Implementing Continuous Chromatography into DSP of Bio-Molecules

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Objective

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

In particular due to

- introduction of biosimilars,
- increased fermentation titers, thus purification becoming a ”bottleneck”
- adapting single-use, disposable technology
- tighter regulation for nutraceuticals
Outline

Introducing Continuous Chromatography

Its advantages and implementation into downstream processing (DSP)

Technical challenges

- Bioseparation
- Equipment and packing material
- Bioprocessing

Regulatory aspects
Introduction – *Downstream Purification*

Block diagram of generic downstream process

Block diagram of integrated continuous DSP
Introduction – *Downstream Purification*

**Block diagram of integrated continuous DSP**

**Integrated continuous DSP facilitates:**

- Closed system without hold points but smaller footprint
- Scale-up through multiplication of flexible multi-product facility
- Risk reduction
- Implementing single-use, disposable technology; thus, simplified cleaning and process validation procedures and shorter turnarounds
- Full advantage when applying continuous chromatographic steps
Introduction - Multi-Column Continuous Chromatography

Special requirements due to multi-component separation of bio-molecules, CIP/ SIP and single, sanitary on-off valves.

Parallel or sequential processing

Partially counter-current processing
Introduction - Multi-Column Continuous Chromatography

Current status

- SMB is established technology for manufacturing of synthetic API: chiral or achiral separation.

- Modeling and control tools are limited when process modifications needed

- R&D activities in industry and academia for 20 years, also for non-conventional separations as needed for DSP.
  - (Publications from Delft, ETH, Magdeburg, Dortmund and Purdue about the MC\textsuperscript{5} with solvent gradient, CIP, and SEC)

- Limited implementation in the manufacturing of bio-molecules: e.g. food, nutraceuticals, commodity industry … however none in biopharmaceutical industry
Challenge: DSP of Bio-Molecules

Multiple chromatographic unit operations in manufacturing processes to capture, purify, and polish polypeptides, proteins, enzymes, and antibodies

Multi-component mixture purified by different retention mechanisms: IEX, SEC, HIC, Affinity

Complex retention behavior; therefore, solvent and salt modifications

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

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

Variability of feed composition and concentrations
Challenge: DSP of Bio-Molecules

Low feed concentrations (solubility, aggregation)

Linear or step gradients - ON/OFF mechanisms:

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# Process Design - MCC

<table>
<thead>
<tr>
<th>Column I</th>
<th>Column II</th>
<th>Column III</th>
<th>Column IV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inlet:</strong></td>
<td><strong>Inlet:</strong></td>
<td><strong>Inlet:</strong></td>
<td><strong>Inlet:</strong></td>
</tr>
<tr>
<td>Regenerated</td>
<td>Loaded</td>
<td>Washed</td>
<td>Washed</td>
</tr>
<tr>
<td>Equilibrated</td>
<td></td>
<td>Eluted</td>
<td>Eluted</td>
</tr>
<tr>
<td><strong>Outlet:</strong></td>
<td><strong>Outlet:</strong></td>
<td><strong>Outlet:</strong></td>
<td><strong>Outlet:</strong></td>
</tr>
<tr>
<td>Waste</td>
<td>Waste</td>
<td>Product</td>
<td>Waste when no pure product</td>
</tr>
<tr>
<td></td>
<td>After breakthrough</td>
<td></td>
<td>Optional - non-pure product to next column (I)</td>
</tr>
<tr>
<td></td>
<td>to next column (III)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Scheduling of column switch

**Column LOADING**

Defined by the feed flow rate

Breakthrough Curve is function of thermodynamic (adsorption)

\[ t_{r,i} = t_o \left( 1 + \frac{F}{d_i} \frac{dq_i}{dc_i} \right) \]

and kinetic (mass transfer and diffusion) behavior of molecules

\[ t_{load} = t_{r,i} + 3 \sigma \]

Flow rate limitation due to pressure drop limitation of packing

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

Total number of columns depends of other tasks can be performed in time frame
\[ t = t_{\text{wash}} + t_{\text{elution}} + t_{\text{regen}} + t_{\text{equilib}} \]

due to the pressure limitation to all columns, especially important when connected

Define: CV for Wash
  CV for Elution
  CV for Regeneration (CIP)
  CV for Equilibration

Note: Pressure drop also depends on viscosity of solution
  Feed solution is significant more viscous as some other buffers
  Regeneration step can also be performed in reverse flow
Continuous Sequential Purification

Periodic Counter-Current Chromatography PCC from GEHC

Sequential Multi-Column Chromatography SMCC from NovaSep
Continuous Sequential Purification

Cost – Performance - Risk Assessment

At least two pumps:
1. Feed pump
2. Pump for wash, elution (low pressure mixing when gradient), CIP, regeneration, and equilibration

At least two columns with smaller dimension
1. better packing efficiency
2. better separation performance
3. less packing material
4. better utilization of packing

CaptureSMB
from ChromaCon

www.chromacon.com
Multi-column continuous counter-current chromatography for three compound separation with CIP section

- High solvent strength/Re-equilibration solvent
- Solvent
- Recycle from IV

Main component and late eluting impurities
- Extract
- Recycle to II

Product/Waste
- Raffinate

Implementing Continuous Chromatography

\[
t_{r \text{ raf,imp1}} > t_{\text{switch}} > t_{r \text{ raf,protein}} \\
t_{r \text{ ex,protein}} > t_{\text{switch}} > t_{r \text{ ex,imp2}} \\
t_{r \text{ regen,imp2}, t_{\text{equal/regen}}} > t_{\text{switch}}
\]
Challenge: Design and Processing

Preferred operating conditions:

- Isocratic or step gradient (mixing effects)
- Continuous to reduce start-up and shut-down
- Counter-current is optimal use of packing

2 Step Gradient (SG-SMB)

Challenge: Design and Processing

Determine operating parameters based on thermodynamics (adsorption isotherm) and kinetics (mass transfer).

Limited scheduling and modelling option (ChromWorks, Aspen …).

Implementation of PAT tools: online/inline UV detectors, pH and conductivity meters.

Establish control-strategy during process design.
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.
Challenge: MCC Equipment

Risk Assessment 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 and Knauer

High risk of internal leakage but small external volumes.

Dead volumes within valves but more importantly between valves if there are on/off valves (avoid cross-contamination between the different bio-molecule streams; IMPORTANT with CIP).
Challenge: MCC Equipment

Columns

- Low/medium pressure columns with differences between analytical and preparative columns (packing procedure, wall effects and distribution system).

- Mechanical and chemical stability of packing material (valve switching and caustic wash) and packing characteristics (shrinking and expanding).

- MCC uses with smaller columns with better performance compared to large process column, possible implementation of pre-packed single-use columns.

- Increased loadibility – packing life time limited by load or cleaning cycle.

- Cleanability during 24/7 operation: How to define the cleaning requirements (load per time?) and how to check?

- Leachables and extractables (control strategies).
Regulatory Aspects

API of biopharmaceutical processes created in fermenter, not in the last process of synthetic route.

Transition from batch to continuous 24/7 processing:

- Exposure time of molecule to process conditions causing denaturation or aggregation; therefore, immunogenic reactions
- Developing of control strategy to monitor critical process parameters (implementation of PAT)
- Initially, process development performed on continuous scale-down model using extensive control strategy
- Critical process attributes comparable for batch and continuous process, or deviates

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

Validation of the MCC process for cGMP environment:

- Definition of batch size (based on the fermenter), and therefore, batch integrity.
- CIP protocol for continuous process (based on batch definition, column load, residence time, cleaning cycle ...).
- Long-term testing on scale-down model to guarantee the process stability and the equipment cleanability.
- DoE to evaluate sensitivity of CPPs.
- Qualification of equipment.

Collaboration between Regulatory Agencies, Pharma and vendors.
How to overcome the challenges?

By implementing Continuous Chromatography into the downstream purification of bio-molecules.
ありがとうございます！
Thank you!
Danke!

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