Integrated Continuous Downstream Processing - an Enabling Approach

(That will Break the Bottleneck)

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LEWA Process Technologies
16 April 2015

Connecting a World of Pharmaceutical Knowledge

Abstract

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.

White Paper: ***Integrated Downstream Processing – An Enabling Manufacturing Approach***, Part 1 and 2, K. Mihlbachler

Biography

Dr.-Ing. Kathleen Mihlbachler
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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.

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Twin-column Equipment Platform

Lab-scale Biopharma
Medium-pressure modular system for biopharmaceutical R&D

Pilot/Production-scale (GMP)
Twin column cyclic and continuous chromatography

ChromaCon

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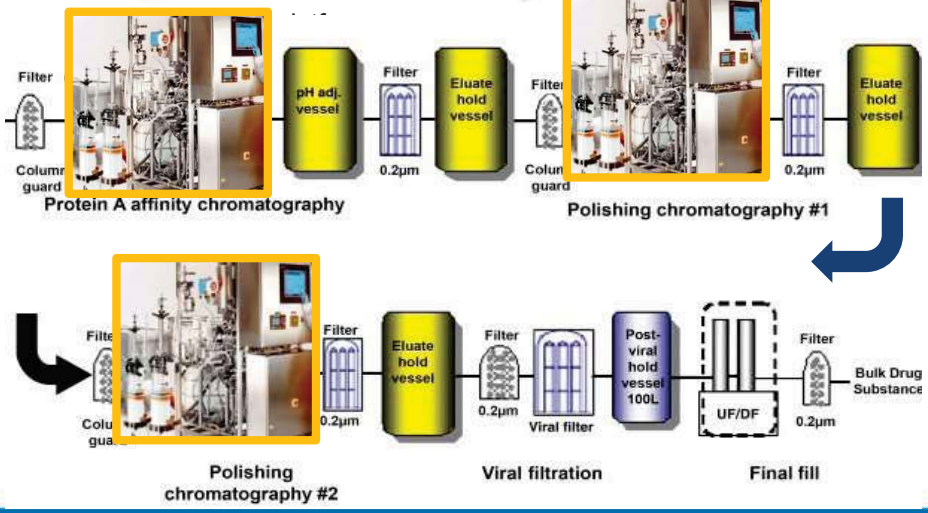


Agenda

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

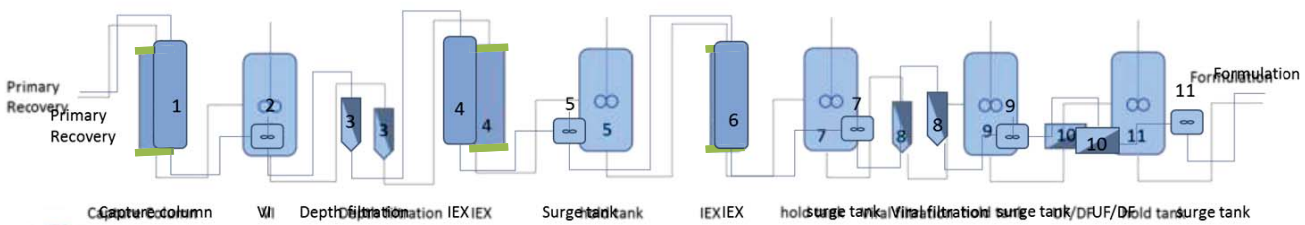
Drivers

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

Introduction – Downstream Purification

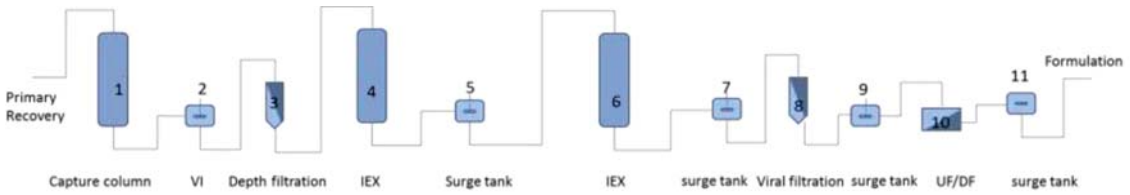
Capture	Purification	Polishing
Isolation Concentration	bulk impurity removal: variants, DNA, HCPs, and endotoxins	removal of trace impurities
Affinity/Protein A IEX, HIC and filtration	IEX, SEC, and membranes	IEX, RP, SEC and membranes

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?

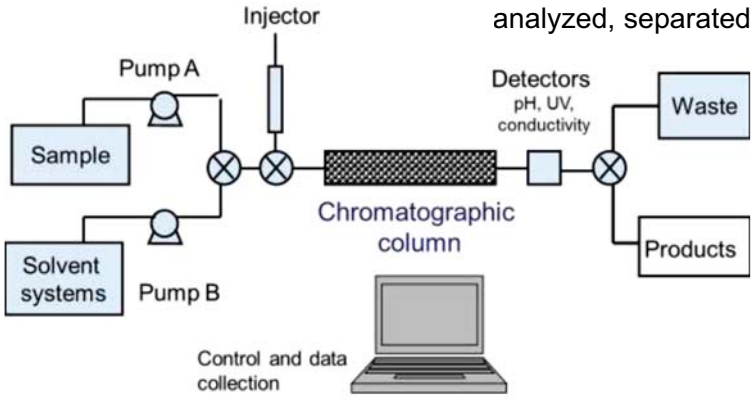
What are the technical challenges, especially for chromatographic process steps?

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

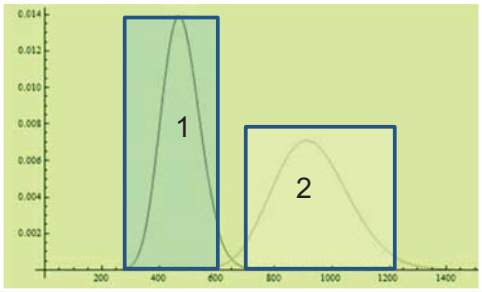
What are the regulatory/quality challenges and control strategies?

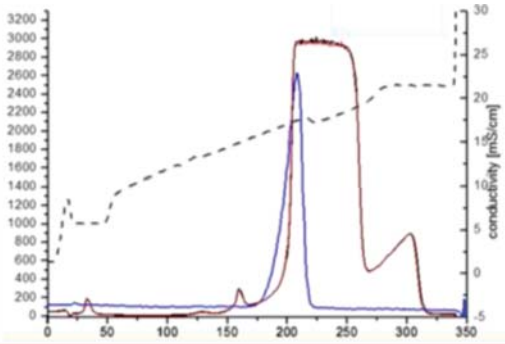
Introduction – Batch Chromatography

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



Chromatograms

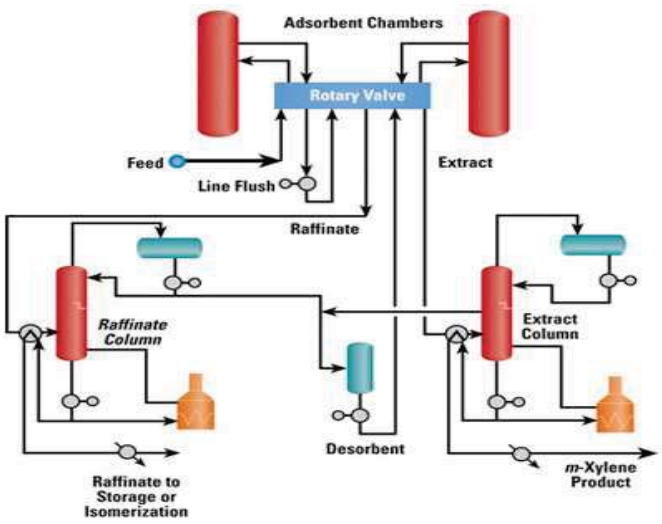


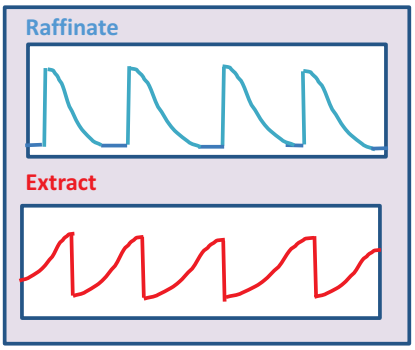


Introduction – Continuous Chromatography

- Continuously feeding of compound mixture into chromatographic unit,
- Continuously separating / purifying of this mixture and
- Continuously (cyclic) collecting of the product streams

Sorbex™ UOP





Introduction - Multi-Column Continuous Counter-Current Chromatography

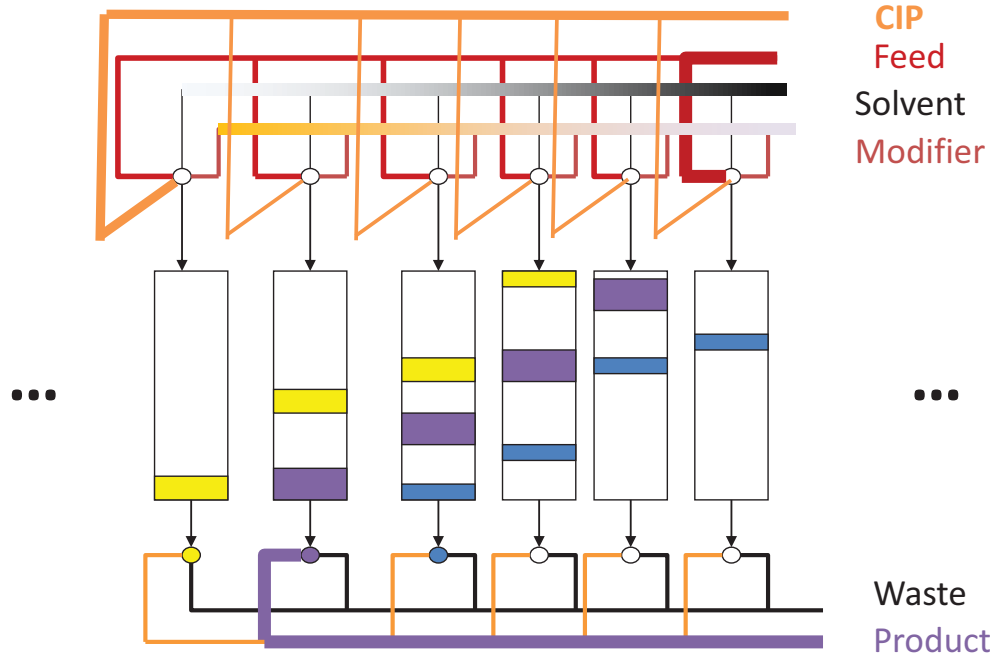
Petro-Chemicals:
ethyl benzene, m-xylene, indene from alkyl aromatics, p-chloro nitrobenzene, toluene di-isocyanate, p-toluidine

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™

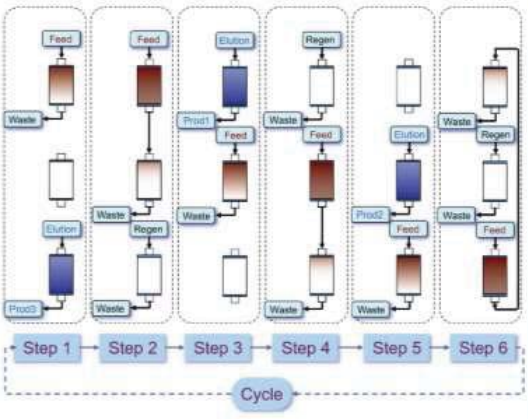
Introduction – Multi-Column Continuous Chromatography using parallel separation of mixture



Introduction – Multi-Column Continuous Chromatography

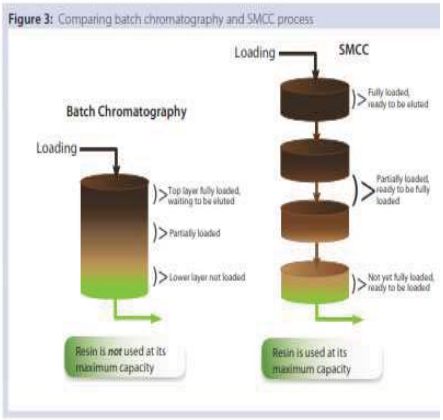
Overview commercially available systems

PCC from GE



Biotechnology and Bioengineering, Vol. 109, No. 12, December, 2012

SMCC from NovaSep



76 BioProcess International SEPTEMBER 2008

CaptureSMB by ChromaCon

www.chromacon.com

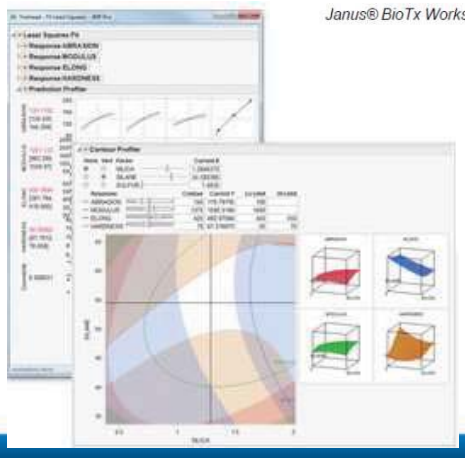
Process Design

Conventional approach based on batch processes

Structure evaluation and 96-well plate or column screening :

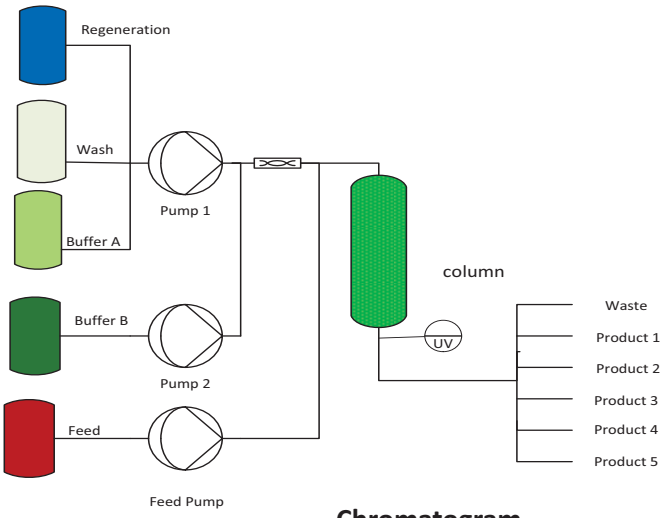
- High-through put screening
- setting DoE for different conditions (pH, conductivity, salts, media ...)
- Sharp breakthrough curves and high column capacity for DSP
- High solubility in buffer.

Janus® BioTx Workstations (Perk

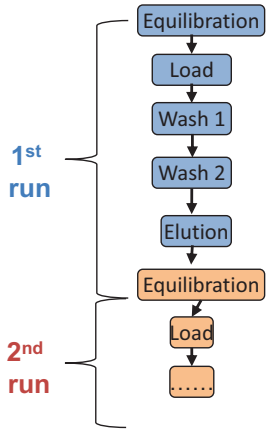


Process Design – MCC

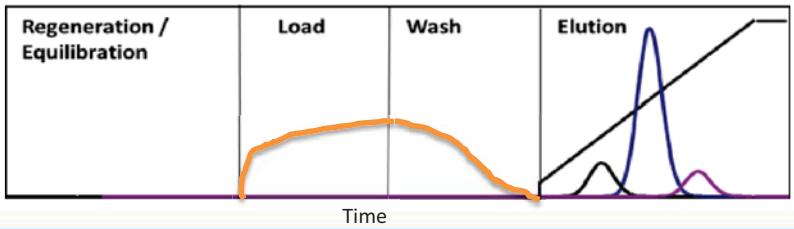
Single column batch chromatography



Process Recipe / Sequence



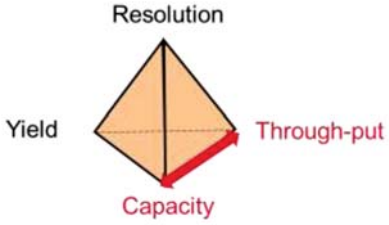
Chromatogram



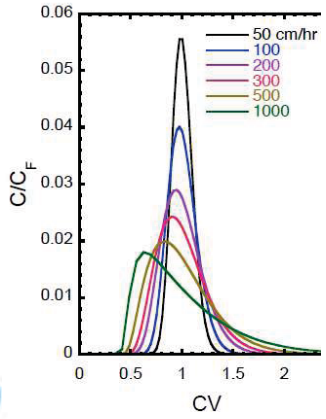
Process Design – MCC

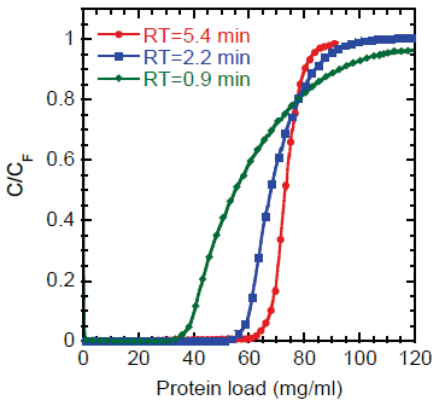
Capture/Affinity: Bind-Elute or Flow-Through mode

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



Optimal flow rate





Tao, Chen, Carta, Ferreira, Robbins, AIChE J., 58 (2012) 2503

Pressure drop :

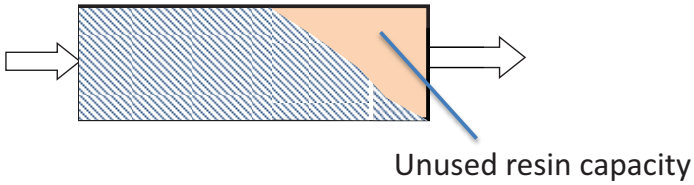
$$\Delta P = L_c \frac{\eta \cdot u \cdot}{d_p^{\,2}} \cdot \frac{(1-\varepsilon)^2}{\varepsilon^3} \cdot 150$$

Linear velocity:

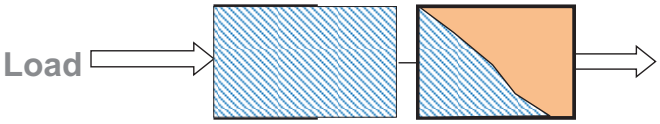
$$u_l = \frac{L_C}{t_0} = \frac{\dot{V}}{\pi/4\, d^2 \varepsilon_{total}}$$

Process Design – MCC for Capture

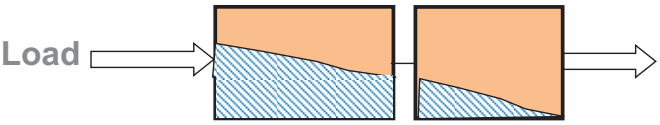
Single column batch chromatography

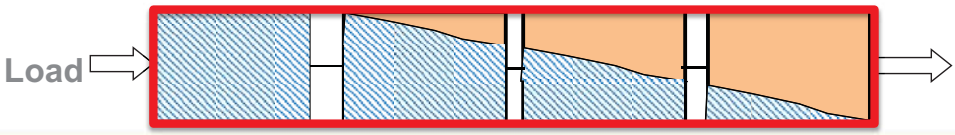


Ideal case (sharp breakthrough)



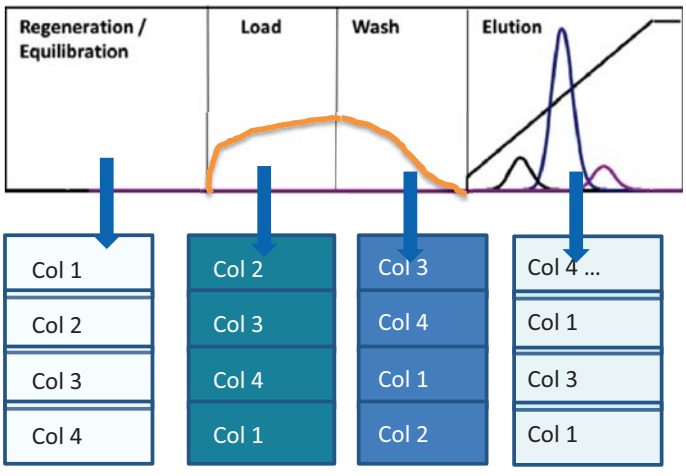
Broad breakthrough





Process Design –MCC

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,

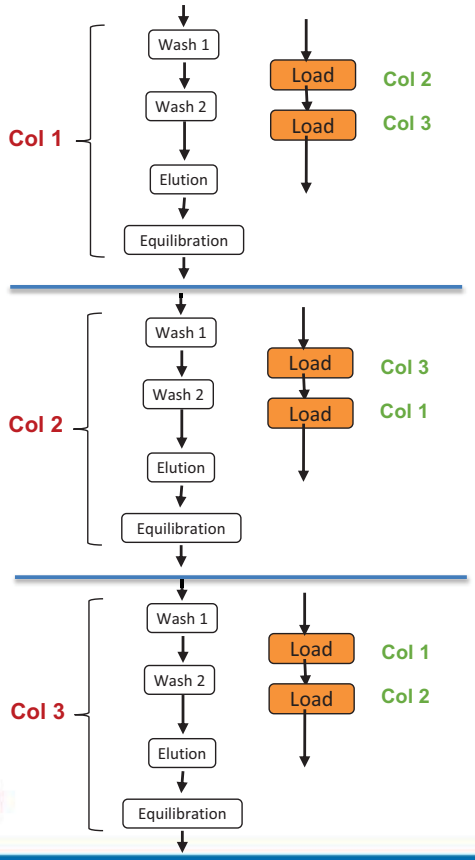
but not full advantage of sequential loading / counter-current principles

Scheduling of column switch

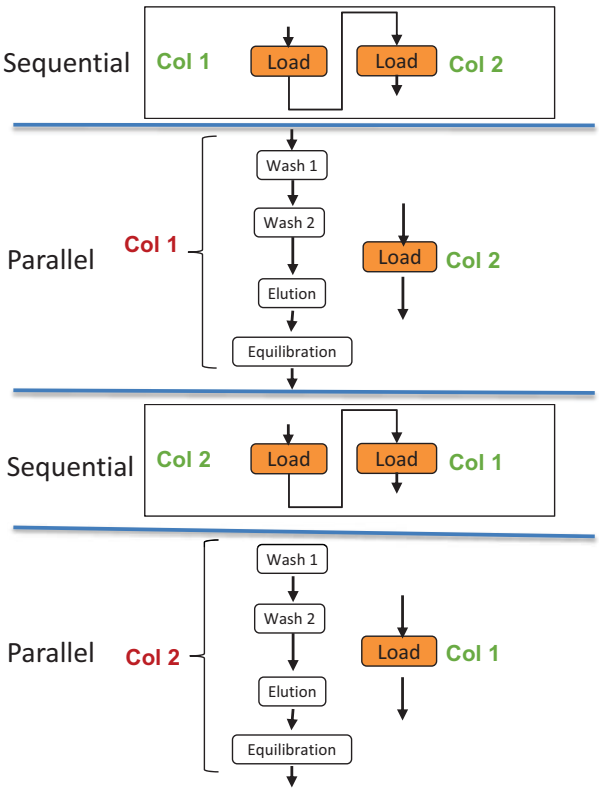
$$t = twash + telution + tregen + tequilib$$

- Combining recovery and regeneration steps
- Determine flow rate of steps (pressure and residence time limitations).
- Keep steep breakthrough curves to avoid losses during loading.

3- Column Parallel Process



CaptureSMB

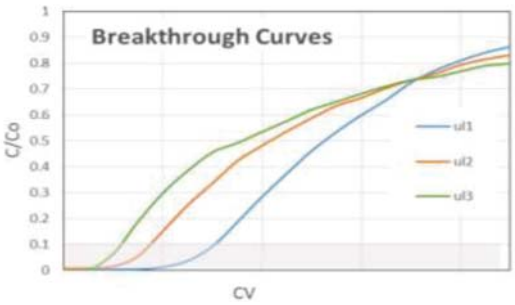


Process Design Example – MCC for Capture

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

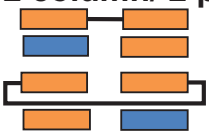


column length [cm]	10
column ID [cm]	2.5
Cross section [cm2]	4.91
column volume [mL]	49.09
linear velocity [cm/h]	200
vol flow rate [mL/min]	16.36
BT 0% in CV	15
CV elution and regen	25
cycle time [min]	120

Feed concentration [g/L]	2.5
load per cycle [g]	1.84
load per column [kg/Lres]	0.0375
load per h [g/h]	0.92
productivity [kg/Lres/d]	0.45
buffer consumption [L/gprod]	0.667

Process Design Example – MCC for Capture

2 column/ 2 pumps

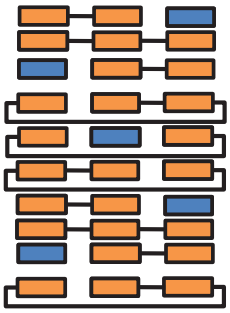


step		time	CV step	Load CV at end on col #1	Load CV at end on col #2
1	load	15	5	5.00	0.00
	elute	45	15	5.00	15.00
2	load	15	5	0.25	19.75
	elute	45	15	15.25	19.75
3	load	15	5	19.99	0.26
	elute	45	15	19.99	15.26
4	load	15	5	0.26	20.00
	elute	45	15	15.26	20.00
5	load	15	5	20.00	0.26
	elute	45	15	20.00	15.26
6	load	15	5	0.26	20.00
	elute	45	15	15.26	20.00

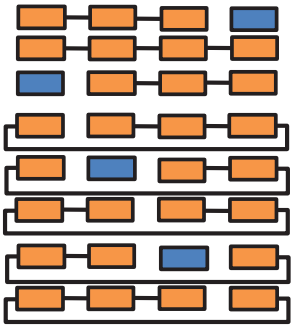
column length [cm]	10	
column ID [cm]	2.5	
Cross section [cm2]	4.91	
column volume [mL]	49.09	
linear velocity [cm/h]	200	batch
linear velocity [cm/h]	200	connected
BT 0% in CV	15	for 200 cm/h
loading single	15	
CV elution and regen	25	
loading connected	5	
vol flow rate [mL/min]	27.27	elution/reg
vol flow rate [mL/min]	16.36	connected
linear velcosity [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



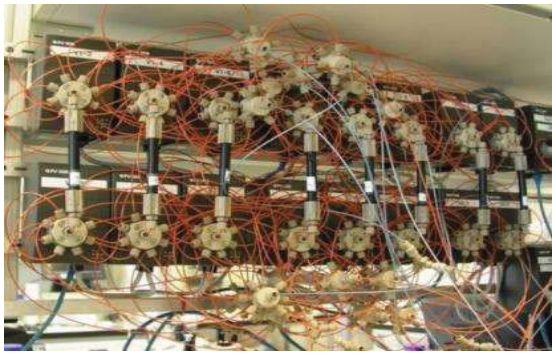
4 column / 2 pumps



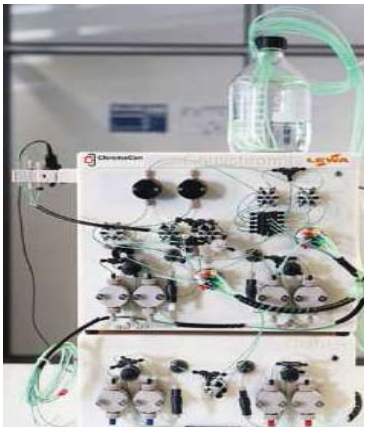
# of col	L sin CV	L con CV	uL sing [cm/h]	uL con [cm/h]	Cycle [min]	load per cycle [g]	L per col [kg/Lres]	L per h [g/h]	prod [kg/Lres/d]	buffer [L/gprod]
1	15	0	200	0	120	1.84	0.038	0.92	0.45	0.67
2	15	5	200	200	60	2.45	0.050	2.45	0.60	0.50
3	15	20	200	200	105	4.30	0.029	2.45	0.40	0.29
3	25	0	200	0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

Advantage of 2-column processes

8 column multicolumn process



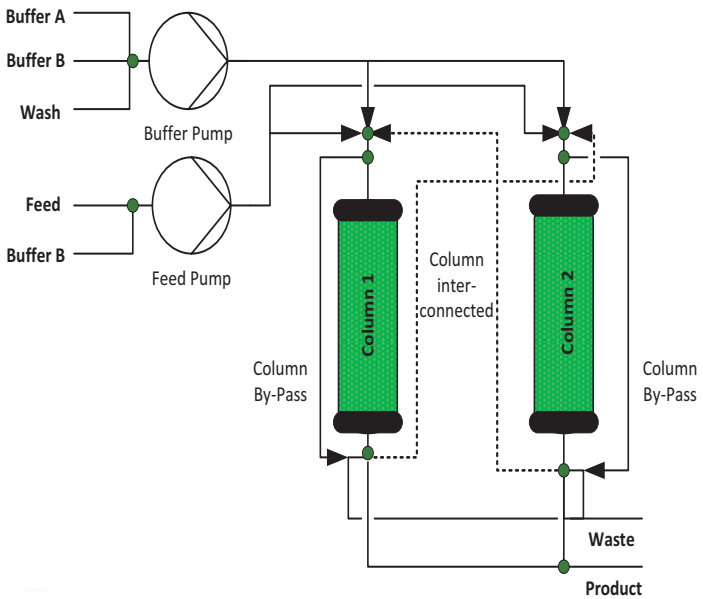
2 column CUBE + from ChromaCon



- 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

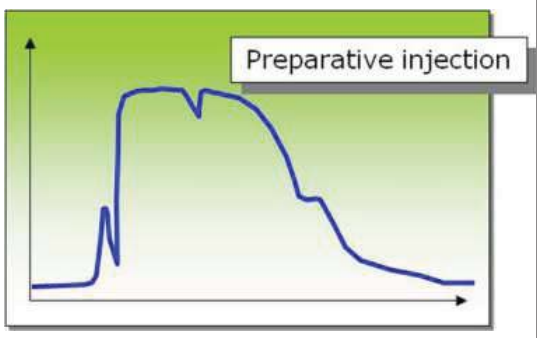
Description	Probablitiy	Severity	Impact (GMP, GAMP5 …)	Detectable	Comments Complexity, Novelty … Detectable	Risk Control Measures
General Risks CaptureSMB						
Process: batch vs continuous	medium	medium	medium	yes	same process steps only feed continuously using multiple column, possible longterm operation (24 h to 6 weeks), preception that different process due to advertisment	monitoring using PAT,process and cleanability verification on benchtop scale, adjusted automation
Skid: batch vs continuous	medium	medium	medium	yes	very similar design that is capable to run two column parallel or sequential	Verification of design (see below) no dead legs or back mixing
mechanical and chemical stability of bio-molecules	medium	medium	high	yes	novel continuous process, longterm stability data needed under this operating conditions	Longterm feasibility studies, control strategies, PAT implementation, equipment cleanablity studies
mechanical and chemical stability of resin	low	medium	medium	yes	novel continuous process, longterm stability data needed under this operating conditions	Longterm feasibility studies, control strategies, PAT implementation, equipment cleanablity studies
…						
Skid Design						
compexity	high	medium	medium	yes	more complex design with additional parts, need for more complex automation and control stategie	rigorous design to avoid any dead volumes, monitoring CPP, implementing cleaning procedure,
…						
Valves						
multiple port valve	medium	high	high	yes	When one fails more potential negative	double valves, feedback from valves
single on-off valves	high	medium	high	yes	Large number of valves but the effect of one failing is not as tramatically	double valves on important points, valve feedback
…						
Columns						
two	medium	medium	medium	yes	two column but smaller design, more robust and efficient	testing of columns, pressure monitoring, cleaning of skid and
…						

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

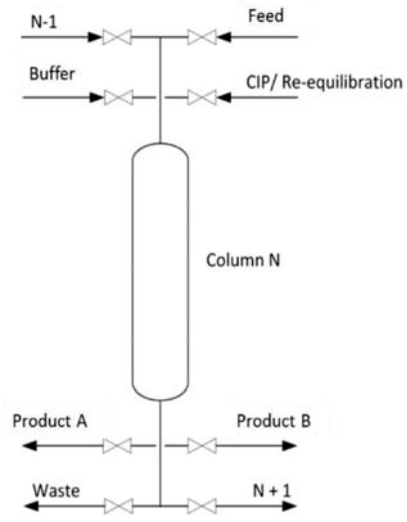
High initial capital investment for skid and multiple pumps and columns

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

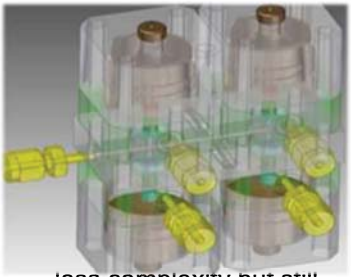
Valve

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

CSEP design from Calgon Carbon

high risk of internal leakage but internal dead volumes

Block valve based on BTS technology



less complexity but still small dead volumes

Commercial block arrangement

reduction of dead volumes

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

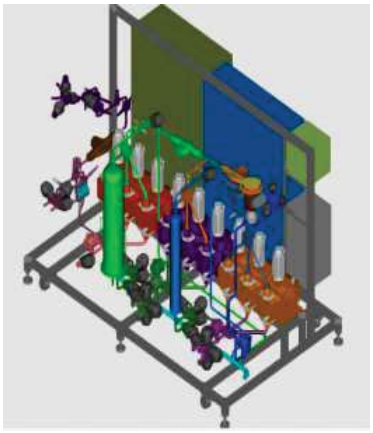
Challenge: Equipment for Integrated Continuous DSP

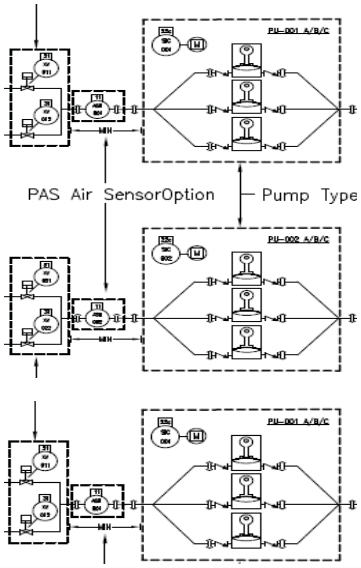
Buffer In-Line Dilution System - LEWA Intellidrive Approach

Stand-alone or as part of chromatographic skid

⇨ 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

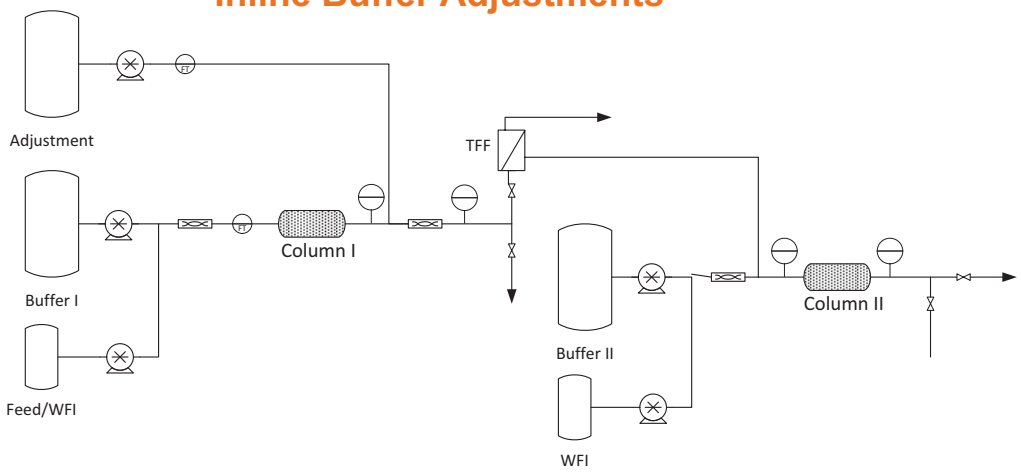
very accurate and reproducible

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 bio-molecules (existing processes vs process design for new molecules)

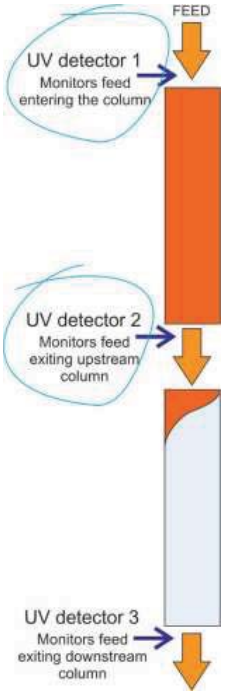
Control Strategy example: analytical tools monitor during processing

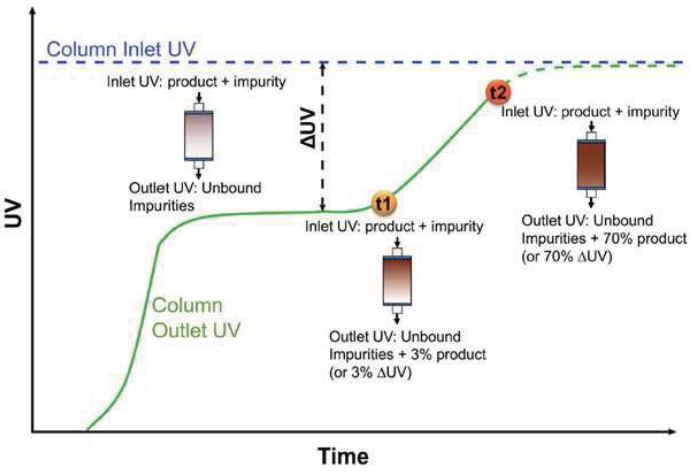
Protein determination:	Bradford protein assay, UV-spec at 280 nm (including HCP)
Identity:	Peptide mapping, HPLC C18, SEC, SDS-Page with Western Blot
DNA determination:	UV spec at 260 nm
Yield:	ELISA, HPLC and SEC
Purity:	HPLC C18, SDS-Page
Aggregate and Fragment:	SEC

Challenges of Integrated Continuous DSP

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



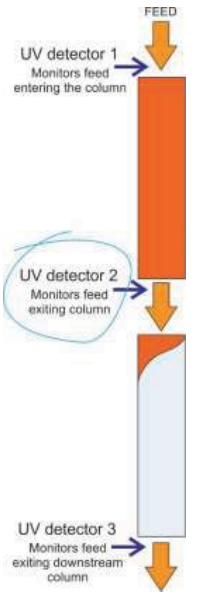


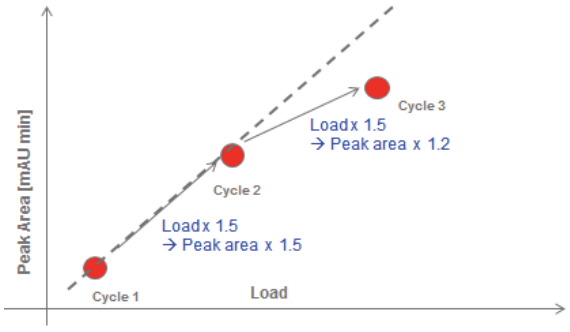
**Source, Figure: Warikoo, V .et al. (2012), Integrated continuous production of recombinant therapeutic proteins. Biotechnol. Bioeng., 109: 3018–3029. doi: 10.1002/bit.24584*

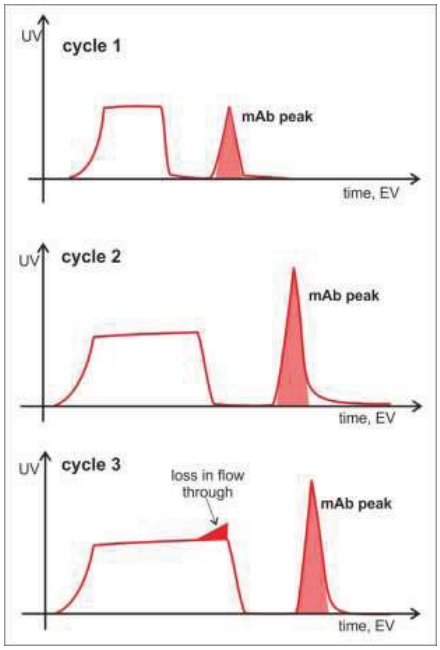
Challenges of Integrated Continuous DSP

Control: Elution Peak Area*

Only one UV detector (eliminating calibration accuracy issues)







linear correlation load and peak area:
- while load < dynamic capacity,
- 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

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

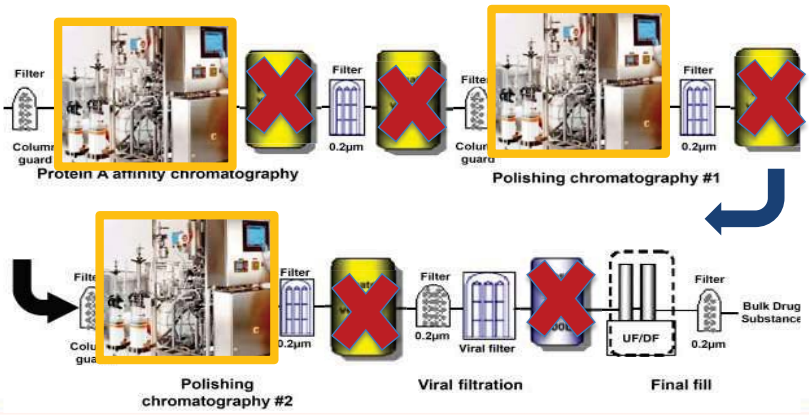
Conclusions

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 .

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