Applications and Properties of New Polymeric Mixed Mode Cation Exchange Chromatography Media

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PREP-2004, Baltimore
Introduction

Properties and Advantages of Mixed Mode Cation Exchangers

- Unique separation capability
- High mechanical strength
  - High linear velocity
  - Column packing and unpacking
- Chemical stability
  - Wide range of operation condition
  - Long lifetime
  - Regeneration and cleaning
- Optimal particle and pore geometry
Polymer Properties

- Hydrophilic - Mechanical Strength – Porosity
- Particle Size: 35um and 90um and Pore Size: 500 A
- High Exclusion Limit of $10^6$ MW - Ligand-Pore : Accessibility

Mixture of Thyroglobulin, IgG, BSA and Lysozyme
Physico-Chemical Characterization of Weak Cation Exchanger (Poly-ABx)

- Cation Exchange Capacity 0.23 mM/ml
- Anion Exchange Capacity 0.12 mM/ml
- Starting Anion Exchange Capacity 0.36 mM/ml

Mixed Mode functionality: Frontal Chromatography
Effect of Linear Velocity and Mobile Phases on Pressure Drop

Pressure Profile on PolyABx

- Water
- 6M Urea
- 1.5 M NaCl
- 1 M NaCl, pH 5.6

Particle size: 35 µm
Column dimension: 14 cm X 1.6 cm ID

Rigid mechanical strength and insignificant mobile phase effect
Compressibility of PolyABx in Various Solutions

<table>
<thead>
<tr>
<th>Media</th>
<th>Water</th>
<th>6 M urea</th>
<th>0.1 N NaOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resin Volume (%)(^1)</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Packing Velocity (cm/hr)</td>
<td>1074</td>
<td>1074</td>
<td>1074</td>
</tr>
<tr>
<td>Packed Column Volume (%)(^2)</td>
<td>87.29</td>
<td>86.44</td>
<td>86.44</td>
</tr>
<tr>
<td>Expanded Column Volume (%)(^3)</td>
<td>89.83</td>
<td>88.14</td>
<td>92.37</td>
</tr>
</tbody>
</table>

The packing pressure in all three cases is about 46-61 psi.

1 In storage solution bottle.
2 All the columns were packed using a slurry ratio 1:12.
3 Packed volume after removal of adaptor pressure.

Column dimension: 20 cm X 1.6 cm ID, particle size: 35 µm.
Particle Size Distribution of Polymeric Mixed Mode Cation Exchanger

- 401. Initial sample in storage solution.
- 402. Initial sample stirred and washed with excess distilled water in a sintered glass funnel up to 20 times for imitation column packing and unpacking.
- 404. Supernatant collected during the column packing from the reservoir.
- 405. Supernatant from the initial sample.

No fragments found after excessive stirring (column packing)

No fine particles found in supernatant
Effect of Linear Velocity on Lysozyme Capacity: Weak Cation Exchanger (ABx)

Column = 4.6X50 mm, 1mg/ml Lysozyme in 20mM NaOAc pH 6.2
Effect of Linear Velocity on IgG Capacity of PolyABx

Breakthrough Capacity, mg/ml
Saturation Capacity, mg/ml (Incomplete up to 70%)

Column = 4.6X50 mm, 1mg/ml IgG in 20mM NaOAc pH 5.6
Reproducibility of PolyABx Column

- Reproducibility of protein separation

Sample: 0.25 mg IgG and 0.12 mg lysozyme

Red: 1st injection; Blue: 50th injection
Stability of Baker PolyABx Column

Column: PolyABx 10X10 mmID washed with 0.1 M NaOH at 1 ml/min under room temperature (0.13 CV/min, or 76 cm/hr).

Sample: 0.25 mg IgG and 0.12 mg lysozyme

Red: initial column; Blue: 24 hours; Green: 48 hours

Stability to NaOH
Stability of Baker PolyABx Column

Column: PolyABx 10X10 mmID washed with 10 mM H₃PO₄ at 1 ml/min under room temperature (0.13 CV/min, or 76 cm/hr).
Wash: 360 CV (180Cycles)
Sample: 0.25 mg IgG and 0.12 mg lysozyme
Red: initial column;
Blue: 48 hours;

Stability to H₃PO₄
Stability of Baker PolyABx Column

- **Capacity (mg/ml polymer)**
  - **Breakthrough**
    - Fresh: 50.6
    - H$_3$PO$_4$ washed: 50.3
    - NaOH washed: 45.4
  - **Saturation**
    - Fresh: 61.6
    - H$_3$PO$_4$ washed: 62.6
    - NaOH washed: 53.5

- **IgG adsorption capacities comparison (mg per ml PolyABx)**

- **Column:**
  - PolyABx
  - 10X10 mmID

- **Total Washed Volume:**
  - 360 CV, or
  - 180 cycles
Application of Cation Exchangers: Separation of P8 Protein

- **P8 Protein**
  - Important functional protein
  - Molecule Weight ~50,000, PI ~4.9

- **Comparison of Resins**
  - **PolyCSx**
    - A mixed mode strong and weak cation exchanger
  - **Other Strong Cation Exchangers**
    - Conventional exchangers A, B, C and D
Comparison of Conventional Cation Exchangers: Separation of P8 Protein

- P8 Protein separated on conventional exchangers A (left) and B (right)
- Binding: 0.02 M TEA/HAc pH 4.7. Elution: 1 M NaCl in binding buffer
- Sample: 0.3 mg P8 protein per injection
Comparison of Conventional Cation Exchangers: Separation of P8 Protein

- P8 Protein separated on conventional exchangers C (left) and D (right)

  Binding: 0.02 M TEA/HAc pH 4.7. Elution: 1 M NaCl in binding buffer

  Sample: 0.3 mg P8 protein per injection
Separation of P8 Protein Using PolyCSx

- P8 Protein separated on PolyCSx mixed mode cation ion exchanger
  - Binding: 0.02 M TEA/HAc pH 4.7. Elution: 1 M NaCl in binding buffer
  - Sample: 0.5 mg P8 protein per injection
Separation of P8 Protein Using PolyCSx

- P8 Protein purified on PolyCSx mixed mode cation ion exchanger
  Sample 1 – cleaned broth
Separation of P8 Protein Using PolyCSx

- P8 Protein purified on PolyCSx mixed mode cation ion exchanger
  Sample 2 – cleaned broth stored and fragmented
Conclusion

High mechanical strength
   No fragments found after repeating packing and unpacking column

Wide range of application
   pH 1-13 usage condition

Uniformity of hydrophilic polymer structure
   Less non-specific adsorption

Homogeneity in slurry
   Convenience in column packing and unpacking

Polymeric stationary phases exhibits excellent chemical stability
   Broad separation conditions
   Acid/base cleaning/regeneration
Two novel mixed mode cation exchangers were developed and demonstrated

PolyABx weak cation and anion
PolyCSx strong and weak cation

PolyABx can be operated at high linear velocity for various column dimensions to achieve a high throughput

Throughput up to 1 g h⁻¹ cm⁻²
Effective linear velocity up to 722 cm/h

High reproducibility in protein separation
No ligand leakage
Minimum irreversible protein adsorption

Unique separation performance
Separation and purification of P8 protein and fragments