Continuous end-to-end manufacturing with Contichrom®

January 2017
Our Unique Selling Proposition

- The most comprehensive automated and continuous chromatography process technology

- The least complex (Twin) and most productive multi-column technology

- Bio-Process Portfolio
  - Unique portfolio of countercurrent capture and polish processes that can be combined on a standard Contichrom® hardware platform with ChromIQ operating system to provide tailored continuous DSP trains for mAbs and sensitive glycoproteins
  - Fully continuous end-to-end downstream train linked to perfusion culture with ChromIQ® DSP operating control

- Scale-up: FPLC/HPLC Contichrom® Equipment and GMP pilot & process-scale Ecoprime® Twin skids from our partner LEWA
Continuous processes and process control

Continuous Chromatography

PILLAR 1
Continuous Capture
CaptureSMB® (2C-PCC)

PILLAR 2
Continuous Polish
Connected 2-step batch
MCSGP Flow2®

PILLAR 3
Process Control
AutomAb® MControl®

ChromaCon is the only player having all three elements
The Contichrom® CUBE / Combined FPLC System

- A modular system for batch and continuous processes
- Contichrom® modules can be combined to provide a fully continuous DSP train

Flowrates: 36 and 100 ml/min
Up to 100 bar
## Contichrom® Process Portfolio

<table>
<thead>
<tr>
<th>Batch and Connected Batch</th>
<th>CaptureSMB® (2C-PCC)</th>
<th>Flow2® (2C-PCP)</th>
<th>MCSGP (2C-PCP)</th>
<th>N-Rich®</th>
</tr>
</thead>
</table>

- **In-line dilution**
- **Two sequential batch purification steps without a stop**
  - **Fast capture process and full capacity utilization of Protein A**
  - **Capturing impurities and letting the product flow through**
  - **Obtain both high yield and purity with difficult separations**
  - **Isolate product-related impurities overnight**
Combining Contichrom® modules for continuous processes

Design an optimal automated continuous process for your purification challenge by combining the suitable Contichrom modules together

- MAb standard platform process – continuous with batch
- MAb standard platform process – continuous with PCC capture and polish (bind/elute)
- MAb standard platform process – continuous with PCC capture and polish (membrane FT)

- Linked to standard batch/fed-batch fermentation culture
- Linked to perfusion culture for sensitive glycoproteins with high antennary glycostructures
Contichrom®: a toolbox for your optimized process

2C-PCC (CaptureSMB®) Capture and Connected 2-step batch polish (CIEX, AIEX) for automated standard mAb platform process

2C-PCC Capture and MCSGP polish for automated purification of tagged proteins from microbial expression

Fully continuous mAb platform process 2C-PCC Capture & Flow2® CIEX membrane polish & Flow2 AIEX membrane polish Automated process with extremely high productivity
Case Study

End-to-end fully continuous manufacturing of a biosimilar mAb

Implemented at ETH Zurich
End-to-end fully continuous manufacturing (PD scale)

Perfusion connected to continuous DSP train at the laboratory of Prof. Morbidelli, ETH Zurich

Offers the advantage of a fully automated Biologics production

- Small footprint, high product output
- Can be used in restricted area barrier systems reducing infrastructure costs
- High product quality / homogeneity due to production at steady state
- Continuous production is supported by FDA quality initiatives

Perfusion Culture of a biosimilar monoclonal antibody

- Continuous **feed** of nutrients/ **removal** of inhibitory metabolites (LAC, AMM)
- **Cell retention** device to up concentrate cells

**With respect to fed-batch:**
- prolonged culture at higher VCD
- short residence time: favorable for “unstable” proteins
- control of metabolite concentrations in the reactor
- steady state: improved product quality homogeneity
- **Small equipment footprint:** low CAPEX
- **Large media consumption:** large COG
- Simpler **GMP/FDA** operation

Data from the Group of Prof. Morbidelli, ETH Zurich
Perfusion Culture: Stable Operation

- **Cell environment:**

![Cell density & viability graph](image1)

- **Metabolism:**

![Metabolite concentrations graph](image2)

Data from the Group of Prof. Morbidelli, ETH Zurich
Perfusion culture: Constant Product Heterogeneity

Constant charge isoform profile over 4 weeks of culture of a biosimilar monoclonal antibody

Data from the Group of Prof. Morbidelli, ETH Zurich
Continuous Capture: 2C-PCC Capture Step (CaptureSMB®)

- Employs only 2 chromatography columns to create a periodic countercurrent capture purification step.
- The least complex and most robust multi-column capture process.*
- The loading process is optimized to make full use of the capacity of the expensive Protein A affinity resin.
- Can be operated with AutomAb® dynamic process control for long process times, keeping the process at an optimum.

*Reference:
Continuous Polish: Several Process Options

- MCSGP is a cyclic countercurrent process allowing to obtain high yield AND purity at an up to 10-fold higher productivity than batch.

- MCSGP is controlled by MControl, the dynamic process control algorithm, keeping the MCSGP process always at an optimum for prolonged continuous process times.

- The 2-step connected batch process can be run with two IEX steps in sequence with in-line dilution ad isocratic/gradient elution, the typical polish step sequence of the mAb platform process.

- The Flow2® process is a cyclic countercurrent flowthrough process retaining the impurities and letting the product flow through. Through the choice of resins and / or membranes the impurity removal (aggregates, DNA, HCP) can be customized. The Flow2 process achieves very high productivities.
End-to-end fully continuous manufacturing

Combination of Contichrom Modules having different continuous process options allows to tailor the best continuous downstream train for:

✓ Highly complex glycoproteins with sensitive antennary structures produced in continuous perfusion culture and connected to a continuous DSP purification train

✓ Standard mAb platform processes turning from batch to continuous mode
Steady-state manufacturing: Impact on product quality

- **Drug substance is a heterogeneous mixture**
  - N-Glycosylation, charge Isoforms, aggregates / fragments

- **Reduce heterogeneity**: through perfusion and continuous DSP processes

- **Modulate heterogeneity**: through continuous DSP processes (MCSGP, N-Rich)
Product Quality Comparison: USP/DSP Capture

Charge isoform profile is more homogeneous from continuous USP/DSP capture operation

Data from the Group of Prof. Morbidelli, ETH Zurich
Product Yield Comparison: USP/DSP Polish

Perfusion → Cont. Capture → MCSGP
Fed-batch → Batch capture → MCSGP
Perfusion → Cont. Capture → Batch Polish
Fed-batch → Batch capture → Batch Polish

Perfusion makes polishing steps easier and MCSGP is more efficient than batch polishing

Data from the Group of Prof. Morbidelli, ETH Zurich
Constant Product Quality and Concentration

Data from the Group of Prof. Morbidelli, ETH Zurich

- HCP < 4 ng/mL
- Leached Protein A < 0.5 ng/mL
- DNA < 1 ng/mL
Scenario 1: Continuous integrated process for clinical trial manufacturing

End-to-end manufacturing of mAbs for clinical trials using Contichrom processes and Ecoprime equipment
Integrated process for clinical trial manufacturing (mAb)

Fed batch fermentation:
- 2000 L at 5 g/L
- output: 10 kg mAb
- Total transit time to API: 2.5-4h

### Table: DSP steps

<table>
<thead>
<tr>
<th>Step</th>
<th>Parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clarification</td>
<td>Depth filter 20 min transit time</td>
</tr>
<tr>
<td>Protein A</td>
<td>2x25 cm i.D.X 10cm bed height, MS Sure, product residence time 60 min, loading flow rate 600 cm/h</td>
</tr>
<tr>
<td>Virus Inactivation</td>
<td>30 min at low pH in hold tank, pH adjustment (in-line adjustment optional)</td>
</tr>
<tr>
<td>Cation exchange</td>
<td>40 cm i.D. i.D.X 10cm bed height, salt step elution, in-line adjustment of eluate. Product residence time 100 min</td>
</tr>
<tr>
<td>Anion exchange:</td>
<td>540 ml Q Membrane Cartridge (Flow through mode) at 10 membrane volumes/min. Product residence time: 1 min</td>
</tr>
<tr>
<td>Virus filtration and UF/DF</td>
<td>Single pass TFF with product residence time: 30 min</td>
</tr>
<tr>
<td>Total</td>
<td>Total product residence time in continuous DSP: 4 hours If Cation exchange step was done with Flowthrough using Flow2 step: further reduction by 1.5 hours</td>
</tr>
</tbody>
</table>
Integrated process for GMP clinical trial manufacturing (mAb)

Fed batch fermentation
2000 L at 5 g/L
output: 10 kg mAb
Total transit time to API: 2.5-4h

**Ecoprime Twin 500 (LEWA)**

- Required skid size: 5.5 L/min (operated at 600 cm/h)
- Flow capacity of Ecoprime 500: 0.06 L/min to 9.0 L/min

An Ecoprime 500 has the ideal size to process the required volumes. The Ecoprime systems can accommodate all processes. Two Ecoprime 500 systems and auxiliary systems would be needed to run a fully continuous process.

Advantage for CMOs: increasing project turnover, improving plant Capacity utilization, saving CAPEX and OPEX
Scenario 2: continuous integrated process for manufacturing (pilot / mfg scale)

End-to-end manufacturing of proteins for clinical trials and market supply using Contichrom processes and Ecoprive equipment
### Continuous integrated process (pilot / mfg scale)

Continuous fermentation
200 L at 1.5 g/L
1 React. Vol/day
Output: 0.3kg/d

<table>
<thead>
<tr>
<th>Step</th>
<th>Parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clarification</td>
<td>ATF and 0.2 µm filter</td>
</tr>
<tr>
<td>Protein A</td>
<td>2x7 cm i.D. x 10 cm bed height, MS Sure, product residence time 125 min,</td>
</tr>
<tr>
<td></td>
<td>loading flow rate 300 cm/h</td>
</tr>
<tr>
<td>Virus Inactivation</td>
<td>30 min at low pH in hold tank, pH adjustment (in-line adjustment optional)</td>
</tr>
<tr>
<td>Cation exchange</td>
<td>6 cm i.D. i.D.X 10 cm bed height, salt step elution, in-line adjustment</td>
</tr>
<tr>
<td></td>
<td>of eluate. Product residence time 100 min</td>
</tr>
<tr>
<td>Anion exchange:</td>
<td>75 ml Q Membrane Cartridge (Flow through mode) at 10 membrane volumes/</td>
</tr>
<tr>
<td></td>
<td>min. product residence time: 1 min</td>
</tr>
<tr>
<td>Virus filtration and</td>
<td>Single pass TFF with product residence time: 30 min</td>
</tr>
<tr>
<td>UF/DF</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>Total product residence time in continuous DSP: 4.75 hours</td>
</tr>
<tr>
<td></td>
<td>If cation exchange step was done with Flowthrough using Flow2 step and:</td>
</tr>
<tr>
<td></td>
<td>further reduction by 1.5 hours</td>
</tr>
</tbody>
</table>
Continuous integrated process (pilot / mfg scale)

Process details: Surge Tank Sizing and pump sizing

- The surge tank balances inflow from ATF and outflow to Capture step.
- Surge tank inflow: 140 mL/min
- Surge tank outflow: Average 140 mL/min:
  - 194 mL/min in sequential loading phase, 61 min
  - 99 mL/min in batch loading phase, 63 min

Recommended surge tank size: 10 L (Leaves 1h to react on USP issues)

Skid size: The max flow rate occurs in 2C-PCC:

- 200 mL/min, 300 cm/h, optimized scenario
- Flow capacity of Ecoprase 100 fits: 4 mL/min to 600 mL/min
Continuous integrated process (pilot / mfg scale)

2C-PCC versus batch process economics for annual (300 days) production:

<table>
<thead>
<tr>
<th></th>
<th>2C-PCC process</th>
<th>2C-PCC process</th>
<th>Batch process</th>
</tr>
</thead>
<tbody>
<tr>
<td>flow rate</td>
<td>[cm/h]</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>bed height</td>
<td>[cm]</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>scale up factor</td>
<td>[x]</td>
<td>197</td>
<td>277</td>
</tr>
<tr>
<td>total resin volume</td>
<td>[L]</td>
<td>0.8</td>
<td>1.1</td>
</tr>
<tr>
<td>column i.D.</td>
<td>[cm]</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>number of cycles</td>
<td>[-]</td>
<td>4.8</td>
<td>5.5</td>
</tr>
<tr>
<td>resin costs (1 packing)</td>
<td>[USD]</td>
<td>10'234</td>
<td>14'388</td>
</tr>
<tr>
<td>Resin packings per year</td>
<td>[-]</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td>annual resin costs</td>
<td>[USD]</td>
<td>153'515</td>
<td>258'975</td>
</tr>
<tr>
<td>annual buffer demand</td>
<td>[1000 L]</td>
<td>37.8</td>
<td>45.5</td>
</tr>
<tr>
<td>max feed flow</td>
<td>[L/min]</td>
<td>0.2</td>
<td>0.3</td>
</tr>
</tbody>
</table>
Comparison of PCC Processes

2C-PCC

3C-PCC

4C-PCC
Multicolumn PCC process performance

Prolonged CIP times Comparing different PCC Processes

- Multicolumn process comparison of 15-60 min CIP time. Experimental and simulation data were computed. The Typical CIP time is 15 or 30 min
- 2C-PCC and 3C-PCC have similar performance at high capacity utilization for all CIP times
- 4C-PCC is inferior as it dramatically declines at CIP time 60 min.
## PCC processes: hardware complexity

The number of hardware components is proportional to number of columns

<table>
<thead>
<tr>
<th>PCC system</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>6</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Pumps</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Number of detectors</td>
<td>2</td>
<td>4*</td>
<td>4</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Number of Valves</td>
<td>16</td>
<td>24</td>
<td>32</td>
<td>48</td>
<td>64</td>
</tr>
<tr>
<td>Risk of failure</td>
<td>reference</td>
<td>+ 50%</td>
<td>+ 100% (2x)</td>
<td>+ 200% (3x)</td>
<td>+ 400% (4x)</td>
</tr>
</tbody>
</table>

- Risk of batch failure in continuous processes can be expensive
- Risk of failure is additive! Assuming the same hardware components equipment with more columns has a much larger chance of failure!
- Use of “deltaUV”-control requires additional detector in 3C-PCC
# PCC dynamic process control for capture processes

- PCC is controlled through online UV signal feedback control
- Only 2C-PCC and 3C-PCC dynamic process controls are known

<table>
<thead>
<tr>
<th></th>
<th>2C-PCC AutomAb control</th>
<th>3C-PCC deltaUV control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control parameter</td>
<td>Breakthrough (pre-load)</td>
<td>Breakthrough (delta-UV)</td>
</tr>
<tr>
<td>Number of detectors</td>
<td>1 detector post column</td>
<td>2 detectors (pre-/post column)</td>
</tr>
<tr>
<td>Calibration</td>
<td>No detector calibration required</td>
<td>detector calibration required</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>High impurity baselines / low titers can be problematic</td>
<td>High impurity baselines / low titers can be problematic</td>
</tr>
<tr>
<td>Features</td>
<td>Accounts for feed titer variations and loss of resin capacity (aging)</td>
<td>Accounts for feed titer variations and loss of resin capacity (aging)</td>
</tr>
</tbody>
</table>

**Diagram:**
- **AutomAb (ChromaCon):**
  - UV1
  - UV2
  - Stop loading and switch
  - Pre-load area
  - Elution Volume

- **DeltaUV (GE):**
  - Column load
  - Column exit UV
  - Pre-load area
  - Elution Volume
  - Time

Confidential © ChromaCon 2016
Scalability of PCC processes

- 10 cm bed height can be packed reliably through all scales
- Using 10 cm bed heights in process development allows direct scale-up
- 2C-PCC can use a direct scale up as both in process development and scale up 10 cm bed heights are possible. Thus the 2-PCC process is scalable to production scale
- 3C-PCC and 4C-PCC processes cannot use the direct scale up with 10 cm bed height because their systems would not withstand the increase pressure with more than 2 columns!
- Watch for bed heights in small scale studies, consider the final scale (clinical trial mfg., commercial scale?)

<table>
<thead>
<tr>
<th>PCC Type</th>
<th>Bed Heights</th>
<th>Direct Scale-up Possible</th>
</tr>
</thead>
<tbody>
<tr>
<td>2C-PCC</td>
<td>10 cm 10 cm</td>
<td>Direct scale-up possible</td>
</tr>
<tr>
<td>3C-PCC</td>
<td>6.7 cm 6.7 cm 6.7 cm</td>
<td>No scale-up to commercial scale possible</td>
</tr>
<tr>
<td>4C-PCC</td>
<td>5 cm 5 cm 5 cm 5 cm</td>
<td>No scale-up to commercial scale possible</td>
</tr>
</tbody>
</table>
Conclusion

The 2C -PCC process (CaptureSMB) offer a number of advantages compared to other multi-column processes:

- Higher productivity than competitive 3, 4 column processes

- Least complex process and hardware configuration reducing the breakdown risk for long continuous processes

- Superior dynamic process control algorithm and software

- The only multicolumn process principle with direct scaleability from process development to pilot/process scale
Moving fast with twin-processes