Multi-Column Continuous Chromatography

Increasing productivity and decreasing cost in the downstream process

Built on patented & licensed technology from LEWA Bioprocess Technologies Group, Devens, MA USA
Agenda

- Who we are, what we do
- Drivers for multi-column capture chromatography
- Our approach to multi-column capture chromatography
- Additional features increase flexibility
- Simple design, simple software, simple set up
- Case studies – capture step
- MCSGP polish step technology
Who is LEWA?

65+
Years in business

14,000+
Pumps in process scale chromatography

5,400+
Global Staff

$1.3B
Corp Revenue
Who we are, what we do

LEWA Bioprocess Technologies Group

A group of ~ 60+ professionals dedicated to GMP scale bioprocess systems’ design and manufacture.

Part of the ~ 6,000 employees of Nikkiso Corp. – a diversified company with $1.3B in revenue.

Location
Devens, MA USA

Date of Establishment
2000

Ownership
Acquired in 2014, we are now a group within LEWA-Nikkiso America, Inc.

About LEWA
The LEWA group ($280M US) is a manufacturer of metering pump headquartered in Leonberg, DE. LEWA was acquired by Nikkiso in 2008. Both Nikkiso & LEWA founded ~65 years ago.

GMP systems engineering, manufacturing & automation;
• Chromatography
• Buffer In-line Dilution
• TFF
• Downstream process skids

Markets:
• Biopharmaceutical
• Pharmaceutical
• OEM (bio/pharma)

Video Link Here
A sampling of systems we produce at LEWA Bioprocess Group

Capture 2 column LPLC

MCSGP 2 column LPLC & HPLC

Simulated Moving Bed HPLC

Buffer In-Line Dilution Systems

Continuous Multi-column Chromatography Systems

Buffer / Formulation Systems

HPLC Systems

Cell Harvest Systems

HMI / Software Development

Blending Systems

Control Systems

TFF Systems
We partner with ChromaCon AG using their IP for twin-column processes.

LEWA manufactures pilot & production scale multi-column chromatography systems.
EcoPrime® Twin process capabilities

CaptureSMB (2C-PCC)
Fast capture process and full capacity utilization of Protein A

Batch
Traditional purification

Sequential Process
High throughput Purification

MCSSGP
Obtain both high yield and purity with difficult separations

Utilize the full loading capacity of your column

Capture and polish or two polishing steps

Recycle impure product, extract pure product

40% Protein A cost savings
2 x faster processing

Bind/elute or flow-through

Run two chromatographic steps without a stop

In-line dilution or buffer modification

Recycle until it’s pure!

more pure product

Capture and polish or two polishing steps

© LEWA Bioprocess Group and
Easy scale-up, scale-down; multiple formats

Contichrom CUBE

ChromaCon

LPLC

HPLC

EcoPrime Twin LPLC Capture

EcoPrime Twin MCSGP Polish

EcoPrime Twin MCSGP HPLC
Agenda

– Who we are, what we do
– **Drivers for multi-column capture chromatography**
– Our approach to multi-column capture chromatography
– Additional features increase flexibility
– Simple design, simple software, simple set up
– Case studies
– Conclusions
Benefits of *continuous* manufacturing

- Less documentation
- Lower inventory
- Improved safety
- Smaller footprint
- Less steps
- Higher quality
- Consistent quality
- Integrated process

**Aspen Alert Survey 2017**

- Yes: 84%
- No: 16%
Why use continuous chromatography for capture?

Reduce process steps, hold time and risk
Reduce cost, increase productivity

Batch

- Set Up
- Run
- CIP
- Cleaning Validation
- Set Up
- Run
- CIP
- Cleaning Validation
- Set Up

Continuous Processing

- Set Up
- Run
- CIP
- Cleaning Validation

Breaking the "bottleneck" in downstream processing
Why use continuous chromatography for capture?

Under utilization of resin capacity in Protein A step

- Protein A resin costs: $10,000 – $15,000 / L
- Size of Protein A columns used in manufacturing: 100 – 250 L
- One packed column worth $1 – $2.5 M, but utilization is only 40-60%!

Feed (clarified harvest)

Batch single Protein A column

Unused resin capacity, typically 40-60%

Flow-through
Why use continuous chromatography for capture (2C-PCC*)?

Batch capture

Load

40-60% unused resin capacity

Continuous capture

Load

Improved resin utilization significantly decreases cost. Savings for production column savings more than $0.5M US

*2C-PCC: 2-Column Periodic Countercurrent Chromatography
Why use continuous chromatography for capture?

High reward for intensifying the first downstream step

- Reduction of large buffer and hold-up tanks
- Smaller size of LPLC systems / columns

![Graph showing productivity and buffer consumption comparison between Batch and Continuous processes.](image)

**Better space use / smaller footprint**
- Less buffer consumption
- Less proteinA consumption (cost out)
- Higher productivity
Agenda

– Who we are, what we do
– Drivers for multi-column capture chromatography
– **Our approach to multi-column capture chromatography**
– Additional features increase flexibility
– Simple design, simple software, simple set up
– Case studies
– Conclusions
**ChromaCon / LEWA Partnership**

LEWA is the global GMP scale up partner for ChromaCon two column technology. Control logic for operating two columns continuously on a single system.

<table>
<thead>
<tr>
<th>ChromaCon</th>
<th>LEWA</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Process know-how</td>
<td>• Scale-up equipment</td>
</tr>
<tr>
<td>• Intellectual property</td>
<td>• Process solutions</td>
</tr>
<tr>
<td>• Entry-stage equipment</td>
<td>• Continuous manufacturing</td>
</tr>
<tr>
<td></td>
<td>• Worldwide footprint</td>
</tr>
</tbody>
</table>

- Technical, marketing and sales collaboration.
- Patented technology
Continuous capture with LEWA EcoPrime® Twin LPLC

Huge productivity
10 cm ID Protein A columns – more than 1 kg mAb purified/day (500 L feed)

Simplicity
Two column design minimizes complexity and secures maximum uptime

Easy scale up
Process design on the Contichrom CUBE, fast scale-up to EcoPrime Twin
Simple and robust design

8 column process
~240 Valves

CUBE from ChromaCon®

LEWA EcoPrime Twin
~40 Valves

The 2-column approach reduces risk

- Accelerates validation
- Simplifies maintenance
- Lessens costly downtime
- Minimizes hold-up and operating expenses
- Streamlines implementation; easy-to-understand and use

Same productivity as systems with 3 or more columns, in a simple 2-column design.
Easy scale-up, scale-down

CUBE is a simple tool for scaling-up and scaling down.
• Develop new processes
• Troubleshoot existing processes

EcoPrime Twin mirrors CUBE operation with continuous capture, batch, and sequential process functionality.

Contichrom CUBE

EcoPrime Twin 100

EcoPrime Twin 500+

EcoPrime Twin models to 33 LPM
Seamless scale-up of a continuous capture process

UV signals of four cycles (column 1 and column 2)
Continuous capture dramatically improves productivity

<table>
<thead>
<tr>
<th>System/Model</th>
<th>Column ID Range (cm)</th>
<th>Feed per day (L/day)</th>
<th>Product recovered* (kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EP Twin 100</td>
<td>2.5 10</td>
<td>23 360</td>
<td>0.11 1.7</td>
</tr>
<tr>
<td>(0.004 – 0.6 L/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EP Twin 250</td>
<td>5 20</td>
<td>92 1,460</td>
<td>0.44 7.0</td>
</tr>
<tr>
<td>(0.02 – 3.0 L/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EP Twin 500</td>
<td>8 45</td>
<td>206 7,420</td>
<td>1.0 35.0</td>
</tr>
<tr>
<td>(0.06 – 10.0 L/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Process dependent

EcoPrime Twin systems cover range from process development to large production scale.
EcoPrime Twin LPLC design

EcoPrime Twin 100

1. Symmetric design
2. Minimum hold-up volume
3. Simple piping design

2 Flow meters
Column inlet valve block
Column outlet valve block
Pump A (Buffer) with 6 inlets
Pump B (feed) with 4 inlets
Outlet valve block
pH probe
2 UV detectors
2 conductivity and temperature
2 ecodos pumps
Single outlet valve
Column inlets and outlets and their by-passes

© LEWA - Bioprocess Group and...
Agenda

– Who we are, what we do
– Drivers for multi-column capture chromatography
– Our approach to multi-column capture chromatography
– Additional features increase flexibility
– Simple design, simple software, simple set up
– Case studies
– Conclusions
Multi-function systems - not just multi-column capture.

Base EcoPrime Twin includes patented multi-column functionality
Multi-function systems – conventional batch

Twin Column CaptureSMB

Batch
single or “flip-flop” column chromatography

Col. 1 or 2

CEX

AEX

Single Column

“Flip-Flop” batch
Multi-function systems – Sequential process functionality available

Twin Column CaptureSMB

Sequential polish chromatography

Batch
single or “flip-flop” column chromatography

CEX

AEX
Multi-function systems – buffer from concentrates

- Twin Column CaptureSMB
- Sequential polish chromatography
- Batch single or “flip-flop” column chromatography
- Buffer In-line Dilution
- CEX
- AEX

© LEWA-Nikkiso America Inc.
Multi-function systems - not just continuous capture

Risk reduction, process intensification, reduce footprint and manufacturing flexibility
We have optimized to link directly to a single use environment – while maintaining an aseptic flow path.
Automation flexibility

Allen-Bradley
Newest version PAC

Rockwell Automation
• ControlLogix
• FactoryTalk

DELTA
V
direct control by
DeltaV based
program supplied
by LEWA

Customer can elect either control platform
Agenda

– Who we are, what we do
– Drivers for multi-column capture chromatography
– Our approach to multi-column capture chromatography
– Additional features increase flexibility
– **Simple software, simple set up, robust design**
– Case studies
– Conclusions
Information-rich, graphical user interface

HMI – main screen (EcoPrime Twin LPLC System)

Process flow chart with sensors to monitor and control process.

Trends, Sequences and Recipes, Reporting and Alarms …

User trained on day 1 and up and running on day 2
Recipe-driven process configuration

- Easily create recipes from preconfigured sequences
- ‘Development’ and ‘Production’ recipes with access control
- All data stored in a database for audit trail
Simple design, simple to set up & run

Set up / run system <1 day*

LEWA EcoPrime® Twin LPLC

* EP Twin 100 model GMP scale demo system
Robust design for extended run time performance

Long-term study over 210 loads (13 days) (EcoPrime Twin 100)

Monitoring feed flow rate and pump discharge pressure
(Shown - last 24 hours only)

Consistent and robust performance
Reproducibility during continuous operation on EcoPrime Twin 100

Conductivity monitored at the outlet of each column

Consistent & reproducible performance
Agenda

– Who we are, what we do
– Drivers for multi-column capture chromatography
– Our approach to multi-column capture chromatography
– Additional features increase flexibility
– Simple design, simple software, simple set up
– Case studies
– Conclusions
Continuous Capture using the 2-column system

CaptureSMB Process
Patent by ChromaCon®
Case Study #1

Customer “A”
Simple transfer from batch to continuous capture

Customer A - process development run comparison with 1g/L titer mAb

**Batch Capture**
Total CV 7.85 L

<table>
<thead>
<tr>
<th>Process Step</th>
<th>CV</th>
<th>Linear [cm/h]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column Equilibration</td>
<td>3</td>
<td>250</td>
</tr>
<tr>
<td>Load</td>
<td>40</td>
<td>150</td>
</tr>
<tr>
<td>Wash 1</td>
<td>2</td>
<td>150</td>
</tr>
<tr>
<td>Wash 2</td>
<td>2</td>
<td>250</td>
</tr>
<tr>
<td>Wash 3</td>
<td>3</td>
<td>250</td>
</tr>
<tr>
<td>Elution</td>
<td>3</td>
<td>250</td>
</tr>
<tr>
<td>post wash 1</td>
<td>3</td>
<td>250</td>
</tr>
<tr>
<td>post wash 2*</td>
<td>2</td>
<td>250</td>
</tr>
<tr>
<td>Regen*</td>
<td>3</td>
<td>250</td>
</tr>
</tbody>
</table>

**Continuous Capture**
Total CV 1.6 L

<table>
<thead>
<tr>
<th>Process Step</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Load Start-Up</td>
<td>15</td>
</tr>
<tr>
<td>Load Connected</td>
<td>27</td>
</tr>
<tr>
<td>Load Parallel</td>
<td>22</td>
</tr>
<tr>
<td>Wash 1 connected</td>
<td>1</td>
</tr>
<tr>
<td>Wash 1 Parallel</td>
<td>1</td>
</tr>
<tr>
<td>Wash 2</td>
<td>2</td>
</tr>
<tr>
<td>Wash 3</td>
<td>3</td>
</tr>
<tr>
<td>Elution</td>
<td>3</td>
</tr>
<tr>
<td>Post-Wash1</td>
<td>2</td>
</tr>
<tr>
<td>Post Wash 2</td>
<td>1</td>
</tr>
<tr>
<td>Regeneration</td>
<td>3</td>
</tr>
<tr>
<td>Re-Equilibration</td>
<td>5</td>
</tr>
</tbody>
</table>

Essentially same process steps = simple process transfer
Continuous capture has advantages

Customer A - process development run comparison with 1g/L titer mAb

Batch Capture
Total CV 7.85 L

<table>
<thead>
<tr>
<th></th>
<th>CV</th>
<th>Linear [cm/h]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column Equilibration</td>
<td>3</td>
<td>250</td>
</tr>
<tr>
<td>Load</td>
<td>40</td>
<td>150</td>
</tr>
<tr>
<td>Wash 1</td>
<td>2</td>
<td>150</td>
</tr>
<tr>
<td>Wash 2</td>
<td>2</td>
<td>250</td>
</tr>
<tr>
<td>Wash 3</td>
<td>3</td>
<td>250</td>
</tr>
<tr>
<td>Elution</td>
<td>3</td>
<td>250</td>
</tr>
<tr>
<td>post wash 1</td>
<td>3</td>
<td>250</td>
</tr>
<tr>
<td>post wash 2*</td>
<td>2</td>
<td>250</td>
</tr>
<tr>
<td>Regen*</td>
<td>3</td>
<td>250</td>
</tr>
</tbody>
</table>

Continuous Capture
Total CV 1.6 L

<table>
<thead>
<tr>
<th></th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Load Start-Up</td>
<td>15</td>
</tr>
<tr>
<td>Load Connected</td>
<td>27</td>
</tr>
<tr>
<td>Load Parallel</td>
<td>22</td>
</tr>
<tr>
<td>Wash 1 connected</td>
<td>1</td>
</tr>
<tr>
<td>Wash 1 Parallel</td>
<td>1</td>
</tr>
<tr>
<td>Wash 2</td>
<td>2</td>
</tr>
<tr>
<td>Wash 3</td>
<td>3</td>
</tr>
<tr>
<td>Elution</td>
<td>3</td>
</tr>
<tr>
<td>Post-Wash1</td>
<td>2</td>
</tr>
<tr>
<td>Post Wash 2</td>
<td>1</td>
</tr>
<tr>
<td>Regeneration (CIP)</td>
<td>3</td>
</tr>
<tr>
<td>Re-Equilibration</td>
<td>5</td>
</tr>
</tbody>
</table>

Process time: 9 h
Cycle time: 4 h at linear velocity of 250 cm/h

Same amount of material can be processed in 50% less time

Higher load volume 40 vs 49 CVs ➔ higher resin utilization & reduce buffer consumption
Shorter residence times ➔ faster loading & shorter processing time
**Strong continuous capture reproducibility**

Customer A using EcoPrime Twin 100 – 1g/L titer run results at 40 g/L load

At steady state, variability in peak area < 0.25 %. Steady state can be reached with first elution.

<table>
<thead>
<tr>
<th>Peak</th>
<th>Area column1</th>
<th>Area column2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.153</td>
<td>0.192</td>
</tr>
<tr>
<td>2</td>
<td>0.170</td>
<td>0.194</td>
</tr>
<tr>
<td>3</td>
<td>0.170</td>
<td>0.193</td>
</tr>
<tr>
<td>4</td>
<td>0.170</td>
<td>0.193</td>
</tr>
<tr>
<td>5</td>
<td>0.170</td>
<td>0.194</td>
</tr>
<tr>
<td>6</td>
<td>0.170</td>
<td>0.194</td>
</tr>
<tr>
<td>7</td>
<td>0.170</td>
<td>0.194</td>
</tr>
<tr>
<td>Average</td>
<td>0.170</td>
<td>0.193</td>
</tr>
<tr>
<td>error %*</td>
<td>0.1764</td>
<td>0.2169</td>
</tr>
</tbody>
</table>

* does not include the first cycle
Continuous capture reproducibility

Customer A using EcoPrime Twin 100 –1g/L titer mAb: 50 g/L load

At steady state, variability of peak area is <1 %. Steady state can be reached with first elution.
Comparison batch to continuous capture

Customer A - process development run: 1g/L titer mAb

**Productivity - Customer A**

- Batch: 4.2 g/L resin/h
- EcoPrime Twin: 13.5 g/L resin/h

**Buffer Consumption - Customer A**

- Batch: 0.526 L/g
- EcoPrime Twin: 0.38 L/g

Triple productivity or 67% less resin and ~30% buffer reduction
Scale-up estimate

Customer A – 1 g/L titer estimate for 100-kg process scale-up

- **Batch**: 80x20-cm column (100.5 L)
- **Resin costs [§K]**
  - 900 $K resin savings
- **Process Days [d]**
  - 22 days savings
  - EcoPrime Twin 2 - 45x10-cm column
  - Batch 45x20-com column (31.8 L)

**Savings**
- Up to $900K in resin
- Or 22 days in processing time
- 14,600 L estimated buffer savings
Case Study #2

Customer “B”

**BioProcess Technical**

Scale-Up of Twin-Column Periodic Counter-Current Chromatography for MAb Purification

James Angelo, John Pagano, Thomas Müller-Späth, Kathleen Mihlbachler, Srinivas Chollangi, Xuankuo Xu, Sanchayita Ghose, and Zheng Jian Li
User data shows >2X productivity and ~50% buffer savings.

Scale-Up of Twin-Column Periodic Counter-Current Chromatography for MAb Purification

James Angelo, John Pagano, Thomas Müller-Späth, Kathleen Mihlbachler, Srinivas Chollangi, Xuankuo Xu, Sanchayita Ghose, and Zheng Jian Li

Successful 100X scale-up. Now proceeding to production scale.

Seamless transfer from batch to continuous capture

**Customer B – pilot plant process comparison; 5 g/L titer mAb**

### Batch capture
- Load 50 g/L resin
- Total CV 17.3 L

<table>
<thead>
<tr>
<th>Step</th>
<th>CV</th>
<th>linear [cm/h]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Sanitization</td>
<td>3</td>
<td>300</td>
</tr>
<tr>
<td>Equilibration</td>
<td>3</td>
<td>300</td>
</tr>
<tr>
<td>Load</td>
<td>10</td>
<td>150</td>
</tr>
<tr>
<td>Wash 1</td>
<td>2</td>
<td>300</td>
</tr>
<tr>
<td>Wash 2</td>
<td>5</td>
<td>300</td>
</tr>
<tr>
<td>Wash 3</td>
<td>3</td>
<td>300</td>
</tr>
<tr>
<td>Elution</td>
<td>5</td>
<td>300</td>
</tr>
<tr>
<td>CIP</td>
<td>3</td>
<td>300</td>
</tr>
<tr>
<td>Neutralization 1</td>
<td>3</td>
<td>300</td>
</tr>
<tr>
<td>Sanitization</td>
<td>3</td>
<td>300</td>
</tr>
<tr>
<td>Neutralization 2</td>
<td>3</td>
<td>300</td>
</tr>
<tr>
<td>Storage</td>
<td>3</td>
<td>300</td>
</tr>
</tbody>
</table>

### Continuous capture
- Load 80 g/L resin
- Total CV 1.6 L

<table>
<thead>
<tr>
<th>Step</th>
<th>CV</th>
<th>linear [cm/h]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Sanitization</td>
<td>3</td>
<td>400</td>
</tr>
<tr>
<td>Equilibration</td>
<td>3</td>
<td>400</td>
</tr>
<tr>
<td>Load</td>
<td>4.6</td>
<td>100</td>
</tr>
<tr>
<td>Load interconnected</td>
<td>11.4</td>
<td>150</td>
</tr>
<tr>
<td>Wash 1</td>
<td>2</td>
<td>400</td>
</tr>
<tr>
<td>Wash 2</td>
<td>5</td>
<td>400</td>
</tr>
<tr>
<td>Wash 3</td>
<td>3</td>
<td>400</td>
</tr>
<tr>
<td>Elution</td>
<td>3.5</td>
<td>400</td>
</tr>
<tr>
<td>CIP</td>
<td>3</td>
<td>400</td>
</tr>
<tr>
<td>Re-equilibration</td>
<td>3</td>
<td>400</td>
</tr>
<tr>
<td>Sanitization</td>
<td>3</td>
<td>400</td>
</tr>
<tr>
<td>Neutralization 2</td>
<td>3</td>
<td>400</td>
</tr>
<tr>
<td>Storage</td>
<td>3</td>
<td>400</td>
</tr>
</tbody>
</table>

Essentially same process steps = simple process transfer
Continuous capture has advantages

Customer B – pilot plant process comparison; 5 g/L titer mAb

### Batch capture
- Load: 50 g/L resin
- Total CV: 17.3 L

<table>
<thead>
<tr>
<th>Step</th>
<th>CV</th>
<th>Linear [cm/h]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Sanitization</td>
<td>3</td>
<td>300</td>
</tr>
<tr>
<td>Equilibration</td>
<td>3</td>
<td>300</td>
</tr>
<tr>
<td>Load</td>
<td>10</td>
<td>150</td>
</tr>
<tr>
<td>Wash 1</td>
<td>2</td>
<td>300</td>
</tr>
<tr>
<td>Wash 2</td>
<td>5</td>
<td>300</td>
</tr>
<tr>
<td>Wash 3</td>
<td>3</td>
<td>300</td>
</tr>
<tr>
<td>Elution</td>
<td>5</td>
<td>300</td>
</tr>
<tr>
<td>CIP</td>
<td>3</td>
<td>300</td>
</tr>
<tr>
<td>Neutralization 1</td>
<td>3</td>
<td>300</td>
</tr>
<tr>
<td>Sanitization</td>
<td>3</td>
<td>300</td>
</tr>
<tr>
<td>Neutralization 2</td>
<td>3</td>
<td>300</td>
</tr>
<tr>
<td>Storage</td>
<td>3</td>
<td>300</td>
</tr>
</tbody>
</table>

**Process time:** 4.5 h

### Continuous capture
- Load: 80 g/L resin
- Total CV: 1.6 L

<table>
<thead>
<tr>
<th>Step</th>
<th>CV</th>
<th>Linear [cm/h]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Sanitization</td>
<td>3</td>
<td>400</td>
</tr>
<tr>
<td>Equilibration</td>
<td>3</td>
<td>400</td>
</tr>
<tr>
<td>Load</td>
<td>4.6</td>
<td>100</td>
</tr>
<tr>
<td>Load interconnected</td>
<td>11.4</td>
<td>150</td>
</tr>
<tr>
<td>Wash 1</td>
<td>2</td>
<td>400</td>
</tr>
<tr>
<td>Wash 2</td>
<td>5</td>
<td>400</td>
</tr>
<tr>
<td>Wash 3</td>
<td>3</td>
<td>400</td>
</tr>
<tr>
<td>Elution</td>
<td>3.5</td>
<td>400</td>
</tr>
<tr>
<td>CIP</td>
<td>3</td>
<td>400</td>
</tr>
<tr>
<td>Re-equilibration</td>
<td>3</td>
<td>400</td>
</tr>
<tr>
<td>Sanitization</td>
<td>3</td>
<td>400</td>
</tr>
<tr>
<td>Neutralization 2</td>
<td>3</td>
<td>400</td>
</tr>
<tr>
<td>Storage</td>
<td>3</td>
<td>400</td>
</tr>
</tbody>
</table>

**Cycle time:** 2.5 h

- Higher load 10 vs 16 CVs ➔ higher resin utilization & reduce buffer consumption
- Faster linear velocity ➔ shorter processing time
Reproducibility and scalability

Customer B – 5g/L titer mAb: 80 g/L load

EcoPrime Twin run with 14-h process time

Contichrom Cube run with 12-h process time

<table>
<thead>
<tr>
<th>peak #</th>
<th>Column 1 peak area</th>
<th>Column 2 peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.239</td>
<td>0.276</td>
</tr>
<tr>
<td>2</td>
<td>0.255</td>
<td>0.283</td>
</tr>
<tr>
<td>3</td>
<td>0.259</td>
<td>0.285</td>
</tr>
<tr>
<td>4</td>
<td>0.260</td>
<td>0.286</td>
</tr>
<tr>
<td>average</td>
<td>0.2583</td>
<td>0.2849</td>
</tr>
<tr>
<td>error %*</td>
<td>1.1</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Steady state can be reached with first elution.

Variability of peak area is ~1 % at steady state.
Comparison batch to continuous capture

Customer B - process development run: 5 g/L titer mAb

> 2X productivity or 50% less resin and ~50% buffer reduction
Scale-up estimate

Customer B – 5 g/L titer estimate for 100-kg process scale-up

Savings up to $320K in resin or 6.1 days in processing times
37,000 L estimated buffer savings
Case Study #3

Customer “C”
Reproducibility testing on mAb over multiple cycles

Customer C  EcoPrime Twin run with 30-h process time

Col 1 and 2 UV profiles - Consistent performances over 16 cycles
Control state reached at second cycle.
Reproducibility testing on mAb over multiple cycles

Customer C  EcoPrime Twin run with 30-h process time

Met purity requirements.
Variability of peak area less 0.3% at steady state
Agenda

– Who we are, what we do
– Drivers for multi-column capture chromatography
– Our approach to multi-column capture chromatography
– Additional features increase flexibility
– Simple design, simple software, simple set up
– Case studies

– Conclusions – What’s next?
Customer experience on mAb shows Twin column capture delivers:

- Significant productivity gains
  
  2-3 times

- Substantial cost or time savings
  
  Better Protein A utilization
  Faster loading
  Shorter processing time
  Reduced buffer consumption ..30-50%

- Seamless scale up
  
  Same process steps
  Proven reproducibility

- Simple 2-column approach and system
  
  Accelerate implementation
  Minimize risk
Free Modeling Tool for Batch to Multi-column Capture Processes

<table>
<thead>
<tr>
<th></th>
<th>Batch Process</th>
<th>Continuous Process</th>
</tr>
</thead>
<tbody>
<tr>
<td>Throughput (g/h)</td>
<td>1,744</td>
<td>7,449</td>
</tr>
<tr>
<td>Productivity (g/L resin/h)</td>
<td>17.3</td>
<td>74.1</td>
</tr>
<tr>
<td>Buffer Consumption (L/g protein)</td>
<td>0.60</td>
<td>0.40</td>
</tr>
</tbody>
</table>

Congratulations!

77% increase in Productivity
With a 33% decrease in Buffer

https://www.lewabiosolutions.com/twin
WE ARE PLEASED TO ANNOUNCE THE 2017 ASPEN AWARD WINNERS.

The annual Aspen Awards recognize the two most innovative new products that have made the greatest contribution to the advancement of bioprocessing.

https://aspenxchange.com/aspen-award
What’s next?
Dynamic Control Strategies
to Accommodate Process Variabilities

AutomAb control strategies implemented as already on Contichrom CUBE

Compensates:
• Variability in incoming feed titer due to process variability in upstream cell-culture
• Reduction of Protein A binding capacity due to exposure to caustics
What is next?

**Batch and Sequential**
High throughput Purification

**CaptureSMB (2C-PCC)**
Fast capture process and full capacity utilization of Protein A

**MCSGP**
Obtain both high yield and purity with difficult separations

**CaptureSMB**

- Recycle impure product, extract pure product
- Have the full loading capacity of your column
- Run two chromatographic steps without a stop

**MCSGP**

- Two polishing steps Capture and Polish
- In-line dilution
- High throughput Purification
- Two polishing steps Capture and Polish
- Have the full loading capacity of your column
- Run two chromatographic steps without a stop

**For high resolution separations in traditional single column batch chromatography gradients can be used. However, if there still are overlapping impurities, there is a trade-off between yield and purity. The MCSGP (Multi-column Counter-current Solvent Gradient Purification) process resolves this issue.**

40% Protein A cost savings
2 x faster processing

**Next Innovation:**
Yield and Purity Gain

**Applications:**
ADC, Peptides & Bispecifics

**Technology:**
Continuous twin column polish system.

**When:**
Production scale 2018
Multi-Column Continuous Polish Chromatography

A focus on the MSCGP technology providing both Yield and Purity

LEWA Bioprocess Technologies Group, Devens, MA USA
We partner with ChromaCon AG using their IP for twin-column processes.

LEWA manufactures pilot & production scale multi-column MCSGP chromatography systems.
EcoPrime® Twin process capabilities

**CaptureSMB (2C-PCC)**
- Fast capture process and full capacity utilization of Protein A
- Utilize the full loading capacity of your column
- 40% Protein A cost savings
- 2 x faster processing

**Batch**
- Traditional purification
- Bind/elute or flow-through

**Sequential Process**
- High throughput Purification
- In-line dilution or buffer modification
- Capture and polish or two polishing steps

**MCSGP**
- Obtain both high yield and purity with difficult separations
- Recycle impure product, extract pure product
- Recycle until it’s pure!

© LEWA Bioprocess Group and CaptureSMB (2C-PCC)
Easy scale-up, scale-down; multiple formats

Contichrom CUBE

ChromaCon

LPLC

HPLC

EcoPrime Twin LPLC Capture

EcoPrime Twin MCSGP Polish

EcoPrime Twin MCSGP HPLC
Combining the intellectual property of ChromaCon with 4 decades of low and high pressure GMP scale chromatography to deliver *yield and purity* at production scale.
EcoPrime Twin MCSGP precision and pressure powered exclusively by ‘gold standard’ in pump industry

LEWA Ecoflow Hygienic
100 bar

LEWA Ecodos Hygienic
7.5 bar
Ecoprime Twin® MCSGP Scale-up

Select features*

- Ability to run batch and MCSGP
- Platform design
- Integrated Buffer In-line Dilution
- Scale-up method conversion from CUBE to EcoPrime methods
- Allan-Bradley Rockwell and DeltaV operating systems
- Enables compliance with 21CFR part 11, and others
- Alarm and event logs, access control
- Drain & blow dry
- CIP & and aseptic single use connectivity (LPLC)
- Cleanability of all wetted parts
- Flow accuracy: 0.5% - 1.0% variation.
- Gradient accuracy: 0.5% - 1.0% variation
- Pressure rating: 7.5 bar (up to 100 bar for ATEX non-GMP MCSGP units)
- Flow path: stainless steel
- Equipment payback time of < 1 year

Example of MCSGP LPLC system ranges*

```
<table>
<thead>
<tr>
<th>Type</th>
<th>Min L/min</th>
<th>Max L/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ecoprive 100</td>
<td>0.004</td>
<td>0.6</td>
</tr>
<tr>
<td>Ecoprive 250</td>
<td>0.02</td>
<td>3.0</td>
</tr>
<tr>
<td>Ecoprive 500</td>
<td>0.06</td>
<td>10</td>
</tr>
</tbody>
</table>
```

Example of MCSGP HPLC system ranges*

```
<table>
<thead>
<tr>
<th>EcoPrime Twin HPLC</th>
<th>Flow rate range* [L/h]</th>
<th>Column ID range [cm]</th>
<th>Linear velocity range [cm/h]</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>0.5</td>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td>250</td>
<td>2</td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td>500</td>
<td>7.5</td>
<td>20</td>
<td>24</td>
</tr>
<tr>
<td>1000</td>
<td>17.5</td>
<td>30</td>
<td>25</td>
</tr>
</tbody>
</table>
```

* Low and High Pressure MCSGP Twin models may vary in features noted
MCSGP delivers High Purity and High Yield

• Recycle until it’s pure.  
  Internal recycling of product-containing side fractions

• Compared to a typical batch process  
  Yield improvements of up to 90%  
  10-fold more productive  
  Consumes 70% less solvent

• Analytical resolution at preparative scale  
  Uses smaller columns with smaller particle size resin

• Seamless method transfer from batch process  
  Uses the same solvents/buffers and resins

© LEWA - Bioprocess Group and
Thank you! Any questions?

Creating innovative solutions for downstream bioprocess from the leader in precision, accuracy and reproducibility

Chromatography Systems

Batch or Continuous Systems
Flow accuracy <0.5% from 1 to 99% linear gradient
- EcoPrime LFLC
- EcoPrime Twin
- EcoPrime BID
- EcoPrime HPLC

Analytical Performance at Pilot and Production Scale

ChromaCon
Contichrom® FPLC & HPLC Preparative Chromatography

Batch and continuous processes with dynamic process control
GMP Process Scale Solutions

EcoPrime® Twin