Evaluation and Application of Polymer-based Novel Ion Exchange Chromatographic Media

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Enabling Characteristics

- **Unique Properties and Performance**
  - Mixed mode ion exchange characteristics
  - Improved separation capability
  - More application options

- **Higher Stability**
  - Demonstrated for operation, regeneration, cleaning and storage
  - Strong acid and base solutions useable for cleaning/sanitization
  - Optimal polymeric backbone, chemistry, structure and ligand density

- **Higher Throughput**
  - High processing velocity and capacity
  - Mechanically stable polymer beads for easy in-column packing and operation
Novel BAKERBOND™ Polymeric Products for Ionic Exchange Chromatography

<table>
<thead>
<tr>
<th>Name</th>
<th>Functionality</th>
<th>Ion Exchange Capacity, meq/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>PolyPEI</td>
<td>Weak anion exchanger</td>
<td>0.3-0.4 (anion)</td>
</tr>
<tr>
<td>PolyABx</td>
<td>Mixed mode- Primarily weak cation exchanger with weak anion exchange sites</td>
<td>0.15-0.25 (cation) 0.05-0.12 (anion)</td>
</tr>
<tr>
<td>PolyCSx</td>
<td>Mixed mode- Primarily strong cation exchanger with weaker cation and anion exchange sites</td>
<td>0.15-0.25 (strong cation) 0.15-0.25 (weak cation) 0.05-0.12 (anion)</td>
</tr>
<tr>
<td>PolyQUAT</td>
<td>Mixed mode- Primarily strong anion exchanger with weaker anion exchange sites</td>
<td>0.15 (weak anion) 0.25 (strong anion)</td>
</tr>
</tbody>
</table>

- Product series covers most application areas of ion exchange chromatography
Novel BAKERBOND™ Polymeric Products for Ionic Exchange Chromatography

- Schematic ligand chemical structure on PolyPEI (Upper left), PolyABx (Upper right), PolyCSx (Lower left) and PolyQUAT (lower right)
Mechanical Stability -- Macropore Size and Narrow Particle Size Distribution

Mixture of Thyroglobulin, IgG, BSA and Lysozyme

- Uniform Macro Inner Pore Structure

- Particle Size Distribution and Mechanical Stability against destruction and breakage during mechanical processing

Red: Initial polymer slurry (in 20% ethanol)
Pink: polymer slurry (1:2) after recirculated 80 cycles through a diaphragm pump at 1220 ml/min
Green: polymer slurry (1:4) after recirculated 80 cycles
Mechanical Property – Easy and Reproducible Column Packing

PolyABx Columns evaluated by 1% acetone (left) and 1 M NaCl (right)
PolyABx was packed in a XK16 column (120X16 mmID) with 1:4 slurry under 500 cm/hr

Reproducibility in PolyABx Columns packing (test by NaCl)

<table>
<thead>
<tr>
<th>Test</th>
<th>efficiency</th>
<th>asymmetry</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9391</td>
<td>1.14</td>
</tr>
<tr>
<td>2</td>
<td>10551</td>
<td>1.11</td>
</tr>
<tr>
<td>3</td>
<td>10790</td>
<td>1.18</td>
</tr>
<tr>
<td>4</td>
<td>10820</td>
<td>1.02</td>
</tr>
</tbody>
</table>
Stability Determination and Evaluation

Test

- Stability in binding buffer
- Stability in storage solution
- Stability in alkali and acid
- Static (storage) stability
- Dynamic (usage) stability
- Stability at 47°C
- Stability at room temperature
- Stability at 4°C
- Biologic stability and clearance

Method

- pH monitoring
- UV spectrum
- FT-IR spectrum
- Lyophilization
- Particle size analysis
- Total organic carbon
- Elementary analysis
- Protein binding capacity
- Separation performance
- Visual check
Stability of PolyPEI in Various Conditions – Separation Performance and Protein Binding Capacity

Testing Conditions (one year)

Sample 1: PolyPEI in proprietary storage solution at 4°C for 11 months
Sample 2: PolyPEI in proprietary storage solution at room temperature for 11 months
Sample 3: PolyPEI in 10 mM phosphoric acid at room temperature for 11 months
Sample 4: PolyPEI in 0.1 M sodium hydroxide at room temperature for 11 months
Sample 5: PolyPEI (as in sample 1) washed dynamically with 0.5 M NaOH for 48 hours at 1.0 ml/min.

- Separation Performance and BSA Binding Capacity of PolyPEI under various Conditions
Stability of PolyABx in Various Conditions -- IgG Binding Capacity

- Monitoring of Immunoglobulin Binding Capacity of PolyABx Stored in Phosphoric Acid for up to 4 months
  - Blue: 0 week; Red: 1 week; Green: 4 weeks; Cyan: 16 weeks

- IgG Binding Capacity of PolyABx under various Storage Conditions
  - A: Initial; B: storage solution 4°C for 10 months; C: 0.1 M sodium hydroxide 10 months; D: 10 mM phosphoric acid 4 months; E: storage solution 4°C 24 months; F: 10 mM phosphoric acid 24 months; G: 0.1 M sodium hydroxide 24 months.
Stability of PolyABx – Separation Performance after Dynamic Wash

**Column**: PolyABx 100X10 mmID washed with 10 mM H₃PO₄ at 1 ml/min at room temperature (0.13 CV/min, or 76 cm/hr).

- **Red**: initial column; **Blue**: 48 hours;

**Column**: PolyABx 100X10 mmID washed with 0.1 M NaOH at 1 ml/min at room temperature (0.13 CV/min, or 76 cm/hr).

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

- **Stability to H₃PO₄ (left) and NaOH (right) washing**

**Determination**: 1.5 ml/min flow rate, Binding buffer: 0.05 M MES-NaOH pH 5.6, Elution buffer: 1 M NaCl in binding buffer.
The PolyQUAT column/polymer can go through the following treatments and maintain its structure and protein binding capacity unchanged:

1. On site washed with 10 mM H₃PO₄ for 10 CV;
2. On site washed with 10 mM H₃PO₄ 10% HAc for 10 CV;
3. On site washed with 0.1 M NaOH for 10 CV
4. On site washed with 0.5 M NaOH for 10 CV
5. On site washed with 1.0 M NaOH-2.0 M NaCl for 10 CV
6. On site washed with 1.0 M NaOH-2.0 M NaCl for 10 CV at 50°C
7. Incubated in 0.5 M NaOH at 80°C for 4 hours
8. Unpacked and washed in 30 CV 0.5 M NaOH at 90°C for 3 hours with stirring

The PolyQUAT column/polymer can go through the following treatments and maintain its structure and protein binding capacity unchanged:
Stability of PolyQUAT -- Minimized Irreversible Adsorption

**Column:** PolyQUAT 50 X 4.6 mm ID

**Sample:** 2.0 mg/ml α-lactalbumin in binding buffer

**Injection:** #1 to #5

**Flow rate:** 1.0 ml/min

**Binding buffer:** 0.05 M CAPS/NaOH pH 10

- Reproducibility of protein binding on PolyQUAT (5 injections)
Enhanced Performance of PolyABx – IgG Processing at High Velocity

IgG Adsorption Capacity under Different Linear Velocities
Column: 50X4.6 mm ID; Sample: 1.0 mg/ml IgG in pH 5.6 20 mM NaAc buffer

IgG Purification Throughput for Various Column Dimensions at Different Linear Velocities
Enhanced Performance of PolyCSx – IgG Binding at High Velocity

Effect of Flow Rate on IgG Binding Behavior

Breakthrough point was determined as 25 mAU at 280 nm (about 10% of maximum for 1.0 mg/ml IgG)
Red: 1.0 ml/min (119.4 cm/h); Pink: 3.0 ml/min (358.3 cm/h); Green: 5.0 ml/min (596.8 cm/hr); Blue: 7.0 ml/min (835.6 cm/h)
Reproducibility of PolyQUAT Column

Reproducibility of PolyQUAT Column Tested by Multiple Sample Injections

Column: 100 X 10 mmID
Flow rate: 5.0 ml/min
Binding buffer: 50 mM Tris-HCl pH 8.5;
Elution buffer: 1.0 M NaCl in binding buffer
Sample: Cytochrome c 74.1 mg, rabbit globulin 49.2 mg, β-lactoglobulin 178.9 mg, dissolved in 250 ml binding buffer. Injection volume: 3.9 ml.

Cytochrome c, rabbit globulin and β-lactoglobulin
Biological Stability -- Inhibition of Bacterial Growth

- Bacterial Growth in PolyABx / Equilibration Buffer (pH 5.6) Slurry

- Spiked Bacterial (*E. coli*) Counting in PolyABx / Equilibration Buffer (pH 5.6) Slurry (Inhibition Effect)
Application of PolyQUAT – Separation of Recombinant Protein from Cell Culture

For comparison
Conventional strong anion exchanger exhibited lower binding capacity and lower recovery in product

Separation of a recombinant protein with PolyQUAT column from cell culture
Sample: cell culture media, dialyzed against binding buffer before loading
Application of PolyCSx – Purification of Recombinant Protein from Q Column

- Scale-up purification of a recombinant protein on PolyCSx mixed mode cation exchanger

- Red: 10 mg; Blue: 20 mg; Green: 40 mg; Cyan: 60 mg; Pink: 80 mg protein. On a 7.8 ml column (100 X 10 mmID)
**DNA/Endotoxin Clearance during Protein Separation/Purification**

- **DNA clearance with PolyABx column (DNA only)**
  - Column: C10/10 7.5 ml in volume; Flow rate: 153 cm/hr (2.0 ml/min)
  - Chromatogram (upper), DNA by fluorescence (lower)
  - Log Clearance = 3.1 (Elution 1 step, DNA load 16876 µg)

### Chromatogram

- **Step** | **Description** |
  | Load | pH 5.5 50 mM sodium acetate, 20 CV |
  | Wash 1 | pH 5.5 50 mM sodium acetate, 6 CV |
  | Wash 2 | pH 7.0 50 mM sodium phosphate, 4 CV |
  | Elution 1 | pH 7.0, 50 mM sodium phosphate 0.5 M NaCl 6 CV |
  | Elution 2 | pH 7.0, 50 mM sodium phosphate 1.0 M NaCl 4 CV |
  | Clean | 0.1 M NaOH, 10 CV |
  | Recond | pH 5.6, citrate, 10 CV |
DNA/Endotoxin Clearance during Protein Separation/Purification

DNA clearance with PolyABx column
Column: C10/10 7.5 ml in volume; Flow rate: 153 cm/hr (2.0 ml/min)
Chromatogram (upper), DNA by fluorescence (lower)
Log Clearance = 3.2 (Elution step, DNA load 20880 µg)
DNA/Endotoxin Clearance during Protein Separation/Purification

- DNA clearance with PolyABx column
  - Column: C10/10 7.5 ml in volume; Flow rate: 153 cm/hr (2.0 ml/min)
  - Chromatogram (upper), DNA by fluorescence (lower)
  - Log Clearance = 3.2 (Elution step, DNA load 20880 µg)

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<td>Elution</td>
<td>pH 7.0, 50 mM sodium phosphate 0.5 M NaCl 6 CV</td>
</tr>
<tr>
<td>Regener</td>
<td>pH 7.0, 50 mM sodium phosphate 1.0 M NaCl 4 CV</td>
</tr>
<tr>
<td>Clean</td>
<td>0.1 M NaOH, 10 CV</td>
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<tr>
<td>Recond</td>
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DNA/Endotoxin Clearance during Protein Separation/Purification

DNA clearance with PolyABx column (DNA and protein mixture)

Total Proteins: BSA 33 mg, IgG 34 mg; Chromatogram (upper), DNA (lower)

Log Clearance = 2.8 (Elution 1 step, DNA load 8790 µg)
Conclusion -- Advantages of Mixed Mode Polymer Chromatographic Media

- **Stability enables various applications and operations**
  - Polymer can be used in a wide pH range (strong acid or base)
  - Inhibition to bacterial growth
  - Long term storage and sanitization using NaOH or H₃PO₄ without noticeable property changes

- **Mechanical stability to high throughput processing and manufacturing**
  - High throughput with larger allowable linear velocity
  - Highly uniform in slurry, easier for column packing and unpacking

- **Synthesized structure facilitates enhanced chromatographic performance**
  - Minimum unwanted/irreversible adsorption, effective DNA/endotoxin removal
  - Improved separation efficiency
  - Repeated up to 50-injections without any change in protein separations
  - Applications in separation and purification of recombinant proteins from cell culture

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