**Highlights**

* Design and validation of a control scheme for the semi-continuous MCSGP separation process, based on mp-MPC techniques.
* Use of the integral concentrations as outputs in the control problem, creating a solid basis for continuous control throughout the process cycle, enabling easier online measurements.
* The control scheme inherently suggests a periodic input profile that could allow cyclic steady state to be achieved.
* Good agreement between the computational (control) results and the experimentally optimised profiles as provided by ETHZ.

**Assisting continuous biomanufacturing through advanced control in downstream purification**

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# Abstract

Aiming to significantly improve their processes and secure market share, monoclonal antibody (mAb) manufacturers seek innovative solutions that will yield improved production profiles. In that space, continuous manufacturing has been gaining increasing interest, promising more stable processes with lower operating costs. However, challenges in the operation and control of such processes arise mainly from the lack of appropriate process analytics tools that will provide the required measurements to guarantee product quality. Here we demonstrate a Process Systems Engineering approach for the design a novel control scheme for a semi-continuous purification process. The controllers are designed employing multi-parametric Model Predictive Control (mp-MPC) strategies and the successfully manage to: (a) follow the system periodicity, (b) respond to measured disturbances and (c) result in satisfactory yield and product purity. The proposed strategy is also compared to experimentally optimised profiles, yielding a satisfactory agreement.

# Industrialisation of monoclonal antibodies

## The market challenge

Monoclonal antibodies (mAbs) are known for their targeted selectivity, which makes them a very powerful and attractive method for the treatment of cancer, various autoimmune diseases or organ transplantations (Bai, 2011). That together with the continuously increasing understanding of diseases at a molecular level has driven a rapid advancement in mAb research and has led to a remarkable market growth (Pavlou and Reichert, 2004). Currently, mAbs act as the leading product in the rapidly increasing market of high value biologics and their sales are increasing twice as fast as other biotechnological drugs. According to market forecasts, the value of the mAb market is expected to increase up to 75 billion USD p.a. by 2025(Mabion S.A., 2018).

Nevertheless, the high price tag of antibodies (approximately $35000 p/a per patient for mAbs treating cancer conditions) (Farid, 2007), as well as their upcoming patent expiration (Konstantinov and Cooney, 2015), underline the need for significant improvements in mAb manufacturing. The application of novel, cost-effective processes that will secure patents on the manufacturing process, may decelerate the emergence of similarly manufactured drugs. Moreover, a shift towards more cost-effective solutions and smart manufacturing will allow higher process yield, cheaper end-products and shorter production times, enabling mAbs to maintain a significant percentage of the pharma sales pie (Blackstone and Fuhr, 2012; Xenopoulos, 2015).

## From production to approval

The production of mAbs consists of two main parts: (a) the upstream processing (USP) and the downstream processing (DSP). The former refers to the culturing of the cells in bioreactors and the production of the targeted product, while the latter involves a sequence of separation/purification steps responsible for the isolation of the antibody from the upstream harvest. Currently, mAb biomanufacturing considers mainly fed-batch cell culture systems and batch separation processes (Xenopoulos, 2015). Figure 1 illustrates a standard process sequence followed for the production of mAbs. The process steps illustrated here may run either in batch or continuous mode. The USP usually uses mammalian cells (mostly CHO, NS0 or Sp2/0 cell lines) as the expression system that are cultured in suspension in large bioreactors (5-25 thousand litre) (Kelley et al., 2009; Wurm, 2004).



Figure 1 Indicative production process of mAbs. Upstream processing steps are depicted in the white area, while the grey area illustrates the downstream purification steps (adapted by Kelley (2009) and Liu et al. (2017)).

The DSP cascade involves various separation technologies, including different chromatographic purification steps. (Carta and Jungbauer, 2010)

Monoclonal antibodies are associated with stringent regulations that define their purity and composition. According to the ICH Q6B note on “Specifications: Test Procedures and Acceptance Criteria for Biological/Biotechnological Products” (ICH, 1999), for a drug to be considered acceptable for its intended use it needs to comply with pre-defined specifications. The latter correspond to “a list of tests, references to analytical procedures, and appropriate acceptance criteria which are numerical limits, ranges, or other criteria for the tests described”. Based on regulatory announcements (ICH Q6B) as well as information provided in the open literature (Carta and Jungbauer, 2010; Eon-Duval et al., 2012; Shukla et al., 2007) impurities are classified into two main categories: (a) product-related and (b) process-related impurities. The first one relates to impurities that result from the product, such as aggregates, while process-related impurities evolve from the process itself, such as Host Cell Proteins (HCPs).

Table 1 Critical Quality Attributes (CQAs) commonly observed in biopharmaceutical proteins (adapted by Eon-Duval et al. (2012), ICH Q6B & EMA “Guideline: Development, production, characterization and specifications for monoclonal antibodies and related products” (2016)).

|  |  |  |
| --- | --- | --- |
| Product-related impurities  and substances | Process-related impurities | Contaminants |
| Aggregation | Residual DNA | Adventitious agents (e.g. bacteria, mycoplasma, viruses) |
| Fragmentation | Residual Host Cell Proteins (HCPs) | Endotoxins |
| C- and N- terminal modifications | Raw material-derived impurities (e.g. leached Protein A) |  |
| Oxidation |  |  |
| Deamidation/isomerization |  |  |
| Glycosylation |  |  |
| Glycation |  |  |
| Conformation |  |  |
| Disulfide bond modifications/free thiols |  |  |

Critical Quality Attributes (CQAs) are considered to highly affect product safety and efficacy, hence it is suggested that they are closely monitored (Table 1).Limits on other types of impurities may depend on their type and/or effect on the drug activity, efficacy and/or safety and are therefore decided upon characterization and collection of preclinical/clinical data (Eon-Duval et al., 2012). Given the tight regulations on certain product and/or process impurities, thorough process monitoring becomes eminent. In that respect, control schemes that manage to track the individual level of impurities can be of great potential.

## From batch to continuous operation: The shift and challenges

To address the increasing competition, recent trends in biopharmaceutical processing are investigating the possibility of operating the process steps in a continuous fashion, envisioning the development of a fully integrated, continuous bioprocess. Continuous biomanufacturing is currently a promising solution towards: (i) improved productivity, (ii) decreased Costs of Goods (CoGs), (iii) reproducible product quality and (iv) decreased process times. The shift to continuous operation will offer the opportunity to reduce the capital cost through significant decrease in the equipment size and footprint (e.g. small bioreactors and chromatography columns, elimination of hold tanks) (Konstantinov and Cooney, 2015).

Process intensification through continuous operation has already been successfully applied to various industries (Anderson, 2001; Laird, 2007), aiming to decrease the environmental footprint, while increasing process productivity and product quality. Recent presentations and reports by the US FDA show that regulatory authorities have started to embrace the shift from batch to continuous operation in mAb manufacturing, pointing out its potential and needs (Chatterjee, 2012). Regulatory bodies embrace technological advances that can lead to more efficient processes of lower cost. The latter is supported by recent announcements from the FDA (2004; 2017) . urging manufacturers to make a step towards innovative pharmaceutical manufacturing. Moreover, the benefits of novel Process Analytic Technologies (PAT) technologies (such as reduction of production cycles and increased automation to minimize human error) are acknowledged and their development is encouraged(FDA and CDER, 2017).

Such a ground-breaking change in the status quo requires thorough consideration and investigation of several factors that affect both process counterparts (USP and DSP). The vision of a fully integrated bioprocess can also be facilitated through gradual development of each process independently, leading to hybrid intermediates (e.g. continuous upstream and batch downstream) (Godawat et al., 2015; Konstantinov and Cooney, 2015).Technological advances are therefore required in both USP and DSP in order to ensure that the “new” bioprocess will bring substantial improvements. Perfusion systems are already being used to a certain extent for the production of recombinant proteins (Hernandez, 2015) by leading manufacturers. Contrary to the USP, downstream processing is still facing significant challenges. Recent studies identify the latter as the most costly and limiting factor of the bioprocess. DSP is associated with approximately 80% of the manufacturing costs in bioprocessing (Girard et al., 2015; Hunt et al., 2001) and currently handles limited amount of volumes, thus limiting further improvements in the upstream process (Chon and Zarbis-Papastoitsis, 2011; Dunnebier et al., 2001; Gronemeyer et al., 2014; Strube et al., 2012). Issues regarding process performance and capacity utilization need to be addressed as they significantly affect product purity, production costs and yield. Besides this, process robustness is one of the main challenges, as batches out of specification due to variations need to be avoided (Degerman et al., 2009; Jungbauer, 1993; Thomas Mueller-Spaeth et al., 2013). Moreover, the need of technological advances in continuous viral inactivation and UF/DF as well as PAT is also reported (DePalma, 2016; Konstantinov and Cooney, 2015).

# The role of Quality by Design (QbD) and the application of PSE approaches

Quality by Design (QbD) was introduced by the FDA in 2004 as the method that allows the development of manufacturing processes that consider product quality as integral part of the process design. Developing a process, where it’s design ensures product quality requires thorough process understanding and a design that is based on scientific, risk-based, proactive approaches (Rathore and Winkle, 2009). The latter is usually achieved through offline experimentation, where critical process parameters and their effect on the quality attributes are identified and taken into consideration. QbD can assist both regulators and manufacturers to establish a more holistic communication, moving away from empirical solutions. By principle, QbD approaches are based on the identification of the product performance at an early stage, identifying all key attributes CQAs). The manufacturing process is then designed aiming to meet those specifications. It is evident that such procedures require thorough process understanding that will lead to identification of the sources and consequences of variability and will allow the development of control strategies to maintain the process within the optimal design space.

Although QbD offers pre-identification of the design space that will lead to the desired product quality, process understanding and scale up remain an open challenge. In that space the development and use of advanced computational tools promise to provide a low-cost experimentation platform that will assist process development. The design of mathematical models that describe the process at hand, offers a risk-free alternative to perform experiments of lower cost, in order to investigate the system dynamics. In addition to that, such models may allow the development and testing of optimisation and/or control policies that will yield improved operating profiles. Furthermore, the use of computational platforms in tandem with the online process, provides an intermediate solution to bypass points where online measurements are not readily available. Particularly in intensified (continuous) processes we often encounter the lack of online feedback as a major challenge in the application of advanced control strategies. This can be therefore tackled through intermediate measurements received from the mathematical model, until the offline or at-line experimental measurement is available. In this framework, the application of Process Systems Engineering (PSE) approaches in pharmaceutical manufacturing gain increasing attention (Bano et al., 2018; Diab and Gerogiorgis, 2018; Ierapetritou et al., 2016; Jolliffe and Gerogiorgis, 2018; Rossi et al., 2017).

Focusing on continuous processes, the need to design of a systematic approach that will minimize investment costs and potential risks increase. A stable, optimised continuous process has the potential to return products of consistently high quality, decreasing the risk of batch-to-batch variability. A robust, integrated experimental/computational approach can serve as the tool to obtain the information required for the design of an efficient, continuous bioprocess. However, currently, there are no standardized methods indicating the nature and the amount of the experiments that will provide the essential information needed for the optimization of the system. As a result, the information coming from experiments is often overwhelming and requires costly and time-consuming procedures to be retrieved. To complement and facilitate experimentation, comes the design of advanced computational tools that provides a solid basis for cost-free simulations, comparisons of different operating scenarios, as well as design of tailor-made experiments, thus minimizing labour cost and time (Royle et al., 2013). In this space, here, we are focusing on the design of an advanced control strategy for a semi-continuous separation process aiming to: (i) overcome challenges stemming from unavailable online measurements, (ii) maintain high product purity and yield and (iii) tackle variability in the composition of the feed stream.

# Case study: End-to-end process control for a semi-continuous chromatographic separation process

Improvements in cell culture titers, along with new regulatory directives (such as QbD and PAT) and the emergence of novel therapeutics of lower production costs (Cramer and Holstein, 2011) are driving the current state-of-the-art in downstream processing towards ground-breaking changes. In this direction several advances have been made both in the materials used, as well as in the operating strategies of purification processes. Several works have demonstrated chromatographic separation processes of increased capacity, achieved by either changes in the purification procedure or the use of novel technologies (e.g. single use technologies). Although the recovery yield is one of the dominant factors that characterize the efficiency of downstream processing, it is not the only criterion. Particularly in the case of mAbs, process efficiency is also evaluated by the purity of the end-product that is coupled with strict regulations (Kurz, 2007) . The combined objective of high purity and yield that follow a reverse analogy, gave rise to novel chromatographic separation processes of semi-continuous nature. The shift from batch to semi-continuous and eventually to continuous processing in chromatographic processes offers the opportunity for standardized procedures, common for all biopharmaceutical products and therefore stable product quality. In addition, the continuous nature promises significant reduction in equipment size and consequently in the capital costs (Konstantinov and Cooney, 2015).

There have been several works in the open literature examining the design of advanced control strategies for such processes, aiming to achieve high recovery yield and product purity. Most of the contributions, employ PID (Krättli et al., 2013) or ‘cycle-to-cycle’ (Grossmann et al., 2010; Suvarov et al., 2014) approaches that consider purity and yield as the control outputs. Several of the main challenges discussed in the open literature are: (a) the achievement of Cyclic Steady State (CSS) that will ensure process stability (lack of periodic input strategy), (b) lack of online feedback measurements of the tracked outputs, (c) efficient handling of disturbances and (d) simplification of the often computationally expensive optimization and/or control problem. In this work we develop and propose a control strategy, based on the tracking of the outlet concentrations of the mixture components that manages to: (a) lead to periodic input profiles, (b) high recovery yield and (c) high product purity.

## The system and process

Here we consider the twin-column Multicolumn Countercurrent Solvent Gradient Purification (MCSGP) (Aumann and Morbidelli, 2007; Krättli et al., 2013) used for the separation of a three-component mixture, containing an IgG1 monoclonal antibody as the product of interest, weak and strong impurities that correspond to aggregates and fragments of the IgG1. Currently, purification equipment based on the MCSGP process is commercially available by ChromaCon AG and LEWA Bioprocess Technology Group and has been used by several manufacturers (e.g. Bristol-Myers Squibb, Clariant, Merck Serono, Novartis) (Aumann et al., 2011; ChromaCon, n.d.; Krättli et al., 2013; Muller-Spaeth et al., 2011; Ströhlein et al., 2006). The work presented in this manuscript is based on a case study separation performed using the ContiChrom HPLC unit used for the separation of a monoclonal antibody from product-related impurities (ChromaCon, n.d.).

The semi-continuous setup (Figure 2) consists of two, identical ion-exchange columns that alternate between batch and interconnected mode, with the latter facilitating the recycle of the impure stream fractions from the column on the right-hand side of the setup to the one on the left. During the first interconnected (continuous) phase (I1), column 2 starts empty. Simultaneously, the outlet flow of column 1 enters column 2 mixed with an additional fraction of adsorbing eluent (or modifier) (E). Based on the principles of ion-exchange chromatography, the modifier is the salt used in the process to induce mixture separation (Guiochon and Trapp, 2012). After the completion of I1, the two columns start operating in batch mode (B1 phase). During this B1 phase, feed (F) is introduced to column 2, while column 1starts eluting the product (P), loaded from the previous process cycle. In I2 phase the recycling stream containing the impure fraction of product and strong impurities (S) exits column 1 and enters column 2. By the end of I2 phase, column 2 starts eluting weak impurities (W) (B2 phase). B2 phase finishes when the overlapping region of weak impurities and product reach the exit of column 2. At this point the first switch (Switch 1) has been completed and the two columns swap positions, by essentially applying the applying the input strategy of column 1 (eluent gradient and flow rate schedule) to column 2 and vice versa. Therefore, column 1 will go through the recycling and feeding tasks as described above, while column 2 will continue with the gradient elution (Krättli et al., 2013).



Figure 2 The twin-column MCSGP setup as presented by Krättli (2013)

The mathematical model used to describe the events taking place during the purification of the mAb using the MCSGP process has been previously developed and validated by the Morbidelli Group (ETH Zürich) (Melter et al., 2008; Ströhlein et al., 2006). The model equations are described in detail in Appendix A and have been previously discussed in (Papathanasiou et al., 2016). The model comprises two main parts: (a) the equation set describing the mass balances for the mixture components and the adsorption isotherms (Guiochon, 2002) and (b) the mass balances around the columns. During one process cycle, an input strategy is imposed, where the modifier concentration follows a gradient profile with minimum value 2 mg/mL and maximum value 12 mg/mL. The inlet flow rate varies between 0.1 mL/min and 1.5 mL/min approximately *(Due to confidentiality reasons the exact values cannot be disclosed and therefore only estimates are provided)*.

## Control concept for the twin column MCSGP process

In this work, we present a novel control concept for the twin column setup, under which the two columns are monitored independently, in a continuous fashion. The monitoring strategy is based on continuous measurements from both columns in real time. We use the mathematical model (Appendix A) and process setup (Figure 2) designed and validated by the Morbidelli group (Aumann and Morbidelli, 2007; Krättli et al., 2013; Müller-Späth et al., 2008) for the development and testing of the control scheme as presented below.

Output selection: *The concept of the integral*

According to standard practice, the optimization and control studies performed on chromatographic systems consider the maximization of recovery yield under purity constraints. However, purity and yield are calculated based on the average concentrations of the eluted mixture components (Equations 1 and 2).

|  |  |
| --- | --- |
|  | *Equation 1 Product purity* |
|  | *Equation 2 Recovery yield* |

|  |  |
| --- | --- |
|  |  |

Where, and correspond to the average purity and recovery yield over a process cycle, cav,s,j is the average concentration of the mixture components at the end of each cycle, indicates the feed concentration of the targeted product, 𝑗 indicates the cycle index and s the outlet stream

Consequently, that renders them “discrete-type” outputs that leads to ‘cycle-to-cycle’ control strategies. Furthermore, the calculation of purity and yield, usually requires an offline run of a separate separation cycle, thus adding a significant delay to the measurement. The latter approach may render tight control schemes infeasible to apply, especially for processes where parts are operated in a continuous fashion. The selection of continuous variables as outputs, would facilitate the derivation of tailor-made control laws and tighter process monitoring, as the controller would receive feedback in a continuous (or semi-continuous) fashion. Moreover, the outputs chosen for the formulation of the control problem should be ideally measurable or their measurements should not require lengthy experimental procedures. Aiming to tackle the aforementioned challenges, we suggest tracking of the integral of the outlet concentrations of the mixture components with respect to time (Figure 3b). Currently the quantities of the eluted components can be identified using standard UV detectors, used in such processes. The latter can be translated into the concentration of the eluted quantity using a linear relationship between the total mass injected in the column and the area below the peak of the chromatogram (Equation 3).

|  |  |
| --- | --- |
|  | Equation 3 Linear relationship between the total mass injected in the column and the area below the peak of the chromatogram |

Where is the quantity of the eluted component i, is the absolute response factor for component i and is the area below the peak on the chromatogram.

This linear relationship holds within a certain range (approximately up to 1000 AU, a representative range for such separation processes). Current setups are equipped with computational systems able to provide within seconds accurate, automated information on the area below the elution peak and therefore the eluted component quantity. This information can be used for the calculation of the integral of the eluted concentration via commercially available software (such as MATLAB®) and returned to the controller as feedback. The use of the proposed output offers continuous monitoring of the outlet stream throughout the process cycle and therefore allows the controller to act in a timely fashion in case of disturbances and/or deviations of the assigned setpoints. In addition, continuous (or pseudo-continuous at the range of seconds) tracking of the system offers the flexibility to use a series of measurements as controller feedback and minimize the risk of failure. The control scheme developed in this work is based on the principles of model-based control and therefore allows the use coming from the knowledge of the process model. Consequently, at points where measurements are not available or they require significant time to be computed, the mathematical process model can serve as an intermediate to provide the controller with the required feedback. Once the original measurement is obtained, this information can be updated.

Input selection: *Sensitivity tests*

The system originally, looks into 5 inputs and 3 outputs. The input set comprises the modifier concentration, the inlet flow rate and the feed composition. Similarly, the output set includes the integral concentrations of the mixture components. Given the complexity of the mathematical model, it is essential to reduce the computational burden of the control studies. Therefore, the control problem formulation needs to be simplified in a meaningful manner, following a rigorous procedure that will ensure the interactions between the inputs, namely the modifier concentration, the product, weak impurity, and strong impurity concentrations at the feed, and the inlet flow rate and outputs, namely the integrated product, integrated weak impurity concentrations, and integrated strong impurity concentrations are considered and the controller will return satisfactory results. Consequently, the 5 input–3 output system (5x3) is subjected to sensitivity tests, where the inputs are varied within the permitted range and their effect on the output behaviour is monitored.

Figure 3 illustrates the strategy applied to the inputs, where we excite within the allowed range: (i) the modifier concentration, (ii) the flow rate and (iii) the feed composition. Each input is varied separately in order to identify the individual effect it has on the monitored outputs (synergetic/antagonistic effects are not considered here). Based on the experimental procedures followed for the operation of the MCSGP the three variables are excited within the ranges shown in Table 2 and are representative for applications in mAb purification (Grossmann et al., 2010; Krättli et al., 2013). The illustrated bounds are in line and have been defined experimentally by the Morbidelli Group (ETH Zürich). Due to confidentiality agreement with the Morbidelli Group (ETH Zürich) the exact values cannot be disclosed and therefore only estimates are provided.



Figure 3 Pulse input strategy as applied for the sensitivity tests and the development of the state space models for (a) the modifier concentration (mg/mL), (b) feed composition (mg/mL) and (c) the inlet flow rate (mL/min).

Table 2 Upper and lower bounds used for the sensitivity tests. In the case of the feed composition: the concentrations of weak impurities, the product and strong impurities are varied within ± 10% from the base case values (0.07, 0.4 and 0.04 mg/mL respectively)

|  |  |  |  |
| --- | --- | --- | --- |
| Variable | Upper bound | Lower bound | Units |
| Modifier concentration (Figure 3a) | 4 | 2 | mg/mL |
| Inlet flow rate  (Figure 3c) | 1 | 0.2 | mL/min |
| Feed composition  (Figure 3b) | +10% from the base case value | -10% from the base case value | mg/mL |

Each input is varied independently and therefore synergetic/antagonistic effects are not considered here. The pulse strategy applied here is in line with the input strategy applied in the industrial operation of MCSGP and therefore it is preferred to a multisine approach. In order to allow an objective handling of the system, the time points and the duration of the pulses are randomly generated and chosen for each variable. Firstly, the modifier concentration is perturbed from its starting value (2.48 mg/mL), following 3 sequential changes (+8%, -19% and +61%) starting at the 60th, 136th and 200th minute respectively with 10 min duration (Figure 3). It is important to underline that between changes the system is let free to reach steady state. The latter allows unbiased monitoring of each perturbation independently. In this fashion, during changes in the modifier concentration the system is operated under constant flow rate (0.6 mL/min) and constant feeding with predefined composition (0.07, 0.4 and 0.04 mg/mL for weak impurities, product and strong impurities respectively). As depicted in Figures 3 and 4 the modifier concentration affects significantly the eluting component quantities.

More specifically, we observe that in the beginning when the modifier concentration is constant the eluted component quantities are constant as well. However, the first increase in the modifier concentration (60th minute) results in an increase in the eluted quantities. In particular we observe a 72%, 67% and 43% increase in the eluted concentrations of weak impurities, product and strong impurities respectively. From a physicochemical perspective it is expected that an increase in the modifier concentration will result into higher eluted quantities, as the modifier is the salt that induces the separation. Moreover, it is observed that the perturbation in the modifier concentration affected the weak impurities the most. The latter is also in line with the physics of the system as such kinds of impurities are characterized by decreased charge and are the ones that are relatively easy to separate and elute.

Conversely, the strong impurities require significantly increased modifier concentration in order to be eluted and thus are less affected by small changes. Following that, the modifier concentration is decreased (-19%) and re-stabilized to its original value after 10 min (Figure 3a). These two consecutive changes firstly allow the components to be accumulated in the column (during low modifier concentration) and then rapidly washed out resulting in the peaks observed at the 143rd minute (Figure 4). Lastly, the modifier concentration is increased to 4 mg/mL in order to cover the whole range of concentrations used in mAb purification (Figure 3a) that results into elution at the 200th minute (Figure 4). This jump in the modifier concentration leads to >100% increase in the eluted quantities that is, however, a cumulative effect stemming both from the increase in the modifier concentration as well as in the accumulation of the latter within the column. Contrary to the modifier concentration, the inlet flow rate does not seem to affect the quantities of the eluted components. In particular the inlet flow rate follows 3 consecutive perturbations between the 266th and the 470th minute that result in no significant change in the quantity of the eluted concentrations (Figure 4). This in accordance both with the mathematical model and the experimental procedure as the modifier concentration is used to calculate the Henry constant in a highly nonlinear equation, while the flow rate participates in a linear fashion in the general mass balance Appendix A) that results into lower impact on the total amounts of the eluting components. According to the basic chromatographic principles, the modifier concentration affects the liquid-solid equilibrium in a highly nonlinear fashion and consequently the accumulation and/or elution of the components at the end of each process cycle. On the other hand, the flow rate, for this process, affects the speed of the elution (i.e. the process kinetics) but not the thermodynamics of the chromatographic system.

In the case of the feed composition, the concentrations of the mixture components are varied simultaneously as they are present in the upstream harvest and therefore cannot be controlled independently. More specifically, the feed composition follows 2 perturbations between the 530th and the 610th minute (Figure 3). The first change corresponds to a +10% increase from the base case value (Table 2) and the second to a -10% decrease respectively, while the system is left to stabilize in between. The variation window ±10% has been experimentally predefined by the Morbidelli Group and corresponds to repetitive variations that result from the upstream process used for the production of the mAb studied in this work. The changes applied to the feed composition result in <10% changes in the eluted quantities.

To summarize, it is observed (Figure 4) that the most significant deviation in the output profiles occurs from changes in the modifier concentration. This is followed by changes in the feed composition, while the flow rate seems to have no significant effect on the quantity of the eluted components. It should be underlined that both the modifier concentration and the inlet flow rate can be controlled by the operator. Conversely, the feed composition depends on the upstream process and cannot be controlled. Nevertheless, in standardized processes, the variation range of the composition of the feed stream can be experimentally predefined, thus allowing us to treat it as measured disturbance with known bounds. Consequently, based on the sensitivity tests presented here, the system is reduced to a 1 x 3 input – output system with the modifier concentration as the sole input and the integrals of the outlet concentrations of the three mixture components as outputs.



Figure 4 Output profiles as resulted from the sensitivity tests for the outlet concentration of (a) the weak impurities, (b) the product and (c) the strong impurities.

Following the above presented studies, we use a reduced 1 input–3 output system (1x3) (Figure 5), considering the modifier concentration as the sole input and the integral concentrations of the three mixture components as outputs. The feed composition is considered as a set of measured disturbances, as it is resulting from the upstream process and cannot be controlled by the user. For the computational experiments, a variation window of ±10% is used that has been experimentally predefined by the Morbidelli Group (exact values are not provided here due to confidentiality reasons) and corresponds to repetitive variations that result from the upstream process used for the production of the mAb studied in this work.

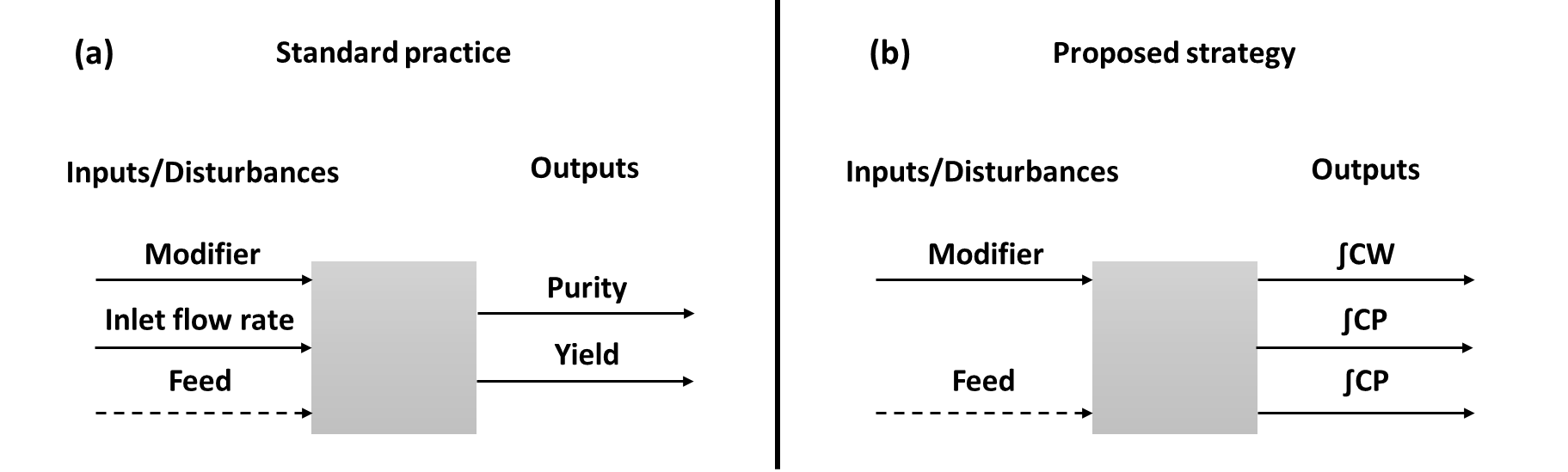


Figure 5 Selection of input, disturbances and outputs according to (a) standard practice and (b) the proposed strategy. , and refer to the integrals of the outlet concentrations of weak impurities, product and strong impurities respectively. Dotted lines refer to disturbances, while continuous lines correspond to inputs and/or outputs.

Control scheme

The setup (Figure 2) consists of two identical columns in terms of physicochemical properties and geometrical characteristics. During the online operation, the columns alternate between batch (B-phases) and continuous/interconnected (I-phases) mode, with half a cycle difference. We design and employ two multi-parametric MPC controllers based on the same formulation for each column throughout the process cycle (Figure 6). Because each column is identical in design and symmetric in operation, the mp-MPC controllers share the same formulation. During B-phases, both columns operate in batch mode, independent from one another. Therefore, the controller operates under the predefined set points that are estimated from offline experiments, monitoring the integral concentrations of the mixture components at the column outlet. During the B-phases, each controller obtains measurements for the introduced feed (labelled disturbance in Figure 6) which comes from the upstream process. The input signal to the controller changes for the introduced feed when switching from the B-phase to the I-phase, where the outlet of the left column becomes the introduced feed to the right column.

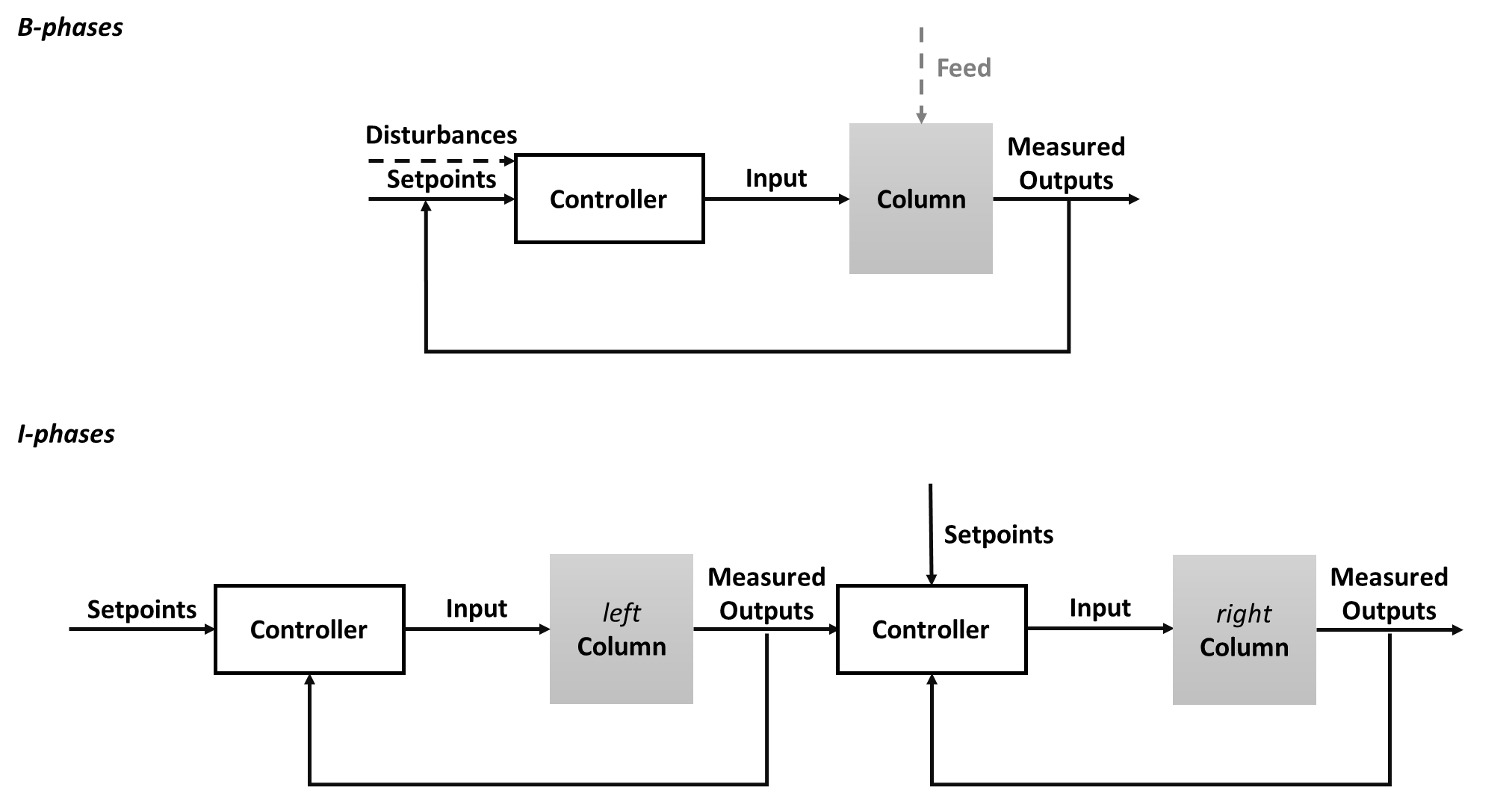


Figure 6 Control scheme for the MCSGP process for B- and I- phases.

The setpoints are user defined and can be estimated from offline experiments. The system is characterised by an inherent delay that is dependent on the operating flow rate. The delay for each process phase is calculated and shown in Table 3. The time delay (*or residence time*) can be calculated using the column volume and the operating flow rate (Equation 4) (Guiochon, 2002).

|  |  |
| --- | --- |
|  | Equation 4 Residence time as a function of the column volume and operating flow rate. |

Where *t* is the residence time (or time delay), *V* is the column volume and *Q* the operating flow rate. The time delay can be calculated a priori at each time point, as both the column volume and the flow rate are known. From an optimization standpoint, the flow rate is one of the most important factors that highly affect the product purity and the elution bands. Here, we pre-calculate the residence time for the given flow rate value and we set the setpoint accordingly to compensate for the delay (“*setpoint shift* strategy”) (Papathanasiou et al., 2016). In that way we create two sets of setpoints: (a) the ones that are shifted backwards in time and are the ones used by the controller for the input generation and (b) the setpoints that follow the elution profiles.

Table 3 summarizes the entities considered as inputs, disturbances and outputs for the formulation of the control problem for each column, based on the setup configuration and the column position. The control problem is formulated and solved following the PAROC framework (Pistikopoulos et al., 2015) (Appendix B).

Table 3 Selection of inputs, outputs and disturbances based on the process phase and the column configuration.

|  |  |  |  |
| --- | --- | --- | --- |
| **Property** | **B-phases** | **I-phases** | |
| ***Left column*** | ***Right column*** |
| **Input** | Modifier concentration | Modifier concentration at the connection point | Modifier concentration |
| **Disturbances** | Feed composition  (B2-phases only) | Impure fractions recycled from the right column (CW, CP, CS) | N/A |
| **Outputs** | Integrals of the outlet concentrations of weak impurities, product and strong impurities  (, , ) | Integrals of the outlet concentrations of weak impurities, product and strong impurities  (, , ) | Integrals of the outlet concentrations of weak impurities, product and strong impurities  (, , ) |

Control scheme validation and performance

The designed control scheme is validated in silico, against the process model as described in Steps 3 and 4 of the PAROC framework (Appendix B). The process model is simulated for 10 consecutive cycles, as per standard practice for such loads, under the operation of the mp-MPC controllers as shown in Figure 6. It is important to underline that original process as presented above is replicated for the closed-loop simulation. Therefore, for the first part of the cycle Column 1 is placed on the right-hand side, while Column 2 on the left (Figure 2). Following that, the two columns swap positions. It should be underlined that even though the controller is considering the modifier concentration as the sole input, the closed-loop validation is performed under the entire range of flow rate as per the original process. For the purposes of this work, switching times are considered fixed and equal to the experimentally optimized process. Due to confidentiality reasons, exact values on flow rates and switching times cannot be provided here.

Table 4 summarizes the ranges used for the inlet flow rates, while Figures 7 and 8 show the feeding strategy applied for each of the columns. Due to confidentiality reasons, exact values for the flow rate, feed composition and phase duration cannot be disclosed.

Table 4 Details of the system operation based on the position of the column as shown in Figures 2 and 4 (indicative values/ranges).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Phase** | **Flow rate** (mL/min) | | **Delay** (min) | | **Feed** (Figures 7 and 8) | |
| *Left column* | *Right Column* | *Left column* | *Right Column* | *Left*  *column* | *Right Column* |
| **I1** | Maximum | Average | 15 | 8 | - | - |
| **B1** | Maximum | Maximum | 5 | 5 | Feed:  CW, CP, CS | - |
| **I2** | High | Low | 6 | 26 | - | - |
| **B2** | Maximum | Maximum | 5 | 5 | - | - |

Figures 7 and 8 illustrate the feed composition (disturbance profile) for Column 1 and Column 2 respectively throughout the 10 cycles. It should be underlined that the feed composition, including weak impurities, product and strong impurities, results from the upstream process and cannot be controlled by the user. The three components are found in the same mixture and thus are fed through a single stream. However, for the sake of clarity in the visualisation, their concentrations are represented in separate graphs. Based on the phase of the process, the concentrations of the mixture components are either zero, where no feed is introduced, or equal to a base case value (B2-phases).



Figure 7 Feed strategy for Column 1 for 10 consecutive cycles. Composition of the feed stream containing: (a) weak impurities, (b) product and (c) strong impurities. This profile is used as measured disturbance for Controller 1.



Figure 8 Feed strategy for Column 1 for 10 consecutive cycles. Composition of the feed stream containing: (a) weak impurities, (b) product and (c) strong impurities. This profile is used as measured disturbance for Controller 2.

Figure 9 illustrates the modifier concentration (input profiles) as generated by the controllers for column 1 and 2, respectively. A clear periodicity in both inputs is observed that is inherently suggested by the controller and is not user-imposed. The latter is of key significance for the achievement and maintenance of Cyclic Steady State (CSS) during online operation of the controller. The observed profile is a result of the integral tracking that allows both for continuous controller output and periodic change in the setpoint.



Figure 9 Input profile (modifier concentration) as suggested by the controllers for: (a) column 1 and (b) column 2 for 10 consecutive separation cycles.

Aiming to evaluate the performance of the controller with respect to the original system, we compare the modifier concentration at the inlet of the two columns with and without the operation of the control scheme (Figure 10). It is observed that the two profiles are almost identical to each other with negligible deviations towards the end of the simulation (cycles 8, 9 and 10). In addition to that, the controller tends to reach slightly higher concentrations of modifier at the respective peaks. Furthermore, the controller anticipates its action at every cycle aiming to optimise for the residence time and the associated delay in the elution. It could be concluded that the controller is capable to mimic the experimentally optimised input profile, as well as the periodicity.



Figure 10 Comparison of the modifier concentration at the column inlet for: (a) column 1 and (b) column 2 - under the operation of the controller (continuous black line) and in open loop, applying the experimentally optimized input profile (modifier concentration) (dotted grey line).

Figure 11 illustrates the elution profiles of the three mixture components (weak impurities, product and strong impurities) for both columns over a 10-cycle period, under the operation of the controllers. It is evident that the proposed scheme manages to return an input profile that allows separation to be achieved in the correct time points (B2-phases). Although the outlet concentrations demonstrate a clear periodicity, it is observed that towards the last 3 cycles (cycles 8, 9 and 10) the eluting concentration of the weak impurities increases, while the amount of product leaving the column decreases. Moreover, the input profile suggested by the controller over these cycles deviates slightly from the experimentally optimised one (Figure 11). A total deviation between the controller input and the experimentally optimised modifier concentration of approximately 4 min over 10 cycles is observed (Figure 10). This corresponds to 0.4 min of deviation per cycle, which is a total time for which the controller is optimising (output horizon of four 0.1 min steps) (please refer to Appendix B for the control problem formulation). Therefore, the controller aims to compensate for this, anticipating its actions as the process moves into the next cycle. As a result, the controller is anticipating the action in time, thus leading to the illustrated deviations. Nevertheless, a similar deviation in the profile of weak impurities is not observed. The latter is a result of the physicochemical properties of the system, as weak impurities are the first to be removed and are more sensitive to changes in the modifier concentration. These are followed by the product and finally by the strong impurities that require a high modifier concentration in order to be removed from the column.



Figure 11 Elution profiles for: (a) column1 and (b) column 2 over a 10-cycle period under the operation of the controllers, subjected to the input as shown in Figure 8.

As mentioned earlier, the assessment of the process performance is highly dependent on purity and yield. The latter are calculated based on the elution profiles shown in Figure 11. Figures 12a and b illustrate the profiles for yield and purity respectively, over the 10-cycle operation. The setup under the operation of the controller achieves a high percentage of purification, with a minimum of 97.3% and a maximum of almost 99%. Overall, the process could be characterised efficient, with 60% minimum yield (excluding the first cycle, where not both columns are loaded). Nevertheless, it is observed that the process yield gradually decreases after the 6th cycle, while it starts increasing again at the final cycle. Similarly, purity seems to follow a negative slope during the last 3 cycles. This behaviour is in accordance to the results presented in Figure 11, where the quantity of the eluted product decreases over cycles 8, 9 and 10. However, despite the slightly poorer behaviour of the controller in the last three cycles, the overall system performance can be considered satisfactory.



Figure 12 Profiles for: (a) yield and (b) purity over 10 consecutive cycles under the operation of the proposed control scheme (continuous black line) as compared to the experimentally optimised profile (dotted black line). Values are calculated based on the profiles shown in Figure 9.

Figure 12 looks also into the comparison of the system performance in open loop (dotted line) versus the closed loop operation (continuous line). In the case of the recovery yield (Figure 12a), the controller seems to outperform the open loop system up to the 6th cycle, while it demonstrates an inferior behaviour thereafter. The dotted line indicates that the setup manages to reach a plateau at 78% yield under open loop operation, whereas the system under the operation of the controller reaches 91% yield at the 5th cycle and drops after that. However, based on the overall average yield of the two operations, we observe that the open loop system returns 70.6%, while the system under the operation of the controller results into 71%. Therefore, based on the performance of the yield, the controller behaviour is considered satisfactory and very close to the experimentally optimised system. On the other hand, product purity (Figure 12b) seems to be significantly improved under the operation of the controller (continuous line), reaching 99%.

# Conclusions

In this work, a novel control strategy for the periodic, twin-column MCSGP chromatographic process is presented. Based on mp-MPC principles, two identical controllers are designed, considering: (a) the modifier concentration as input, (b) the feed composition as disturbance and (c) the integrals of the outlet concentrations as outputs.

As illustrated in the presented results, the proposed scheme manages to achieve high product purity (97.3%-99%) and average to high yield (>60%, excluding the first cycle, where both columns are not loaded), throughout the 10-cycle operation. Employing the integral outlet concentration of the mixture components as outputs, allows continuous monitoring, throughout the process cycle and encourages inherent periodicity in the input profile (modifier concentration) that could lead to cyclic steady state (CSS) during the online operation. Moreover, a control strategy based on the integrals of the outlet concentrations could be seamlessly applied during online operation, obtaining measurements from existing equipment. The behaviour of the proposed scheme is also compared to the experimentally optimised profile as provided by ETH Zurich and a very good agreement is observed (Figure 10). The controller suggests an input profile (modifier concentration) almost identical to the one proposed by the experimentally optimised process, with slight deviations in the maximum reached values, as well as the profiles of the last 3 cycles. The matching profiles of the open loop and closed loop performance is a positive indication that the controller can find use in an environment with process and/or measured disturbances. Therefore, the proposed controller design lays the foundation for future work.

Unlike other works in the open literature, the suggested scheme does not consider the flow rate as part of the control problem, while the duration of each phase and the switching times remain fixed to their experimentally optimised values. Based on the sensitivity tests presented in this work, the inlet flow rate is shown to affect the elution times, but not the eluting amount of the components. Therefore, aiming to reduce the computational complexity of the control problem, the flow rate is excluded from the input set. Nevertheless, the “setpoint shift” strategy presented by Papathanasiou et al. (2016) is employed here, according to which the output setpoints are set prior in time to compensate for the inherent time delay. The latter depends highly on the inlet flow rate value and is not constant throughout the process cycle. The suggested strategy manages to successfully handle the delay under the whole range of operating flow rates, leading to component elution within the predefined windows. Although the duration of the phases and the switching time are assumed to be fixed to their experimentally optimised values, it would be interesting to investigate alternative solutions that could potentially improve the current profile. However, it should be underlined that considering any of those as an optimisation variable will significantly increase the computational complexity of the problem. In this case, offline optimisation experiments could be conducted, in order to decrease the online computational expense.

Lastly, the presented control scheme considers variations in the feed stream as measured disturbances, based on experimentally predefined ranges. Given that the composition of the feed stream usually comes from the upstream process and cannot be controlled by the user, considering it as disturbance in the formulation of the control problem, allows the controller to efficiently handle possible variations. As illustrated in the presented results, the controllers can successfully handle the periodic profile of the feed that ranges from 0 mg/mL (equivalent to no feed) to the maximum concentration (when feed is introduced to the column). The controller is trained to handle variations in the feed stream that range from 0 mg/mL up to +10% from the base case value. Current and future research is focusing on improvements of the control problem formulation to compensate for the system delay and the integral behaviour, as well as testing the controller under variations in the feed composition and unmeasured disturbances that may arise from signal failures.

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# Appendix A

## Mathematical model of the MCSGP process

The mathematical model describing the MCSGP process has been developed by ETH Zürich and has been tested for various case studies ((Muller-Spaeth et al., 2011; Müller-Späth et al., 2011, 2010, 2008)). The model comprises Partial Differential and Algebraic Equations (PDAEs) and describes the main events taking place in in the chromatographic column.

## Physical and Mathematical Quantities

|  |  |  |
| --- | --- | --- |
|  | Average concentration of species i in stream s | mg/mL |
| Acol | Column cross section | cm2 |
| c(z,t)i,h | Liquid phase concentration of species i in column h | mg/mL |
| c0i,h | Initial concentration of species i in column h | mg/mL |
| cfeedi | Feed concentration of species i | mg/mL |
| cini,h | Inlet concentration of species i in column h | mg/mL |
| couti,h | Outlet concentration of species i in column h | mg/mL |
| Hi,h | Overall Henry constant of species i in column h | mg/mL |
| HIi,h | Henry constant for the adsorption site 1of species i in column h | mg/mL |
| HIIi,h | Henry constant for the adsorption site 2of species i in column h | mg/mL |
| Hmod | Henry constant of the modifier | mg/mL |
| ki | Lumped mass transfer coefficient of species i | min-1 |
| Lcol | Column length | cm |
| ncol | Number of columns | - |
| ncomp | Number of components | - |
| noutlet | Number of outlet streams |  |
| q(z,t)i,h | Solid phase concentration of species i in column h | mg/mL |
|  | Average purity at cycle j | - |
| q\*(z,t)i,h | Equilibrium solid phase concentration of species i in column h | mg/mL |
| Qh | Flow rate of column h | mL/min |
| qIi,h | Saturation capacity for the adsorption site 1of species i in column h | mg/mL |
| qIIi,h | Saturation capacity for the adsorption site 2of species i in column h | mg/mL |
|  | Yield at cycle j | - |
| αi,1, …, αi,8 | Coefficients | - |
| εi | Column porosity for component i | - |

## Subscripts

|  |  |
| --- | --- |
| h | Column index |
| i | Species index |
| j | Cycle index |
| s | Stream index |
| t | Time |
|  | Cycle duration |
| z | Space coordinate |

Table A.1 Model equations for the twin column MCSGP setup.

|  |  |  |
| --- | --- | --- |
| **Index** | **Equation** | **Description** |
| **E1** |  | Liquid phase concentration |
| **E2** |  | Solid phase concentration |
| **E3** |  | Solid phase concentration at equilibrium |
| **E4** |  | Henry constants |
|  |
|  |
| **E5** |  | Saturation capacities |
|  |
| **E6** |  | Solid equilibrium phase concentration (modifier) |
| **E7** |  | Product purity |
| **E8** |  | Recovery yield |

Table A.2 Initial and boundary conditions.

|  |  |  |
| --- | --- | --- |
| **Index** | **Equation** | **Description** |
| **C1** |  | Initial Condition |
| **C2** |  | Boundary conditions at the column inlet |
| **C3** |  | Boundary conditions at the column outlet |

Table A.3 Model parameters and the respective order of magnitude (data obtained by ETHZ, group of Prof. Morbidelli). Due to confidentiality reasons the exact values cannot be disclosed and therefore only estimates are provided.

|  |  |  |  |
| --- | --- | --- | --- |
| Parameter | Significance | Value (range) | Units |
| Acol | Column cross-section | 0.2-0.5 | cm2 |
| Lcol | Column length | 10-16 | cm |
| ki | Lumped mass transfer coefficient of species *i* | 20-100  [Species dependent] | min-1 |
| Dax | Effective axial dispersion coefficient | 0-0.001 | cm2/min |
| ε | Column porosity for component *i* | 0.5-0.8  [Species dependent] | Dimensionless |
| Hmod | Henry constant for the modifier | 0.2 | Dimensionless |
| αi | Species dependent constants | Species dependent | Dimensionless |

# Appendix B

## The PAROC framework and software platform

The PARrametric Optimization and Control Framework (PAROC) is a method to develop multiparametric solution to optimization problems based on a high-fidelity model that governs a system of interest.

Step 1: Modelling and Simulation

The first step in the PAROC framework is to develop a mathematical model that accurately represents the system of interest. This high-fidelity model is based on either first principles (i.e. (partial) differential algebraic equations) or data-driven modelling techniques

Step 2: Model Approximation

Due to the complexity associated with the high-fidelity model, optimization based decision making may require the use of an approximate model to ease the computational burden. Developing the approximate model to represent the high-fidelity model is addressed with either system identification or model reduction techniques. In this work, we utilize MATLAB System Identification Toolbox™ to develop the linear discrete time state space approximate model that is used to represent the high-fidelity model developed in Step 1. The state space model developed in this step is an intermediate step to facilitate the formulation of the multiparametric programming problem. The approximate model is developed by exciting the high-fidelity model via the inputs and disturbances to the system while tracking the output of the system. This input/output profile is used to develop the resulting state space model of the following form:

where are the states and have no physical meaning, is the input to the system (modifier concentration), is the disturbance to the system (feed composition), and is the output of the system (integral of outlet concentration) at time . The state space model is validated against the high fidelity model to ensure quality open loop performance.

Step 3: Multiparametric Programming

In this step, the approximate model developed in Step 2 is utilized in an optimization formulation where initial conditions, disturbances, and references are considered as uncertain parameters. Solving this optimization formulation in a multiparametric manner enables the offline explicit solution of the objective function and optimization variables as expressions of these uncertain parameters. Multiparametric programming yields critical regions where within a given region the expression for the optimization variable and objective function remains the same. The solution and categorization of the multiparametric problem is accomplished via the-state-of-the-art Parametric Optimization toolbox (Pistikopoulos et al., 2015).

Step 4: Closed Loop Validation

The explicit control laws developed in Step 3 are validated *in-silico* against the high fidelity model by monitoring the set point tracking performance. If the performance of the resulting closed loop response is adequate, the developed control laws can be utilized as needed. However, if the closed loop performance is poor, either (i) a new approximate model in Step 2 is developed, or (ii) the control formulation in Step 3 is retuned.

## Application of the PAROC framework on the MCSGP Process

Step 1: Modelling and Simulation

The high fidelity dynamic model (Appendix A) is simulated in the gPROMS® ModelBuilder environment with 50 spatial discretization points following a finite difference method, yielding 823 differential equations and 3308 algebraic relations (Papathanasiou et al., 2016).

Step 2: Model Approximation

The input strategy presented in Table B1 is used for the design of a linear state space model of the characteristics presented in Table C.1.

Table B.1 Characteristics of the SIMO state space model with the feed as disturbance.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Input  Variable | Output Variable  (Integrals of the outlet concentration) | Measured Disturbance | Number of States | Sampling Time (s) |
| Modifier  Concentration | Weak impurities,  Product and Strong impurities | Feed composition | 4 | 6 |

The state space model is validated against the mathematical, process model and results into: 94.88%, 94.93% and 93.06% fit for the three outputs respectively. Figure B.1 illustrates the comparison between the state space model (dotted line) and the process model (continuous line). The matrices A, B, C and D correspond to the ones presented and discussed above for the state space formulation.



Figure B.1 Comparison of the state space model simulation (---) against the high-fidelity process model (─) for (a) the weak impurities, (b) the strong impurities and (c) the product, with 94.88%, 93.06% and 94.93% fit respectively.

Step 3: Model based control

The developed state space model is used in a multiparametric formulation to develop an explicit offline Model Predictive Controller to maintain a user defined target value for the outlet concentrations. The controller maintains a manipulated action in the form of the modifier concentration, measured disturbance from the concentrations of components in the feed stream, and set point tracking of the integrated outlet concentrations. The tuning matrices, output horizon, and control horizon for the multiparametric model predictive controller are presented in Table C.2. To ensure quality set point tracking from the controller, a large penalty is applied to the deviation of the integrated concentration of the product from its desired set point, as seen by the second term in the QR matrix. The multiparametric solution was determined by using the state-of-the-art POP® toolbox (Oberdieck et al., 2016).

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Table B.2 Design parameters of the mp-MPC controller

|  |  |
| --- | --- |
| **Design Parameter** | **Value** |
| Output horizon | 4 |
| Control horizon | 2 |
| QR (Quadratic matrix for tracked outputs) |  |
| R (Quadratic matrix for manipulated variables) |  |

The MPC formulation exhibits standard set point tracking for the inputs and outputs based on a quadratic objective function with linear constraints. A mismatch term, *e*, is incorporated due to the inherent plant model mismatch between the linear state space model used to represent the highly nonlinear process (Katz et al., 2018; Ziogou et al., 2013). Box constraints are imposed on the states, inputs, outputs, and parameters of the system, where the upper and lower bounds for the inputs, outputs, and parameters are derived from real physical quantities. The input is defined as the modifier concentration, the output is the integrated concentration for the weak impurities, product, and strong impurities, and the disturbances are the feed compositions of the weak impurities, product, and strong impurities at the inlet of the chromatograph.

Step 4: Closed Loop Validation

The designed controller is validated performing an *in-silico* test against the high fidelity dynamic model. The closed loop performance utilized the multiparametric solution and the gPROMS® software in conjunction with gO:MATLAB, the interface between the gPROMS® environment (high fidelity model) and the MATLAB® environment (control solution). The results of the closed-loop validation are discussed in the main text for completeness, Figures B.2 and B.3 illustrate the behaviour of the integrals of the outlet concentrations that are used as the controller tracked outputs. In all cases, the integrals of the outlet concentrations remain constant for the time periods that no elution is taking place, while they increase following a gradient at the dedicated elution windows. The gradient profile is imposed through the setpoint in order to ensure that components are leaving the columns following a gradient elution profile. For the major part of the simulation, a good agreement between the user-defined setpoint and the process model output is observed (<2%). Nevertheless, in the case of strong impurities an increasing mismatch is observed past the 300th minute for both columns. Although this is significantly away from the 2% acceptable threshold, its impact on the general process efficiency is not significant. Strong impurities are eluted last from the column and the main objective is to clean the column from any remaining impurities. From a process standpoint, strong impurities (Figure B.2) should be: (a) maintained at low concentration during product elution and (b) removed from the column by the end of every cycle. According to the results shown in Figure 11 in the main body of the manuscript, both those targets are achieved and therefore we assume that the controller performs efficiently for the purposes of this work, despite the presented offset.



Figure B.2 Comparison of the predefined setpoint (---) and the output of the process model simulation (─) for column 1 as resulted from the controller closed-loop validation for (a) weak impurities, (b) product and (c) strong impurities over.



Figure B.3 Comparison of the predefined setpoint (---) and the output of the process model simulation (─) for column 2 as resulted from the controller closed-loop validation for (a) weak impurities, (b) product and (c) strong impurities over.