Over the last decade, the expression levels have tremendously increased in the upstream fermentation; thus, the downstream processes (DSP) became the "bottleneck" in manufacturing process of bio-pharmaceuticals, especially for monoclonal antibodies. Additionally, biosimilars/biobetters are introduced to the market which demands new downstream approaches that are cost and time effective by retaining the properties of the biomolecules. Consequently, different integrated DSP and/or multi-column continuous chromatographic technologies are investigated that show promising results in reducing manufacturing costs. Only recently, the first integrated downstream process was reported at the production scale. What are the remaining barriers when implementing the approaches into the downstream processing? This presentation will outline the integrated continuous downstream process by focusing on the continuous chromatography and highlight major barriers and how to overcome them in the GMP environment.

Biography

Dr. Mihlbachler has worked in the field of process chromatography for almost 20 years. Currently, she is the Global Director of Separations Development at LEWA Process Technologies. She is responsible for the development of separation technologies for synthetic and biological molecules, in particular for continuous processing. Prior to joining, Dr. Mihlbachler was a consultant to LEWA-NIKKISO where she has supported the technical transfer of process chromatographic technology and consulted in customer projects. Dr. Mihlbachler worked 10 years as Sr. Researcher in pharmaceutical industry. She was involved in the development, scale-up and manufacturing of purification/separation processes for chiral and non-chiral compounds, peptides and proteins, in particular to implement continuous processes, at BMS, Eli Lilly and Pfizer. From 2011 to 2013, Dr. Mihlbachler has taught undergraduate courses for chemical and biomedical students at New Jersey Institute of Technology.

LEWA - Nikkiso --- A Global Supplier
Agenda

Introduction to Downstream Processing

Multi-Column Continuous Chromatography
Process Design
Example

Implementation Barrier:
Process
Technical
Risks and Control Strategies
Regulatory

Conclusions
**Objective**

Improve the economical, ecological and safety aspects of biopharmaceutical manufacturing by implementing a continuous processing platform.

**Drivers**

- Continuous upstream processing,
- Increased upstream titers, thus purification becoming a "bottleneck"
- Adapting single-use, disposable technology
- Multicomponent facilities, especially for CMOs
- Introduction of biosimilars/biobetter
- Tighter regulation for nutraceuticals

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**Introduction – Downstream Purification**

<table>
<thead>
<tr>
<th>Capture</th>
<th>Purification</th>
<th>Polishing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolation</td>
<td>bulk impurity removal: variants, DNA, HCPs, and endotoxins</td>
<td>removal of trace impurities</td>
</tr>
<tr>
<td>Concentration</td>
<td>IEX, SEC, and membranes</td>
<td>IEX, RP, SEC and membranes</td>
</tr>
<tr>
<td>Affinity/Protein A</td>
<td>IEX, HiC and filtration</td>
<td></td>
</tr>
<tr>
<td>IEX, HiC and filtration</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Block diagram of integrated continuous DSP**

Block diagram of generic downstream process
Integrated Continuous Downstream Purification

Block diagram of integrated continuous DSP

Close system without or minimal hold points
Scale reduction, thus, smaller footprint
Scale-up through multiplication
Flexibility for multi-product facilities

Risk reduction

Implementing single-use technology; thus, simplified cleaning and process validation procedures and shorter turnarounds
Full advantage when applying continuous chromatographic steps

Integrated Continuous Downstream Purification

**Continuous chromatography**
Feed continuously. Steady state in operating parameters.

However, feed characteristics and processing conditions might caused product variability. Plus, cyclic product collection with variability in composition.

How to combine several semi-continuous processes into one integrated continuous Downstream Processing scheme?

What is the time frame for the continuous operation? 24 h or 6 wk?

What are the technical challenges, especially for chromatographic process steps?

How to implement parallel operations (e.g. filtration steps), buffer exchanges and to eliminate/reduce hold points.

What are the regulatory/quality challenges and control strategies?
Introduction – Batch Chromatography

Single injections of compound mixture to be analyzed, separated or purified

Introduction – Continuous Chromatography

- Continuously feeding of compound mixture into chromatographic unit,
- Continuously separating / purifying of this mixture and
- Continuously (cyclic) collecting of the product streams
Introduction - Multi-Column Continuous Counter-Current Chromatography

**Petro-Chemicals:**
- ethyl benzene, m-xylene, indene from alkyl aromatics, p-chloro nitrobenzene, toluene di-isocyanate, p-toluidine

**Food:**
- Fatty Acids, mono-/tri glycerides, Sugars (500T/d)

**Bio-Molecules:**
- Citric Acid, Phenylalanine, Lactic acid and API’s (?)

**Synthetic Molecules:**
- Chiral and achiral Separation, Impurity Removal, SMB Mining™

---

Introduction – Multi-Column Continuous Chromatography using parallel separation of mixture

![Diagram showing multi-column continuous chromatography](chart.png)
Introduction – Multi-Column Continuous Chromatography
Overview commercially available systems

PCC from GE

SMCC from NovaSep

CaptureSMB by ChromaCon

Process Design

Conventional approach based on batch processes

Structure evaluation and 96-well plate or column screening:

- High-through put screening
- Setting DoE for different conditions (pH, conductivity, salts, media ...)
- Sharp breakthrough curves and high column capacity for DSP
- High solubility in buffer.
Process Design – MCC

Single column batch chromatography

Capture/Affinity: Bind-Elute or Flow-Through mode

High resolutions and generally high yields balance between through-put and capacity as well as buffer consumption

Optimal flow rate

Pressure drop:
\[ \Delta P = L_c \frac{\eta \cdot u \cdot (1 - \varepsilon)^2}{d_p^2 \varepsilon^3} \cdot 150 \]

Linear velocity:
\[ u_j = \frac{L_c}{t_0} = \frac{\dot{V}}{\pi/4 d^2 \varepsilon_{total}} \]

Tao, Chen, Carter, Ferreira, Robbins, AIChE J., 58 (2012) 2503
Process Design – MCC for Capture

Single column batch chromatography

Ideal case (sharp breakthrough)

Broad breakthrough

Transforming batch into continuous chromatography

Transfer sequential batch steps to parallel columns

Parallel batch chromatography → incremental performance improvement due to smaller equipment design and improved column performance, but not full advantage of sequential loading / counter-current principles

Scheduling of column switch: $t = twash + telution + tregen + tequilb$

- Combining recovery and regeneration steps
- Determine flow rate of steps (pressure and residence time limitations).
- Keep steep breakthrough curves to avoid losses during loading.
Process Design Example – MCC for Capture

Traditional Batch Recipes

Resin: Protein A
Sample: 2.5 g/L

Equilibration: 5 CVs
Load: 20 CVs
Wash Low Salt: 5 CVs
Wash High Salt: 5 CVs
Elution: 5 CVs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>column length [cm]</td>
<td>10</td>
</tr>
<tr>
<td>column ID [cm]</td>
<td>2.5</td>
</tr>
<tr>
<td>Cross section [cm²]</td>
<td>4.91</td>
</tr>
<tr>
<td>column volume [mL]</td>
<td>49.09</td>
</tr>
<tr>
<td>linear velocity [cm/h]</td>
<td>200</td>
</tr>
<tr>
<td>vol flow rate [mL/min]</td>
<td>16.36</td>
</tr>
<tr>
<td>BT 0% in CV</td>
<td>15</td>
</tr>
<tr>
<td>CV elution and regen</td>
<td>25</td>
</tr>
<tr>
<td>cycle time [min]</td>
<td>120</td>
</tr>
<tr>
<td>Feed concentration [g/L]</td>
<td>2.5</td>
</tr>
<tr>
<td>load per cycle [g]</td>
<td>1.84</td>
</tr>
<tr>
<td>load per column [kg/Lres]</td>
<td>0.0375</td>
</tr>
<tr>
<td>load per h [g/h]</td>
<td>0.92</td>
</tr>
<tr>
<td>productivity [kg/Lres/d]</td>
<td>0.45</td>
</tr>
<tr>
<td>buffer consumption [L/gprod]</td>
<td>0.667</td>
</tr>
</tbody>
</table>
Process Design Example – MCC for Capture

2 column/2 pumps

<table>
<thead>
<tr>
<th>Step</th>
<th>Time</th>
<th>CV Step</th>
<th>Load CV at end on col #1</th>
<th>Load CV at end on col #2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>5</td>
<td>5.00</td>
<td>0.00</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>5</td>
<td>0.25</td>
<td>19.75</td>
</tr>
<tr>
<td>3</td>
<td>45</td>
<td>15</td>
<td>15.25</td>
<td>19.75</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>5</td>
<td>0.26</td>
<td>20.00</td>
</tr>
<tr>
<td>5</td>
<td>45</td>
<td>15</td>
<td>20.00</td>
<td>0.26</td>
</tr>
<tr>
<td>6</td>
<td>15</td>
<td>5</td>
<td>0.26</td>
<td>20.00</td>
</tr>
</tbody>
</table>

Column length [cm]: 10
Column ID [cm]: 2.5
Cross section [cm²]: 4.91
Column volume [mL]: 49.09
Linear velocity [cm/h]: 200 batch
Linear velocity [cm/h]: 200 connected
BT 0% in CV: 15 for 200 cm/h

Loading single: 15
CV elution and regeneration: 25
Loading connected: 5

Vol flow rate [mL/min]: 27.27 elution/reg
Vol flow rate [mL/min]: 16.36 connected
Linear velocity [cm/h]: 333 elution/reg

Feed concentration [g/L]: 2.5
Load per cycle [g]: 2.45
Load per column [kg/Lres]: 0.050
Load per h [g/h]: 2.45
Productivity [kg/Lres/d]: 0.600
Buffer consumption [L/gprod]: 0.500

---

Process Design Example – MCC for Capture

3 column / 2 pumps

<table>
<thead>
<tr>
<th># of col</th>
<th>L sin CV</th>
<th>L con CV</th>
<th>uL sing [cm/h]</th>
<th>uL con [cm/h]</th>
<th>Cycle [min]</th>
<th>Load per cycle [g]</th>
<th>L per col [kg/Lres]</th>
<th>L per h [g/h]</th>
<th>prod [kg/Lres/d]</th>
<th>buffer [L/gprod]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>0</td>
<td>200</td>
<td>0</td>
<td>120</td>
<td>1.84</td>
<td>0.038</td>
<td>0.92</td>
<td>0.45</td>
<td>0.67</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>5</td>
<td>200</td>
<td>200</td>
<td>60</td>
<td>2.45</td>
<td>0.050</td>
<td>2.45</td>
<td>0.60</td>
<td>0.50</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>20</td>
<td>200</td>
<td>200</td>
<td>105</td>
<td>4.30</td>
<td>0.029</td>
<td>2.45</td>
<td>0.40</td>
<td>0.29</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>0</td>
<td>200</td>
<td>0</td>
<td>75</td>
<td>3.07</td>
<td>0.021</td>
<td>2.45</td>
<td>0.40</td>
<td>0.40</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>18.75</td>
<td>200</td>
<td>150</td>
<td>150</td>
<td>5.37</td>
<td>0.027</td>
<td>2.15</td>
<td>0.26</td>
<td>0.23</td>
</tr>
</tbody>
</table>

4 column / 2 pumps

---

4/15/2015
Advantage of 2-column processes

- More robust operations with less risks due less complexity in process and equipment.
- Fewer hardware components (pumps, valves, piping) → less risk for breakdown
- Lower CapEx investment and footprint!

Multi-Column Continuous Purification

Cost – Performance - Risk Assessment

Feed pump
Recovery pump for wash, elution, CIP, regeneration, and equilibration

Two columns
- smaller dimension
- better packing efficiency
- better separation performance ⇔ productivity
- less packing material
- better utilization of packing
- less equipment and process complexity
- Higher flow rates ⇔ productivity
- but higher buffer consumption
Multi-Column Continuous Purification

Cost – Performance - Risk Assessment

<table>
<thead>
<tr>
<th>Description</th>
<th>Probability</th>
<th>Severity</th>
<th>Impact (GMP, GAMP5 ...)</th>
<th>Detectable</th>
<th>Comments</th>
<th>Risk Control Measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>General Risks Capture/SMB</td>
<td>medium</td>
<td>medium</td>
<td>medium</td>
<td>yes</td>
<td>Complex mixture as feed from upstream bioreactors</td>
<td>Monitoring using PAT, process and cleanability verification on benchtop scale, adjusted automation</td>
</tr>
<tr>
<td>Process: batch vs continuous</td>
<td>medium</td>
<td>medium</td>
<td>medium</td>
<td>yes</td>
<td>Same process steps only feed continuously using multiple column, possible long-term operation (24 h to 6 weeks), perception that different process due to advertisement</td>
<td>Verification of design (see below) no dead legs or back mixing</td>
</tr>
<tr>
<td>Skid: batch vs continuous</td>
<td>medium</td>
<td>medium</td>
<td>medium</td>
<td>yes</td>
<td>Novel continuous process, long-term stability data needed under this operating conditions</td>
<td>Long term feasibility studies, control strategies, PAT implementation, equipment cleanability studies</td>
</tr>
<tr>
<td>Skid Design</td>
<td>low</td>
<td>medium</td>
<td>medium</td>
<td>yes</td>
<td>Novel continuous process, long-term stability data needed under this operating conditions</td>
<td>Long term feasibility studies, control strategies, PAT implementation, equipment cleanability studies</td>
</tr>
<tr>
<td>complexity</td>
<td>high</td>
<td>medium</td>
<td>medium</td>
<td>yes</td>
<td>More complex design with additional parts, need for more complex automation and control strategy</td>
<td>Rigorous design to avoid any dead volumes, monitoring CPP, implementing cleaning procedure,</td>
</tr>
<tr>
<td>Valves</td>
<td>medium</td>
<td>high</td>
<td>high</td>
<td>yes</td>
<td>When one fails more potential negative</td>
<td>Double valves, feedback from valves</td>
</tr>
<tr>
<td>single on-off valve</td>
<td>high</td>
<td>medium</td>
<td>high</td>
<td>yes</td>
<td>Large number of valves but the effect of one failing is not as dramatically</td>
<td>Double valves on important points, valve feedback</td>
</tr>
<tr>
<td>Columns</td>
<td>two</td>
<td>medium</td>
<td>medium</td>
<td>yes</td>
<td>Two column but smaller design, more robust and efficient</td>
<td>Testing of columns, pressure monitoring, cleaning of skid and</td>
</tr>
</tbody>
</table>

Challenges of Integrated Continuous DSP

Complex mixture as feed from upstream bioreactors

Multiple chromatographic steps to capture, purify, and polish using different retention mechanisms: IEX, SEC, HIC, Affinity

Very weakly and strongly bound components, however, some are closely related to the molecule of interest

Buffer and salt modifications

Sensitivity of bio-molecule to mechanical (pressure, flow, mixing …) and chemical (solvents and salt modifications) stress

Variability of feed composition and concentrations
Challenges of Integrated Continuous DSP

Chromatographic resins (and filters/membranes):

Mechanical and chemical stability of resin (caustic wash) as well as its characteristics (shrinking and expanding) and batch-to-batch variability

Reproducible packing of multiple chromatographic columns: What is allowed variability? How to measure variability?

Increased loadibility (concentration step on column), however, due to the continuous operation longer/higher loads – packing life time

Frequency for cleaning depending on load or time?

Cleaning regiment depending on residence time or volume?

24/7 operation – cleanability (CIP/SIP and re-equilibration) and life time

Challenge: MCC Equipment

High initial capital investment for skid and multiple pumps and columns

Skid

integrated CIP system (coupled or decoupled) with additional tubing, valves and tanks (avoid cross-contamination with bio-molecule streams)

critical ratio of extra column volume to hold-up volume (reduced tubing length but symmetry)

mechanical and chemical stability and bio-comparability of tubing, valves, and diaphragm pumps

EcoPrimeTwin FlowChart
Challenge: MCC Equipment

Piping Design Optimization:
CFD modeling ensures performance:
• System pressure (per step as required)
• Mixing (Reynolds number, flow velocity)
• Pressure drop

Results:
• Minimum hold-up volume
• Efficient mixing - tubing volume to ensure gradient accuracy
• Optimal piping design
• Optimal pipe design for pump inlet and outlet to avoid cavitation and to allow optimal mixing, respectively
• Implementing pressure regulator

Challenge: MCC Equipment

Valve

single multi-port valve, multiple multi-port block valves (# of columns), or multiple on/off valves (over 100 valves !!!)

CSEP design from Calgon Carbon
Block valve based on BTS technology
Commercial block arrangement

Reduction of internal and external dead volumes to avoid cross-contamination between bio-molecule streams and CIP
**Challenge: MCC Equipment**

**Metering Diaphragm Pumps**

Deliver accurate but more importantly reproducible

**LEWA ecodos pumps**
- Four layer diaphragm sandwich with rupture monitoring
- Robust design across a large flow rate range using one to triplex heads
- Suitable for pressures up to 10 bar
- Hygienic as well as CIP’able and SIP’able

**IntelliDrive technology** with single or multiple servo motors
- Gradient operation accuracy below +/- 1%
- Larger flow rate range provided by turndown of 100:1 or even 150:1
- Digital stepping motor design for greater accuracy and reproducibility

---

**Challenge: Equipment for Integrated Continuous DSP**

**Buffer In-Line Dilution System - LEWA Intellidrive Approach**

Stand-alone or as part of chromatographic skid
\( \Rightarrow \) reduces need for tanks and their sizes

dilution of contracted buffers with WFI at the point of use

**3 Pump Configuration**
- Servo motor per pump or head
- Three heads per pump

**Buffer In-line Dilution**
- Integrated system with PAT to control very accurate and reproducible flow rate, thus, pH and/or conductivity adjustments
- Reduced footprint
Challenge: Equipment for Integrated Continuous DSP

Inline Buffer Adjustments

Control systems and strategies needed that incorporated in the overall control

- Rigorous process design needed
- Determination of the appropriate critical process and product attributes
- Robust instrumentation with online calibration capability
- Reliable sample fractionation with fast analyses (online or offline PAT)

Challenges of Integrated Continuous DSP

Implementing Control Strategies by using PAT tools: online/inline UV detectors, pH and conductivity meters

Limited experience in transfer batch to continuous operation for biomolecules (existing processes vs process design for new molecules)

Control Strategy example: analytical tools monitor during processing

- **Protein determination:** Bradford protein assay, UV-spec at 280 nm (including HCP)
- **Identity:** Peptide mapping, HPLC C18, SEC, SDS-Page with Western Blot
- **DNA determination:** UV spec at 260 nm
- **Yield:** ELISA, HPLC and SEC
- **Purity:** HPLC C18, SDS-Page
- **Aggregate and Fragment:** SEC
Control solution: Breakthrough

- Upstream column loaded until % breakthrough
- Monitor column inlet (feed) and outlet signals ‘live’
- %breakthrough determined by comparison of the UV signals from the outlet of the upstream column and the inlet (feed) signal


Challenges of Integrated Continuous DSP

Control: Elution Peak Area*

Only one UV detector (eliminating calibration accuracy issues)

linear correlation load and peak area:
- while load < dynamic capacity, non-linear → product losses
- once load > dynamic capacity, non-linear → product losses

* ChromaCon at ACS Meeting 2015
Regulatory Challenge

“FDA supports continuous processing for pharmaceutical manufacturing.”

“There are no regulatory hurdles for implementing continuous manufacturing, but there is lack of experience”.

… “offers potential quality advantages in both development and manufacturing”.

Based on the 21th century quality initiative …
leads to agile, flexible and geographically independent manufacturing processes that deliver at high product quality and low production costs

Dr. Janet Woodcock at the International Symposium on Continuous Manufacturing of Pharmaceuticals at MIT, 2014

Regulatory Challenge

API of biopharmaceutical processes created in upstream bioreactor, not in the last step of the processing scheme of synthetic molecules.

Transition from batch to continuous 24/7 processing

Exposure time of molecule to process conditions causing any denaturation, association, or aggregation; therefore, immunogenic reactions

Risk assessment of the product, process and equipment based on ICH Q9

FDA provided the regulatory frame work through ICH guidelines implementing Control Strategies and Risk assessments …
“Demonstrably under-control processes can lead to decreased regulatory oversight.”

Dr. Janet Woodcock at MIT, 2014
Regulatory Challenge

QC/QA (impurity profile), product and process comparability, deviations – enable Real Time Release

Validation of the MCC chromatography and Integrated Continuous DSP in cGMP environment.
- CIP protocol for continuous process
- Long-term testing to guarantee the cleanability
- Definition of batch size and Batch integrity

FDA in 21 CFR 210.3: “Lot - a batch, or a specific identified portion of a batch, having uniform character and quality within specified limits; or, in the case of a drug product produced by continuous process, it is a specific identified amount produced in a unit of time or quantity in a manner that assures its having uniform character and quality within specified limits.”

“Batch” refers to quantity of material and not to mode of operation.

Economic Evaluation of CaptureSMB

- **CMOs**- Higher productivity allows balancing between two market goals
  
  ![Scales Diagram]

  - Maximization of number of projects
  - Increased profit

  Reduction of Operating costs
  - Increased margins for CMOs

- **Commercial manufacturing** - Reduced operating costs (time, space, resin, buffers) is major benefit of higher productivity but also reduced initial capital investments
Conclusions

Multi-Column Continuous Chromatography enables Integrated Continuous DSP and Single-Use Technologies

higher productivity – size reduction – elimination of hold tanks

technical and process challenges for implementing MCC Chromatography and Integrated DSP

however, there are business and regulatory drivers to implement

Thank you for your attention!
Vielen Dank!
ありがとうございます。

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e-mail: kmihlbachler@lewapt.com