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New choices for protein purification

- **MEP HyperCel:**
  - Mixed-Mode Chromatography Sorbent.
  - It is a good choice for purifying Mabs and is particularly effective to replace conventional HIC.

- **HyperD Ceramic resin:**
  Ceramic HyperD® is the result of rigid composites and can be described as an established innovation.

- **Q and S HyperCel:**
  - Enhanced productivity due to high dynamic binding capacity at high flow rates (> 150 mg/mL at 2 minutes Residence time) and distinctive salt sensitivity.
Dynamic Binding Capacity (DBC)

- How much target protein will bind to the column.
- Specific to resin, protein, conditions.
- Conditions include flow rate (residence time), bead size (number of active sites, HETP), buffer chemistry (ionic strength, pH, etc), chemistry of target, concentration of target, gradient, etc.
Pall BioSepra
A part of Pall Corporation

- 4.000m² (43.000ft²) facility
- Audited to ISO9001
- Strong and diversified sorbent technology platforms
  - HyperD®, HyperCel™, HyperZ™, Trisacryl®, Ultrogel®, Spherodex
- Unique Process Proteomics & Chromatography Services
  - Accelerates process development and supports industrial customers
Pall Mixed-Mode Sorbents

HCIC:
Hydrophobic Charge Induction Chromatography

- Effective to replace conventional HIC.
- A new choice for purifying Mabs

HIC: Hydrophobic Interaction Chromatography
Mixed-Mode Ligand Family
All based on the robust HyperCel matrix.

- MEP HyperCel ➔ Binding principally by hydrophobic &/or affinity interaction.
- PPA HyperCel
- HEA HyperCel

Eluting by electrostatic charge repulsion

- Exploits multiple protein-ligand interactions to adsorb target proteins or impurities.
- Bring capture or intermediate/polishing separation options where other conventional methods (IEX, HIC) fail ▶
  - Where feedstream conductivity is too high for efficient capture on traditional ion exchange resins.
  - Purifications by Hydrophobic Interaction (HIC) that would require massive addition of salt.
  - Separations where affinity ligands too expensive.
  - Different selectivities for Mab and other protein separations.
Hydrophobic Interaction Chromatography

Covalently attached methyl, butyl, phenyl, or octyl group

Apply gradient of decreasing [salt]

Protein bound to mildly hydrophobic matrix at high salt concentration

Protein detaches from matrix at low salt concentration
Pall HyperCel™ Mixed-Mode Sorbent Family

Mabs and recombinant proteins

Alternatives to HIC, no-salt or low-salt

- MEP HyperCel
  - 80-125 µeq/mL
  - 4-Mercapto-Ethyl-Pyridine (4-MEP)
  - pKa = 4.8

- PPA HyperCel
  - 58-80 µeq/mL
  - Phenylpropyl substituent

- HEA HyperCel
  - 50-84 µeq/mL
  - n-hexyl substituent

- Ligand density > 50 µmoles /mL
- Critical working ligand pKa = 8

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**Mixed-mode Chromatography (HCIC)**

- Proteins bind by hydrophobic interaction and other interactions.
- No salt needed to promote adsorption
- Desorption is achieved by decreasing the pH of the mobile phase
Interaction of 4-MEP Ligand with Antibody

- Adsorption at near-neutral pH
  - pKa = 4.8

- Desorption at pH 4.0 – 5.8
  - Desorption at pH 4

<table>
<thead>
<tr>
<th>pH</th>
<th>% in (+) Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.8</td>
<td>50%</td>
</tr>
<tr>
<td>5.8</td>
<td>10%</td>
</tr>
</tbody>
</table>

Hydrophobic interaction

Electrostatic Repulsion

phenyl group
Optimization of Elution-pH on MEP HyperCel

- pH 5.5: Elution of relatively basic proteins.
- pH 5.2: Elution of relatively hydrophilic proteins.
- pH 4.9: Elution of relatively basic proteins.
- pH 4.6: Elution of relatively acidic proteins.
- pH 4.3: Elution of relatively hydrophobic proteins.

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Mixed-Mode Applications

- Mixed-mode is an alternative to conventional hydrophobic interaction, with no or less salt addition.
- Mixed-Mode as a replacement for affinity chromatography.
- MEP HyperCel can be used for Mab capture or intermediate step. HEA HyperCel can be used also for intermediate Mab purification or even capture step in some cases.
- Cost-effective vs. Affinity on protein A sorbents
- Increasing number of « non antibody » applications. (aggregate, DNA and HCPs removal).
Case 1: MEP HyperCel™ as HIC replacement

Advantages of Mixed-Mode Chromatography

Used as an HIC resin alternative to purify a protein produced in E. coli, Pall MEP HyperCel sorbent gave excellent results. Capacity and yield were high (around 100 mg/ml), the protein active, and the method truly scalable. We appreciate the MEP dual-mode separation mechanism, and we are now systematically including MEP and other PALL mixed-mode sorbents in our resins screening.
### Case 2: Compared with Conventional HIC

**140mPBS, pH 7.4**

<table>
<thead>
<tr>
<th>Resin</th>
<th>Protein</th>
<th>Injection (mg)</th>
<th>FT (mg)</th>
<th>Binding (mg)</th>
<th>Elution (mg)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEA</td>
<td>BSA</td>
<td>20</td>
<td>0</td>
<td>20</td>
<td>18.5</td>
<td>98</td>
</tr>
<tr>
<td>PPA</td>
<td>BSA</td>
<td>20</td>
<td>0</td>
<td>20</td>
<td>19.5</td>
<td>98</td>
</tr>
<tr>
<td>Hexyl HIC</td>
<td>BSA</td>
<td>20</td>
<td>14.8</td>
<td>5.2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Phenyl HIC</td>
<td>BSA</td>
<td>20</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>不結合</td>
</tr>
<tr>
<td>HEA</td>
<td>Albumin</td>
<td>20</td>
<td>0</td>
<td>20</td>
<td>16.6</td>
<td>83</td>
</tr>
<tr>
<td>PPA</td>
<td>Albumin</td>
<td>20</td>
<td>0</td>
<td>20</td>
<td>14</td>
<td>70</td>
</tr>
<tr>
<td>Hexyl HIC</td>
<td>Albumin</td>
<td>20</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>不結合</td>
</tr>
<tr>
<td>Phenyl HIC</td>
<td>Albumin</td>
<td>20</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>不結合</td>
</tr>
<tr>
<td>HEA</td>
<td>α-淀粉酶</td>
<td>20</td>
<td>16.8</td>
<td>3.2</td>
<td>3.8</td>
<td>100+</td>
</tr>
<tr>
<td>PPA</td>
<td>α-淀粉酶</td>
<td>20</td>
<td>0</td>
<td>20</td>
<td>20</td>
<td>100</td>
</tr>
<tr>
<td>Hexyl HIC</td>
<td>α-淀粉酶</td>
<td>20</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>不結合</td>
</tr>
<tr>
<td>Phenyl HIC</td>
<td>α-淀粉酶</td>
<td>20</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>不結合</td>
</tr>
</tbody>
</table>

**Chymotrypsin**
Case 3: Purification of Intracellular zinc metaloenzyme

PEI-Carried Supernatant from 100 L Fermentation (~4 g/L) Applied to 15 L Column of MEP HyperCel

Equilibration & Initial Post-Load Wash: 25 mM Na phosphate, 0.5 M Am$_2$SO$_4$, pH 7.2

Purity > 95%, Recovery 75 – 116%

Courtesy of Dr. Andrew Lees, Fina Biosolutions, Rockville, MD
Case 4: Purification of Hydrogenase by HEA HyperCel and Phenyl Toyopearl 650 M

- **HEA HyperCel**
  - **Injection:** PBS + **1M NaCl**, pH 7.2
  - **Elution:** pH steps: pH 5.0, 4.0, 3.0. (+ 50 mM Na acetate) 2-3CV/pH.
  - **Result:** no Flowthrough ~ 95% recovery pH 4.0, 50mM NaAc

- **Phenyl Toyopearl 650 M**
  - **Injection:** PBS + **1.5M (NH4)2SO4**, pH 7.2
  - **Elution:** PBS from 1.5 M to 0 M (NH4)2SO4 24 CV
  - **Result:** No Flowthrough Salt Concentration: 0.4 M (NH4)2SO4
**Mixed-Mode Chromatography used as alternative to HIC**

*Typical findings from various case studies*

- Accomplish binding at reduced salt concentration vs. conventional HIC.
- Use low-cost, environmentally-friendly salt
- Recover product in dilute buffer, or buffer of moderate conductivity.
- Control chromatography based on both pH and salt concentration.
- High product recovery and often better capacity.
Mixed-Mode Applications

- Mixed-mode is an alternative to conventional hydrophobic interaction, with no or less salt addition.
- Mixed-Mode as a replacement for affinity chromatography.
- MEP HyperCel can be used for Mab capture or intermediate step. HEA HyperCel can be used also for intermediate Mab purification or even capture step in some cases.
- Cost-effective vs. Affinity on protein A sorbents
- Increasing number of « non antibody » applications. (aggregate, DNA and HCPs removal).
Case 5: Mixed-Mode as a replacement for affinity chromatography

- New challenging chromatographic method for the purification of recombinant proteins fused with maltose-binding protein in *E. coli*


- EA4135, ESTBB, Université Victor Segalen Bordeaux2, 146 rue Léo Saignat, 33076 Bordeaux, France

Mixed-mode for the purification of recombinant proteins fused with maltose-binding protein (MBP) in E. coli

<table>
<thead>
<tr>
<th>Resin</th>
<th>MBP-LacZ</th>
<th>MBP-Leap2</th>
<th>MBP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextrin agarose</td>
<td>3.6</td>
<td>3.9</td>
<td>4.5</td>
</tr>
<tr>
<td>Amylose resin</td>
<td>4.2</td>
<td>4.0</td>
<td>3.3</td>
</tr>
<tr>
<td>Amylose resin HF</td>
<td>4.4</td>
<td>4.1</td>
<td>3.4</td>
</tr>
<tr>
<td>Mixed-mode PPA</td>
<td>24.7</td>
<td>25.2</td>
<td>26.1</td>
</tr>
<tr>
<td>Mixed-mode HEA</td>
<td>22.5</td>
<td>23.2</td>
<td>24.2</td>
</tr>
</tbody>
</table>
## Recovery

<table>
<thead>
<tr>
<th>Resin</th>
<th>MBP-LacZ</th>
<th>MBP-Leap2</th>
<th>MBP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextrin agarose</td>
<td>60%</td>
<td>61%</td>
<td>62%</td>
</tr>
<tr>
<td>Amylose resin</td>
<td>49%</td>
<td>51%</td>
<td>51%</td>
</tr>
<tr>
<td>Amylose resin HF</td>
<td>50%</td>
<td>52%</td>
<td>53%</td>
</tr>
<tr>
<td>Mixed-mode PPA</td>
<td>82%</td>
<td>83%</td>
<td>85%</td>
</tr>
<tr>
<td>Mixed-mode HEA</td>
<td>81%</td>
<td>81%</td>
<td>83%</td>
</tr>
</tbody>
</table>
Mixed-Mode Applications

- Mixed-mode is an alternative to conventional hydrophobic interaction, with no or less salt addition.

- Mixed-Mode as a replacement for affinity chromatography.

- MEP HyperCel can be used for Mab capture or intermediate step. HEA HyperCel can be used also for intermediate Mab purification or even capture step in some cases.

- Cost-effective vs. Affinity on protein A sorbents

- Increasing number of « non antibody » applications. (aggregate, DNA and HCPs removal).
**MEP in Antibody Harvest and Purification**

- **Direct Immunoglobulin Capture from a Variety of Feedstocks**
  - cell culture supernatant (CCS) with FBS/Protein-free CCS/Ascites fluid
- **Concentration of dilute samples is not necessary**
  - No significant variation in capacity: 50 μg/mL to 5 mg/Ml
  - Efficient capture as 50 to 100 μg IgG/mL
- **IgG Elution in Mild Conditions:**
  - IgG is typically eluted in the pH 5.5 to 4.0 range.
  - Milder elution compared to Protein A (pH < 5.0 down to 2.5)
- **Can purify all MAbs like (IgG, IgA, IgM)**
- **Allows selective desorption of Mab from contaminants (HCPs)**
- **Separate monomeric IgG from aggregates**
- **No protein A leakage**
- **4x the life time of Protein A (Because it be cleaned with 1M NaOH)**
  - Allow stringent clean-in-place using 1M NaOH
- **Lower sorbent cost vs. affinity on Protein A/G**

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One-step Purification of IgG1 from Albumin-rich CHO Cell Culture Supernatant
(Rat IgG in 15% Fetal Bovine Serum content)

Equilibration/ Wash : PBS pH 7.4
Elution: E1  : 100 mM Na Ac pH 5.5, NaCl 0.5 M
E2  : 50 mM Na Ac pH 5.0
E3; E4 : 50 mM Na Ac pH 4.0; 3.0
Flow rate : 80 cm/h (Residence time = 7.5 min.)

Optimized elution step at pH 5.0 leads to IgG Purity 97% and yield of 93% (albumin eluted at pH 5.5 in the presence of 0.5 M NaCl)

Courtesy of S. Allen, ABBOTT Diagnostics Division, IL, USA and S. Chaudoreille, Pall Applications R&D Group, Cergy (F)
Efficient capture of antibody from highly dilute feedstock (0.04 mg/mL), without preliminary concentration.

Column: 5.0 cm ID x 17.5 cm H (V = 343 ml)
Equil / Wash: PBS pH 7.4
Load: 28 Liters (1200 mg))
Flow rate: 230 cm/h (loading) to 300 cm/h (wash + elution)
Residence time: 3.5 to 4.6 minutes

Purity and Yield 97%
Capture and Purification of Mab from Clarified Cell Culture Supernatant

Equilibration & Load: sodium phosphate, pH 7
Elution: pH Gradient, 50 mM sodium acetate, pH 5.8 – 3.8

After Optimizing:
- ~98% yield MAb
- ~410 ng/mg HCP


Web Link: http://www.biopharminternational.com/biopharm/article/articleDetail.jsp?id=423189
Example 1: Analytical data

<table>
<thead>
<tr>
<th></th>
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<th></th>
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</thead>
<tbody>
<tr>
<td>Load</td>
<td>0.14</td>
<td>183</td>
<td>-</td>
<td>17668</td>
<td>96,7</td>
<td>199</td>
</tr>
<tr>
<td>Flow through</td>
<td>0.15</td>
<td>1</td>
<td>0.5</td>
<td>5535</td>
<td>6362</td>
<td>ND</td>
</tr>
<tr>
<td>Wash 1 + 2</td>
<td>0.076</td>
<td>-</td>
<td>-</td>
<td>3240</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Elution pH 5.2</td>
<td>0.036</td>
<td>119</td>
<td>65</td>
<td>230</td>
<td>1.9</td>
<td>&lt; 50</td>
</tr>
<tr>
<td>Elution pH 3.0</td>
<td>0.028</td>
<td>7</td>
<td>4</td>
<td>983</td>
<td>133</td>
<td>&lt; 50</td>
</tr>
</tbody>
</table>

The MEP HyperCel pH 5.2 eluate showed a reasonable reduction of HCP and DNA and could be applied directly to CM Ceramic HyperD after adjustment of the pH. HCP was further reduced on the IEX column by a factor 2 (not shown).
MEP HyperCel™ Mab related communications
(more on Pallnet chromatography section!)

- A Two-Column Process to Purify Antibodies Without Protein-A

- Comparison of standard and new generation hydrophobic interaction chromatography resins in the monoclonal antibody purification process

  MEP can not only capture mAb from crude CHO fermentation supernatant but also substantially enhance mAb purification process flow efficiency when serving as a polishing role

- Development of a MEP HyperCel IgG Purification process for a Commercial Polyclonal Antibody Product.

- “Mixed-mode“ chromatography materials in downstream process development
  Felix Oehme, Klaus Kaiser, Jürgen Lenz, Jörg Peters, Bayer HealthCare AG, Global Biological Development, Wuppertal, Germany
  SPICA 2008 Zurich, CH.
Opportunities for improvements of Purification Platforms

- A unique separation mechanism and selectivity for protein separations
- A no-salt/low-salt alternative to Hydrophobic Interaction Chromatography (HIC)
- Monoclonal and polyclonal IgG capture and intermediate purification (aggregate, DNA and HCP removal)
  - Replace Protein A Capture step or implement post-Protein A purification
- Enhanced process economics

  - Saving of unit operations:
    - such as diafiltration or ultrafiltration
  - Long service life (0.5 to 1 M NaOH, 30 to 60 minutes contact time)

MEP HyperCel:
Commercial scale production of antibodies

Presented by Dr. Richard Francis, SCI Mixed Mode Conference, London, June 2007

Courtesy of Protherics, UK

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Ceramic HyperD® Ion Exchange Sorbents

<<The pioneers of high-productivity ion exchange sorbents >>
Lets Look At The Performance…

- Capacity
- Dynamic Binding Capacity (DBC) & flow rates
- Resolution studies.

AcroSep IEX Columns offer -High DBC at higher flow rates.
Better resolution at higher flow rates.

Blue dye coming out on left half
Ceramic HyperD® Ion Exchangers

- Truly rigid, not compressible
- Work 2-5 times faster than conventional resin with higher Dynamic binding capacities.
- CM grade has unique salt tolerant behaviour, can bind proteins at 15-19mS/cm, meaning limited dilution, or saving of diafiltration/TFF.
- Improves throughput, decrease column size, make more shifts per day with the same equipment…
- Saves buffer and WFI (smaller columns).
Key determinant of performance of liquid chromatography

- Composition:
  - Support matrix: rigid solids, hard gels, or soft gels
  - Particle size and structure: spherical vs. irregular
  - Pore structure: porous vs. superficially porous
  - Bonded phase (surface chemistry):
Support matrix

- Soft bead
  - High capacity
  - Compressible
  - Cellulose, dextran, and agarose

- Rigid bead
  - Non-compressible
  - Low capacity
  - Porous silica, glass, hydroxyapatite, alumina, and zirconium

- Composite bead
  - Non-compressible
  - High capacity
  - Ceramic beads filled with hydrogel
What happens if I run fast?
Increase flow rate with *conventional* gels

Flowing faster = shortens the purification time, less risk of protein degradation

Low linear flow
- Spherical bead
- Good capacity

High linear flow
- Bead compresses
- Loss of capacity!
Ceramic HyperD® - ‘Gel in a Shell’

- Porous, non-compressible ceramic bead
- >0.2 μm (2000 Å) ‘pores’
- *In situ* polymerization to form hydrogel bead, containing the functional groups
- Keep capacity at high flow rates

---

*In situ* polymerization
Ceramic HyperD® - Cross-section

- **Black**
  - Ceramic Backbone

- **White-Gray**
  - Hydrogel

- **Gray spots**
  - Gold-labeled albumin
## Ion Exchange Dynamic Binding Capacity (DBC)

<table>
<thead>
<tr>
<th>Media</th>
<th>1 ml/min</th>
<th>5 ml/min</th>
<th>10 ml/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>HyperD® Q-20</td>
<td>106.0 mg/ml</td>
<td>91.5 mg/ml</td>
<td>82.5 mg/ml</td>
</tr>
<tr>
<td>HyperD F DEAE</td>
<td>101.5</td>
<td>87.5</td>
<td>77.5</td>
</tr>
<tr>
<td>HyperD F S</td>
<td>80.5</td>
<td>61.5</td>
<td>53.5</td>
</tr>
<tr>
<td>HyperD S-20</td>
<td>98.0</td>
<td>89.5</td>
<td>83.5</td>
</tr>
<tr>
<td>HyperD F CM</td>
<td>108.0</td>
<td>87.5</td>
<td>73.5</td>
</tr>
</tbody>
</table>

- Anion Exchange: 5mg/ml BSA in 25 mM Tris pH 8.5
- Cation Exchange: 5 mg/ml Lysozyme in 10 mM MES-NaOH pH 5.8
- 1 ml column, run on ÄKTA Explorer
Performance: DBC

AcroSep Columns Provide 1.5-5.6 Times Higher DBC

1 mL AcroSep Column Average Dynamic Binding Capacity (10% Breakthrough)

- **Q**: 116 mg/mL
- **DEAE**: 62 mg/mL, 69 mg/mL
- **S**: 128 mg/mL
- **CM**: 167 mg/mL, 30 mg/mL

Conventional Columns

Flow Rate: 1.0 ml/min
Performance: DBC

At High Flow Rates, AcroSep Columns Continue to Deliver High DBC's

- AcroSep (3.56 mL/min)
- Conventional Columns

3.56ml/min

mL AcroSep Average Dynamic Binding Capacity (10% Breakthrough)
Resolution Comparison of Acrosep Columns with Q HyperD F and Conventional Q

Resolution: Ability to separate proteins from one another

Resolution at 1 mL/min
- AcroSep Q Hyper D
- AcroSep Q Hyper D
- AcroSep Q Hyper D
- Conventional Q

Flow Rate of: 1 ml/min

% Elution Buffer

BSA and Conalbumin

Flow rate: 1 mL/min.
Flow rate: 4 mL/min.

Resolution at 4ml/min
- AcroSep Q Hyper D
- AcroSep Q Hyper D
- AcroSep Q Hyper D
- Conventional Q

Break Through
% Elution Buffer

Flow Rate of: 4ml/min
CM Ceramic HyperD®: Salt-Tolerant Antibody Capture

- A salt tolerant cation exchanger.
- Direct antibody capture at moderate conductivity (15-19 mS/cm). About 180mm NaCl.
  - Limits feedstock dilution (no diafiltration).
- Combines easily to other capture or intermediate steps (e.g. mixed-mode on MEP HyperCel sorbent)
- Mab capacity 40-100 mg/mL depending on feedstock, binding pH and antibody type.
- Rigid: High flow rates and mechanical stability.
Summary Ceramic HyperD® Ion Exchangers

- Truly rigid, not compressible, >15 years on the market.
- High Dynamic binding capacities: Work 2-5 times faster
  - Rapid harvest of large volumes of feedstock
    - Decrease shift time or make more shifts per day
    - Facilitate operation using columns of moderate volume, decrease WFI and buffer consumption.
- **Result = Process economics benefits**
- CM grade has unique **salt tolerant behaviour**, can bind proteins at 15-19mS/cm, meaning limited dilution, or saving of diafiltration/TFF.
- Improve throughput, decrease column size, make more shifts per day with the same equipment…
Q and S HyperCel™ Ion Exchange Sorbents

« Increase Productivity and distinctive selectivities for Bioseparations »
### Q and S HyperCel Sorbents

<table>
<thead>
<tr>
<th></th>
<th>Q HyperCel</th>
<th>S HyperCel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle size (µm)</td>
<td>60-90</td>
<td>60-90</td>
</tr>
<tr>
<td>Ionic groups (µeq/mL)</td>
<td>99 - 138</td>
<td>54 - 84</td>
</tr>
<tr>
<td>Dynamic binding capacity (mg/mL)</td>
<td>160-198 (^{(1)})</td>
<td>134-190 (^{(2)})</td>
</tr>
<tr>
<td>Working pressure (^{(3)})</td>
<td>&lt; 1.2 bar (17 psi)</td>
<td>&lt; 1.3 bar (19 psi)</td>
</tr>
<tr>
<td>Working pH</td>
<td>1 - 13</td>
<td></td>
</tr>
<tr>
<td>Cleaning pH</td>
<td>1 - 14</td>
<td></td>
</tr>
<tr>
<td>Recommended cleaning conditions (^{(4)})</td>
<td>0.5 - 1 M NaOH</td>
<td></td>
</tr>
</tbody>
</table>

- \(^{(1)}\) Determined using 5 mg/mL BSA in 50 mM Tris-HCl, pH 8.5 at 2 min residence time.
- \(^{(2)}\) Determined using 5 mg/mL hIgG in 50 mM sodium acetate, pH 4.7.
- \(^{(3)}\) At 1,000 cm/h in a Pall LRC column (1.5 cm ID x 20 cm) in above buffers.
- \(^{(4)}\) Injection of 5 column volumes (CV) of 0.5 - 1 M NaOH, 1 hour contact time.
Selectivity of Q and S HyperCel™ Sorbents
Distinctive Protein Selectivity and Salt Sensitivity

Anion exchangers:

Protein Mix:

Q sorbents:
Cytochrome C, Human Transferrin, Bovine Serum Albumin

Cation exchangers

S sorbents:
Cytochrome C, Lysozyme, Ovalbumin
Chromatography of *E. coli* Lysate on Three Anion Exchange Sorbents

- **Rigid Agarose Q and Q Ceramic HyperD F sorbents:**
  - GFP begins eluting at 0.1 M NaCl. Essentially complete by 0.2 M NaCl

- **Q HyperCel sorbent:**
  - GFP eluted at 0.1 M NaCl. Nearly complete elution at 0.1 M NaCl.
Q and S HyperCel™: Summary

- Q and S HyperCel have distinctive selectivity and specific salt sensitivity that bring new purification options.
- They offer enhanced Productivity due to their high Dynamic Binding Capacity and excellent Flow rate properties.
- 1 M NaOH, 300 cycles for CIP.
Sorbents: Supporting Your Teams Efforts

Thank You
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