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Technical State-of-the-Art and Risk Analysis on Single-Use Equipment in Continuous Processing Steps

DSP model guidelines antibody risk intensification production technologies mitigation upstream state-of-the-art mAb implementation biopharmaceuticals manufacturing CHO management FMEA scale equipment downstream continuous single-use regulatory productivity strategies processing cultivation GMP perfusion analysis integration USP

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1 Introduction

Biopharmaceutical manufacturing has traditionally been performed with batch and fed batch manufacturing concepts. In recent years, continuous manufacturing has been gaining momentum as the biopharmaceutical industry has realized its significant potential for process intensification [1]. Continuous bioprocessing improves process productivity by shortening the overall processing time and reducing the size of unit operations, which, in turn, translates to buffer, resin and consumable savings. Furthermore, it enables the coverage of larger capacity demands with relatively small single-use devices and provides enhanced flexibility and improved process consistency.

Multiple biopharmaceutical manufacturers have explored the impact of continuous manufacturing technologies and the integration thereof [2–5], and several end users have already performed technical runs in a good manufacturing practice (GMP) environment during 2019 [6]. Finally, the first biosimilar monoclonal antibody (mAb) from continuous downstream processing (DSP) has been approved for phase 1 clinical trials in early 2019 [7].

With biopharmaceutical companies implementing continuous technologies at manufacturing scale under current GMP (cGMP) conditions, the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guideline Q9, which requires the industry to address the risks associated with continuous processing, necessitates closer consideration.

The present document investigates technical risks and questions associated with continuous biomanufacturing in both upstream processing (USP) and DSP using single-use equipment. A systematic risk analysis and prioritization through a failure mode and effects analysis (FMEA) is performed for a monoclonal antibody process at 500 L production scale. For every processing step, the technical state-of-the-art, most prominent risks and mitigation strategies are discussed. The mitigation strategies summarize suggestions from industrial and academic end-users, as well as suppliers and take statements from regulatory authorities and existing guidelines from further industry collaborations into consideration [8,9]. The authors aim to harmonize efforts towards the implementation of continuous processing technologies.

2 Risks and Mitigation Strategies

This docment systematically assesses and prioritizes technical risks associated to various stages of continuous USP and DSP through an FMEA. An FMEA allows to systematically evaluate and quantify the risks associated with continuous processing and follows the ICH guideline Q9 on quality risk management [10]. A model process for a Chinese hamster ovary (CHO) cell-based mAb production was defined to set the scope of the risk analysis. The process steps shown in Figure 1 were identified according to current state-of-the-art mAb production. It was defined, that the mAb production is operated over a 30 day period using a 500 L production bioreactor and employing only single-use equipment in both USP and DSP.

An FMEA, limited to risks that are of a technical character, was performed for every process step defined in the model process. Risks were included if either (1) they are of greater significance in continuous processing than in batch processing or (2) they are unique to continuous processing. Risks associated to general batch processing were excluded and represent the reference on which the FMEA is based.

The risks assessed concern general operations or life cycles present during initiation, as well as throughout the continuous process. The intention is to address risks associated with the dynamic or cyclic nature of the process, its instrumentation, equipment and process control.

This publication provides an overview of the continuous working principles, presenting the state-of-the-art technical solutions and discussing the main risks identified for every USP and DSP step of the model mAb production process. It needs to be acknowledged, that certain risks cannot be directly linked to a single processing step but may concern multiple steps of the overall model process. These topics are summarized in section 3.

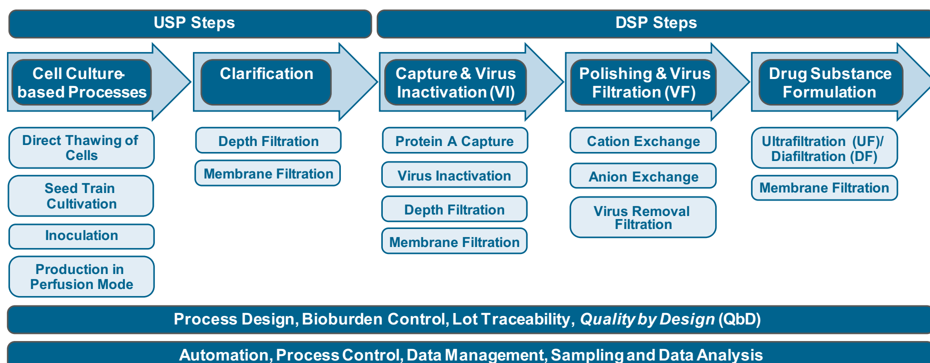


Figure 1: Defined USP and DSP process steps for the continuous manufacture of a mAb (model process) and points to consider for the overall process.

2.1 Continuous USP

Continuous USP aims to produce a cell-free supernatant containing product (in this case the mAb) using a perfusion culture of mammalian (CHO) cells. Unlike continuous DSP, continuous USP is a reality and already several commercial perfusion-based protein products can be found on the market today [11]. Such cultivations are performed in suspension culture according to the scheme shown in Figure 2 and require a stable cell line suitable for perfusion cultivations.

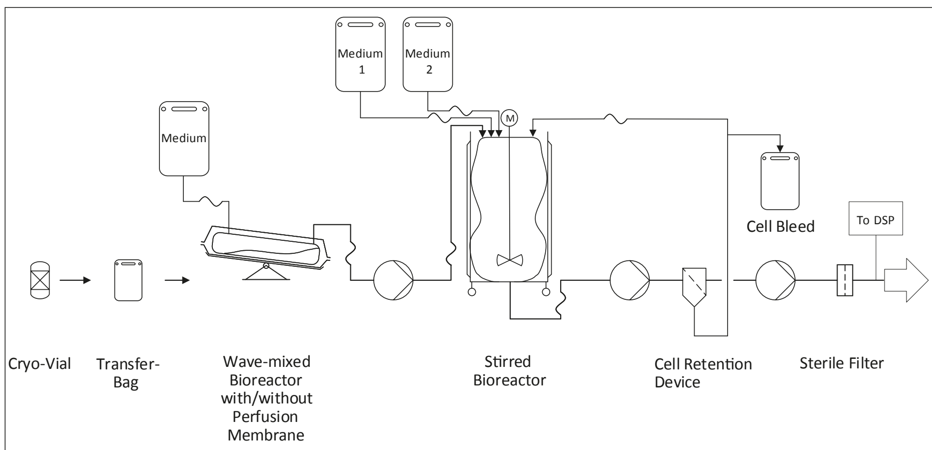


Figure 2: mAb production: Continuous USP process using single-use equipment.

Transfer of the cells into a suitable single-use bioreactor can take place immediately after thawing using a transfer device or containment. Alternatively, the cells may also be cultivated in shake flasks and shaken in an incubator for 2 to 3 weeks. The inoculum generated in this manner is then transferred to a wave-mixed or stirred single-use bioreactor for 7 to 9 days (step N-x), where a high cell density cultivation, using a cell retention device, may be undertaken. If a continuous perfusion process is to be employed during the seed train, the corresponding bioreactor has to be equipped with additional pumps and a cell retention device. Additional sensors like single-use biomass probes for automated seed transfer to inoculate the next n-stage can provide an additional level of process control for perfusion processes. Frequently used cell retention devices are: (1) continuous crossflow filtration systems such as hollow fiber systems, tangential flow filtration (TFF) systems or the alternating tangential flow filtration (ATF) system, (2) built-in perfusion membranes in wave-mixed bags, (3) continuously operating centrifuges, (4) acoustic cell retention devices such as low frequency standing wave cell retention systems, (5) hydro-cyclones and (6) gravity sedimentation devices such as inclined settlers.

After the seed train, the mAb production process again relies on a single-use bioreactor for a high cell density cultivation using one of the cell retention devices mentioned above. Just as with the seed train perfusion process, the single-use bioreactor must be equipped with additional pumps, vessels and a cell retention device to ensure a well-controlled perfusion mode. Here, sensors like single-use biomass probes can support automated cell bleed. Depending on the cell retention device used, supernatant can either be directly transferred to the DSP or requires an additional clarification step by depth and membrane filtration.

The FMEA identified specific risks that are to be associated with the individual processing steps of thawing, seed train production, inoculation during the seed train cultivation and the mAb production in perfusion mode. In addition, the FMEA showed further risks surrounding equipment suitability, especially that of exhaust gas and perfusion filters, equipment performance over extended processing times and bioreactor design.

2.1.1 Thawing

Appropriate cryo-vials or cryo-bags containing cells from the working cell bank are used to inoculate the seed train bioreactor. This process is a batch operation and not considered as a continuous unit operation. The risks and risk mitigations are identical to that of a batch process and are not within the scope of this study.

2.1.2 Seed Train Cell Cultivation with Perfusion

The transfer of cells from cryo-vials or shake flask cultivations represents a batch operation. The perfusion culture is initiated after this transfer. A high-density perfusion at the N-x stage enables high-density inoculation of the production bioreactor which reduces the costly operation time of the production bioreactor, meaning more production campaigns can be run throughout the year and the mAb titer may be increased.

The bioreactor is inoculated with a defined cell density, based on specific strategies, when a perfusion is started. The primary goal of the seed train cultivation is the generation of viable cell biomass, therefore the supernatant is generally not collected. Product retention in the seed train bioreactor is, thus, not as critical as it is in the mAb production bioreactor.

Typically, only one perfusion medium is used to reduce the complexity of the process and a cell specific perfusion rate (ramped feeding vs. capacitance-controlled feed) is set. Critical process conditions, which must be ensured, are: (1) constant working volumes, (2) dissolved oxygen (DO), pH, temperature set points and (3) the duration of cultivation.

Criteria for the transfer of the generated inoculum to the mAb production bioreactor are the viable cell density and the physical status of the cells, which must be in the exponential growth phase. The increased process duration in continuous operation mode is typically associated with: (1) an increased

culture media shelf life with coinciding pH stability as media is stored connected to the bioreactor over the duration of the process, (2) a need for prolonged stable sensor performance, (3) a prolonged performance of single-use pumps and further single-use components and (4) 8 to 100 times higher cell densities, compared to traditional seed train productions. Consequently, the pH control during seed train cultivation is of high importance. The pH of the perfusion medium has to be identical or close to the process set point to avoid the use of high quantities of pH correction solutions. For further mitigation of risks, the authors refer to sections 2.1.3, 2.1.4 and 2.1.5.

2.1.3 Inoculation During Seed Train Cultivation and mAb Production

As described in section 2.1, the seed train consists of various stages, which each include manual handling, sampling and transfer steps to the next stage. Mitigation strategies for risks associated to these operations are summarized in section 3 of this publication.

The condition of the inoculum, concerning viability and exponential growth, is a critical parameter, requiring consistent and reliable observation of the culture using capacitance probes, off-gas analyzers or online monitoring of key substrates. Compared to batch processing, automated process control gains importance and it is expected that the choice of sensors and options for automated control will increase in the near future.

2.1.4 mAb Production: Stirred Single-Use Bioreactor with External Cell Retention

During the continuous production of mAb in the production bioreactor, the required process conditions are almost identical to the perfusion during the seed train cultivation, with few relevant differences: the perfusion rate is not only a function of cell density but may be influenced by substrate, metabolite or product concentrations. It is desired to keep the USP perfusion rate and mAb titer as constant as possible to facilitate harmonization of subsequent DSP unit operations. A strategy to support this requirement is the use of multiple media with adjusted and more complex compositions. Culture media specifically designed for perfusion operations have become available, facilitating the adoption of the continuous bioreactor mode, but the use of multiple media remains a reality [12]. Also, cell bleeding can be used to control the perfusion cultivation.

In addition to the specific features already mentioned in section 2.1.2, the following three are associated with the continuous mode in the production bioreactor:

1. Interactions between different medium concentrates (possible precipitation of components) since perfusion cultivation can use different concentrated media feeds to reduce perfusion volume

2. Product concentrations are identical to bioreactor concentration in the permeate flow (product sieving should be avoided for membrane-based cell retention devices)

3. Transport of proteases and lipases and other enzymes out of the bioreactor (avoidance of product degradation in the bioreactor and following DSP).

2.1.5 Equipment Suitability

In continuously operated single-use bioreactors, a critical point is seen in the plastic material robustness and mechanical stability, e.g. of plastic layers or single-use impellers becoming brittle and prone to breakage during long process times. The long-term usage of the single-use equipment in general was identified as the main reason for the risk. Single-use equipment was originally designed to be used in batch or fed batch processes, with a duration of 7 to 21 days. Therefore, the equipment manufacturers had to find a best practice way of identifying plastics that have sufficient durability in long process runs and survive e-beam and gamma sterilization, which leads to material stress and may impair the shelf life of the single-use equipment. In addition, the selected materials need to be free of cell-growth inhibiting leachables which becomes relevant for storage bags for perfusion media.

Several companies such as Wuxi Biologics have published successful perfusion runs of more than 30 days in stirred and wave-mixed single-use bioreactors [13]. In this context, material degradation through e-beam and gamma sterilization merely represented a minor factor. The suitability and stability of the single-use equipment over extended processing times of more than 30 days remains a low risk. However, especially for single-use sensors it can be advisable to perform an equipment characterization under worst conditions.

Connectivity with aseptic connections remains a potential issue with small-scale bioreactors as it displays the basis for aseptic handling: bioreactors for process development may use simple connections which can be prone to leaks. It is advisable to work with more sophisticated connectors also at small scale. In GMP processing, high efforts were made by equipment manufacturers to provide simple and validated aseptic connectors or weldable and sealable thermoplastic tubes. When trained in proper usage, these connections enable aseptic and stable connections during shipping, irradiation, filling and operation, even under higher pressures.

A topic that should not be underestimated in the risk discussion is filter blocking of the exhaust gas filter. While the inlet gas filters are adequately designed to the required capacities, exhaust gas filters may suffer from blocking risk. For long-term processes, the exhaust gas filters require special attention to defining the right dimensions. Exhaust gas filters in standard configurations may be undersized for withstanding the longer exposure time and higher biomass load in the outlet gas. The method and optimization of the exhaust gas cooling or heating to reduce possible biofilm generation in the exhaust gas filter needs to be addressed.

Reduced filter permeability for media and breakdown of the perfusion rate, resulting from perfusion filter blockage, were further identified as high risks. Reasons for this may be wrong pore size, filter type or fil-

ter size. Further reasons are the appearance of filter fouling, due to biofilm formation during the process run and/or low pump rates through the filter. A possible solution is to replace the filter during the run. The regeneration of filters by back-flushing during the process run might also represent an option but is rarely done as this implies new risks. For mitigation, good scale-down models for perfusion processes are essential for an optimized sizing of the filter, allowing predictable process runs.

With continuous cultivations, heat transfer is addressed as a potential risk for two reasons: firstly, continuous addition of fresh medium at room temperature requires the dedicated control system to constantly adjust the temperatures. Secondly, temperature loss from external pump circuits can add to the challenge of temperature control, while simple systems to heat external tubing can facilitate constant temperature control. Temperature control in single-use bioreactors has been shown to be as accurate as in stainless-steel or glass bioreactors and optimization of bioreactor design with usage of heat-blankets for perfusion filters may provide possible solutions.

In conjunction with high cell densities achieved in continuous cell culture processes, high demands are made on mass transfer, especially with respect to oxygen transfer and CO_2 removal. In single-use bioreactors for perfusion cultivation, impeller and sparger design should be carefully selected to support the higher k_La demand and allow adequate CO_2 stripping capacity. It is advisable to select bioreactor equipment with both micro- and macro-sparger designs and to work with impeller size and design, which provides sufficient power input at moderate agitation levels. Reports indicate that high volumetric mass transfer coefficients and transfer rates up to k_La values over 15 h^{-1} are required, when assuming an oxygen uptake of 5.14 – 5.77 pmol/CHO cell*day, to maintain up to 150 to 200 million viable cells per mL, which have been reported for single-use bioreactors [14-16].

It is a fact that equipment manufacturers are steadily optimizing their single-use bioreactor systems to ensure k_La/OTR and CO_2 evolution rate (CER) values which are more appropriate for long-term perfusions. This requires optimized mixing and sparger systems. In order to ensure effective mass transfer with sparger systems and gassing under harsher conditions, it is stringently required to address foaming risks and related mitigation techniques. Foam control can be achieved using foam sensors and the automatic addition of antifoam chemicals or mechanical dispersion. Furthermore, customized versions of single-use foam separators for biomanufacturing processes are available for purchase from equipment manufacturers.

2.1.6 Clarification

Following mAb expression in the production bioreactor, clarification by depth and membrane filtration may need to be performed, depending on the retention technology used in the perfusion. Depth filters and sterilizing grade filters are well-established in batch productions, with the process risks associated therewith being generally well-understood. The FMEA has identified risks associated to filter exchange procedures, bioburden growth, and variations of flow and product quality as main discussion points.

Risks associated with filter exchange protocols are primarily linked to the dead-end nature of filtration, which requires a repetitive exchange of blocked filter capsules. In sterilizing grade filters, the prolonged exposure time comes along with the additional risk of microbial penetration of the sterility barrier, which also requires a periodical replacement of the filter capsule. A procedure thereof is described in more detail in section 2.2.4. A further consideration may be to employ pre-filters or filter trains to reduce the risk of filter breakthrough and to implement breakthrough monitoring.

A process risk associated with the filter replacement is bioburden control. In depth filtration, these risks can be partially mitigated by mild sanitization or autoclaving the small depth filter capsules. The subsequent bioburden control filter provides an extra level of bioburden control in clarification. To further mitigate the risk, manifolds with multiple filter assemblies should be considered, permitting the switch of filters without breaching the closed system.

Variations in flow rate or product and impurity content may result in pressure fluctuations and potential differences in the flow resistance profile during filtration. It is recommended to perform a process characterization of the filtration, taking into account variations of feed quality and flow rate when designing the worst-case scenarios.

At this point, the authors would like to point out that filtration steps are also part of DSP steps, typically after low-pH virus inactivation or after final ultrafiltration and diafiltration.

2.2 Continuous DSP

Continuous DSP aims to manufacture the mAb drug substance while focusing on high product yield and purity and high process productivity while increasing capacity. The authors will discuss aspects of continuous bind-elute chromatography, virus inactivation, flow-through chromatography, virus removal filtration and final ultrafiltration as well as diafiltration below.

2.2.1 Bind-Elute Chromatography

Chromatography steps, to remove impurities and to ensure a reliable product quality, play a key role in DSP. For example, bind-elute chromatography is used in the protein A capture step and in the cation exchange polishing step of the model process.

In recent years, several companies have developed continuous chromatography systems which operate with two to eight parallel columns at GMP manufacturing scale. The chromatography sequence in principle is similar to a normal batch mode process (with steps such as loading of product, washing, elution of product, regeneration and equilibration of the column) with the exception that in the steady state condition of a continuous chromatography, several parallel columns are simultaneously in different phases

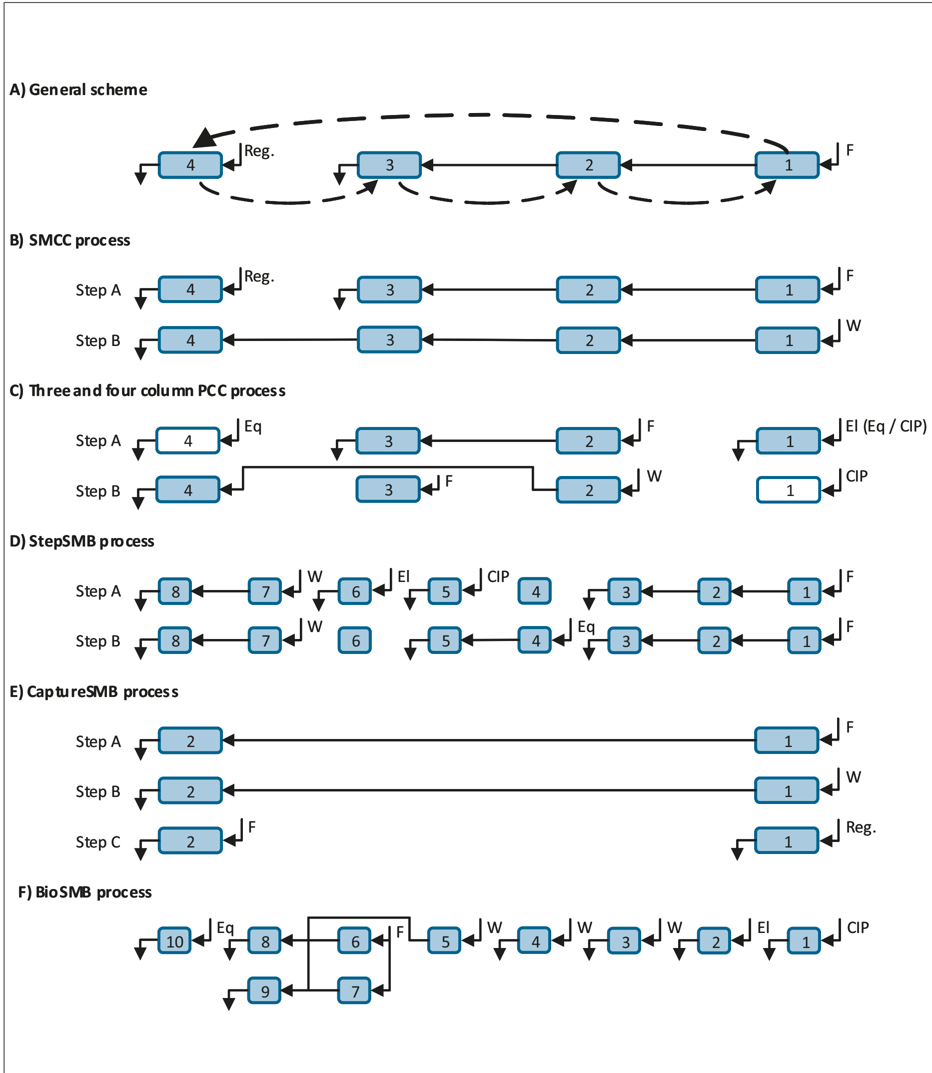


Figure 3: General difference between continuous chromatography concepts (modified after [17,18]. The general scheme of multicolumn chromatography (A) shows feed flow through columns 1-3 while column 4 is taken offline for regeneration. While the liquid moves from right to left, the columns move against the direction of liquid flow. Schemes B-F show different modes of multicolumn chromatography applied in todays processes. ***CIP cleaning in place, El product elution, Eq equilibration, F feed, P Product, PCC periodic counter-current chromatography, Reg Regeneration, SMB simulated moving bed, SMCC sequential multicolumn chromatography, W Wash.***

of the process. As the loading of the column is often time consuming, this sequence can be optimized by running two or more columns at the same time in parallel. Two columns can be connected in series during loading where the second column captures the breakthrough of the first. Consequently, a continuous chromatography approach can enable higher loading capacities with shorter residence times and therefore increased productivity. Figure 3 illustrates the principles of continuous chromatography and the different approaches that have been developed so far.

It is obvious that continuous or continual multicolumn chromatography (MCC) has an impact on the chromatography system as it requires a more complex flow path with a higher number of valves, pumps and a sophisticated process control. Different companies offer solutions from process development to manufacturing scale using two to eight columns.

The FMEA showed that the majority of risks in continuous bind-elute chromatography are associated with the extended operating time and changes or inconsistencies that can occur during the process. In addition, column packing consistency and virus safety have been identified as critical points.

2.2.1.1 Column and Process Consistency

In the area of column and process consistency, main topics involve packing consistency, column fouling and dynamic changes in feed quality.

Packing Homogeneity

In continuous DSP, the risk of column consistency alterations [19–24] might be higher due to the longer processing times and the multiple columns which are used in the process. Therefore, a check for consistency before, during and after the process might be necessary for all columns.

End users of chromatographic columns have both used self-packed and pre-packed columns for single-use operations. Pre-packed columns are mostly provided together with a dataset, containing data on the height equivalent of a theoretical plate (HETP), asymmetry factor (Af) and pressure drop [25, 26]. A pre-use consistency check might then not be necessary. However, to have a full set of data available and account for risks of transportation, it is recommended to execute a pre-use check in addition to the checks during and after the process step.

In chromatography, the elution profile and baseline of columns can already serve as a first indication of column health. In addition, the cyclic nature of MCC can allow one or multiple columns which are not in the protein load sequence of a cycle, to undergo HETP and Af analysis. This requires a long non-loading step in which the columns can be checked. The use of acetone in a GMP environment is undesirable; also, the use of an additional solution (i. e. 0.8 M NaCl) would mean additional hardware resources (i. e. an additional system inlet/pump/valves). Instead, the existing buffers can be used for the health check of columns through transition analysis. Some buffer changes within the process, e. g. from wash buffer to sanitization buffer or from sanitization buffer to equilibration buffer, will result in a change of the

outlet conductivity, pH or UV signal in the chromatogram. The transition curves can be used to analyze the column health comparable to the conventional HETP and Af analysis. It is worth mentioning that recently, advanced methods have been described to better align the results from transition analysis with conventional methods [27, 28].

Pressure drop represents an important measure by which columns can be evaluated [29, 30]. It follows a characteristic relationship as a function of flow rate, medium type and column geometry. Pressure drop can be used to monitor the physical stability of a chromatography. In addition, it gives indications about physical strength, shifted or compressed media, channels and voids as well as bed or frit fouling due to bound product or contaminants.

In a similar way as for the Af measurements when applying acetone or salt, the symmetry of the elution peak can be determined and therefore serves as a measure of consistency and reproducibility of protein elution. It demonstrates consistency of product loading and yield and shows nonspecific binding due to solute buildup or media degradation. Product peak measure can also be used to monitor successful column cleaning.

Online multivariable (multivariate) data analysis is another way to collect and analyze information about column health during the process. Data which are already available from the chromatography systems sensors, e. g. pressure drop, conductivity and pH are combined and analyzed. Typically, two data sets (e. g. column pressure and pH or pH and maximum UV signal) are used for this sort of analysis [31]. The combination of several process data gives a profound overview about performance shifting, worsening column health or even minor incidents during a run, if the data is analyzed online and in real time.

Recently, more effort has been invested into the investigation of the influence of poorly packed chromatographic beds on the separation performance and binding capacity drifts on protein A capture columns [32]. It was shown, that the pressure flow behavior is a reliable measure for packing variations. If the pressure flow variations are kept below 20%, the impact on the performance in a multicolumn affinity chromatography process is insignificant, irrespective of the column configuration. On basis of these findings, the need of a sensitive measurement for column health check during a continuous MCC run might not be of high importance.

Column Fouling

If column performance changes or general alterations on any critical product parameter are observed, a column swap out can be considered. Investigations for the exchange of a single column or a complete column set without interruption of the chromatography process are ongoing. Strategies should consider maintaining the aseptic barrier of the unit operation. Possible strategies could include the use of back-up-columns or an exchange of columns at specific time windows during the chromatography process.

Dynamic change of feed quality

In perfusion-based USP, the product and impurity concentrations are expected to vary over the course of the process. Section 2.2.2 describes main impacts and mitigation strategies in more detail. Especially in case of protein A bind-elute chromatography, different strategies including a dynamic load based on UV signals [33] or a conservative volume-based loading [2] have been implemented. Further input signals such as concentrations, measured by ultra-performance liquid chromatography (UPLC), in the load step have also been evaluated and could be implemented in dynamic loading strategies [3].

2.2.1.2 Process Interruptions

An interruption of the process has to be considered for any step of a fully continuous process, but for a bind-elute chromatography the likelihood can be regarded as significant due to the complexity of this step. Interruptions can be necessary due to unplanned events such as leakages, equipment problems, reduced column performance or fouling and interruptions of the chromatography`s up- and downstream steps. The following three aspects and risk mitigations have to be taken into account for process interruptions:

1. In case of an interruption the columns are situated in different zones of the chromatography step. The acceptable duration of that hold needs to be investigated in process development. Special emphasis has to be put on critical steps like elution, especially in gradient elutions of a cation exchange or sanitization steps. In addition, a potential impact on virus removal has to be evaluated.

2. If a process interruption impacts product quality, measures for detection need to be in place with the option to quarantine the affected product. Multivariate data analysis has been described as a means to detect small deviations early in the process [31].

3. To provide flexibility for handling process interruptions, surge tanks used up- and downstream of the chromatography step acting as a surplus residence volume can be implemented. The possibility to send product to waste or a dedicated surge container can be considered for worst-case scenarios.

2.2.1.3 Virus Safety

A key regulatory requirement for all biopharmaceuticals is virus safety, which is assured by combining studies and virus clearance with purification unit operations. Viral clearance is assessed in small-scale tests, and regulatory guidance documents provide a general framework for how these tests should be performed [34, 35]. Even with guidance, demonstrating an appropriate level of viral clearance can be challenging for manufacturers of biopharmaceuticals.

The question of the ability of continuous multicolumn operations to clear viruses as sufficiently as batch operations has been raised [36]. Viral clearance validation of the continuous MCC or SMB shows the impact on the log reduction value, relative to a similar batch process. Moreover, many aspects of the characteristics of a continuous process (altered column geometry, lower flow rates, more cycles) should be mimicked in a scale-down model, in order to evaluate any impact on the virus reduction capabilities [31, 37].

Viral Clearance of MCC

For two-column chromatographic processes without full saturation of the first column and capture of the breakthrough at the second column, conventional virus clearance validation studies [34] can be performed. The principles and chemistries are the same as for single-column batch chromatography and do not require advanced study designs.

Advanced small-scale virus clearance validation studies are required for MCC systems, where the operating binding capacity is close to the static binding capacity and a subsequent column captures the breakthrough from the first column. It is helpful to simplify process optimization for virus clearance by emulating continuous operation in batch, or to find surrogate markers for virus removal in small-scale studies. Results from surrogate markers such as bacteriophages must then be confirmed with mammalian viruses [38].

Design Strategies on Small-Scale Virus Clearance Studies

To facilitate small-scale virus clearance studies, a simplified protocol has been defined [38] (see Figure 4). This involves loading a primary column with virus-spiked feed solution. The effluent of this primary load column is directed to a secondary load column. Once the primary load column has received the desired amount of feed solution, it is discarded, and the secondary load column becomes the primary load column. This column will continue to receive virus-spiked feed solution until the desired volume of feed solution has also been applied on this column. This column is then subjected to all non-load steps until the product is eluted. Based on the eluted product, the virus clearance capability of the capture step can be expressed in terms of a log-reduction value (LRV).

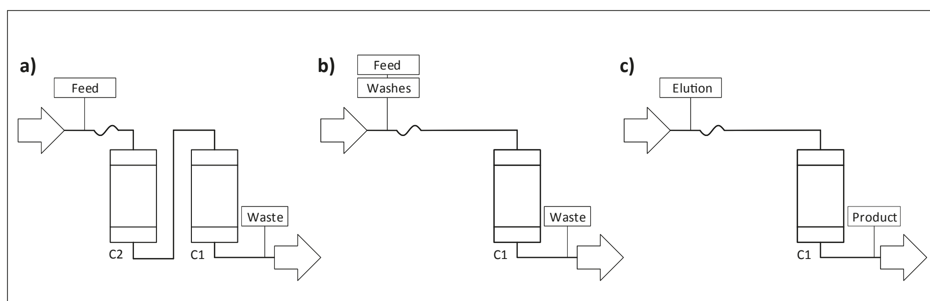


Figure 4: Simplified scheme for small-scale virus clearance validation studies for a MCC process using a simplified protocol on only one column. In the first step of a multicolumn chromatography, the column typically receives break-through material of another column (C2) that is currently loaded. This is mimicked in step a): the target column (C1) is receiving break-through of virus-spiked feed material. In the second step b), the target column C1 is loaded with virus-spiked feed material while C2 is taken offline. C1 is then washed according to the chromatography protocol. In the last step c) the column is eluted to determine the virus clearance.

Since each column in the MCC process goes through the same series of events for every cycle, a scale-down model based on the simplified scheme (Figure 4) with two columns is considered adequate for virus clearance studies. The scale-down model reflects the process conditions that each individual column goes through in every cycle. This includes the use of a spare column (C2) to provide a feed solution that is (partially) depleted from antibody but still contains virus. M. Schofield et al. [38] indicated, that results support the hypothesis that the viral clearance capabilities of a multicolumn continuous protein A system can be evaluated using an appropriately scaled-down single-column process and equipment.

2.2.1.4 Sampling and Data Management

A well-defined control of process steps is based on a combination of sampling and process parameter monitoring. In case of batch bind-elute chromatography often one or few sub-cycles are performed to process a batch. This translates to a limited set of data to be controlled for interventions by the personnel. Even a sampling of each sub-cycle and analytical verification is often doable before combining the eluates for the following step.

In continuous manufacture, a multitude of cycles, even up to several hundred cycles, could be collected in one batch, depending on process design and a risk-based batch definition strategy. This implies more and new requirements for the automated process control as the amount of data is significantly higher, the available reaction time is shorter and sampling and analytical control is limited.

The following three aspects can be considered when defining the data-based control and sampling strategy:

1. Implementation of a highly sophisticated automated control of all quality relevant parameters like UV curve shape, pH values and conductivity for each cycle is mandatory. Due to the high number of cycles and the short time windows for potential operator intervention, a deviation from pre-defined ranges ideally leads to an automated rejection of the affected product stream.

2. Sampling can support the process control but analytical control of the intermediate product quality between unit operations is more challenging than in batch operation, especially when intermediate surge containers are omitted. However, sampling at strategic points following a pre-defined frequency based on a residence-time distribution model delivers supportive data for batch release.

3. Additional PAT tools to measure critical quality attributes (CQAs) instantly or directly in the product stream would be helpful to ensure suitable process control and reliable product quality. Development is progressing rapidly in this field but in particular for downstream processes the technology hurdles are quite high, see section 3.1.

Continuous processing inevitably requires a sophisticated data management system in order to combine process parameters with analytical control and PAT tools. Regardless, a strategy has to be defined how to

deal with deviations from pre-defined ranges or limits in a continuous process flow. It has been seen that current continuous processes can operate successfully with today's standard analytical tools.

2.2.2 Virus Inactivation

Viral safety is essential for animal cell culture-based production of mAbs. Therefore, viral clearance procedures are mandatory and highly regulated, so that production processes require two orthogonal viral reduction steps. One is usually virus inactivation at low pH conditions. In contrast to the viral filtration step, which removes viruses from the product by size-exclusion, the low pH treatment focuses on enveloped viruses, which are susceptible to inactivation, including the endogenous retroviruses. The low pH virus inactivation step is usually performed after the protein A capture step. The pH of the protein A eluate varies between pH 3.0 to pH 4.1 (depending on the purification process of the mAb) and is very close to the pH value required for an efficient viral inactivation of the product intermediate. For the low pH

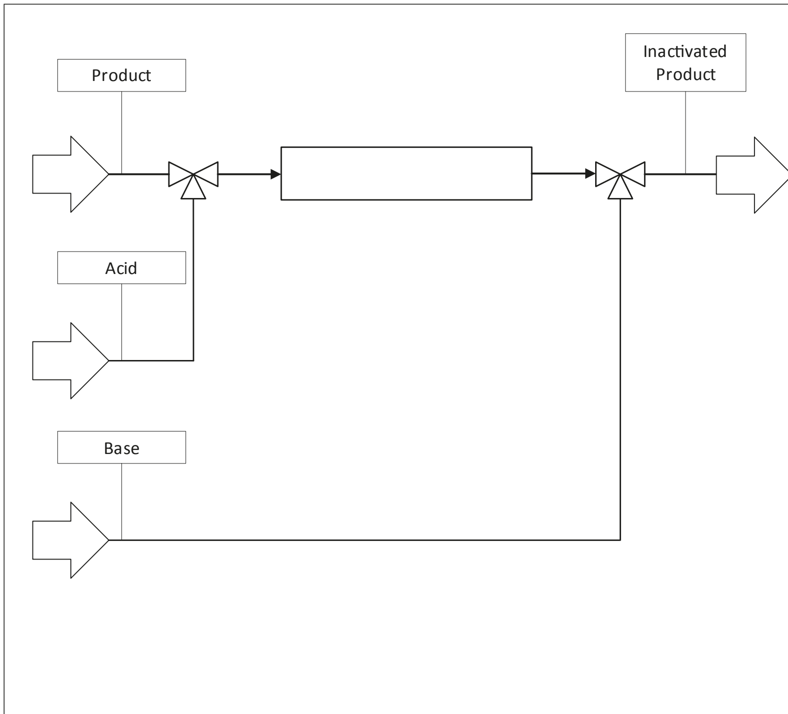
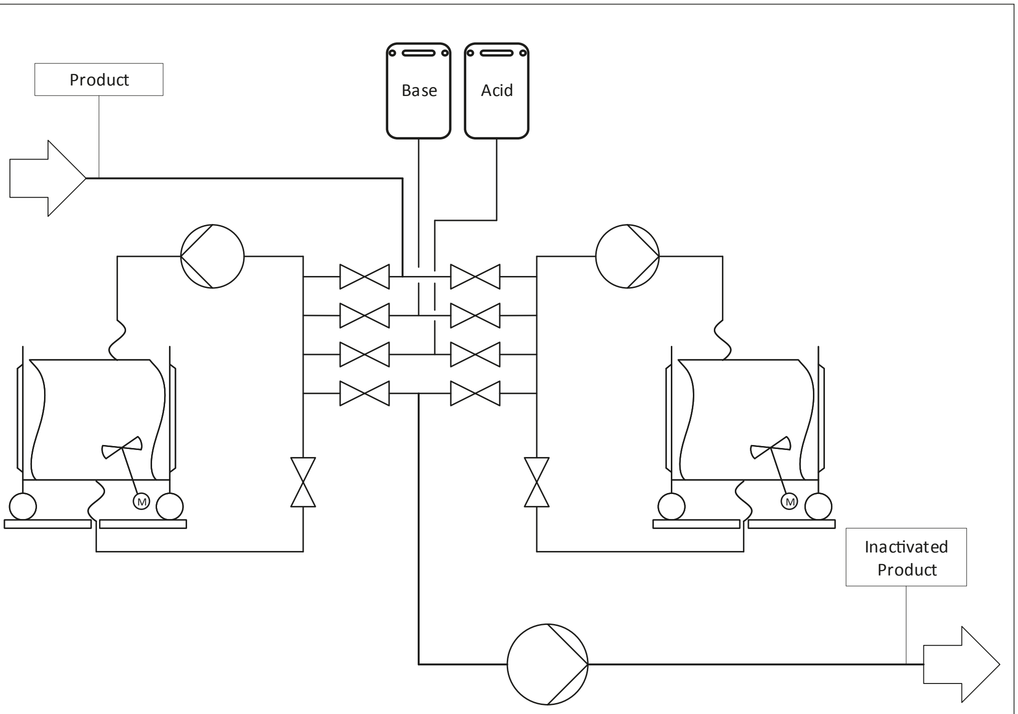


Figure 5: Schematic overview of plug-flow virus inactivation unit operation (left) and two container mix and hold virus inactivation unit operation (right).

virus inactivation of for example retroviruses the 2012 ASTM Standard Practice describes the following conditions: pH ≤ 3.6, hold time of 30 min at ≥ 15 °C in a product specific buffer at the specified pH. This provides 5 log reduction value [8,34,39–42].

In a continuous manufacturing process, the virus inactivation is a challenging step due to standard procedures and fixed residence times. There are two methods that have been proposed to transition from batch to continuous low pH virus inactivation: (1) a continuous tubular reactor and (2) a semi-continuous repetitive batch, where the product is continuously pooled in surge tanks (Figure 5) [39].

Using a plug-flow reactor for virus inactivation, protein A eluate needs to be pooled and pH adjusted before it is pumped through the tubular reactor which ensures the target inactivation time. At the reactor outlet the process fluid is mixed with base to finish the inactivation process. In this concept the inactivation time is a function of the liquid velocity and the reactor length. Ideally, the process fluid flows through the reactor



as a series of infinitely thin coherent "plugs". Each of the plugs have a uniform composition. The plugs travel in an axial direction to the reactor and are perfectly mixed in the radial direction, but not in the axial direction (neither at the upstream nor at the downstream element). However, a certain degree of dispersion will always be present resulting in a broader residence time distribution of the fluid elements [8, 41, 43].

In the two-container mix and hold, the collection of products, low pH titration, hold of low pH, and high pH titration are all performed in the same mixing container. Each container has a mixing unit and a pH probe to monitor the acid and the base addition. The containers are connected to a control system that directs the liquid flow. One container collects the protein A eluate, while the second container performs the low pH titration, hold and high pH titration of the product. As soon as the second container has completed the inactivation step and has transferred out the product, it is ready to collect protein A eluate again. At the same time the first container is starting with the inactivation of the collected product [8]. Alternative system designs using up to three mixers in a cascaded inactivation approach have also been presented [44].

The FMEA has identified main challenges to be associated with system control and design to assure sufficient virus inactivation and avoid carry-over as well as bioburden control and sensor stability.

2.2.2.1 Plug-Flow Reactor

System Control

One of the main risks with plug-flow for virus inactivation is seen with system control like adjustment of pH and control of hold time at the target pH. Not meeting the design space of these parameters may result in insufficient virus inactivation and increased risk for human health. Since the eluate stream from the protein A chromatography step comes with a variation in composition and pH (due to peak profile of mAb elution and mixing of wash and elution buffer) [45], seamless connection to plug-flow for virus inactivation requires fast and accurate inline measurement and adjustment of pH, resulting in high demands for measurement and control technology or a predetermined sequence of different mixing ratios of eluate and acid stream. In this connection, any variation in acid solution pH jeopardizes virus inactivation.

Suitable sensors and appropriate control technology must be applied. To mitigate the risk of not reaching the required pH, a titration line with several points for pH control could be implemented prior the plug flow reactor. To check and monitor the inactivation process, several pH sensors should be placed up- and downstream of the tube as well as at intermediate position. An option that has been reported for smoothing peaks in pH signal is homogenization of the eluate pool of a protein A cycle before subjecting it to virus inactivation [45], combined with inline static mixer for pH adjustment [8, 46]. Moreover, solutions for pH adjustment need to be prepared carefully and released after quality control. Titration methodologies must be established by detailed characterization studies. Likewise, control of hold time or residence time distribution should be demonstrated for a flow rate range for the given tubing dimensions [41, 47]. To monitor and/or control flow rates, the use of a flow meter is recommended. Mitigation for pump excursions should also be considered [8, 48]. For the mitigation of sensor stability risks and bioburden control the reader is referred to section 3 of this document.

Process Interruption

A further issue are process upsets concerning stop of flow, which could occur both in unit operations upstream and the virus inactivation step itself, jeopardizing inactivation or product quality. Therefore, the implementation of plug-flow for virus inactivation needs to provide a robust process operation, including assessment of failure modes and definition of a strategy for process interruptions. To mitigate the risk of unexpected stop of product feed flow, an intermediate surge tank for protein A eluate could be used to balance out flow rate changes [8] until normal operation mode is restored, or the process is shut down in a controlled manner. This intermediate hold could be combined with the homogenization functionality proposed in the section “system control”. To ensure the required hold time for product that is already at target pH, stop of product feed flow might be compensated by chasing liquid out of the tube with buffer or air. However, potentially different liquid flow properties and foam formation must be taken into account. In addition, the maximum tolerable wait time (in surge tank or tube), which still provides product with an acceptable quality, should be examined for any relevant pH values, as well as the possibility to re-feed insufficiently inactivated material back into the virus inactivation process. If sufficient virus inactivation cannot be ensured, the affected product volume must be isolated and discarded to guarantee patient safety.

2.2.2.2 Two-Container Mix and Hold

System Design and Control

With this inactivation concept, the hold-up volumes, the single-use container emptying, robustness of pH probe, collection of products and addition of acid as well as base need to be considered, according to the FMEA. During the virus inactivation step the collection of process fluid, low pH titration, and hold (inactivation) takes place in a single container. One container receives new product and at the same time in the other container the low pH titration and low pH hold are performed. There is a potential risk that some liquid, which has not been treated, may make contact with already inactivated product (common concern with top down entry). To avoid contamination with active virus the single-use container should be designed with low point product entry to prevent splashing, hanging drops, foaming, and hold-ups. Additionally, the mixing should ensure using a zero-hold-up recirculation loop. Another aspect is the design of the single-use container. If it’s not possible to fully drain the container, there is a potential risk to over-incubate the product. It has been shown that this risk can be mitigated by single-use system design [49].

2.2.2.3 Process interruption

As described in 2.2.2.1, a process interruption can be a risk to virus inactivation and thus patient safety. A strategy must be defined how to proceed with product which is directly affected, for example by independently proceeding with the inactivation or neutralization process even if previous or following unit operations are stalled or alternatively evacuating or quarantining the product in a surge container. To minimize the risk of a process interruption due to untimely emptying of the single-use container, the next unit operation should operate with a surge vessel, which needs to be placed between the two unit operations to normalize the flow rate [8].

2.2.3 Flow-Through Chromatography

Flow-through chromatography aims to reduce product or process related impurities through binding while the target product passes. Flow-through chromatography operates under continuous flow condition, which can be well adapted to a continuous processing platform. While the typical setup for a standard flow-through chromatography is comprised of a skid for one adsorber or column, a continuous flow-through chromatography skid requires a few additional considerations.

As the load on the flow-through column or adsorber is limited, the column/membrane adsorber needs regular regeneration throughout the process. To maintain continuous flow at all times, the regeneration can be scheduled in parallel to the loading process through implementing multiple adsorber units and pumps. Compared to continuous bind-elute chromatography, loading in series is typically not beneficial in flow-through mode of operation which reduces complexity of skids for these applications. A simple setup for two adsorbers is shown in Figure 6.

The described setup allows a cyclic processing with permanent loading (Figure 7). The timing of the load step needs to be equal or longer than the time needed for all regeneration steps. If this process design is not feasible, breaks can be implemented in the faster cycle, thus the solution becomes feasible, as the duration of the regeneration steps is shorter. The equilibrated adsorber will remain on hold in equilibration buffer, until the other adsorber has completed the loading. Ergo, if the load cycle is shorter than the

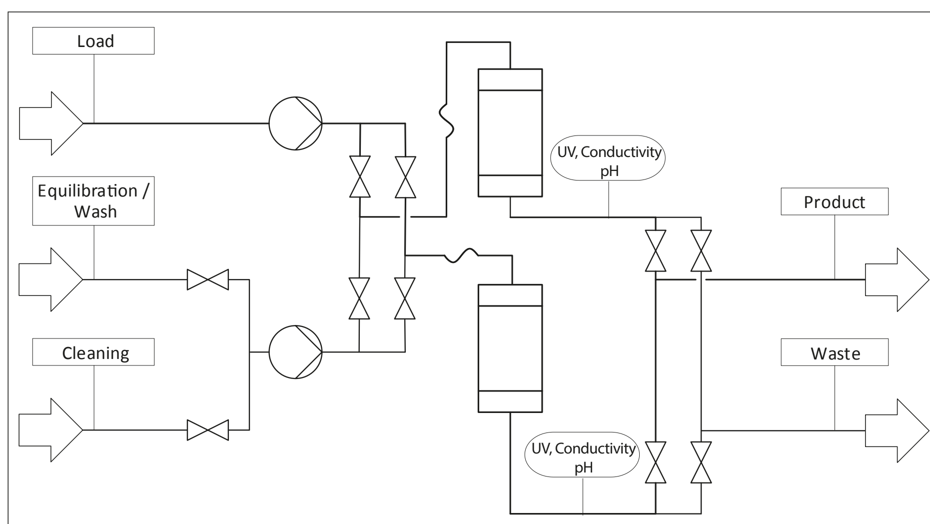


Figure 6: Schematic overview of a continuous flow through chromatography setup with two adsorbers for repetitive batch operation.

regeneration, a hold is not ideal. Alternative designs with three or more adsorbers can be considered. In this case, the longest phase can be extended over multiple sub-phases and the process duration can be matched, although this requires more complex equipment design or identical chromatography skids as used in bind-elute chromatography. The third option is implementing one membrane adsorber scaled large enough to last throughout the manufacturing campaign. System complexity can be reduced but the impact of the low flux on flow distribution needs to be evaluated.

2.2.3.1 Column/Adsorber Lifetime

If a flow-through chromatography is designed as a cyclic operation, incomplete regeneration is seen as a risk to impurity removal, especially if the step is applied for virus reduction. As in batch processing, small-scale studies evaluating potential carry-over of virus need to be performed, for more information see chapter 2.2.1.

In addition, the column/adsorber ages with each loading cycle. Regeneration of the column/adsorber is applied to restore the binding capacity, however, ageing effects cannot be avoided and regeneration cycles in continuous chromatography are often designed faster and for more frequency than in batch processing. To mitigate this risk, a column lifetime study needs to be performed (same as in batch processing) as part of the validation activities, in order to gain a clear picture of the binding capacity or impurity removal based on cycle count. The limit of cycles for the column has to be determined and must trigger replacement. With this data, two mitigation strategies can be implemented. First, the column loading per cycle is reduced to a safe level to avoid breakthrough of impurities in the final cycles. The disadvantage of this strategy is that the column capacity is not reached in the initial cycles and thereby the amount of product processed with the column over its lifetime is lowered. Second, the load amount can be varied over the lifetime of the column/adsorber within its design space. This mitigation allows for the full usage of the column but asks for a more complex control strategy which adds to validation challenges.

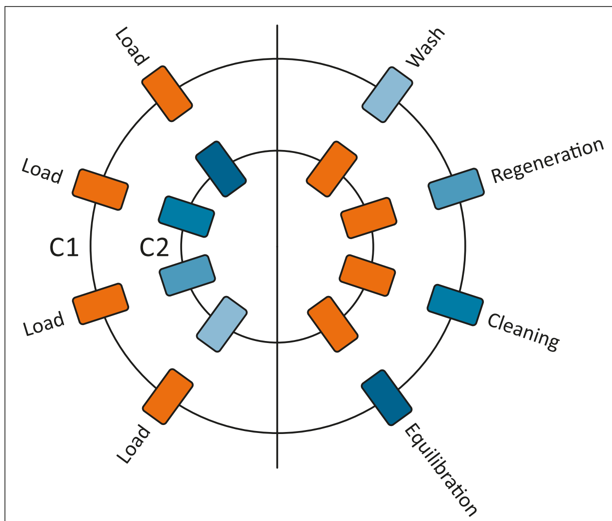


Figure 7: Process overlap in continuous flow-through chromatography. C1: Column 1, C2: Column 2.

2.2.3.2 Dynamic Process Changes

Typically, flow-through chromatography is applied towards the end of the DSP process, therefore the feed for this step normally originates from a bind-elute chromatography step. During every elution of the aforementioned step, the feed stream will change over time in titer, pH, conductivity and impurity profile, especially if the previous step involves a gradient elution. All these parameters can influence the binding capacity of the flow-through chromatography and can therefore lead to: (1) column overloading with impurity breakthrough, (2) underloading of the column or (3) binding of the product on the column. An appropriately sized surge container placed prior to the flow-through chromatography can homogenize the elution peak to a certain extent prior to loading and reduce the feed variability.

2.2.3.3 Column/Adsorber Variability

Especially in case of manually packed columns, differences in the flow pattern of the columns may be identified. The most common test for the flow pattern is the HETP analysis which can be performed after packing and after a defined number of production cycles. HETP testing only detects differences in the flow pattern, dissimilarities due to resin/adsorber functionality differences cannot be spotted. Differences in the column are closely associated with earlier breakthrough and varying breakthrough patterns resulting in variable impurity concentrations. To mitigate this issue, regular HETP test should be considered. To have columns with most similar HETP characteristic, either pre-packed columns can be used, or automatic column packing can be applied. As chemical differences cannot be detected by HETP testing, it can be considered to only use resin/adsorbers from the same manufacturing lot for one process. More information is provided in chapter 2.2.1.1.

2.2.4 Virus Removal Filtration

Virus reduction in biopharmaceutical manufacture relies on a minimum of two orthogonal steps as required by the ICH Q5A guidelines [34, 50], with filtration-based virus clearance being applied in most biotechnological products originating from mammalian cell cultures [51]. Virus removal modules consist of a 15 to 20 nm filtration membrane and are generally validated to be implemented as single-use devices.

In batch processes, virus removal filters are commonly operated under constant pressure and operation is limited by flow decay or breakthrough due to clogging of the filter. Virus removal filtration in continuous processing needs to operate under constant flow conditions. Methods for a continuous adaptation of virus removal filtration have been presented, including automated switch-in and -out systems, which describes a principle where filters that reached their validated throughput are exchanged with new virus removal filters [52]. The system consists of a minimum of two filters staged in parallel and relies on a cumulative throughput control to switch to a new filter once the validated throughput or maximum pressure is reached (Figure 8).

The FMEA has identified the uncertainties of virus removal filtration to be associated with the general life cycle of filters, dynamic process changes or interruptions and changes in operating and validation principles.

2.2.4.1 Filter Lifetime

The dead-end nature of virus removal filtration causes the flow resistance to gradually increase which leads to rising pressure differentials. It has been discussed that one large unit operation can be used in continuous processing instead of cycling multiple smaller units. Implementing one large virus removal filter with enough capacity to last over the whole campaign introduces certain risks. The large filter will operate at very low flux and thus small pressure differentials, which induces challenges in flow distribution. Even though the single device allows operation with simpler hardware and controls, it implies further disadvantages, as discussed in section 2.2.3.

Alternatively, the parallel switch-in and -out system comes with a higher level of complexity, but higher process robustness and safety, as well as improvements in deviation management and traceability that provide significant benefits. The automated parallel flow filtration system has been tested in a proof-of-

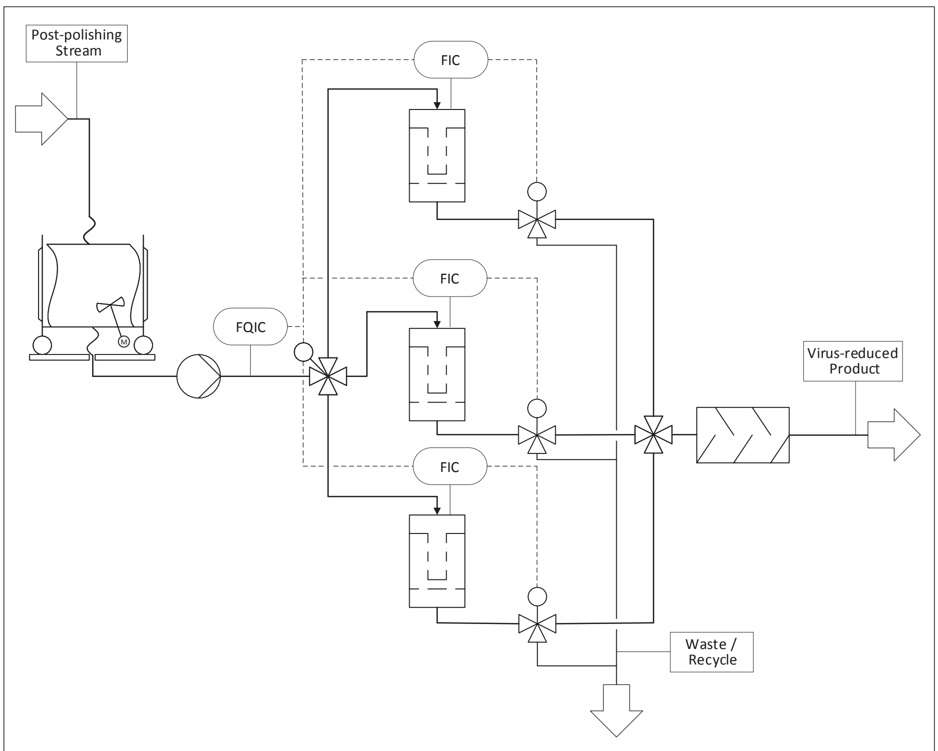


Figure 8: Setup of parallel switch-in/-out for continuous virus removal filtration (modified after [52]).
FIC Flow Indicator Controller, FQIC Flow Indicator with Totalizer.

concept study by the Food and Drug Administration (FDA) using traditional batch viral spiking methodologies [53]. To achieve bioburden-controlled filter switches, possible solutions include the implementation of filter banks early-on in the system design stage, to allow for tube welding, aseptic connection or sanitization where required. Modifications to the system design likely require small-scale spiking studies to ensure viral retention under the given design principles.

A risk that remains for any system design is that of virus removal filters not passing the post-use integrity test. In this case, the downstream fluid assembly is potentially contaminated with virus and needs to be exchanged to guarantee patient safety. Alternatively, dedicated tubing assemblies may be used for each filter. A pre-use integrity test is recommended to reduce the risk of filter failure and minimize the likelihood of having to exchange single-use assemblies.

2.2.4.2 Dynamic Process Changes

Dynamic process changes originate from variability in feed quality. While batch processes typically hold and homogenize product prior to virus removal filtration, there is minimal to no hold time in continuous process designs. The shortened residence time is expected to generally decrease fouling in filtration, however it also means that small variabilities in pH, conductivity or protein concentration are more pronounced. Variability in protein concentration can result in premature clogging, which lowers the throughput due to earlier increase in back pressure or pressure spikes. The robustness of the virus removal filter towards expected variation in concentration, pH or conductivity should be assessed and taken into consideration in the virus clearance validation study. Studies have shown that virus retention can tolerate a wide range of variations of protein, pH or conductivity [54].

Process interruptions present a temporary pressure pause or pressure release with the risk of reduced virus retention, mainly influenced by diffusion. Such interruptions can be caused by power outages, mechanical failures or failed synchronization and appear during the final product recovery flush. Additionally, the implementation of buffer flushes, for example before switching in a new filter, constitutes the possibility of loss in filter retention due to pressure variation [55]. If the continuous virus removal filtration needs to be paused or reactivated it is recommended to assess the robustness of the virus removal filter for process interruptions. Studies with virus removal filters aimed for continuous processing have shown no impact in log reduction values or flux after multiple several-hour stops [56].

2.2.4.3 Operating and Validation Principle

A focal challenge for continuous virus removal filtration entails the change of operating condition from constant pressure to constant flow. Furthermore, small-scale virus validations are typically performed under constant pressure. The application of maximum throughput and filter resistance data generated at constant pressure to a process operated at constant flow presents a potential risk. Initial data comparing the relation of resistance to the throughput for both constant flow and constant pressure scenario suggests high similarities but extensive data for justification of this concept was requested by regulatory authorities [57].

An alternative approach defining critical process parameters for the continuous filtration design space can be considered. The design space in continuous viral filtration shifts to a low-pressure and low-flux process with extended operating times and the risk for process interruptions. It is essential to reflect this design space in the characterization and define a worst-case scenario. In addition, the continuous system design, protein aggregation and virus stability needs to be accounted for in the long-term spiking study design. Different validation strategies for continuous virus removal filtrations have been published [57].

In addition, product-specific parameters for the expected concentration ranges need to be considered including buffer pH, conductivity, pressure or flow rates as they may affect virus retention. Concerning these parameters, it has been recommended to adapt existing batch protocols when performing validation studies for continuous processing [52].

2.2.5 Final Ultrafiltration and Diafiltration

This section focuses on the ultrafiltration, diafiltration, and membrane filtration; key bioprocess unit operations to set the final concentration and buffer condition. During TFF, the transmembrane-pressure drives the solution through the membrane, the crossflow velocity (defined as the rate of the fluid flow through the feed channel and across the membrane) assures that the molecules are swept away from the membranes surface. A drawback of the TFF process is the low conversion per cycle, requiring a recirculation loop and bulky equipment, such as a feed pump and recirculation tank. The majority of TFF processes are operated in batch mode, however single-pass tangential flow filtration (SPTFF) for concentration or volume reduction has been adapted in industry over the past few years [58, 59].

In SPTFF the continuous feed flow is concentrated as it passes through the SPTFF module. The flow path design enables up to 30x concentrations in one module passage [58]. A recirculation loop is not necessary, which decreases aggregation problems, eliminates mixing, and allows the SPTFF step to be coupled with other DSP steps such as virus removal filtration and chromatography steps (Figure 9). Additionally, SPTFF results in lower flow rates, hold-up volumes and buffer requirements and translates to higher recoveries.

Various design principles in single-use format have been developed. They include modules with staged flow-paths or standard TFF cassettes operated at lower flow rates for higher feed-to-permeate conversion rates. Diafiltration can be achieved by staged flow paths consisting of repetitive concentration and dilution or co- and counter-current diafiltration in flat sheet membrane devices [60]. Furthermore, recent studies have shown early development work showing how simultaneous concentration and diafiltration is achievable in a single SPTFF module [61]. The FMEA has identified risks in final concentration and diafiltration linked to module regeneration, equipment availability and suitability as well as dynamic process changes.

2.2.5.1 Module Lifetime

Over extended operating times the SPTFF modules likely need to be regenerated. A mitigation strategy to address this risk involves the use of alternating SPTFF devices where one device is in use while the

other is regenerated. During process characterization, suitable regeneration conditions need to be established. The use of alternating smaller modules also reduces the hold-up volume in the filtration steps and allows a faster propagation of drug substance through the individual unit operations corresponding to shorter hold time. Alternatively, SPTFF modules can be exchanged at strategic points in time, for example to correspond with one product sub-lot. Thereby, bioburden control needs to be assured, similar to the requirements for a switch-in/-out system described in 2.2.3.

2.2.5.2 Dynamic Process Changes

In single-pass concentration, modules or systems control the volumetric concentration factor through the ration between feed and retentate flow rate. It is critical to reach the targeted volumetric concentration factor to avoid potential aggregation and reach the targeted concentration factor or diafiltration efficiency. During start-up and shut-down, the ultrafiltration/diafiltration (UF/DF) is not in a steady-state and effects on product quality need to be evaluated. To accommodate potential feed flow and feed quality variability throughout the operation, it is recommended to perform small-scale studies to evaluate proven acceptance ranges for feed and retentate flow rates and evaluate the impact of drug substance concentration on the concentration factor and process pressure. A similar evaluation is recommended for the critical process parameters of the diafiltration step in the SPTFF module. The characterization described above would result in a design space or proven acceptable ranges and the normal operating range for feed flow, retentate flow, diafiltration buffer flow and process pressure. Thereby, it can be considered that some variance in final concentration may be acceptable given that the drug substance is afterwards diluted in drug product formulation.

2.2.5.3 Operating Principle

In process design, consideration needs to be given to the formulation step, which follows all the previous unit operations and that it needs to match given processing times or mass capacity. Low flow rates typically seen in continuous formulation processes and extended processing times in continuous TFF applications may lead to membrane fouling and increase the risk of aggregation. It is therefore critical to size the UF/DF modules accordingly, especially in applications with high protein concentrations and viscosity. Various module sizes, from process development to processing scale, with modular designs, cut-offs and membrane material are available today, which enable scale-up and scale-down within certain boundaries. Successful operation requires control of UF/DF with suitable sensors for pressure, flow rate and conductivity in automated skids.

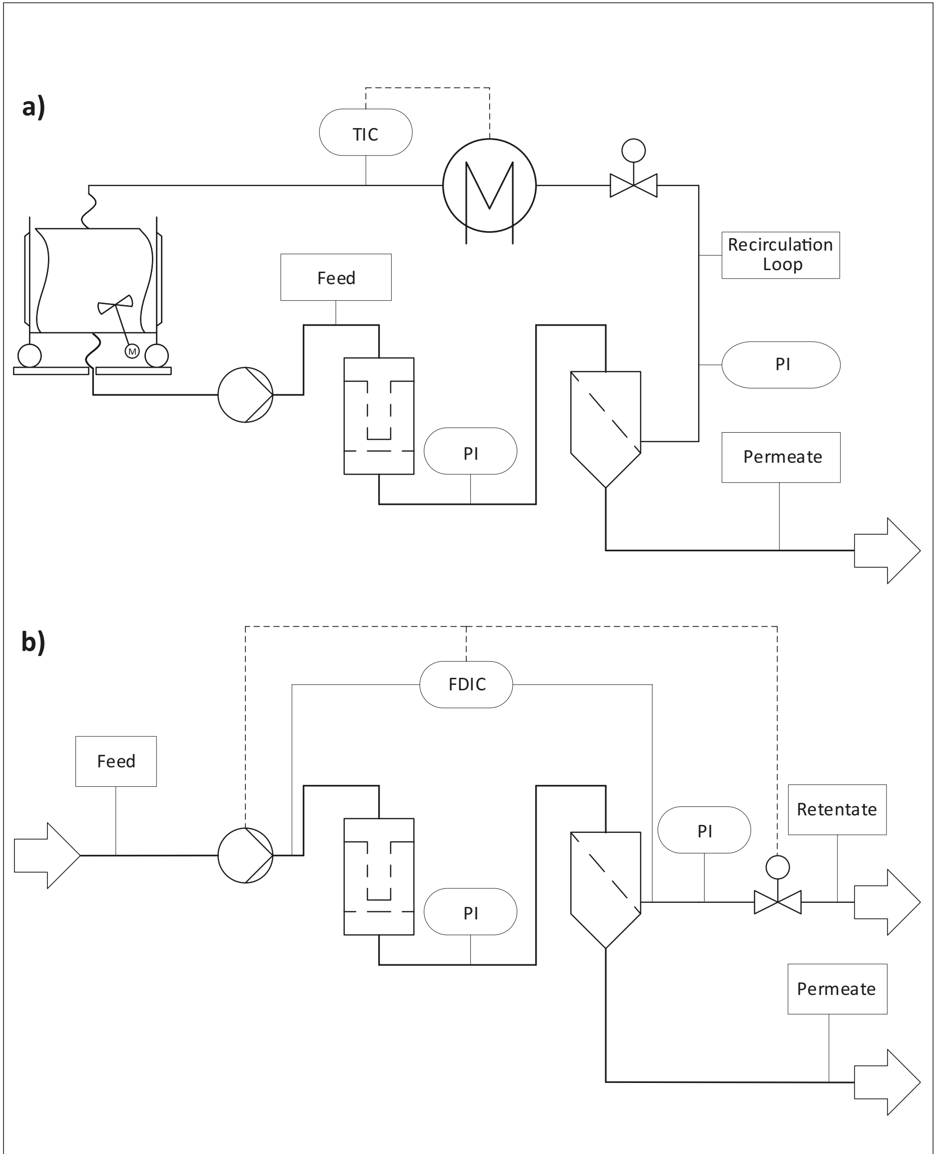


Figure 9: Comparison of a) conventional TFF and b) SPTFF setup (modified after [58]). ***PI Pressure Indicator, TIC Temperature Indicator Controller, FDIC Flow Difference Indicator Controller.***

3 Overall Processing Risks

The FMEA has identified various general processing risks that apply to several of the processing steps described above. These topics include the question of process control and data management, using suitable equipment for the extended process duration and questions linked to regulatory topics such as bioburden control or lot definition. This section aims to give a brief overview of the risks and mitigation strategies linked to the overall continuous process.

3.1 Process Control and Data Management

In the dynamic field of continuous operation with its series of interconnected unit operations, a seamless operation requires a strong degree of process control through automation. Bayer (DE) and Merck (US) have presented the first fully automated continuous platforms operating on Emerson DeltaV or Siemens PCS7, with projects currently ongoing [62, 63]. However, due to the complexity of the individual unit operations, it is likely, that local control is combined with supervisory control system. While the knowledge concerning automation is already present in the industry, the greater challenge is adequately defining the automation strategy.

Regardless of the chosen strategy, the supervisory control system should allow the control of the liquid flow management and balance flow deviations between unit operations based on, for example, input from weight cells. In addition, the control system needs to be able to react to the interruptions, warnings or alarms of individual unit operations, for example by evacuating liquid into a surge container or by initiating a cascaded line shutdown. Apart from the control function, the control system can be used to collect raw data for online data management. Likewise, the supervisory control system could be used to control automated sampling systems in the continuous platform.

Data management in continuous processing comes with challenges but also opportunities, thanks to the large amount of data that is generated. The multi-cycle nature of several of the unit operations in the process create a large pool of data over the course of a batch which allows close monitoring of the process performance over time. This also opens the possibilities for advanced data evaluation by employing multivariate data analysis, such as principal components analysis. This form of data analysis has shown to be very powerful in the early detection of small process variances in continuous chromatography [62]. More examples on multivariate data analysis are given in chapter 2.2.1.1. Even though the industry is moving towards process analytical technologies, which will allow for real time release in the future, the current control systems are expected to support today`s commonly accepted release strategies.

3.2 Equipment Suitability

In continuous processing all equipment, from tubing or bags to filtration devices or sensors, needs to be able to withstand longer processing times. Here, two main points were identified in the FMEA, namely the impact of (1) extractables and leachables and (2) sensor stability.

Theoretical and practical studies on the impact of extended processing times on the leachable profile of continuous DSP unit operations have shown little risk [64, 65]. Predictive calculation of process equipment related leachables furthermore indicate a lower risk compared to standard batch production due to the higher volumetric throughput through the systems [65]. Nonetheless, it is critical to characterize leachables in the final drug product in a toxicological risk assessment to assure patient safety. Furthermore, understanding potential interactions between leachables and the drug product is necessary. For all continuous platform unit operations, it is therefore recommended to utilize a quality by design (QbD) approach for leachable characterization following current recommendations from BioPhorum Operations Group [8].

Another challenge of equipment suitability concerns the stability of single-use sensors throughout the entire process duration. The sensors must meet the requirements of potential bioburden reduction treatments and remain sufficiently accurate throughout the process [48]. Fouling and bleaching can be observed, and especially optical pH sensors are prone to signal drift over time. Working in closed systems does not allow for recalibration of sensors, only offline reference measurements could be taken to adjust the probes. Therefore, suitability and stability of sensors under specific process conditions should be assessed upfront. Installation of redundant sensors can further reduce the risk of material failure. Additionally, sensor exchange and recalibration strategies could facilitate continuous manufacturing processes, however, there are very few solutions readily available. Alternatively, greater use of quality by design strategies may help mitigate issues related to sensor stability [8].

3.3 Regulatory Aspects

Regulatory communities, industry groups and suppliers have given support through the drafting of different strategies for process validation, in order to address regulatory uncertainties. These strategies cover possible solutions for e.g. control of bioburden, virus safety, lot definition, lot traceability or QbD [31]. Ongoing collaborations such as a virus removal study for MCC, in which regulatory agencies are actively involved, support the implementation of these strategies [38]. The following sections give a brief overview over the three main regulatory questions mentioned above.

3.3.1 Bioburden Control

Introduction of bioburden and microbial growth is a risk that may result in rejection of product and is therefore rated as significant in most of the USP and DSP processing steps. On one hand, the bioburden risk is generally reduced as a result of the reduction of product residence time in the process, but due to

the extended operating times in perfusion-based processes, appropriate control measures are required. The strategies recommended to ensure bioburden safety resemble the preventive measurements seen in today's batch operations, namely:

1. Implementation of gamma-irradiated, pre-sterilized and closed single-use flow paths
2. Aseptic connectors or weldable tubing for aseptic connections between manifolds
3. Bioburden control filters at strategic points of operation and periodic change-out thereof
4. System suitability test before use
5. Optional: System sanitization or periodic exchange of equipment

Continuous bioprocessing is generally viewed as more complex than batch or fed batch processes. Manual interactions are reduced through a high grade of automation that needs to be consistent, even if single-use equipment is used during continuous bioprocessing.

Main risks for USP are identified in reduced effectivity of filtration (exhaust gas and perfusion), single-use sensors and single-use components operating under extended process conditions. Current developments such as digitalization are aimed at fully controlled and streamlined continuous single-use upstream processes while considering the predominant risks. This may lead to more fully single-use upstream process facilities, compared to the current hybrid structures, which combine single-use equipment in seed train cultivations and stainless steel at production size. However, to determine critical needs and drivers across industry, further collaborations are required.

In DSP, further measures can be taken in chromatography: single-use systems and columns can typically be sanitized after installation. Pre-treatment of chromatography resin can further reduce the risk given that the resin provides the chemical stability. Considering the limited stability of protein A resin towards cleaning reagents, sanitization with peracetic acid has been described and data for a multi-day run with a low-bioburden closed fluid path and gamma-irradiated protein A columns has been demonstrated to be a successful alternative [2]. It is aspired to fully isolate DSP steps from the upstream operations by implementing surge tanks or check valves. With rapid microbiological testing methods available, early detection of growth becomes feasible. A regular monitoring strategy cannot avoid a bioburden issue but allows a timely intervention to isolate the contamination and reduce the risk for the connected steps and the product.

3.3.2 Batch Definition

The FDA defines a lot as a batch with uniform character and quality within specified limits and has shared, that they will be applying the same definition to continuous bioprocesses [66]. Concerning products originating from continuous operations, the lot is identified by an amount of product produced in a unit

of time or a given quantity that has uniform character and quality within specified limits. A lot could also be defined based on the cyclical nature of the process or raw material lots. The somewhat open definition gives end users the flexibility in defining a batch.

It is recommended to base the rationale behind batch or lot definition on a risk assessment. By associating the batch definition to the most eminent risk in the continuous bioprocessing platform, namely the risk of a batch rejection, the definition and amount of product affected can be linked in a way, that the business impact of potential batch rejections can be minimized. Examples for defining end user lots include the correlation of the batch with the exchange of processing equipment, such as the upstream perfusion module [5].

3.3.3 Product Traceability

With continuous bioprocessing being based on integrated flow between unit operations, a deviation in one-unit operation will propagate on its way downstream, likely before the deviation has been detected. A strategy to assess of how potential process upsets travel through the cascade of unit operations is to determine the propagation velocity and residence time distribution of the product over the unit operations. This residence time distribution model allows to determine affected product lots retrospectively, but also allows for the quarantine or redirection of product to a surge tank during operation [31]. Instead of determining the residence distribution for every single unit operation, certain unit operations can be combined in the evaluation: based on the likelihood of a deviation and the likely locations of detection, a risk-based approach would allow the combination of individual contributors of the residence time distribution.

4 Conclusion

Aimed at developers and realizers of continuous processing steps in biopharmaceutical manufacture as well as facility planners and designers, this paper provided an overview of the current state-of-the-art, technical risks and mitigation strategies of single-use continuous processing steps. An FMEA performed for a mAb model process at 500 L scale using single-use technologies in all up- and downstream processing steps systematically identified the most prominent risks.

The analysis rated risks according to their severity, occurrence and detection and identified challenges in USP mainly to be associated with the extended duration and higher complexity of the perfusion cell cultivation. Strategies to address these challenges include design options and means to evaluate system suitability or improve process control. For continuous downstream unit operations, it was evaluated that addressing process interruptions and dynamic process changes are most critical. In this context, different design options and recommendations for small-scale process characterization were described. Overall challenges that concern both continuous upstream and downstream steps were seen mainly in process control, data management and equipment suitability over extended processing time. Suggested mitigation strategies include the use of quality by design, advanced data evaluation methods or process analytical technologies.

The mitigation strategies summarize the suggestions from industrial and academic end-users, knowledge from current adopters of continuous processing, information from suppliers and include recommendations from regulatory authorities and guidelines from further industry collaborations. While for many questions addressed in this paper, strategies are already clear, other areas require the continued collaboration between end-users, universities, suppliers and regulatory authorities.

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