POROS™ Chromatography Resin: High Performance Tools for Downstream Purification

Column Packing

The world leader in serving science
Outline

• Column Packing Procedures
  • Technical Recommendations & Best Practices
  • Procedural Outline
  • Column Qualification Points to Consider
  • Application Examples
• Addendum
  • Qualification Study Results
  • POROS XQ Qualification Study
  • Pressure vs Flow Curves
  • Particle Size Distribution Examples
  • Radial Flow Example
POROS® Chromatography Resin: Product attributes

- Polystyrene-Divinylbenzene Backbone
  - Rigid, Incompressible
  - Easy Handling
  - Robust Chemical Stability
- Perfusion Chromatography
  - Pore Structure with Large Throughpores
  - Unlocks Interior of Bead
  - Increased Convective Flow, Reduced Diffusional Limitations
  - Improved Mass Transfer, More Efficient Purification
- 50 Micron Particle Size
  - Superior Resolution
  - Excellent Pressure-Flow Properties
  - Fully Scalable
Column Packing and Scaling with POROS resins

- Beads are mechanically rigid and incompressible
- Can be packed in low-pressure glass columns or in high pressure stainless steel columns
- Lack of wall support with increasing column diameter has minimal impact: beads support themselves
- Flexible column-packing approaches and consistent and robust results
- Beads do not desiccate, therefore non-traditional methods can be used to exchange the shipping solution
- Willing to conduct on-site packing demonstrations
Flexible Column Packing Approaches and Consistent Robust Results

- Packing methods are flexible
  - Pack-in-Place/Stall Pack: Up to pressure limits of the column
  - Traditional Flow Pack: Target flow rate 50% greater than process maximum
  - Axial Compression: Up to pressure limits of column, bed will dictate axial compression limits

- A range of packing solutions can typically be used, i.e. water, sodium chloride, hydroxide

- Numerous customers have stated that POROS resin is the easiest media to pack at large scale. Packing is straightforward and robust leading to a well defined, reproducible chromatography process
Technical Recommendations and Best Practices

- Materials supplied as a 55% slurry in 20% ethanol or buffered ethanol
  (1.8 L slurry = 1 L packed bed, ~60% gravity settled)
- Packing factor: 1.06 (1.08 for POROS XQ)
  - Recommended to account for the difference in bed volume between a gravity-settled bed and a 3 bar pressure-packed bed
- Recommended frit size: 10-23 µm
- For best results, use a column tube or column fitted with an extender large enough to contain the entire slurry so that the bed can be packed all at once. Funnel-like column packing devices do not work well for packing POROS resins
Technical Recommendations and Best Practices

• Condition the column in upflow during packing, if the column will be run in upflow during the process for any step

• POROS beads have a skeletal density similar to the density of water and do not settle rapidly. Do not allow the resin to gravity-settle in the column before packing

• Avoid mixing that will result in shear
  • Magnetic stir bars
  • Mixing unslurried material with force or grinding
1. Exchange Packing Solution
   • Allow resin to settle >4 hours between exchanges
   • Decant supernatant and replace with equal volume of packing solution (Recommend 0.1 M NaCl)
   • Repeat 2x

2. Determine Slurry Ratio (SR)
   • Determine slurry ratio after final exchange by sampling 100 ml of slurry from vessel into a graduated cylinder and allowing to settle
   • Volume of resin/ Total Volume = Slurry Ratio (SR)
3. Calculate Required Slurry Volume (RSV)
   - Required Slurry Volume (RSV) = Target Column Volume / Slurry Ratio (SR) x Packing Factor (PF) of 1.06
   - Example: To pack a 25L Column (40cmD x 20cmL)
     - RSV = 25L / 0.57 (SR) x 1.06 (PF) = 46.5L Required Slurry Volume
   - Packing Factor is the difference between the volume of loose gravity settled media and the dimensional volume of a 3 bar packed column

4. Adjust top flow adapter as required
5. Pour or pump to deliver the required slurry to the column

6. Pack as required

7. Adjust top flow adapter to desired bed height depending on column hardware, if required

8. Flow condition column with 3-5 CVs of packing buffer
   - If the column will be run in upflow during the manufacturing process, condition the bed in upflow with an additional 2-3 CVs of packing buffer
   - Initial 1-2 CVs of column effluent may be turbid

9. Qualify column
Column Packing with POROS Resin:

Flow pack followed by Axial Compression in Pack-in-Place Column

Fix top adaptor about 2x above desired bed height and fill column with slurry solution.

 Deliver slurry to column at a slow flow rate, ~100cm/H.

Flow Pack column at desired LFR up to pressure limits of column/system.
Column Packing with POROS ® Resin:

Flow pack followed by Axial Compression in ‘Pack-in-Place Column

Move top adaptor to final bed height

Unpack with exchange of slurry solution through top and bottom nozzles

Continue to unpack
To qualify the integrity of a packed column, determine HETP and asymmetry using a non-binding analyte (a “plug”).

**Column Qualification: Points to Consider**

**Common plug solutions**

<table>
<thead>
<tr>
<th>Solution</th>
<th>Concentration for Pulse</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium chloride</td>
<td>0.5-1.0 M</td>
<td>Sodium chloride concentrations ≥2M NaCl are not recommended for column qualification because a shoulder will be detected on the backside of the peak and will yield erroneous results</td>
</tr>
<tr>
<td>Sodium hydroxide</td>
<td>0.5-1.0 M</td>
<td></td>
</tr>
<tr>
<td>Sodium nitrate</td>
<td>50–200 mg/mL</td>
<td>Add 1.0 M NaCl to the nitrate solutions if running on anion exchange resins</td>
</tr>
<tr>
<td>Acetone</td>
<td>1-50%</td>
<td>Use acetone only for POROS R150 and R250 resins. Do not use for POROS ion exchange resins or Protein A resins. Acetone binds to POROS resins in the absence of high organics, therefore add acetone only to an acetonitrile solution (for example, 80–90%)</td>
</tr>
</tbody>
</table>
Column Qualification: Points to Consider

• Many variables effect the column qualification results (not specific to POROS resin):
  • Injection mode
  • Flow rate
  • Qualification solutions (running and plug/pulse)
  • Injection volume
  • Column hardware
  • System configuration (tubing diameter/length, pumps, detectors)
• The results are dependent on scale and the chromatography system
• POROS resin is efficient so how the plug is introduced onto the column is how the plug will move through the column and be detected
• Performing consistent column qualification methods is critical
  • If implemented column issues can be detected (headspace formation)
  • The method can be used to study pack to pack reproducibility and reuse performance
Column Qualification: Points to Consider

- Determine the variability of your chromatography system, automated/manual method, injection mode, buffer system, column type, etc.
- Setting specifications:
  - After you define a column qualification procedure for a given system (column plus chromatography system), base the qualification acceptance criteria for reproducibility and consistency on historical values and ranges rather than on theoretical qualification results.
- Performing consistent column qualification methods is critical:
  - If implemented column issues can be detected (headspace formation).
  - The method can be used to study pack to pack reproducibility.
- Monitor peak height, width at ½ height, and peak shape in addition to asymmetry, HETP and plates.
Column Qualification: Points to Consider

• Ensure uniform column plumbing:
  • Avoid using reducers to connect different tubing sizes
  • Minimize and keep consistent the column tubing lengths between the plug solution to the column inlet and the column outlet to the detector(s)

• Use:
  • Plug volume: 1–3% of the total column volume
  • Plug concentration: 5–10 times the mobile phase concentration (for example 0.1 M sodium chloride mobile phase with a 1 M sodium chloride plug)
  • Use process equilibration buffer or 0.1 M sodium chloride as the mobile phase

• Monitor:
  • Conductivity for sodium chloride and sodium hydroxide
  • Absorbance monitored for sodium nitrate and acetone
Column Qualification: Points to Consider

- Execute at the flow rate defined for the intended unit operation, typically 100–300 cm/hr
- Equilibrate with at least 2 CVs of equilibration buffer before injection
- For small scale, i.e. AKTA, inject using a sample loop on the Injection pump and flow entire run through post injection
Recommended Column Qualification Conditions

- Flow rate: operating flow rate (cm/hr)
- Equilibration buffer: 0.1 M sodium chloride
- Plug solution: 1.0 M sodium chloride
- Plug volume: 2% of column volume

POROS XQ Only Recommendations

- Flow rate: operating flow rate (cm/hr)
- Equilibration buffer: 0.5 M sodium chloride
- Plug solution: 2.0 M sodium chloride
- Plug volume: 4% of column volume
A few examples of large scale POROS columns in-place

- **US Biotech/Pharma**
  - 1mD, 3bar limit, Operated at 300cm/H
  - 1.4mD, 3 bar limit, Operated at 250cm/H
  - 80cmD, 3 bar limit, Operated at 500cm/H
  - 1.8mD, 3 bar limit, Operated at 200-250cm/H

- **EU Biotech/Pharma**
  - 1.6mD, 3 bar limit
  - 80cmD, 3 bar limit, Operated at 500cm/H
  - 1.8mD, 3 bar limit, Operated at 200cm/H

- **Asia Biotech/Pharma**
  - 2mD, 3 bar limit
## Column Packing with POROS Chromatography Resin

<table>
<thead>
<tr>
<th>Packing Description</th>
<th>Column Diameter (cm)</th>
<th>Packing Buffer</th>
<th>Slurry Ratio (%)</th>
<th>Initial Packing Flow Rate (cm/H)</th>
<th>Final Packing Pressure (bar)</th>
<th>Final Bed Height (cm)</th>
<th>HETP</th>
<th>As</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow Pack/ Axial Compression</td>
<td>40</td>
<td>0.15M NaCl</td>
<td>57</td>
<td>580</td>
<td>2.8</td>
<td>26</td>
<td>0.018</td>
<td>0.97</td>
</tr>
<tr>
<td>Axial Compression</td>
<td>40</td>
<td>0.15M NaCl</td>
<td>50</td>
<td>700</td>
<td>2.8</td>
<td>18.4</td>
<td>0.032</td>
<td>1.0</td>
</tr>
<tr>
<td>Axial Compression</td>
<td>40</td>
<td>0.5M NaCl</td>
<td>47</td>
<td>1000</td>
<td>2.5</td>
<td>19.3</td>
<td>0.047</td>
<td>1.1</td>
</tr>
<tr>
<td>Stall Pack, 3 bar Constant Pressure</td>
<td>60</td>
<td>0.15M NaCl</td>
<td>60</td>
<td>2000</td>
<td>3</td>
<td>20</td>
<td>0.015</td>
<td>1.18</td>
</tr>
<tr>
<td>Flow Pack/ Axial Compression</td>
<td>200</td>
<td>Water</td>
<td>57</td>
<td>250</td>
<td>2</td>
<td>30</td>
<td>0.037</td>
<td>1.24</td>
</tr>
</tbody>
</table>

**Flexible Column Packing Approaches and Consistent Column Qualification Results**
Effect of Gravity Settling POROS on Particle Size Distribution

Gravity settling causes a gradient in particle size distribution which may effect chromatographic performance.
POROS Column Qualification Study
Summary of Column Qualification Data Set

Study Design

**Study Goal:** To determine the parameters that affect qualification of POROS HS50 packed columns

- 10 Columns were packed
  - Column Formats: GE XK16 and Omnifit 15 (~20 cm bed height)
  - Resin: POROS HS and Agarose bead
  - Packing Buffer: 0.1M NaCl
  - Packing Flow Rate: 500 cm/hr
  - Slurry Concentration: 68.9%, buffer exchanged 3x
- 150 Qualifications runs were performed
- 5 Different running buffers studied
  - Water
  - 0.1M NaCl
  - 0.15M NaCl
  - 0.5M NaCl
  - 1M NaCl
• 3 Different salt solutions used for plug/pulse
  • 0.5M NaCl
  • 1M NaCl
  • 2M NaCl
• 4 Different plug/pulse volumes: 1%, 2%, 3%, and 4% CV
• 5 Different flow rates: 30, 60, 100, 200 and 300 cm/hr
• 3 AKTA configurations for injection (column in/out of line)
  • Sample loop for injection as well as run
  • Sample loop for injection only
  • A1 system pump injection
Multiple Datapoints were Collected to Better Understand the Effect of the Qualification Method

- The following parameters were evaluated:
  - Asymmetry
  - Retention Volume
  - HETP
  - Plates/m
  - Width at 1/2 Height
  - Peak Height
  - Peak Shape
Summary of Column Qualification Data Set: Results

- The qualification results are dramatically affected by the following parameters (in no particular order):
  - Injection mode
  - Flow rate
  - Qualification solutions (running and plug/pulse)
  - Injection volume
  - Column hardware
  - System configuration (tubing diameter/length, pumps, detectors)
Injection Mode Observations: Column In-line

- **Goal:** To determine the effect of different AKTA configurations for injection and flow rates on the diffusion of the salt plug through an XK16 column.
- **30 cm/hr flow on AKTA allows for more consistency due to data collection timing, pump sensitivity and programming delays.**
- **Plug injection through the sample loop is significantly more consistent than plug injection through the A1 system pump.**
- **Less variability is seen when entire run is executed through sample loop especially at higher flow rates.**

<table>
<thead>
<tr>
<th>Injection Mode: 2% Spike (0.8 ml)</th>
<th>Running / Plug Solutions</th>
<th>Min Flow: 30 cm/hr</th>
<th>Max Flow: 300 cm/hr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Asymmetry</td>
<td>Retention Volume (ml)</td>
<td>HETP</td>
</tr>
<tr>
<td>Loop for Injection &amp; Run</td>
<td>0.1M NaCl/1M NaCl</td>
<td>1.61</td>
<td>30.75</td>
</tr>
<tr>
<td></td>
<td>Water/2M NaCl</td>
<td>0.98</td>
<td>30.93</td>
</tr>
<tr>
<td>Loop for 0.8ml Injection Only</td>
<td>0.1M NaCl/1M NaCl</td>
<td>1.62</td>
<td>30.64</td>
</tr>
<tr>
<td></td>
<td>Water/2M NaCl</td>
<td>1.13</td>
<td>30.61</td>
</tr>
<tr>
<td>A1 Pump Injection</td>
<td>0.1M NaCl/1M NaCl</td>
<td>Not Run</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Water/2M NaCl</td>
<td>Not Run</td>
<td></td>
</tr>
</tbody>
</table>
Effect of Flow Rate on Column Qualification

- **Goal:** To determine the effect of the flow rate on the diffusion of the salt plug through an XK16 column.
- **As the qualification flow rate increases:** the qualification is less efficient on both POROS HS and Agarose Bead (system dependent).
  - Width at ½ height \(\uparrow\)
  - Peak height \(\downarrow\)
  - HETP \(\uparrow\)
  - Plates/M \(\downarrow\)
    - 54% \(\downarrow\) on POROS HS
    - 47% \(\downarrow\) on Agarose Bead
  - Asymmetry \(\uparrow\)
- **Flow rate may have even more of an effect at the larger scale:** on asymmetry with a larger tubing diameter than observed on the AKTA.
- **Therefore,** qualification results most meaningful when run at operating flow rate.
Effect of Running/Spike Solution, %CV of Spike

• Goal: To determine the effect of running/spike solution and %CV of spike on the column qualification
• An XK16 column was packed with POROS® HS
• 48 Different conditions were tested
  • 12 Solution combinations
  • 4 Injection volumes
• 72 Qualification tests were performed
• Result Summary
  • HETP
    – Min: 0.014
    – Max: 0.080
  • Plates/M
    – Min: 1242
    – Max: 7032
  • Asymmetry
    – Min: 0.45
    – Max: 2.28

<table>
<thead>
<tr>
<th>Running Buffer</th>
<th>Salt Spike: NaCl Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1M NaCl</td>
<td>1 M, Beginning</td>
</tr>
<tr>
<td></td>
<td>0.5 M</td>
</tr>
<tr>
<td></td>
<td>2 M</td>
</tr>
<tr>
<td>Water</td>
<td>0.5 M</td>
</tr>
<tr>
<td></td>
<td>1 M</td>
</tr>
<tr>
<td></td>
<td>2 M</td>
</tr>
<tr>
<td>0.1M NaCl</td>
<td>1 M, Middle</td>
</tr>
<tr>
<td>0.5MNaCl</td>
<td>1 M</td>
</tr>
<tr>
<td></td>
<td>2 M</td>
</tr>
<tr>
<td>1M NaCl</td>
<td>2 M</td>
</tr>
<tr>
<td>0.15M NaCl</td>
<td>0.5 M</td>
</tr>
<tr>
<td></td>
<td>1 M</td>
</tr>
<tr>
<td></td>
<td>2 M</td>
</tr>
<tr>
<td>0.1M NaCl</td>
<td>1 M, End</td>
</tr>
</tbody>
</table>

Table 1: Inputs each run at 1, 2, 3 and 4% CV
Column Qualification Study Summary

• Specific column qualification assay conditions resulted in wide data ranges; The observations were consistent over 10 column packs:
  • Water/0.5M NaCl Injection, 1% Injection Volume
    • Lowest HETP
    • Highest Plates
    • Severe Fronting (~0.19-0.50)
  • 0.1M NaCl/ 2M NaCl Injection, 4% Injection Volume
    • Highest HETP
    • Lowest Plates
    • Severe Tailing (>2) and Shoulder Formation
• Control conditions:
  • 0.1M NaCl/ 1.0M NaCl Injection, 2% Injection Volume
Resulting Salt Peaks from Varying Qualification Conditions are NOT Specific to POROS Resin

Both POROS® and Agarose columns were affected by the Min, Max and Control conditions similarly.
POROS HS50 is More Efficient than Agarose Resulting in Taller Narrower Peaks

Qualification Conditions:

- Running Buffer: 0.1M NaCl
- Injection Buffer: 1M NaCl
- Flow Rate: 298 cm/hr
- Injection Volume: 2%

<table>
<thead>
<tr>
<th>Type of Resin</th>
<th>POROS HS</th>
<th>Agarose Bead</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymmetry</td>
<td>1.45</td>
<td>0.71</td>
</tr>
<tr>
<td>Retention Volume (ml)</td>
<td>29.32</td>
<td>30.53</td>
</tr>
<tr>
<td>HETP</td>
<td>0.032</td>
<td>0.062</td>
</tr>
<tr>
<td>Plates/M</td>
<td>3124</td>
<td>1610</td>
</tr>
<tr>
<td>Width at 1/2 Height (ml)</td>
<td>2.78</td>
<td>4.29</td>
</tr>
<tr>
<td>Peak Height (ms/cm)</td>
<td>36.39</td>
<td>25.80</td>
</tr>
</tbody>
</table>
POROS Column Qualification Example
Same packed column, different qualification parameters

Different qualification methods will yield different results on the same packed column

0.5M NaCl Running Buffer
2M NaCl salt injection

0.1M NaCl Running Buffer
1M NaCl salt injection
Qualification Results after Reuse had Normal Variability

Asymmetry Distribution on POROS HS in XK16 over 72 Runs

<table>
<thead>
<tr>
<th>Run Number</th>
<th>As</th>
<th>HETP</th>
<th>Plates/M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Run Number</td>
<td>17</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>Average</td>
<td>1.44</td>
<td>0.034</td>
<td>3021</td>
</tr>
<tr>
<td>Min/Max Range</td>
<td>1.24-1.63</td>
<td>0.026-0.041</td>
<td>2441-3774</td>
</tr>
<tr>
<td>1 SD, 67%</td>
<td>0.09</td>
<td>0.004</td>
<td>402</td>
</tr>
<tr>
<td>1SD Range</td>
<td>1.25-1.62</td>
<td>0.029-0.038</td>
<td>2619-3423</td>
</tr>
<tr>
<td>3 SD, 99%</td>
<td>0.28</td>
<td>0.013</td>
<td>1206</td>
</tr>
<tr>
<td>3 SD Range</td>
<td>1.16-1.71</td>
<td>0.020-0.047</td>
<td>1814-4227</td>
</tr>
<tr>
<td>%CV</td>
<td>6.4%</td>
<td>13.3%</td>
<td>13.3%</td>
</tr>
</tbody>
</table>

POROS HS in XK16
1.6cmD x 20 cmL (1 Column)
Running Buffer: 0.1M NaCl
Injection Buffer: 1M NaCl
Flow Rate: 298 cm/hr
Injection Volume: 2%
Beginning, Middle and End Qualification Results were Comparable Over the 72 Runs

- No trends were observed over the 72 qualification runs
- Results were within normal variability of the system

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Asymmetry</th>
<th>Retention Volume (ml)</th>
<th>HETP</th>
<th>Plates/M</th>
<th>Width at 1/2 Height (ml)</th>
<th>Peak Height (ms/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beginning</td>
<td>2</td>
<td>1.50</td>
<td>29.40</td>
<td>0.026</td>
<td>3774</td>
<td>2.58</td>
</tr>
<tr>
<td>Middle</td>
<td>26</td>
<td>1.45</td>
<td>29.32</td>
<td>0.032</td>
<td>3124</td>
<td>2.78</td>
</tr>
<tr>
<td>End</td>
<td>60</td>
<td>1.57</td>
<td>29.02</td>
<td>0.029</td>
<td>3492</td>
<td>2.60</td>
</tr>
<tr>
<td>Post 10 Cycles</td>
<td>72</td>
<td>1.43</td>
<td>28.79</td>
<td>0.032</td>
<td>3113</td>
<td>2.73</td>
</tr>
</tbody>
</table>
Mock Run Cycling had No Effect on Qualification Results

Consistent Peak Formation and No Trends were Observed Over 10 Cycles

**POROS HS in XK16**
1.6cmD x 20 cmL

**Mock Run Cycle:**
- 5 CV 0.1M NaCl
- 5 CV 1M NaCl
- 5 CV 1M NaOH
- 3 CV 0.1M NaPO4, pH 7.0
- 5 CV 20% Ethanol

**Qualification Conditions:**
- Running Buffer: 0.1M NaCl
- Injection Buffer: 1M NaCl
- Flow Rate: 298 cm/hr
- Injection Volume: 2%
Conclusions

• Many test variables effect the column qualification results
• The results are dependent on scale, chromatography system, and consistency the qualification method is run
• POROS resin is efficient so how the plug is introduced onto the column is how the plug will move through the column and be detected
• Column reuse and mock runs do not affect the qualification results
• Qualification tests should be run at the operating flow rate to be most meaningful
• Variability of a given system needs to be determined in order to interpret the results from run to run
• Specifications set by historical data allow for the detection of deviations which could be indicative of a problem; A result could shift out of historical norms, but the qualification data may still fall within “theoretical specification”
POROS™ XQ Integrity Testing

The world leader in serving science
POROS XQ: Column Qualification Study Overview

- **Goal:** To determine the effect column qualification parameters on the results
  - XK16 column packed with POROS XQ
    - 20.1 cm bed height, 40 mL CV
- **36 Different conditions were tested**
  - 6 Solution combinations
  - 3 Injection volumes
  - 2 Flow rates
- **42 Qualification tests were performed**
  - **Result Summary**
    - HETP
      - Min: 0.017
      - Max: 0.076
    - Plates/M
      - Min: 1315
      - Max: 6082
    - Asymmetry
      - Min: 0.09
      - Max: 1.41

<table>
<thead>
<tr>
<th>Running Buffer</th>
<th>Salt Spike: NaCl Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 M NaCl</td>
<td>1.0 M</td>
</tr>
<tr>
<td></td>
<td>2.0 M</td>
</tr>
<tr>
<td></td>
<td>3.0 M</td>
</tr>
<tr>
<td>0.5 M NaCl</td>
<td>2.0 M</td>
</tr>
<tr>
<td></td>
<td>3.0 M</td>
</tr>
<tr>
<td>1 M NaCl</td>
<td>3.0 M</td>
</tr>
</tbody>
</table>

Table 1: Inputs each run at 1, 2, and 4% CV and 100 and 300 cm/hr
POROS XQ column integrity testing with different qualification solutions at 300 cm/hr

The increased ionic capacity on POROS XQ requires higher concentrations of NaCl to reduce the charge interaction during the qualification.
POROS XQ column integrity testing at different flow rates: 100 and 300 cm/hr

The flow rate of the qualification study can impact the results. This is typically most impacted by the system plumbing and tubing diameter.
POROS XQ: Column Qualification Study Summary

• Higher conductivity integrity testing solutions are required for POROS XQ column packs to achieve acceptable results
  • High ionic surface charge requires increased ionic concentration to prevent ligand/solute interaction during testing

• Peak fronting and low asymmetry values are expected with low conductivity testing solutions

• Recommended conditions:
  • Flow rate: operating flow rate (cm/hr)
  • Equilibration buffer: 0.5 M sodium chloride
  • Plug solution: 2.0 M sodium chloride
  • Plug volume: 4% of column volume

• Consistency in the setup, solutions, and operating conditions is key to successful and reproducible results
POROS® Pressure vs Flow Curves
POROS® Chromatography® Resin Pressure Flow: 40 cm Diameter Column, 0.15M NaCl

![Graph showing linear relationship between linear flow rate and column inlet pressure](image)

**POROS HS250-413 PERMEABILITY**

40cmD PALL Euroflow Column
20um Frits

- Linear Flow Rate (cm/H) vs. Column Inlet Pressure (bar)
- Blue line: Flow/Axial Pack 26cmL, $y = 0.0046x$, $R^2 = 0.9881$
- Green line: Stall Pack 20cmL, $y = 0.003x$, $R^2 = 0.9851$
POROS® Chromatography® Resin Pressure Flow: 60 cm Diameter Column, 0.15M NaCl

Note: The system pressure was not subtracted from data
POROS® Chromatography® HS Resin
Pressure Flow: 200 cm Diameter Column, water

Note: The system pressure was not subtracted from data
POROS® Chromatography® HQ Resin
Product Attributes: Pressure vs. Flow Curves

Note: The system pressure was not subtracted from data.
POROS® Perfusion Chromatography® HQ Resin
Product Attributes: Pressure vs. Flow Curves

Note: The system pressure was not subtracted from data
POROS® XS Pressure vs Flow
6.2 cm Diameter Column

POROS XS Pressure Flow Curve
Vantage 6.2cmDx19.8cmL, 12um frits, 0.1M NaCl, 3 bar pack

Pressure (bar)

Linear Flow Rate (cm/hr)

Linear pressure response
POROS® XS Pressure vs Flow
30 cm Diameter Column

**POROS XS Pressure Flow Curve**
GE BPG 30 cmD x 20.3 cmL, 23 um frits, 0.1 M NaCl, flow pack

* System pressure not subtracted
POROS® XS and HS Pressure vs Flow
1 cm Diameter Column

Pressure Flow Curve:
1cm x 20 cm, packed at 1000 cm/hr

Flow Rate (cm/hr)

Pressure (bar)

Linear pressure response
POROS® XS Pressure vs Flow
2.5 cm Diameter Column in 0.1M NaCl and water

Pressure Flow Curve:
2.5cmD x 20cmL, 20um frits, packed at 730 cm/hr

Pressure (bar)
Linear Flow Rate (cm/hr)

0.1M NaCl
dH2O

Linear pressure response
POROS™ MabCapture™ A Chromatography Resin
Pressure vs Flow Response

POROS MabCapture A PERMEABILITY
6.2cmD x 23cmL; 3bar Axial Compression Pack

**Linear Pressure Response**

$y = 0.0036x$

$R^2 = 0.9959$
POROS™ MabCapture™ A and MabCapture A Select Pressure vs flow response

1.6 cmD XK, 0.1M NaCl, 3 bar pack (1000 cm/hr)

Pressure (bar) vs Linear Flow Rate (cm/hr)

* MCA Select 22 cmL, MCA 22 cmL
POROS® XQ Performance
Pressure vs flow curve compared to POROS® XS

6.2 cmD x 20 cmL, 12 um frits, 0.1M NaCl, 3 bar pack

• Linear and predictable pressure – flow response

• Ability to operate under high linear flow rates while maintaining < 3b backpressure

ThermoFisher Scientific
Under buffer/solution conditions of low ionic strength (< 5 mS/cm), higher backpressures can be realized due to the nature and density of the anionic functional group used.
POROS® Particle Size Distribution Slides
POROS® HS Chromatography Resin
Particle size distribution

- 50um Bulk Resin: Tight Particle Size Distribution

<table>
<thead>
<tr>
<th>Population Size (n)</th>
<th>32</th>
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<tbody>
<tr>
<td>Average Particle Size (um)</td>
<td>51.6</td>
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<tr>
<td>1SD, 67%</td>
<td>11.33</td>
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<tr>
<td>1SD Range (um)</td>
<td>40 - 63</td>
</tr>
<tr>
<td>2SD, 95%</td>
<td>22.66</td>
</tr>
<tr>
<td>2SD Range (um)</td>
<td>29 - 74</td>
</tr>
<tr>
<td>Span (um)</td>
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</table>
POROS® XS Chromatography Resin
Particle size distribution

• POROS® XS Resin: Tight Particle Size Distribution

<p>| | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Population Size (n)</td>
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<tr>
<td>Average Particle Size (µm)</td>
<td>51.8</td>
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<tr>
<td>1 SD, 67%</td>
<td>11.6</td>
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<tr>
<td>1 SD Range (µm)</td>
<td>40-63</td>
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<tr>
<td>2 SD, 95%</td>
<td>23.2</td>
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<td>2 SD Range (µm)</td>
<td>29-75</td>
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<tr>
<td>Span (µm)</td>
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</table>
POROS® Chromatography Resin Attributes: HQ 50

- HQ 50 Bulk Resin Particle Size Distribution

<table>
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<tr>
<th>Population Size (n)</th>
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<tbody>
<tr>
<td>Average Particle Size (um)</td>
<td>47.1</td>
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<td>1SD, 67%</td>
<td>9.4</td>
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<td>1SD Range (um)</td>
<td>38 - 56</td>
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<td>2SD, 95%</td>
<td>18.7</td>
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<td>2SD Range (um)</td>
<td>28 - 66</td>
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<tr>
<td>Span (um)</td>
<td>38</td>
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</tbody>
</table>
POROS® Radial Flow Example
High Performance Radial Flow Chromatography

Efficiency vs. Bed Height

**Easy packing**
- Efficient packing in minutes
- Concentrated unpacking

**HP-RPC Column Formats**
- 750 L  20 cm  Ø 1.4 m  (vs. 2.40 m)
- 350 L  15 cm  Ø 1.0 m  (vs. 2.0 m)
- 30 L    6 cm  Ø 0.4 m  (vs. 1.0 m)
Innovative Processing Approaches

Combine Efficient Resin with Efficient Hardware

Increased Efficiency and Capacity at Low Pressure
References

