Orthogonal Separation Process for Manufacturing Proteins from Vegetable Derived Culture Media

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PREP-2005, Philadelphia
Objective

- To develop optimal purification processes for the of manufacturing proteins
  - Resulting from vegetable-derived media
  - Applicable to bacterial, mammal and other cell culture processes.

- Select optimal chromatographic media by comparing performance
# Comparison of Animal and Vegetable Derived Media

<table>
<thead>
<tr>
<th>Component</th>
<th>Animal Media</th>
<th>Vegetable Media</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Nitrogen (%)</td>
<td>7.8 -- 9.6</td>
<td>8.3 -- 10.1</td>
</tr>
<tr>
<td>Amino Nitrogen (%)</td>
<td>4.1 -- 5.1</td>
<td>3.3 -- 4.1</td>
</tr>
<tr>
<td>Total Carbohydrate (mg/g)</td>
<td>68.4 -- 83.6</td>
<td>302.6 -- 369.8</td>
</tr>
<tr>
<td>Sodium (mg/g)</td>
<td>39.6 -- 48.4</td>
<td>27.0 -- 33.0</td>
</tr>
<tr>
<td>Alanine (%)</td>
<td>5.3 -- 6.5</td>
<td>3.2 -- 4.0</td>
</tr>
<tr>
<td>Aspartic Acid (%)</td>
<td>1.2 -- 1.4</td>
<td>0.2 -- 0.22</td>
</tr>
<tr>
<td>Glycine (%)</td>
<td>1.7 -- 2.1</td>
<td>2.0 -- 2.4</td>
</tr>
<tr>
<td>Isoleucine (%)</td>
<td>2.1 -- 2.5</td>
<td>2.3 -- 2.8</td>
</tr>
<tr>
<td>Lysine (%)</td>
<td>1.5 -- 1.9</td>
<td>2.3 -- 2.9</td>
</tr>
<tr>
<td>Histidine (%)</td>
<td>0.7 -- 0.9</td>
<td>1.2 -- 1.4</td>
</tr>
<tr>
<td>Tyrosine (%)</td>
<td>0.5 -- 0.7</td>
<td>0.7 -- 0.9</td>
</tr>
<tr>
<td>Tryptophan (%)</td>
<td>0.4 -- 0.44</td>
<td>0.1 -- 0.11</td>
</tr>
<tr>
<td>Valine (%)</td>
<td>2.4 -- 3.0</td>
<td>2.5 -- 3.1</td>
</tr>
</tbody>
</table>
Types of Contaminants

- Proteins
  - HCP
- Endotoxin
- DNA
- Media components
- Modified biopolymers (non-protein)
  - Modified carbohydrates
Approach

- Evaluate components clearance during different chromatographic steps
  - Cation exchange
  - Anion exchange
  - Hydroxyapatites
  - HIC

- Process Criteria
  - Overall process yield over 60%
  - Protein purity greater than 98%
  - Protein dry weight assay greater than 98%
Chromatographic Process Evaluation Scheme

Clarified Culture Media

- PolyCSx
- PolyQUAT
- PolyQUAT
- PolyQUAT
- HIC

PolyQUAT

PolyCSx

HA

HA

PolyCSx
Chromatographic Process Evaluation Scheme Objectives

- **First step**
  - Protein purity greater than 95%
    - Four fold purification
  - Protein yield greater than 80%

- **Second step**
  - Removal of endogenous and other non-protein contaminants
    - Achieve dry weight purity objective
  - Improve protein purity to greater than 98%
Optimized Process

1. Harvest Cell Culture Media
2. Filtration/Diafiltration
3. PolyQUAT
4. PolyCSx
5. Diafiltration/Lyophilization
Investigated Chromatography conditions

<table>
<thead>
<tr>
<th>Buffers</th>
<th>MES, Tris, Acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ionic Strength</td>
<td>Binding: 1 – 10 mS/cm</td>
</tr>
<tr>
<td></td>
<td>Washing: 3 – 15 mS/cm</td>
</tr>
<tr>
<td></td>
<td>Elution: 15 – 40 mS/cm</td>
</tr>
<tr>
<td>pH</td>
<td>4.5 – 9.0</td>
</tr>
<tr>
<td>Flow rate</td>
<td>100 – 400 cm/hr</td>
</tr>
<tr>
<td>Sample load</td>
<td>1 – 10 gm/L</td>
</tr>
</tbody>
</table>
# PolyQuat and PolyCSX Media Characteristics

<table>
<thead>
<tr>
<th>Name</th>
<th>Functionality</th>
<th>Ion Exchange Capacity, meq/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>PolyQUAT</td>
<td>Mixed mode – Primarily Strong Anion exchanger with weak anion exchange sites</td>
<td>0.15 (Weak anion)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.25 (strong anion)</td>
</tr>
<tr>
<td>PolyCSx</td>
<td>Mixed mode- Primarily Strong cation exchanger with weak cation and anion exchange sites</td>
<td>0.15-0.25 (strong cation)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.15-0.25 (weak cation)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.05-0.12 (anion)</td>
</tr>
</tbody>
</table>
PolyQuat and PolyCSX Media
Characteristics

• Hydrophilic Polymeric Backbone
  • Good Mechanical Properties and Ease in Handling
  • Wide Operating Range and Stability
    – pH range of 2-12
    – Pressure up to 150 PSI and Flow up to 1200 cm/hour

• Chemistry and Functionality
  • Polymer With Hydrophilic Spacer*
  • Covalently Linked PEI (MW 600 - 100,000)
    – Functionalize From PEI Spacer*
      » Weak and Strong Anion, Cation and Mixed Exchange sites
  • Mixed mode characteristics
    – Capacity, Selectivity and Separation Efficiency

* Patent pending
Particle Size Distribution of Polymeric Mixed Mode Ion Exchanger

- 401. Initial sample in storage solution.
- 402. after column packing-unpacking simulation (20 cycles)
- 404. Supernatant collected during the column packing from the reservoir.
- 405. Supernatant from the initial sample.

No fragments generated after simulated column packing unpacking
No fine particles in supernatant
Separation of P8 Protein Using PolyQUAT: First Step

Sample: cell culture media, dialyzed against binding buffer before loading
Flow rate: 115 cm/hr
Sample load: 10 gm/L
Comparison of Media in First Step

- Conventional strong anion exchanger exhibited lower binding capacity and lower level in product purity as compared to PolyQUAT
  - (left) Conventional strong anion exchanger and
  - (right) PolyQUAT strong anion exchanger
Second Purification Step with PolyCSx

Binding: 7 mS/cm acetate pH 4.7. Elution: 1 M NaCl in binding buffer
Sample: 5.1 g/L P8 protein. Flow rate: 300 cm/hr

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Specialty Products

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Gradient and Step Elution Comparison

<table>
<thead>
<tr>
<th>Method</th>
<th>Procedure</th>
<th>Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gradient</td>
<td>0 to 100% elution buffer (1.0 M NaCl) in 10 CV</td>
<td>&gt; 98%</td>
</tr>
<tr>
<td>Step</td>
<td>(1) 50 mM NaCl for 5 CV. (2) 200 mM NaCl for 5 CV. (3) Elution buffer</td>
<td>95%</td>
</tr>
</tbody>
</table>

- Injection 5.1 g/L P8 protein with 7.12 mS/cm
- Flow rate 300 cm/hr
Selectivity Comparison of Different Cation Exchangers

Separation of P8 protein isomers on PolyCSx
Sample load: 1.5 gm/L. Flow rate: 115 cm/hr
Selectivity Comparison of Different Cation Exchangers

- P8 Protein separated on conventional exchangers A (left) and B (right)
  - Binding: 1 mS/cm acetate pH 4.7. Elution: 1 M NaCl in binding buffer
  - Sample: 1.5 gm/L P8 protein per injection
Selectivity Comparison of Different Cation Exchangers

- P8 Protein separated on conventional exchangers C (left) and D (right)
  - Binding: 1 mS/cm acetate pH 4.7
  - Elution: 1 M NaCl in binding buffer
  - Sample: 1.5 gm/L P8 protein per injection

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Conclusions

- Change in culture media components requires different purification strategy

- Optimized process produced highly purified protein products
  - Step I - PolyQUAT - Protein purity >95%, yield > 80%
  - Step II - PolyCSx - Protein purity >98%, yield > 70%

- Improved process throughput resulting from higher capacity and selectivity of PolyQUAT and PolyCSx
  - High flow rate (>300 cm/hr)
  - High Sample Loading ( greater than 10gm/L)
  - Direct loading in second step at high ionic strength