number of gaps to drive changes to:

- Minimize the impact of problems with one piece of equipment affecting others and the overall production
- Optimize the performance of each piece of equipment
- Keep consistent throughput on the production line.

Table 18: Gaps in process optimization

Gaps or perceived gaps	Importance for continuous processing	Expected implementation date			
		Current	2022	2025	2030
Need to report and communicate critical unit problems or interruptions to downstream units to support performance adjustments	H	■	■	■	■
Need for weight and/or mass measurements of all key SU surge tanks and relay this data to support downstream unit performance adjustments	M	■	■	■	■
Need for measurement and closed-loop control of SU pH/conductivity in all key unit operations and surge vessels	M	■	■	■	■
Lack of measurement and closed-loop control via modeling on protein concentrations in all key SU unit operations	M	■	■	■	■
Require real-time, multi-variate modeling of unit yield, production throughput and production cycle time to optimize continuous line throughput	M	■	■	■	■

Key:
L = Enhancement of capability, M = Less efficient workarounds available, H = Must have for operation with high benefit

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6.3 Operator/operations management requirements

Operator engagement and management of the downstream process will change with a move to CP, see Table 19 for identified gaps. While fewer operators will typically be needed to operate each piece of equipment, continuous 24-hour operations may require more shifts if moving from a 12 to a 24-hour operation.

Operator tasks and activities will also likely change as more of their traditional work becomes automated and cleaning activity is reduced (especially for SU applications), but there may be more set-up required.

Table 19: Gaps in operations management

Gaps or perceived gaps	Importance for continuous processing	Expected implementation date			
		Current	2022	2025	2030
Need for more operator interfacing capability via control rooms and mobile devices to facilitate flexible operations	M				
Requirement to convert paper processes, such as logbooks, to electronic systems for recording operational and equipment activity	M				
Need to expand the scope of operator training and certification as their 'span of control' increases with more continuous production	M				
Provision of electronic dashboards required showing individual equipment, as well as overall production performance	M				

Key:
L = Enhancement of capability, M = Less efficient workarounds available, H = Must have for operation with high benefit

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6.4 Material consumption/waste tracking requirements

Material allocation, movement, tracking and traceability requirements change when applying The BioPhorum First Edition Technology Roadmap (Automated Facility) to continuous downstream processing[40]. Moving materials and consumables should be automated to ensure the proper material is delivered to production at the correct time/location. Systems need to automatically verify the correct material is being used at the point of consumption. Switching from batch to continuous production changes how material traceability is performed to support consumption and regulatory reporting. Organizations need to determine how to define the start and the stop of batches for reporting purposes in a continuous process (see Section 8.1).

Table 20 identifies a number of gaps to drive changes to:

- Avoid deviations by using the correct materials at the appropriate time during production
- Ensure proper reporting of all work-in-progress inventory to help minimize carrying costs
- Provide proper traceability and genealogy for all required reporting and troubleshooting
- Support RTR.

Table 20: Gaps in material consumption and waste tracking

Gaps or perceived gaps	Importance for continuous processing	Expected implementation date			
		Current	2022	2025	2030
No provision for automated material allocations and electronic pick lists (including confirmation capabilities) or integrating enterprise inventory systems with plant floor operations	M				
No validated residence time distribution models to determine lot composition and start and stop times during CP	M				
Need for integration of serialization tracking system with continuous labeling system to make it easy to track materials	L				

Key:
L = Enhancement of capability, M = Less efficient workarounds available, H = Must have for operation with high benefit

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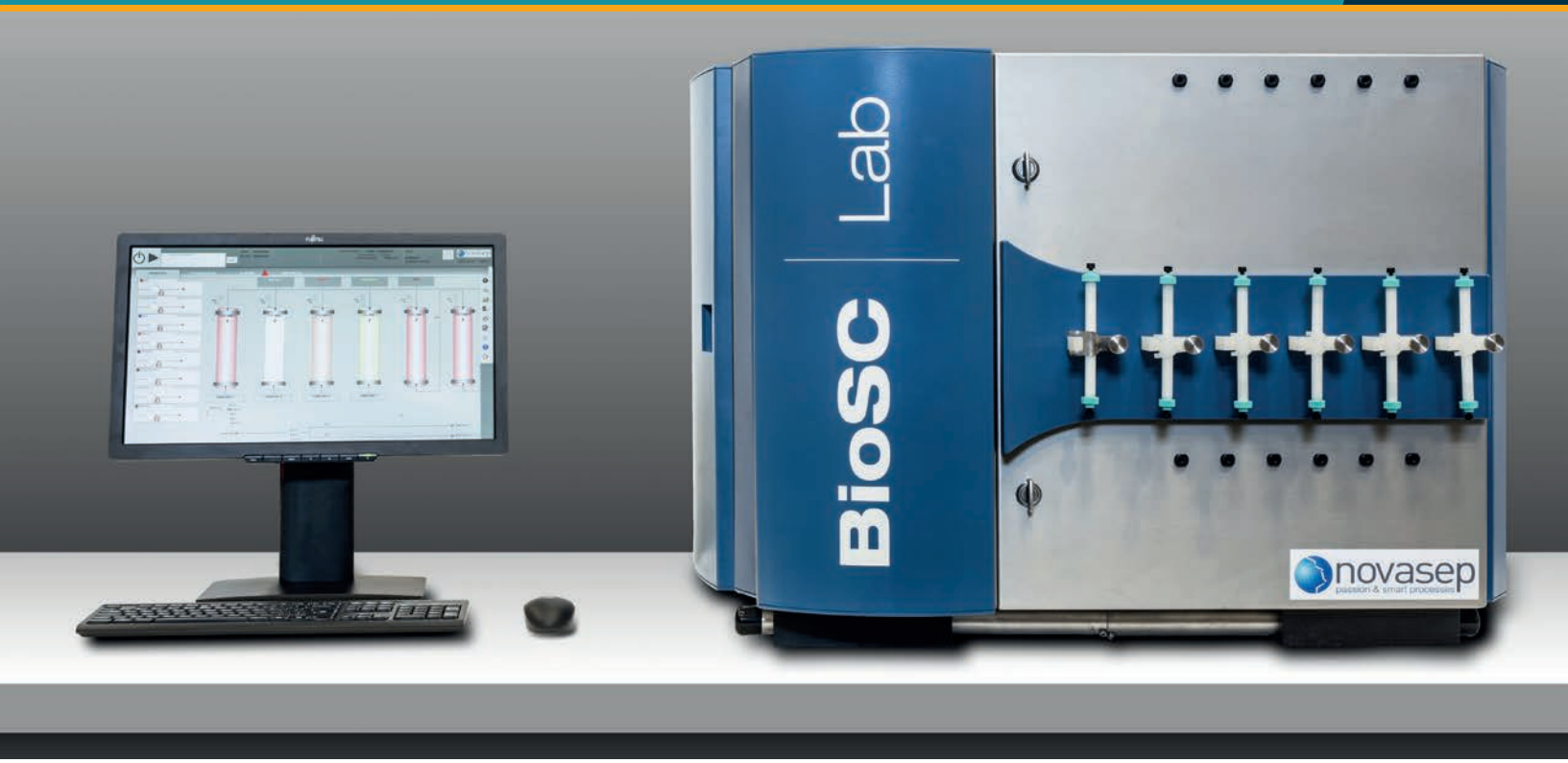

6.5 Production set-up and changeover

New production facilities will require more flexibility in their set-up and change-over as they will not be dedicated to single products. Switching between products will require additional controls that are not needed in static systems. SU applications are a growing trend for continuous production and require set-up and tracking of the SU consumables.

Table 21 identifies gaps in automation to enable more flexible facilities in the future with enhanced material tracing capabilities. Modular plug-and-play approaches are critical to the success of rapid changeovers and are currently being worked on by the BioPhorum Plug and Play team.

Table 21: Gaps in production set-up and changeover

Gaps or perceived gaps	Importance for continuous processing	Expected implementation date			
		Current	2022	2025	2030
Need for automated assembly and electronic tracking of SU assemblies and material	M				
Requirement for modular, moveable production skids supporting a flexible manufacturing strategy tracked via skid warehouse management. Modular equipment does not use full plug-and-play capability for equipment and automation systems, so equipment is not automatically available and ready to operate when moved to production	H				

Key:
L = Enhancement of capability, M = Less efficient workarounds available, H = Must have for operation with high benefit

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6.6 Batch record requirements

The BioPhorum First Edition Technology Roadmap (Automated Facility)[40] recommends using comprehensive electronic batch records, which replace current paper batch records, can be retroactively applied to existing batch processes and should be designed into new continuous production processes. Table 22 identifies gaps to drive changes to:

- Avoid production deviations/out-of-specification product during the production run
- Enable electronic RTR of each production run
- Capture all required production information for later analysis and/or troubleshooting.

The following types of data should be automatically and electronically collected during continuous DSP to support these goals:

- Process parameters
- Alarms and events
- Environmental conditions
- Critical process parameters
- CQAs
- Laboratory samples and their results (during production and post-production)
- Materials used and their status
- Equipment/consumables used and their status
- People used in production and their qualification status
- Product recipe/formulization/sequences used.

Table 22: Gaps in batch records

Gaps or perceived gaps	Importance for continuous processing	Expected implementation date			
		Current	2022	2025	2030
Need for automatic creation of deviation alerts during the manufacturing process and an associated collaboration environment for manufacturing, quality and technical operations to: • Evaluate the deviation • Determine if there is any impact on the current production run • Take any required actions • Provide a formal record and sign-off that everything has been resolved for electronic product release and sign-off	M				
Lack of connectors able to connect and disconnect aseptically or perform multiple connections/disconnections aseptically	H				

Key:
L = Enhancement of capability, M = Less efficient workarounds available, H = Must have for operation with high benefit

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7.0

Economic process modeling

The BioPhorum First Edition Technology Roadmap[1] modeled a continuous biomanufacturing process encompassing the upstream and downstream unit operations at two operational scales that indicated a cost benefit to moving to continuous manufacturing. Previous work by other authors has shown that pre-commercial biomanufacturing activities benefit from using semi-continuous downstream processing[12], suggesting full use of each unit operation is a key driver in achieving process efficiencies.

Analyzing the DSP operation separately from the upstream has allowed us to better understand and analyze the impact of process configurations so that, for a given throughput, the following factors need to be considered and standardized before comparing the impact of alternative technologies. A preliminary model was developed by Biopharm Services' BioSolve Process economic modeling software package[41] and a summary of the assumptions and conclusions are provided here:

- Buffer preparation strategy: when prepared on a batch basis, the scale and number of solution preparations will be linked to the number and scale of batches. This has a direct impact on labor and capital. To standardize the comparison between batch and continuous processing the buffers were prepared on a time basis (every 3.5 days) so that the buffer system was kept constant between the scenarios and would only be impacted on if a scenario required less buffer for a given throughput
- For a fixed throughput and titer, as the number of batches per year increases the size decreases. Modeling has shown that this a significant parameter and can influence cost outcomes for a given process, i.e. can move the COG by +/-20%. In this study, the harvest frequency was standardized for fed-batch upstream. One significant difference for perfusion upstream is that the pooled feed volume supplying the downstream is larger than in the case of a fed-batch upstream (for fed-batch it was 2.5 and 3.5 days, for perfusion upstream it was 5 and ten days). It might be argued that this could be normalized for fed-batch by using a larger upstream pool, reducing the batch frequency
- A batch cost breakdown suggests the maximum impact targets are technical innovations, such as buffer stock blending[33]. A baseline breakdown for batch or continuous downstream highlights the key point of consumable costs dominating the COG. In a continuous operation, the proportion of costs associated with consumable components is reduced through a higher dynamic binding capacity and extended use of SU components. It indicates that while labor and capital are important, any technology improvements must address consumables costs if they are to have a radical impact on the COG (Protein A, VF and UF/DF).

Evaluation and modeling of downstream operations are not simple and many unknowns remain around the operating strategies and accurate assumptions for feeding these models. Many of the current modeling assumptions are founded on batch-processing knowledge and isolation of just the downstream unit operations has picked out modeling issues that are not seen when determining a cost analysis of a fully integrated USP and DSP process. It is clear that there are still several areas that need further exploration, including:

- Evaluation of different modalities, such as connected processing and duplex columns
- Alternative automation strategies that look at integration operations (the current models assume islands of automation for each unit operation)
- Incorporation of quality control tests and other support operations
- Optimization of the key unit operations.

Table 23 lists the current gaps in our understanding of how continuous biomanufacturing should be modeled to better reflect potential benefits.

Table 23: Gap analysis of modeling approach

Gaps or perceived gaps	Importance for continuous processing	Expected implementation date			
		Current	2022	2025	2030
Impact of upstream configuration and harvest strategy on DSP is not fully understood	H				
Buffer supply strategy impact on DSP economics is not understood	H				
Lack of operational experience in CP to constrain modeling assumptions and operating parameters	H				
Metric for quantifying facility flexibility through CP is not defined (e.g. are surge vessels more flexible than fixed pool tanks?)	M				
Identification of appropriate sensitivity ranges to drive improvements in continuous over batch processing is required for: • Automation • Facility footprint • Labor • PAT • Batch size • Operating time	H				
The current state of technologies and assumptions are based on batch knowledge. True influence of CP on QC/QA, operator or facility costs needs to be demonstrated	H				
It is unknown if automated analytics (PAT) and complete process automation significantly reduces operational staffing levels	H				

Key:
L = Enhancement of capability, M = Less efficient workarounds available, H = Must have for operation with high benefit

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The initial modeling in this paper showed that benefits could be realized from continuous biomanufacturing. The business case can further be improved by addressing the gaps noted in Table 23 to provide the solutions to ensure the upstream and downstream parts of a continuous process contribute to the overall cost reductions desired in The BioPhorum's First Edition Technology Roadmap.

8.0

Regulatory considerations

Innovation in pharmaceutical manufacturing, such as CP, is a key interest of industry and regulators globally.

Regulatory agencies such as the FDA, European Medicines Agency and Pharmaceuticals and Medical Devices Agency have developed internal teams focused on driving new technologies and innovative approaches to pharmaceutical manufacturing:

- FDA Emerging Technology Team promotes the adoption of innovative approaches to pharmaceutical product design and manufacturing. Industry representatives can engage with the team to discuss the development and implementation of new technologies (https://www.fda.gov/AboutFDA/CentersOffices/OfficeofMedicalProductsandTobacco/CDER/ucm523228.htm)
- European Medicines Agency's Process Analytical Technology (PAT) Team includes assessors and inspectors supporting PAT and 'quality by design' activities (http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/document_listing/document_listing_000162.jsp)
- Pharmaceuticals and Medical Devices Agency's Innovative Manufacturing Technology Working Group (IMT-WG) includes members from the Office of New Drugs, Office of Manufacturing/Quality and Compliance, and Office of Regulatory Sciences.

These agencies provide a pathway for industry engagement in support of new technologies and approaches, such as continuous and semi-CP before formal regulatory submissions. The current regulatory landscape (ICH Q8-Q11) in general supports the adoption of continuous or semi-continuous manufacturing concepts[42]. However, the lack of clear regulatory guidelines and global harmonization are potential hurdles to robustly implementing a global continuous manufacturing and lifecycle management strategy. The proposed ICH Q13 (Continuous Manufacturing for Drug Substances and Drug Products) guidance[43] provides an opportunity to develop high-level regulatory expectations concerning continuous manufacturing of small and large molecules.

The current regulatory framework provides a path for continuous manufacturing implementation. Regulatory expectations for batch vs continuous under the current framework are the same. However, these criteria may be met differently for continuous vs batch. Gaps or perceived gaps are summarized in Table 24.

The following sections discuss the regulatory considerations for continuous manufacture concerning batch definition, validation strategy and control strategy/PAT of a downstream continuous or semi-continuous process.

Additional reference material:

- Technical and Regulatory Considerations for Pharmaceutical Product Lifecycle Management[44]
- Regulatory Perspectives on Continuous Pharmaceutical Manufacturing: Moving From Theory to Practice[45]
- Current Regulatory Considerations for Continuous Manufacturing of Pharmaceuticals in Japan[46]

"How do you define a batch in CP?"

Table 24: Gaps in Regulatory

Gaps or perceived gaps	Importance for continuous processing	Expected implementation date			
		Current	2022	2025	2030
Acceptance of batch definition for continuous and semi-continuous processes by regulatory agencies	H	■	■	■	■
Lack of experience in validation of a continuous process to meet current regulatory expectations	M	■	■	■	■
Requirement to demonstrate viral reduction established throughout the process meeting regulatory clearance requirements	M	■	■	■	■
Need for PAT technologies to support robust in-line monitoring	M	■	■	■	■

Key:
L = Enhancement of capability, M = Less efficient workarounds available, H = Must have for operation with high benefit

■ Research ■ Development ■ Manufacturing

8.1 Batch definition

The definition of a batch (or lot) noted in ICH Q7 (Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients Q7[47]) is valid for a continuous/semi-continuous process:

"A specific quantity of material produced in a process or series of processes so that it is expected to be homogeneous within specified limits. In the case of continuous production, a batch may correspond to a defined fraction of the production. The batch size can be defined either by a fixed quantity or by the amount produced in a fixed time interval."

The approach to set the batch or lot size will differ from traditional batch manufacturing, where final product testing is used to support the release. Continuous manufacturing could generate more than one batch, making both in-process and final product testing important for batch release. This is key to ensure batch or lot numbering will maintain material traceability through the process. Batch or lot size can be set based on various factors as defined in Table 25.

Table 25: Batch definition

Bioreactor configuration*	Harvest process	DSP process	Batch definition approaches
n-perfusion	Continuous harvest into downstream	Continuous downstream process (refer to Section 3)	• Run time and throughput speed • Amount of drug substance produced
Intensified	Batch harvest into downstream	Continuous downstream process (refer to Section 3)	• Charge amount of raw material (USP harvest) • Amount of drug substance produced
n-perfusion	Continuous harvest into downstream	Semi-continuous downstream process (refer to Section 3)	• Charge amount of raw material (USP harvest) • Amount of traditional hold step • Amount of drug substance produced
Intensified	Batch harvest into downstream		

*See process descriptions in Table 4

8.2 Validation

The validation of continuous processes, as described in FDA's *Draft Guidance (Small Molecules): Quality Considerations for Continuous Manufacturing*. [34], must include the ability to evaluate real-time data to maintain operations within established criteria, which consistently produces drug substance that meets specified product quality criteria. The overall process validation strategy for a downstream process includes elements of characterization and consecutive validation batches. Traditional approaches to process characterization and validation, inclusive of plant protection and quarantine, may need to be adjusted for continuous manufacturing processes.

8.2.1 Characterization strategy

Batch processes typically allow for the discrete isolation of unit operations and, subsequently, greater control over conditions that exist between unit operations. Thus, characterization of a batch process may be deemed more approachable. In contrast, the characterization of a continuous manufacturing process needs to account for the connectivity between steps. In this situation, performing 'design of experiments' over the entirety of the process using partition designs may be more representative compared to testing isolated unit operations. Early implementation of PAT in process development would help meet regulatory expectations, but comparability assessments for characterizing continuous against batch manufacturing should be proactively discussed with regulatory agencies. Unit operations tailored to continuous manufacturing, such as periodic counter-current or simulated moving bed, will present unique challenges in designing small-scale characterization studies. Specifically, failure modes, lifetime and other difficult-to-control variables should be assessed to support characterization expectations from the regulators.

Additional reference material on PAT:

- *Q8(R2): Harmonised Tripartite Guideline Pharmaceutical Development*[48]
- *Q10: Harmonised Tripartite Guideline Pharmaceutical Quality System*[49]
- *FDA Guidance for Industry: PAT – A Framework for Innovative Pharmaceutical Development, Manufacturing and Quality Assurance*[50]
- *The BioPhorum First Edition Technology Roadmap (In-line Monitoring and Real-time Release)*[36]

8.2.2 Qualification and validation strategy

The qualification of integrated equipment and automated control systems are an essential part of the overall qualification and validation strategy. This information is a key input into the process parameter qualification (PPQ) validation strategy.

The traditional three-batch PPQ approach may prove challenging for continuous manufacturing processes due to constraints of time and scale. Thus, the validation strategy for a continuous process could initially demonstrate overall process consistency over the duration of a single batch, assuming sufficient data exists for a single batch to justify such a claim followed by a continued process verification strategy. PPQ run time for a single batch should capture expected process variability. In contrast to batch processes, continuous manufacturing may lend itself to the natural evaluation of the 'limit of *in vitro* cell age for production' due to the duration of the sustained cultures.

8.2.3 Viral clearance validation strategy

The guidance for viral safety and viral clearance are the same as those for batch-manufactured drug products. The process and the approach needed to meet the guidance criteria are different. Virus- or pathogen-safety concepts are based on what is commonly referred to as the 'safety tripod'. This relies on the principles of:

1. Selecting well characterized and, wherever possible, low-risk material for biomanufacturing
2. Extensive testing in all production stages for endogenous or adventitious pathogens by different methods
3. Extensive virus reduction over the DSP, e.g. by inactivation- (pH or detergent) or removal- (virus filter, chromatography or fractionation) methods.

It should be noted that the contribution of virus reduction (point 3) to overall safety is generally greater by several orders of magnitude, due to known limitations in selecting and testing (points 1 and 2, respectively).

The modes for virus removal in DSP, including worst-case parameters for removal, are generally well understood. Many deductions can be made from the classical approach that applies to CP but the challenge lies in generating a valid downscale model for virus clearance studies as a given DSP unit operation is likely to deal with significantly greater fluctuations in process conditions, such as pH, conductivity and protein concentration.

For these reasons, the main challenge lies in how to realistically simulate/validate what a unit operation 'sees' during a cycle or its time of utilization (see Section 4.3 and 4.4).

Additional guideline reference materials on viral clearance:

- *Code of Federal Regulations Title 21, subchapter F - biologics*[51]
- *Guideline on plasma-derived medicinal products*[52]
- *Note for Guidance on Virus Validation Studies: The Design, Contribution and Interpretation of Studies Validating the Inactivation and Removal of Viruses* [53]
- *Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use*[54]
- *Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals*[55]
- *Preparation of Virus Spikes Used for Virus Clearance Studies TR47*[56]
- *Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin*[57]
- *Quality of biotechnological products: viral safety evaluation of biotechnology products derived from cell lines of human or animal origin*[58]
- *Guideline on Virus Safety Evaluation of Biotechnological Investigational Medicinal Products*[59]
- *Guidelines on viral inactivation and removal procedures intended to assure the viral safety of human blood plasma products*[60]
- *Minimizing the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human or Veterinary Medicinal Products*[61]

8.3 Batch vs continuous

Regulatory quality requirements for batch vs continuous are equivalent but the approach taken to demonstrate validated processes and ensure control will differ. Table 26 summarizes differences in approach for batch vs continuous manufacturing processes.

Table 26: Comparison of batch and continuous processing

	Batch	Continuous/semi-continuous
Batch definition	• Defined batch based on fixed input/output material that meets quality criteria	• Batch definition based on run time, charge amount of input materials and/or drug substance produced
Validation strategy	• Characterization of unit operations • Traditional three-batch PPQ • Demonstrate viral clearance at unit operation in the downstream process	• Challenges regarding characterization studies and demonstrating viral clearance of unit operations • Traditional three-batch PPQ approach may not be feasible; thus, continued process verification is a critical aspect of the overall validation
Control strategy	• Overall control strategy may or may not include PAT	• PAT is a critical aspect of the overall control strategy and a key component to the validation strategy

A change from a batch process to a continuous one will be considered a major change and require a prior approval supplement in the US. Considerations regarding changes to unit operations, equipment, process parameters and control strategy should be evaluated utilizing a risk-based approach. Also, when switching between batch and continuous manufacturing processes, or vice versa, comparability of the product quality would need to be assessed. It is recommended that the bridging strategy is discussed with the agency before implementation.

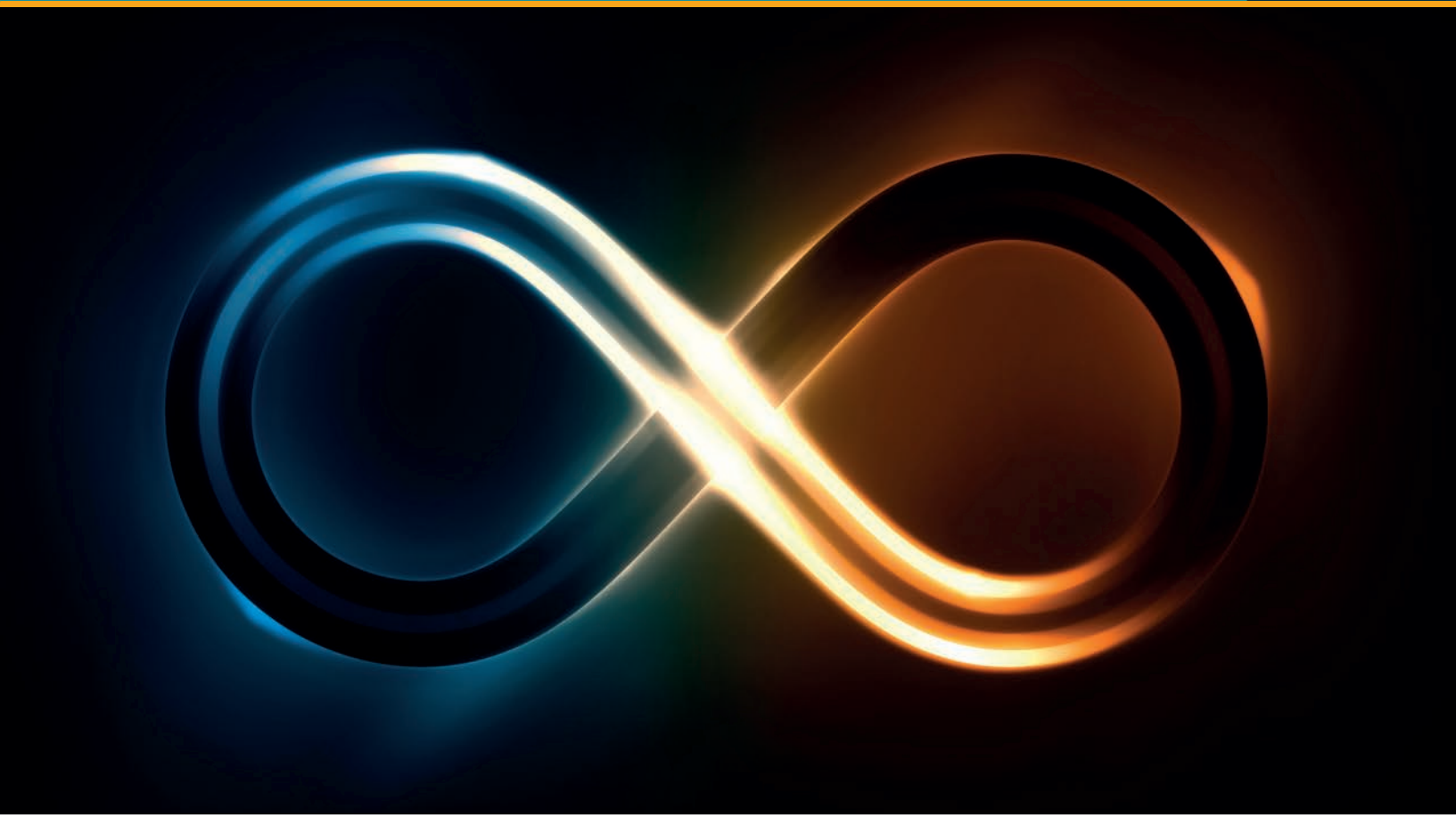
9.0

Leveraging benefits of continuous bioprocessing for other therapies

While the focus of this document is on mAbs DSP, the promise of increased process productivity, improved process economics, reduced facility footprints and biomanufacturing flexibility by transitioning from batch to continuous bioprocessing applies to all biotherapies. The lessons learned from implementing CP for mAbs should then accelerate a broad adoption for many other classes of biotherapeutics and, therefore, benefit all of the biopharmaceutical industry. Without going into specific details, this section will highlight how non-mAb therapies could benefit from the application of continuous bioprocessing.

Manufacturing processes for new antibody modalities (e.g. antibody drug conjugates, bi-/tri-specific antibodies or antibody fragments), recombinant proteins, plasma-derived therapies, vaccines, and gene and cell therapies are different, but still rely on a common architecture. Especially on the downstream side, all processes use similar separation techniques with multiple steps of purification using filtration, precipitation and/or chromatography techniques. The technologies specifically developed for mAbs continuous downstream processes are therefore applicable to other processes. The multi-column chromatography systems that have been recently commercialized are not dedicated to a given class of biomolecule and all types of chromatography resins, membranes and monoliths can be used to purify all types of proteins, vaccines and viral vectors. The SPTFF used to continuously concentrate and diafilter is also applicable to non-mAbs processes when product concentration and/or buffer exchange is required.

When considering other biomanufacturing processes, the existing cascade-purification process used for human albumin, intravenous immunoglobulin and other plasma-derived products makes them good candidates for the application of continuous DSP, where multiple sub-processes would continuously produce multiple products in parallel. The flexibility brought by continuous bioprocessing could completely change the blood fractionation industry, potentially with small manufacturing facilities installed close to blood collection centers to continuously produce plasma-derived therapeutics. A continuous process is beneficial for unstable recombinant proteins with minimized processing times and with no need for intermediate storage.
In the example of viral vector processes for gene therapy, the opportunity for CP is even greater since no robust batch-based commercial manufacturing process exists, allowing this area to help innovate CP, analytics and the regulatory framework.



“The automation developed for continuous process monitoring and control will facilitate the regulatory compliance for all other biotherapies.”

The knowledge generated from the application of continuous mAb processes can therefore be leveraged for the development of continuous commercial viral vector manufacturing processes for gene therapy.

The regulatory structure taking shape for continuous mAb manufacturing will inform and guide the regulatory framework for other therapies. All the developments related to the definition of a lot, process safety with RTR, and process control strategies can be transferred from mAbs to other molecules. The automation developed for continuous process monitoring and control will facilitate the regulatory compliance for all other biotherapies. Since in-line, at-line and off-line PAT strategies are being developed for continuous mAbs processing, the same strategies, even if using different analytical techniques, can be applied for other biotherapeutics.

Regarding process economics, the trend for process cost reduction is happening all across the biopharmaceutical industry. Thanks to the implementation of continuous bioprocessing for mAbs, a target manufacturing cost below $10/g is considered achievable. We can anticipate that such implementation could reduce the cost of manufacturing of intravenous immunoglobulin significantly below $10/g and many vaccines below $1/dose. Market pressure to reduce costs is even greater with respect to gene therapies.

10.0

Conclusion

This paper uses the mAb purification platform as the vehicle to discuss the current barriers to the adoption of continuous downstream GMP bioprocessing in the biopharmaceutical industry. However, the manufacturing processes for many different biotherapeutics use identical or similar purification technologies, process monitoring and control, and regulatory frameworks, such that the continuous purification discussions in this paper apply to other protein therapies. The lessons learned from implementing CP for mAbs will, therefore, accelerate its adoption for other recombinant medicines, vaccines and new therapies, such as cell and gene therapies. The technologies developed and the regulatory framework created for continuous downstream mAb processes can be leveraged to realize the measured benefits in terms of decreased cost, reduced time to market, flexible manufacturing and increased product quality and safety across the biopharmaceutical industry.

For the industrial implementation of this emerging technology, the technical and regulatory hurdles and 'road blocks' need to be reduced or removed, and its economic benefits clearly articulated. More generally, there is a requirement to develop a consensus on continuous bioprocessing that will help establish expectations among manufacturers, suppliers, regulators and academics to facilitate the implementation and improvement of continuous biomanufacturing. The modeling in The BioPhorum First Edition Technology Roadmap[1] demonstrated an economic benefit for continuous biomanufacturing. The data presented here focused on downstream processing to act as a benchmark for evaluating downstream continuous biomanufacturing technologies and strategies. The key learning point was that downstream processing currently enables the benefits of upstream perfusion, but has yet to significantly impact on bioprocessing, providing an opportunity to innovate downstream operations and realize the economic advantages.

The analysis of the current state of continuous downstream mAb purification in this paper has identified 118 gaps, of which 44 were considered as already having a solution or the benefits of addressing the gap was insufficient to focus effort on devising a solution. The remaining 74 gaps, whose solutions are essential and provide high benefits or simplify current workaround solutions, were grouped into several areas and have been ranked from most impactful to least in Table 27. There are 38 gaps identified as significantly and positively impacting continuous bioprocessing and 36 that will simplify continuous operations. A summary is given in the table, with specific details found within the body of the document, to provide end-users, vendors, integrators and academics with a framework to rapidly address the implementation hurdles to continuous biomanufacturing.

Table 27: Summary of identified gaps

Sensors	
7 high importance gaps	Requirement for accurate (absolute value and fast response) monitoring/controlling sensors of process intermediates, buffers, additives and excipients concentrations, especially responding to changes in process flow rates and product stability for the duration of the process. Need for sensors in closed flow paths that are pre-calibrated, can withstand sterilization methods, shipping and pre-use storage, and can be checked while in service and/or changed-out during processing without compromising the closed system. Limited range of measurement technologies covered by current disposable SU sensors for process monitoring and control, and absence of in-line, real-time bioburden monitoring for prolonged operation.
5 medium importance gaps	Key analytical measurements required in- or at-line to provide closed-loop quality control through PAT technologies that support robust monitoring and measuring of a greater range of product attributes. Need for modeling on protein concentrations for closed-loop control in all key SU unit operations. Need for sensor compatibility with calibration standards and process fluids.
Automation	
6 high importance gaps	Integration of modular, moveable production skids with manufacturing execution systems, and linkages to surge vessels and/or other unit operations, to create an automated, unified process with material tracking models, critical process parameters and batch-reporting tools is required. Need for common, real-time, in-/on-line analyzers for closed-loop quality control of continuous processes with multi-variate statistical process control to ensure regulatory approval.
7 medium importance gaps	Development of residence time distribution and multi-variate data analysis models is needed to provide a comprehensive understanding of lot composition, start and stop times, yield of continuous unit operations, production throughput and production cycle time to optimize continuous line throughput. Requirement for automatic deviation alerts plus an environment for manufacturing, quality and technical operations to evaluate the deviation, take any required actions and provide a formal record for electronic product release and sign-off. Implementation of comprehensive equipment effectiveness monitoring to confirm proper ongoing equipment processing is necessary. Absence of plug-and-play-style SU systems or electronic tracking of SU flow paths and material.
Modeling	
6 high importance gaps	Lack of operational experience of continuous bioprocesses limits assumptions to batch based knowledge and current continuous technology solutions. True benefit of continuous processing requires further knowledge of operating parameters and sensitivities ranges for automation, facility footprints, utility costs, QC/QA needs, labor, PAT, batch size and operating times. Nor is the impact of upstream configuration, harvest and buffer supply strategies on downstream processing economics fully understood.
1 medium importance gap	Metric for quantifying facility flexibility through continuous processing is not defined (e.g. are surge vessels more flexible than fixed pool tanks?).
Validation	
6 high importance gaps	Lack of experience and no standardized approach to validating continuous processes and their unit operations (e.g. viral clearance). Limited or no validation data on SU systems for continuous timescales (e.g. up to three months).
7 medium importance gaps	Development of residence time distribution and multi-variate data analysis models is needed to provide a comprehensive understanding of lot composition, start and stop times, yield of continuous unit operations, production throughput and production cycle time to optimize continuous line throughput. Requirement for automatic deviation alerts plus an environment for manufacturing, quality and technical operations to evaluate the deviation, take any required actions and provide a formal record for electronic product release and sign-off. Implementation of comprehensive equipment effectiveness monitoring to confirm proper ongoing equipment processing is necessary. Absence of plug-and-play-style SU systems or electronic tracking of SU flow paths and material.

Medium importance gap = Less efficient workarounds available

High importance gap = Must have for operation with high benefit

Process	
4 high importance gaps	There were 14 gaps identified within the paper that were considered to fall with the process category, where the process product or technologies impacts on the operating approach. The lack of understanding of the impact of process fluctuations and interactions in continuous bioprocessing and determining relevant mitigation strategies to maintain control were identified as critical gaps. Gaps associated with operational improvements focused on specific equipment (e.g. performance ranges/capabilities), technologies (e.g. resin function) and their implementation (e.g. filter integrity testing) in a continuous process.
10 medium importance gaps	
Sampling	
3 high importance gaps	No simple and robust method for obtaining representative samples from the process for control and monitoring (e.g. bioburden contamination) or as inputs to testing RTR assays and in-line sensors for PAT.
1 medium importance gap	Need to devise accurate mass balances when product pools are not collected.
In-line concentration/in-line dilution	
2 high importance gaps	Scale-down model validation is difficult with the current modular membrane format and continuous diafiltration buffer requirements can be significantly greater than a batch process.
5 medium importance gaps	Lack of user-friendly skids that integrate into process automation systems. Limited filter configurations may not fit the application or scale of interest. Lengthy cleaning and regeneration cycles do not seamlessly fit into a continuous paradigm without having multiple membrane modules. Viscosity changes at high protein concentrations may limit continuous ultrafiltration capabilities. Start-up and shutdown sequences where a product is out-of-specification requires additional process controls.
Single-use	
2 high importance gaps	Robustness of SU components concerning chemical (leachables and extractables) and mechanical stability for a long duration operation is not yet understood.
3 medium importance gaps	Lack of standardized connectors for connection into a single production line and that can perform one or multiple connect(s) and disconnect(s) aseptically. Low-pressure rating of some SU components limits the ability to pressure integrity check the end-to-end flow path.
Scale-down	
2 high importance gaps	Lack of experience and no standardized approach to validating continuous processes and their unit operations (e.g. viral clearance). Limited or no validation data on SU systems for continuous timescales (e.g. up to three months).
3 medium importance gaps	Scalability of systems is not proven.
Buffer preparation	
2 medium importance gaps	Limited understanding of bioburden control for continuous buffer preparation and supply to unit operations, and implementation of continuous buffer preparation for continuous manufacturing (e.g. number of units required).

11.0

Acronyms and abbreviations

Term	Definition
AEX	Anion exchange chromatography
BDS	Bulk drug substance
CFTP	Continuous flow-through processing
CIP	Clean-in-place
CMCC	Continuous multi-column chromatography
COG	Cost of goods
CP	Continuous processing
CPP	Critical process parameter
CQAs	Critical quality attributes
DF	Diafiltration
DSP	Downstream processing
EMA	European Medicines Agency
FDA	US Food and Drug Administration
GMP	Good manufacturing process
HC UFP	High-concentration drug ultrafiltration product
ILDF	In-line diafiltration
LC UFP	Low-concentration drug ultrafiltration product
mAb	Monoclonal antibody
PAT	Process analytical technology
PPQ	Process parameter qualification
QA	Quality assurance
QC	Quality control
RTR	Real-time release
SEC	Size-exclusion chromatography
SIP	Steam-in-place
SPTFF	Single-pass tangential flow filtration
SS	Stainless steel
SU	Single-use
SUM	Single-use mixer
TMP	Transmembrane pressure
UF	Ultrafiltration
UFP	Ultrafiltration product
USP	Upstream processing
VF	Viral filtration
VI	Viral inactivation

References

1. **BioPhorum.** *The BioPhorum First Edition Technology Roadmap for the Biopharmaceutical Manufacturing Industry.* [Online] 2017. https://www.biophorum.com/executive-summary/
2. **Elvidge, S.** *FDA urges move from stop-start to continuous manufacturing.* [Online] 2019. https://www.biopharmadive.com/news/fda-urges-move-from-stop-start-to-continuous-manufacturing/549453/
3. **Ecker D. M., Davidovits T., Ransohoff T. C.** *The Value of Single-Use and Other Flexible Technologies. BPI.* [Online] 2017. https://bioprocessintl.com/manufacturing/continuous-bioprocessing/forecasting-demand-demonstrates-value-single-use-flexible-technologies/
4. **Rader R.A., Langer E.S.** *Worldwide Biopharmaceutical Manufacturing Capacity Analysis: Growth Continues Across the Board.* BPI, 2018
5. **BioPlan.** *Concentration of global biopharmaceutical manufacturing.* Top100bio. [Online] 2019. http://top1000bio.com/
6. **Amgen.** *Next generation biomanufacturing.* [Online] 2017. https://www.youtube.com/watch?v=A2_a13bTyu4
7. **Konstantinov K. B., Cooney C. L.** *White Paper on Continuous Bioprocessing*, Journal of Pharmaceutical Sciences, 2015, J. Pharm. Sci, Vol 104: pp. 813-820
8. **Arnold L., Lee K., Rucker-Pezzini J., Lee J.H.** *Implementation of Fully Integrated Continuous Antibody Processing: Effects on Productivity and COGm.* Biotechnology Journal, 2019, Biotechnology Journal
9. **Klutz S., Holtmann L., Lobedann M., Schembecker G.** *Cost evaluation of antibody production processes in different operation modes.* Chemical Engineering Science, 2016, Chemical Engineering Science, Vol. 141: pp. 63-74
10. **Zydney, A. L.** *Continuous Downstream Processing for High Value Biological Products: A Review.* Biotechnology and Bioengineering, 2016, Biotechnology and Bioengineering, Vol. 113: pp. 465-475
11. **Pollock J., Bolton G., Coffman J., Ho S.V., Bracewell D.G., Farid S.S.** *Optimising the Design and Operation of Semi-Continuous Affinity Chromatography for Clinical and Commercial Manufacture.* 2013, Journal of Chromatography, Vol. 1284: pp. 17-27
12. **Pollock J., Coffman J., Ho S.V. and Farid S.S.** *Integrated continuous bioprocessing: economics, operational and environmental feasibility for clinical and commercial antibody manufacture.* Biotechnology Progress, 2017, Vol. 33 pp. 854-866
13. **Walther J., Godawat R., Hwang C., Abe Y., Sinclair A., Konstantinov K.** *The Business Impact of an Integrated Continuous Biomanufacturing Platform for Recombinant Protein Production.* Journal of Biotechnology, 2015, Vol 213: pp. 3-12
14. **Warikoo V., Godawat R., Brower K., Jain S., Cummings D., Simons E., Johnson T., Walther J., Yu M., Wright B., McLarty J., Karey KP., Hwang C., Zhou W., Riske F., Konstantinov K.** *Integrated Continuous Production of Recombinant Therapeutic Proteins.* Biotechnology and Bioengineering: Issue 109: 3018-29, 2012, Biotechnol Bioeng
15. **Lavoie A., Di Fazio A., Blackburn K., Carbonell R. and Menegatti S.** *Peptide-toyopearl adsorbents for removing CHO host cell proteins from cell culture supernatants.* 2018, Abstracts of Papers of The American Chemical Society, Vol. 256, pp. 1155
16. **Orozco R., Godfrey S., Coffman J., Amarikwa L., Parker S., Hernandez L., Wachuku C., Mai B., Song B., Hoskatti S., Asong J., Shamlou P., Bardliving C., Fiadeiro M.** *Design, Construction, and Optimization of a Novel, Modular, and Scalable Incubation Chamber for Continuous Viral Inactivation.* Biotechnology Progress, 2017, Biotechnology Progress, Issue 33: pp. 954.
17. **Gillespie C., Holstein M., Mullin L., Cotoni K., Tuccelli R., Caulmare J., Greenhalgh P.** *Continuous In-Line Virus Inactivation for Next Generation Bioprocessing.* 2018, Biotechnology Journal.
18. **Coffman J., Goby J., Godfrey S., Orozco R., Vogel J. H.** *Germany, USA WO 2015/158776 A1 Patent . PCT/EP2015/058172* 2017.
19. **Xenopoulos A.** *US9809799B2, EP2867359A4, JP6182210B2, CN104411820B, WO2014004103A1 Patent.* 2017.
20. **Parker S. A., Amarikwa L., Vehar K., Orozco R., Godfrey S., Coffman J., Shamlou P., Bardliving C. L.** *Design of a Novel Continuous Flow Reactor for Low Ph Viral Inactivation.* Biotechnol. Bioeng, 2017, Biotechnology and Bioengineering.
21. **Amarikwa L., Orozco R., Brown M., Coffman J.** *Impact of Dean Vortices on the Integrity Testing of a Continuous Viral Inactivation Reactor*. Biotechnology Journal, 2019, Biotechnology Journal.

22. **Klutz S., Lobedann M., Bramsiepe C., Schembecker G.** *Continuous Viral Inactivation at Low pH Value in Antibody Manufacturing.* Chemical Engineering and Processing - Process Intensification, 2016, Issue 102: pp. 88-101.

23. **David L., Maiser B., Lobedann M., Schwan P., Fröhlich M., Ruppach H., Waldschmidt L. M., Schembecker G.** San Mateo 2017. Charles River 2017 Viral Safety and Viral Clearance Summit.

24. **Bolton G. R., Selvitelli K. R., Iliescu I., Cecchini D. J.** *Inactivation of viruses using novel protein A wash buffers. 2015*, Biotechnology Progress, Issue 31: pp. 406-413.

25. **Soice N., Hubbard J. D., Zhang Y., Hamzik J.** *C07K 1/22 (20060101); (20060101), C07K16/00; B01D 15/08 (20060101) Patent. Protein For Affinity-Separation Matrix United States, 2011.*

26. **Johnson S.A., Brown M. R., Lute S.C., Brorson K.A.** *Adapting viral safety assurance strategies to continuous processing of biological products.* Biotechnology and Bioengineering., June 2017, Biotechnol Bioeng., Vol. 114, pp. 1362. doi: 10.1002/bit.26245. PMID: 28425635.

27. **Roush DJ.** *Integrated Viral Clearance Strategies-Reflecting on the Present, Projecting to the Future.* Current Opinion in Biotechnology, 2018, Vol 53: pp. 137-143.

28. **Yamamoto A., Hongo-Hirasaki T., Uchi, Y., Hayashida H., Nagoya F.** *Effect of Hydrodynamic Forces on Virus Removal Capability of Planova- Filters.* AIChE Journal, 2014, AIChE Journal, Vol. 60, pp. 2286–2297.

29. **Hongo-Hirasaki T.** *Optimization of Virus Removal Filtration Processes: Design of Process Parameters and Virus Clearance Studies.* Prague : Planova Workshop, 2015.

30. **Strauss, D.** *Challenges of Implementing Virus Filtration into Continuous Manufacturing.* Florence, Italy, 2018. PDA Virus Safety Forum.

31. **Burnham M., Schwartz A., Vyas E., Takahashi N., Nemitz P., Strauss D., Hirotomi N., Hughes J.** *Advanced Viral Clearance Study Design: A Total Viral Challenge Approach to Virus Filtration.* BPI, March 2019, BioProcess International, Issue 16: p. 3.

32. **Heise, C.** *Evaluating TFF Capsules for SPTFF in mAb Production.* Molsheim, France, 2018, Purification Expert Meeting.

33. **BioPhorum.** *Buffer Preparation project.* [Online] 2019. https://www.biophorum.com/buffer-preparation/.

34. **FDA.** *Draft Guidance (Small Molecules): Quality Considerations for Continuous Manufacturing.* [Online] Feb 2019. https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM632033.pdf.

35. **FDA.** *FDA Guidance for Industry: PAT - A Framework for Innovative Pharmaceutical Development, Manufacturing and Quality Assurance.* [Online] 2014. http://academy.gmp-compliance.org/guidemgr/files/PAT-FDA-6419FNL.PDF

36. **BioPhorum.** *The BioPhorum First Edition Technology Roadmap for the Biopharmaceutical Manufacturing Industry (In-line Monitoring and Real-time Release).* [Online] 2017. https://www.biophorum.com/in-line-monitoring-and-real-time-release/

37. **Lee, S. L., O'Connor, T. F., Yang, X., Cruz, C. N., Chatterjee, S., Madurawe, R. D., Woodcock, J.** *Modernizing Pharmaceutical Manufacturing: from Batch to Continuous Production.* Journal of Pharmaceutical Innovation, 2015, Journal of Pharmaceutical Innovation, Vol. 10: pp. 191-199

38. **Biopharm Services.** *Manufacturing Technology Roadmap Tool.* [Online] 2017. https://biopharmservices.com/industry-roadmap/.

39. **BioPhorum.** *Extractables Protocol & Leachables Best Practice Guide.* [Online] 2018. https://www.biophorum.com/best-practices-guide-for-evaluating-leachables-risk-from-polymeric-single-use-systems-used-in-biopharmaceutical-manufacturing/.

40. **BioPhorum.** *The BioPhorum First Edition Technology Roadmap for the Biopharmaceutical Manufacturing Industry (Automated facility)* [Online] 2017. https://www.biophorum.com/automated-facility/

41. **Biopharm Services Ltd.** *BioSolve Process.* [Online] 2019. https://biopharmservices.com/ and https://biopharmservices.com/industry-roadmap/

42. **ICH.** *Q8-Q11: Development and Manufacture of Drug Substances (Chemical Entities and Biotechnological/Biological Entities).* [Online] 2012. https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q11/Q11_Step_4.pdf

43. **ICH.** *Q13: Continuous Manufacturing of Drug Substances and Drug Products.* [Online] 2018. https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q13/Q13EWG_ConceptPaper_2018_1115.pdf

44. **ICH. Q12: Technical and Regulatory Considerations for Pharmaceutical Product Lifecycle Management.** [Online] 2017. https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q12/Q12_Draft_Guideline_Step2_2017_1116.pdf

45. **Nasr M. M., Krumme M., Matsuda Y., Trout B. L., Badman C., Mascia S., Cooney C., Jensen K. D., Florence A., Johnston C., Konstantinov K., Lee S. L.** *Regulatory Perspectives on Continuous Pharmaceutical Manufacturing: Moving From Theory to Practice.* 2016, Journal of Pharmaceutical Sciences, 2017, Journal of Pharmaceutical Sciences, Vol. 106: pp. 3199-3206

46. **Takayama I., Matsuda Y., Katori N.** *Current Regulatory Considerations for Continuous Manufacturing of Pharmaceuticals in Japan.* [Online] 2018. http://www.pmda.go.jp/files/000223907.pdf#page=1

47. **ICH.** *Q7: Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients.* [Online] 2000. https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q7/Step4/Q7_Guideline.pdf

48. **ICH.** *Q8(R2): Harmonised Tripartite Guideline Pharmaceutical Development.* [Online] 2009. https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q8_R1/Step4/Q8_R2_Guideline.pdf

49. **ICH.** *Q10: Harmonised Tripartite Guideline Pharmaceutical Quality System.* [Online] 2008. https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q10/Step4/Q10_Guideline.pdf

50. **FDA.** *FDA Guidance for Industry: PAT – A Framework for Innovative Pharmaceutical Development, Manufacturing and Quality Assurance.* [Online] 2004. https://www.federalregister.gov/documents/2004/10/04/04-22203/guidance-for-industry-process-analytical-technology-a-framework-for-innovative-pharmaceutical

51. **FDA.** *Code of Federal Regulations Title 21, Subchapter F - Biologics,* [Online] 2018. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=600

52. **EMA.** *Guideline on Plasma-Derived Medicinal Products EMA/CHMP/BWP/706271/2010.* [Online] 2011. https://www.ema.europa.eu/documents/scientific-guideline/guideline-plasma-derived-medicinal-products_en.pdf

53. **EMA.** *Note for Guidance on Virus Validation Studies: The Design, Contribution and Interpretation of Studies Validating the Inactivation and Removal of Viruses. 1996.* [Online] 1996. https://www.ema.europa.eu/documents/scientific-guideline/note-guidance-virus-validation-studies-design-contribution-interpretation-studies-validating_en.pdf

54. **FDA.** *Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use Food and Drug Administration 1997.* [Online] 1997. https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/OtherRecommendationsforManufacturers/UCM153182.pdf

55. **FDA.** *Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals.* [Online] 1993. https://www.fda.gov/downloads/BiologicsBloodVaccines/SafetyAvailability/UCM162863.pdf

56. **PDA.** *Preparation of Virus Spikes Used for Virus Clearance Studies. Technical report 47.* [Online] 2010. https://store.pda.org/TableOfContents/TR4710_TOC.pdf

57. **ICH.** *Q5A(R1): Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin.* [Online] 1999. http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q5A_R1/Step4/Q5A_R1__Guideline.pdf

58. **EMA.** *ICH Q5A (R1) Quality of biotechnological products: viral safety evaluation of biotechnology products derived from cell lines of human or animal origin.* [Online] 1997. https://www.ema.europa.eu/en/ich-q5a-r1-quality-biotechnological-products-viral-safety-evaluation-biotechnology-products-derived

59. **EMA.** *EMEA/CHMP/BWP/398 498/2005: Guideline on Virus Safety Evaluation of Biotechnological Investigational Medicinal Products.* [Online] 2006. https://www.ema.europa.eu/documents/scientific-guideline/guideline-virus-safety-evaluation-biotechnological-investigational-medicinal-products_en.pdf

60. **WHO.** *Guidelines on Viral Inactivation and Removal Procedures Intended to Assure the Viral Safety of Human Blood Plasma Products. WHO Technical Report, Series No. 924, 2004, Annex 4.* [Online] 2004. http://apps.who.int/medicinedocs/en/m/abstract/Js19651en/

61. **EMA.** *CPMP /CVMP/410/01, Rev. 2 /268 /95: Minimizing the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human or Veterinary Medicinal Products.* [Online] 2011. https://www.ema.europa.eu/en/minimising-risk-transmitting-animal-spongiform-encephalopathy-agents-human-veterinary-medicinal

62. **BioPhorum Drug Substance** *Single-use user requirements* https://www.biophorum.com/resource/single-use-user requirements/