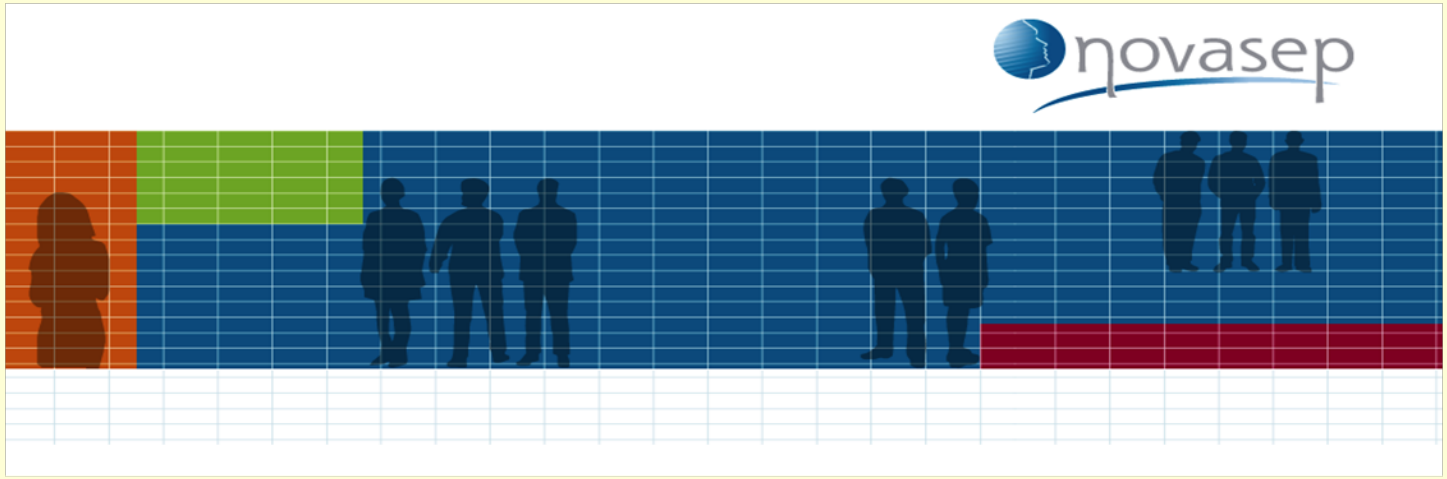
ISPE-CASA PRESENTS "CLEAN & GREEN"

CLEAN AND GREEN TECHNOLOGIES FOR PURE WATER AND PROCESS TECHNOLGIES



Sequential Multi-Column Chromatography

ISPE, April 13, 2009

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Sales Manager
Novasep, Inc.

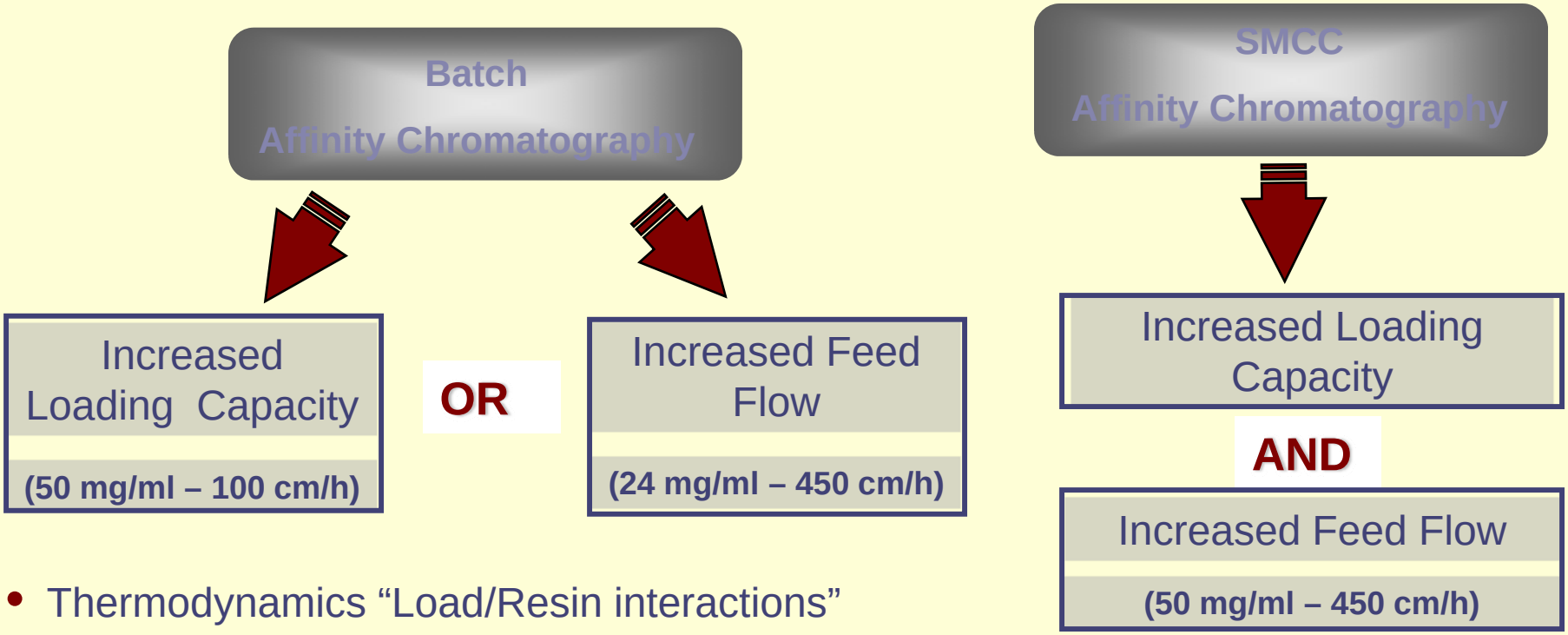
Presentation Outline

- Purpose and Applications of SMCC
- Functional Principles
- Case Study including Economic Evaluation
- Equipment Product Specification
- Practical & Regulatory Considerations
- Conclusion

Sequential Multi-Column Chromatography : Purpose & Application

- "Debottlenecking" Downstream Purification Processes
  - **Improving Efficiency of all Chromatography Applications**
    - Capture or Flow Through
    - Affinity, IEX, HIC; practically all types of stationary phases
    - Applicable to Low, Medium and High Pressure Chromatography
  - **Applicable to all Types of Biomolecules**
    - MAb's
    - rTherapeutics
    - Peptides
    - Blood Fractions
    - Vaccines

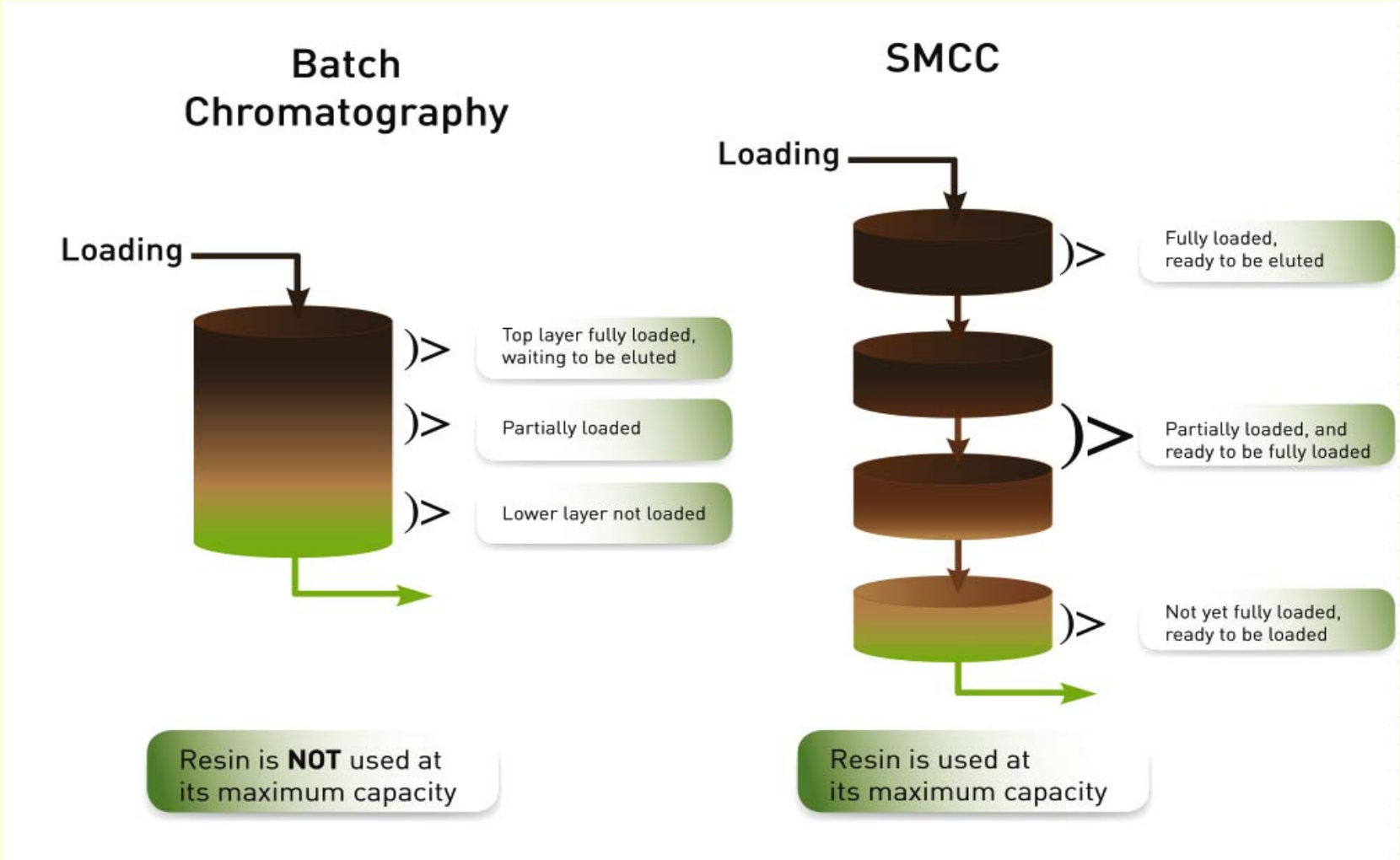
Current Chromatography Productivity Paradigm
Affinity Capture Example



- Thermodynamics “Load/Resin interactions” Vs Kinetics “Flow Rate”
- Minimum process flow rate requirements compromise maximization of productivity

- Significantly higher loading flow rates
- Loading on multiple smaller columns
- Minimizes impact of kinetics ⇒ Increased dynamic resin capacity (g/L)

Sequential Multi-Column Chromatography Functional Principles



Sequential Multi-Column Chromatography
Batch / SMCC Comparison

Batch Chromatography

- **Wasted Resin Capacity**
  - Resin capacity not used 100%
- **Wasted Water**
  - BV's for wash, elute, regeneration, etc based on actual bed volume, not working bed volume
- **Wasted Time**
  - Top layer is waiting to be eluted
  - Lower layer is waiting to be loaded

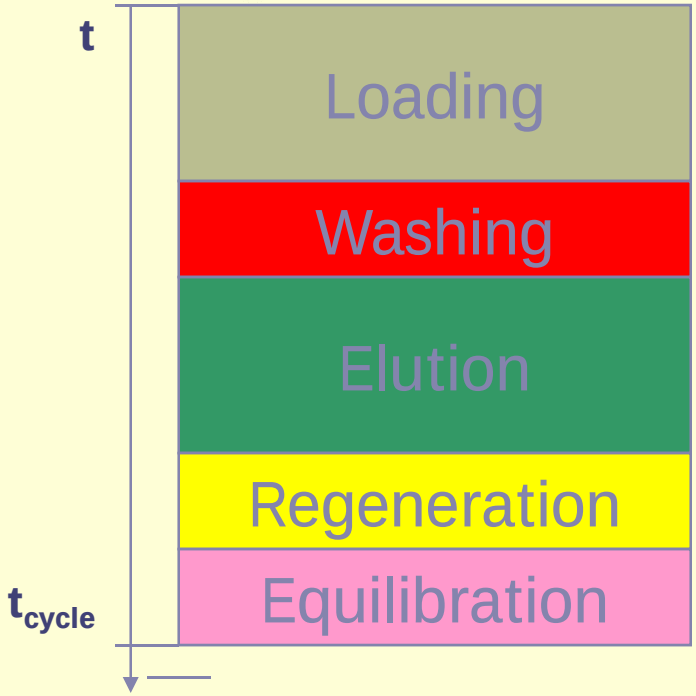
SMCC

- **Less Resin**
  - 3-6 Smaller Ø columns
  - Typically >40% resin volume reduction
  - Smaller space requirements
- **Less Water**
  - Smaller bed volumes
  - Same BV elute ratio
  - Less buffers
- **Faster Processing Time**
  - Higher productivity

Sequential Multi-Column Chromatography

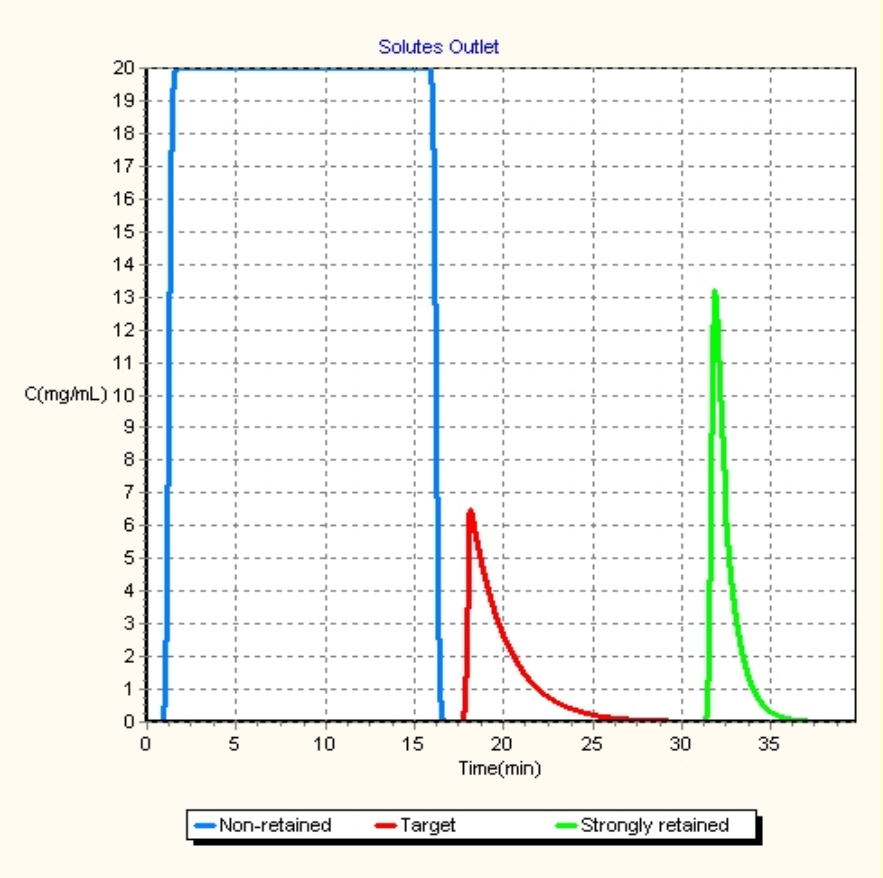
Cycle on 1 column

Chronogram



Batch Process

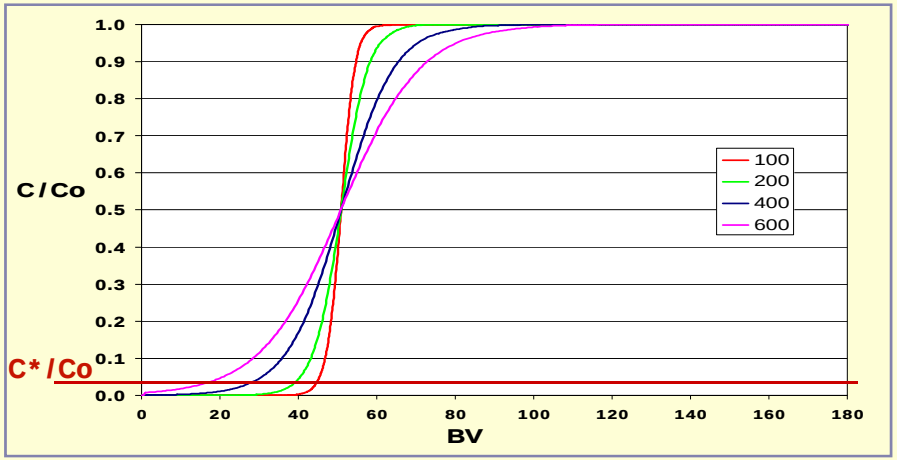
Chromatogram



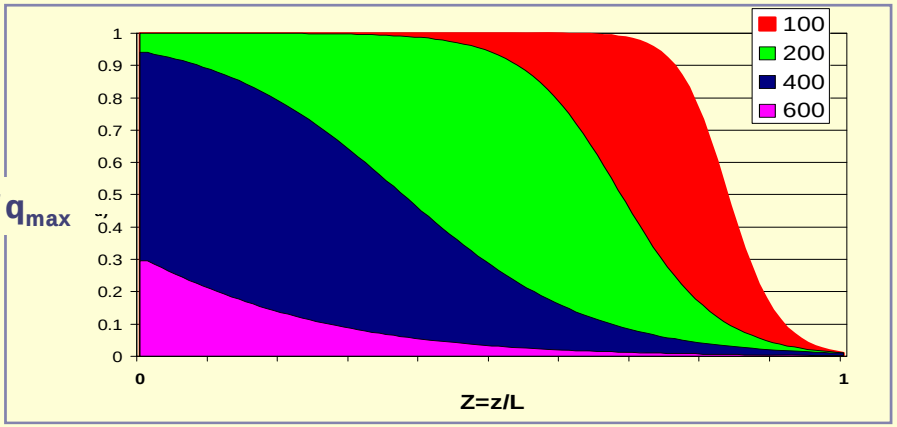
Sequential Multi-Column Chromatography

Batch Characterization 100 to 600cm/hr

Breakthrough Curves



Loading Capacity

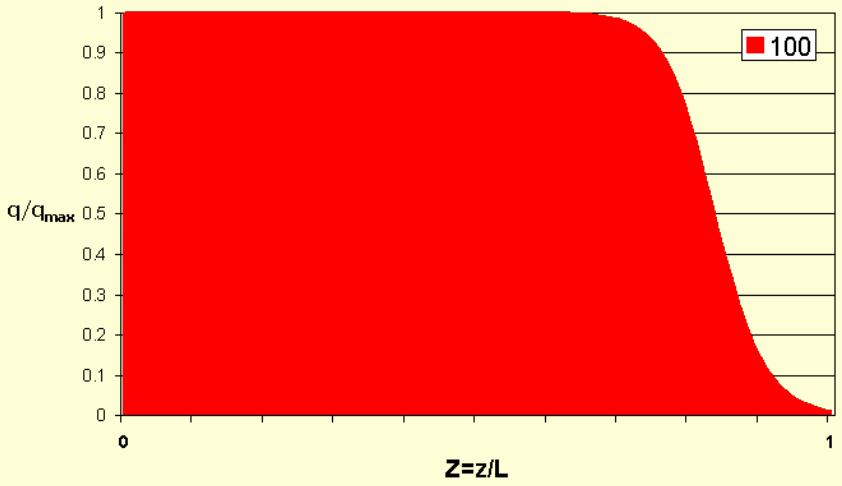


Sequential Multi-Column Chromatography

Loading Capacity

Multicolumn Process

Single Batch Column @ 100cm/hr

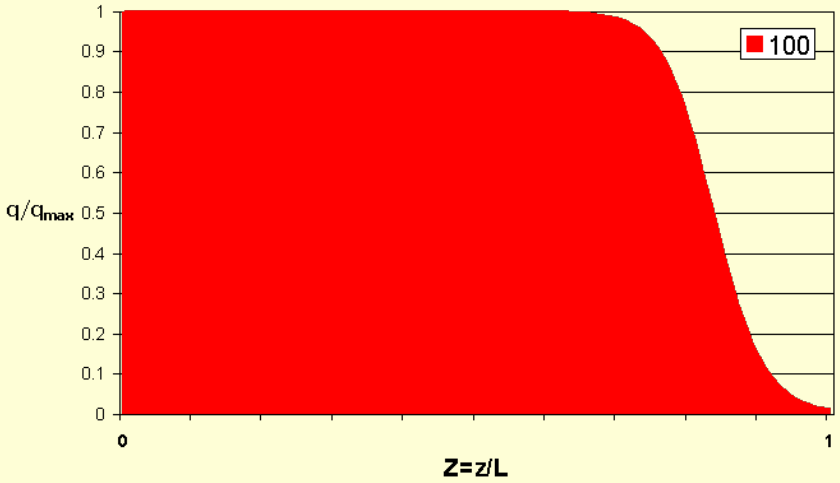


Sequential Multi-Column Chromatography

Loading Capacity

Multicolumn Process

Single Batch Column @ 100cm/hr

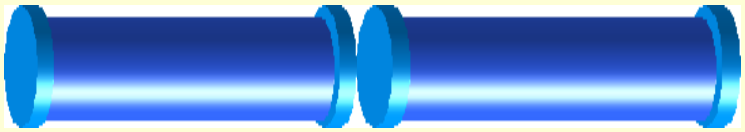


Sequential Multi-Column Chromatography

Loading Capacity

Multicolumn Process

Single Batch Column @ 100cm/hr



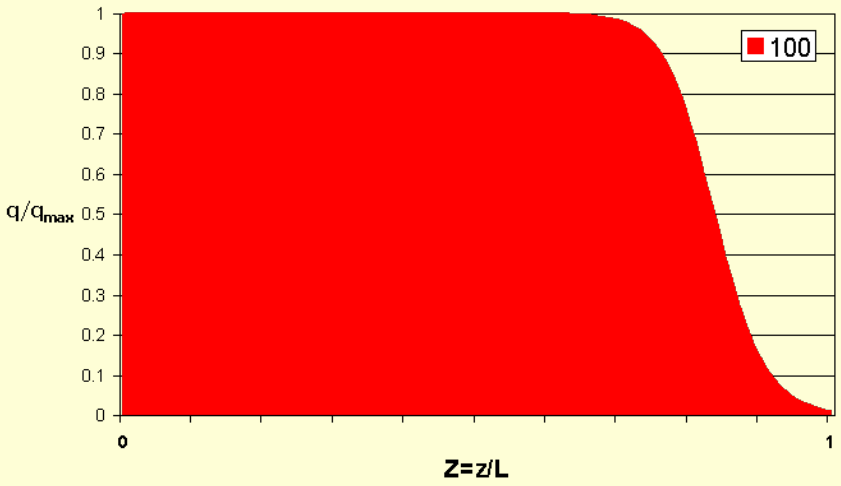
BioSC –2 Column SMCC Process @ 100cm/hr

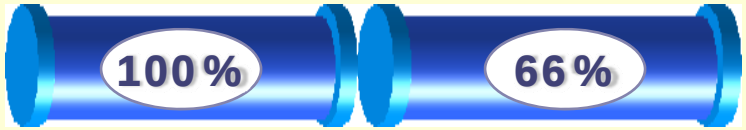
Sequential Multi-Column Chromatography

Loading Capacity

Multicolumn Process

Single Batch Column @ 100cm/hr



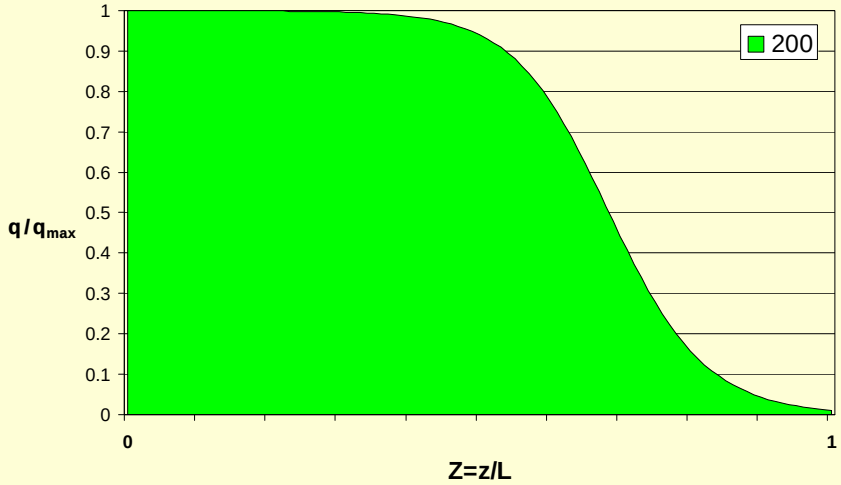


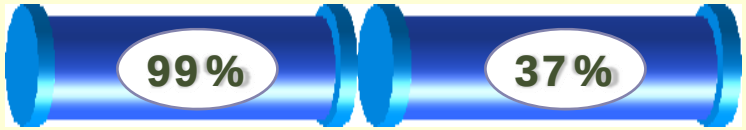
BioSC –2 Column SMCC Process @ 100cm/hr

Sequential Multi-Column Chromatography

Column Loading Capacity Profiles

Single Batch Column @ 200cm/hr



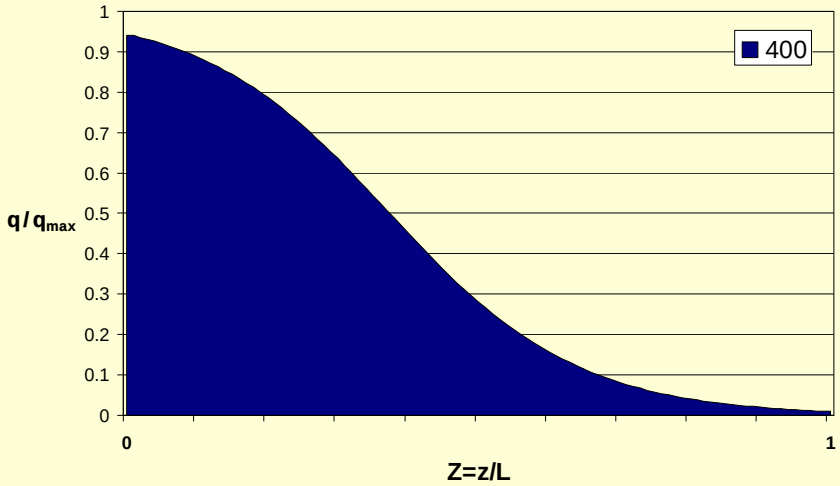


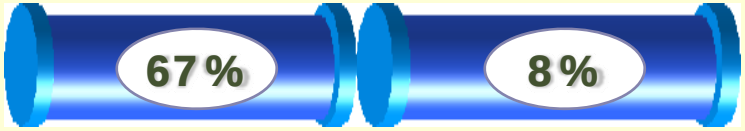
BioSC –2 Column SMCC Process @ 200cm/hr

Sequential Multi-Column Chromatography

Column Loading Capacity Profiles

Single Batch Column @ 400cm/hr



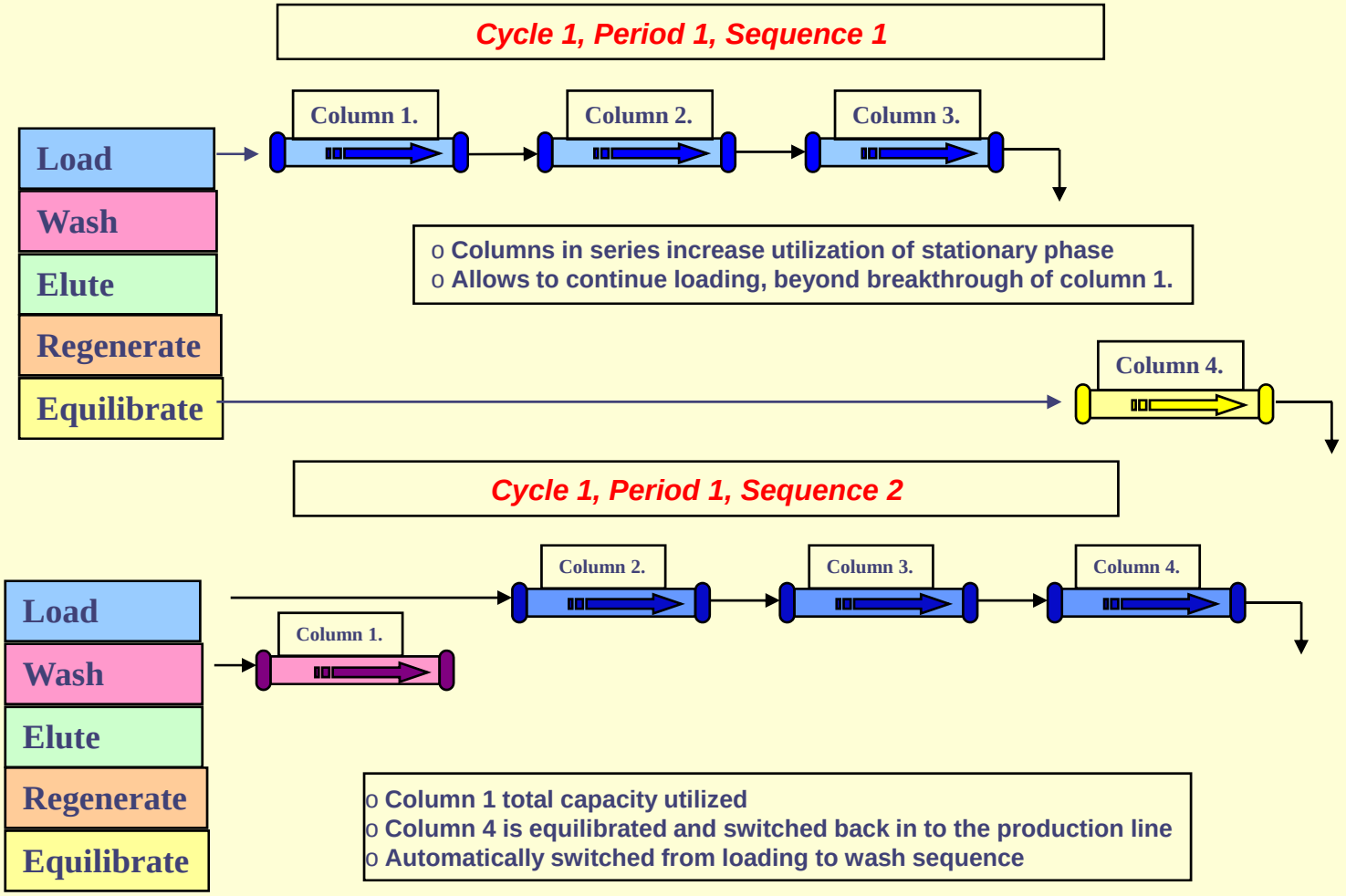


BioSC –2 Column SMCC Process @ 400cm/hr

BioSC –3 Column SMCC Process @ 400cm/hr

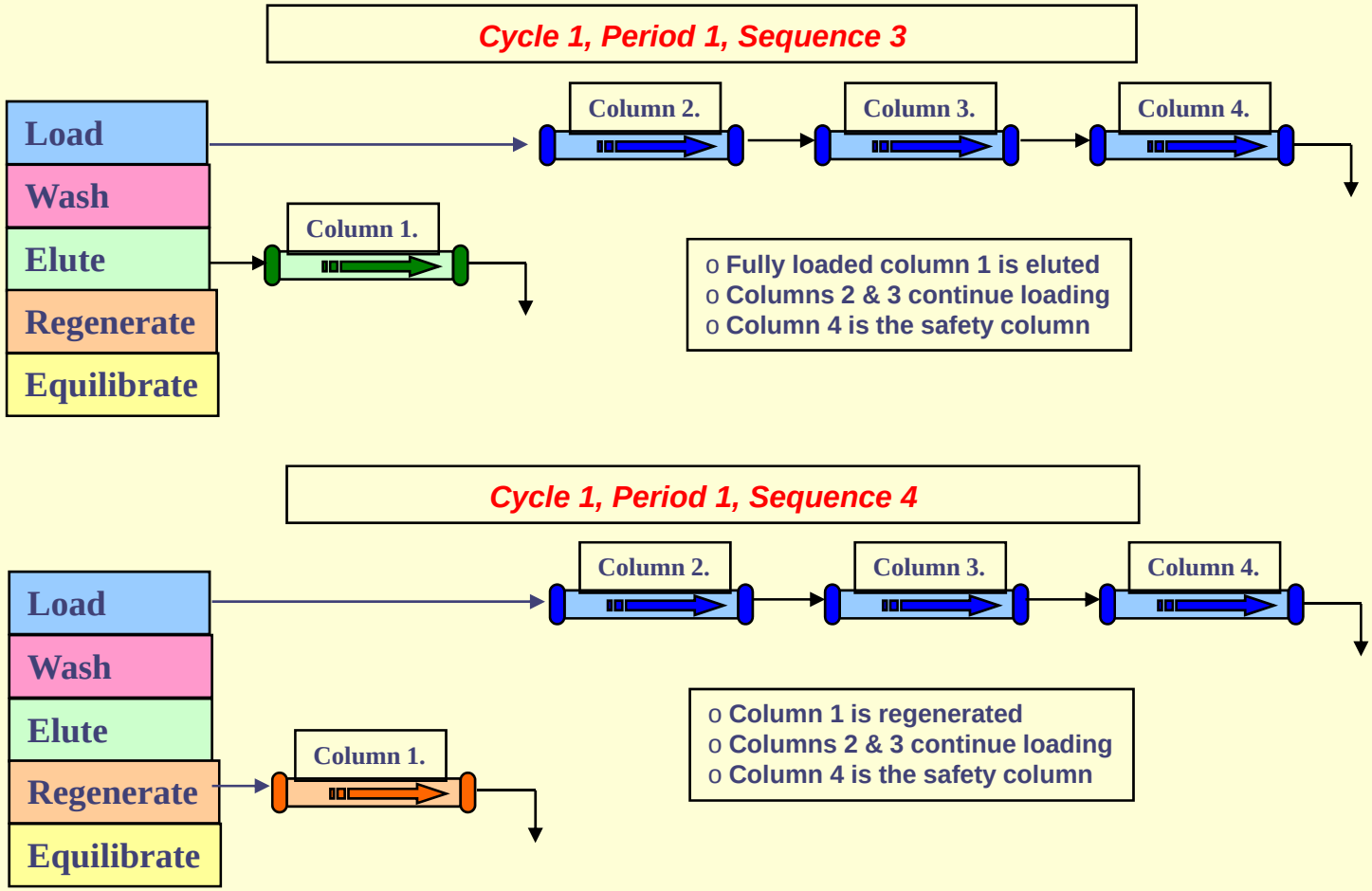
Sequential Multi-Column Chromatography

BioSC 4 Column SMCC Flow Schematic - *1/4*



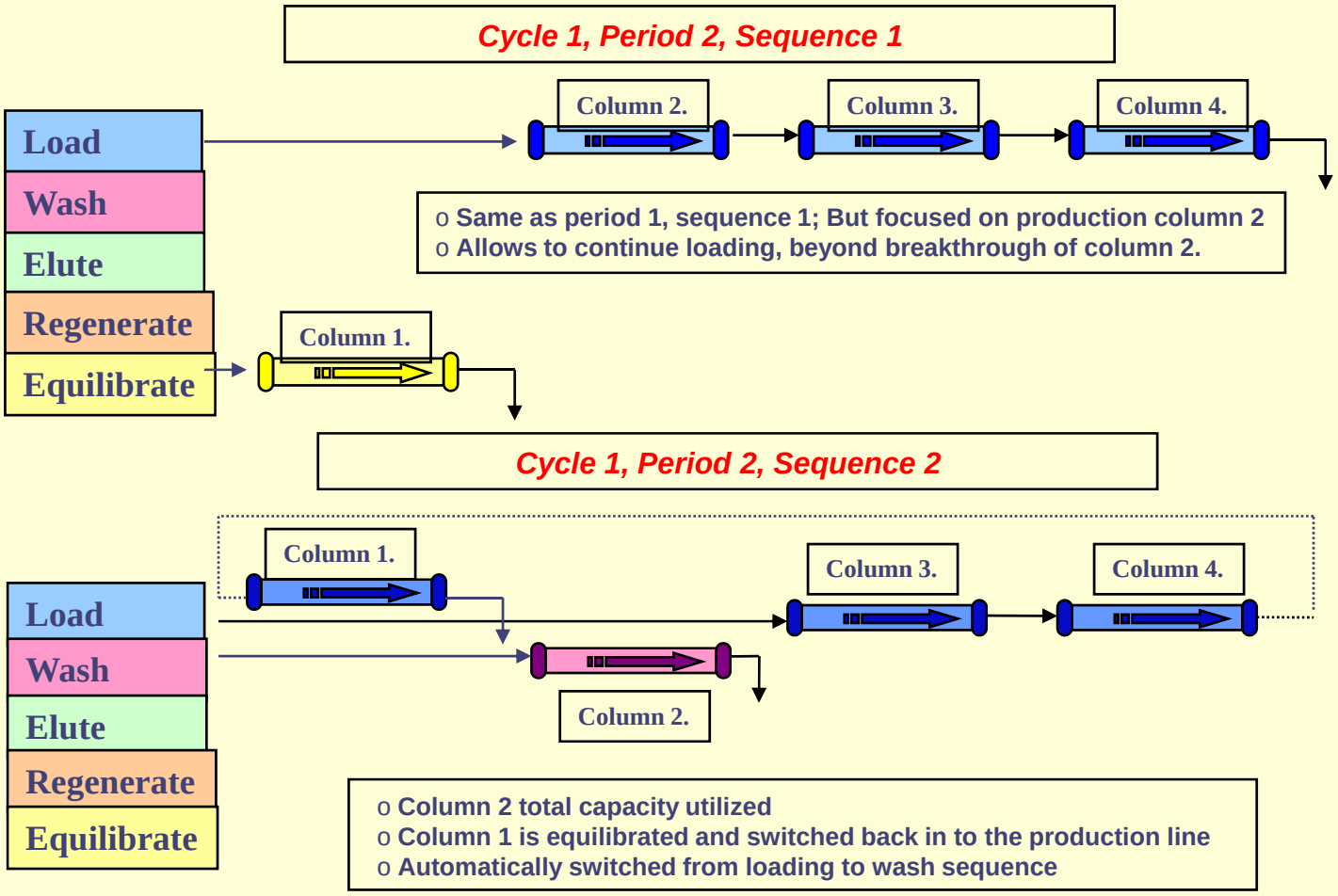
Sequential Multi-Column Chromatography

BioSC 4 Column SMCC Flow Schematic - 2/4



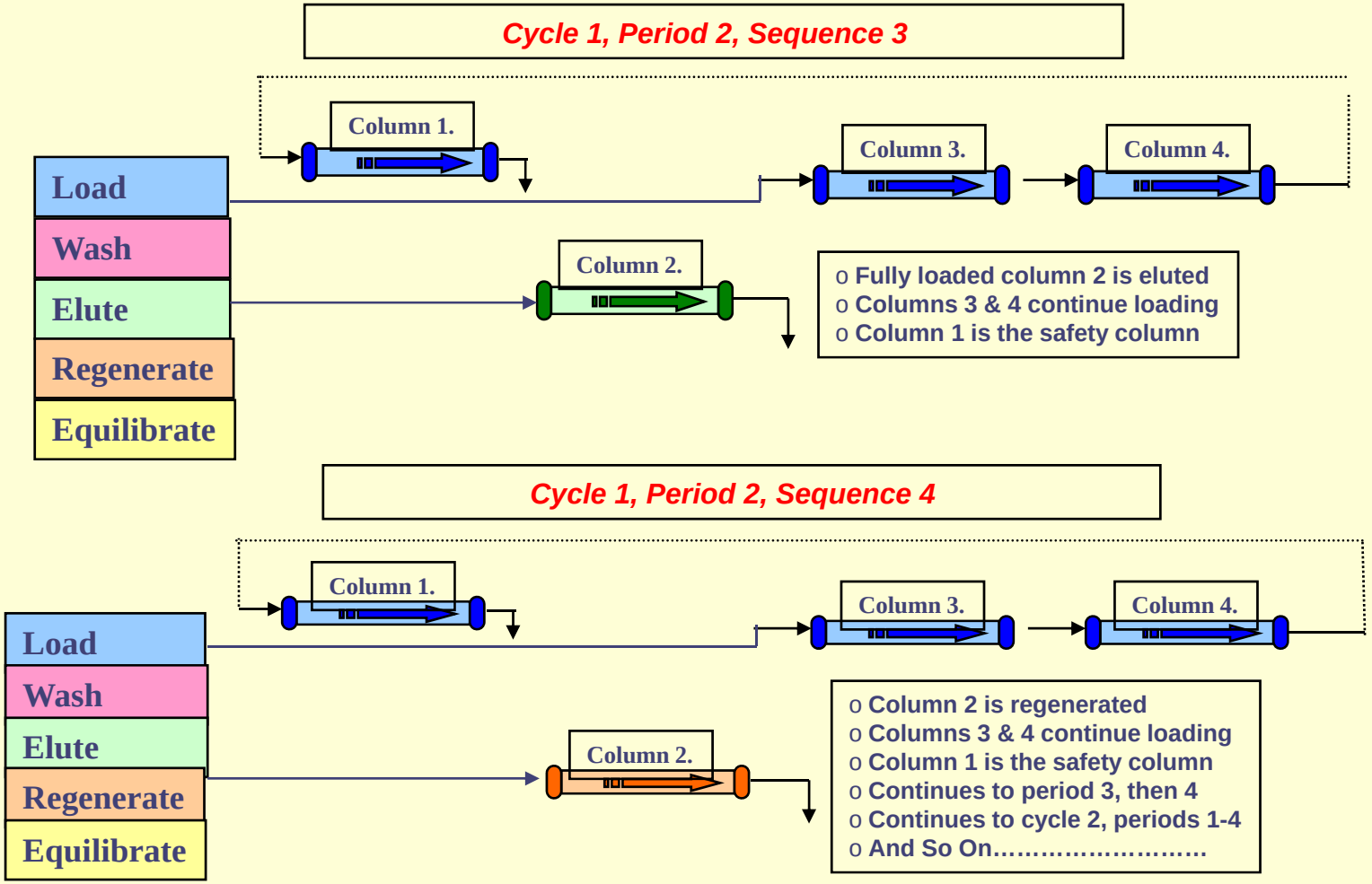
Sequential Multi-Column Chromatography

BioSC 4 Column SMCC Flow Schematic - 3/4



Sequential Multi-Column Chromatography

BioSC 4 Column SMCC Flow Schematic - 4/4



Sequential Multi-Column Chromatography
Lab Scale Affinity Capture – Mab @ 1g/L

		Experimental Data		Simulation
		Single Column Batch	BioSC 3 Column	BioSC Simulation
# Columns		1	3	3
Resin Volume (ml)		60	60	60
Dynamic Capacity (g mAb/L resin)	300cm/hr	25 g/L		40 g/L
Buffer Consumption (ml/g mAb)		1200 ml/g		675 ml/g
Productivity (g mAb/L resin/day)		200 g/l/day		215 g/l/day
Dynamic Capacity (g mAb/L resin)	450cm/hr	20 g/L	40 g/L	40 g/L
Buffer Consumption (ml/g mAb)		1500 ml/g	830 ml/g	675 ml/g
Productivity (g mAb/L resin/day)		220 g/l/day	310 g/l/day	320 g/l/day
Dynamic Capacity (g mAb/L resin)	600cm/hr	15 g/L	40 g/L	40 g/L
Buffer Consumption (ml/g mAb)		2000 ml/g	675 ml/g	685 ml/g
Productivity (g mAb/L resin/day)		240 g/l/day	430 g/l/day	445 g/l/day

- *44% or 1.8 fold increase in productivity*
- *2.9 Fold decrease in water usage*
- *Purity 99% in each case*
- *Yield 98% in each case*

- *Excellent agreement between simulation modeling & empirical data*
- *Implications for development & optimization of continuous processes*

Commercial Protein A Capture Process – Simulation @ Const. Resin Volume

		Batch mAb capture	BioSC SMCC Modelling Data		BioSC SMCC Modelling Data
		Single Column Batch Process	3 Columns	4 Columns	4 Columns
	Number of columns	**1**	**3**	**4**	**4**
	Bed Height (cm)	**20**	**20**	**20**	**14**
300 cm/h	Dynamic capacity (g mAb/L resin)	16.8 g/L			
300 cm/h	Buffer Consumption (mL/g mAb)	1500 mL/g			
300 cm/h	Productivity (g mAb/L resin/day)	386 g/L/day			
750 cm/h	Q'max Capacity (g mAb/L resin)		40.8 g/L		
750 cm/h	Buffer Consumption (mL/g mAb)		630 mL/g		
750 cm/h	Productivity (g mAb/L resin/day)		786 g/L/day		
1200 cm/h	Q'max Capacity (g mAb/L resin)			40.8 g/L	
1200 cm/h	Buffer Consumption (mL/g mAb)			630 mL/g	
1200 cm/h	Productivity (g mAb/L resin/day)			939 g/L/day	
950 cm/h	Q'max Capacity (g mAb/L resin)				**40.8 g/L**
950 cm/h	Buffer Consumption (mL/g mAb)				**630 mL/g**
950 cm/h	Productivity (g mAb/L resin/day)				**969 g/L/day**

- ***Best Scenario:***
  - ***4 Columns with resin volume as batch process***
  - ***Smaller diameter columns***
  - ***2.6 Fold increase in productivity***
  - ***2.4 Fold reduction in buffer usage***

Generic Commercial Protein A Capture Step

Best Batch Scenario Single Step Purify 10 kg-1 Cycle

L/Resin/day for kg at 386 g/L	26 L
L/Resin for 10 kg mAbs	**624 L**
Column Size for 20 cm BH	**2.0 meter**
Daily production	10 kg
Assuming 200 Regeneration Cycles	**3.4 kg per lifetime Liter**

SMCC Scenario Single Step Purification 10 kg-1 Cycle

L/Resin/day for kg at 969 g/L	10 L
L/Resin for 10 kg mAbs	**250 L**
Column Size (4 columns with 14 cm BH)	**75 cm**
Daily production	10 kg
Assuming 200 Regeneration Cycles	**8.0 kg per lifetime Liter**

Economic Implications of SMCC process

Batch - 1.7meter diameter with 20cm bed height, DBC 20mg/ml, 300 cm/hr

SMMC – (4) 80cm diameter with 10cm bed height, DBC 45 mg/ml, 600cm/hr

	Batch MAb Capture	SMCC MAb Capture	Potential Savings
Volume of Protein A Media	455L	200L	>$3,000K
Process Time	4 hours	2 hours	50% savings
Buffer Usage	18,000 L	5,800 L	66% savings

BioSC™ Hardware Features
BioSC™ Pilot System

Capacity & Equipment

- 6 x5 - 15 cm columns (Glass, Acrylic or Stainless Steel)
- In-Line Buffer Dilution
- Isocratic, Gradient or Step gradients
- Analog «mass flow meter» controlled
- Up to 4 fraction collections as standard
- Up to 8 inlet, load, buffer lines
- Fully Automated
- SIP option
- On Line/At Line Monitoring
- Footprint : 2 m x 2 m x 1 m

ASME/BPE Engineering Design

Materials:

- 316 L Stainless Steel
- Teflon, EPDM, Glass or Acrylic
- Sanitary Design for Assured CIP
- Diaphragm valve
- Rotating piston pumps
- >1% pipe slopes
- Fully drainable
- No dead legs
- Column bypass loops
- TC connections

Control Platform

- Chromsoft user friendly interface
- Intellutions iFix control systems
- 21 CFR Part 11 compliant
- GAMP 4 compliant

- ***Small Production Foot Print***
  - *– 2m x 2m x1m*
- ***LabScale System Available***
  - *– 0.5 – 2.6 cm Ø Columns*
- ***Process Scale***
  - *– Specification to Client URS*

Regulatory and Quality Considerations for SMCC

- **Regulatory and Quality Questions:**
  - Still Chromatography using the same buffers …

- **What is a batch?**
  - Typically defined by the process strategy, by the starting material (e.g. the fermentation batch) or by the formulation quantity and even sub lots.
  - The Overall DSP process remains batch even if 1 chromatography step is changed to sequential.

- **On Line Control:**
  - Critical quality attributes can be controlled On or At Line with BioSC™, compliant with PAT initiatives.

- **Quality Profile of a continuous or "steady state" process is much more homogenous than batch**
  - Stationary phase usage is more homogenous.
  - Less overloading, less fouling, less cleaning.
  - Improved performance of chromatography resin.
  - Scalable process

Regulatory and Quality Considerations for SMCC

- **Typical Strategy for SMCC Process Validation**
  - Characterization of the batch process (Breakthrough curves with the purified molecule & the load)
  - Process Simulation
  - Lab tests to verify the predicted process
  - Resin life study
  - Test of the robustness / definition of PAR of the process -> Design Space

- **Implementation at the industrial scale including IQ, OQ, PQ of equipment**

- **Process Validation**

***Note*: SMCC uses the same buffers, CIP solutions and stationary phases as batch processes**

Regulatory and Quality Considerations for SMCC

FDA Approved API Process

- Continuous, fully automated systems
- DAC columns
- 1 meter in diameter
- Approved by FDA
  - Varicol ®

SMCC Processing Using BioSC™ In Conclusion

- **Established scientific principles**
  - Applicable to most chromatography types
- **Utilizes same product contact materials**
  - Resins, Buffers, Eluents, etc.
- **Delivers significant economy of scale**
  - 2-4 Fold increase in productivity
  - 2-4 Fold reduction in water usage
    - Positively impacts facility CapEx, foot print, utilities, tanks, etc.
  - 2-4 Fold increase in process speed
- **Manageable implementation and learning curve**
  - NOVASEP Expertise
    - Running FDA regulated continuous processes
    - Simulation & modeling capability
    - NOVASEP Biopharma PPD™ expertise for contract process development

Acknowledgements

- **Margit Holzer, Laurent David, Jean Guillerm & H. Osuna-Sanchez**

  Novasep Process

Thank you for your attention!

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