WORKSHOP 2

New Modalities, Enabling Technologies and Unit Operations
"Mocha Dick or the d----l [devil]," said I, 'this boat never sheers off from any thing that wears the shape of a whale."

The book portrays destructive obsession and monomania, as well as the assumption of anthropomorphism.
Integrated Continuous Biomanufacturing

October 20-24, 2013
Gran Hotel Rey Don Jaime
Castelldefels, Spain - near Barcelona
Part 1: Continuous Downstream
Practical examples and things to consider

1. “Improving Adenovirus Purification by Simulated Countercurrent Size Exclusion Chromatography” José Paulo Mota

2. “A Simple Strategy for Continuous Viral Inactivation” Mark Brower

3. “Robustness and Regulatory Considerations in the Development of a Continuous Bioprocess Unit-Operation” Ajoy Velayudhan

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Improving adenovirus purification by two-column, simulated countercurrent, size-exclusion chromatography

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Objective

- Adenoviruses are one of the most suitable platforms for producing viral vaccines & gene therapy vectors
- Downstream processing is increasingly important for a reliable production process with high purity and yield, and, also importantly, cost efficiency.

What we have done:

- Streamline the downstream processing of Ad5
- Focus on the chromatography steps since they are the most expensive
- Demonstrate advantages of (partially) changing the Ad5 purification train to (semi-)continuous operation
Objective

• Streamline the DSP train by boosting the performance of the SEC step

Use a dirtier bulk
Target: veterinary vaccine
Strategic decision

- Basic process design choice
  - Product (Ad5) + impurities (HCP, DNA, ...)

Center-cut separation
  (more complex)

Ad5 has intermediate elution profile

Minimizes Ad5 residence time
Minimizes Ad5 dilution (SEC only)

Two-fraction separation
  (simpler)

Ad5 elutes first

Ad5 elutes last
Strategic decision

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Ad5 elutes last

Preliminary requirement: availability of suitable SE medium
Basic assumptions

• **(1)** Efficient clarification & concentration of the bioreaction bulk to produce a virus solution in which the virus particles are the largest component.

• **(2)** Choose the **SEC medium** so that (a) virus particles are bigger than the widest pores of the SEC matrix and (b) impurities, which are smaller, can penetrate, to larger or lesser extent, into the pores of the matrix.

• This way, the **virus particles are completely excluded from the pores of the matrix and therefore elute in the void volume**, whereas the **impurities elute at later times** according to differences in their molecular size.
(Clarification & concentration)

- Triton & Benzonase treatment
  - 0.1% Triton X100, incubation for 2 hours at 37°C with 50 U/ml of Benzonase

- Microfiltration with Sartopore 2 membrane
  - 0.8 μm prefilter + 0.45 μm filter

- Concentration × 10 times & diafiltration × 5 times with Sartorius cassette prototype with average cut-off of 500-750 kDa
• **Sepharose 4 Fast Flow** (S4FF, GE Healthcare) average particle size of 90 μm (range of 45–165 μm).
  – S4FF is based on a highly cross-linked 4% agarose matrix, which gives good physical stability and chromatographic qualities; its exclusion limit for globular proteins is ca. 3×10⁷.

• **Buffer** = 20 mM Tris & 150 mM NaCl at pH 8 (for final delivery formulation)
SEC medium

- Ad5 particles are excluded from the SEC matrix
Prep columns (XK 26/20)

Col 1
$L = 10.4 \text{ cm}$

Col 2
$L = 10.8 \text{ cm}$

Cols 1+2

Exp.
UV
Model
UV
Ad5
impurities
Process design

• Two-column configuration
  – Simplicity, small footprint

• Open-loop configuration
  – Simple as batch system; very robust; less pumps

• Simulated countercurrent operation
  – Cycle divided into 2 equal switch intervals ($\tau+\tau$)
  – Each column undergoes the same sequence of steps but phased out in time by one switch interval

![Diagram showing the sequence of steps in a two-column configuration with simulated countercurrent operation.](image)
Process design

• **Switching interval** = sequence of elementary steps

  ![Diagram](image)

  (a)  
  (b)  
  (c)  
  (d)  
  (e)  
  (f)

• Discard (e) partial splitting of an exit stream and (f) closed-loop recirculation
Process design

• 3 key components:
  – Ad5 (product, fastest component)
  – Fastest eluting impurity (Ad5’s neighbor)
  – Slowest eluting impurity (defines cycle time)

• Design parameters
  – Column geometry, retention factors, HETPs,
  – Process constraints: pump flow rates, max ΔP, etc.
  – Quality constraints & objective:
    • For a given target purity maximize yield
Process design

- Hide design complexity from end-user

Parameters + specifications → Cycle design model based-optimization

5 × 2-way valves per column
Optimum SEC cycle

- Elute at upstream end • collect product at downstream end • Inject feed at the middle • discard waste fractions in between

(1st half cycle)                          (2nd half cycle)
Optimum SEC cycle

- Predicted outlet concentration profiles at each column outlet over the 1st switch interval
Analytics

• **Quantification of total viral particles**
  – DNA extraction and no. of viral DNA copies determined by real-time PCR with LightCycler system
  – Total particle concentration confirmed by Nanosight NS500

• **Protein profile analysis** in 4-12% NuPage gradient pre-cast gels (Invitrogen)

• **HCP** determined using ELISA kit for HEK293 cell line

• **DNA** quantified by Quant-iT PicoGreen Assay kit
Pilot-plant run

- UV signal at the outlet of column 2 for the 1st 5 cycles: stable and fast cyclic steady state

Product collection windows
Pilot-plant run

- TVP, HCP, and DNA concs in the prod fraction collected at each switching interval

Stable cyclic steady state
2C-SCC vs Batch

- Performance comparison against batch for the same amount of SEC medium

<table>
<thead>
<tr>
<th></th>
<th>Productivity (L/min)</th>
<th>Virus Yield (%)</th>
<th>DNA Clearance (%)</th>
<th>HCP Clearance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>2-column, open-loop SCC-SEC</strong></td>
<td>0.67 × 6</td>
<td>86.3 + 50%</td>
<td>90</td>
<td>89</td>
</tr>
<tr>
<td><strong>Single-column SEC</strong></td>
<td>0.11</td>
<td>57.4</td>
<td>94</td>
<td>94</td>
</tr>
</tbody>
</table>
Conclusions

• Ad5 DSP train was streamlined and improved by converting the SEC single-column batch step to semi-continuous, two-column SCC operation. *Purification & polishing* in single step with buffer for final formulation

• Productivity increased 6 fold and yield increased by 50%

• Main drawbacks of SEC—low productivity and low product titer—minimized by our novel 2-column system that recycles the mixed fractions inside the system while operating in open-loop as the batch process

• Future work: plan to adapt core bead technology to the SEC step ➔ increase retention capacity for impurities
Thank you...

• Co-authors

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Cristina Peixoto (Head, DSP @ IBET)
Paula M. Alves (CEO, IBET)
Manuel J.T. Carrondo (Vice-president, IBET)

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A Simple Strategy for Continuous Viral Inactivation

Mark Brower

BioProcess Technology & Expression
Bioprocess Development
Kenilworth, NJ
Continuous Processing DSP

- Single-Use Centrifugation
  - Surge Bag
    - Depth / BRF Filtration
      - Surge Bag
        - Anion Exchange Membrane
          - Continuous Viral Inactivation
            - BRF
              - BioSMB Protein A
                - Polishing Step
                  - BRF
                    - Viral Filtration
                      - Surge Bag
                        - Continuous UF
                          - Formulation: BRF/DiaF

*Single-Use Bioreactor
Classical Inactivation Methods

- Low pH
- UVC Light
- Detergent
- Precipitation
- HTST* (*high temperature short time)
- MVM
- *Boscetti 2003

Chemical Compounds:
- Triton X-100
- tri(n-butyl) phosphate
Borrowing Concepts from Bioreactors

Tubular Holding Loop

Spiral Holding Loop

Bulk Media

Sterilized Media
Residence Time Distribution Analysis

\[ E(t) = \frac{C(t)}{\int_0^\infty C(t) \, dt} \]

\[ \mu_1 = t_m = \int_0^\infty t \cdot E(t) \, dt \]

\[ \sigma^2 = \mu_2 = \int_0^\infty (t - t_m)^2 \cdot E(t) \, dt \]

\[ \mu_3 = s^3 = \frac{1}{\sigma^{3/2}} \cdot \int_0^\infty (t - t_m)^3 \cdot E(t) \, dt \]

\[ \frac{\sigma^2}{t_m^2} = \frac{2}{Pe} + \frac{8}{Pe^2} \]

\[ Pe = \frac{\text{Convection}}{\text{Dispersion}} \]

\[ X(\zeta = 1, \Theta) = \frac{1}{\sqrt{4\pi \Theta Pe}} e^{-\frac{(1-\Theta)^2}{4\Theta Pe}} \]

\[ \zeta = \frac{z}{H} \quad \Theta = \frac{t}{t_m} = \frac{tv}{H} \quad X = \frac{c}{c_0} \]

Fogler 1999
Tubular Reactor Based Viral Inactivation

\[ \tau > 2t_m \]

Feedback control loop
Continuous Processing Case Study

pH of Pro A Elution Peak

pH

Elution [CV]

Time [Min]

VI Feed

AEX Feed
Questions?
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Part 2: Continuous Bio-manufacturing

Panel discussions: Ajoy Velayudhan, Mark Brower, José Paulo Mota,

1. Technology gaps
   • Sensors
   • Control strategies
   • Auxiliary technologies

2. Future directions
   • White papers
   • PDA Technical reports
   • Standardization
Chromatography at its **very** best

**Better resins**
- optimized pore structure
- composite constructs
- improved/new ligands

**Smart engineering**
- improved hardware usage
- new operating principles
- better understanding

**Process intensification**
- semi- and/or continuous processes
- smaller/more efficient columns/systems
- control algorithms

**Good**
- already here (past)

**Better**
- better understanding (present)

**The best**
- strict rules (future)
Definition of Process Intensification

A set of often radically innovative principles ("paradigm shift") in process and equipment design, which can bring significant (more than factor 2) benefits in terms of process and chain efficiency, capital and operating expenses, quality, wastes, process safety, etc.

(European Roadmap of Process Intensification, 2007)
Robustness and regulatory considerations in the development of a continuous bioprocess unit-operation

Ajoy Velayudhan, Spiros Konstantinidis, and Jayan Senaratne
Department of Biochemical Engineering
University College London
Outline

• Introduction
• Process Development
• Control: Rapid adjustments to variations
• Quality: Fault diagnosis
• Regulatory: Batch definition
• Conclusions
Introduction

• Clear advantages to continuous DSP
  – Better utilisation of adsorbent; reduced consumption of mobile phase; more difficult separations ($\alpha \sim 1.5$) feasible

• Opportunities remaining
  – If the entire process is continuous, a global design is required
    • Must match equipment/process time constants
    • Long-range interactions increase failure/deviation modes
    • Control strategies must become more sophisticated
  – Any continuous step requires batch/lot definition
Downstream Process Development

• Batch
  – Each unit operation is usually developed separately
  – Any optima found are therefore only local
  – Unexpected interactions among steps may ensue

• Continuous
  – If the entire process is continuous, must be developed holistically
  – More likely to result in a robust process
  – Multiple modalities available
Operational Modes in Continuous DSP

- Multiple operational modes in the literature
- Optimisation methods can select operational mode as well as parameter values
  - Kawajiri and Biegler, 2006
- Multi-objective optimisation has been used
  - Many two-objective examples
  - Four objectives have been optimised (yield, purity, throughput, solvent consumption)
    - Hakanen et al, 2007
Case study—ternary separation by stepwise elution in 6-zone SMB/TMB

D1 ($C_m^1$)  
E1 (Enriched in C)

Zone I

D2 ($C_m^2$)  
E2 (Enriched in B)

Zone II

R (Enriched in A)  
F ($A, B, C, C_m^3$)

Zone III

Zone IV

Zone V

Zone VI

Direction of adsorbent movement
Base-Case Result

~0% ~0% >99%
~93% ~7% 20% 20%
~99% ~0.5% ~7%
Robustness of SMB

• Robust against time-invariant changes for 4-zone SMB
  – Step changes in porosities and binding capacities: Wang, 1998
  – Column homogeneity: Guiochon, 2001
  – Feed variations: Wankat, 2002
  – Switching time: Wang, 2003

• Time-dependent changes for 6-zone TMB/SMB
  – Gradual loss of binding capacity
  – Fluctuating flow rates and modulator levels
Gradual decrease of resin binding capacity

• Consequences
  – Gradual worsening of purity, yield
  – Eventual failure of CQAs
  – Control-based adjustment of flow rates insufficient
  – Run must be stopped
  – Fate of lot?
Decrease of flow rates from set points

• Quite easily detectable in simple systems
• Will this become more difficult for complex systems?
• What rapid response is available?
Resin failure mode
Pump failure mode
Combined failure mode
Assessment

• If failure modes cannot be mapped uniquely into first causes, adjusting to variations on-the-fly will become problematic.

• As more unit operations are combined, the complexity of the overall model will inevitably increase.

• Will such non-uniqueness arise?

• If so, may need more complex models and more sophisticated analytics to recover a unique mapping.
Regulatory Definitions of Batch and Lot

• 21 CFR 210.3

• **Batch**: Specific quantity of drug [...] intended to have uniform character and quality [...] and is produced during a single manufacturing order during the same cycle [...] 
  • Allows for continuous processing
• **Lot**: a batch, or specific identified portion of a batch [...] ; or [if] produced by a continuous process, a specific amount produced in a unit of time or quantity [...] that assures its having uniform character or quality [...]
Semi-Continuous Manufacturing

- Continuous Cell Culture
- Continuous Capture
- Holding Tank
- Viral Inactivation
- BDS
- Viral Filtration
- Continuous Polishing

Continuous steps

Batch steps
Batched (semi-continuous) operation

• Clear advantages
  – Can address process deviations
    • Replace resin or other raw material
    • Recalibrate pumps, etc
  – Clear definition of batches and lots
    • Limited lots in jeopardy because of deviations
    • Facilitate the addressing of deviations

• Disadvantages
  – Start-up and shutdown can be long
  – Mismatch with the rest of the process if it is truly continuous

• Shutdown example
  – How much material can be collected during shutdown?
Conclusions

• (Semi-)Continuous operations will certainly be widely tested for biologics.

• Caveat:
  – Complex sequences of steady-states may encapsulate complex dynamics.
  – A deeper understanding of our unit-operations, and interactions among them, may be essential to success.
  – Better analytics, as well as more frequent sampling, may be needed.
Inside the iPhone 5

The new model is estimated to cost Apple about $9 more than the predecessor, due to the larger display and added wireless technology.
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