

The world leader in serving science

Outline

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 - Column Qualification Points to Consider
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 - Addendum
 - Qualification Study Results
 - POROS XQ Qualification Study
 - Pressure vs Flow Curves
 - Particle Size Distribution Examples
 - Radial Flow Example



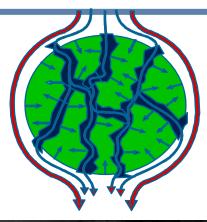
POROS[®] Chromatography Resin: Product attributes

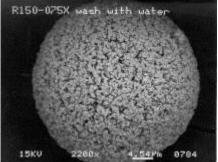
Polystyrene-Divinylbenzene Backbone

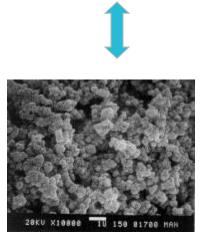
- Rigid, Incompressible
- Easy Handling
- Robust Chemical Stability
- Perfusion Chromatography
 - Pore Structure with Large Throughpores
 - Unlocks Interior of Bead
 - Increased Convective Flow, Reduced Diffusional Limitations
 - Improved Mass Transfer, More Efficient Purification

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 to 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 gravitysettled 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



POROS Column Packing Procedure

- 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)



POROS Column Packing Procedure

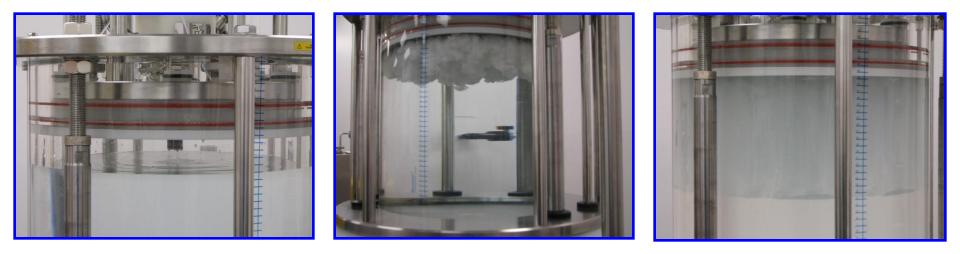
- 3. Calculate Required Slurry Volume (RSV)
 - Required Slurry Volume (RSV) = Target Column Volume / Slurry Ratio (SR) x Packing Factor (PF) of 1.06
 - Example: To pack a 25L Column (40cmD x 20cmL)
 - RSV = 25L / 0.57 (SR) x 1.06 (PF) = 46.5L Required Slurry Volume
 - Packing Factor is the difference 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

POROS Column Packing Procedure

- Pour or pump to deliver the required slurry to the column
- 6. Pack as required
- 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").

Common plug solutions

Solution	Concentration for Pulse	Comment
Sodium chloride	0.5-1.0 M	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
Sodium hydroxide	0.5-1.0 M	
Sodium nitrate	50–200 mg/mL	Add 1.0 M NaCI to the nitrate solutions if running on anion exchange resins
Acetone	1-50%	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%)

- 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

- 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



- 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

- 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



POROS Chromatography Resin: Large Scale Implementation

- 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



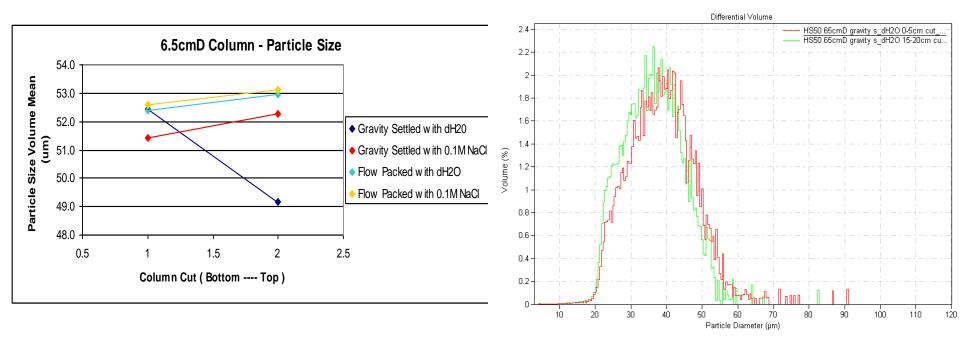
Column Packing with POROS Chromatography Resin

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

Flexible Column Packing Approaches and Consistent Column Qualification Results

Thermo Fisher

Effect of Gravity Settling POROS on Particle Size Distribution



Gravity settling causes a gradient in particle size distribution which may effect chromatographic performance

SCIENTIFIC

Addendum



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

Summary of Column Qualification Data Set: Study Design Continued

- 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

Injection Mode: 2% Spike (0.8 ml)	Running / Plug Solutions	Min Flow: 30 cm/hr				Max Flow: 300 cm/hr							
		Asymmetry	Retention Volume (ml)	HETP	Plates/M	Width at 1/2 Height (ml)	Peak Height (ms/cm)	Asymmetry	Retention Volume (ml)	HETP	Plates/M	Width at 1/2 Height (ml)	Peak Height (ms/cm)
Loop for Injection & Run	0.1M NaCl/ 1M NaCl	1.61	30.75	0.022	4465	2.37	40.95	1.62	31.50	0.034	2968	2.98	36.19
	Water/ 2M NaCl	0.98	30.93	0.027	3654	2.64	56.63	0.91	31.80	0.047	2147	3.54	44.60
Loop for 0.8ml Injection Only	0.1M NaCl/ 1M NaCl	1.62	30.64	0.018	5462	2.14	41.68	1.46	31.16	0.022	4428	2.42	29.29
	Water/ 2M NaCl	1.13	30.61	0.026	3787	2.57	54.73	0.75	31.24	0.028	3632	2.68	35.00
A1 Pump Injection	0.1M NaCl/ 1M NaCl	Not Run						1.68	33.68	0.030	3377	2.99	15.80
	Water/ 2M NaCl	Not Run						0.5	33.02	0.024	4088	2.66	10.3

Thermo Fisher

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 ↑
 - Peak height \downarrow
 - HETP ↑
 - Plates/M \downarrow
 - 54% \downarrow on POROS HS
 - 47% \downarrow on Agarose Bead
 - Asymmetry ↑
- 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
- \bullet An XK16 column was packed with POROS $^{\ensuremath{\mathbb{R}}}$ 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 1: Inputs each run at 1, 2, 3 and 4% CV

Running Buffer	Salt Spike: NaCl Concentration				
	1 M, Beginning				
0.1M NaCl	0.5 M				
	2 M				
	0.5 M				
Water	1 M				
	2 M				
0.1M NaCl	1 M, Middle				
	1 M				
0.5MNaCl	2 M				
1M NaCl	2 M				
	0.5 M				
0.15M NaCl	1 M				
	2 M				
0.1M NaCl	1 M, End				

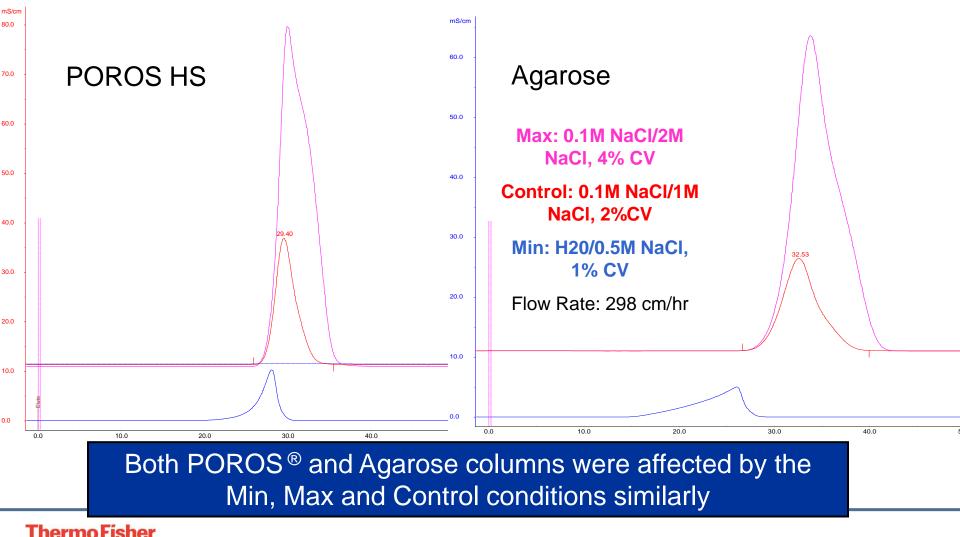
Thermo Fisher

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

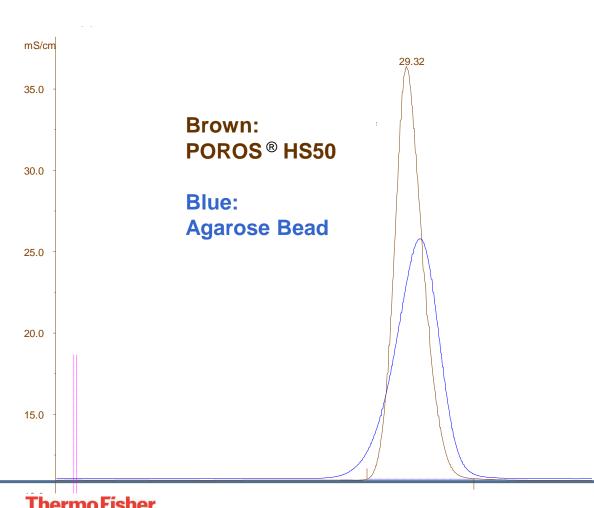


Resulting Salt Peaks from Varying Qualification Conditions are NOT Specific to POROS Resin



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POROS HS50 is More Efficient than Agarose Resulting in Taller Narrower Peaks



CIENTIFIC

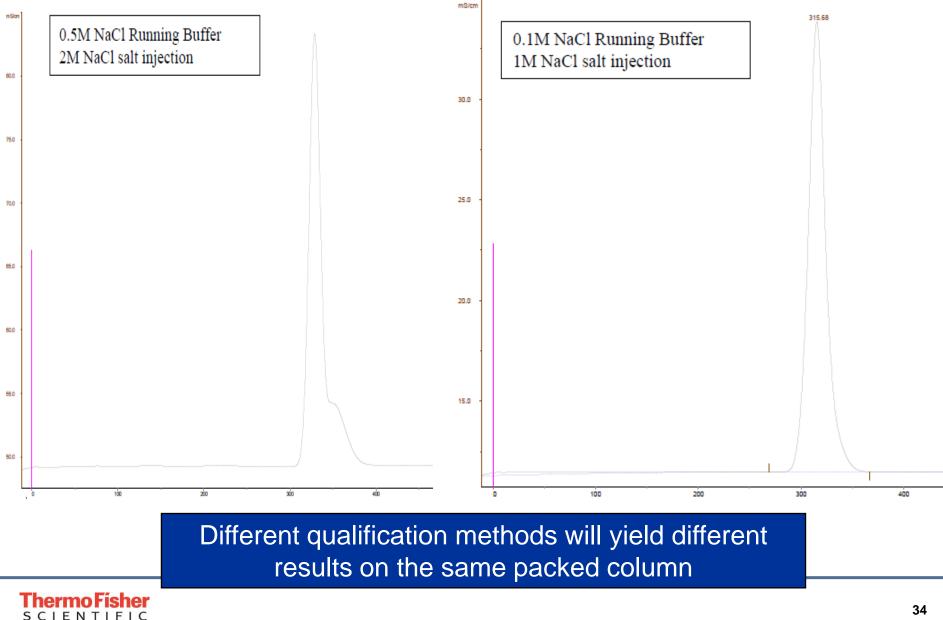
Qualification Conditions:

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

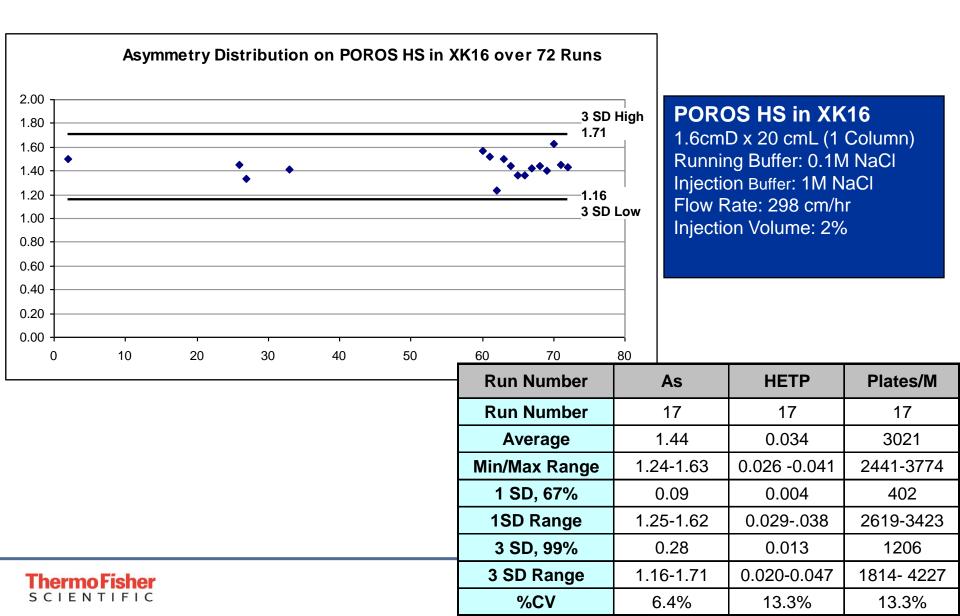
Type of Resin	POROS HS	Agarose Bead		
Asymmetry	1.45	0.71		
Retention Volume (ml)	29.32	30.53		
НЕТР	0.032	0.062		
Plates/M	3124	1610		
Width at 1/2 Height (ml)	2.78	4.29		
Peak Height (ms/cm)	36.39	25.80		

POROS Column Qualification Example

Same packed column, different qualification parameters



Qualification Results after Reuse had Normal Variability



Beginning, Middle and End Qualification Results were Comparable Over the 72 Runs

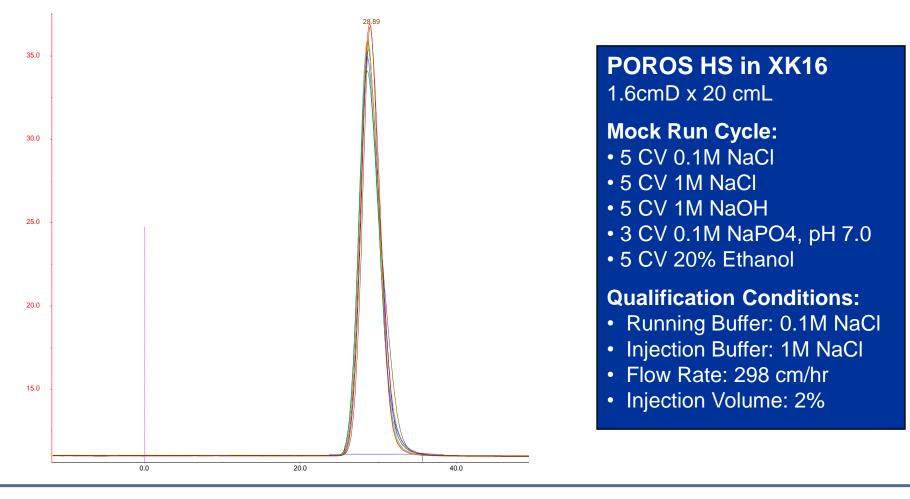
- No trends were observed over the 72 qualification runs
- Results were within normal variability of the system

	Run Number	Asymmetry	Retention Volume (ml)	HETP	Plates/M	Width at 1/2 Height (ml)	Peak Height (ms/cm)
Beginning	2	1.50	29.40	0.026	3774	2.58	36.95
Middle	26	1.45	29.32	0.032	3124	2.78	36.39
End	60	1.57	29.02	0.029	3492	2.60	36.56
Post 10 Cycles	72	1.43	28.79	0.032	3113	2.73	35.57



Mock Run Cycling had No Effect on Qualification Results

Consistent Peak Formation and No Trends were Observed Over 10 Cycles

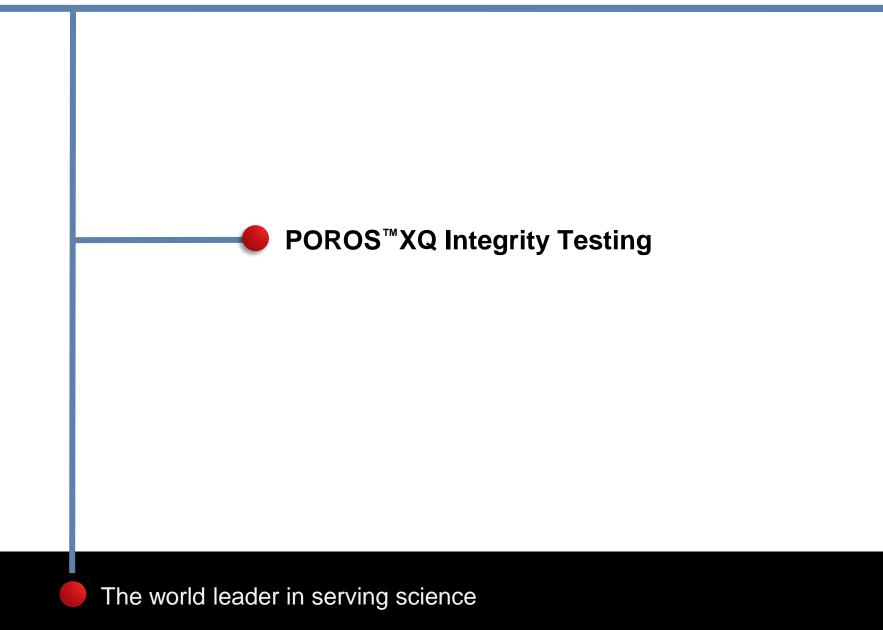




Conclusions

- 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: Column Qualification Study Overview

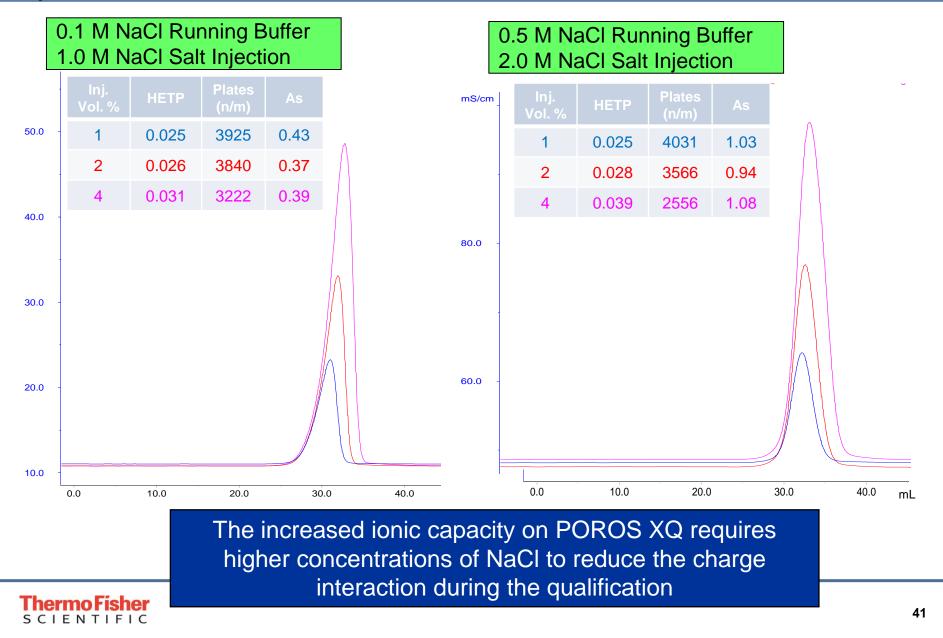
• Goal: To determine the effect column qualification parameters on the results

- XK16 column packed with POROS XQ
 - 20.1cm 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

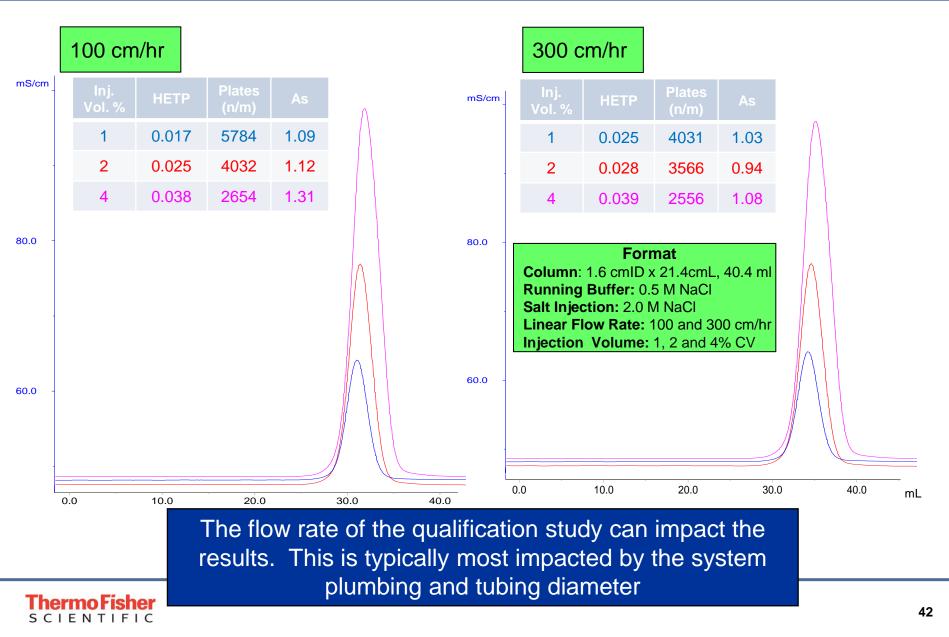
Table 1: Inputs each run at 1, 2, and 4% CV and 100 and 300 cm/hr

Running Buffer	Salt Spike: NaCl Concentration
0.1 M NaCl	1.0 M
	2.0 M
	3.0 M
0.5 M NaCl	2.0 M
	3.0 M
1 M NaCl	3.0 M

POROS XQ column integrity testing with different qualification solutions at 300 cm/hr



POROS XQ column integrity testing at different flow rates: 100 and 300 cm/hr



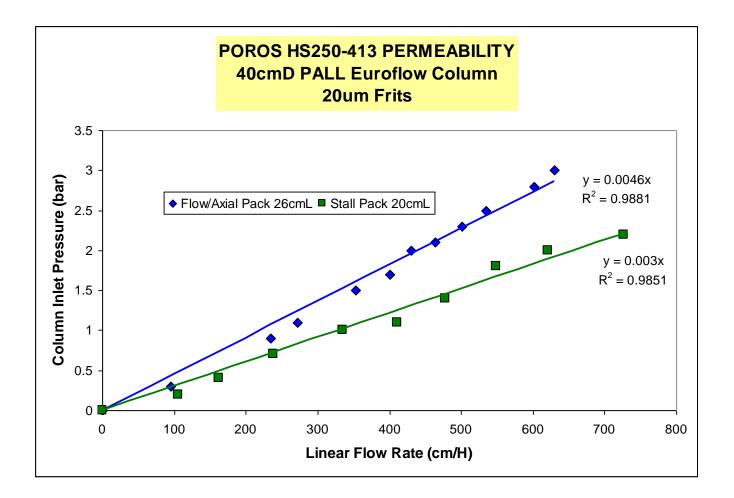
POROS XQ: Column Qualification Study Summary

- 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

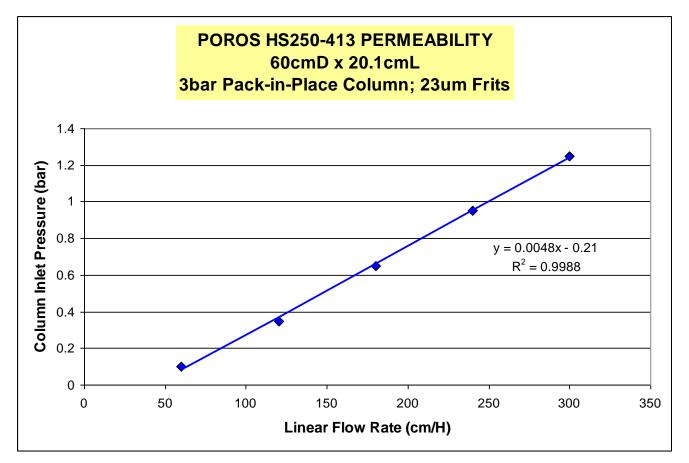


POROS[®] Chromatography[®] Resin Pressure Flow: 40 cm Diameter Column, 0.15M NaCl





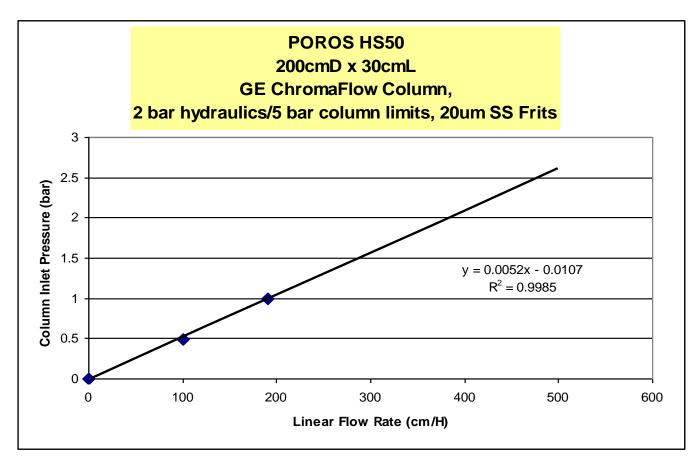
POROS[®] Chromatography[®] Resin Pressure Flow: 60 cm Diameter Column, 0.15M NaCl



Note: The system pressure was not subtracted from data



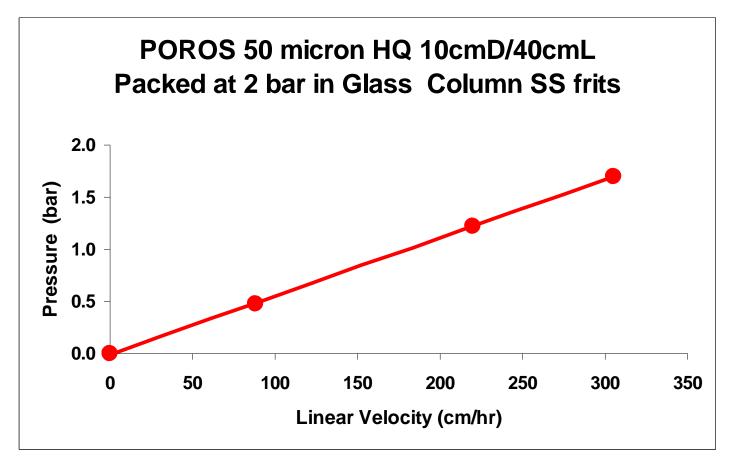
POROS[®] Chromatography[®] HS Resin Pressure Flow: 200 cm Diameter Column, water



Note: The system pressure was not subtracted from data



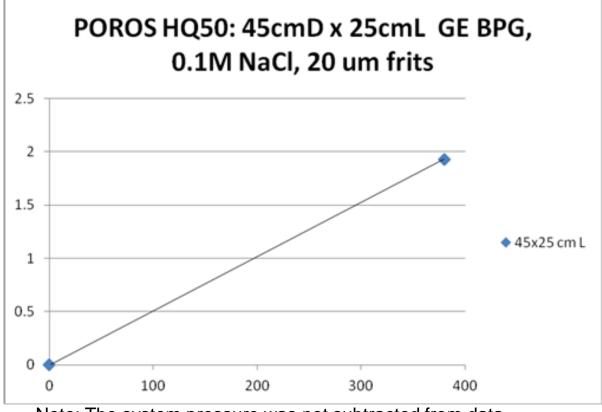
POROS[®] Chromatography[®] HQ Resin Product Attributes: Pressure vs. Flow Curves



Note: The system pressure was not subtracted from data.



POROS[®] Perfusion Chromatography[®] HQ Resin Product Attributes: Pressure vs. Flow Curves

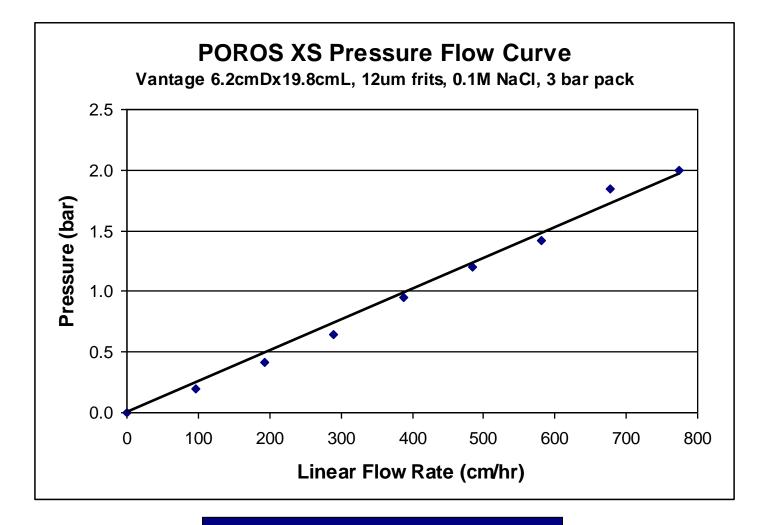


Note: The system pressure was not subtracted from data



POROS[®] XS Pressure vs Flow

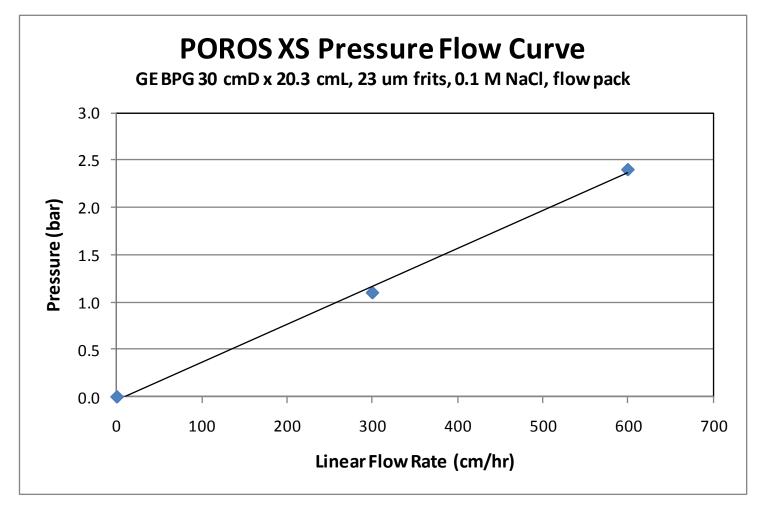
6.2 cm Diameter Column



Linear pressure response

POROS[®] XS Pressure vs Flow

30 cm Diameter Column



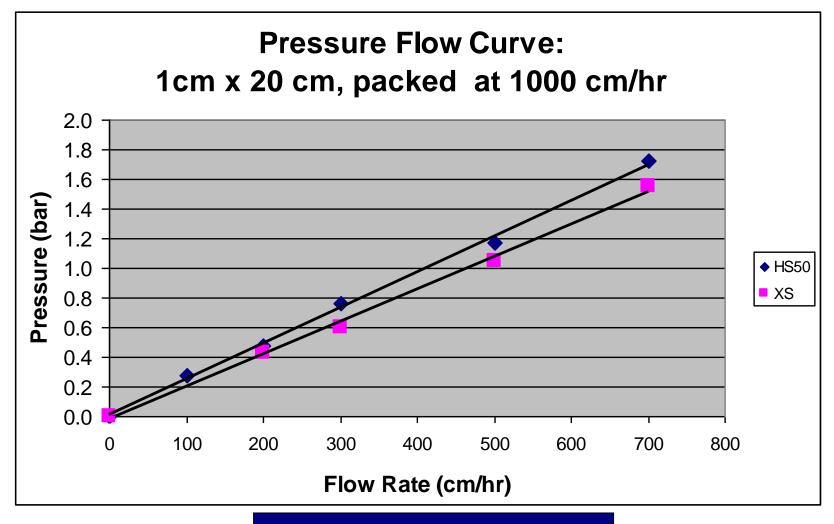
* System pressure not subtracted

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Linear Pressure Response

POROS[®] XS and HS Pressure vs Flow

1 cm Diameter Column

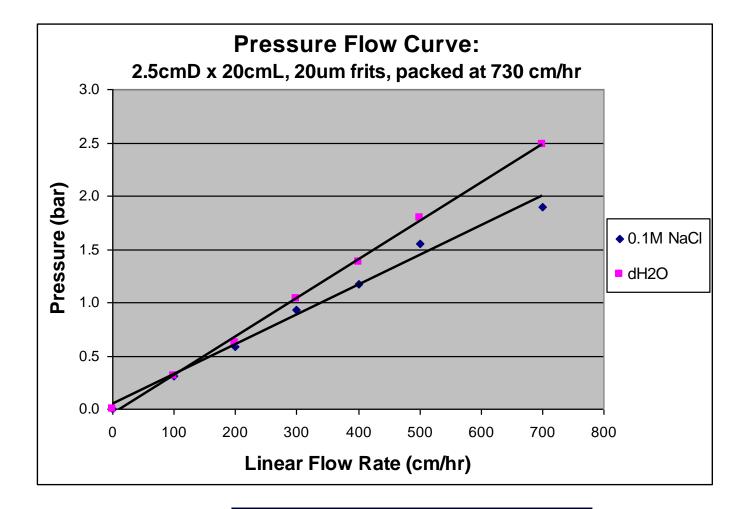


Linear pressure response



POROS[®] XS Pressure vs Flow

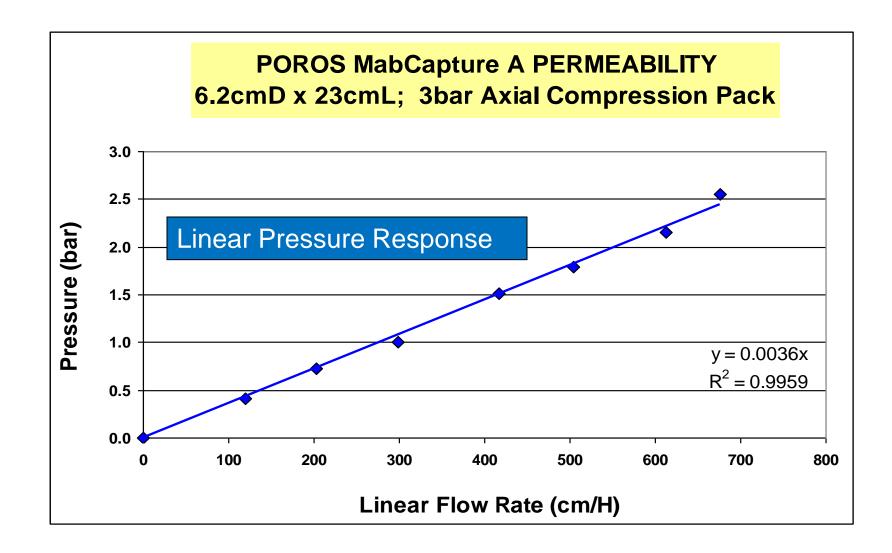
2.5 cm Diameter Column in 0.1M NaCl and water



Linear pressure response

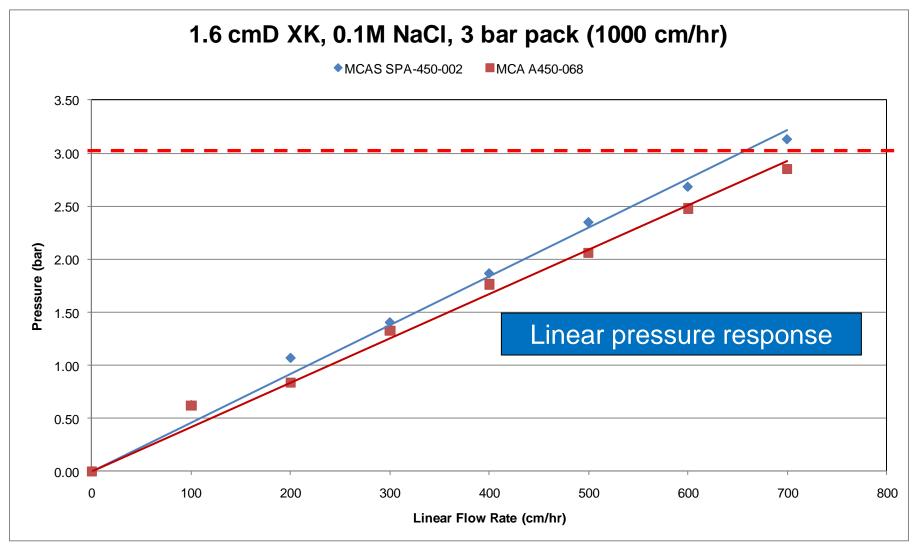


POROS[™] MabCapture[™] A Chromatography Resin Pressure vs Flow Response



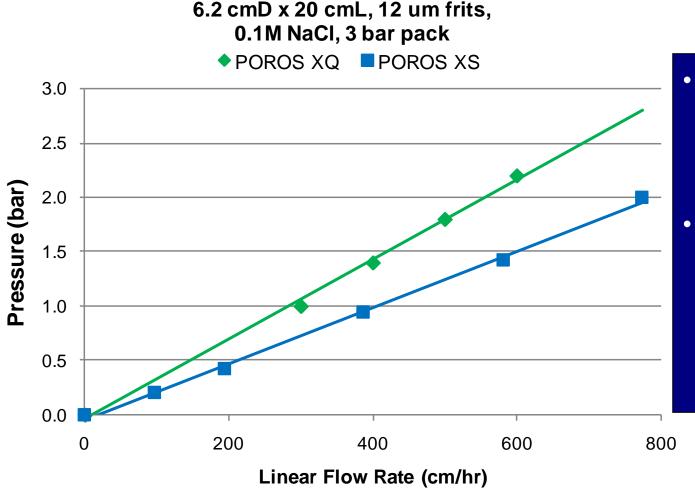


POROS[™] MabCapture[™] A and MabCapture A Select Pressure vs flow response



* MCA Select 22 cmL, MCA 22 cmL

POROS[®] XQ Performance Pressure vs flow curve compared to POROS[®] XS

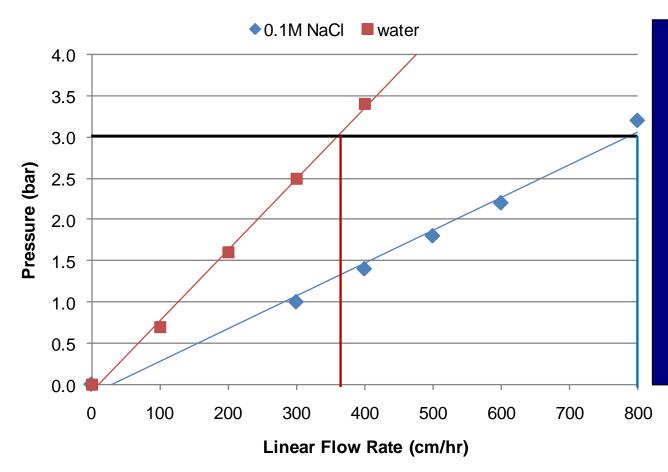


- Linear and predictable pressure – flow response
 - Ability to operate under high linear flow rates while maintaining < 3b backpressure

POROS[®] XQ Performance

Pressure vs flow curve

POROS XQ, 6.2 cmD x 19.6 cmL, 12 um frits, 3 bar pack



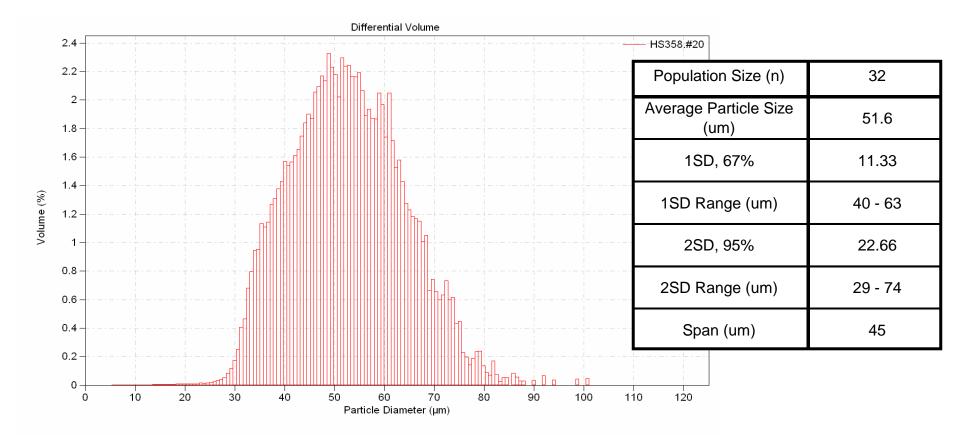
Under buffer/solution conditions of low ionic strength (< 5 mS/cm), higher backpressures can be realized due to the nature and density of the anionic functional group used

POROS[®] Particle Size Distribution Slides



POROS[®] HS Chromatography Resin Particle size distribution

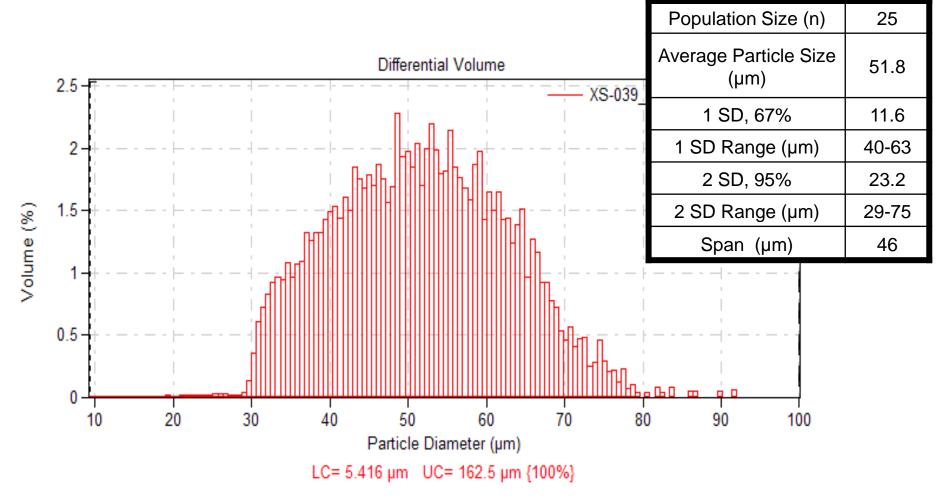
50um Bulk Resin: Tight Particle Size Distribution



POROS[®] XS Chromatography Resin

Particle size distribution

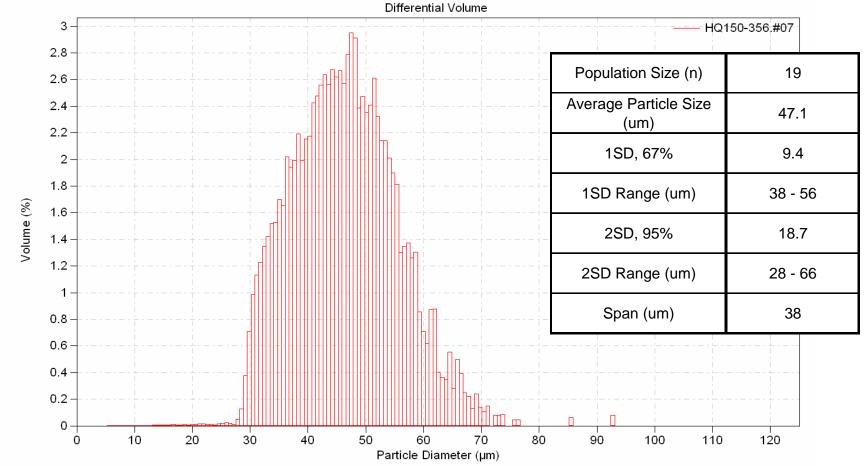
POROS[®] XS Resin: Tight Particle Size Distribution





POROS[®] Chromatography Resin Attributes: HQ 50

• HQ 50 Bulk Resin Particle Size Distribution

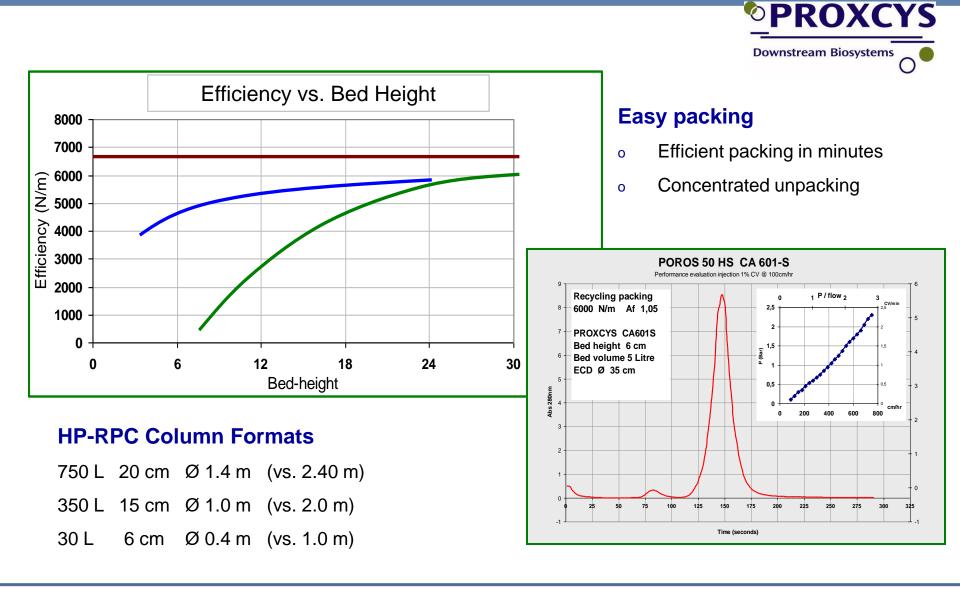




POROS[®] Radial Flow Example



High Performance Radial Flow Chromatography



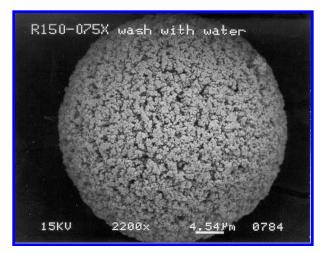
Thermo Fisher

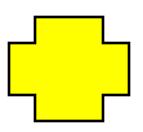
SCIENTIFIC

Innovative Processing Approaches



Combine Efficient Resin with Efficient Hardware









HP-RFC

Increased Efficiency and Capacity at Low Pressure



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