

The Influence of Scale of Operation on Purification Process Design

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Introduction

- Product Requirement
- Modelling
- Equipment Considerations
- Yield
- Manual Handling
- Buffers
- Matrix Re-Use
- Process Time
- Conclusions

Product Requirement

- Three things determine the amount of product required
 - Size of Dose
 - Number of Doses per Patient
 - Number of Patients
- From a Big Pharma's Perspective the best product
 - Small Dose
 - Many Doses
 - Many Patients
- A Biochemical engineer would add...
 - High Expression
 - Ease of Purification
 - Stability

A Tale of Two Products

	Plasmid	MAb
Dose (mg)	1	1,000
Expression (g/L)	0.10	2.50
Doses pa	1,000,000	1,000,000
Mass Required (g)	1,000	1,000,000
Process Yield (%)	60	60
Litres Required (L)	16,667	666,667
Batches pa	30	30
Buffer Requirement	3,000	200,000
Fermenter Size (L)	556	22,222

The Importance of Models

- A complex enough model will allow you to:
 - Determine the effect of process changes on:
 - ◆ Unit op times, cycles etc.
 - ◆ Buffer requirements
 - ◆ Raw materials; chemicals, filters, etc.
 - ◆ Costs
 - ◆ Campaign schedule

- Feasibility can be assessed immediately

Equipment Considerations

- Physical limitations can apply:
 - Column diameters
 - Pump sizes
 - Head pressures
- This leads to:
 - Increased no. of chromatography cycles
 - Large product volumes
 - Slower filtration processes
- Which means:
 - Long processing times
 - Large buffer requirement
 - Process bottlenecks

Process – Laboratory Scale



- CV ~ 10's mL
- Area ~ 0.01 m²
- Buffer ~ 10's L

Process – Small Scale



- CV ~ 1's L
- Area ~ 0.1 m²
- Buffer ~ 100's L

Process – Pilot Scale



- CV ~ 10 's L
- Area $\sim 1 \text{ m}^2$
- Buffer $\sim 10,000$'s L

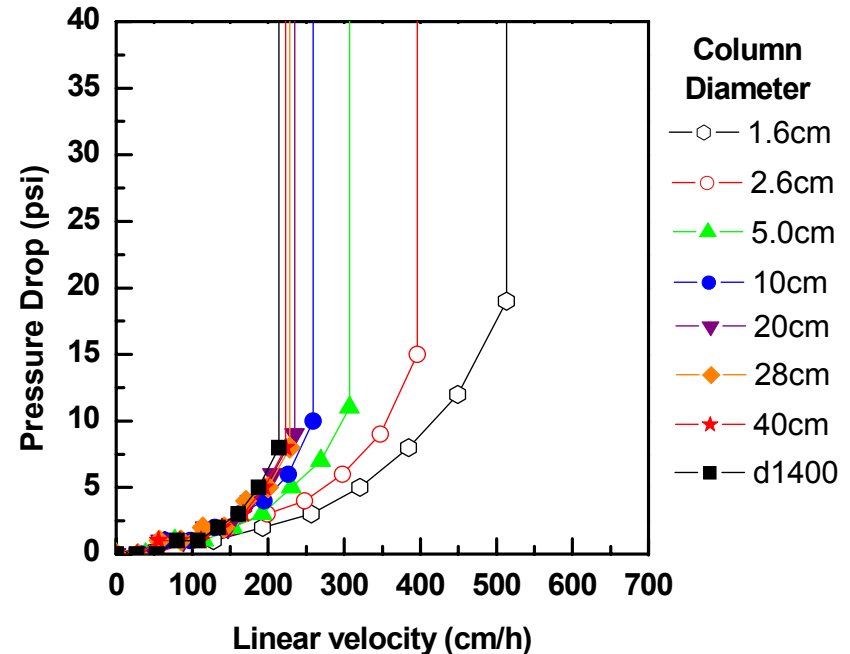
Process – Manufacturing Scale



- CV ~ 100's L
- Area ~ 10's m²
- Buffer ~ 100,000's L

Equipment Limitations Example

- Chromatography scale-up usually achieved by increasing diameter only
- Bed height & linear velocity kept constant
- This reduces supportive wall effects
- Results in increased pressure drop at same flow velocity (compressible matrices)
- Imposes severe limits to usable bed heights and flow rates



Pressure Drop versus Flowrate

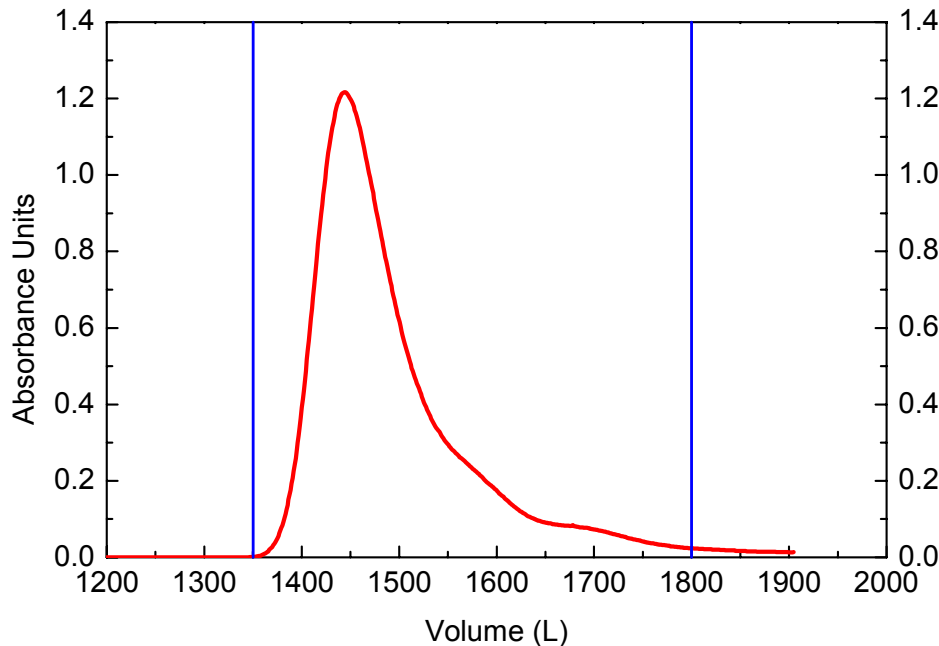
Packed bed Height = 15cm
 Matrix = Sepharose 4 Fast Flow
 Buffer = PBS @ 22°C

Stickel JJ, Fotopoulos A.

Process Solutions to Equipment Problems

- Maintain focus of development
 - Model manufacturing scale operation
 - Product progression and timelines
- Drive processes harder
 - Dynamic binding capacities
 - Increased concentration during UF
- Consider campaign yield
 - Unit op yield is important
 - Extra batches may give more product p.a.

Campaign Yield vs. Unit Op Yield



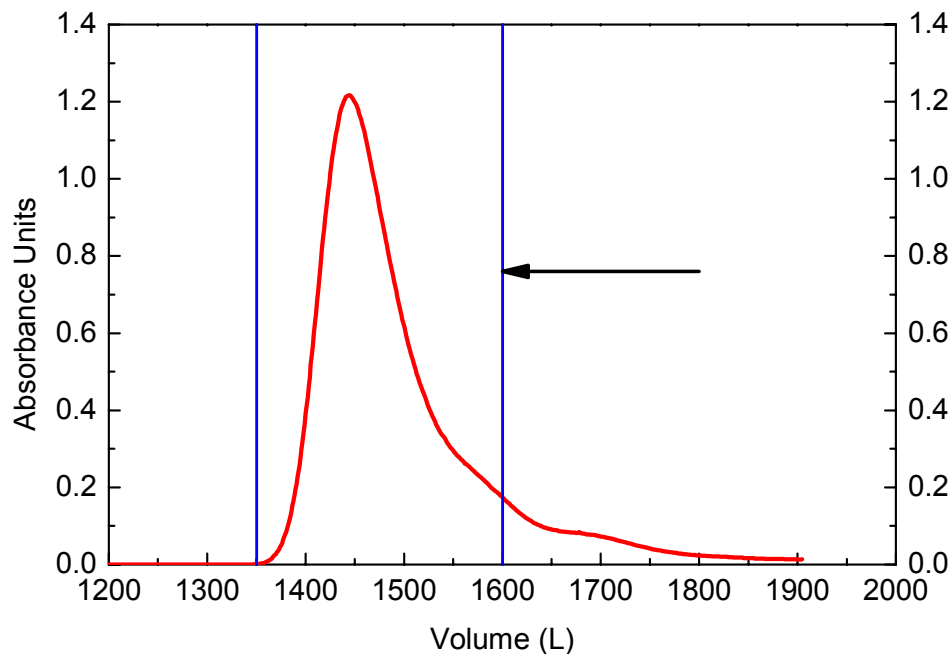
■ Process:

- 20,000 L
- 2.5 g.L⁻¹
- 5 cycles

■ Peak collection

- 1,350 L to 1,800 L
- Elution Vol = 450 L
- Total Vol = 2,250 L
- Yield = 96 %

Campaign Yield vs. Unit Op Yield



- New peak collection
 - 1,350 to 1,600
 - Elution Vol = 250 L
 - Total Vol = 1,250 L
 - Yield = 92 %
- New process saves 2 days
 - C/Tog shorter
 - UF shorter
 - No tank back-filling
- Allows 20 batches pa instead of 18
- Product pa after this step increased by 56 kg (7 %)

Materials Handling

- Trivial concentrations in the lab can be crippling at scale
 - 2 m column, $H_o = 25$ cm, $CV = 820$ L
 - 2 CV elution, 5 cycles, buffer req = 8,200 L
 - Elution buffer = 1 M $(NH_4)_2SO_4$
- This would require:
 - Over one metric tonne of salt
 - ◆ Storage
 - ◆ Transfer
 - Disposal
 - ◆ High BOD
 - ◆ Typical US plant limit 250 L per day
 - ◆ ∴ Tankers required for removal

Materials Handling cont^d

- Any reduction in buffer conc during development is beneficial
- In the lab it is tempting to:
 - Use round numbers
 - Play it 'too' safe
- Reducing $(\text{NH}_4)_2\text{SO}_4$ concentration to 0.9 M saves:
 - >800 L buffer
 - >100 kg salt
- For a 40 batch campaign
 - >30 m³ buffer
 - >4 tonnes salt
- This is just one buffer in one unit operation

Buffer

- There are 3 phases to any buffer prep:
 - Make-up
 - Sterilisation
 - Storage
- Make-up
 - Adding of raw materials
 - Mixing to homogeneity
 - Final adjustments to achieve specification
- Sterilisation
 - Filtration, HTFF, UV, SIP
- Storage
 - Store in make-up vessel? – sterilisation method
 - Maximum hold time

Buffer problems

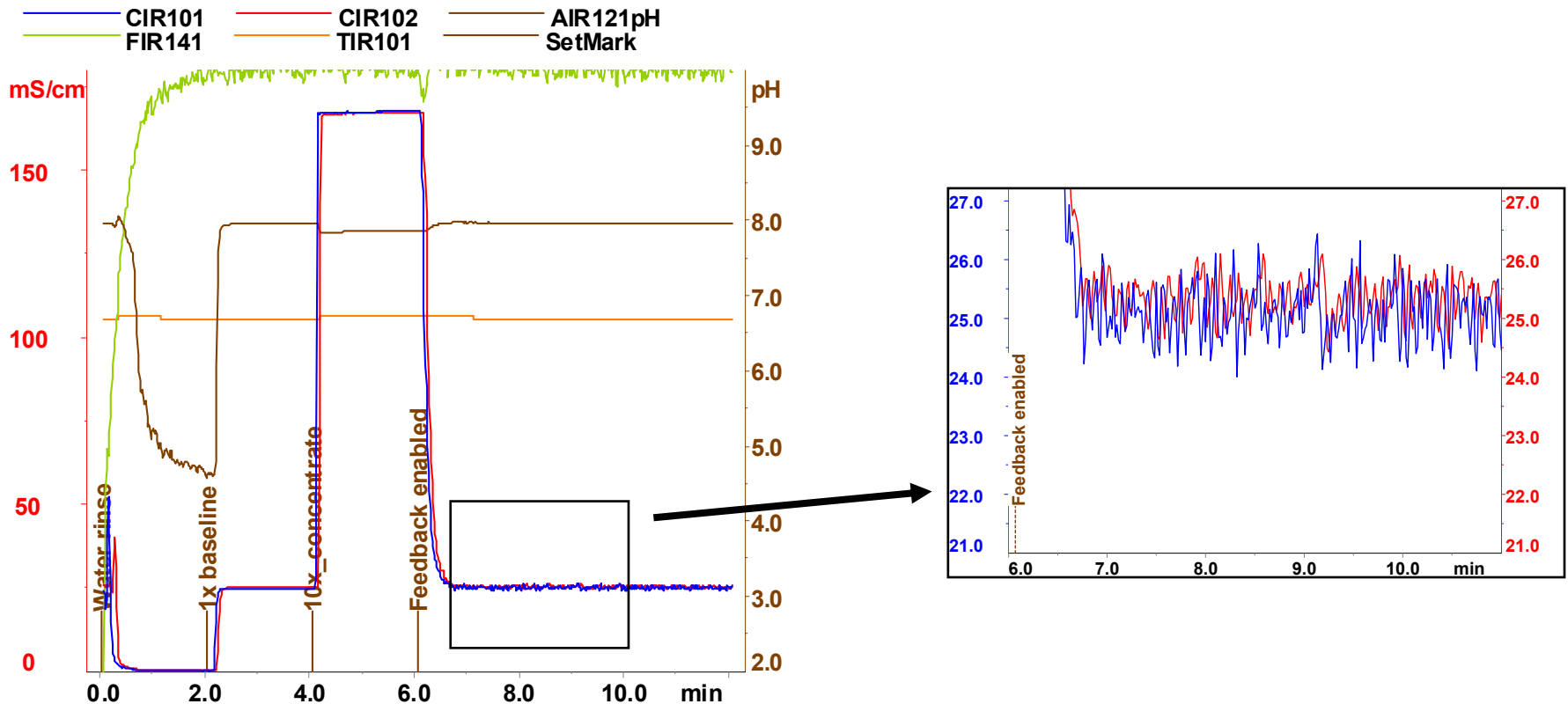
- Costs
 - Chemicals
 - Services; WFI, sterilisation
 - Time
- Bottlenecks
 - GMP materials storage
 - Tank capacities
 - Storage tank capacities
- Concentrations
 - High molarity means handling problems
- Flexibility
 - Disposables up to Pilot scale allow for greater flexibility than manufacturing scale fixed tanks

Solutions

- In-line dilution
- UF Operation
- Minimise chemical concentrations
- Minimise diversity of buffers

Inline Dilution

- LONZA has successfully implemented in-line dilution for chromatography buffers
 - Reduced buffer prep requirements by up to 10x



Inline Dilution

- Requires a WFI break tank / heat exchanger to maintain temp
 - WFI re-circulates at 80 °C
 - Generation must meet peak demand or large tank

- Ensure the concentrate is soluble
 - Don't expect to concentrate a 1 M salt buffer 10x

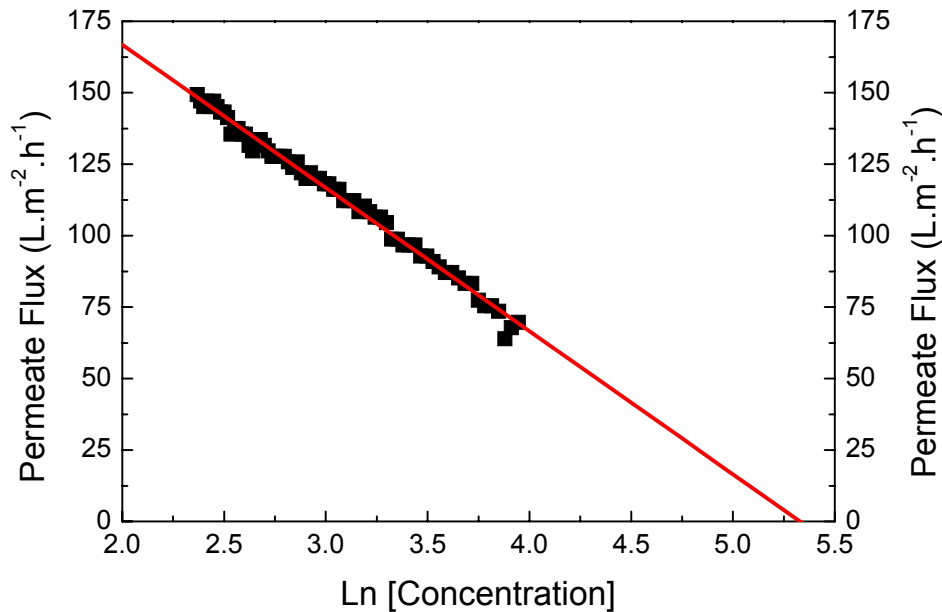
- Programme skid to put column in line only after spec met
 - Some buffer is sent directly to waste
 - Typically < 1 % CV

UF Operation

- Ultrafiltration
 - Concentration Step
 - Diafiltration Step
 - ◆ Prepares product for next process unit op

- Process Model
 - 7,300 L process stream
 - 5.24 g.L⁻¹ initial concentration
 - 5 volume diafiltration
 - 40 m² membrane area
 - Process time calculated as a function of conc at diafiltration

Concentration and Diafiltration Optimisation



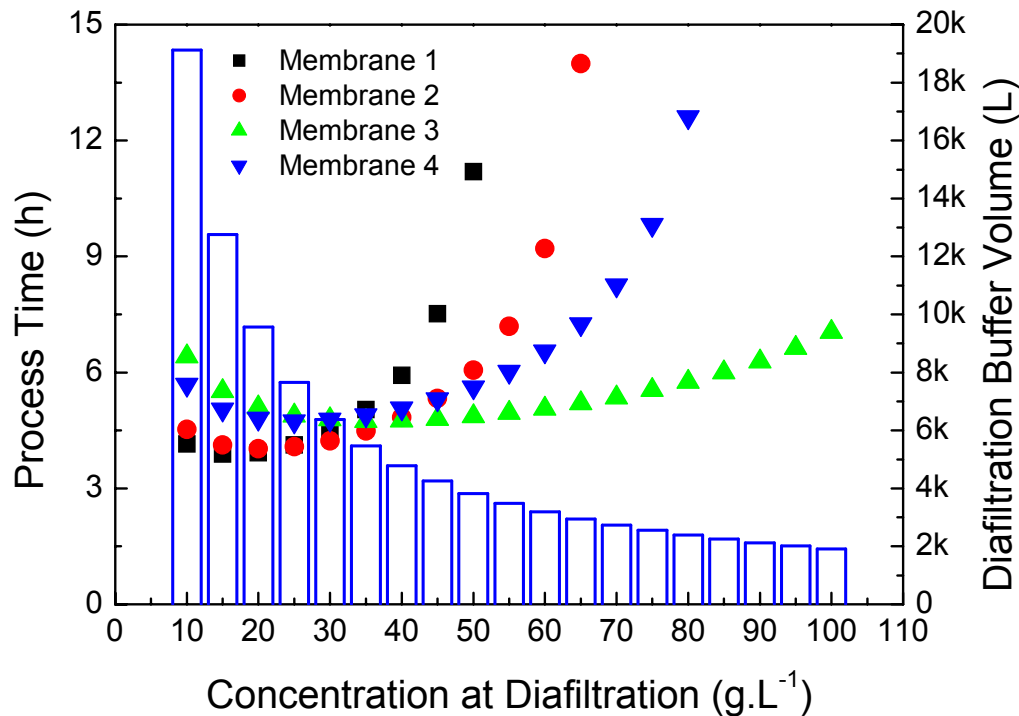
- Rig in conc mode
- Use optimal conditions
- Measure flux as conc ↑
- From:

$$J = k \ln\left(\frac{C_g}{C_b}\right)$$

- When $J = 0$, $C_b = C_g$
- Optimal Cdf wrt time:

$$C_{df} = \frac{C_g}{e}$$

Choosing membranes and conditions



- Buffer consumption can be minimised by choice of:
 - operating conditions
 - membrane
- Product must be capable of withstanding required concentration
- This is product and buffer specific

Matrix Re-Use: Column Size and Cycles

- For in market supply the number of cycles is crucial to cost
- 10,000 L fermentation
 - 1.0 g.L⁻¹
 - Fed-Batch operation
- Affinity Chromatography Capture Step
 - 30 g.L⁻¹ dynamic binding capacity
 - 15 cm bed height
 - 70 cycle life expectancy
 - £5,000 per Litre
- Choices
 - 140 cm column – 2 cycles per batch
 - 80 cm column – 5 cycles per batch

Matrix Re-Use: Column Size and Cycles

- 140 cm column
 - 231 L column volume
 - Each batch of matrix
 - ◆ Will cost £1.2 million
 - ◆ Will last 35 fermentation batches
 - ◆ This equals 350 Kg product (gross)
 - ◆ Matrix costs per gram : £3.30 (72 % utilisation)
- 2 cycles at 6.8 h each = 13.6 hours
 - 2 operators = £1,088
 - This costs £0.11 per gram
- Fewer cycles means less risk
- Op-Ex costs per gram : £3.41

Matrix Re-Use: Column Size and Cycles

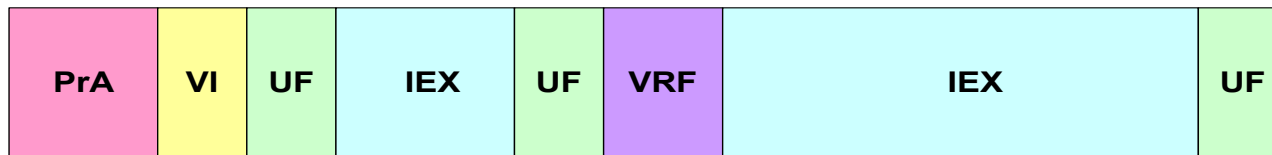
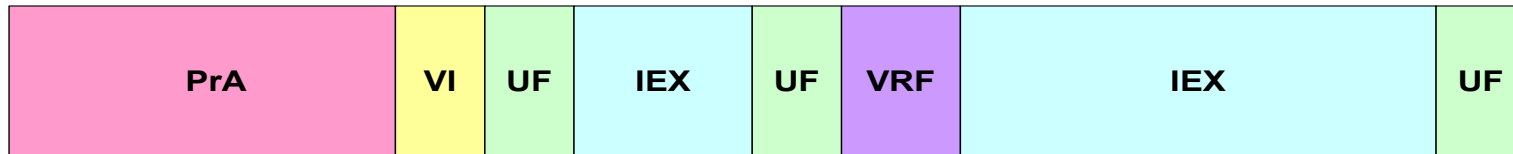
- 80 cm column
 - 75 L column volume
 - Each batch
 - ◆ Will cost £0.4 million
 - ◆ Will last 14 fermentation batches
 - ◆ This equals 140 Kg product (gross)
 - ◆ Matrix costs per gram : £2.70 (88 % utilisation)
- 5 cycles at 7.3 h each = 36.5 hours
 - 2 operators = £2,920
 - This costs £0.29 per gram
- Op-Ex costs per gram : £2.99
- Operational Expenditure is £0.42 per gram lower
- But... process takes 23 hours longer

Process Time

- Numerous strategies are available
 - One route is to upgrade compressible matrices to high flow rate rigid matrices
 - Will improve schedule but not buffer consumption
 - Targeted initial two column steps for matrix upgrade evaluation.
 - Imposed constraint.....chromatography buffer chemistry should remain the same despite change in matrices.
 - ◆ Reflects a typical rapid development project

Effect of Affinity upgrade on process throughput

- First target for high throughput upgrade
 - Implement rigid matrix for affinity step
- Maintained column geometry (& cost)
 - Increased number of cycles (4 to 5)
 - Decreased affinity step time (by 60%)
 - Increase in buffer consumption (by 25%)



← **-15%**

- Overall productivity improvement of 15% throughput

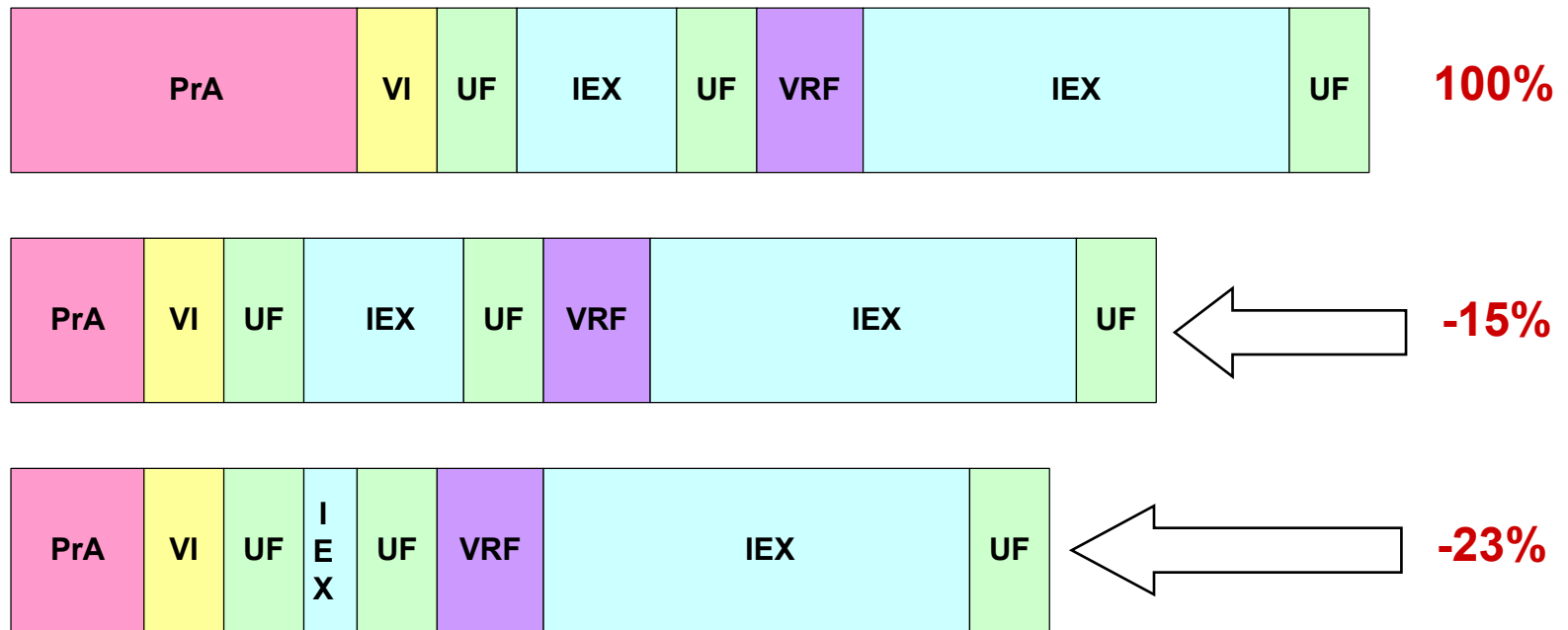
Upgrade IEX productivity

- Evaluated impurity and contaminant clearance of
 - Sepharose based IEX step vs.
 - Rigid IEX matrix

- Experimentally verified...
 - Equal clearance of contaminants, impurities
 - Equal protein purification
 - Equivalent product quality and step yield

- Major improvement...
 - 3x increase in step productivity due to 3x increase in linear velocity for same column volume.

Effect on production schedule of PnA and IEX rigid matrix upgrades



- 23% Productivity increase was achieved using the upgraded high throughput process
- No detrimental effect on purification, yield or product quality observed

Conclusions

- High volume processing faces certain challenges
- Full and accurate process model
- Equipment / consumables may face physical limitations
- Buffer constraints
 - Fixed plant may not be able to be changed
 - In which case, sufficient
 - ◆ materials handling capacity
 - ◆ buffer preparation
 - ◆ Buffer storage
- Re-Use of resins and membranes will have an impact on scale of operation and therefore costs and time.
- Decide whether time or cost is limiting, in general:
 - Time is primary concern for in market supply
 - Cost is limiting when supply exceeds demand

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