Current challenges and recent advances on the path towards continuous biomanufacturing

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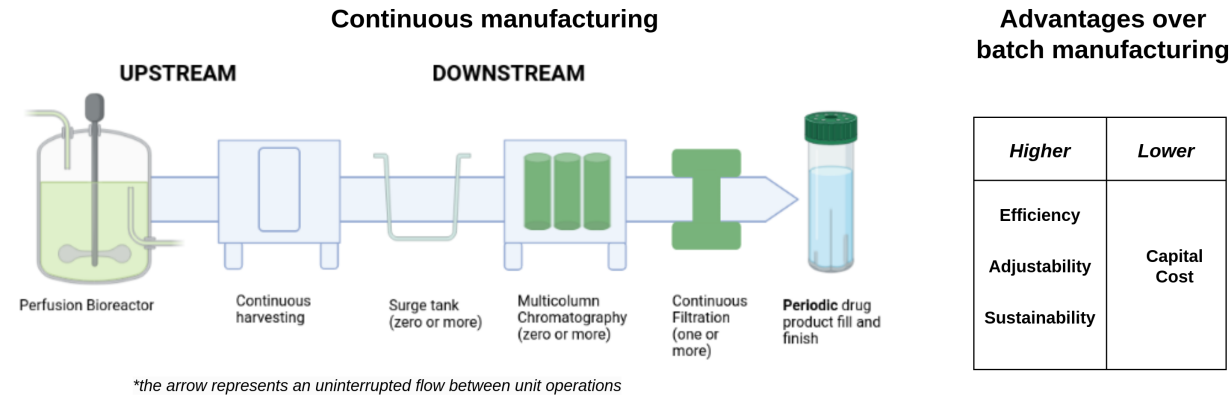
July 7, 2023



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Abstract

Continuous biopharmaceutical manufacturing is currently a field of intense research due to its potential to make the entire production process more optimal for the modern, ever-evolving biopharmaceutical market. Compared to traditional batch manufacturing, continuous bioprocessing is more efficient, adjustable, and sustainable and has reduced capital costs. However, despite its clear advantages, continuous bioprocessing is yet to be widely adopted in commercial manufacturing. This paper provides an overview of the technological roadblocks for extensive adoptions and points out the recent advances that could help overcome them. In total, three key areas for improvement are identified: Quality by Design (QbD) implementation, integration of upstream and downstream technologies, and data and knowledge management. First, the challenges to QbD implementation are explored. Specifically, process control, process analytical technology (PAT), critical process parameter (CPP) identification, and mathematical models for bioprocess control and design are recognized as crucial for successful QbD realizations. Next, the difficulties of end-to-end process integration are examined, with a particular emphasis on downstream processing. Finally, the problem of data and knowledge management and its potential solutions are outlined where ontologies and data standards are pointed out as key drivers of progress.

Keywords: continuous biomanufacturing; process intensification; Quality by Design; process integration; knowledge management;

Introduction

Traditionally, the biopharmaceutical industry is oriented towards batch manufacturing, while the utilization of continuous manufacturing has been quite limited. Batch manufacturing involves multiple discrete steps, where each subsequent step begins only after the previous one is finished. This inter-step dependency typically leads to multiple holding periods that can significantly prolong production, especially when all steps are not performed in the same manufacturing facility. On the other hand, in continuous manufacturing, flow between individual steps is uninterrupted, eliminating hold periods, which in turn may increase production efficacy (Figure 1).[1,2]

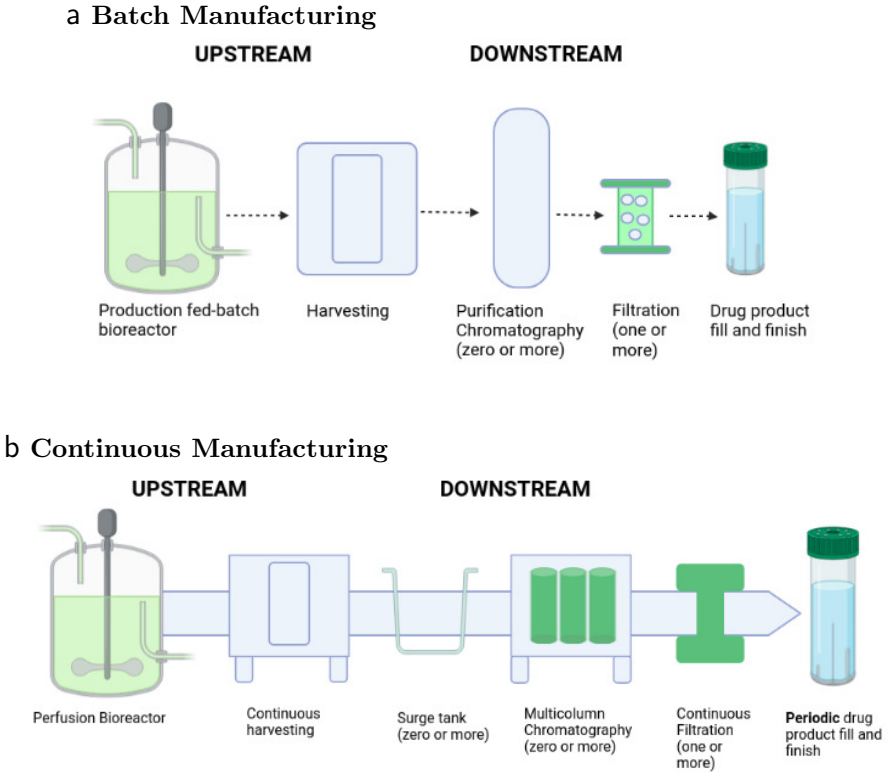

Figure 1: Schematic representation of **(a)** batch biopharmaceutical manufacturing and **(b)** continuous biopharmaceutical manufacturing.

The cause for the strong emphasis on batch manufacturing lies in the fact that the economic benefits within the biopharmaceutical industry have been primarily achieved by product rather than process development. The relatively low number of products that reach

the commercial stage also implies that cost control is imperative, especially in the early stages. In other words, the primary market driver was to minimize production development time and initial investments for which batch production is more suitable than continuous.[3]

The better suitability of batch originates from its discrete nature. Discrete operations can be controlled individually and, as such, require less sophisticated and precise control mechanisms. As a consequence, less process knowledge is required for development and validation, making initial process development faster and set-up investments lower.[3]

Since the first FDA-approved continuous perfusion product in 1993, the primary and dominant use of continuous manufacturing has been restricted to manufacturing protein products that undergo degradation in prolonged culture conditions (e.g., types of growth hormones and blood-related products). In these cases, the employment of perfusion bioreactors (a continuous manufacturing technology) is imperative as it warrants perpetual harvesting and an uninterrupted purification stream, which minimizes the exposure to degradative conditions and thus ensures that product quality is sustained.[4]

Biomanufacturing has, however, come a long way from its "humble roots" of producing recombinant versions of natural proteins, starting with recombinant insulin approval in the early 1980s.[5] The diversity of products has dramatically increased, along with the overall share in the pharmaceutical market, with global sales reaching US$ 336 billion in 2021.[6,7] The constantly growing market implies an ever-growing demand to produce larger product quantities. The market competition (e.g., from the advent of biosimilars) and the pressure to drive down product pricing are also increasing.[8–10] These new market trends indicate that the economic benefit of many biomanufacturing processes (e.g., manufacturing of mAbs) is becoming more reliant on large-scale production efficiency and long-term cost-effectiveness, areas in which continuous manufacturing has an advantage over batch. That is, the integrated nature of continuous manufacturing enables a higher product quantity to be produced during a given time interval and lowers the manual labor required, which also leads to a reduction in facility footprint.[3,11–14] As such, there is a growing interest in widening the utilization of continuous manufacturing.

Another important trend that might enable faster adoption of continuous manufacturing is the shift towards platform processing. Platform processes can be defined as a collection

of distinct parts, components, or modules that are recurrent in the manufacturing of a set of products with common characteristics.[15,16] The recurrent units permit efficient leveraging of prior knowledge when optimizing the production of a new product with those same characteristics. Thus, platform processing has the potential to reduce efforts in production development - one of the critical hindrances of continuous manufacturing implementation.[16,17] The adoption of continuous manufacturing is, nevertheless, proceeding slowly which can be attributed to three challenges: business, regulatory and technological. Business and regulatory challenges are outside the scope of this paper and have been extensively reviewed elsewhere.[14]

The rest of this paper focuses on the major technological challenges in shifting to continuous manufacturing and reviews the current research to overcome them. Three areas are pointed out as crucial for a successful realization of continuous biomanufacturing. The first area represents the obstacles associated with implementing the QbD paradigm, essential for more flexible and cost-efficient production. The second area revolves around the integration of unit processes, where advances in downstream processing technologies are especially significant as they are lagging compared to the upstream ones and are still essentially batch in nature. The final area discussed is data and knowledge management and its role as an enabler of inter-unit communication and perpetual manufacturing improvement.

Quality control and process monitoring

In the early 2000s, quality control in the biopharmaceutical industry was mostly done by the principle of quality-by-testing (QbT). At the end of a processing step, the product is tested per the predefined quality criteria. In case the criteria are not met, the entire batch might be discarded. Consequently, any process corrections may only be introduced for future batches. That is, in QbT the entire manufacturing procedure is rigid, process improvements are slow, and significant losses may occur.[18]

Process stiffness imposed by the QbT severely limits the possibility of responding to any variations in materials or operating conditions. The process flow in continuous manufacturing is uninterrupted, which means that, by adhering to the QbT paradigm, any significant

deviations in input materials or cellular conditions can lead to extended periods of complete production halt. The inability to efficiently respond to changes in operating conditions prolongs the time required to adapt a continuous manufacturing process to changes in product demand. These limitations imply that if maximal continuous manufacturing benefits are to be achieved, shifting from the rigid QbT paradigm becomes imperative. The rest of this section discusses the paradigm shift in process control from QbT and its enabling components to increase productivity, flexibility, and consistency.

Quality by design

The US FDA realized that instead of defining more constraints/tests *per se*, an overhaul of the entire process design procedure was needed, and thus a quality by design (QbD) paradigm was outlined.[18,19] The key idea behind QbD is that process design directly stems from product features. To achieve this aim, attributes that determine the desired clinical performance first need to be identified, namely critical quality attributes – CQA and then connected to the influencing production parameters, namely critical process parameters - CPP.[19–21]

Prior to CQA and CPP identification, a quality target product profile (QTPP) is established. QTPP is a "prospective summary of drug product quality characteristics that should be achieved to ensure desired quality taking into account safety and efficacy".[22] Based on the QTPP, CQAs are identified by risk assessment, as specified in the ICH guidance Q8 and Q9.[23,24] The result of this step should provide a ranking of the CQAs by order of importance and link them to product safety and efficacy. Next, CPPs are usually elucidated by using mechanistic principles or through the design of experiments. CPPs are then further analyzed to identify acceptable operating ranges. Finally, a control strategy is established, which involves risk assessment that considers the criticality of the CQA and process capability.[20] A successfully realized QbD pipeline enables the production process to be more cost-effective and flexible as changes of operating conditions within the specified ranges do not need to be resubmitted for regulatory approval.[20,21]

While the QbD paradigm is beneficial for batch manufacturing, this approach becomes paramount in the case of continuous manufacturing, as detailed process knowledge is an

absolute necessity in creating a holistic process design. Simultaneously, continuous manufacturing maximizes the benefits of the QbD paradigm as it enables integrated, real-time control of CPPs and continuous CQA monitoring, thereby ensuring seamless continuous process verification (CPV) and process improvement over a product lifecycle.[18]

Therefore, intensive research is being conducted to enhance QbD application on a wide production scale. Main areas of focuses are the following:

- Formulation of novel control strategies
- Discovery of critical process parameters
- Advancements in process analytical technology (PAT)
- Mathematical models for process design, control and monitoring

Quality by control

Quality by control (QbC) is the next paradigm shift based on QbD, tailored to continuous manufacturing needs. It augments the QbD by placing active process control as the focal point to create a solid framework for integrated control of a continuous process. Active and integrated process control entails identifying dynamic relationships between critical material attributes (CMAs) and CPPs with CQAs.[18,25] Such connections then allow for the design of control systems with quantitative and predictive capabilities that can, for instance, minimize the effects of upstream perturbations on downstream processing.[18]

Advancement from QbD to QbC is a crucial enabler for end-to-end continuous manufacturing as real-time control of CPPs and CQAs over the entire process would be permitted. Accordingly, this enables the possibility of real-time release testing (RTRT).[26] RTRT leverages real-time measurement data, integrated control systems, and enhanced product understanding for real-time process correction.[18,27] RTRT has the potential to minimize the need for end-product testing, thereby ensuring a faster product release.[27] Although RTRT is also possible for batch processes, this notion can truly be perfected in continuous manufacturing. The integrated nature of the process ensures a holistic approach to control and

enables a more precise controlling mechanism, thus ensuring sustainable production quality over prolonged periods.

In summary, QbC introduces three critical advantages: 1) reduction of process cycle times, 2) higher process reliability and 3) increase in process robustness (insensitivity to variation in process inputs or process parameters).[18] However, the advantages of adopting such a principle in biopharmaceutical manufacturing have not been fully realized. Most operations in continuous processes are controlled individually, their interconnections remain limited, and utilization of dynamic control strategies is low.[18,28]

Dynamic control strategies are able to handle significant process perturbations with minimal manual intervention and production interruption, consequently reducing labor costs and increasing productivity and system robustness. The key idea behind dynamic control strategies is that the control system adapts to the changing environment based on feedback from process measurements.[29] Nevertheless, despite these advantages being demonstrated in other industries (e.g., chemical industry), the adoption of dynamic control in biomanufacturing is still in its infancy.

The primary challenges of implementing dynamic control in biomanufacturing stem from insufficient process understanding and overall process complexity, lack of regulatory clarity on control validation, and in some cases, potentially high computational costs.[30] However, there has been a surge in publications that indicate the feasibility and high future adoption benefit of dynamic control. For example, neural network-based control has demonstrated its effectiveness under system perturbations and parametric uncertainties.[31,32] Different variations of model predictive control have also shown excellent performance for various unit operations.[29,33–36] Additionally, several methodologies for reducing computation burdens are emerging such as model linearization, and reinforcement learning.[37–39]

To fully implement the QbC, an additional requirement is to develop a systematic framework that enables the integration of operation control based on hierarchical process automation principles. An example of a 3-level hierarchical control structure is shown in Figure 2.[18] Level 0 usually controls single unit processes, while level 1 encompasses several of them and enables feedforward/feedback control to minimize process disturbance. Finally, level 2 contains mathematical models that validate measurements, predict the effect of the CPPs

on the CQAs, detect faults and intensify process operations. Potential advantages of implementing this system are better production robustness, rapid perturbation response, a high degree of automation, and continuous process improvement.

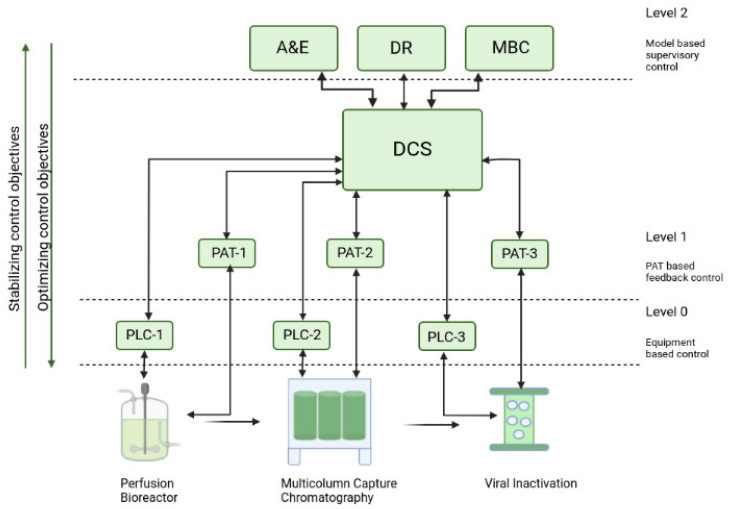

Figure 2: Schematic representation of a simplified three-level hierarchical control architecture for a part of a continuous biomanufacturing process. Abbreviations depicted: PAT – Process Analytical Technology, PLC – programmable logic controller, DCS – distributed control system, A&E–alarm and emergency, DR – data reconciliation, MBC – model-based control.

Feidl et al. demonstrated an implementation encompassing level 0 and level 1 of the control system for end-to-end manufacturing of antibodies.[40] Supervisory control and data acquisition (SCADA) system was used to collect and store data from unit operations, and a monitoring and control system was developed in MATLAB. Level 2 has not been implemented. Nevertheless, over the tested time, the system demonstrated robustness to perturbation, stable production performance, and consistent product quality. Specifically, the control system demonstrated that even though the inherent instability of the cell line caused a 30% titer decrease over the run time, the control system was able to adjust the loading length of the Capture step such that constant mass loading was achieved. This resulted in optimal resin utilization without yield loss and constant input for subsequent units. The system could also respond to pH disturbances in the viral inactivation step and reduce the

amount of High Molecular Weight species(from roughly 11% to less than 1%) in the subsequent polishing steps. Thus, during the entire integrated run-time, the system kept the yield constant at 75% and maintained satisfactory product quality requirements under process disturbances.[40]

An implementation case study of a full 3-level hierarchical QbC system has been presented in Su et al. 2019.[18] The case study (although about producing tablets, a non-biopharmaceutical product) demonstrated that the hierarchical control system could reach the targeted weight set points steadily and automatically. Additionally, the control system was able to shorten the period of diversion of off-spec products under set point changes and process disturbances, thus achieving higher process robustness. This case study also showed that Process Analytical Technology (PAT) and the proper identification of CPPs played essential roles in enabling the paradigm. Next, we discuss CPP discovery and PAT in the context of biopharmaceutical production.

Discovery of critical process parameters

Identification of CPPs is one of the fundamentals of QbD and QbC. Compared to batch manufacturing, active and holistic process control capability is significantly increased in continuous manufacturing. As such, more parameters can be actively influenced to ensure consistent product quality in response to media and processing conditions variation. While physical and chemical parameters are mainly elucidated, understanding how media compounds affect certain CQAs is still an ongoing effort.[41–43] Understanding media compound impact is challenging yet vital, as it could improve the product yield and make the process more robust.[1] The challenge stems from the inherent intricacy of living systems, making a thorough understanding of the mechanisms and inner cell regulatory strategies that affect product formation quite difficult. Understanding media component impact is even more vital in media optimization for perfusion processes as media utilization is one of the significant contributors to perfusion operational costs and footprint. That is, a perfusion process warrants the enabling of the growth of a high-density and high-viability cell culture while minimizing the amount of perfusion media.[44,45]

Recent studies showed the importance of regulating the concentrations of trace metals. In

particular, *Markert et al.* demonstrated the effects of a mix of trace metals and their specific concentration ranges on antibody N-glycosylation patterns, titer, and biomass formation in a series of high-throughput screening experiments.[46] *Radhakrishnan et al.* demonstrated the importance of also understanding the components' temporal effects, as MnCl2 has a varying influence on mAb glycosylation patterns during different growth stages.[47]

Other small molecules can also influence the product quality. An extensive study across multiple scales and different cell lines by *Lobrich et al.* showed the effect of nine small molecule media compounds (cytidine, galactose, glucosamine, uridine, fucose, manganese, ManNAc, glycerol, NANA) as well as copper on the glycosylation pattern in monoclonal antibody production.[48] The impacts of pyruvate on glycosylation profile as well as cell diameter drift, have also been recently explored.[49]

While determining the optimal CPP operating ranges is a primary concern, gaining a better understanding of the mechanism by which CPP affects a specific CQA is crucial for ensuring consistent product quality. For example, a case study demonstrated that glucose predominantly impacts charge heterogeneity by extracellular glycation and not by changing glycosylation patterns or cellular metabolism alterations.[50]

Finally, it is important to note that most research regarding CPPs has been conducted on fed-batch reactors and that further optimization on continuous reactors needs to be performed to achieve an effective QbD and/or QbC. Currently, progress is being made to create efficient transfer protocols from fed-batch platforms to continuous systems. Namely, *Janocheck et al.* developed a protocol whereby a fed-batch process is used as a basis for semi-perfusion shake flask process development and optimization, which was then transferred to a small-scale bioreactor.[51] The reported transfer to a semi-perfusion process implies that cell culture dynamics of truly continuous perfusion are not fully captured by such an approach. Thus, the approach is primarily applicable for initial screenings and establishing acceptable parameter ranges.

In addition to transfer protocols from fed-batch to continuous, dedicated scale-down systems are required for perfusion process development and optimization. Due to batch dominance in the biopharmaceutical industry, historically, scale-down perfusion systems have been lacking. With the increasing interest in continuous manufacturing, specialized high

throughput methodologies, and systems are being created to screen operating parameters and media formulations for continuous culture systems.[52,53] It is important to note that experiments in the cited literature were performed in pseudo-perfusion or semi-perfusion. As previously noted, this limits their utility in fully understanding the cell culture dynamics. For a detailed overview of the challenges of semi-perfusion systems, see *Schwarz et al.*[54]

Microbioreactors connected to cell retention devices at up to several hundred mL scale are also becoming available for optimization and screening. As an example, the ambr® 250 perfusion system was demonstrated to be an adequate scale-down model in terms of viable cell density and volumetric productivity.[55] Also, DASbox bioreactor coupled with a hollowed fiber filter, was demonstrated to be able to support stable culturing of HEK293 at high cell density over a period of several weeks.[54] In terms of downstream processing, dedicated high throughput systems are also emerging. A detailed overview of the current state of the art of downstream high throughput scale down model availability and challenges is given in *Silva et al.* and *São Pedro et al.*[56,57]

For an effective control system, the identification of CPPs and their relationship with CQAs is vital. Additionally, the system needs to have an efficient monitoring strategy of the CQAs throughout the process such that well-timed adjustments of CPPs can be performed. Conversely, the CPPs themselves need to be monitored (e.g., the concentration of amino acids and glucose in the media). This is enabled by the employment of various process analytical technology analyzers, which are outlined next.

Process analytical technology analyzers

Process analytical technology (PAT) is defined as "a system for designing, analyzing, and controlling manufacturing through timely measurements (i.e., during processing) of critical quality and performance attributes of raw and in-process materials and processes, with the goal of ensuring final product quality."[58] In addition to process understanding, there are three critical components to successful PAT implementation: 1) reliable and timely analyzers, 2) signal deconvolution and data analysis methods, and 3) integration with process control systems.[58,59] Control strategies were reviewed in the QbC section, and signal deconvolution and data analysis will be covered in the next section. As such, the rest of this section will

focus on PAT analyzers.

PAT is applicable to both batch and continuous manufacturing. However, the latter can really maximize PAT's potential.[60] Batch manufacturing is driven by tight, predefined recipe specifications as well as acceptance criteria evaluation. Therefore, there is limited opportunity for the utilization of measurement data for real-time integrated process optimization. On the other hand, continuous manufacturing has the potential to replace tight recipe bounds with proactive and holistic control whose success is highly dependent on reliable process monitoring.[18] By being run in a "quasi-steady state," continuous manufacturing also has comparatively more time-invariant properties,[61] thereby making the usage of PAT for process monitoring and control more amenable than in batch manufacturing. However, the very nature of continuous processing imposes additional requirements for PAT analyzers:

1. Compared to batch manufacturing, continuous manufacturing processes are run over extended periods. Hence, employed PAT analyzers need to have long-term stability. Long-term stability is particularly vital for on-line sensors as they directly interface with the bioprocess and, thus, must be resilient to fouling.[61,62]

2. Continuous flow necessitates alignment of scale and throughput between unit operations. As a consequence, PAT analyzers employed for measuring process parameters related to mass and volume flow need to be robust and have a high degree of quantification accuracy.[63]

Most physical and chemical parameters (e.g., pH, conductometry, UV, oxygen levels) are reliably monitored in upstream and downstream processing. On the other hand, monitoring of the quality attributes of biopharmaceuticals (e.g., glycosylation, glycation aggregation, oxidation) is limited chiefly to offline testing, which limits its utilization in dynamic control and poses difficulties for RTRT.[59] Developing analyzers that could accurately monitor these attributes on-line or in-line is thus paramount. Alternatively, sterile sampling coupled with at-line[1] rapid analysis represents another option, given that it can provide data

[1] **On-line monitoring:** measurement where the sample is diverted from the manufacturing process, and may be returned to the process stream.

In-line monitoring: measurement where the sample is not removed nor diverted from the process stream

sufficiently fast for the control system in place. The upstream unit processes usually have a larger timeframe, which makes this part of the process more tractable for at-line PAT implementation.[59]

The most promising sensor technologies include spectroscopic sensors, and *in-situ* biosensors.[64,65] With the advancements in automatic sterile sampling, on-line process monitoring with High- or Ultra-Performance Liquid Chromatography (HPLC/UPLC) columns coupled with a PDA (Photodiode-Array Detection) or UV-Vis detector have become another feasible option, especially in downstream processing, where reaction times need to be faster.[70] Several examples of emerging technologies have been summarized in Table 1. Nevertheless, industrial-scale utilization is still lacking mainly because of stringent documentation needed for analytics in the Good Manufacturing Practice (GMP) environment, lack of highly-trained personnel, high cost of some of the equipment, and the relative newness of most biosensor technology. For a detailed review of this topic, see *Gargalo et al.*[64]

Mass spectrometry (MS) is a very attractive measurement tool to be incorporated into at-line and on-line monitoring. Its main advantages are excellent sensitivity, speed of analysis, the possibility of simultaneous analysis of multiple components and attributes of a heterogeneous biomolecule, and the feasibility of coupling to different separation techniques. Several different mass spectrometry coupled to LC or HPLC systems are currently in development for monitoring different aspects of antibodies.[72] One of the difficulties with MS protein analysis at the upstream stage of the process is that, unless adequately purified, compounds present in the cell media might lead to noisy measurements. Thus, rapid protein purification and sample clean up step prior to the analysis would improve measurement reliability. High throughput protein A purification system coupled with LC-MS has recently been tested on a small-scale to monitor glycation and glycosylation antibody profiles, with the protein purification step contributing to increased sensitivity and the robustness of the procedure.[42] Another promising strategy is the peptide mapping approach based on digestion followed by LC-MS/MS analysis, as it demonstrates good performance on multiple bioreactor scales and

and can be invasive or non-invasive.

At-line: Measurement where the sample is removed, isolated from, and analyzed in close proximity to the process stream.

Table 1: Examples of emerging analyzer technologies for monitoring product-related attributes and their reported application area

Analyzer	**Monitored Product-related Attribute**	**Process Phase**	**References**
Raman spectroscopy	N-glycosylation of bispecific antibodies	Upstream	[66]
Water proton NMR	Flow parameters (concentration and aggregation)	Downstream	[67]
Near-infrared spectroscopy (NIRS)	Antibody concentration	Downstream	[68,69]
Ion-exchange Liquid Chromatography (IEX LC)	Charge heterogeneity	Downstream	[71]

types.[73]

Besides having information about the actual product, monitoring media compounds is also of fundamental importance as they directly or indirectly affect the product itself or the biomass viability and yield.[74,75] Several electrochemical and optical sensors for on-line and in-line analysis show promise for the monitoring of small molecules.[75,76] For at-line analysis Rebel analyzer is a promising solution as it is able to analyze over 30 media components (amino acids, vitamins and biogenic amines).[77] Despite the outlook of current technologies, further improvement is needed as the problems of small molecule analysis are similar to those previously mentioned for product characterization sensors.[65,76]

Mathematical models for process design, control and monitoring

Connecting process parameters to critical quality attributes is indispensable for a robust process design that ensures product quality over time.[78] The standard approach for discovering these relationships is the design of experiments (DoE). DoE is defined as "a structured and organized method for determining the relationships between input factors affecting one

or more output responses, through the establishment of mathematical models." Experimental designs can be further divided into screening designs where the goal is to find which of the selected parameters affect the quality attribute and optimization designs where the goal is to find the range of effect on a specific CQA outcome.[79] While several different DoE methodologies have been created[79,80], factorial, central composite or Doehlert designs are still predominantly used in practice because of their ease of handling.[46,81–84]

DoE methodologies are a powerful approach for an initial systematic exploration of the design space, especially in cases where prior process knowledge is low. Nevertheless, the experimental effort could be potentially high for process optimization based solely on a DoE approach.[85] Batch manufacturing is discrete, and as such, optimizing unit operations independently of each other is a feasible strategy. On the other hand, the holistic nature of continuous manufacturing implies that to achieve the optimal results the individual units must be optimized together. Holistic process optimization implies that the experimental burden needed with DoE is even higher in continuous manufacturing, leading to prolonged development times and high capital investments.[62] Moreover, a higher number of process parameters in continuous manufacturing makes it extremely challenging to find an optimal set of operating conditions by a purely empirical approach.[62,85]

Systematic and simultaneous unit operation optimization is well-established in the majority of continuous chemical manufacturing processes. In continuous chemical manufacturing, empirical approaches are typically augmented by mathematical models that enable an *in sillico* process characterization and optimization to reduce experimental efforts, costs, and development.[86,87] Mathematical models for process optimization and understanding have also demonstrated significant value in the continuous manufacturing of pharmaceuticals.[88–90] However, widespread adoption in process optimization for continuous biomanufacturing is still lacking and is currently at the stage of active research. Based on the amount of system knowledge required, mathematical models can be classified into data-driven (black box), mechanistic and hybrid (Table 2).[91,92]

Data-driven modeling offers the advantage when there is a lack of detailed process understanding. This advantage is simultaneously its biggest disadvantage as it means that predictions are only reliable within the experimental conditions used to optimize the model.[92]

Table 2: Overview of different mathematical model types and their relative characteristics

Model Type	**Process Knowledge Required**	**Model Development Time**	**Model Interpretability**	**Interpolation Capability**	**Model Transferability**
Data-driven Models	*Low*	*Low*	*Low*	*Low*	*High*
Mechanistic Models	*High*	*High*	*High*	*High*	*Low*
Hybrid Models	*Medium*	*Medium*	*Medium*	*High*	*Medium*

Hence, their primary utilization is in the early stages of process development and exploration of high-dimensional datasets.[93] For instance, data-driven models which augment the traditional DoE have demonstrated their value in media formulation.[53,94] Among different data-driven techniques, machine learning (ML) tools seem to show the most promise for elucidating complex, non-linear relations, especially in cases where datasets are inherently noisy.[95] For example, a reinforcement learning approach has demonstrated the capability to reduce the experimental effort in finding an optimal flow rate for continuous chromatography.[96] Nevertheless, while showing excellent prediction capabilities, pure machine learning models suffer from low interpretability, and variability of biases across differing data batches.[97]

Mechanistic models use physical and biochemical principles to simulate the analyzed system and infer the causality between input and output variables.[98,99] Advantages of this modeling approach are high accuracy, good extrapolation, and physical and biochemical interpretation of parameters which is why it has been a predominant approach for cases with sufficient process knowledge. On the other hand, mechanistic models require a high level of mathematical expertise and system knowledge, leading to high model development time and an extensive experimental effort to parametrize the given model.[91,92,99] The two main strategies used in biopharmaceutical process modeling are kinetic-based and flux-based modeling.[99,100]

Kinetic-based modeling represents the system via coupled differential equations.[99,100]

Based on the modelling of the intracellular dynamics, kinetic models of the upstream process phase can be split into unstructured and structured models. Unstructured models represent the system as a function of abiotic variables (e.g., metabolite and nutrient concentrations) and treat the inner cellular dynamics as a "black box." Given that such an approach requires multiple simplifications and assumptions, unstructured models are most suitable for modeling the dynamics of 1) cell viability and density, 2) nutrient and metabolite concentrations, and 3) product titer.[101] On the other hand, structured kinetic models explicitly represent the intracellular dynamics of the cells, such as enzyme kinetics within a particular cellular compartment (e.g., Golgi apparatus). They can thus be suitable for modeling factors such as antibody glycosylation.[100,101] Structured models are usually constructed by first defining a model for the macroscopic system characteristics such as growth kinetics and process-related values. Next, this model's variables are used as initial and boundary conditions of the microscopic model that describes the product synthesis or modification process within the cells.[102,103] An example of such a kinetic modeling technique is described in the glycosylation of antibodies modeling in a perfusion bioreactor by *Karst et al.*[102] Downstream kinetic models try to capture the solute transport from the inlet to the outlet. Based on the model assumption and characteristics, they can be divided into mass transfer models and adsorption kinetic models. For a detailed review of this topic, along with an extensive list of literature examples, see *Shekhawat et al.*[104]

Flux-based models represent the system as a reaction network that defines the consumption and reaction stoichiometry of each species. In general, they require less experimental data for parameter determination than kinetic models. This type of model's central assumption is that the system is in a steady state, which is also its main flaw, as the steady-state assumption cannot correctly capture the dynamic shift inherent to cell culture systems. Also, accurate reaction rate estimation requires having analytical measurements of key metabolites. Flux-based models can therefore be only reliably applied at discrete time points. Due to these limitations, the application of flux-based models to process control is limited. Nevertheless, when the steady-state assumption holds, flux-based models do present a valuable tool for process design and optimization, as has been shown in multi-perfusion bioreactor modeling.[105]

Models that combine flux-based and kinetic models are also emerging. The advantage of combining the two models is that flux-based estimation of metabolites can replace some of the complex kinetic reactions, thereby reducing the number of kinetic parameters that are difficult to estimate or might vary during the upstream process.[106] As an example, *Sha et al.* utilized a flux-based model to estimate the intracellular concentrations of nucleotide sugar donors (NSDs). The estimated NSD concentrations within the Golgi apparatus were then used as an input to the glycosylation kinetic model to estimate the antibody glycosylation profile. The model was able to accurately predict the glycosylation profile impacts of process conditions such as pH shift, temperature shift, inoculation density, and feed composition.[107]

Hybrid models attempt to augment a mechanistic (fully parametric) with data-driven approaches (statistical and machine learning), to enable capturing of phenomena that are highly-nonlinear or not well understood (e.g., the impact of variability in raw material on product CQAs).[108,109] Advantages of hybrid models over traditional mechanistic approaches include higher adaptability to process variations and data heterogeneity, higher capability to be transferred to similar processes and an overall reduction in necessary process understanding.[101,110] Hybrid models demonstrated excellent performance in rapid evaluation and optimization of complex downstream processes.[111] A hybrid modeling approach has demonstrated superior accuracy and robustness compared to a Lumped kinetics mechanistic model to predict breakthrough curves.[112] Also, a hybrid modeling approach has been successfully applied to Single-pass tangential flow filtration (SPTFF) to reduce the overall experimental effort required for method optimization.[113]

In contrast to downstream processing the application of hybrid modeling to perfusion bioreactors is lacking and most applications up to date have only been tested on fed-batch systems. Nevertheless, the principles tested out for fed-batch bioreactors could potentially be extended and adapted for perfusion systems. For example, a combination of a hybrid model and an iDoE was applied to the process characterization of *E.coli* recombinant human superoxide dismutase production, achieving reduced process characterization time and development costs.[114] *Luo et al.* proposed a hybrid system where a mechanistic or semi-mechanistic process model is augmented with a supplemental data-driven model to mitigate low inter-process transferability and lower development time. Such a set-up enables faster

adaptation to a new process as the base structure remains unchanged, while the supplemental model captures the perturbation introduced by process alterations.[103] Also, a hybrid model that combines a macroscopic mechanistic model with a machine learning model for Golgi apparatus glycosylation has demonstrated to be more robust and adaptable to system perturbations compared to a fully parametrized mechanistic model of the glycosylation process.[115]

Finally, process design and model building should be performed synergistically.[85] The model can be utilized to guide the empirical experimentation required, while on the other hand newly obtained experimental results can be used to advance the capabilities of the models.[116] Consequently, a virtuous cycle of perpetual improvement can be created.

In addition to process optimization, mathematical models have become indispensable for employing advanced control strategies. Namely, in continuous manufacturing, there is a greater opportunity to modulate the process outcome by real-time or near-real-time variation of process parameters for which dynamic control strategies are imperative.[18,28] In downstream processes mechanistic first-principle models have been successfully applied for model predictive control (MPC) and model based adaptive control.[30] For instance, *Steinbach et al.* demonstrated the feasibility of adaptive control with respect to different feed rates and resin aging in continuous capture steps.[117] Also, mechanistic multiparametric MPC controller has demonstrated superior performance for a Counter Current Solvent Gradient Purification process in mAb manufacturing.[118] In upstream processes mechanistic model predictive control has also been established.[30] However, due to the high process complexity and many variable parameters, control strategies that are based on data-driven (primarily ML) or hybrid models are also gaining traction. For example, a controller based on a radial basis function neural network has shown adequate performance for time varying parameters, uncertain non-linear disturbances and unmodeled dynamics in a fed-batch bioreactor.[119] Also, a hybrid flux-balance analysis model has demonstrated the ability to reliably capture system dynamics and serve as a basis for feed modulation in baby hamster kidney cell cultivation.[120]

While the process models of unit operations bring benefits to both design and control, the very integrated nature of a continuous process means that holistic models are needed to

truly represent the process in question. Advantages of introducing holistic models include a more comprehensible and quantitative manufacturing risk assessment and deviation detection, a solid foundation for a cost-of-goods model, improved plant-wide control, and better continuous improvement.[99] Moreover, such models can provide a basis for a digital twin of the entire process, which has the simulation capabilities to provide additional insight and help with process design, control, personnel training, and optimization.[121–123]

Two strategies for integrating individual unit operations are present. The first being the unit-by-unit approach, where the unit operations are all simulated individually and are connected in an inlet-outlet manner. Such an approach enables high modularity as individual unit operations are easily switched, and each unit can be optimized independently. However, this approach's sequential nature means that optimization covering several unit operations can be challenging. On the other hand, in a time-step approach the propagation of the entire inlet profile is evaluated across all the unit operation at each time step, enabling genuinely integrated control at the cost of higher computational time and usage of more complex software.[124]

The effectiveness of control or process design models relies heavily on having an ample amount of accurate measurements. Sensors such as Raman or near infrared spectroscopy however provide complex outputs that can not be utilized directly. Therefore, mathematical models are also imperative for deconvoluting the sensor signal and correlating it with desired measurement parameters.[64,91]

The approaches primarily applied for spectroscopic signals are data-driven and are typically multivariate data analytics (MVDA) techniques such as principal component analysis (PCA) and partial least squares regression (PLSR).[76,92] For example, PLSR has been successfully tested for obtaining measurements of small molecules with Raman spectroscopy in cell culture media in a perfusion bioreactor.[125] Machine learning approaches are also gaining importance, especially when the parameters monitored by sensors are highly non-linear.[126] For instance, support vector regression has demonstrated superior performance to PLSR for obtaining measurements of different glycoforms with NIR spectroscopy.[127] Additionally, machine learning has a self-learning capability that could enable real-time sensor calibration and, therefore, potentially increase the robustness of sensors to process drifts and raw

material variability.[128]

In addition to converting raw data, mathematical models are an integral part of soft sensors. Soft sensors are a combination of a mathematical model and one or more traditional sensors, whereby the physical measurements made by the traditional sensors are utilized in the model to infer variables of interest.[85] On-line or in-line soft sensors can indirectly estimate the values of parameters that would otherwise be measured offline.[85,129]

Data-driven and hybrid models are the predominant model types employed for soft sensors. For example, an in-line gas probe in combination with a multilinear regression model was utilized as a soft sensor for biomass estimation in perfusion bioreactors.[130] Also, a soft sensor based on a combination of multi-wavelength fluorescence spectroscopy and a hybrid model demonstrated good performance in estimation of cell concentration, titer, glucose and ammonia.[131] However, it should be noted that several challenges are associated with developing soft sensors: 1) variable process lengths, 2)multiple process phases, and 3) physical sensor faults. As extensively reviewed by *Brunner et al.*, several methods have emerged that can mitigate the aforementioned challenges.[129] Nevertheless, this can introduce further development complexity and, thus, induce further costs and potentially lead to protracted development. Additionally, the accuracy, frequency, and cost of the underlying physical sensor may impact the overall utility of the soft sensor. Therefore, the decision to develop and deploy a soft sensor and any associated design choices should be guided by the purpose and requirements of the application. For instance, an online soft sensor for biomass estimation is particularly beneficial when there is a 1) demand for frequent measurements imposed by factors such as cell-line specific characteristics or processing conditions and control strategy requirements (e.g., utilizing biomass measurements to adjust the bleed rate to minimize the variability in viable cell concentration during prolonged cultivation periods),[132] 2) a need to minimize sampling due to the risk of compromising the sterile barrier (e.g., biomass estimation in continuous aseptic closed systems) or 3) it has demonstrated higher robustness to process fluctuations and background compared to already existing physical sensors (e.g., capacitance probe).[133,134]

Mathematical models, at the heart of soft sensors, can also be applied to detect and reduce the measurement error of physical sensors. Namely, in order to reliably utilize mea-

surement results to achieve more holistic, robust, and proactive process control, as well as RTRT, data reconciliation is required. Data reconciliation can be defined as "a mathematical approach applied to correct imperfect measurement data to satisfy a mathematical model of the process, generally based on mass and energy balances, material property relations, process variance, and dynamics, or correlation between variables." [135] In principle, measurement errors can be split into two broad categories: random errors and systematic errors. Random errors are caused by small changes within the system (e.g., electrostatic fluctuations and air movements). Their correction is relatively well established (e.g., use of simple median filters or polynomial filters).

On the other hand, systematic errors occur due to factors such as sensor fouling and calibration errors (e.g., for Raman or NIR spectroscopy). Compared to random error minimization, correction and detection of systematic errors is more challenging and typically requires the utilization of mechanistic or hybrid models. [131,134,136,137] Data reconciliation methods for the reduction of systematic errors are already relatively well established in other industries, such as the chemical industry. [136,138] However, applying advanced data reconciliation methods in the biopharmaceutical industry is still a developing field, especially in the case of mammalian cells. Nevertheless, with the increased availability of mechanistic and hybrid models, progress is being made. Recently, *Narayanan et al.* demonstrated the potential utilization of a hybrid model with an extended Kalman Filter (EKF) to increase the accuracy of results derived from spectroscopic signals, such as glucose and lactate concentration. [136]

Finally, data reconciliation methodologies should be applied to the soft sensors themselves as the measurement error stemming from the physical sensor can propagate in the soft sensor model and thereby reduce the overall accuracy of prediction. For instance, Steinwandter et al. developed a framework to correct for various sources of errors for a biomass soft sensor. [134] Also, *Ohadi et al.* used an EKF based on a combination of a dynamic mechanistic model and a fluorescence-based soft-sensor to increase the soft-sensor prediction accuracy. [131] For a more detailed review on data reconciliation the reader should look at *Su et al.* and *Brunner et al.* [129,135]

Synchronization of upstream and downstream processes

Regardless of the final product in question, every bioprocess can be split into two major phases: upstream and downstream.[1] Upstream processing is the starting phase of production where the targeted therapeutic is synthesized by prokaryotic or eukaryotic cells,[139] or, in the case of cell therapy, where the cells are expanded to reach the necessary production scale.[140] Downstream processing represents a collection of purification steps necessary to recover the final drug substance with the required purity level from the complex matrix obtained at the end of the upstream process.[141]

Most of the work so far has been predominantly focused on making the upstream phase continuous as it has been proven multiple times that it can lead to higher cell density and, therefore, higher rates of production.[141,142] Over the decades, significant progress has been made in upstream process design and optimization, including cell banking, inoculum expansion, cell retention and separation devices, and bioreactor design.[142–145] For example, modern day single-use bioreactors have the benefit of removing sterilization and clean-up steps along with lower capital costs due to using non-stainless-steel materials and the reduced equipment size.[141,146]

Comparatively, progress in continuous downstream processing has been slow. The most probable reason is that continuous purification systems have begun to be available only recently, and as such, the experience with using them, especially on a manufacturing scale, is limited. Additionally, the heterogeneity between different biopharmaceutical classes makes the number of purification steps, and the type of equipment needed diverse.[1] In addition to technological advancements, a successfully realized continuous process warrants the harmonization between the upstream flow rate and the subsequent downstream purification flow rate. Considering these statements, the rest of the section is split into the following topics:

- Advances in downstream technology
- Integration of upstream and downstream processing

Advances in downstream technology

The predominant techniques for purification are various chromatography techniques, as they possess unrivaled robustness, resolution, and flexibility in both scale and types of separation criteria.[141,142] One of the main factors determining the costs of a chromatography process is the capacity a system needs to have to separate a product stream with a specific concentration efficiently. As the advances in upstream processing in both cell lines and bioreactor designs are enabling the production of, for example, antibodies to reach new titer levels, downstream processes need an adequate capacity boost to process the new material. Thus, the overall process cost is shifted more towards the purification steps.[141] Additionally, for efficient continuous manufacturing implementation, equipment reliability is required over prolonged periods.[1] Therefore, dedicated continuous chromatography systems are needed that can achieve higher capacity and productivity, lesser operating costs, and prolonged functionality.[142,147] Several different methods have been developed to achieve this, including continuous annular chromatography, counter-current chromatography, and expanded bed chromatography.[141,148–151] Also, single use chromatography is in expansion due to flexibility and lower capital costs.[149] For a more comprehensive list of techniques along with their performance indication, see *Rathore et al. 2015* and *Rathore et al. 2018*.[142,149]

During continuous chromatography purification, elution streams stemming from different columns in multicolumn systems are typically pooled. This means that a single defective column could significantly damage the overall quality of the product output unless adequately addressed.[152] More stringent and robust monitoring and feedback mechanisms are necessary to prevent such a failure in a timely manner. The monitoring technologies and control models under development have been discussed in the previous section. However, the additional valves, monitoring devices, pressure gauges, and control hardware needed to ensure product quality of multicolumn systems significantly increase the operational and process characterization complexity. This complexity is also positively correlated with the number of columns. A balance between maximizing production output and operational simplicity is thus necessary.[153] Examples of such column systems that seem to manage to increase the output with minimal added complexity are ChromaCon's Eco Twin GMP and GE health-

care's periodic counter current technology.[153,154] The incorporation of single-use technologies into these systems also presents an attractive alternative option.[146,147,153,155]

In order to fully utilize the advances in upstream productivity, alternatives to chromatography are also emerging. Methods employing monoliths and membranes with large pores have the potential to circumvent conventional chromatography's mass transfer limitation, thereby providing reduced purification time and improved productivity.[156,157] Although the focus is still primarily on adsorptive methods, the interest in aqueous two-phase extraction (ATPE) is also rising due to the method's scalability and productivity.[12] Despite the lower resolution and operational maturity compared to conventional chromatography, the higher throughput and reduced cost make the described technologies potent candidates for integration into downstream purification pipelines.

In addition to purification technologies, continuous viral clearance and filtration systems are necessary. Viral clearance is an essential step to ensure the safety of biologics produced in human or animal cell lines, which, if not handled properly, can lead to prolonged facility shut down for decontamination.[153,158] For proteins that are transiently stable at low pH, a robust method for viral inactivation involves exposing the eluate after a chromatography step to a low pH buffer for a pre-specified time interval. However, continuous chromatography systems involve collecting eluates from multiple columns at varying time points, and an inherent risk exists of over or under exposure to low pH conditions leading to ineffective inactivation or protein aggregation.[158]

To circumvent the problem described above, the discrete concept of incubation time should be translated into residence time distribution (RTD), which is a probability distribution that describes how a material travels within a unit operation of a continuous system.[159] The viral inactivation step should aim to have a narrow RTD as this minimizes the probability of under or over incubation. While existing technologies still have limited commercial utilization, several solutions that demonstrate a narrow RTD are emerging; including a continuous packed bed reactor and a coiled flow inversion reactor.[160,161]

Other essential parts of the purification pipeline are membrane-based filtration systems that adjust the product concentration or buffer exchange.[162] Traditionally, this was done using tangential flow filtration (TFF) over multiple passes until the target volume or buffer

composition was achieved. However, it is challenging to integrate TFF in a continuous process as multiple passes cause a substantial retention time and would thus create a bottleneck in the production stream.[163] Consequently, for continuous manufacturing TFF, was adapted to be single-pass by increasing the length of the flow path and the membrane surface area. Examples of successful implementation include buffer exchange in multiple stages of antibody purification[164] and antibody concentration after anion exchange chromatography polishing.[165]

Finally, pre-formulation and formulation processes of biopharmaceuticals include generating lyophilized solid formulations and high-concentration liquids. For administration and production, high-concentration liquid formulations (HCLF) are preferred compared to lyophilized formulations. However, this approach has challenges, such as aggregation, gelation, phase separation, and high solution viscosities.[166] Various ultrafiltration and diafiltration strategies are employed to enable continuous concentration of products and buffer exchange for the formulation step.[12] For example, in cases of antibody production, some techniques, such as countercurrent diafiltration systems, are used for continuous product formulation.[167] Most often, lyophilization in the biopharmaceutical industry is performed using conventional batch freeze-drying.[168] Although conventional freeze-drying has been a golden standard for manufacturing solid biopharmaceuticals, drawbacks such as high capital cost, processing time variations, and high energy consumption have been recognized. Furthermore, the risk of discarding the entire batch in cases of a process failure is present. Additionally, depending on the dryer design, container closure, and load condition, some concerns related to heat and mass transfer and process scale-up have been reported.[169] Various continuous manufacturing drying techniques emerged as an alternative to conventional batch freeze-drying. One example is the continuous Spin freeze-drying developed by Corver et al. (RheaVita, Ghent, Belgium), where all freeze-drying process steps are integrated into a continuous production line, thereby reducing production time and cost while avoiding scale-up issues.[170] While this methodology was successfully used for more stable biopharmaceutical products, further product-specific evaluations are necessary for determining the effects Spin-freezing has on labile biopharmaceuticals. Moreover, a publication by Lamoot et al. (2023) demonstrates the lyophilization of mRNA lipid nanoparticles using a continuous

lyophilization process.[171] Even though they are still not widely accepted in the biopharmaceutical industry, some novel continuous drying technologies have also appeared, such as Spray-drying and continuous Freeze-drying of suspended vials. For a more detailed review of the emerging drying technologies and their performances, see *Sharma et al.*[169]

Integration of upstream and downstream processing

Along with technological advancements in downstream and upstream technology, another critical aspect to a successful transition is integrating the upstream with the downstream processing.[152] After cell harvesting, a capture step is performed where the goal is to reduce process volume and separate the biomolecule of interest from the harmful materials present in its surroundings.[172]

Integration between the cell harvesting and capture step necessitates synchronizing the upstream perfusion flow rate with the subsequent downstream purification flow rate. To ensure this, employment of membrane chromatography or multicolumn systems might be necessary. An example of a lab-scale implementation of a two-column capture system integrated with a perfusion bioreactor is given by *Steinebach et al.*[173] As an alternative to multicolumn strategy, a single column continuous capture system has been demonstrated by *Kamga et al.*[174] While the single column system has benefits over the multicolumn one because of the reduction in implementation cost and ease of control, an upstream media optimization is needed, as lower perfusion rates are required to make the system feasible.[175] In addition to flow rate synchronization, to avoid process disruptions caused by, for instance, filter clogging, some redundancies might have to be employed, such as surge tanks and backup columns.[152]

It should be noted that surge tanks might also be needed between further downstream steps to maintain lower operating pressure and average out the variability stemming from different process streams. However, care must be taken to decide where surge tanks are placed and how they are controlled as they can increase operational cost and complexity. Thus, a set of general guidelines for surge tank placement and control to maximize their effectiveness has been proposed by *Thakur et al.*[175] Finally, the employment of sterile barriers between upstream and downstream processing and additional contamination control is needed.[152]

In addition to the flow rate synchronization, variations in the content of the flow streams are essential to consider when integrating the upstream process with the downstream. For instance, harvest titer measurement is used to determine the loading of the subsequent capture step, thereby ensuring that the column capacity is adequately utilized and that column underloading (underusing expensive resin) and overloading (wasting product as flow-through (FT)) do not occur. In the case of batch manufacturing, only one titer measurement is sufficient as the discrete nature of batch processing yields a single homogenous harvest pool. In contrast, the flow between unit operations in continuous manufacturing is uninterrupted. Variations during the upstream phase in factors such as cell density and cell-specific productivity can thus cause the titer to vary during the column loading.[176] As such, efficient titer monitoring and a strategy to modulate the operating parameters of the capture step adequately in response to titer changes are required. For instance, *Ramos et al.* used a protein A bindable assay to monitor titer and then modulate load duration and frequency of elution for a dual load capture step to ensure constant mass load. This strategy enabled achieving a constant concentration downstream of the capture step, regardless of the variability in titer during the run.[177] *Karst et al.* demonstrated a feedback control system that modulates the capture step operating parameters of a countercurrent two-column capture based on at-line HPLC titer measurements.[178]

Another key factor to consider when integrating upstream and downstream is the amount and composition of impurities generated during the upstream process. The impurities can be classified into two categories: 1) product-related components and 2) process-related components. Product-related components include variants of the target product, such as precursors, degraded products, and aggregates. Process-related components include cell components (e.g., DNA, RNA, host cell proteins) as well as residual media or digested components (e.g., carbohydrates, amino acids, salts, and lipids). Out of the listed impurities, host cell proteins (HCP) represents the biggest challenge. Namely, the amount and similarity of HCP with the product can cause complications in downstream processing in terms of increased process time, material consumption, and costs. Variation in culturing conditions (outlined in detail in *Gronemeyer et al.*[179]) can shift the HCP profile towards being more similar to the product in characteristics such as pI, molecular weight, and hydrophobicity.[179,180] The increase

in the amount of HCP is often more pronounced in processes that have higher titers.[179] As such, upstream process optimization, such as media composition, is required to reduce the burden on downstream operations. In many cases, such an optimization needs to consider the cost-benefit trade-off between high titer and reduced HCP generation. The HCP profile and amount can also dictate the choice of adequate equipment for the harvesting step, as demonstrated in *Gronemeyer et al.*[179,181] The HCP profile should likewise be considered when choosing the appropriate downstream equipment regarding equipment type, downstream operation sequence, column size and resin capacity, as well as the possible need for duplicity in certain operations.[182] Finally, the understanding of the change in upstream processing conditions that cause impurity shifts should be included in the downstream control strategy such that operating conditions in downstream can be modulated if an upstream perturbation occurs. Conversely, detection of a change in the impurity profile during downstream processing (e.g., increased amount of protein aggregates) should trigger an adequate adjustment in upstream processing.[183]

An alternative to end-to-end integrated manufacturing might be a hybrid system, where only a subset of the production process is run in continuous mode.[1] For instance, *Ötes et al.* made the protein A capture column continuous, while the rest of the downstream purification remained in batch mode. The lower buffer expenditure and higher product yield demonstrated that such a solution is a potential intermediate step before end-to-end continuous processes become a reality.[184] A hybrid system is also easier to integrate with current systems. Additionally, economic analysis demonstrated that depending on the production scale, in some cases, fed-batch bioreactor coupled with partially or fully continuous downstream processing offers the benefit of increased operational simplicity and reduced ecological footprint.[185,186] Another explored option is periodic continuity, where equipment is operated in periodic pulses with scheduled hold and flow steps. While a periodic continuity system has lower specific productivity and increased consumable utilization when compared to a fully continuous system, it benefits a lower facility cost and reduced operational complexity.[187]

Data and knowledge management in continuous biomanufacturing

The QbD paradigm has led to the realization that it is the knowledge and not just the sheer volume of data that should reinforce science-based submission and evaluation of a biomanufacturing process characterization.[188] Consequently, through process understanding, continual improvement can be achieved throughout the entire process lifecycle.[18,59] For example, by obtaining and connecting knowledge from data gathered during research, development, and manufacturing, it is possible to increase understanding of the propagation of variations throughout different lifecycle phases, which can guide the identification and improvement of control objectives as well as reduce process development time.[188,189] Thus, regulatory authorities suggest introducing knowledge management about the product and the process from early conceptualization up to product discontinuation.[188,190]

Knowledge management consists of methods to capture, create, transfer, document, retrieve and reuse knowledge. This is a particularly daunting task in biopharmaceutical manufacturing as a typical process has numerous process parameters and unit steps.[188] In continuous manufacturing, knowledge management becomes particularly challenging as the units are interconnected, and the volume of data generated from process monitoring is increased. The variability in raw materials also has to be documented and stored as it can impact the end-product.[191] Finally, knowledge from ongoing developmental studies and technology transfer needs to be incorporated as well.[188] The rest of the section will analyze biopharmaceutical knowledge management with respect to its three distinct components:

- Data collection and exchange standards
- Recipes and data management systems
- Data contextualization and metadata management

Data collection and exchange standards

The first step toward knowledge management is to develop an effective system to collect and exchange data within the system at all levels of operation and process research

and development phases. Currently, data obtained during biopharmaceutical manufacturing comes in diverse proprietary and non-standardized file formats that vary across vendors.[192] This is particularly problematic for continuous manufacturing as cross-unit communication is needed to enable integrated end-to-end control and decision making.[152] The data obtained in the research and development phases also typically comes in a different format and has different or additional characteristics and features, making it challenging to utilize in technology transfer and data-informed decision-making. Hence, it is essential to have standardized communication protocols and interfaces to achieve integration in production and laboratory environments.[193,194]

Various communication protocols exist in practice for communication between SCADA and individual devices. Furthermore, modern facilities need to enable efficient integration across devices, SCADA systems, manufacturing execution systems (MES), and enterprise-level systems.[195] Currently, the most applied standardized integration protocols for process industries and their supply chains include MIMOSA, B2MML, PackML, BatchML, OAGIS, open platform communications (OPC), and OPC UA (unified architecture).[196] Overview of standards for both types of communication are given in Table 3.

In the analytical and development laboratories, either OPC or Standardization in Laboratory Automation (SiLA) standards are typically used for equipment integration.[193] In other words, it is not that standards do not exist. There are actually quite a few of them. However, no industry-wide consensus has been created, making equipment and systems integration case-specific, costly, and protracted on all levels. Therefore, having a ubiquitous or connected suite of standards that will be universally used in biomanufacturing is paramount for progress.

In addition to standardized interfaces and communication set-ups for an established production system, the ability to interchange equipment during production is gaining importance.[197] Namely, with the increasing use of single-use equipment, prolonged uninterrupted periods of production typical for continuous manufacturing, and the need for fast adaptation to changing market demands, a way to integrate equipment into an existing production line in a streamlined manner is required.

A promising standard for integrating manufacturing equipment has been developed by

Table 3: Overview of current standards for communication between SCADA and individual devices

Standard	**Description**
IEC 61158	The standard specifies industrial communication networks – Fieldbus including ControlNet and Profibus
IEC 61784	This standard is used in design of communication devices and defines a set of protocol specific communication profiles built on the IEC 61158 series and real-time ethernet communication profiles.
IEEC 62591	The standard specifies Wireless communication network and communication profiles – WirelessHART
IEC/PAS 62030 (Modbus)	Modbus standard provides serial communication protocol to connect industrial electronic devices; it is often used for connection between remote terminal units (RTUs) or PLC and SCADA.
MQTT	An exceptionally light-weight publish and subscribe messaging transport for connections with remote locations where small code footprint and/or network bandwidth are of utmost importance.
IEC 62541	OPC Unified Architecture – an industrial Machine-to-Machine (M2M) communication protocol for interoperability developed by the OPC foundation.

Namur under the name VDI/VDE/NAMUR 2658.[198] The key idea behind this standard is the module type package (MTP) and that all units involved in processing should be based on it (both software and hardware) such that all necessary information for integration (e.g., such as communication, services, a human-machine interface (HMI) description, and maintenance information) is already contained within the MTP of the units. Also, work has been done to integrate MTP into higher-level communication based on OPC UA.[197] This setup could potentially enable seamless integration and cross-unit interoperability in a plug-and-play fashion.[197,198]

In the modern manufacturing environment, the role of models and data analysis in process optimization and control is ever-increasing.[199] Consequently, the impact of efficient data utilization is also becoming higher. In recent years, more and more data are generated but need to be post-processed including transforming, connecting, and contextualizing into information and knowledge – the forms easily used by domain experts.[200] Much historical data is also still stored in legacy systems, which increases the difficulty of long-term maintainability and future integration. That is, raw data are difficult to analyze and hence underutilized; and post-processing is difficult and costly. To that end, a unified data framework would be beneficial as it would ease data integration, reduce data post-processing time and associated errors and lead to better data utilization.

For various analytical measurements in laboratories, two frameworks are used. The first is Allotrope that is stable for over a decade now and can cover various techniques and scales but is a for-fee license product.[201] On the other hand, AnIML is easy to integrate and open source but has a lower number of supported equipment.[202] Both have the potential to be adopted at an industrial scale, however, neither of them has good semantic coverage for manufacturing, control, system modeling, and simulation, and neither has been evidently adopted by manufacturers to this date.

Another critical issue in biomanufacturing is that many essential documents such as certificates of analysis (CoA) and batch records are still in many cases paper-based.[199,203] That means a manual entry of data is needed for the information contained within them to be computer usable and hence utilizable for modeling, visualization, and data analysis. This process is error-prone, cumbersome, and it makes checking the validity of the information

more difficult.[199] Thus, an efficient strategy for document digitalization is needed.

Several industry leaders have started the adoption of electronic CoAs, and batch records. For electronic CoAs, an XML-based standard has been created by ASTM (E3077 - 17). The advantage of utilizing this standard and switching to electronic format include fast data transfer, a more streamlined business, and reduced cost of implementation.[204] However, the ASTM standard is primarily tailored for chemical entities and not complex assembled consumables such as certain types of single-use equipment. Hence, further developments of the standard or the emergence of a complementary standard that could handle the previously mentioned complexity are required. Also, a wider adoption across the industry is needed. Finally, the standard is a one-off standard. Therefore, integration with other larger standards, such as B2MML or OAGIS, should take place.

Recipes and data management systems

In addition to data format and communication standardization, a modular and scalable data management system is needed to deal with data integration, assembly, and contextualization efficiently.[191,200] Some of the benefits that a standardized data management system would bring include system reusability and lower implementation costs.[205] In batch manufacturing industries, the basis of data management systems is the utilization of standards defined by the Instrumentation, Systems, and Automation Society (ISA) for batch execution and planning/modeling (ISA-95, ISA-88).[200,206,207]

ISA 95 defines a manufacturing functional hierarchy through a 4-level automation pyramid (Enterprise, Manufacturing operations management, SCADA, and device). ISA 95 is production type neutral and hence can be utilized directly for both batch and continuous manufacturing.[207]

The ISA-88 is batch process specific. It defines the terminology, and provides the data structure as well as the architecture in terms of a physical and functional model.[206,208] ISA-88 standard has gained popularity across different manufacturing sectors because of its intuitive recipe representation, which eases understanding and implementation throughout the entire lifecycle. What makes ISA-88 especially attractive for bioprocess data models are the modular design philosophy and the separation of process requirements from equipment

capability.[209]

The aforementioned advantages are enabled by usage of hierarchical recipes. The recipe is defined in ISA-88 as "an entity that contains the minimum set of information that uniquely defines the manufacturing requirements for a specific product."[206] Given the varying amount of information required by different parts and hierarchy of an enterprise, four different levels of recipes are established, namely general, site, master, and control, that drive from the more generic process requirements down to the specific equipment requirements. These levels are related, and are compatible with the ISA-95 hierarchy. Information in each level varies with respect to the amount of production details present. The ISA-88 also outlines the logical paths of how to transform a recipe in one level to another.[200,206]

Recipes could be used as a hierarchical data structure to assemble data generated from various parts of the biopharmaceutical manufacturing process.[206] In addition to ubiquitous recipe data model, an efficient system for data management that includes storage, dissemination, and analysis is needed.[210] In the same vein, *Fermier et al.* integrated the ISA-88 recipes with data warehousing to create a modular and scalable system for data management throughout the entire product lifecycle.[200] The recipe data warehouse model was recently built upon by Cao et al. in order to adapt the system to continuous manufacturing. Specifically, the enhanced system encompassed more levels of control compared to traditional batch manufacturing as well as a laboratory recipe system to be able to accommodate both on-line and offline measurements. The recipe data warehouse framework was able to capture and transform data from different process levels and locations and therefore aid the process control and decision making. Although pilot tests were based solely on tablet manufacturing, the system has the potential to be extended to continuous biomanufacturing processes.[191] As such, there seems to be a trend to focus on the further evolution of ISA-95 and ISA-88 in the context of continuous biomanufacturing, instead of an attempt to develop a new set of standards. In other words, retaining the intuitiveness of ISA-95 and ISA-88 while also addressing specific aspects of continuous manufacturing should be emerging as one of the critical drivers of progress.

Data contextualization and metadata management

An essential aspect of knowledge management is domain contextualization and an unambiguous and universal representation of concepts that enables cross-system interoperability. Metadata needs to be preserved, contextualized and connected to properly utilize the information available in a dataset. Therefore, universal, explicit, and platform-independent vocabulary is also required to enable smooth knowledge flow, transfer, and generation.[210]

Ontologies enable the representation of domain knowledge in an interoperable way with a logical description of data and information. An ontology can be defined as a controlled vocabulary consisting of a consensus-based common set of terms that enable a standardized description of entities in a domain of interest and their mutual interconnections. In addition to providing a common vocabulary, ontologies are enriched with the logical representation of terms, enabling machine understanding, consistency checking, and inference.[211] Hence, the utilization of ontologies enables knowledge to be standardized, transferable and both machine and human usable. Ontology has also been shown to assist in feature and model selection, causal inference,[212] and automated reasoning[209] in research related to other industries.[212–215]

So far, in the biomedical field and pharmaceutical industry, several prominent ontologies have emerged that cover various aspects of product development and manufacturing. For instance, Ontology for biomedical investigations (OBI) can be used to log biological and clinical investigations.[216] *Coakley et al.* presented an ontological approach to describe a biopharmaceutical manufacturing process with particular emphasis on the CQAs and CPPs involved in each process step,[217] and *Gueblitz et al.* created a QbD compliant ontology for risk management.[218] The Allotrope foundation has also created an ontology called Allotrope Foundation Ontology (AFO) that describes laboratory analytical processes with an accompanying data model that enables data interoperability in practice.[201] *Muñoz et al.* built an enterprise ontology model based on the ISA-88 recipe model and demonstrated ontological approach to recipe management that showed improvement in plant process communication.[208,219] The Industrial Ontology Foundry (IOF) aims at producing a highly axiomatized suite of ontologies to enable the connectivity of information throughout the digital thread of a product.[220,221] While these ontologies are not necessairly compatible, fortunately there

is a trend to converge on the top-level ontology called Basic Formal Ontology (BFO).[222] Hence, there should be an emerging unified ontology for biomanufacturing that would serve as a grounding point for integration and that would assure interoperability across distinct domains and activities involved in biomanufacturing.

To that end, Figure 3 enhances the information management outlined in *Fermier et al.*[200] (the bottom box – high-performance data processing and retrieval) with the knowledge management and exploration layer using ontology (the top box – domain expert-friendly knowledge graph tool). This top layer provides scientists or other domain experts, such as process engineers, easy access to the full digital thread of data throughout the product life cycle using graph data technology and standardized terminology via ontology. Graph data provide readily connected data so scientists can explore without relying on IT support. The dotted lines represent the type of personnel needed to perform the data management function. The data management functions outlined in *Fermier et al.*[200] require IT specialists to develop them so scientists can use pre-built data analysis functionalities in the bottom orange box. This stifles innovations from data because scientists cannot directly interact with the data. With the knowledge graph layer, scientists can visualize and understand ontology. They can also interactively explore data represented according to the ontology in the graph mart (note that the color dots in the graph mart are instance data of the corresponding terms in the ontology) and then experiment with the data using analysis tools compatible with graph data. Once they know what they want, they can communicate requirements (data analysis) to IT specialists who can implement them in higher-performance systems (than knowledge graph systems) for real-time adaptive control or performance monitoring.

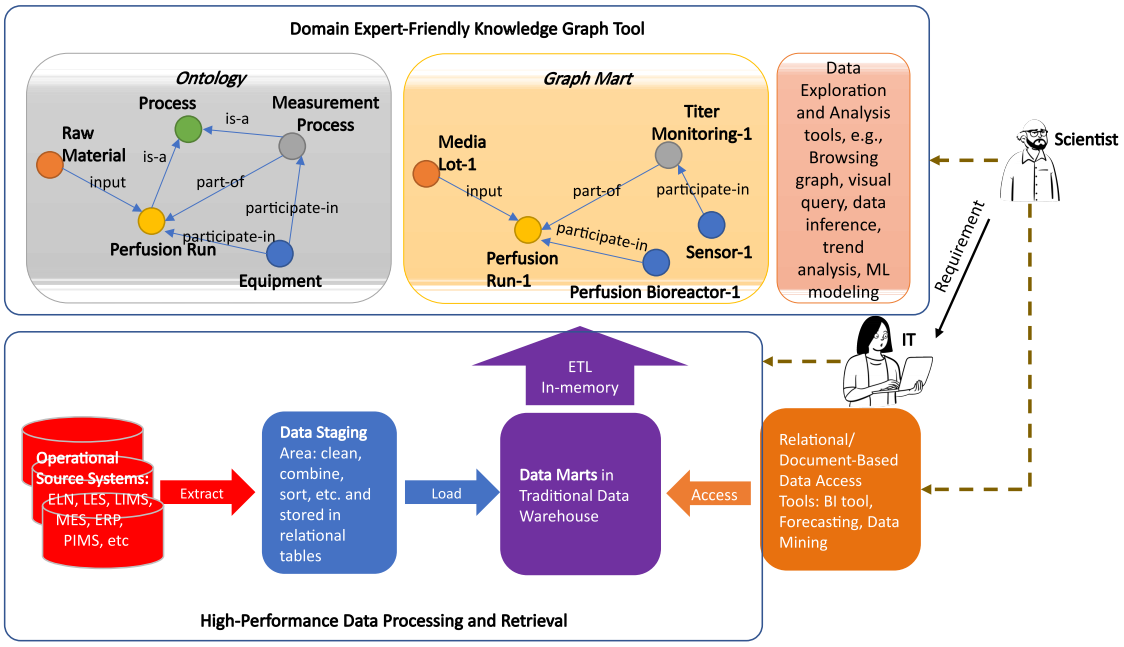

Figure 3: Notional data and knowledge management architecture supporting continuous manufacturing development and execution

Conclusions and future perspective

Biotechnology has been recognized as an industry of increasing economic impact and potential for innovation, with the biopharmaceutical industry being its integral, rapidly growing part.[223] Thus, innovations and advancements in biopharmaceutical manufacturing could bring far-reaching benefits to the entire global economy. Transitioning from batch to continuous biomanufacturing has been acknowledged in both industry and academia as an essential driver of progress in the modern biopharmaceutical market. However, this process is still in its infancy, and multiple hurdles need to be surmounted. Primarily, improvements associated with QbD and the PAT initiatives are needed, along with advancements in equipment, information and knowledge integration.

Quality by Design has become one of the cornerstones of biopharmaceutical manufac-

turing because its implementation reduces product variability and eases any post-approval changes, given that they are within the submitted design space. Due to its integrated nature, continuous biomanufacturing has the potential to build upon the QbD paradigm and enable real-time release. For this to become a reality, advancements in the active process control and process monitoring technologies are required.

Process control is shifting towards increased utilization of model-based control strategies, with a mechanistic or hybrid data-driven model at the core. Significant breakthrough was achieved at the level of unit processes. Moreover, the lacking holistic approaches are actively researched. However, one of the hindering factors for adoption is the need for more regulatory clarity, especially in the case of hybrid models. To mitigate this challenge several possible efforts could be undertaken. Namely, companies should work with regulatory agencies through channels such as the FDA Emerging Technology Team (ETT). The ETT provides a "safe space" to identify and resolve any regulatory or technical issues before filing a regulatory submission.[224] Consortia-based efforts should also be undertaken to provide community-based proof of principle demonstrations that can 1) raise awareness of a need for, as well as demonstrate the value potential of a particular technology and 2) be used as a basis for further regulatory discussions (e.g., through means such as the NIIMBL RCC).[225,226] An example of such an ongoing project is the NIIMBL Big Data model-based adaptive control project.[227] Finally, to further facilitate adoption, more studies that benchmark the performance of advanced control strategies compared to more traditional control should be performed.

Regarding process monitoring, minimizing reliance on offline analysis is paramount. Consequently, as part of the PAT initiative, innovative devices and methods for on-line, in-line, and at-line monitoring are being developed. The most promising technologies include spectroscopy techniques, mass spectrometry, and soft sensors. Nevertheless, widespread adoption has not occurred because of the lack of trained personnel, strict GMP documentation requirements, and in some cases, relatively high cost of implementation. To facilitate further adoption, investing in dedicated workforce development programs is needed.[226] Also, more studies that address the business value, return on investment, and limitations of a particular PAT technology should be conducted. An example of such a study and its benefits is

provided in the recent PAT benchmarking survey conducted by BioPhorum Development Group.[228]

Connecting the equipment to allow for uninterrupted flow between unit processes is fundamental for achieving continuous biomanufacturing. Upstream continuous technologies are relatively better developed and are available on a commercial scale. On the other hand, downstream continuous manufacturing remains an active research problem. The most notable advancements made are novel continuous chromatography and viral inactivation systems. Also, in both upstream and downstream manufacturing, single-use equipment has been significantly gaining traction. Finally, synchronization of upstream and downstream phases of the process remains a primary concern, where balancing the trade-off between cost and complexity with process robustness is particularly difficult and should be further explored. As a viable alternative, partially continuous (hybrid) systems have emerged, allowing greater flexibility and easier adaptation of current production lines.

Cost effective and efficient systems, information, and knowledge integration is one important axis to enable continuous biomanufacturing. While there are advancements in all three fronts of integration, ubiquitous or well-connected suite of standards is yet to be available. Projects at NIST's System Integration Division and NIIMBL are underway to both improve efficiency around standard development/deployment and create new and better standards that are more domain expert friendly.[221,229,230]

Disclaimer

Certain commercial software products are identified in this paper. These products were used only for demonstration purposes. Their use does not imply approval or endorsement by NIST, nor does it imply these products are necessarily the best available for the purpose.

Conflicts of Interest

All authors have no conflicts of interest to disclose.

Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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