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Moving to CoPACaPAnA: Implementation of a continuous protein A capture process for antibody applications within an end-to-end single-use GMP manufacturing downstream process

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ABSTRACT

For the first time to our knowledge the implementation of a continuous protein A capture process for antibody applications (CoPACaPAnA) embedded in an end-to-end single-use 500 L GMP manufacturing downstream process of a multispecific monoclonal antibody (mAb) using a single-use SMB system was conducted. Throughout the last years, a change concerning the pipelines in pharmaceutical industry could be observed, moving to a more heterogeneous portfolio of antibodies, fusion proteins and nanobodies. Trying to adjust purification processes to these new modalities, a higher degree of flexibility and lower operational and capital expenditure is desired. The implementation of single-use equipment is a favored solution for increasing manufacturing agility and it has been demonstrated that continuous processing can be beneficial concerning processing cost and time. Reducing protein A resin resulted in 59% cost reduction for the protein A step, with additional cost reduction also for the intermediate and polishing step due to usage of disposable technology. The downstream process applied here consisted of three chromatography steps that were all conducted on a single-use SMB system, with the capture step being run in continuous mode while intermediate and polishing was conducted in batch mode. Further, two steps dedicated to virus inactivation/ removal and three filtration steps were performed, yielding around 100 g of drug substance going into clinical phase I testing. Therefore, in this study it has been demonstrated that employing a continuous capture within a GMP single-use downstream processing chain is feasible and worthy of consideration among the biotech industry for future application to modality-diverse pipelines.

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1. Introduction

Throughout recent years, the diversity of biologics has significantly increased as not only monoclonal antibodies (mAbs), but also nanobodies and multispecifics have become of major interest for future therapy approaches. Impacting the process design regarding purification strategies, these new developments also lead to a varying range of product demands from 50 to more than 500 kg/year [1,2]. In order to adjust to these new requirements, the implementation of single-use technologies might be a suitable option leading to reduced process cost and time. In contrast to the fixed size of stainless steel equipment, single-use materials allow for higher flexibility and improved utilization of capacities [3,4]. Further, no cleaning procedure is required, which reduces complexity and time for each unit operation or time between batches and provides the potential to simplify the infrastructure of the facility. Employing single-use bags instead of stainless steel vessels can lead to a reduction in hardware associated cost and therefore a reduction in total cost as hardware and maintenance cost are supposed to exceed the increase in raw material cost [5]. Currently, many companies tend to employ single-use equipment rather in upstream than in downstream processing. However, this is likely going to change in the next years because of these benefits [1].

Leachables and extractables are often mentioned as critical issues when employing single-use materials. While leachables are substances dissolving from material surfaces into the product solution and might affect cell growth or activity, extractables display chemicals which can be removed from the product

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containing surface by specific cleaning agents or solvents [6]. Therefore, appropriate testing must be performed and documented, proving that levels of leachables and extractables remain below certain values during the process. Relying completely on equipment obtained from vendors inevitably brings a high degree of dependency regarding the supply chain and quality consistency [4]. However, a reliable supply chain and a single-use purification process definitely embody the potential to reduce time to market [7].

Continuous processing is another promising solution for future products. Running a process in continuous mode eliminates holding steps and therefore leads to reduced process time and increased productivity. Godawat et al. [8] demonstrated the feasibility of an end-to-end continuous process for a mAb using a PCC-system while achieving an increase of 25% regarding resin capacity utilization and 20% buffer reduction at consistent product quality (Godawat et al. [8]). Further, it was shown recently that the simulated moving bed technology is another suitable option for the continuous capture step [9]. As the production plant is used more efficiently, reductions in capital expenditure and facility size are within reach 10,11]. Further, continuous processing is favored by authorities like the Food and Drug Administration (FDA), as steady state operations should in theory generate constant product quality [12]. Moreover, running end-to-end continuously in a closed process reduces the risk of bioburden [8]. It should be noted that a high level of process understanding and control are essential in order to assure consistent results. Despite appearing highly attractive and desirable, it of course depends on the respective process and product as to which extent the implementation of single-use equipment and continuous unit operations is truly reasonable [13]. Klutz et al. [14] observed that implementing a fully continuous process is not economically advantageous because of the high costs of perfusion culture. Regarding downstream, they found that continuous processing is slightly beneficial as cost of goods decrease from 12 €/g to 6–9€/g when employing continuous protein A chromatography [14]. Similar findings were obtained by Hammerschmidt et al. [15], pointing out that a hybrid process results in the lowest cost of goods for annual production of a protein [15].

The downstream process for a mAb which is described in this study is an end-to-end single-use process that was implemented in a facility dedicated for single-use process operations. Here, we describe partly the preparation and execution of the process and focus on benefits arising from the combination of single-use equipment and continuous processing in a GMP environment.

2. Materials and methods

The process being described in this manuscript was performed under GMP conditions yielding clinical trial testing material. Therefore, the amount and depth of actual process data is limited due to legal and IP constraints. However, our lab scale process development has been described intensively in previous publications [9,16] and since then the process has not been changed significantly. The authors would like to refer to these publications in order to allow for the appropriate degree of process details although being unable to do so in this very manuscript.

2.1. Facility

The downstream process was conducted in a GMP facility. Two individual production lines with a shared buffer preparation area and a cleanroom air lock connecting the two cleanroom C status areas (according to EG-GMP-guideline Annex 1) were set up on about 1000 m^2. Being connected by two closed bridges, harvest was performed in a separate building and the container was transported via a DONKEY-S-HL-1206-Lasernav system by Donkey motion (Stöckmatten, Germany) to the cleanroom area. Using this transportation system, the harvest container is prevented from touching the ground.

2.2. Consumables

The main components including bags and filters were all purchased from Sartorius-stedim (Göttingen, Germany) and Pall Medistad BV (Medemblik, The Netherlands). All necessary documents were distributed by the vendors, including BSE-TSE certificates. General study results regarding leachables and extractables were distributed, but no specific testing of the consumables used in this report was carried out as this is not required by authorities in early phase manufacturing. However, this is anticipated for late phase manufacturing.

2.3. Equipment

Specific user requirements for all systems were validated during factory acceptance test (FAT) and site acceptance test (SAT). Here, installation qualification (IQ) and operational qualification (OQ) were part of the SAT, while a first test run was performed for performance qualification (PQ). The following systems were used for the downstream process. An additional magnetic mixing system by Sartorius-stedim was used for buffer preparation (Table 1).

As the single-use SMB system was used for three purification steps and was the sole continuous unit operation, more details are provided in the following.

2.4. Single-use SMB system

The employed single-use SMB system (Cadence BioSMB 350 system by Pall) is a continuous chromatography GMP-ready unit employing a fully disposable flow path. The system is able to perform simulated moving bed chromatography (SMB), although in this study a sequential chromatography approach was used. The principle is based on switching valves automatically between multiple interconnected columns in a way to achieve an optimized utilization of the resin which ultimately reduces the amount of resin needed and leads to an increase in productivity. The system can be

Table 1
List of systems used for purification steps.

Purification step	System	Manufacturer
Continuous protein A chromatography	Cadence™ BioSMB 350	Pall Medistad BV (Medemblik, The Netherlands)
Virus inactivation (VI)	Magnetic Mixer system	Pall Medistad BV (Medemblik, The Netherlands)
Depth filtration	Pilot Scale Holder for Pod configurations	Merck Millipore (Jaffrey, NH, USA)
Intermediate chromatography	Cadence™ BioSMB 350	Pall Medistad BV (Medemblik, The Netherlands)
Polishing chromatography	Cadence™ BioSMB 350	Pall Medistad BV (Medemblik, The Netherlands)
Virus filtration (NF)	Quattroflow™ 1200SU	QuattroFlow™ Fluid systems (Duisburg, Germany)
Ultra-/Diafiltration	Allegro™ SU TFF CS1000	Pall Medistad BV (Medemblik, The Netherlands)

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operated with up to 8 columns, whereas it can also be operated with one column only (conventional batch chromatography). The columns for protein A had a volume lower than 1000 mL. There are 7 inlets connected to flow meters and air detectors and 4 outlets connected to conductivity, pH and UV sensors each. Before implementing the system, user specific requirements were defined and validated during FAT and SAT whilst performing IQ, OQ and PQ.

2.5. The downstream process

The mAb used in this study was a multispecific mAb cultivated in fed batch mode at 500 L scale using Chinese Hamster Ovary cells as expression host. The downstream process consisted of a continuous three column protein A capture step and subsequent viral inactivation by low pH and followed by depth filtration. Afterwards, a batch mode intermediate chromatography step was carried out in flow through mode. A batch mode polishing chromatography step was followed by virus filtration. At last, ultra-/ diafiltration (UFDF) and formulation were performed. All chromatography steps were conducted on a single-use SMB system. After the intermediate chromatography step, the product solution was diluted 1:2 using a stock solution in order to adjust molarity equivalently to the equilibration buffer of the polishing step. The complete sequence can be seen in Fig. 1.

Protein A chromatography was used as capture step. Three pre-packed columns containing MabSelect Sure resin by GE Healthcare were employed using a single-use SMB system. The columns had an inner diameter of 12.6 cm and 8 cm bed height. Viral inactivation was achieved by low pH incubation for 60 min. Afterwards, pH was shifted to neutral again for further processing by depth filtration. For the intermediate chromatography step, a self-packed column of 14 cm diameter and a bed height of 20 cm was connected to the BioSMB 350 system and run in flow through mode. The polishing chromatography step was conducted in bind elute mode using the same single-use SMB system as well, employing one column with 30 cm inner diameter and 14 cm bed height. This was followed by virus filtration (NF), while the sequence of the UFDF step was a single concentrating step being followed by 10-fold buffer exchange replacing the polishing step elution buffer with the formulation buffer. In a final formulation step, an excipients stock solution was added to dilute the mAb product solution to the final drug substance target concentration.

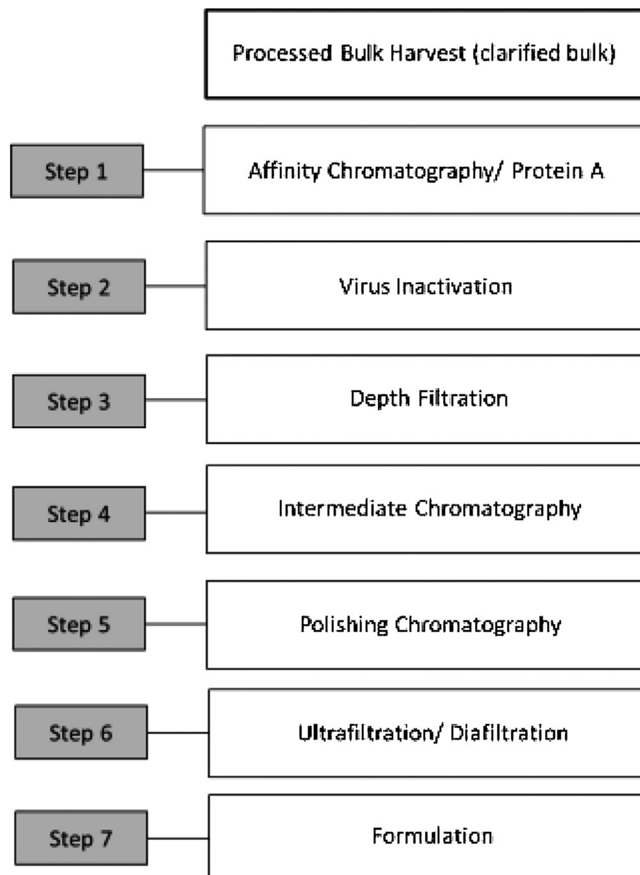

Fig. 1. Downstream process scheme.

2.6. Sampling

For in-process monitoring several samples were drawn before and after each purification step and analyzed for biosafety (bioburden and endotoxin), removal of impurities and purity. The results depicted in this study display a selection of these data.

3. Results and discussion

3.1. Analytical evaluation

The following data show relative percentages of a specific target value representing the specification setting set for the drug substance. Therefore, 100% indicates that the target value, i.e. drug substance specification is reached or even exceeded. As mentioned above, downstream processing started with clarified mammalian cell culture supernatant (processed bulk harvest - PBH). After continuous protein A chromatography, virus inactivation (VI) and depth filtration were carried out. Two chromatographic steps were followed by virus filtration (NF) and ultra-/ diafiltration (UFDF) to reach drug substance level (DS).

In mAb purification, the reduction of high and low molecular weight fractions (HMWs/ LMWs) is a major target. It can be seen that protein A chromatography has some influence on monomer concentration, shifting content from 60% to 73%. The following steps have rather no influence on removal of HMWs or LMWs as monomer concentration remains constant between 73% and 77%. The target level of monomer concentrations reached after polishing chromatography and stays rather constantly at around 102% until drug substance level (Fig. 2).

Slightly different results regarding purity can be seen by non-reduced CE-SDS analysis (Fig. 3). Starting from 56.0% at PBH level, protein A chromatography already increases purity to 97.1%, while a steady increase can be seen until the target value, i.e. drug substance specification is reached at drug substance level (DS).

Besides product quality, removal of impurities is an essential task within a downstream process. As protein A binding to mAbs is highly selective, this step already leads to a major depletion in DNA content reaching 44% of the target, i.e. drug substance specification value (Fig. 4). From this step onward, virus inactivation coupled with depth filtration were the only unit operations that led to a measurable decline in DNA content, as afterwards all samples analyzed already reached the target value for drug substance and were even below detection limit.

Removal of HCP shows rather similar results, as the target value, i.e. drug substance specification for residual HCP levels is reached already after depth filtration (Fig. 5). The first two purification steps show a rather low impact on HCP removal which was expected to be differently, as only about 5% of the target value is reached after virus inactivation. However, during the following unit operations a steady reduction of HCP can be seen while values are 12 fold lower than the specification settings for residual HCPs in drug substance after polishing chromatography and beyond.

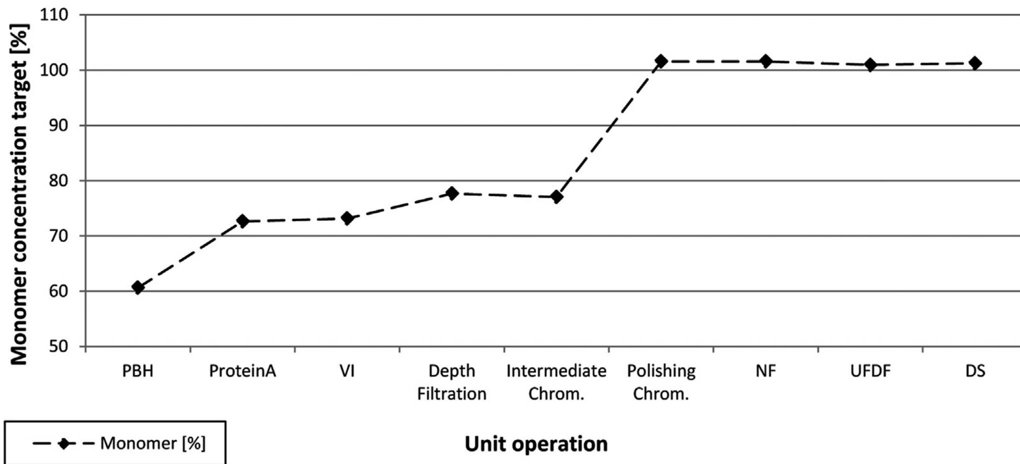

Fig. 2. Size exclusion chromatography results. Monomer content in percent is indicated by black symbols. The values are given in percent of drug substance specification, indicating that the target/ drug substance specification is reached at 100%.

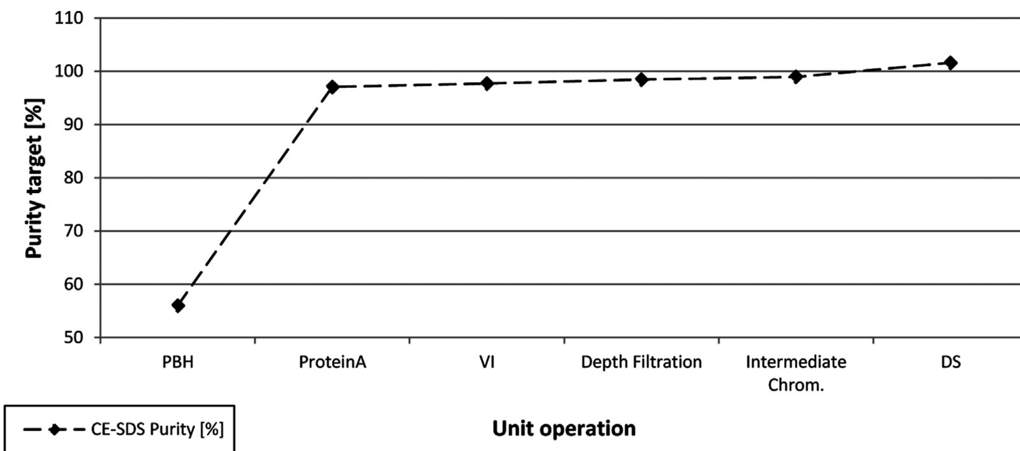

Fig. 3. mAb purity analyzed by non-reduced CE-SDS. Data is given as connected black symbols for the different purification steps. The values are given in percent of drug substance specification, indicating that the target/ drug substance specification is reached at 100%.

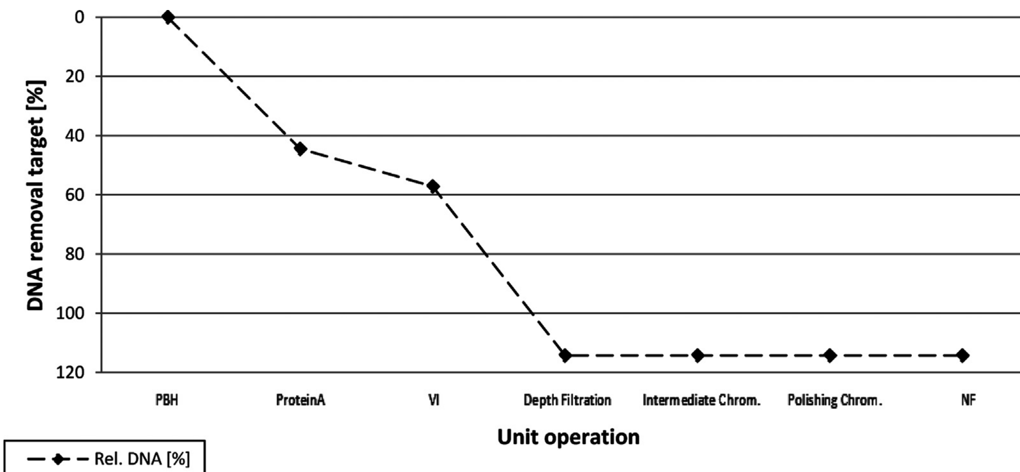

Fig. 4. DNA content during the purification process. The remaining DNA concentration is given for the different unit operations. The values are given in percent of drug substance specification, indicating that the target/ drug substance specification is reached at 100%.

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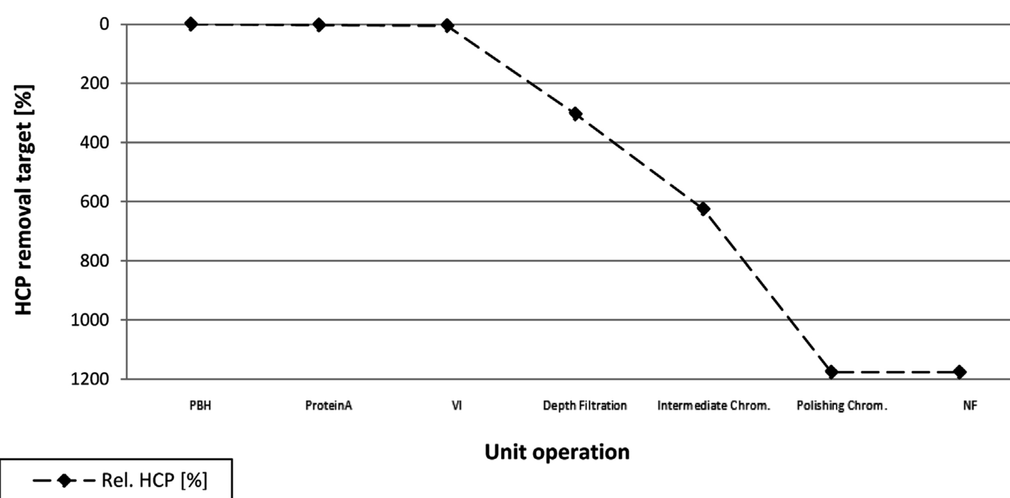

Fig. 5. HCP levels displayed as HCP removal over the course of the downstream process. The values are given in percent of drug substance specification, indicating that the target/ drug substance specification is reached at 100% and final HCP levels for polishing chromatography and beyond are 12 fold lower than drug specification.

3.2. GMP process performance and assessment

3.2.1. Robustness and performance

The process described in this study was a GMP batch, processing material until drug substance which was dedicated to be used in clinical phase I trials. Throughout the GMP process, no major issues leading to a potential impact on product quality could be observed. However, some details should be mentioned for future processes. The manifold being installed in the single-use SMB system was sanitized with 1 M NaOH for 60 min after a test run before GMP use. Despite being exposed to caustic for the whole incubation time, no sensor failed during or after the sanitization. However, some T-pieces employed as tubing connectors started to leak after the procedure. The connectors were replaced and a new assembly of the manifold was proposed for the GMP process. No leakage was observed during or after the GMP process. Using manifolds and tubing that must mainly be installed or assembled externally of the employed system consequently makes handling more complicated. Especially connecting the manifold of the single-use SMB system is prone to errors as there are various inlets and outlets that must all be connected correctly. However, we could show that employing an accurate but simple labeling and connection procedure, errors could be prevented. Moreover, handling of the Quattroflow pump which has disposable pump heads was regarded to be even easier than common systems.

Additionally, it was proposed that developing an adequate waste treatment system including an appropriately dimensioned garbage compactor and logistics for process consumables would be another time saving instrument, as this is currently based on man power. For future processes, it is anticipated to employ an automated virus inactivation system and a bag holder system which is connected to a load cell for monitoring elution volumes.

It was mentioned earlier that employing continuous steps and single-use materials or systems might have several benefits in terms of process cost, time or footprint [4,12,3,1,16]. We could not observe a benefit in footprint as most systems were the size of their corresponding stainless steel variant. However, such savings are highly dependent on the respective process and equipment used. Regarding the protein A step, there is a reduction in column diameter but using multiple columns clearly weakens the saving in footprint. On the other hand, storage capacity for the columns is not needed as they are disposables. Then again, more storage capacity for consumables is required as some disposable sensors tend to fail more often and spare parts are required to ensure consistent process performance. This is also of high importance because of the high dependency on the vendors ́ supply chain as mentioned by Konstantinov et al.

3.2.2. Economical and ecological benefits

In terms of process time, the single-use GMP process was slightly faster which can mainly be linked to the continuous capture step that enabled the purification of the entire harvest. Compared to the protein A step in batch mode, productivity based on grams of purified mAb per liter resin and time, productivity could be increased by factor 4–5, depending on individual settings. Experimental settings are described in previous publications, as the process itself was not changed significantly [9,16]. In conventional mode due to limitations in column sizes, multiple subcycles would need to be manufactured. Being able to employ less resin allows for ordering pre-packed columns which is of course another time saving aspect and further increases performance consistency of the equipment used. However, it should be mentioned that running in continuous mode means running multiple cycles on each column during one subcycle, leading to a higher depreciation compared to batch mode. The impact of these process conditions was previously discussed by Ötes et al. [9]. All other process steps took roughly the same time compared to corresponding stainless steel processes. However, in former purification processes like protein A chromatography around 235 L of 1 M NaOH were used for sanitization, followed by 2000 L of purified water (WFI) and another 90 L of 0.1 M NaOH for storage. This procedure (except storage) is performed before and after each process step, summing up to 3760 L of 1 M NaOH and 3200 L of WFI for an eight-step process. These amounts could consequently be saved in the present process as no cleaning procedure was required. The importance of reducing the amount of water and eliminating cleaning procedures has been pointed out before in recent studies by Budzinski et al. [17] *and* Madabhushi et al. [18], while evaluating the process mass intensity (PMI) of pharmaceutical manufacturing processes. Using PMI as an instrument to evaluate the efficiency and environmental impact of a process, *Madabhushi* observed that water makes up about 93% of the overall PMI, while 34–54% of these are linked to chromatography steps. Further, protein A chromatography accounts for 98% of the chromatography PMI because of its low binding capacity compared to the amount of resin. Therefore, it is suggested to employ protein

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A resin at higher capacity to make processes more environmentally beneficial [18]. This is in line with the findings of Budzinski et al. [17], pointing out that 75% of total PMI can be linked to downstream processes, wherein chromatography operations display the major driver. Proposing that industry should focus on increasing the resin binding capacity in order to lower the environmental footprint of mAb production, it is suggested to implement single-use equipment. While employing disposable equipment consequently leads to a higher PMI of consumables, the PMI of water strongly decreases while being one or partly even two orders of magnitude higher than the PMI of consumables.

Moreover, each of the sanitization steps takes around 2.5 h including preparation, incubation time and post-treatment. Therefore, a time saving of about 40 h or one Full Time Equivalent (FTE) for one business week can be realized while employing the single-use process the way it is being described in this study. Here, one person out of a team of six can follow other tasks due to this time saving (-17%). This is roughly the same percentage of employees as estimated by Levine et al. [10] for drug substance manufacturing, but of course such estimations highly depend on the specific process. The execution and analysis of cleaning verifications and determining clean and dirty hold times for the systems are additional time consuming aspects that are not necessary in this process. Besides from economic benefits, eliminating cleaning steps can also be ecologically advantageous. Comparing single-use and conventional process technologies, Pietrzykowski et al. [19] found that employing single-use technologies and materials leads to substantial reductions in environmental impact categories like global warming potential and cumulative energy demand. Here, it is pointed out that the main contributors of conventional processes are CIP/SIP procedures and protein A chromatography while processes relying on single-use materials show a significant decrease in WFI and steam usage [19]. Further, we could also observe a much shorter time that is needed for buffer preparation, as the cleaning procedure of the vessel becomes obsolete. A disposable bag for buffer preparation can be installed within 5–10 min, while sanitization and neutralization would take roughly 1.5 h.

3.2.3. Detailed cost analysis

Based on cost assumptions for buffer, labor and resin cost taken from a publication by Pfizer [20] the cost savings which arise from our new process approach switching to a single-use process and continuous protein A chromatography that was previously described were calculated and depicted in Fig. 6. It was assumed that buffer costs are around 10€ per liter and purified water around 1€ per liter. Further, labor cost for one employee working in a shift between 7–8 h is around 1100€ and resin cost for protein A resin is around 10,000€ per liter. As pointed out by Farid et al., replacing protein A might be an additional option for cost reduction in downstream processing, although being rather unlikely because of its binding selectivity leading to high yields and high purity. Therefore, resin reuse becomes an important issue when striving to reduce processing cost [5]. It should be mentioned that the cost analysis and its potential benefits are only valid for the respective process conditions which are described here. Total cost of the conventional stainless steel process were found to be reduced by 80% when employing the single-use process approach. Walther et al. [11] analyzed the impact of implementing continuous mAb and non-mAb manufacturing processes on operational and capital expenditure (OPEX, CAPEX) in an approach based on process economic modeling and Monte Carlo simulations. They found a 23% reduction in OPEX and 47% reduction in CAPEX for implementing a fully continuous mAb process compared to a conventional batch process. They further pointed out that resin cost is the main OPEX reduction contributor, which could be reduced by about 30% while total cost could be reduced by 55% [11]. These findings are in line with the ones from the present study, examining the cost saving distribution in Fig. 6. As assumed before, the main part of the savings is linked to the protein A capture step by using the single-use SMB system and the reduced amount of protein A resin, resulting in a cost saving share of 59.5%. In terms of single-use driven benefits, buffer cost reduction for the entire downstream process sums up to 17.3% as water and caustic can be reduced and sanitization steps become obsolete due to usage of disposable equipment. Finally, labor cost saving makes up 4.0% of the total cost savings, resulting from shorter preparation time and the absence of sanitization steps.

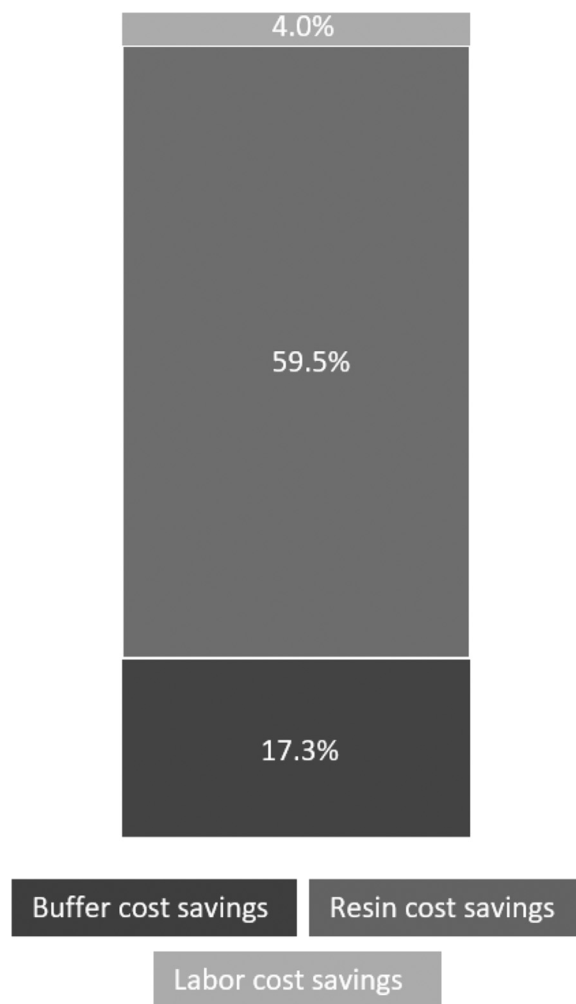

Fig. 6. Distribution of process cost savings arising from the implementation of a single-use process combined with continuous protein A capture. It can be seen that buffer cost savings are 17.3%, while resin cost savings are around 59.5% and labor cost savings were found to be 4.0%. In total, these savings sum up to 80.8% total process cost reduction.

Therefore, it can be concluded that savings in buffer and process time lead to a reduction in process cost for the overall downstream process, but the continuous protein A step clearly has the highest impact as only 3 L instead of 17 L of protein A resin is required. Thus, certain aspects like increased flexibility as mentioned by Sukhija et al. [3] can be confirmed because of faster buffer and equipment preparation due to the use of disposable materials. However, lower cost through the use of single-use

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materials could not be observed as proposed by Pollard et al., but rather by employing a continuous protein A step which was stated before by Zydney et al. Analyzing the economic impact of multi-column chromatography with a periodic counter current chromatography system (PCC), Pollock et al. [21] suggested that a reduction of manufacturing cost can rather be seen in early development and becomes less significant in commercial manufacturing [21]. While using the PCC system as well, but within an end-to-end continuous process, Godawat et al. [8] proposed that the possibility of employing smaller equipment will ultimately lead to smaller facilities and it enables the implementation of single-use materials as these are mostly limited in availability reaching a certain scale. Therefore, they stated that these aspects should lead to a reduction in OPEX and CAPEX [8].

3.2.4. Potential benefits and adjustments for the future

However, transferring continuous processing from the lab to GMP manufacturing requires monitoring of process performance and the definition of acceptance criteria for column performance parameters during resin reuse for example. In our study, we observed constant product quality and consistent process performance throughout the protein A step, but it must be noted that automation systems and validation activities are mandatory for implementing more continuous unit operations. Similar suggestions were made by Godawat et al. [8] as they pointed out that robust in-line monitoring of product quality, data management solutions and validation strategies for continuous processing is required for large scale end-to-end continuous manufacturing [8]. The potential monetary benefits of an end-to-end continuous process for mAb and non-mAb processes were calculated by Walther et al. [11], as they combined CAPEX and OPEX with uncertainty as net-present values (NPV) to generate a holistic evaluation of the manufacturing platforms. A net-present value of 64 $M is calculated for continuous production of mAbs compared to a conventional batch process, while NPV for continuous production of non-mAbs is at 306 $M, whereas CAPEX displayed the main driver as equipment can be reduced in size and number [11].

Further possibilities to reduce CAPEX include the option to perform polishing chromatography in continuous mode as well, yet these savings strongly depend on the process and the chromatography technique itself [11]. This may be a viable option for future processes whilst aiming to run the entire downstream process in continuous mode in a GMP facility. Another option for future processing might be including the continuous protein A step in virus clearance studies. This has not been performed for the current project as the employed polishing steps already ensured significant virus clearance while only moderate contribution was expected from the protein A step per se.

4. Conclusion

In this study we presented a downstream process consisting of end-to-end single-use systems combined with the CoPACaPAnA step which was conducted for the GMP manufacturing of a multispecific mAb. All product quality attributes at drug substance level fulfilled our acceptance criteria. We could not observe benefits in terms of footprint comparing the end-to-end single-use process to previous conventional processes. The main benefit can be regarded as time saving because the implementation of disposable materials eliminates holding steps and reduces time for preparation. It was calculated that the absence of sanitization procedures results in saving up to 40 h, which equals to 1 FTE for 1 week, corresponding to 17% of actual headcount reduction and 4% labor cost saving compared to the conventional process. Buffer savings were calculated to be 3760 L of buffer and 3200 L of WFI (corresponding to 17.3% compared to the conventional process), respectively. Therefore, this leads to a certain reduction in operational costs. Mainly, costs could be lowered because of the continuous protein A step as resin volume was reduced by about 83%. Cost saving was calculated to be around 59% compared to a conventional stainless steel process strategy for the protein A step, mainly due to resin reduction. Total cost savings up to 80% compared to the stainless steel process are anticipated due to usage of disposable equipment also for the intermediate and polishing step, all being performed on the single-use SMB system. However, taking into account that all consumables must be disposed after the process, these cost savings might turn out to be smaller regarding several batches throughout the year. In general, a more automated process is desired, including more than one continuous unit operation but adequate control systems must be installed to assure reliable process control and consistent process quality. Overall, the general feasibility of employing continuous process steps under GMP conditions has been shown, thereby highlighting the advantages of such processes not only at lab scale but also in a GMP environment which might therefore encourage the biotech industry to implement new process approaches in order to rise to the upcoming challenges.

Author statement

Hendrik Flato: Method design. Analysis and interpretation of data.

Cathrin Bernhardt: Supervising manufacturing. Supervising the implementation of technical components. Lead of transfer activities manufacturing (receiving unit).

Kevin Brandt: Person in plant. Method design and technical supervisory.

Florian Capito: Revision of the manuscript. Supervision of experiments.

Otmar Klingler: Downstream process design supervisory. Lead of transfer activities process development (sending unit).

Katharina Landrock: Method design and technical supervisory. Participation in transfer activities.

Verena Lohr: Revision of the manuscript. Supervision of manufacturing process.

Ralf Stähler: Method design and technical supervisory. Participation in transfer activities.

Ozan Ötes: Method design. Writing of manuscript. Data analysis and compilation.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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516 ## Appendix A. Supplementary data

517 Supplementary material related to this article can be found,
518 in the online version, at doi:https://doi.org/10.1016/j.btre.2020.
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