Developments in mixed mode chromatography in biopharmaceutical purification

Dr John Liddell
Avecia Biologics
Billingham
UK

SCI Mixed Mode Chromatography Conference  27th June 2007
Outline

• Avecia background
• Review of mixed mode media chemistries
• Mixed mode behaviour
• Mixed mode application in biopharmaceutical purification processes
• Case studies
• Summary
Businesses

Biologics

Biopharmaceutical API contract manufacturing

Vaccines

Development of Recombinant vaccines

DNA Medicines

Oligonucleotide API contract manufacturing
Microbial and mammalian derived recombinant proteins

**Customer Focus**
- US, Europe, Japan
- Established Biotechs
- Life-cycle involvement

**Compliance**
- MHRA licensed
- FDA inspection ready
- Regular customer audits

**Development**
- Process invention
- Process development
- Characterisation
- Validation

**Facilities**
- Activities aligned on one site
- Pilot scale
- Clinical manufacture (1000L)
- Commercial manufacture (2x 5000L)
- Net Assets > £60m

**Dimensions**
- 25 years experience; >35 biologics developed; >500 employees
### Mixed mode media

<table>
<thead>
<tr>
<th>Media</th>
<th>Type</th>
<th>Ligand</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEP (Pall)</td>
<td>Hydrophobic binding near neutral pH with elution by pH reduction</td>
<td>4-Mercapto ethyl pyridine</td>
</tr>
<tr>
<td>HEA (Pall)</td>
<td>Hydrophobic binding near neutral pH with elution by pH reduction</td>
<td>Hexylamino</td>
</tr>
<tr>
<td>PPA (Pall)</td>
<td>Hydrophobic binding near neutral pH with elution by pH reduction</td>
<td>Phenylpropylamino</td>
</tr>
<tr>
<td>MBI (Pall)</td>
<td>Hydrophobic binding at slightly acid pH with elution by raising pH</td>
<td>2-Mercapto-5-benzamidazole sulfonic acid</td>
</tr>
<tr>
<td>Capto MMC (GEHC)</td>
<td>Cation exchanger with mixed mode functionality</td>
<td><img src="image" alt="Capto MMC" /></td>
</tr>
<tr>
<td>Capto adhere (GEHC)</td>
<td>Strong anion exchanger with mixed mode functionality</td>
<td>N-benzyl-N-methyl ethanolamine</td>
</tr>
<tr>
<td>CHT hydroxyapatite (BioRad)</td>
<td>Ion exchange with hydrophobic component</td>
<td>$(\text{Ca}_5\text{PO}_4)_2\text{OH}$</td>
</tr>
<tr>
<td>CHT fluoroapatite (Biorad)</td>
<td>Ion exchange with hydrophobic component</td>
<td>$(\text{Ca}_{10}\text{PO}_4)_2\text{F}$</td>
</tr>
</tbody>
</table>

Others
- Affinity media can display significant mixed mode character depending on spacer
- Thiophilic media
But you may have been experiencing mixed mode media all the time............
CIEX experimental studies

- Similar experimental configuration
- 80kD protein, pl 8.5
- Tested with four different cation exchange media
- All have same cation functionality
- Same experimental set up in all cases
- Loaded to 11mg/ml at 20mS/cm, pH 7.0
- 10CV linear elution gradient to 1M NaCl
- 14cm bed depth
## CIEX media characteristics

<table>
<thead>
<tr>
<th>Media</th>
<th>Functionality</th>
<th>Matrix</th>
<th>Particle size (micron)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP XL Sepharose</td>
<td>Sulphopropyl</td>
<td>Agarose (6% crosslink, dextran coating)</td>
<td>130</td>
</tr>
<tr>
<td>SP Sepharose</td>
<td>Sulphopropyl</td>
<td>Agarose (6% crosslink)</td>
<td>130</td>
</tr>
<tr>
<td>SP Sepharose HP</td>
<td>Sulphopropyl</td>
<td>Agarose (6% crosslink)</td>
<td>45</td>
</tr>
<tr>
<td>SOURCE 30S</td>
<td>Sulphopropyl</td>
<td>Polystyrene/divinylbenzene</td>
<td>30</td>
</tr>
</tbody>
</table>
Buffer B: 25 mM sodium-phos., pH 7.5, 2 M NaCl
Some observations....

- Capacity: SP FF/SP HP > SOURCE 30S > SP XL
- Resolution: SP HP > SP FF > SOURCE \(\cong\) SP XL
- Variable position of elution in gradient
- Resolution not always related to particle size
- Mixed mode behaviour – matrix and ligand interaction
General biological separation scheme

- **Intracellular (ie E coli)**
  - Cell removal
  - Cell disruption
  - Insoluble product recovery
  - Solubilisation/refolding
  - Soluble protein
    - Filtration
    - Chromatography
- **Extracellular (ie secreted – yeast, animal)**
  - Cell removal
  - Solubilisation/refolding
  - Soluble protein
    - Filtration
    - Chromatography #1
    - Chromatography #2
    - Chromatography #3
    - Chromatography #N

- **Primary separation**
- **Purification**
- **DS formulation**

Number of steps?
Intermediate conditioning?
Analysis of chromatography step order

Based on biopharmaceutical processes at early 2006 (in house data)
Ion exchange as step #1, HIC as step #2 a frequent pattern
Good practical reasons
  - Loading capacity
  - IEX→HIC rather than HIC→IEX

<table>
<thead>
<tr>
<th>Separation basis</th>
<th>Chromatography #1</th>
<th>Chromatography #2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ion exchange</td>
<td>78</td>
<td>52</td>
</tr>
<tr>
<td>Hydrophobic</td>
<td>7</td>
<td>48</td>
</tr>
<tr>
<td>Affinity</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Metal chelation</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>
Mixed mode media and purification processes

- Depends on the mix of the modes
- Provides new process options
- Hydrophobic media which can produce a low ionic strength eluant
- Highly hydrophobic media from which protein can be recovered quantitatively
- Selectivity control through control of binding or elution pH, ionic strength, salt type
- Potentially any chromatography step – advantage of matching to subsequent chromatography step
- Capture steps may have most potential
- Highlighted in some examples
Mixed mode and process development

- More complex development
- Different from IEX and HIC
- More adjustable parameters
  - Elution profile - pH step, pH gradient
  - Load - ionic strength, pH, salt type
  - Elution - ionic strength, pH, salt type
- Experimental design approaches and beyond
Potential for high throughput techniques

Capto MMC
• Evaluation of Pall mixed mode chromatography sorbents in initial capture step for recombinant proteins from *E. coli* homogenate
• Capture the target without conditioning the homogenate
• Elute via a simple pH gradient at low ionic strength
• Provide an eluate suitable for simply pH titration and load onto an IEX column without intermediate UF/DF
• Recombinant protein
• Intracellular expression in E coli
• Expressed as fusion protein
• Initial studies with post cleavage, partially purified protein
  • 37.7kDa
  • pI 5.57
  • Aliphatic index 90.75
  • GRAVY –0.549
1. Screening:
   - Establish loading, washing and elution conditions
   - Sample loaded without feed conditioning, washed with the equilibration buffer
   - Eluted via a 20 CV pH gradient from 7 to 3 into a low conductivity buffer.

2. Binding capacity:
   - As screening, but loading protein until breakthrough is seen.

3. Optimisation:
   - Depending on previous data and observations,
   - Parameters such as load rate, load condition, wash buffer, elution strategy varied systematically
   - Maximise data but minimise experimentation.
### Experimental protocol

<table>
<thead>
<tr>
<th>Step</th>
<th>Buffer</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equilibration</td>
<td>20mM PB pH 7.4</td>
<td>5 CV</td>
</tr>
<tr>
<td>Load</td>
<td>7.0 mg.ml$^{-1}$ product in PBS pH 7.4</td>
<td>1 CV</td>
</tr>
<tr>
<td>Wash 1</td>
<td>20mM PB pH 7.4</td>
<td>4 CV</td>
</tr>
<tr>
<td>Wash 2</td>
<td>Buffer A: 100mM Phosphate-50mMCitrate Buffer pH 7</td>
<td>4 CV</td>
</tr>
<tr>
<td>Linear gradient elution</td>
<td>Buffer A: 100mM Phosphate-50mMCitrate Buffer pH 7</td>
<td>20 CV</td>
</tr>
<tr>
<td></td>
<td>Buffer B: 100mM Phosphate-50mMCitrate Buffer pH 2.6</td>
<td></td>
</tr>
<tr>
<td>Wash 3</td>
<td>Buffer B: 100mM Phosphate-50mMCitrate Buffer pH 2.6</td>
<td>2 CV</td>
</tr>
</tbody>
</table>
## Preliminary results

<table>
<thead>
<tr>
<th>Ligand</th>
<th>pKa</th>
<th>Recovery (%)</th>
<th>Capacity at 10% breakthrough (mg.ml⁻¹ resin)</th>
<th>Elution pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEP HyperCel</td>
<td>4.8</td>
<td>58</td>
<td>Not tested</td>
<td>&lt; 5.5</td>
</tr>
<tr>
<td>HEA HyperCel</td>
<td>6 and 9</td>
<td>88</td>
<td>&gt;&gt;7</td>
<td>&lt; 4.5</td>
</tr>
<tr>
<td>PPA HyperCel</td>
<td>6 and 9</td>
<td>64</td>
<td>66.5</td>
<td>&lt; 3.5</td>
</tr>
</tbody>
</table>

Compare with

Hydrophobic interaction media (phenyl) capacity of 10 mg/ml

AIXE capacity ~20 mg.ml⁻¹
Example 2 - background

- Monomeric
- 26kDa
- pl 10.5
- No cysteines
- Aliphatic index 60.61
- GRAVY –0.509
Original Capture Step arrangement

Cell Bank Seed Vial

Extraction requires Specific pH and NaCl

Inoculum

Recover Cells By Centrifugation

Fermentation

Resuspend

Capture on Cation Exchange Column

Break Cells by Homogenization

NaCl in extraction requires dilution / diafiltration

Clarify Homogenate by Centrifugation / Filtration

Huge filter area required to clarify homogenate

Poor binding from homogenate
Homogenate Clarification

- Polyethylenimine (PEI) used to improve clarification
- Cationic polymer
- Highly effective flocculating agent
- Filter requirements substantially reduced and filter use consistent
- Little loss of product to debris phase
• Effective binding directly from extraction conditions
• High yield ~80%
• Substantial purification
• Low conductivity elution
• High capacity >50g/L
## Added Benefits

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>ENDOTOXIN CONTENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fermenter Culture</td>
<td>&gt;&gt;10 000 EU/ml</td>
</tr>
<tr>
<td>Homogenate</td>
<td>&gt;&gt;10 000 EU/ml</td>
</tr>
<tr>
<td>PEI Conditioned Supernatant</td>
<td>1190 EU/ml</td>
</tr>
<tr>
<td>MEP Eluate</td>
<td>1.8 EU/ml</td>
</tr>
</tbody>
</table>

- Effective endotoxin elimination
Added Benefits

- Excess PEI binds tightly to cation exchange media
- Limits re-use
- Excess PEI cleared from process
- Majority cleared in load flowthrough

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>PEI MASS BALANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Load</td>
<td>100% (0.1% v/v)</td>
</tr>
<tr>
<td>Flow Through</td>
<td>81%</td>
</tr>
<tr>
<td>Wash</td>
<td>5%</td>
</tr>
<tr>
<td>pH 6.4 Wash</td>
<td>2%</td>
</tr>
<tr>
<td>pH 5.2 Elution</td>
<td>0.75%</td>
</tr>
<tr>
<td>Strip</td>
<td>5%</td>
</tr>
</tbody>
</table>
Conclusions

- Mixed mode media covers a range of effects
- Significant scope for application in biopharmaceutical processes
- Highlighted by the examples and increasing seen applied in processes
- Additional parameters compared to single mode media to be characterised in development
- Development likely to be more complex
Acknowledgements

GEHC
• Robert Morenweiser

Pall
• John Woodgate
• John Jenco
• Aurelia Topol
• Alun Fowler

Avecia
• Sam Tinsley
• Jackie Dodson