Received: 30 June 2021 Revised: 30 November 2021 Accepted article published: 6 December 2021 Published online in Wiley Online Library: 5 February 2022

(wileyonlinelibrary.com) DOI 10.1002/jctb.7008

Process analytical technology as key-enabler for digital twins in continuous biomanufacturing

Axel Schmidt,* Heribert Helgers, Lara Julia Lohmann, Florian Vetter, Alex Juckers, Mourad Mouellef, Steffen Zobel-Roos and Jochen Strube

Abstract

Over the last few years rapid progress has been made in adopting well-known process modeling techniques from chemicals to biologics manufacturing. The main challenge has been analytical methods as engineers need quantitative data for their workflow. Industrialization 4.0, Internet of Things, artificial intelligence and machine learning activities up to big data analysis have taken their share in solving fundamental problems like component- or at least group-specific evaluation of spectroscopic data. Besides, concerning inline analytics methods included in process analytical technology concepts the key technology has been the generation of decisive validated digital twins based on process models. This review aims to summarize the methodology to achieve a holistic understanding of process models, control and optimization by means of digital twins using the example of recent work published in this field.

Keywords: biotechnology; modelling; process control; simulation; biochemical engineering

INTRODUCTION

Manufacturing of biologicals is widely based on batch processes. A switch to continuous processing has the potential to improve manufacturing in terms of agility, flexibility, quality, cost and societal benefits.[1,2] Institutions like the US Food and Drug Administration and Center for Drug Evaluation and Research encourage the switch to continuous manufacturing by giving examples of achievable improvements: the expansion of production volumes is enabled without the current problems related to batch scale-up. It becomes possible to quickly increase production in case of shortages or emergencies. Currently, production supply chains are spread globally and therefore vulnerable in many ways. Switching to custom manufacturing also allows for introduction of highly meaningful statistical process control and a decreased initial investment. The environmental impact is generally lower; it triggers the demand for highly trained staff and freed resources can be invested in new products.[1-4] Productivity improvements of such transitions from batch to continuous processing have been reported to be up 100-fold.[5]

Current state of the art of monoclonal antibody manufacturing, for example, is based on a batch platform process.[6,7] This process is divided into upstream and downstream processing. The production of the recombinant target protein is based on cell cultivation in bioreactors during the upstream processing. The objective of the subsequent downstream processing is to isolate the target protein from side components such as host cell proteins, host cell DNA, media components, viruses and endotoxins by using various unit operations such as centrifugation, filtration and chromatography.[7-12]

This platform process includes fed-batch suspension cultivation of mammalian cells up to 20 000 L bioreactor volume, centrifugation and depth filtration as cell harvest, protein A affinity chromatography as capture, cation exchange chromatography as intermediate purification and hydrophobic interaction chromatography as polishing step.[13-15] In addition, orthogonal virus inactivation steps using low pH hold and virus filtration are implemented to minimize immunogenicity.[6,7] Subsequent to the protein A affinity chromatography and low pH hold for virus inactivation, diafiltration needs to be performed to load the subsequent cation exchange chromatography.[6]

Looking at the established platform process, the chromatographic steps will reach their capacity limit due to increasing product concentrations achieved by upstream processing optimization (i.e., cell engineering, media and process parameter optimization). This limitation is widely known as downstream

* *Correspondence to: Axel Schmidt, Institute for Separation and Process Technology, University of Technology Clausthal, Clausthal-Zellerfeld, 38678, Germany. E-mail: schmidt@itv.tu-clausthal.de*

Contribution to the Special Issue on Continuous Biomanufacturing

Institute for Separation and Process Technology, University of Technology Clausthal, Clausthal-Zellerfeld, Germany

bottleneck.[10,16-20] With increasing product concentration, the specific costs (euros per kilogram) of the upstream and downstream processing decline. However, with higher product concentrations the platform downstream process will reach its efficiency optimum. Increasing the product concentration even more leads to a significant shift of cost of goods (COGs) from upstream to downstream processing.[7,21-23]

Continuous bioprocessing circumvents the aforementioned downstream bottleneck and batch scale-up problems by increasing the productivity and flexibility of each unit operation with a simultaneous increase in product quality resulting from continuous product processing (i.e., short hold times).[4,9,16,23-34] The Food and Drug Administration, European Medicinal Agency, International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) and various industrial working groups have started to publish guidelines (ICH Q8–Q11) to increase product quality during manufacturing, thereby promoting continuous bioprocessing.[35-40]

Key technologies and concepts needed to realize the full potential of continuous production are process analytical technology (PAT) and quality-by-design (QbD) guidelines. In contrast to traditional quality-by-testing or trial-and-error methods, a knowledge-based link between process and product attributes can be derived. This allows not only dynamic reaction to process fluctuations, but also flexible maintenance of the process optimum in the case of varying input variables through process adjustments within the approved design space. These developments in the transition from batch-wise to continuous manufacturing ultimately culminate in the digital twin, which, through the combination of QbD-based process development, robust PAT online data and predictive process models, enables the automation of process control and, in the ideal case, real-time release testing.

From this perspective, PAT can be understood as a link that enables the QbD process design and the control strategies derived from it through the exchange of critical process data in real time (see Fig. 1).

There are a number of definitions of this digital twin, which vary depending on their background. The most common definition is that the digital twin is a comprehensive digital representation of a physical object that is capable of bidirectional communication with that object. In the process automation community, on the other hand, the definition is that a digital twin as a comprehensive digital representation of a manufacturing asset responds to the state of the physical asset and modifies its behaviour.[44] In this context,

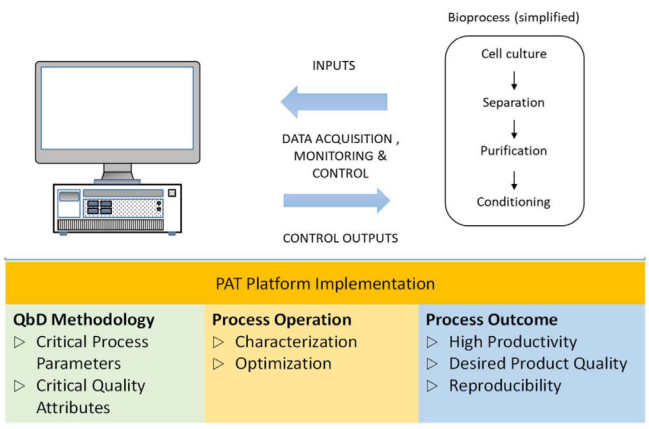

Figure 1. Bioprocess control implementation in the quality-by-design (QbD) paradigm.[41]

Udugama *et al.* proposed a five-step implementation strategy. Starting from simple balance equations to validated process models, the digital twin is ultimately a digital representation of the physical process. With the real-time transmission of measurement data enabled by PAT and model-based advanced process control, the optimization of the physical process is possible in real time. These are arranged according to the complexity of the process model as well as the available data and control options (see Fig. 2).[42]

A detailed illustration of the interaction of process control, data acquisition, data evaluation and modeling is discussed elsewhere.[45]

MONOCLONAL ANTIBODY MANUFACTURING

The following section summarizes a series of studies, which address and highlight the challenges of operational costs and process development for the continuous bioprocessing of a monoclonal antibody by alternative unit operations and the development of a digital twin thereof.[46-50]

Upstream processing

A macroscopic kinetic model based on Monod kinetics for mammalian cell cultivation suitable for digital twin purposes was developed by Kornecki *et al.* and verified according to a general valid model validation workflow.[51] The macroscopic model was verified and validated on the basis of four decision criteria (plausibility, sensitivity, accuracy and precision as well as equality) and subjected to a case study, comprising a Chinese hamster ovary fed-batch cultivation for the production of a monoclonal antibody. It was found that, based on design of experiments and Monte Carlo simulation, the maximum growth rate μ_{max} exhibited the greatest influence on model variables such as viable cell concentration X_V and product concentration. In addition, partial least squares regressions statistically evaluate the correlations between a higher μ_{max} and a higher cell and product concentration, as well as a higher substrate consumption. Details of the upstream process model can be found elsewhere.[51] In the following a short summary and case study for autonomous operation in a continuous manufacturing scenario are given.

The impact of experimental model parameter determination error on the process model, based on error propagation of experimental errors, must be assessed to evaluate model precision. This variance can be included in a Monte Carlo simulation to determine the effect of model parameter errors on the process model. In this case, 100 simulations, varying each parameter equally distributed based on their experimental errors, were conducted.

The model thus validated by Kornecki *et al.* can be used as the basis for a digital twin. For continuous antibody production, there are a number of effects that take on different values with increasing process time and therefore need to be monitored. These include, for example, the decrease in cell-specific productivity, which depends, among other things, on the cell line used. Concentration fluctuations or a continuous monoclonal antibody (mAb) concentration decrease due to decreasing cell-specific productivity over process time have been described.[52] Simulation studies are used to test whether PAT can enable a process control strategy that allows for compensation of titer fluctuations in the subsequent downstream processing (DSP). The scenario for the following simulations is that the mAb concentration and purity (i.e., high- and low-molecular-weight concentrations) are continuously measured in the outstream using a detector array as described by Helgers *et al.*[47] The real-time measurement data

Journal of Chemical Technology and Biotechnology published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry (SCI).

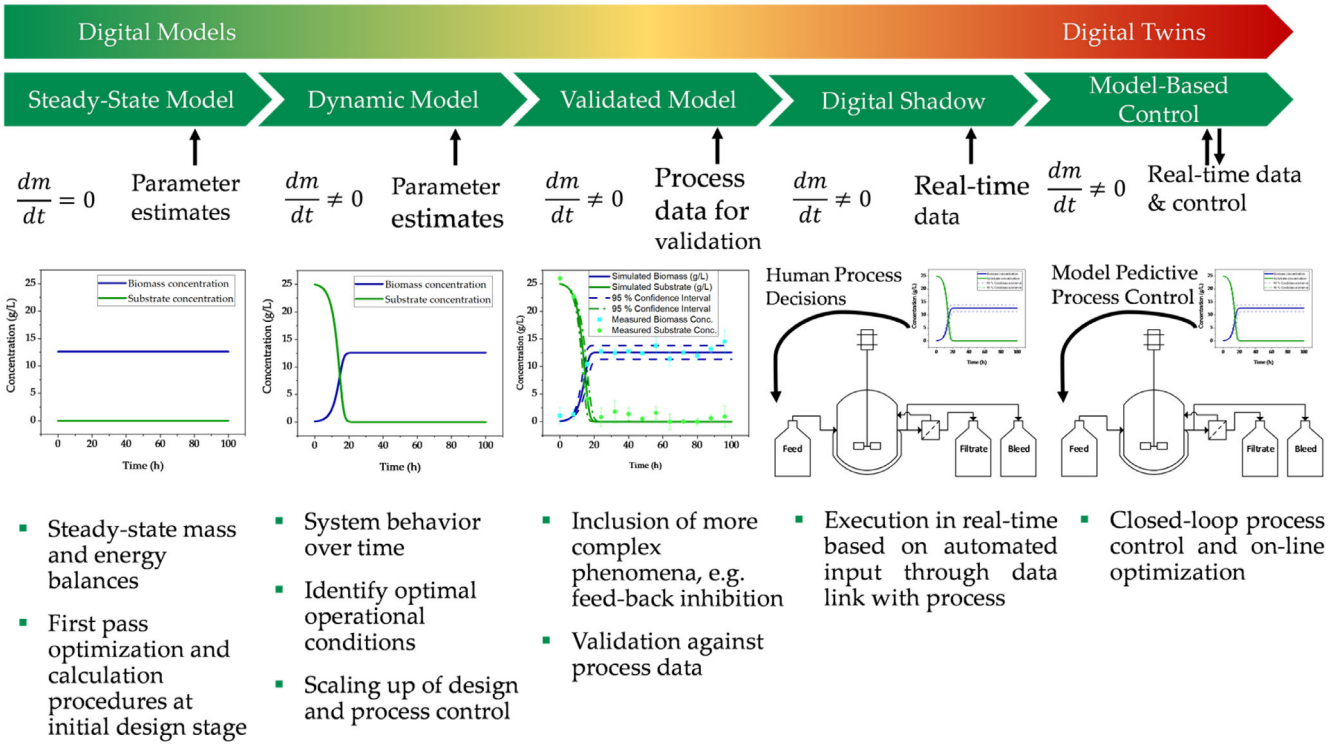

Figure 2. The steps and actions that must be taken for the transition of a steady-state mathematical model to a fully-fledged digital twin.[42,43]

are continuously forwarded to the subsequent unit operation. This information is fed into a process model that calculates the necessary process adjustment to reach either a constant mAb concentration or constant volume flow. The proposed process is a continuous, chromatography-reduced process. Robust process control is possible using a digital twin.

Simulation of viable cell concentration and product concentration in perfusion mode is shown in Fig. 3. The viable cell and product concentration increases over the first 72 h. Then the perfusion is started and the product concentration decreases momentarily since more product is washed out than is produced. Shortly after, the concentration increases again proportionally to the viable cell concentration, until the steady state is reached after approximately 350 h. Product concentration in the steady state reaches 1.8 g L^{-1}. As a basis for the following unit operations, a final steady state concentration of 2 g L^{-1} is assumed.

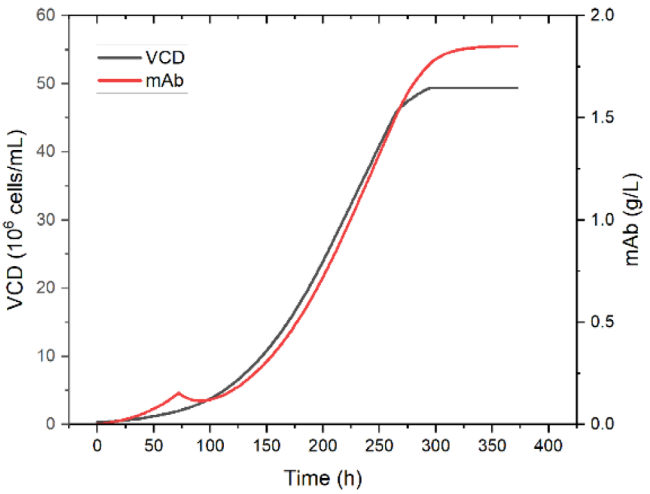

Figure 3. Simulation results of viable cell and product concentration in perfusion.[47]

Figure 4 shows how concentration fluctuations in upstream processing (USP) are processed to a constant concentration in the product-containing light phase. Polymer and salt solutions are mixed based on the phase equilibrium within the same tie-line. Based on the lever-arm rule, either less or more light phase is produced; thereby, concentration is kept constant. Operating on the same tie-line is necessary to ensure constant yield and product phase properties.

As shown in the simulation studies, aqueous two-phase extraction (ATPE can sustain a constant mAb concentration, even when titer in USP fluctuates ±50%. This is achieved by calculating the necessary polymer and salt concentration based on the phase equilibrium. Raman is the recommended spectroscopic technique to analyze mAb concentration in USP and ATPE, and is therefore the primary detector at the inlet and product phase outlet stream, as shown in Fig. 5.

Precipitation

Lohmann *et al*. investigated the feasibility of online measurement techniques of mAb and side components by Raman spectroscopy, attenuated total reflection–Fourier-transform infrared spectroscopy (ATR-FTIR), diode array detection (DAD) and fluorescence for precipitation.[48]

The digital twin is fed with the input data of the ATPE and the online measured spectral data. The model enables control of key process parameters to ensure compliance of the critical quality attributes.

All online measured values are fed back into the digital twin Figure 6 shows a simplified illustration of the described advanced process control (APC) concept. The blue dashed lines represent the signal connection between precipitation unit and digital twin.

Journal of Chemical Technology and Biotechnology published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry (SCI).

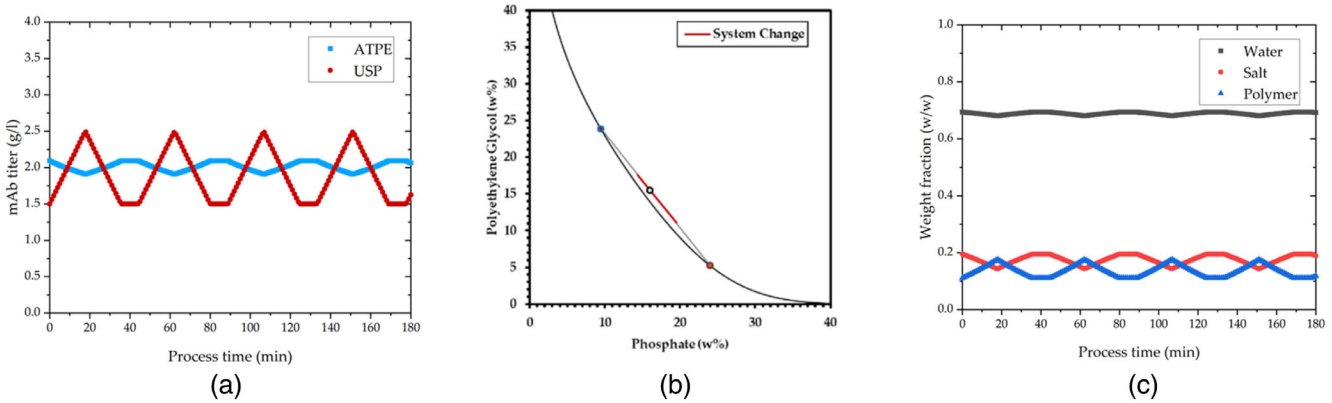

Figure 4. Simulation results for concentration fluctuations (a) and concentration compensation by system adjustment (b,c).[47]

Hereby, the signal direction is indicated by the arrows. The adjacent boxes show the process parameters that are exchanged between the model and the precipitation unit. The online measurement train between ATPE and precipitation unit determines the initial parameters (immunoglobulin G (IgG) concentration, yield and purity) for the precipitation unit. Further, the mass flow of the incoming feed is measured. Based on the mass flow controller (MFC) information the model calculates the mass flow rate of the precipitant (PEG 4000) and the speed of the polyethylene glycol (PEG) pump is adjusted. A conductivity probe in the precipitation tank is used to monitor the ratio between feed and precipitant. In the event of a deviation, the speed of the PEG pump is adjusted to compensate the mass flow variation of the feed. The turbidity probe monitors the precipitation progress and ensures that the precipitation is completed, and no antibody remains dissolved in the supernatant. Once the defined level in the precipitation tank is reached and the precipitation is finished (monitored trough turbidity), valves are opened, and filtration starts with the first module (marked as Subunit 1 in Fig. 7). Filtration is performed in dead-end mode to avoid product loss and to deposit all precipitates on the membrane surface. Pressure sensors are used to measure the transmembrane pressure to prevent pressure peaks beyond the operation range of the filters. At a critical pressure valves are switched, and the dispersion is directed to the next module.

The first loaded module moves on to the next phase, the dissolution of the precipitates. Here, the input variables from ATPE and the specification of the final concentration are used to determine, based on model prediction, the required buffer volume in dissolution. Dissolution is performed in the membrane module by tangentially overflowing the surface area at a high flow rate. Dead-end filtration has compressed the precipitates, which leads to decelerated dissolution kinetics. The experiments have shown that a time of about 14 h is required to reach a yield of >90% antibody recovery after dissolution. The dissolution circuit also contains the measuring equipment described above, which controls the speed of the dissolution buffer pump as well as the valves to the product tank. As soon as the dissolution of the antibody has reached a stationary value, the dissolution is terminated. The module moves on to regeneration, to be ready for the next filtration phase.

The presented simulation study is accomplished with the digital twin presented for one subunit. This study aims to demonstrate that the precipitation unit can react to process variations during manufacturing and is able to provide a constant output concentration.

In the initial simulation study feed titers ranging from 1 to 3 g L^{-1} have been simulated. The final mAb concentration of 3 g L^{-1} is achieved after dissolution. Figure 7(a) shows the

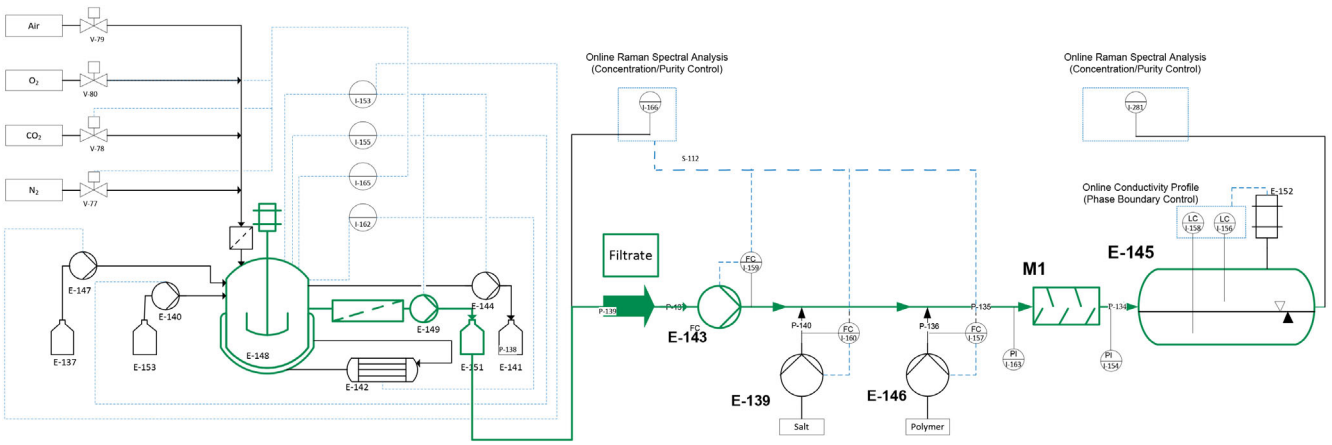

Figure 5. Proposed control strategy for upstream processing (USP) and aqueous two-phase extraction (ATPE). A Raman probe is used as process analytical technology (PAT) at the filtrate outlet of USP and forwards monoclonal antibody (mAb) and side component concentrations to the ATPE, which adjusts the polymer and salt concentrations to produce light phase with a constant mAb concentration, which is then forwarded to the precipitation unit.[47]

J Chem Technol Biotechnol 2022; **97**: 2336–2346 wileyonlinelibrary.com/jctb
Journal of Chemical Technology and Biotechnology published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry (SCI).

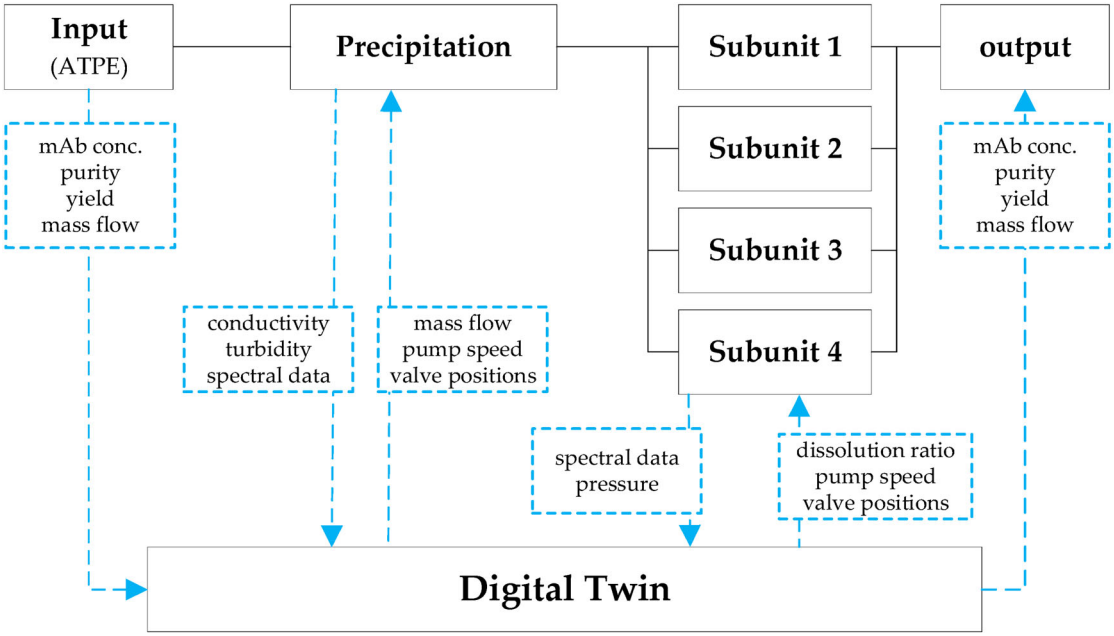

Figure 6. Simplified illustration of proposed advanced process control (APC) for the precipitation unit. Precipitation tanks are merged into one tank and the subunits are filter modules.[48]

concentration decrease in the precipitation step over process time for varying incoming feed concentrations. Complete precipitation occurs within a few seconds after PEG addition. To provide constant output concentration the dissolution ratio is modified (Fig. 7(b)), and an mAb concentration of 2.7 (±0.05) g L^{-1} in the dissolved output can be observed. This was achieved with the previously mentioned adjustment of the dissolution ratio. This simulation study demonstrated that precipitation is able to provide a constant concentration, which is important for subsequent chromatography.

To investigate the response capabilities of the unit operation to eventual process deviations, three theoretical process variations are simulated that are realistic variations for the described process. The simulation studies are labeled as scenarios 1–3:

(1) Concentration variations
(2) Mass flow fluctuations
(3) Purity changes

The first study within scenario 1 includes the proposed APC concept to simulate a process fluctuation during continuous operation. For this purpose, a periodic concentration change is used as input signal, which fluctuates from 2.5 to 1.5 g L^{-1}. A final concentration of 2 g L^{-1} is to be achieved after dissolution. Figure 8(a) demonstrates the simulation results with and without APC. Without APC there are concentration fluctuations after dissolution (grey line). When the dissolution ratio is adjusted through APC the unit can provide a constant mAb concentration of 1.85 (±0.03) g L^{-1} (red line). Figure 8(b) visualizes which parameters must be controlled to ensure a consistent concentration. By adjusting the dissolution ratio (brown columns), either slight concentration of the target component or dilution is achieved, to result in a constant output concentration. Associated with this is the change in the residual PEG content (orange columns), which can become limiting due to thermodynamic equilibrium. This is shown in Fig. 8(c). The grey line depicts the equilibrium concentration of mAb in the presence of an increasing PEG content.

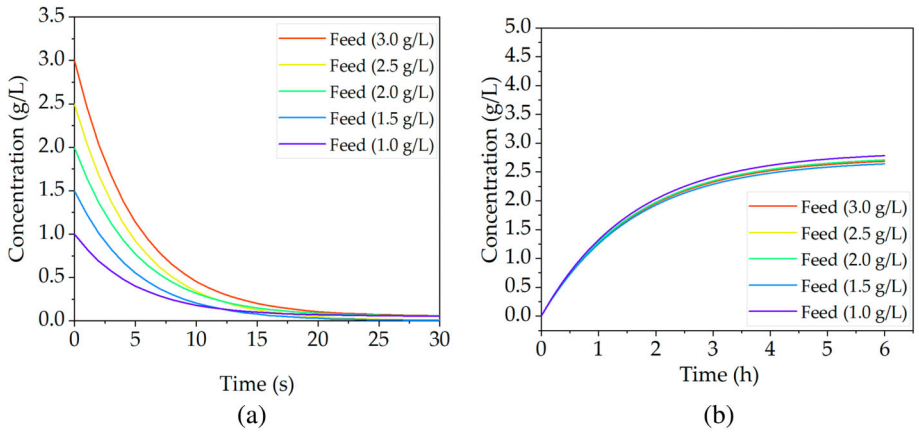

Figure 7. Simulation results of scenario 1 (concentration fluctuations) are presented for precipitation (a) and for dissolution (b).[48]

Journal of Chemical Technology and Biotechnology published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry (SCI).

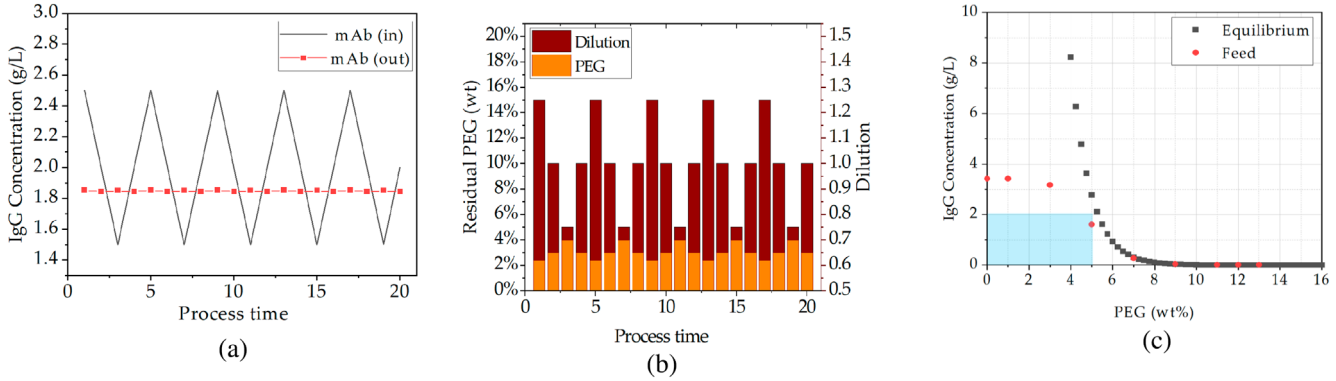

Figure 8. Second simulation study results of scenario 1 (concentration fluctuations). (a) Comparison of incoming concentration and the adjusted immunoglobulin G (IgG) concentration after dissolution because of the integrated advanced process control. (b) Parameter that must be controlled to achieve a constant output concentration of 2 g L^{-1}. (c) Equilibrium concentration of IgG in the presence of polyethylene glycol (PEG). The blue zone in (c) marks the operating window of residual PEG content.[48]

The red line represents the solubility of IgG in the feed at different PEG concentrations. The blue shaded area indicates the thermodynamically given operating window, within which the laboratory process can be carried out. Outside of this operation window limitation by PEG occurs during redissolution, which leads to product loss. A PEG content above 5 wt% limits the resolution of the antibody. In an industrial environment, the remaining PEG in the dissolution product stream should not exceed a proportion of 3 wt%.[53]

In the following section simulation studies regarding scenario 2, which address mass flow fluctuations, are presented. Initial simulations have shown that volume fluctuations mainly affect the precipitation step. This occurs because the supernatant is removed by filtration and is, therefore, not passed on to the subsequent dissolution. The simulations have also shown that an elevated PEG content does not lead to yield loss; hence, here only the simulation results in the case of insufficient PEG content are shown. The potential failure mode with and without the integration of the proposed APC is pointed out. Changes in the mass flow impact the present precipitation conditions in the precipitation tank, which are monitored with the conductivity probe. If this disturbance occurs over a longer period than a few seconds, a wrong ratio between PEG and light phase would be present in the precipitation tank in relation to its residence time. This case is shown in Fig. 9(a). During operation, the required PEG content of 12 wt% can no longer be maintained (orange columns), which leads to an increase in the equilibrium concentration of IgG (light-blue line). As a result, the target component no longer precipitates completely, and a residual portion remains dissolved. This loss is indicated by the decreased yield in the precipitation step (blue columns).

The second simulation study within scenario 2 addresses also the continuous operation with integrated APC. In this case, the PEG mass flow is adjusted as soon as the PEG content is decreasing in the precipitation tank. This results in a brief increase in the equilibrium concentration in relation to the residence time (10 min). Afterwards, the operating point with the correct equilibrium concentration is reached again, and thus no more product loss occurs. For this study the difference in yield loss is about 13%.

Concentration and mass flow changes can both be compensated in the precipitation unit within a short time span of a few seconds. This is reached through the integration of the proposed APC.

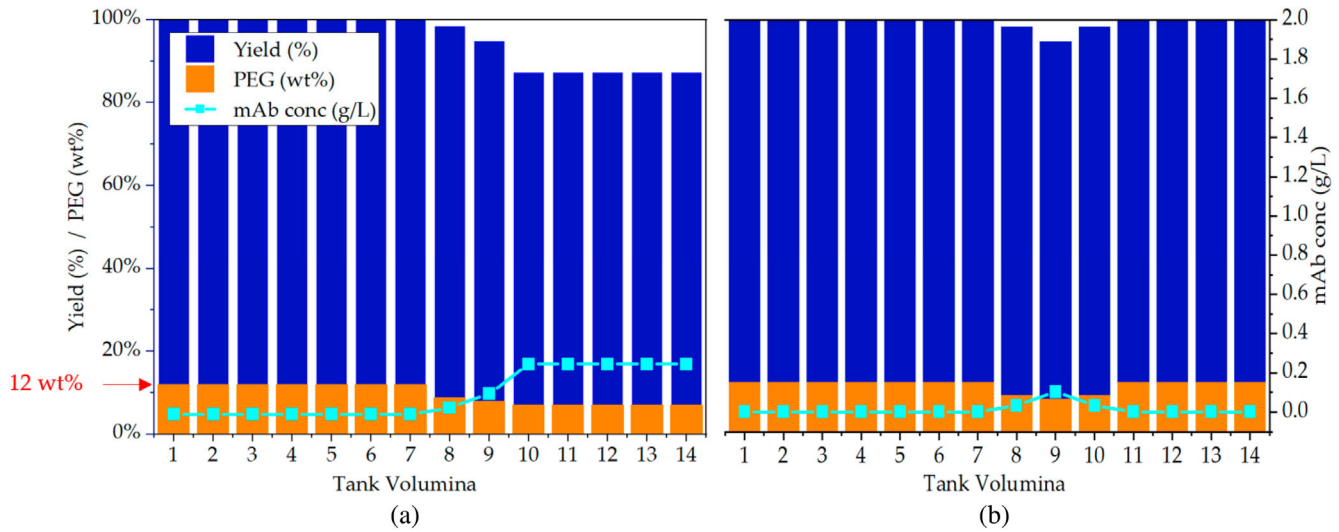

Figure 9. Simulation studies regarding volume deviation during the process. (a) Effect of volume deviations and its impact on process yield. (b) The same scenario but with the integration of the proposed advanced process control.[48]

J Chem Technol Biotechnol 2022; **97**: 2336–2346 wileyonlinelibrary.com/jctb
Journal of Chemical Technology and Biotechnology published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry (SCI).

The feasibility of using online measurement technology has been demonstrated within the framework of the PAT initiative. Best results were achieved by Raman, which reliably detected product concentration and purity. Furthermore, the combination of DAD and Raman has been tested, which is a recommended extension due to the orthogonal measurement methods, resulting in higher process robustness. Finally, in a simulation study, which has been performed with the aid of a digital twin, the online measurement data have been successfully used to control the process. This led to minimized yield losses at potential feed flow or concentration fluctuations.

Chromatography

Vetter *et al*. reported on their work on APC for chromatography.[49] Their study proposes a reliable inline PAT concept for the simultaneous monitoring of different product components after chromatography. The feed for purification consisted of four main components: IgG monomer, dimer, and two lower molecular weight components of 4.4 kDa and 1 kDa. The proposed measurement setup consists of a UV–visible diode array detector and a fluorescence detector. Applying this system, an R^2 of 0.93 for the target component, an R^2 of 0.67 for the dimer, an R^2 of 0.91 for the first side component and an R^2 of 0.93 for the second side component are achieved. The root mean square error for IgG monomer was 0.027 g L^{-1}, for dimer 0.0047 g L^{-1}, for side component 1 0.016 g L^{-1} and for side component 2 0.014 g L^{-1}. The proposed measurement concept tracked component concentration reliably down to 0.05 g L^{-1}. Zero-point fluctuations were kept within a standard deviation of 0.018 g L^{-1} for samples with no IgG concentration but with side components present, allowing a reliable detection of the target component. The main reason inline concentration measurements have not been established yet is the false-positive measurement of target components when side components are present. This problem was eliminated using the combination of fluorescence and UV–visible data for the test system. The use of this measurement system was simulated, allowing an automatic fraction cut at 0.05 g L^{-1}. In this simulation a consistent yield of >99% was achieved. Process disturbances for processed feed volume, feed purity and feed IgG concentration can be compensated with this setup. Compared to a timed process control, yield can be increased by up to 12.5%, if unexpected process disturbances occur.

This simulation study aimed to demonstrate the efficiency of using online measurement data in the process control of chromatographic processes. Furthermore, it aimed to demonstrate that the achieved accuracy is high enough to implement a reliable process control. While process control and process disturbances can be handled with a simple process control, process online optimization has to be employed using model predictive controllers. These are one of the most utilized methods of advanced process control[54] and enable automated process optimization utilizing optimization routines.[54-56] The main problem with model predictive controller-based APC is process drift due to aging, fouling or blocking.[56-58] This drift can be implemented in the simulation using online data, enabling a real-time feedback of the process and allowing for a continuous parameter fit.[57,58]

APC using inline measurements and chemometrics is the most promising way to establish robust continuous downstream manufacturing in the purification of biopharmaceuticals. One of the main reasons for inline spectroscopic data not being widely used for process control is the lack of specificity for a target molecule if other components are present. This problem can be eliminated when a combination of different spectroscopic methods is used. The combination of DAD and fluorescence spectra eliminated the high variation in zero-point fluctuations observed in DAD while simultaneously enabling the measurement of the main and side components.

Employing a digital twin would allow for fractionation control, process optimization and the monitoring of aging processes. The elution times of the main and side components can be calculated. This can then be used to control the fractionation valves. The other control strategy is fractionation control using the inline concentration measurements, which was modeled in this work. The described control system is able to regulate process disturbances of up to 50% variance on the feed concentration and volume, while maintaining a constant concentration and process yield. Compared to a timed fraction cut, the yield could be increased by up to 12.5%. In addition to integrated counter-current chromatography, this detection method can also be used for multicolumn counter-current solvent gradient purification or regular batch chromatography.

In addition to spectroscopic data, other sensor data, such as conductivity, pH and osmolarity, have shown small improvements to the regression of the target component[59] but did not show any significant impact on regression quality in this work.[47]

As a part of a RTRT (real-time-release-testing) as proposed by regulatory authorities, inline concentration and purity measurement will play a large role. Other CQAs (critrical-quality-attributes) as biological efficacy or glycosylation were reportedly possible to control using FTIR.[60-63] Additionally, by controlling these parameters with spectroscopy, semi-automation of process analytics is becoming more viable as an important addition to production of biological efficiency data from inline data via enzyme-linked immunosorbent assay in an acceptable timeframe.[64]

Case study conclusion

In USP and the following direct ATPE, Raman, FTIR, fluorescence and UV–visible spectroscopy have been successfully applied to titer as well as purity prediction. Raman was the most versatile and robust method and is recommended as the primary PAT. In precipitation, similar results were obtained for titer determination. Prediction of purity was challenging for FTIR, fluorescence and UV–visible, but achievable by Raman spectroscopy. In chromatography, the combination of UV–visible and fluorescence spectroscopy was able to overcome difficulties in titer and purity prediction induced by overlapping side component spectra. Raman spectroscopy is especially useful in the early stages of the process, whereas more traditional detector technology concepts, such as DAD, can be used in late process stages. The combination of spectroscopic data improves predictivity, as shown for chromatography.

Continuous operation generates much smaller hold-up volumes than batch processing. This causes much shorter start-up und shut-down times, with smoother systems responses. In addition, system response is much shorter and smoother – that is, nearly constant – around the continuous operation point. Hence, detector signal acquisition times and corresponding sampling scan rates for continuous processing are much lower than for comparable batch operation. Changes due to natural system variances are less steep – that is, continuously near constant system responses. In contrast, only typical gradient elution chromatography operation with fraction cut points at steep chromatogram concentration slopes challenge the accuracy in time resolution. These changes in concentration occur within a few seconds, whereas the feasible number of measuring points is limited by the

Journal of Chemical Technology and Biotechnology published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry (SCI).

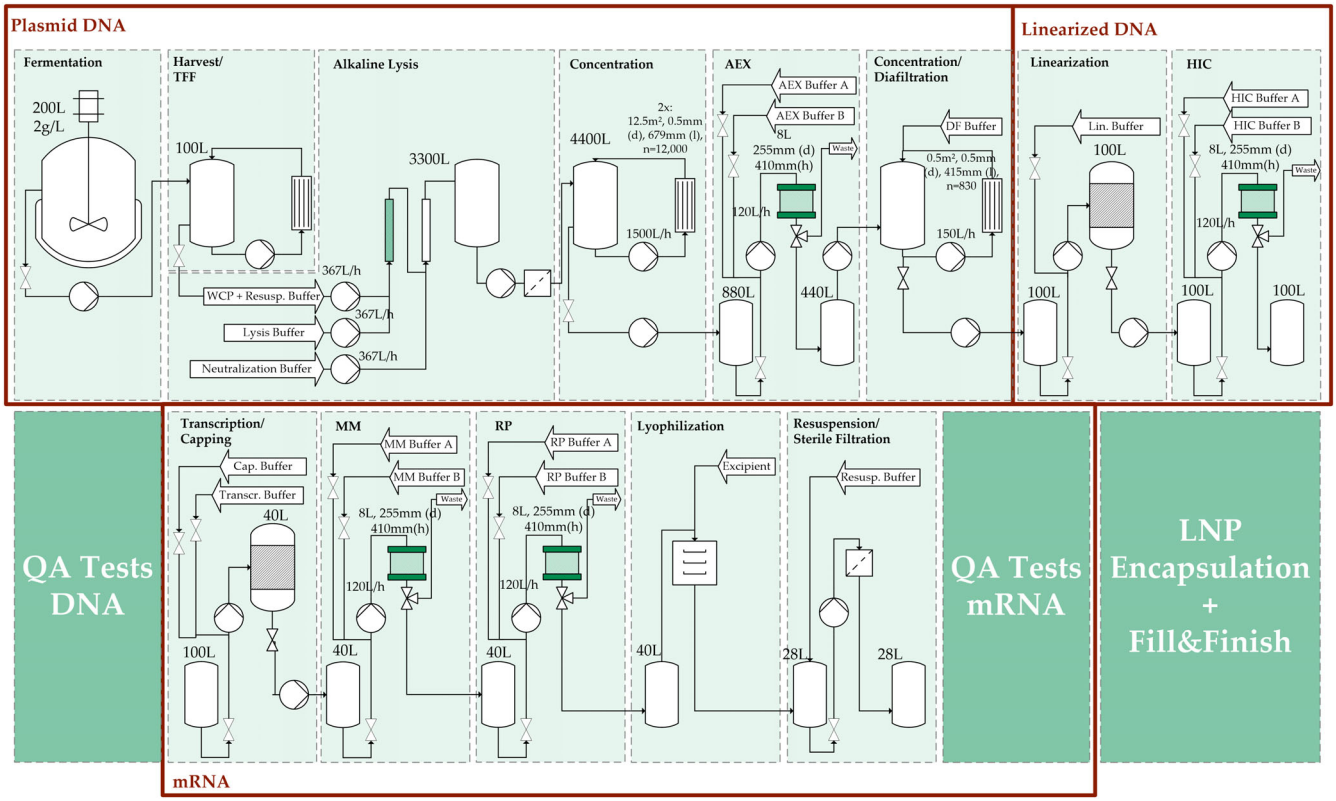

Figure 10. Overview of the plasmid production, quality testing and mRNA manufacturing process. The process consists of three main steps: plasmid production, linearization and *in vitro* transcription. After linearization and mRNA purification, extensive quality testing is required.[66]

sampling rate, which leads to a resolution of 10–50 points per peak. This results in an image of the concentration profile that is not sufficiently accurate for fractionation. Only typical breakthrough curves of flow-through operation mode in capture steps differ, and are also sufficiently describable with higher acquisition times and averaged scans.

Based on the developed spectroscopic predictions, dynamic process control of the unit operations was demonstrated for the total process in sophisticated simulation studies based on validated digital twins available for all unit operations.

Each digital twin uses predictive process models as a basis. While it may seem intuitively obvious that these must differ in detail, process models can be developed according to a consistent and unambiguous validation scheme that takes into account their use as digital twins in regulated industries.[65] There is no fundamental distinction between digital twins for use in USP or DSP, or in batch *versus* continuous operations. However, while in USP process data are often sufficient to achieve control and optimization due to the comparatively slow changes in the system at a low frequency of a few minutes, in highly dynamic processes and rapidly changing concentrations, such as in chromatography, it is necessary to use a much faster sampling rate, of up to a few fractions of a second.

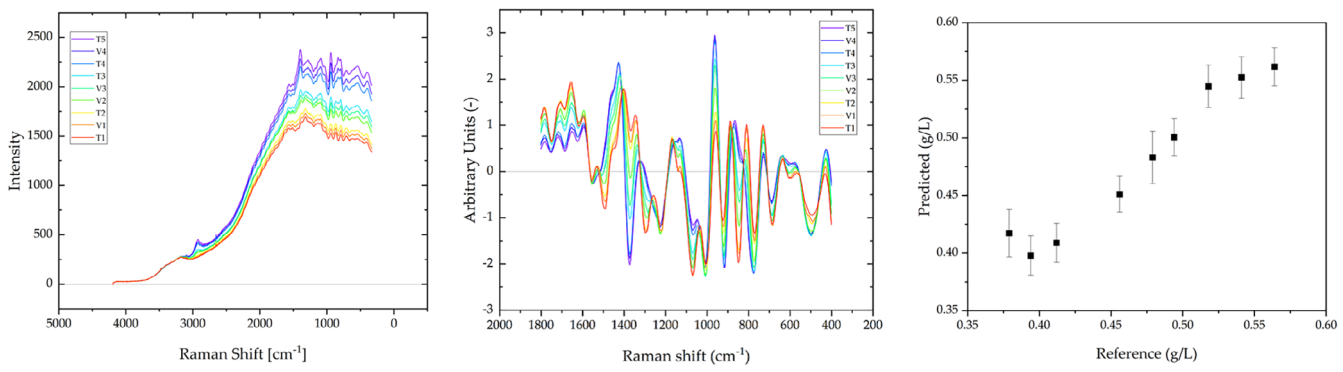

Figure 11. Raman raw (a) and processed spectra (b), and titer prediction *versus* reference (c) after alkaline lysis and clarification.[66]

J Chem Technol Biotechnol 2022; **97**: 2336–2346 wileyonlinelibrary.com/jctb
Journal of Chemical Technology and Biotechnology published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry (SCI).

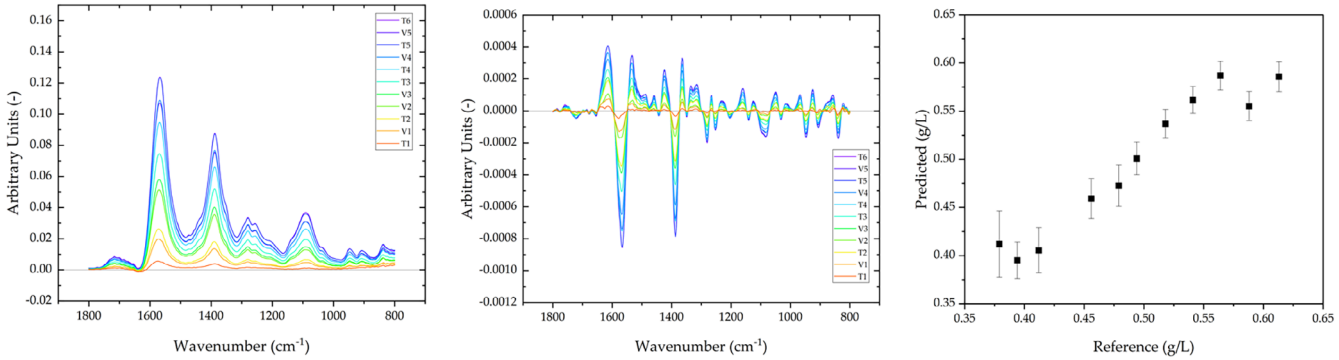

Figure 12. Fourier transform infrared raw (a) and processed spectra (b), and titer prediction *versus* reference (c) after alkaline lysis and clarification.[66]

PERSPECTIVES AND FUTURE TRENDS

The shift from batch-based to continuous production will not only affect the manufacture of established blockbusters such as monoclonal antibodies, but will also have to be reflected as a necessary further development in the provision of vaccines needed in the short term during times of pandemics. The digital twin in continuous biologics manufacturing will require key technologies and concepts such as PAT and QbD (Fig. 10).

In the case of mRNA vaccine production, lengthy quality controls occur first when controlling the linearized DNA, which is the starting material in the production of the actual mRNA drug, and secondly they are necessary before the purified mRNA drug can be encapsulated. This results in holding times of up to several weeks.[66] PAT is a necessary key-enabling technology for continuous biomanufacturing. Most (spectroscopic) sensors are based on chemometric calculations (e.g., partial least square regression, principal component analysis), which are already widely used in the literature. Additionally, there are model-based sensors, which can be based on mass and/or energy balances as well as (extended) Kalman filters, the implementation of which is more time-consuming. However, the last-mentioned sensors provide an extended process understanding, as they can be based on physicochemical effects.

In terms of automation, digital twins in continuous biomanufacturing rely on online process data that update the information fed into the process models in real time.[2,47-49,67] Besides simple process parameters like pressure, conductivity, pH and temperature, concentration of the target component and main impurities is necessary to ensure that the information gathered from the digital twin is reliable. Spectroscopic technologies like Raman, FTIR, UV–visible, fluorescence and circular dichroism have been demonstrated to be suitable detection methods for a variety of biologics manufacturing processes.

To assess the potential of APC enabling techniques, plasmid concentration after alkaline lyses in different concentration and purity ranges were exploratorily investigated by Raman spectroscopy (Raman shift 1800–400 cm^{-1}), FTIR spectroscopy (absorption frequencies from 1800 to 800 cm^{-1}) and DAD (wavelength 200–500 nm) by Schmidt *et al.*

Challenges have to be overcome, such as the methods of spectroscopic and other online measurement techniques for innovative vaccine types such as viruses and virus-like particles, but also oligonucleotides such as mRNA and plasmid DNA, which have only been scarcely researched compared to recombinant proteins. These online measurement techniques are not only necessary to ensure automated process control and thus counteract a possible shortage of qualified operators, but also to introduce real-time release testing of urgently needed drugs in the longer term and thus shorten the prolonged release times caused by time-consuming quality controls (Figs 11–13).

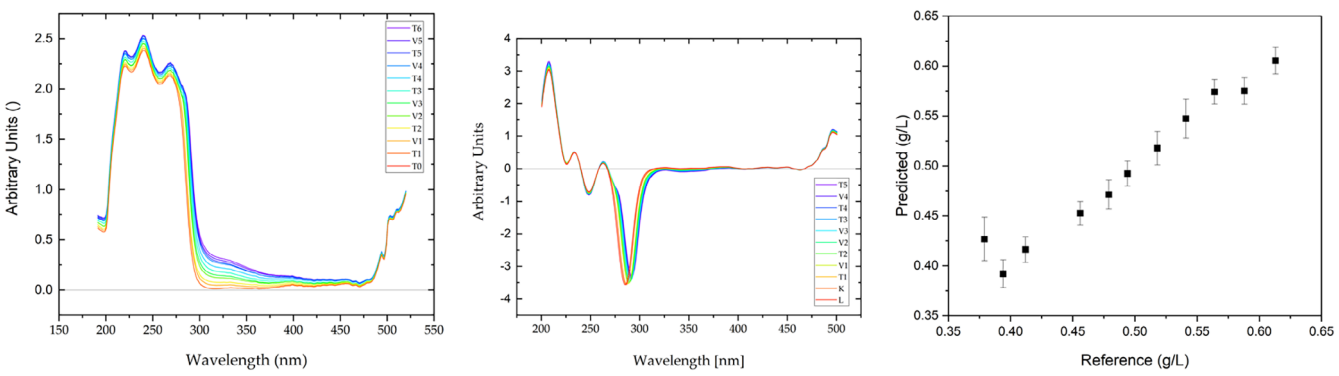

Figure 13. Diode array detection raw (a) and processed spectra (b), and titer prediction *versus* reference (c) after alkaline lysis and clarification.[66]

Journal of Chemical Technology and Biotechnology published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry (SCI).

CONCLUSION

Continuous biomanufacturing studies have proven the potential to harmonize high product quality.[32,46,47,68,69] Continuous production suggests process automation,[70] even if continuous operation by perfusion may be comparably short, of about 2 weeks to 2 months when compared to bulk and petrochemical operation times of up to several months. Autonomous operation reduces product quality variance and keeps the operation state constant around an optimum by advanced process control strategies.[71] Benefits are reduced operation costs, reduction of manufacturing personnel – which is in any case rare nowadays[66] – and significant quality assurance cost reduction by RTRT.[72] The basis of APC are either artificial neural network, rigorous or hybrid models.[72] Digital twins based on process models which need to be distinctively validated for regulatory decisions are combined with PAT via PLS-based data evaluation towards process control strategies.

Digital twins enable a training simulator in combination with the existing process control system, and are a well-established and beneficial procedure in petro-, basic- and fine-chemicals industries. Moreover, operator workload is reduced drastically, as they are enabled to operate different plants in parallel – a most wanted capacity increase option at enhanced product robustness.[66] Digital-twin-based process automation reduces the number of operators required by factor of 2 and lowers their stress level drastically.[73] In addition, product quality is subject to less fluctuation due to the continuous production method and the steady state thus ensured, which has a lower time-to-market due to PAT-supported RTRT as well as lower batch failure rates, which enlarge productivity in the case of mRNA manufacture by about 20%.[73]

This demonstrates the specific competitiveness of the whole continuous biomanufacturing approach on consistent industrialization of digital twins.

REFERENCES

1 Woodcock J. Modernizing pharmaceutical manufacturing: continuous manufacturing as a key enabler, in *MIT-CMAC International Symposium on Continuous Manufacturing of Pharmaceuticals*, Cambridge, MA; 2014.
2 Kornecki M, Schmidt A and Strube J, PAT as key-enabling technology for QbD in pharmaceutical manufacturing: a conceptual review on upstream and downstream processing. *Chim Oggi Chem Today* **36**: 44–48 (2018).
3 Chanda A, Daly AM, Foley DA, LaPack MA, Mukherjee S, Orr JD *et al*., Industry perspectives on process analytical technology: tools and applications in API development. *Org Process Res Dev* **19**:63–83 (2015).
4 Konstantinov KB and Cooney CL, White paper on continuous bioprocessing: May 20–21 2014 continuous manufacturing symposium. *J Pharm Sci* **104**:813–820 (2015).
5 National Academies Press, *Continuous Manufacturing for the Modernization of Pharmaceutical Production: Proceedings of a Workshop*. National Academies Press, Washington, DC (2019).
6 Sommerfeld S and Strube J, Challenges in biotechnology production: generic processes and process optimization for monoclonal antibodies. *Chem Eng Process Intensif* **44**:1123–1137 (2005).
7 Gronemeyer P, Ditz R, Strube J, Trends in upstream and downstream process development for antibody manufacturing. *Bioengineering*. **1**:188–212 (2014). https://doi.org/10.3390/bioengineering1040188
8 Kelley B, Very large scale monoclonal antibody purification: the case for conventional unit operations. *Biotechnol Prog* **23**:995–1008 (2016).
9 Strube J, Ditz R, Kornecki M, Huter M, Schmidt A, Thiess H *et al*., Process intensification in biologics manufacturing. *Chem Eng Process Intensif* **133**:278–293 (2018).
10 Strube J, Grote F and Ditz R, Bioprocess design and production technology for the future, in *Biopharmaceutical Production Technology*, ed. by Subramanian G. Wiley-VCH, Weinheim, pp. 657–705 (2012).
11 Singh N, Arunkumar A, Chollangi S, Tan ZG, Borys M and Li ZJ, Clarification technologies for monoclonal antibody manufacturing processes: current state and future perspectives. *Biotechnol Bioeng* **113**:698–716 (2016).
12 Jain E and Kumar A, Upstream processes in antibody production: evaluation of critical parameters. *Biotechnol Adv* **26**:46–72 (2016).
13 Pollock J, Ho SV and Farid SS, Fed-batch and perfusion culture processes: economic, environmental, and operational feasibility under uncertainty. *Biotechnol Bioeng* **110**:206–219 (2013).
14 Birch JR and Racher AJ, Antibody production. *Adv Drug Deliv Rev* **58**: 671–685 (2006).
15 Al-Rubeai M, *Animal Cell Culture*, Vol. **9**. Springer, Cham (2015).
16 Subramanian G, *Continuous Biomanufacturing: Innovative Technologies and Methods*. Wiley-VCH, Weinheim (2017).
17 Subramanian G, *Biopharmaceutical Production Technology*. Wiley-VCH, Weinheim (2012).
18 Subramanian G, *Bioseparation and Bioprocessing*, 2nd edn. Wiley-VCH, Weinheim (2007).
19 Rita Costa A, Elisa Rodrigues M, Henriques M, Azeredo J and Oliveira R, Guidelines to cell engineering for monoclonal antibody production. *Eur J Pharm Biopharm* **74**:127–138 (2010).
20 Gstraunthaler G and Lindl T, *Zell- und Gewebekultur: Allgemeine Grundlagen und spezielle Anwendungen. 7. Auflage*. Springer, Berlin (2013).
21 Gronemeyer P, Ditz R and Strube J, Implementation of aqueous two-phase extraction combined with precipitation in a monoclonal antibody manufacturing process. *Chim Oggi-Chem Today* **34**:66–70 (2016).
22 Gronemeyer P, Schmidt A, Zobel S and Strube J, Efficient manufacturing of biologics. *ChemIngen Tech* **88**:1329 (2016).
23 Gronemeyer P, Thiess H, Zobel-Roos S, Ditz R and Strube J, Integration of upstream and downstream in continuous biomanufacturing, in *Continuous Biomanufacturing: Innovative Technologies and Methods*, ed. by Subramanian G. Wiley-VCH, Weinheim, pp. 481–510 (2017).
24 Jungbauer A, Continuous downstream processing of biopharmaceuticals. *Trends Biotechnol* **31**:479–492 (2013).
25 Karst DJ, Steinebach F, Soos M and Morbidelli M, Process performance and product quality in an integrated continuous antibody production process. *Biotechnol Bioeng* **114**:298–307 (2016).
26 Karst DJ, Serra E, Villiger TK, Soos M and Morbidelli M, Characterization and comparison of ATF and TFF in stirred bioreactors for continuous mammalian cell culture processes. *Biochem Eng J* **110**:17–26 (2016).
27 Warikoo V, Godawat R, Brower K, Jain S, Cummings D, Simons E *et al*., Integrated continuous production of recombinant therapeutic proteins. *Biotechnol Bioeng* **109**:3018–3029 (2012).
28 Zydney AL, Continuous downstream processing for high value biological products: a review. *Biotechnol Bioeng* **113**:465–475 (2016).
29 Hernandez R, Continuous manufacturing: a changing processing paradigm. *Biopharm Int* **2015**:20–27 (2015).
30 Montgomery A, Scott C and Center A, Continuous chromatography: experts weigh in on the possibilities and the reality. *BioProcess Int* **2019**:1–12 (2019).
31 Thiess H, Zobel-Roos S, Gronemeyer P, Ditz R and Strube J, Engineering challenges of continuous biomanufacturing processes (CBP), in *Continuous Biomanufacturing: Innovative Technologies and Methods*, ed. by Subramanian G. Wiley-VCH, Weinheim, pp. 69–106 (2017).
32 Steinebach F, Ulmer N, Wolf M, Decker L, Schneider V, Wälchli R *et al*., Design and operation of a continuous integrated monoclonal antibody production process. *Biotechnol Prog* **33**:1303–1313 (2017).
33 Kateja N, Kumar D, Sethi S and Rathore AS, Non-protein a purification platform for continuous processing of monoclonal antibody therapeutics. *J Chromatogr A* **1579**:60–72 (2018).
34 Whitford W, GE Healthcare. [Online]. Continuous biomanufacturing: 10 reasons sponsors hesitate (2015). Available: https://www.bioprocessonline.com/doc/continuous-biomanufacturing-reason-sponsors-hesitate-0001. [16 May 2016]
35 ICH Expert Working Group. Pharmaceutical development Q8(R2). [Online]. ICH Harmonised Tripartite Guideline (2009): 1–28. Available: https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q8_R1/Step4/Q8_R2_Guideline.pdf. [29 June 2019]

Journal of Chemical Technology and Biotechnology published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry (SCI).

36 ICH Expert Working Group. Specifications: test procedures and acceptance criteria for biotechnological/biological products Q6B. [Online]. ICH Harmonised Tripartite Guideline 1999; 1–20. Available: https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q6B/Step4/Q6B_Guideline.pdf. [29 June 2019]
37 Food and Drug Administration. Guidance for industry: PAT – a framework for innovative pharmaceutical development, manufacturing and quality assurance, 2004. Available: https://www.fda.gov/downloads/drugs/guidances/ucm070305.pdf. [19 February 2018]
38 Guideline IHT, Quality risk management. *Current Step* **4**:408 (2005).
39 Uhlenbrock L, Sixt M and Strube J, Quality-by-design (QbD) process evaluation for phytopharmaceuticals on the example of 10-deacetylbaccatin III from yew. *Res-Efficient Technol* **3**:137–143 (2017).
40 Alt N, Zhang TY, Motchnik P, Taticek R, Quarmby V, Schlothauer T *et al*., Determination of critical quality attributes for monoclonal antibodies using quality by design principles. *Biologicals* **44**:1–15 (2016).
41 Rathore AS, Mishra S, Nikita S and Priyanka P, Bioprocess control: current progress and future perspectives. *Life* **11**:557 (2021).
42 Udugama IA, Lopez PC, Gargalo CL, Li X, Bayer C and Gernaey KV, Digital twin in biomanufacturing: challenges and opportunities towards its implementation. *Syst Microbiol Biomanuf* **1**:257–274 (2021).
43 Helgers H, Hengelbrock A, Schmidt A and Strube J, Digital twins for continuous mRNA production. *Processes* **9**:1967 (2021).
44 Kritzinger W, Karner M, Traar G, Henjes J and Sihn W, Digital twin in manufacturing: a categorical literature review and classification. *IFAC – PapersOnLine* **51**:1016–1022 (2018).
45 Tsopanoglou A and Del Jiménez VI, Moving towards an era of hybrid modelling: advantages and challenges of coupling mechanistic and data-driven models for upstream pharmaceutical bioprocesses. *Curr Opin Chem Eng* **32**:100691 (2021).
46 Kornecki S, Lohmann H, Mestmäcker K, Huter M, Mestmäcker F, Klepzig L *et al*., Accelerating biomanufacturing by modeling of continuous bioprocessing: piloting case study of monoclonal antibody manufacturing. *Processes* **7**:495 (2019).
47 Helgers H, Schmidt A, Lohmann LJ, Vetter FL, Juckers A, Jensch C *et al*., Towards autonomous operation by advanced process control: process analytical technology for continuous biologics antibody manufacturing. *Processes* **9**:172 (2021).
48 Lohmann LJ and Strube J, Process analytical technology for precipitation process integration into biologics manufacturing towards autonomous operation: mAb case study. *Processes* **9**:488 (2021).
49 Vetter FL, Zobel-Roos S and Strube J, PAT for continuous chromatography integrated into continuous manufacturing of biologics towards autonomous operation. *Processes* **9**:472 (2021).
50 Klepzig LS, Juckers A, Knerr P, Harms F and Strube J, Digital twin for lyophilization by process modeling in manufacturing of biologics. *Processes* **8**:1325 (2020).
51 Kornecki M and Strube J, Accelerating biologics manufacturing by upstream process Modelling. *Processes* **7**:166 (2019).
52 Feidl F, Vogg S, Wolf M, Podobnik M, Ruggeri C, Ulmer N *et al*., Process-wide control and automation of an integrated continuous manufacturing platform for antibodies. *Biotechnol Bioeng* **117**:1367–1380 (2020).
53 Matheus S, Friess W, Schwartz D and Mahler H-C, Liquid high concentration IgG1 antibody formulations by precipitation. *J Pharm Sci* **98**:3043–3057 (2009).
54 Dittmar R, *Advanced Process Control: PID-Basisregelungen, Vermaschte Regelungsstrukturen, Softsensoren, Model Predictive Control*. De Gruyter Oldenbourg, München (2017).
55 Keerthi SS and Gilbert EG, Optimal infinite-horizon feedback laws for a general class of constrained discrete-time systems: stability and moving-horizon approximations. *J Optim Theory Appl* **57**:265–293 (1988).
56 Song I-H, Amanullah M, Erdem G, Mazzotti M and Rhee H-K, Experimental implementation of identification-based optimizing control of a simulated moving bed process. *J Chromatogr A* **1113**:60–73 (2006).
57 Sommeregger W, Sissolak B, Kandra K, von Stosch M, Mayer M, Striedner G, Quality by control: Towards model predictive control of mammalian cell culture bioprocesses. *Biotechnology Journal*. **12**:1600546 (2017). https://doi.org/10.1002/biot.201600546
58 Fanali S, Editorial on 'Simulated moving bed chromatography for the separation of enantiomers' by A. Rajendran, G. Paredes and M. Mazzotti. *J Chromatogr A* **1216**:708 (2009).
59 Felfödi E, Scharl T, Melcher M, Dürauer A, Wright K, Jungbauer A, Osmolality is a predictor for model-based real time monitoring of concentration in protein chromatography. *Journal of Chemical Technology & Biotechnology* (2019). https://doi.org/10.1002/jctb.6299
60 Wang Y, Boysen RI, Wood BR, Kansiz M, McNaughton D and Hearn MTW, Determination of the secondary structure of proteins in different environments by FTIR-ATR spectroscopy and PLS regression. *Biopolymers* **89**:895–905 (2008).
61 Boulet-Audet M, Kazarian SG and Byrne B, In-column ATR-FTIR spectroscopy to monitor affinity chromatography purification of monoclonal antibodies. *Sci Rep* **6**:30526 (2016).
62 Walther C, Mayer S, Jungbauer A and Dürauer A, Getting ready for PAT: scale up and inline monitoring of protein refolding of Npro fusion proteins. *Process Biochem* **49**:1113–1121 (2014).
63 Buijs J, Norde W and Lichtenbelt JWT, Changes in the secondary structure of adsorbed IgG and F(ab′) 2 studied by FTIR spectroscopy. *Langmuir* **12**:1605–1613 (1996).
64 Christler A, Felföldi E, Mosor M, Sauer D, Walch N, Dürauer A *et al*., Semi-automation of process analytics reduces operator effect. *Bioprocess Biosyst Eng* **43**:753–764 (2020).
65 Schmidt A and Strube J, Distinct and quantitative validation method for predictive process modeling with examples of liquid–liquid extraction processes of complex feed mixtures. *Processes* **7**:298 (2019).
66 Schmidt A, Helgers H, Vetter FL, Juckers A and Strube J, Digital twin of mRNA-based SARS-COVID-19 vaccine manufacturing towards autonomous operation for improvements in speed, scale, robustness, flexibility and real-time release testing. *Processes* **9**:748 (2021).
67 Zobel-Roos S, Mouellef M, Siemers C, Strube J, Process Analytical Approach towards Quality Controlled Process Automation for the Downstream of Protein Mixtures by Inline Concentration Measurements Based on Ultraviolet/Visible Light (UV/VIS) Spectral Analysis. *Antibodies*. **6**:24 (2017). https://doi.org/10.3390/antib6040024
68 Steinebach F, Angarita M, Karst DJ, Müller-Späth T and Morbidelli M, Model based adaptive control of a continuous capture process for monoclonal antibodies production. *J Chromatogr A* **1444**:50–56 (2016).
69 Steinebach F, Müller-Späth T and Morbidelli M, Continuous countercurrent chromatography for capture and polishing steps in biopharmaceutical production. *Biotechnol J* **11**:1126–1141 (2016).
70 Geering HP, *Regelungstechnik: Mathematische Grundlagen, Entwurfsmethoden, Beispiele*, 3rd edn. Springer, Berlin (1994).
71 Nordh P. Understand advanced process control [Online]. (2016). Available: https://www.aiche.org/resources/publications/cep/2016/june/understand-advanced-process-control [29 June 2021]
72 Zobel-Roos S, Schmidt A, Uhlenbrock L, Ditz R, Köster D and Strube J, Digital twins in biomanufacturing. *Adv Biochem Eng Biotechnol* **176**:181–262 (2021).
73 Schmidt A, Helgers H, Vetter FL, Juckers A and Strube J, Fast and flexible mRNA vaccine manufacturing as a solution to pandemic situations by adopting chemical engineering good practice: continuous autonomous operation in stainless steel equipment concepts. *Processes* **9**:1874 (2021).

