

ADVANCED MANUFACTURING OF COMPLEX BIOLOGICS: A CBER PERSPECTIVE

Manuel Osorio, FDA/CBER, USA
Manuel.Osorio@fda.hhs.gov

PUSHING THE CLOSED AND CONTINUOUS BOUNDARY: END-TO-END ICB AT THE PILOT SCALE

Kevin Brower, Sanofi Biologics Development, Purification Development US
Kevin.brower@sanofi.com
Michael Coolbaugh, Sanofi
Tarl Vetter, Sanofi
Chad Varner, Sanofi
Emily Davenport, Sanofi
Brad Bouchard, Sanofi
Marcus Fiadeiro, Sanofi
Nihal Tugcu, Sanofi

Key Words: Continuous, End-to-End, Closed, Automation, Multi-column chromatography,

Encouragingly, the biomanufacturing field continues to move towards GMP implementation of integrated and continuous processes. To our knowledge, all implemented ICB processes – including those at Sanofi – do not extend the ICB boundary to drug substance. In fact, questions remain not just as to whether fully continuous is necessary but whether the required process and engineering technologies yet exist to enable fully continuous at a commercially relevant scale. At Sanofi, we have built on our experience developing and implementing ICB technology to achieve an industry-first demonstration of a fully continuous process, including all typical downstream purification steps, to produce kilograms of drug substance. We will present our vision for end-to-end integrated and continuous biomanufacturing including design goals related to closed processing, automation, and continuous unit operations (not fast batch). Operation at the pilot scale, integrated to an intensified 100L perfusion bioreactor, required creative solutions in many aspects of the run design and execution while allowing for identification of true failure modes and, therefore, identification of areas for future development. Overall, we believe that currently available technology may allow for the realization of an end-to-end closed continuous commercial process. Moreover, our results suggest investment in pushing the continuous boundary may inspire disruptive innovation across bioprocessing to meet long-held aspirations for a truly disruptive facility of the future.

PERFORMANCE EVALUATION OF AN AUTOMATED AND CONTINUOUS ANTIBODY PURIFICATION PROCESS IN A SIDE-BY-SIDE COMPARABILITY STUDY

Benjamin Maiser, Bayer AG, Germany

benjamin.maiser@bayer.com

Klaus Kaiser, BAYER AG

klaus.kaiser@bayer.com

Martin Lobedann, BAYER AG

Laura David, BAYER AG

Key Words: MoBiDiK, Modular, Biological, Disposable, Continuous

Continuous manufacturing (CM) introduces the benefits of cost efficiency, reliability and scalability for the manufacturing of biopharmaceuticals. Higher flexibility, smaller facility footprints and cost of goods benefits are advantages of this production mode. It offers high flexibility in regard of demand changes from clinical to launch and for volatile market dynamics. In combination with disposable equipment, faster time-to-market and closed processing seems feasible. Bayer's unique CM platform consists of a series of downstream processing (DSP) unit operations through which the drug substance moves continuously and all unit operations happen more or less in parallel at the same time. The technology offers the potential to make Quality by Design (QbD) a reality (with continuously monitored process parameters and real-time feedback process control to maintain quality-indicating parameters within limits at all times, multi-variate data analysis). Individual unit operations are intelligently integrated and critical process parameters are monitored and controlled in real-time. Conditioning modules allow immediate corrective actions to be executed in an automated fashion to maintain the entire process in a state of control with low batch-to-batch variability. In addition, online sampling and testing functions provide early warning of potential excursions. By reduced manual interference this will also lead to reduction of operator errors and according deviations. Manufacturing facilities will be significantly less capital-intensive (e.g. by simpler layout) than large, traditional batch facilities as disposable technology and aseptic connections offer superior protection against bioburden ingress and other forms of contamination. The presentation also intends to illustrate comparability of CM versus batch processing in a side-by-side approach covering process information, real time analysis as well as quality data from intermediates and final drug substance of an antibody product.

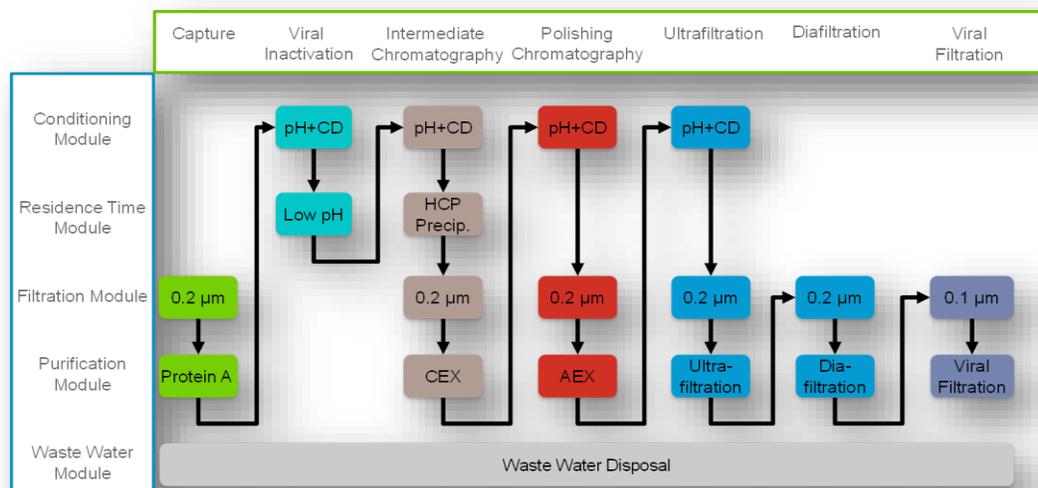


Figure 1 – Bayer's MoBiDiK technology platform for continuous downstream processing

IMPLEMENTING CONNECTED PROCESSES AT-SCALE – CHALLENGES AND OPPORTUNITIES FOR STREAMLINING OPERATIONS

Mark Brower, Merck & Co., Inc., Kenilworth NJ, USA
mark.brower@merck.com

Nuno Pinto, Merck & Co., Inc., Kenilworth NJ, USA
Gregg Nyberg, Merck & Co., Inc., Kenilworth NJ, USA
Eva Gefroh, Just Biotherapeutics, Seattle WA, USA
Rob Piper, Just Biotherapeutics, Seattle WA, USA
Tim Wanek, Just Biotherapeutics, Seattle WA, USA
Mike Vandiver, Just Biotherapeutics, Seattle WA, USA

Key Words: Media Concentrates, Multi-column Chromatography, Single-Use, Connected, Scale-Up

In response to increased demands placed on biopharmaceutical manufacturers to diversify portfolios and to reduce costs, bioprocesses are being intensified to allow for significant protein production in $\leq 2,000\text{L}$ single-use bioreactors. Many biopharmaceutical manufacturers are maturing continuous bioprocessing platforms to meet these demands. Hardware, which was once thought to be novel and high-risk for failure, is now being proven as robust at-scale. When coupled with single-use technology, these processes enable biomanufacturing facilities to be built faster at a lower cost, with more flexibility for reconfiguration and support of regional manufacturing. Many challenges remain to achieving this final vision including liquid management for large-scale perfusion operations, overcoming product sieving decay often encountered in filtration-based cell retention devices, and bioburden control for long duration operations.

In a collaborative case study, Merck & Co., Inc. and Just Biotherapeutics will demonstrate a strategy leveraging fully single-use equipment and connected operations for an extended duration at manufacturing-scale (500L). This presentation will highlight bioreactor performance with the deployment of media concentrate technology to ease the logistical, staffing and space constraints of a traditional perfusion process, as well as implementation of large-scale microfiltration membranes for cell retention with consistently high protein transmission. In addition, data will be presented not only on the performance of a linked continuous multi-column protein A chromatography capture step, but also bioburden monitoring from multiple points in the process. Advancements implemented during this campaign as well as valuable lessons learned will open the door to further expanding the continuous boundary of connected operations and continuous bioprocessing.

INTEGRATED CONTINUOUS BIOPROCESSING: COSTS OF GOODS VERSUS COST OF DEVELOPMENT

Hanna Mahal, University College London, United Kingdom
hanna.mahal.14@ucl.ac.uk
Christos Stamatis, University College London, United Kingdom
Suzanne S. Farid, University College London, United Kingdom

Key Words: Integrated manufacturing, continuous bioprocessing, process economics, monoclonal antibodies.

A significant benefit of continuous manufacture is the potential to provide higher productivities compared to traditional batch processes. Smaller facilities with single-use technology could become preferable offering reductions in the capital expenditure. Hence, continuous bioprocessing could offer savings in the cost of goods (COG). However there are other cost factors that need to be considered when evaluating bioprocess facilities in addition to the COG. The cost of development (COD) is a key cost driver that could affect the decision to adopt new manufacturing methods.

This study aims to carry out a holistic financial assessment of introducing continuous bioprocessing strategies by considering both the COG and the COD. To be able to perform this level of analysis a decisional tool was developed at University College London to evaluate the cost of implementing traditional batch or continuous bioprocessing (end-to-end and hybrid) at various stages of the drug development pathway. A range of scenarios investigated the economics of different manufacturing strategies at various demands, company sizes and stages of manufacture (pre-clinical, clinical and commercial). Therefore, through the analysis it was possible to determine whether the apparent benefits of continuous bioprocessing translate into cost savings, focusing on the development and commercialisation of monoclonal antibodies.

ISKID: FROM INTEGRATED PILOT SCALE RUNS TO GMP IMPLEMENTATION APPROACH

Raquel Orozco, Boehringer Ingelheim, USA
raquel.orozco@boehringer-ingelheim.com
Scott Godfrey, Boehringer Ingelheim, USA
Daisie Ogawa, Boehringer Ingelheim, USA
Aaron Kwong, Boehringer Ingelheim, USA
Joelle Koury, Boehringer Ingelheim, USA
Charles Capron, Boehringer Ingelheim, USA
Zack Kyser, Boehringer Ingelheim, USA
Matt Brown, Boehringer Ingelheim, USA
Eike Zimmerman, Boehringer Ingelheim, USA
Jon Coffman, Boehringer Ingelheim, USA
Jeff Salm, Pfizer, USA
Rob Fahrner, Pfizer, USA
Robert Kottmeier, Pfizer, USA
Matt Stork, Pfizer, USA
Mike Jankowski, Pfizer, USA

One of the most compelling business reasons for integrated processing is the ability to de-risk capital investment due to a significantly more productive process that takes less space and fewer campaigns to generate clinical and commercial material. Boehringer Ingelheim and Pfizer developed the iSKID, a fully integrated and automated system that hydraulically links the perfusion bioreactor with several downstream unit operations (2xProtein A columns, continuous viral inactivation, AEX in flow through mode, and SPTFF). The Protein A elution cycles are discrete and separated by >2hrs, allowing the ability to discard cycles that do not meet process specifications. The discreteness between product cycles and hydraulic linkage enables the sanitization between cycles for a robust bioburden control strategy. Each cycle is captured in a single use mixer (SUM), where the product is pooled in stable conditions until viral filtration, ultrafiltration/diafiltration and final filtration are performed in batch mode.

Identical iSKID prototypes at 100L scale were used at three different sites to generate product quality, process, and bioburden data from three different molecules. The data has been used to understand implementation gaps in GMP facilities and process platforms (CMC1/CMC2). In addition, the team identified specific items to present to the FDA's Emerging Technology Team (ETT). These items include our strategies for batch definition, microbial control, and process control. In this talk, we will use the data generated from the consistency runs to elaborate on the robustness of the process and touch upon the strategies to be presented to the ETT.

DEVELOPMENT AND FUTURE MANUFACTURING OF LIVE BIOLOGICALS

John Aunins, Seres Therapeutics, USA
jaunins@serestherapeutics.com

THE NEXT GENERATION OF THERAPEUTICS FACE DRAMATIC CHALLENGES - IS ICB AN ANSWER?

Joseph Shultz, Head Advanced Process & Manufacturing Technologies, Novartis Pharma AG, Switzerland
Joseph.Shultz@Novartis.com

Integrated and continuous manufacturing strategies take evolving forms, but are driven by similar needs: the ability to meet the market demand, cost reduction, and speed to patient. For example, Amgen's next-generation Manufacturing is reported to achieve a smaller facility footprint with intensification of processes and the use of certain disposable systems. Sanofi, Shire, Jansen publically report applying various extents of continuous and integrated processes. Novartis is currently commercializing its Advanced Integrated Biomanufacturing platform as an end-to-end manufacturing aimed at capacity, speed, cost, and patient access.

While CHO-derived products represent a major component of the current industry portfolio, there are opportunities to utilize integrated and/or continuous techniques to drive benefits beyond productivity. In the case of microbial processes, continuous fermentation approaches may be able to drive quality attributes that are elusive in batch systems. Other emerging aspects of the industry, such as cell or gene therapy, are in the phase where meeting demand is the priority and driver. For these emerging therapeutic areas, integrated processing will likely be necessary to break through to the "cost effective" and/or "fast" stage. While we should not expect to see a direct carry-over of the end-to-end integrated process, that are developed for large proteins, key technology features will certainly be leveraged. We will advance the discussion around the current focus areas and future possibilities.

COMPLEX NEW MODALITIES REQUIRE ADVANCED BIOMANUFACTURING PLATFORMS: THE CASE OF EXOSOME BIOTHERAPEUTICS

Konstantin Konstantinov, Codiak Biosciences
konstantin.konstantinov@codiakbio.com

Exosome-based therapeutics are rapidly evolving as a high potential new modality in multiple clinical areas such as oncology, immuno oncology, neurology and tissue regeneration, among others. As these indications involve large patient populations, the implementation of exosome therapeutics requires robust manufacturing processes yielding large quantities of highly purified material. However, the complexity and heterogeneity of exosomes pose significant R&D challenges. Here, we present the successful development of a large-scale manufacturing process using engineered human cells grown in a high-density continuous culture. The upstream process is followed by a sequence of purification steps yielding material of high purity and quality. The related analytical and characterization methods are also discussed.

DESIGN OF A PERIODIC COUNTER-CURRENT CHROMATOGRAPHY PROCESS FOR EFFICIENT ONCOLYTIC VIRUS PURIFICATION

Ricardo J.S. Silva, iBET - Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal
rsilva@ibet.pt

João Mendes, iBET - Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal
Mikael Berg, GE Healthcare Bio-Sciences AB, Björkgatan 30, 751 84 Uppsala, Sweden
Linda Mathiasson, GE Healthcare Bio-Sciences AB, Björkgatan 30, 751 84 Uppsala, Sweden
Manuel J.T. Carrondo - iBET, Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal
Paula M. Alves - iBET, Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal; Instituto de
Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal
Cristina Peixoto - iBET, Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal; Instituto de
Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal

Key Words: Continuous Chromatography, Downstream Processing, Purification, Oncolytic Virus,

Virus-based biologicals are one of the most promising biopharmaceuticals of the 21st century medicine and play a significant role in the development of innovative therapeutic, prophylactic and clinical applications. These biologicals share between them a high degree of complexity and offer various challenges requiring innovative technologies for their manufacturing. Oncolytic virus manufacturing scale can range from 5L in research and development up to 50L for clinical studies and reach hundreds of liters for commercial scale. The inherent productivity and high integration potential of periodic counter-current chromatography offers a transversal solution to decrease equipment footprint and the reduction of several non-value-added unit operations.

The work to be reported focus on the design of a periodic counter-current chromatography process applied to the intermediate purification of oncolytic adenovirus. Moving away from single-column batch operation towards continuous or semi-continuous, multi-column chromatography creates the opportunity to benefit from synergies of solvent gradients, recycling chromatography, and simulated counter-current movement of the adsorbent and fluid phases, providing substantial reductions in chromatographic resin volume and buffer consumption. The developed ion exchange chromatographic purification method was carried out using a four-column setup, supported by mechanistic mathematical modeling. Obtained virus recoveries (> 60%) and impurity reductions (> 80% DNA, and > 70% total protein) match or overcome batch purification.

The impact of column cycling on column capacity will be presented and the steps taken to minimize it will be discussed, highlighting the optimization of the cleaning-in-place step and the need to include organic solvents to promote the stripping of tighter-adsorbing impurities. Moreover, the robustness of the dynamic control strategy and its ability to overcome perturbations originated in precedent stages will be demonstrated using feeds with different impurity profiles and titers, showing that it is possible to generate elution pools with consistent quality and traceability. Additionally, due to the wealth of data generated through the cycling operations, such as historic columns breakthrough and elution peak profiles, a deeper insight on product quality and process knowledge is gained. Moreover, process automation enables the minimization of errors, maximizing process efficiency, uptime, repeatability, and process replication.

CONTINUOUS MODE OF PRODUCTION FOR TWO CLASSES OF DEFECTIVE INTERFERING INFLUENZA A VIRUS PARTICLES AS ANTIVIRAL CANDIDATES

Marc Hein, Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, Germany
hein@mpi-magdeburg.mpg.de

Felipe Tapia, Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, Germany
Yvonne Genzel, Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, Germany
Sascha Young Kupke, Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, Germany
Udo Reichl, Chair of Bioprocess Engineering, Otto von Guericke University Magdeburg, Germany; Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, Germany

Key Words: Two-stage, MDCK, influenza, defective interfering particles, animal trials

Influenza A virus (IAV) is a major human pathogen with a high mutation rate that causes annual epidemics. Defective interfering particles (DIPs) are naturally occurring IAV mutants that are responsible for low influenza virus yields in continuous passaging. Due to that, previous research suggested that DIPs may be utilized as an antiviral agent [1]. In contrast to infectious influenza standard virus (STV), DIPs typically contain a large internal deletion in at least one of the eight genomic viral RNA (vRNA) segments. For such a DIP, named DI244, protection of ferrets against pandemic influenza A virus was shown [1]. Furthermore, we have recently reported on a novel type of IAV-derived DIP, called OP7 virus, which only contains nucleotide substitutions in segment 7 vRNA instead of large internal deletions [2]. Hence, the focus of this work was to evaluate cell-based production in continuous mode for both DI244 and the newly discovered OP7 DIP.

Madine Darby canine kidney (MDCK) cells were grown in Smif8-CDM as a substrate for virus propagation. The bioreactor used was a cascade of continuous stirred tank bioreactors (CSTRs), where cell propagation and virus replication occur in separated vessels [3]. To improve comparability of DIP dynamics, the bioreactor setup was modified to a parallel two-stage continuous process sharing one CSTR for cell growth (Figure 1). It was shown before that in continuous production the propagation of DIPs leads to oscillations in virus titres [3]. DI244 production showed the expected oscillations. Virus titre as high as $2.4 \log_{10}$ HAU/100 μL were reached with 4×10^9 DI244 copies/mL, sufficient for animal trials. During the production of OP7, instead of oscillations, a low constant virus titre of approximately $1.7 \log_{10}$ HAU/100 μL was observed from 2 to 6 days post infection before other *de novo* generated DIPs arose. The difference in the dynamics between both DIPs could be caused by a strong self-interference of OP7. To increase OP7 virus titres, a media and MOI screening in 125 mL shake flasks in batch mode was conducted. This screening, together with a newly established interfering

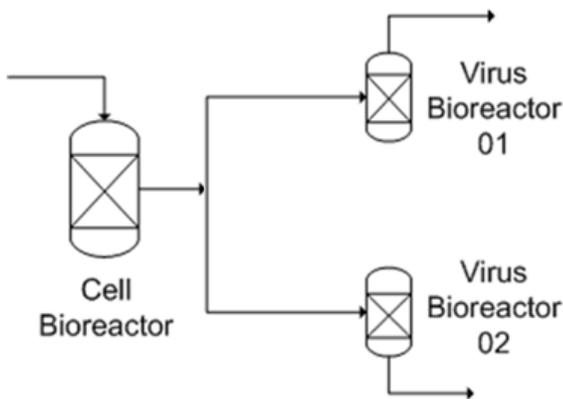


Figure 1 - Diagram of the parallel cascade of CSTRs used for continuous production of defective interfering influenza A viral particles.

assay, showed that the most potent material was produced at an MOI of 1×10^{-2} in chemically defined medium (Xeno-CDM). The remaining STV in the produced DI244 and OP7 material was inactivated by UV light. OP7 was subsequently concentrated by steric exclusion chromatography [4] resulting in highly potent material reducing infectious virus titre by a factor of 7,700 from 7.7×10^8 to 1.0×10^5 PFU/mL in the cell culture-based interfering assay. Both DIPs are currently tested in animal trials (C57BL/6JRj mice).

In summary, continuous production of two types of DIPs, DI244 and OP7, was successfully established in a parallel cascade of CSTRs. DIP propagation dynamics suggested that continuous production might be a good approach for production of certain DIPs, such as DI244. However, OP7 production might be more efficient in other types of continuous systems. Currently, OP7 production using a continuous alternating flow (ATF) bioreactor system is being explored.

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[2] Kupke et al. (2019) J.Virol JVI.01786-18

[3] Frensing et al. (2013), PLOS ONE 8(9):e72288

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TOWARDS CONTINUOUS BIOPROCESSING OF LENTIVIRAL VECTORS

Sven Ansorge, Human Health Therapeutics, National Research Council Canada, Montreal, Canada
Sven.ansorge@nrc.ca

Maurizio Cattaneo, Artemis Biosystems Canada, Inc., Montreal, Canada

Anja Rodenbrock, National Research Council Canada, Montreal, Canada

July Dorion-Thibaudeau, Human Health Therapeutics, National Research Council Canada, Montreal, Canada

Stéphane Lanthier, Human Health Therapeutics, National Research Council Canada, Montreal, Canada

Sonia Tremblay, Human Health Therapeutics, National Research Council Canada, Montreal, Canada

Aziza Manceur, Human Health Therapeutics, National Research Council Canada, Montreal, Canada

Louis-Patrick Gagnon, Human Health Therapeutics, National Research Council Canada, Montreal, Canada

Krishna Raj Tiwari, Human Health Therapeutics, National Research Council Canada, Montreal, Canada

Rénauld Gilbert, Human Health Therapeutics, National Research Council Canada, Montreal, Canada

Key Words: continuous bioprocessing, cell and gene therapy, lentiviral vectors, perfusion-enabled high cell density culture, suspension HEK293

Lentiviral vectors (LV) represent a key tool for cell and gene therapy applications. The production of these vectors in sufficient quantities for clinical applications remains a hurdle, prompting the field toward developing suspension processes that are conducive to large-scale production. Advanced upstream bioprocessing approaches will need to be complemented by appropriate downstream processes in order to reduce overall manufacturing costs and address the current viral vector supply gap.

In this study, stable HEK293 producer cell lines were employed that grow in suspension, thus offering direct scalability, and producing a green fluorescent protein (GFP)-expressing lentiviral vector in the 10^{6-7} transduction units (TU)/mL range in batch culture without optimization. HEK293 stable producer cells were retained in 3 L bioreactors operated in perfusion mode using either a BioSep acoustic cell filter (Applisens), an XCell™ ATF system (Repligen) or a VHU™ Perfusion Filter (Artemis Biosystems). Cultures were grown up to $1 - 1.5 \times 10^6$ cells/mL in batch mode. Perfusion was started at 0.5 volume of medium per reactor volume per day (VVD) and induction was carried out after reaching the targeted cell density of 5×10^6 cells/mL. Perfusion was then continued at 1 VVD with fresh medium containing inducers for 3 – 4 days. In all perfusion runs, harvests were collected and the LV-containing supernatant was kept on ice or at 4°C until clarification (once daily) and subsequently stored at -80°C until quantification using the GTA assay. We are currently working on bioprocess development integrating this upstream process with suitable downstream approaches supported through the use of process development-enabling analytical methods.

Our study demonstrates that LV production in perfusion mode using the VHU filter outperformed our routine perfusion approach using an acoustic cell filter. Cells were retained in the bioreactor while LV particles passed through the filtration device with the harvest. Using this novel device, the cumulative functional LV titers were increased by up to 30-fold compared to batch mode, reaching a cumulative total yield of $>2 \times 10^{11}$ TU/L of bioreactor culture. This approach is easily amenable to large scale production and commercial manufacturing. Purification processes used to manufacture LVs need to be tailored to the unstable nature of LVs to counter vector instability and yields need to be improved through process optimization, such as the application of novel purification methodologies in continuous or semi-continuous mode. We will describe what DSP strategy we will use to most effectively integrate up- and downstream processing for lentiviral vectors. We also expect that our bioprocessing strategy will be transferable to other modalities having similar properties than LV.

THE NEW MANUFACTURING PARADIGM: CHALLENGES AND OPPORTUNITIES OF INTEGRATED CONTINUOUS BIOPROCESSING

Brendan O'Callaghan, Global Head, Biologics Platform
Brendan.O'Callaghan@sanofi.com

Key Words: integrated continuous bioprocessing, digital, GMP facility of the future.

Biologic therapies offer hope to many patients who previously had no effective treatment options for their disease. Whether for treatment of thousands of patients with a rare genetic disease or a rare blood disorder or for millions of patients with cardiovascular disease or diabetes, the number of patients treated with approved biologic drugs continues to increase worldwide.

The uninterrupted supply of these lifesaving medicines depends on the strong expertise and technical and scientific understanding of individuals from many disciplines – molecular genetics and cell biology, upstream and downstream scientists and engineers, bioanalytical scientists, process and plant design engineers, etc.

As production technology and process knowledge has advanced, the opportunity to implement process intensification has likewise increased. While there are many advantages to drug substance process intensification – including opportunities for improve costs – another advantage is the possibility for smaller drug substance manufacturing footprints.

This talk will survey where the industry is heading as well as implementation of integrated continuous processing in a digitally-enabled facility designed for intensified drug substance processes.

GMP IMPLEMENTATION OF CONNECTED AND CONTINUOUS PROCESSING AT AMGEN

Jim Weidner, Amgen Singapore, USA
hewiga@amgen.com

Amgen incorporated Integrated Continuous Biomanufacturing into the design of its Next Generation Biomanufacturing platform. Construction for the first Next Gen facility in Singapore occurred in June 2013 and in Sep 2018 for the second Next Gen facility in Rhode Island. At this point, the Singapore Next Gen facility is approved in major markets for multiple products, is operating at a high run rate, and has additional tech transfers underway. ICB lessons will be shared in the context of what has been executed in the GMP approved facility and processes. While Amgen's Next Generation Biomanufacturing platform extensively deploys single use technology, some lessons are also applicable to facilities of stainless steel design.

The presentation will review lessons of what went well from the start as well as lessons on what could be done better to implement integrated continuous technologies in GMP biological facilities and processes.

CONTINUOUS BIOPROCESSING WITH ULTRA-HIGH PRODUCTIVITY TO EXPEDITE BIOLOGICS DEVELOPMENT

Weichang Zhou, Ph.D. WuXi Biologics, Shanghai, China
weichang_zhou@wuxiapptec.com

Ultra-high productivity continuous bioprocesses have been developed for production of various biologics including monoclonal antibodies, fusion proteins and bispecific antibodies. This enables, for example, 2,000L disposable bioreactors to achieve comparable productivity as traditional 20,000L stainless bioreactors, and to significantly reduce manufacturing cost of goods. This process technology platform consists of continuous cell culture and continuous direct product capture, and is being scaled up and implemented for production of clinical materials. Several case studies, which achieved a cell culture productivity of 2-3 g/L/day, and a similar purification yield of the traditional purification process, will highlight advantages of this integrated continuous process platform in terms of productivity gains and speed to clinic. Scale up and implementation challenges will also be discussed.

DEVELOPING A FLEXIBLE AUTOMATED CONTINUOUS DOWNSTREAM PROCESSING SYSTEM FOR RESEARCH TO CLINICAL SUPPLY

Louise Taylor, Biologics CPI
Louise.taylor@uk-cpi.com
Harvey Branton, Biologics CPI
Daniele Messina, Biologics CPI
John Welsh, Pall
Spyridon Gerontas, Allergan Biologics Ltd
Martyn Hulley, Astra Zeneca Ltd
Richard Krucia-Tran, GlaxoSmithKline Ltd
Tibor Nagy FujiFilm Diosynth Biotechnologies Ltd
Ferran Sanchez, Sciex Ltd

Key Words: Continuous, Downstream, Automation, Control, Flexible

Continuous manufacturing has gained a lot of attention over the last 10-15 years for numerous reasons such as the potential for higher efficiencies, reduced cost of goods, and improved product quality. However, the adoption of these technologies has been slow due to concerns over operating these processes in a GMP manufacturing environment. Some of these concerns relate to the operation of multiple continuous unit operations in an integrated process sequence. This presentation will highlight these concerns and show how these issues were addressed by developing an overarching automated and modular platform which can be easily adapted for processing most products.

The developed automation platform is the result of a project funded by Innovate UK that brings together a number of biopharmaceutical companies including Allergan, AstraZeneca, Fujifilm Diosynth Biotechnologies and GSK to identify and address these issues. One objective of the project is to develop a flexible automated biologics downstream process consisting of multiple unit operations that can be rapidly reconfigured for manufacturing different products. To that end the process has been design with modularity in mind with each module having common inputs and outputs. The automation software has also been developed in a way that most typical downstream processes can be implemented in the system with little to no software updates. The ability to rapidly reconfigure the process has been demonstrated by using the system to produce two products with different process sequences.

Another issue that inhibits the adoption of continuous technologies is the concern over simultaneously operating multiple unit operations. This presentation will detail how the automation software was developed to control both the key unit operations such as chromatography and filtration steps but also intermediate operations such as feed conditioning and viral inactivation steps. The automated system reduces the complexity of downstream processes, which can have in excess of eleven unit operations, to a single user-friendly interface. Implementing this control platform enables a single operator to control the entire process.

This presentation will also detail how the automation strategy has been developed to enable a single operator to deal with start-up/shutdown, perturbations in the process and mid-process equipment turnover. It will highlight the challenges that have been faced when developing this system and how these have been overcome. The aim of this project was to improve efficiency by reducing processing time when compared to the current batch process and this was demonstrated by testing the system with two different products (a MAb and a MAb fusion protein). Furthermore, this presentation will show data from the production of these two products that demonstrates comparability between the continuous process and the original batch processes.

GMP DESIGN OF A SINGLE-USE INTEGRATED CONTINUOUS BIOMANUFACTURING SYSTEM

Robert E. Kottmeier, Pfizer, Inc.
robert.e.kottmeier@pfizer.com
Rob Fahrner, Pfizer, Inc.
Scott Godfrey, Boehringer Ingelheim
Greg Hiller, Pfizer, Inc.
Phil McCormick, Pfizer, Inc.
Raquel Orozco, Boehringer Ingelheim
Jeff Salm, Pfizer, Inc.

Key Words: GMP, Integrated, Continuous, Biomanufacturing, Single-Use

This presentation will show design work by Pfizer and Boehringer Ingelheim on a single-use integrated continuous system for GMP Biomanufacturing including consideration of scale, facility-fit, automation, single-use devices, hardware, and process control and monitoring. The scale of the system needs to be appropriate for the expected quantities of drug substance needed and needs to fit within the constraints of the GMP facility, including physical size and interaction with other facility systems. The system automation needs to control and monitor the entire process, since the upstream and downstream are integrated and the downstream operates semi-continuously. This presentation will discuss the challenges of designing an automation scheme capable of controlling an integrated upstream and downstream process along with the unique features that proved enabling to the integrated system. The single use devices and instruments in the system, including those made with additive manufacturing, are the process contact surfaces, so they need to be compatible with all the process fluids, perform consistently over process cycling and be constructed with sanitary design appropriate for GMP biomanufacturing. The system hardware provides the interface between the automation and the single-use devices controlling operations and the single-use instruments monitoring in-line process data. The design also needs to consider on-line instrument needs and off-line sampling analysis for a continuous flowing process stream.

APPLICATION OF CONTINUOUS PROCESSING IN CELL AND GENE THERAPY: CURRENT STATE AND FUTURE OPPORTUNITIES

Susan Abu-Absi, Ph.D., Pharmaceutical Development & Technology, bluebird bio
sabu-absi@bluebirdbio.com

Lesley Chan, Ph.D., Pharmaceutical Development & Technology, bluebird bio
Ken Kotz, Ph.D., Drug Product Enabling Technologies, bluebird bio

Key Words: gene therapy, lentiviral vector, bedside manufacturing

For CAR-T and autologous ex vivo gene therapies, cells are first collected from patients via apheresis, modified ex vivo with lentiviral vector (LVV) or other gene addition/gene editing methods, expanded in culture, and frozen as a living drug product. With the rapid growth of the cell and gene therapy field, the demand for LVV has increased exponentially. Current LVV production methods using transient transfection are robust but will ultimately be constrained volumetrically and temporally. To overcome these constraints, an alternative production process using stable clonal cell lines may be required to meet future demand. Stable producer cell lines will enable multi-day, continuous and scalable lentiviral vector production. The drug product manufacturing processes for autologous therapies present an ideal opportunity for continuous manufacturing, going beyond decentralized manufacturing to point-of-care or even bedside manufacturing. This presentation will provide an overview of our efforts to advance continuous bioprocessing for LVV and the challenges and opportunities of bedside manufacturing for autologous gene therapy products.

LEVERAGING DEVELOPMENT TO ADVANCE DIGITAL AND PAT TECHNOLOGIES IN MANUFACTURING

Rick St. John, Head of Digital Sciences, PTDU, Genentech, USA
st-john.richard@gene.com

Advancement of biomanufacturing process design and process analytical technologies (PAT) typically begins with process development. Years before a product moves to market, next generation processes are being developed to have greater efficiency and throughput. While developing next generation processes, successful process development organizations also invest in technologies to further improve biomanufacturing efficiency and robustness. Digital and PAT for process monitoring and control are no exception. The ability to mature technologies and move them to manufacturing is essential to remain competitive in today's industry climate. At Roche, we have embarked on a digital transformation in our development organization in order to accelerate pipeline development, deepen process understanding, and prepare for manufacturing processes of the future. Through this initiative, we are focused on increasing our data connectivity, predictive capabilities, and process monitoring sophistication. We expect this initiative to rapidly increase our rate of digital technology maturity and speed the development of digital technologies for biomanufacturing. With greater data connectivity, development will have access to broad data sets that allow for advanced learning, prediction, and modeling. The enhanced process understanding can then be transferred to manufacturing electronically and can be used to both generate instructions as well as build process models for monitoring and deviation resolution. Emerging from our digital transformation, Roche's process development organization will not only be able to deliver pipeline molecules more efficiently, we can do so with sophisticated process understanding enhanced by modeling and digital technologies to support PAT in biomanufacturing.

CONTROL STRATEGIES FOR INTEGRATED CONTINUOUS BIOPROCESSING

Christoph Herwig, Vienna University of Technology, ICEBE, Research Area Biochemical Engineering
 Christoph.Herwig@tuwien.ac.at

Key Words: ICH12, Life Cycle, Established Conditions, Control Strategies, Integrated Process Modelling, Data Science, Digital Twins

Continuous Bioprocessing is perceived to deliver constant product quality and to achieve higher time space yields. This is not new. Other market segments run well established continuous processes successfully since decades. What do we need to do? Just establishing continuous single unit operations (e.g. SMB chromatography) will not suffice. We need to have an integrated look at the entire process chain and stringently use the following control strategies :

- Capture process variability of the preceding unit operation using advanced PAT tools. Just measuring CPPs will not suffice: We need RMAs and intermediate CQAs to be transparent across the process chain.
- Include above variability in the control strategy of the individual unit operations, carry out sound process characterization using data science tools.
- Have clear specifications for intermediate acceptance criteria (IACs), such as distributions of expected outputs of intermediate CQAs
- Apply advanced process control strategies, such as multiple input multiple output controllers to robustly link process chain elements.
- Be aware that additional variability may occur along the life cycle. Hence, establish holistic manufacturing control strategies fulfilling Established Conditions (ECs) along ICH Q12 rationales.

This contribution aims at showing the central role of digital twins and data science in different control strategies: On the one hand, as we cannot measure all RMAs and CQAs of a process, we show how the process knowledge of single unit operations can be characterized by advanced data science tools and captured automatically in Digital Twins. We show how Digital Twins can be deployed for accelerating time to clinic as well as for continuous manufacturing. On the other hand, we can use integrated digital twins in production life cycle management for the identification of intermediate CQAs and can help when experiencing variabilities along Continued Process Verification (CPV) tasks.

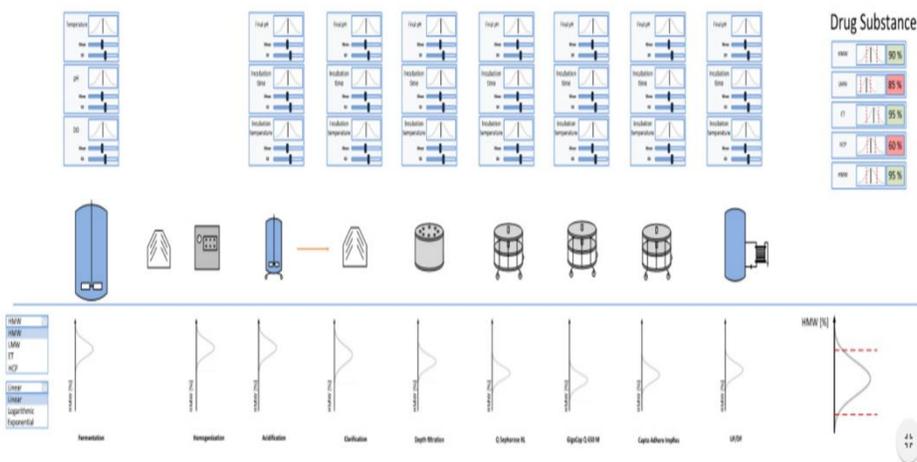


Figure 2 – Integrated Process Model analyzing the effect of CPP variability on the overall process and product quality

DIGITAL TRANSFORMATION IN BIOMANUFACTURING

Amos Lu, MIT, USA
amoslu@mit.edu
Richard Braatz, MIT, USA

Key Words: First-principles Modeling, Data-based Modeling, Machine Learning

This presentation describes ways to leverage and implement digitalization technologies in biopharmaceutical manufacturing. An accelerated process development workflow is described that employs micro-scale technologies, modular unit operations with integrated process control and monitoring systems, systems integration, and full plant automation. The presentation describes how to best develop and transfer knowledge between steps in the workflow via first-principles models, data analytics, and machine learning. Case studies are described that illustrate the application of each of the above methods, including where process equipment was designed digitally by using first-principles models first, then the process equipment was constructed and implemented experimentally, with the experimental results confirming model predictions.

DEVELOPMENT OF SCALABLE SEMI-CONTINUOUS DOWNSTREAM PROCESSES

Xuankuo Xu, Bristol-Myers Squibb, Devens, USA
xuankuo.xu@bms.com
James Angelo, Bristol-Myers Squibb, Devens, USA
Daniel Baur, ETH Zürich, Zürich, Switzerland
Srinivas Chollangi, Bristol-Myers Squibb, Devens, USA
Thomas Müller-Späth, ChromaCon AG, Zurich, Switzerland
Weixin Jin, Bristol-Myers Squibb, Devens, USA
Zizhuo Xing, Bristol-Myers Squibb, Devens, USA
Chao Huang, Bristol-Myers Squibb, Devens, USA
Jie Chen, Bristol-Myers Squibb, Devens, USA
Michael Borys, Bristol-Myers Squibb, Devens, USA
Massimo Morbidelli, ETH Zürich, Zürich, Switzerland
Sanchayita Ghose, Bristol-Myers Squibb, Devens, USA

Key Words: MAb process intensification, Multi-column capture, Low-pH viral inactivation, Integrated pool-less polishing

The goal of this work is to establish an intensified downstream scheme for stable, high-titer monoclonal antibody (mAb) processes to achieve increased manufacturing productivity with short cadence, reduced cost, and small facility footprint. Several continuous manufacturing technologies including multi-column chromatography for capture, automated low-pH viral inactivation (low-pH VI), and integrated pool-less polishing steps were evaluated following consistent development methodologies for several mAbs. This presentation aims to provide an overview of the approaches to developing and integrating these discrete technologies in one cohesive process flow that fits manufacturing requirements in a flexible manner. Development efforts are illustrated in three major areas. First, twin-column continuous capture chromatography (CaptureSMB) was evaluated systematically for equivalency assessment comparing to traditional batch operation for different molecules. Development data showed overall comparable chromatography performance, while certain trends were found to be molecule/process specific. For executing viral clearance studies, scalable models were developed using CaptureSMB and a surrogate system employing standard batch chromatography with flow path modifications to mimic the loading strategy of CaptureSMB. We also introduce a model-assisted process characterization approach toward validation of continuous twin-column capture chromatography owing to increased process understanding. Second, experimental studies and computational fluid dynamics (CFD) modeling were used to reduce the risk of product aggregation in low-pH VI manufacturing operation. For various mixing systems, localized low-pH zones were characterized quantitatively to avoid the undesirable conditions that could cause severe aggregate formation during acid adjustment. The modeling tool integrated with mAb aggregation measurements facilitates the optimization of operating parameters (e.g., titrant addition rate, impeller agitation) and automation strategy to ensure robust VI scale-up performance. Third, various scenarios of integrated pool-less polishing steps operated in flowthrough-flowthrough (FT-FT) or flowthrough-bind/elute (FT-B/E) mode were evaluated with or without inline adjustment between the two steps. Performance and quality attributes are compared for integrated and decoupled polishing steps, with an example describing the development and optimization workflow for a specific mAb process. Finally, implication to process development timelines, scale-up performance and practical challenges to process implementation in the new 2000-L manufacturing facility will be discussed.

ONLINE CONTROL OF SMALL SCALE INTEGRATED DOWNSTREAM PROCESS

Bernt Nilsson, Dept. of Chemical Engineering, Lund University, Sweden
bernt.nilsson@chemeng.lth.se

Simon Tallvod, Dept. of Chemical Engineering, Lund University, Sweden
Joaquin Gomis Fons, Dept. of Chemical Engineering, Lund University, Sweden
Niklas Andersson, Dept. of Chemical Engineering, Lund University, Sweden
Lotta Berghard, Sobi, Stockholm, Sweden

Key words: integrated DSP, online analytics, process control, development platform

Smart downstream processing can be performed with a sequence of integrated purification steps, which minimize the number of storage tanks and reduce hold-up time. The result is an integrated multiple unit operation sequence that performs straight through processing of the target protein, with minimal time from expression to formulation. This downstream processing technique is well suited to be connected to a continuous upstream process based on perfusion. To control the processing additional online detectors and analytics have to be integrated into the processing system to guarantee a successful product for real-time release. To develop these kinds of processes it is important to do studies in small-scale in a convenient way. This paper presents a concept for process development, online analytics and supervisory control of integrated downstream processes in lab-scale.

A general platform is developed for sequential processing of lab-scale integrated downstream processes. The platform is implemented using ÄKTA/UNICORN-systems for demonstration but is not limited to this setup. The modification of the physical setup to handle multiple processing steps in sequence on one single machine makes it possible to study advanced and complex process configurations without a lot of resources. Additional detectors and analytics based on Agilent HPLC-system is integrated to the downstream process for inline measurement and for flow path sampling for online analytics in each processing step. To make it easy to program and run the complicated setup with real-time analytics a supervisory controller is developed on top of the local equipment software. The new controller, called **orbit**, is open, extendable and flexible to handle very different configurations, processes and analytics protocols. To facilitate the usage even further the actual controller code is automatically generated from a high level presentation of the separation problem. Tools for design, control and verification makes it possible to virtual test the concept before making the actual experiment. The



Figure 3: Integrated DSP with analytics

concept is illustrated by an industrial case study based on a downstream process with four chromatography steps and one UF/DF together with online analysis with DAD detector and pool analysis with HPLC. The case study show the opportunities and challenges in lab scale integrated downstream processes with online analytics and control. It also force the development of orbit to handle large amount of generated data and to handle multiple setup with synchronized parallel sequences.

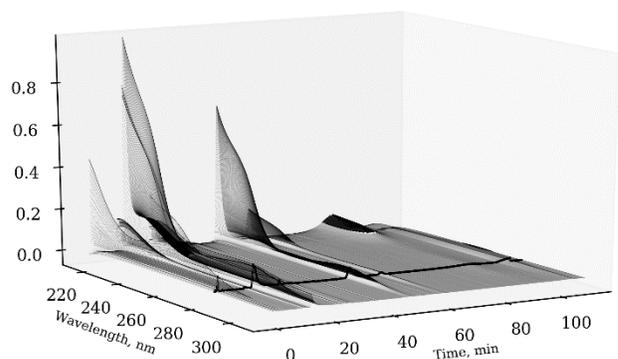


Figure 4: DAD spectra for all separations

PROCESS VALIDATION APPROACHES TO CONTINUOUS/CONNECTED DOWNSTREAM PROCESS

Huanchun Cui, Novartis Pharma AG

huanchun.cui@novartis.com

Mathias Goebel, Novartis Pharma AG

Rui Claudio Dos Reis Rodrigues, Novartis Pharma AG

Joseph Shultz, Novartis Pharma AG

Key Words: connected, discrete, scale down model, viral clearance, process characterization

One of the enabling technologies that Novartis has developed for its small and flexible biomanufacturing plant is the continuous/connected downstream process. The major benefit of continuous/connected downstream process is elimination of large product pool tanks (a few 1000L) by introducing small surge tanks (<100L) which greatly reduces plant foot print. The characteristics of continuous/connected downstream process are: 1) multi-unit operations work together in the same time 2) homogenous intermediate product pools are no longer available for typical offline analytical measurements. These new characteristics require process validation approaches to be reexamined.

This talk covers development of representative scale down models for continuous/connected downstream process to efficiently and robustly support process characterization. Viral clearance validation approaches will be also discussed to reflect the requirement of continuous/connected downstream process. Sampling approaches is an integral part of control strategy which needs to be modified as well to ensure successful process validation.

CONTINUOUS VIRUS INACTIVATION USING A PACKED-BED REACTOR

Duarte L. Martins, ACIB, Vienna, Austria and BOKU, Vienna, Austria
duarte.martins@acib.at

Jure Sencar, ACIB, Vienna, Austria and BOKU, Vienna, Austria

Nikolaus Hammerschmidt, ACIB, Vienna, Austria and BOKU, Vienna, Austria

Björn Tille, Takeda, Vienna, Austria

Johanna Kindermann, Takeda, Vienna, Austria

Thomas R. Kreil, Takeda, Vienna, Austria

Alois Jungbauer, ACIB, Vienna, Austria and BOKU, Vienna, Austria

Key Words: solvent/detergent; residence time distribution; viral inactivation; viral clearance

A critical unit operation in integrated continuous biomanufacturing is continuous virus inactivation. These reactors must provide sufficient minimum inactivation time and must have a narrow residence time. The narrow residence time is required to avoid a too short or too long incubation. Too short incubation may result in insufficient inactivation, too long may result in partial product destruction. We have developed a packed-bed continuous virus inactivation reactor (CVIR, Figure 1) with significant advantages over other continuous processing approaches, namely scalability, ease of operation and being truly continuous with undisrupted mass flow. The residence time distribution of our reactor is smaller compared to a coiled flow inverter or a jig in a box reactor.

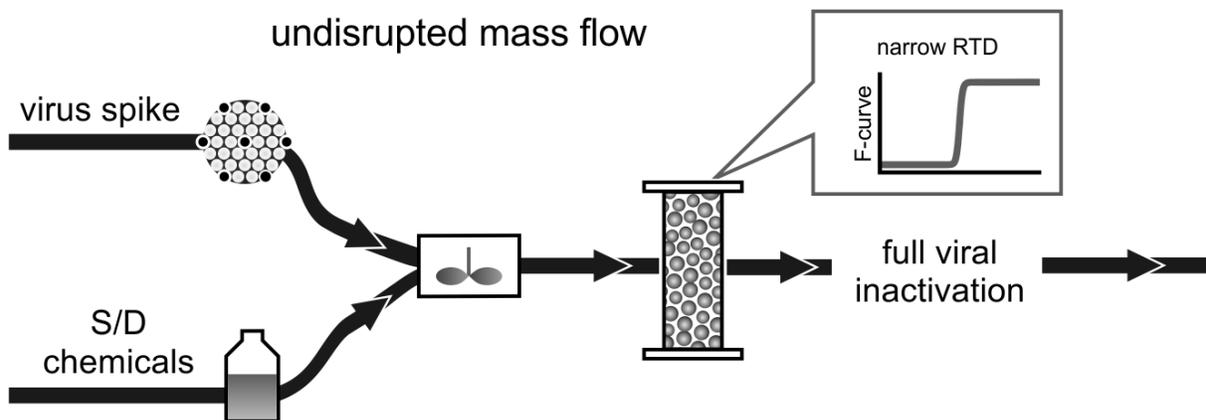


Figure 1: Overview of the continuous virus inactivation setup. The setup consists of a two independent stepper-motors syringe pump, a 2-chamber in-line mixer and the packed-bed CVIR.

Two industry-relevant virus models (X-MuLV and BVDV) were used to demonstrate the effectiveness of the CVIR for solvent/detergent treatment (S/D) unit operation. The CVIR achieved the same virus clearance performance as the traditional batch operation – a requirement for regulatory acceptance. An extensive array of controls proved that the observed virus inactivation was due to the S/D inactivation and not induced by the system. The S/D critical process parameters were subject of independent confirmation. Comparison against batch data showed that the virus inactivation capacity of the solvent detergent step using the packed-bed CVIR is as effective as batch operation and delivered comparable logarithmic reduction values (LRV). A 10-L column can process a stream of 85 L within 24 h.

CONTINUOUS VIRAL INACTIVATION: UNDERSTANDING FUNDAMENTAL MASS TRANSFER ENABLES SIMPLIFIED VIRUS VALIDATION

Matthew Brown, Boehringer Ingelheim, USA
matthew.brown.ext@boehringer-ingelheim.com

As continuous bioprocessing rapidly approaches the opportunity to add to the global drug supply, establishing a robust continuous viral inactivation (CVI) step and associated validation testing is paramount. The use of a plug flow reactor possesses significant advantages over a multiple tank system in that the product spends less time at low pH conditions; it provides the ability to sanitize between cycles; lacks air-liquid interfaces; and enables the 1-point standardization of the pH probe that provides the assurance of reaching the desired target pH. A critical parameter that poses a perceived new challenge is the definition of the exact incubation time of the product stream at production scale, as dispersion effects need to be accounted. Through extensive residence time distribution experimentation, the impact of viscosity, flow rate, and CVI reactor scaling parameters, flow characteristics were identified. This allowed the elucidation of the flow mechanics regarding the transition to weak turbulence inside the reactor due to Dean vortices. Leveraging the Dean vortices influence on dispersion, residence time for the product stream could be calculated allowing for the identification of worst case conditions (i.e. maximum and minimum residence time). The ultimate goal is to use the minimum residence time as the target inactivation time that will allow for a simplified batch based validation strategy in which the worst case conditions for a continuous viral inactivation step could be captured and quantified in a test tube, and be representative of the continuously operated system.

VIRAL CLEARANCE VALIDATION FOR A FULLY CONTINUOUS MANUFACTURING PROCESS FOR PHASE 1 STUDIES

Maarten Pennings, BiosanaPharma, the Netherlands
maarten.pennings@2process.nl
Ard Tijsterman, BiosanaPharma, the Netherlands

Key Words: Viral Clearance, Phase 1 CTM, Validation.

Over the past years, BiosanaPharma has developed a fully continuous process for the manufacturing of antibodies. The objective is to have a continuous production platform for biosimilars. Recently, a phase 1 study was initiated for a biosimilar that was produced using this continuous platform.

Using high cell density perfusion, two BioSMB (chromatography) steps, continuous nanofiltration and UDFD, a production campaign was performed under GMP to produce multiple drug substance batches. Given some unorthodox design choices, validation of the viral clearance capacity of this continuous process revealed some interesting challenges. The viral clearance validation focused on three steps: low pH virus inactivation, a membrane anion exchange step and nanofiltration. In this talk, justification for the scale down model is presented alongside with the results of the viral clearance study to demonstrate the safety of the product for a phase 1 study.

STRATEGY FOR TARGETED DELIVERY OF KEY NUTRIENTS IN HIGH CELL DENSITY PERFUSION

Véronique Chotteau, KTH Royal Institute of Technology, Sweden
veronique.chotteau@biotech.kth.se

Hubert Schwarz, AdBIOPRO, Competence Centre for Advanced BioProduction by Continuous Processing, Sweden; CETEG, Dept. of Industrial Biotechnology, CBH School, KTH, Stockholm, Sweden

Liang Zhang, AdBIOPRO, Competence Centre for Advanced BioProduction by Continuous Processing, Sweden; CETEG, Dept. of Industrial Biotechnology, CBH School, KTH, Stockholm, Sweden

Ye Zhang, AdBIOPRO, Competence Centre for Advanced BioProduction by Continuous Processing, Sweden; CETEG, Dept. of Industrial Biotechnology, CBH School, KTH, Stockholm, Sweden

Caijuan Zhan, AdBIOPRO, Competence Centre for Advanced BioProduction by Continuous Processing, Sweden; CETEG, Dept. of Industrial Biotechnology, CBH School, KTH, Stockholm, Sweden

Matthew Cheeks, Biopharmaceutical Development, AstraZeneca, Cambridge, UK

Richard Turner, Biopharmaceutical Development, AstraZeneca, Cambridge, UK

Andreas Castan, GE Healthcare, Uppsala, Sweden

Francisco Vilaplana, CBH School, KTH, Stockholm, Sweden

Key Words: feed, perfusion process, CHO, HEK293

High cell density perfusion is an attractive way for process intensification. We have developed high cell density processes using CHO cells for the production of monoclonal antibody, or using HEK293 cells for the production non-antibody glycoproteins.

A key aspect in the development of high cell density process is to obtain low culture medium renewal, in order to decrease the medium usage, the volume of harvest and the logistics burden. The concentration of culture medium is one of the tools available to achieve this. We have obtained interesting results of very low cell specific perfusion rate (CSPR) down to one reactor volume medium renewed per day sustaining healthy culture at 100×10^6 cells/mL. The limitation of such an approach is that all the medium components are concentrated generating media close to the precipitation. Beyond the concentration of the whole medium formulation, it is attractive to have a method to deliver selected components in the culture.

Such a method was developed and successfully applied for the delivery of glucose to reduce the lactate production, which drastically impeded the cell growth, in HEK293 cell culture at 80 to 100×10^6 cells/mL for the production of erythropoietin. It was then systematically adopted for all the perfusion cultures in our lab, i.e. using CHO cells or HEK293 cells. The method was then applied to deliver sugars different from glucose in antibody producing CHO cell perfusion process in a study of the effect of different sugars on the glycosylation. Finally, it was applied for the delivery of the amino acids in high density CHO cell perfusion process for the production of antibody at very low CSPR.

PROCESS CHARACTERIZATION FOR DYNAMIC DESIGN SPACE DEVELOPMENT: AN INTENSIFIED DESIGN OF EXPERIMENT METHOD

Moritz von Stosch, GSK, Belgium
MORITZ.X.VON-STOSCH@GSK.COM

In this contribution, an intensified Design of Experiment (iDoE) methodology will be introduced. The iDoE approach is based on the idea that the values of certain factors do not need to be kept constant throughout the experiments. Instead the value of the factors can be changed during the experiments, e.g. after a specified time a step-change from 23 to 30 (°C) can be applied in temperature. In this way a classical Design of Experiment plan can in principle be executed using less experiments.

The iDoE method is applied to industrial and simulated E.coli fed-batch fermentations. A dynamic hybrid modeling method is adopted for the analysis of the data, since the analysis cannot be accomplished with the traditional static statistical methods. The process understanding gathered from the iDoE is compared to DoE results. The results suggest that the number of experiments can be reduced by factor of three to two, meaning less than half of the experiments of a classical DoE are required with the iDoE method. In addition, the understanding of the process dynamics is much improved, which is of particular importance to assess the impact of temporal deviations in the factors on the process response.

HIGH DENSITY PERFUSED BATCH: ROBUSTNESS AND SCALABILITY OF PERFUSION PROCESSES FROM LAB SCALE TO COMMERCIAL SCALE

David Garcia, Advanced Process and Manufacturing Technologies – Novartis Pharma
david.garcia@novartis.com

Ying Jing, Advanced Process and Manufacturing Technologies – Novartis Pharma

Joerg Altekruenger, Drug Substance Development – Novartis Pharma

Joseph Shultz, Advanced Process and Manufacturing Technologies – Novartis Pharma

Key Words: high cell density perfusion, cell retention devices

The advancement on cell retention technologies and downstream processing of the last decades has revived the opportunities for the continuous manufacture of biologics [1]. At Novartis, the vision to make Advanced Integrated Biologics Manufacturing has matured over the past 5 years and it is ready for commercialization. The program is based on novel business strategies, operational approaches and process technologies to reduce time and cost, while increasing flexibility for the different pipeline modalities. As a result, the developed process allows producing commercial quantities in a 10-times smaller manufacturing scale. Over the past 3 years, 3 high-density perfused batch (HDPB) processes have been developed and scaled up showing scaling feasibility and technology robustness.

The HDPB concept has enabled a 1000L disposable bioreactor (reaching 3-4 fold higher cell densities with respect to fed batch) to produce the same mass, or greater, as a traditional ~10,000L bioreactor. The flexible nature of the facility enable a fast turn-around and the use of different cell retention devices (e.g. ATF, TFF). Both cell retention devices were evaluated in our HDPB continuous process at lab and manufacturing scale. The filtration technologies (ATF/TFF) were aligned at lab scale using common engineering criteria resulting in a comparable growth, productivity and product quality, while at manufacturing scale different operation strategies were evaluated in order to improve the process robustness. Overall interchangeability between ATF and TFF was demonstrated, though TFF showed significant advantage when comes to operation flexibility and simplicity. The HDPB processes have been developed at lab scale for multiple products, including 1 NBE (new biological entity) and 2 Legacy molecules. Each of the HDPB processes demonstrated consistent process performance and product quality at manufacturing scale and on average delivered 6-10 fold more product, per liter reactor, relative a commercial fed-batch bioreactor. Additionally, a scale-down model (SDM) was successfully qualified, which enabled the start of process characterization for one of the molecules.

Novartis efforts to keep growing the technology are strong and include SDM development, screening/optimizing cell retention technologies, as well as, improving the operation of the final scale, among others. The next step in our journey to re-imagine the development and manufacturing of biologics is to bring the program to the commercial stage, which ultimately will help us to reach our patients faster and more cost effectively.

[1] E.S. Langer, 11th Annual report and survey of biopharmaceutical manufacturing capacity and production—a study of biotherapeutic developers and contract manufacturing organizations, Rockville, MD, 2014.

SUCCESSFUL SCALE UP OF AN INTENSIFIED PERFUSION PROCESS TO CLINICAL AND COMMERCIAL SCALES

Charles Budde, Sanofi, USA
charles.budde@sanofi.com
Daryl Powers, Sanofi, USA
Jeff Swana, Sanofi, USA
Jean McLarty, Sanofi, USA

Key Words: Cell Culture, Perfusion, Intensification, Scale Up

An intensified perfusion process for production of a therapeutic monoclonal antibody was developed and scaled to 100 L clinical and 500 L commercial scales. The baseline process was developed in 10 L benchtop bioreactors with stainless steel alternating tangential flow (ATF) cell retention systems. The process consisted of a 12 day growth phase followed by a 48 day harvest phase. Cell densities of >120 MvC/mL were sustained with high culture viability. Productivities of >3 g/L·d were maintained throughout the harvest phase. The process was successfully scaled up to a 100 L single use bioreactor with dual ATF6 filters for clinical manufacturing. To verify that the process would perform similarly at commercial manufacturing scale, a proof of concept run was conducted in a 500 L single use bioreactor with dual ATF10 filters. Biomass concentration, culture viability, and productivity were comparable across scales. A full 60 day campaign in a 500 L bioreactor would generate over 70 kg of product in the clarified harvest. These studies demonstrate that intensified perfusion processes developed in benchtop bioreactors can be successfully reproduced at scales relevant for manufacturing.

TAILOR-MADE AQUEOUS TWO-PHASE SYSTEMS FOR APPLICATION IN CONTINUOUS SEPARATION OF POTENT BIOMOLECULES

Christoph Brandenbusch, Laboratory of Thermodynamics, Department of Biochemical and Chemical Engineering, TU Dortmund

christoph.brandenbusch@tu-dortmund.de

Maximilian Wessner, Laboratory of Thermodynamics, Department of Biochemical and Chemical Engineering, TU Dortmund

Fabian Görzgen, Laboratory of Plant and Process Design, Department of Biochemical and Chemical Engineering, TU Dortmund

Bettina Bommarius, School of Chemical & Biomolecular Engineering, Georgia Institute of Technology Atlanta

Andreas Bommarius, School of Chemical & Biomolecular Engineering, Georgia Institute of Technology Atlanta

Gerhard Schembecker, Laboratory of Plant and Process Design, Department of Biochemical and Chemical Engineering, TU Dortmund

Key Words: Thermodynamics, Aqueous Two-Phase Extraction, Process Development, Continuous Separation, Predictive Modeling

Aqueous Two-Phase Extraction (ATPE) using Aqueous Two-Phase Systems (ATPS) has long been shown to be a viable and promising alternative in the work-up of potent biomolecules (e.g. enzymes, proteins, therapeutics) from fermentation broth. Although ATPE has significant advantages over common separation strategies, such as a high biocompatibility, gentle separation profile due to low interfacial tension, good scalability and high efficiencies, industrial applications have not yet been realized.

Reasons typically given are based on the ATPS “physiochemical” properties such as viscosities and low density differences between the phases, which lead to long phase separation times. However, these challenges can be addressed using advanced technology such as the “Tunable Aqueous Polymer-Phase Impregnated Resins” (TAPPIR)-Technology immobilizing one phase of an ATPS inside porous solids, which are then transferred into a chromatography column. The second aqueous phase serves as mobile phase. The main advantage of this technique is the simple and efficient emulsification and liquid–liquid phase separation through the packed-bed column design. In addition, the extraction phases, i.e. both the back extraction phase and the immobilized phase, can be reused enabling a low-waste production process.

The remaining bottleneck for an industrial application is the identification of the “base” ATPS, which enables the desired extraction of the biomolecule with the required yield and purity to be competitive to existing processes. State-of-the-art ATPS design so far is based on a “trial-and-error” based approach identifying ATPS that work for a given task but often perform in suboptimal fashion.

In the present work, we will present a novel thermodynamics-based strategy for the identification and characterization of tailor-made ATPS for the continuous separation of highly potent industrial enzymes by ATPE. By consideration of the molecular interactions in solution, we are able to define potentially suitable ATPS based on a predictive modeling approach using ePC-SAFT, a state-of-the-art equation of state. The objective of this step is to supply a thermodynamically optimized combination of ATPS-phase formers that lead to optimal water condition (low concentration of phase formers, large process window), in principal enabling optimal separation. This initial selection is refined by taking into account molecular interactions of the biomolecule (enzyme), by measuring and modeling biomolecule-biomolecule and biomolecule-phase former interactions. These interactions are experimentally captured using advanced light scattering techniques that are both time and cost efficient. It will be shown that, based on the description of molecular interactions through osmotic virial coefficients (B_{22} and B_{23}) as well as the diffusion interaction parameter (k_D) between the molecules in solution, the phase behavior of the biomolecule in an ATPS can be made accessible, but was previously inaccessible with other phase diagram estimation strategies

One major advantage of our predictive modeling approach is the estimation of the partition coefficient of the biomolecule between the two aqueous phases based on a minimal set of experimental data, i.e. B_{22} , B_{23} , k_D , and phase composition data. Furthermore, the influence of the ATPS phase-formers on protein solubility and stability can be judged qualitatively, an ideal complement in the development of ATPS.

Lastly, we applied the thermodynamics-based strategy to the separation of an industrially relevant dehydrogenase from fermentation broth. The design-driven process development led to the identification of a tailor made ATPS that outperformed the reference ATPS from previous works in terms of solubility and stability of the biomolecule enabling a cost-efficient use of the TAPPIR technology.