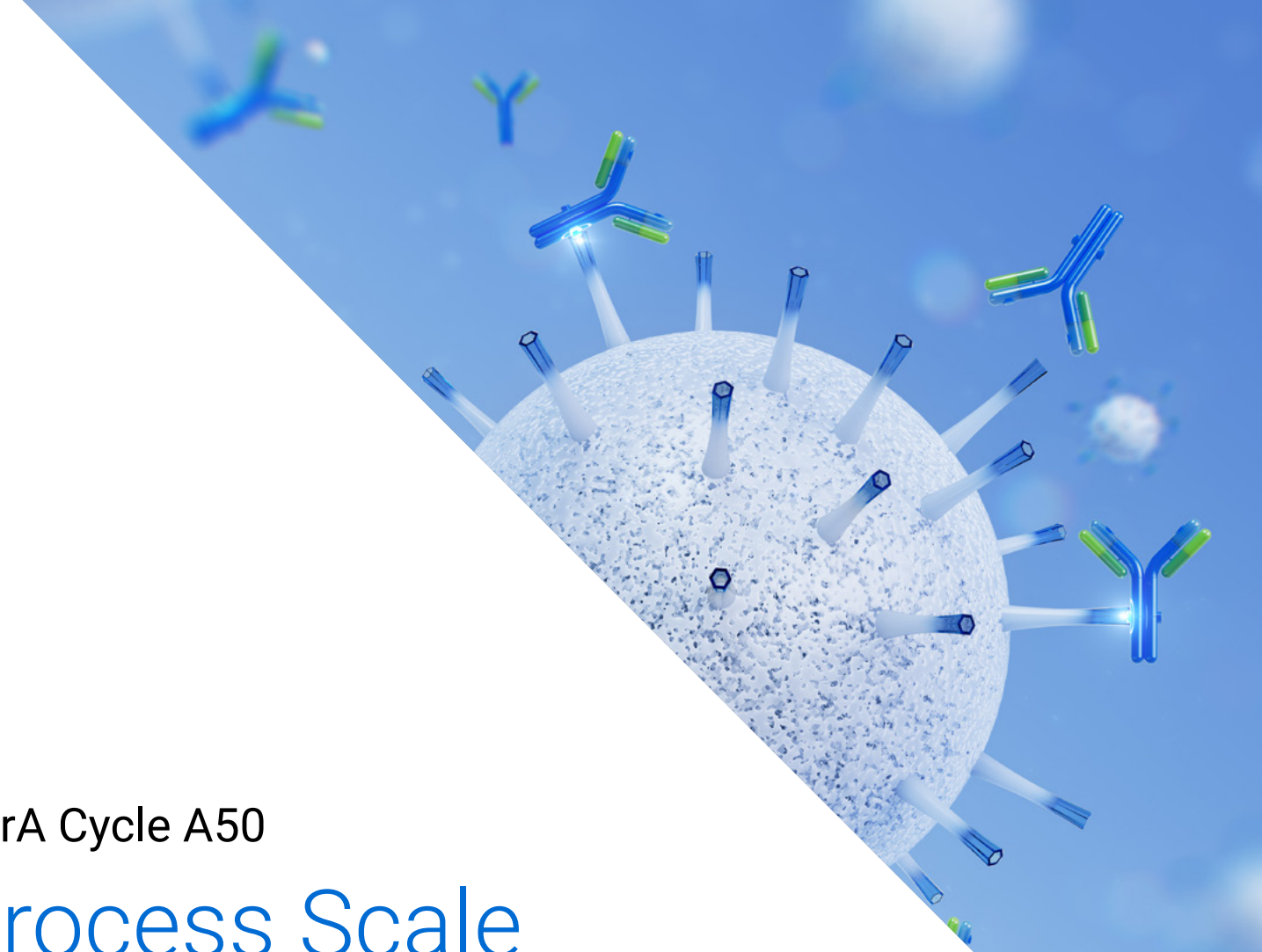
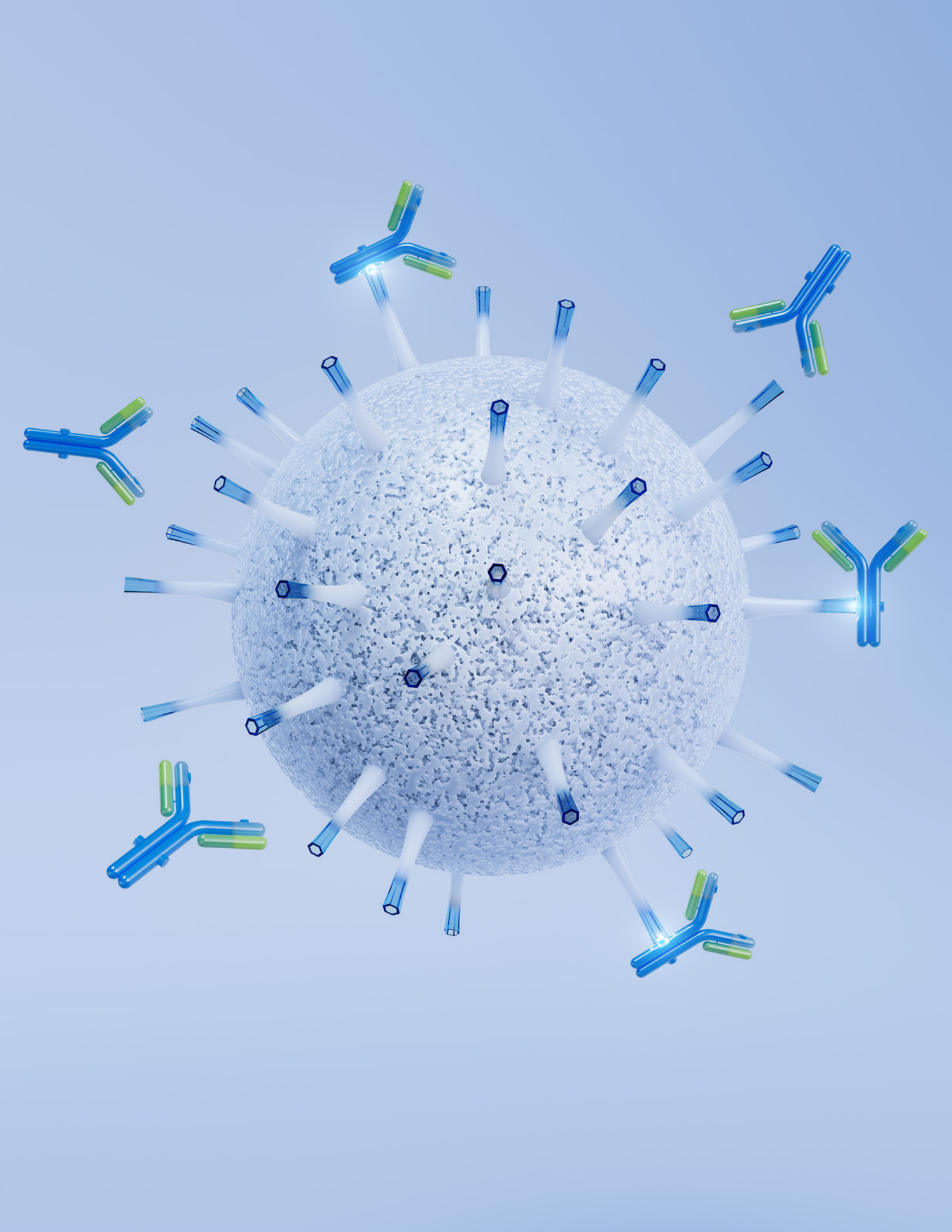


DurA Cycle A50

Process Scale Column Packing Instructions

ECOLAB®



DurA Cycle A50 Process Scale Column Packing Instructions

Contents

Overview

DurA Cycle A50

DurA Cycle A50 is a high capacity, alkaline stable protein A affinity chromatography resin, optimized for downstream processing of monoclonal antibodies and recombinant proteins.

DurA Cycle A50 is a high capacity, alkaline stable protein A affinity chromatography resin, designed to address the challenges in eluting pH sensitive Fc-containing monoclonal antibodies and proteins.

Praesto™ resins are part of the Purolite bioprocessing chromatography resin product family. Based on highly cross-linked agarose, they offer high-capacity with good flow and pressure properties and are readily scalable.

Jetting Technology

Praesto chromatography resins are manufactured using Purolite's patented Jetting technology. Jetting offers a faster, more environmentally-friendly manufacturing process and uniform particle size distribution.

Benefits of Purolite's Jetted Resins

Narrow Bead Size Distrubution
More consistent bead size and minimal variation batch-to-batch

Sustainable Manufacturing
More environmentally friendly than alternative manufacturing methods

Increased Productivity
Faster mass transfer reduces manufacturing costs

Column Packing

Column chromatography is a well-established method for characterisation, purification, and manufacture of a wide range of products, from food to life-saving medications. In biopharmaceutical manufacture, it is critical that the purification process is robust and reproducible from lot to lot. As such, it is vital that chromatography columns are efficiently packed, and able to be qualified within a reasonable time frame.

A well-packed column is essential to achieve maximum efficiency, high product yield and purity. It is important that a homogeneous packed bed is used every time a purification or separation is performed. Irregularities in packing can create an uneven flow within the bed, resulting in peak broadening, lower yield and it can subsequently affect the purity of the product. Essentially, a column that is poorly packed can lead to expensive process disruptions and ultimately, loss of a valuable product.

Herein, we describe packing procedures and parameters for pilot scale columns.

Abbreviations

Slurry percentage (%) = the ratio of resin to surrounding solution

Compression Factor = The level of compression required from a gravity settled bed height to the final packed bed height

Packing Factor = The level of compression required from a consolidated (under low flow) bed height to the final packed bed height

Operational Expectations

Careful consideration needs to be taken when designing a purification process both flow and pressure require careful consideration. Choosing the appropriate bed height with respect to capacity and process economics needs to be designed in at the laboratory bench but also achievable at scale. The details below give an overview of the expected operational windows for use with DurA Cycle A50.

FIGURE 1

Expected operational flow window for DurA Cycle A50 generated at 30 cm inner diameter.

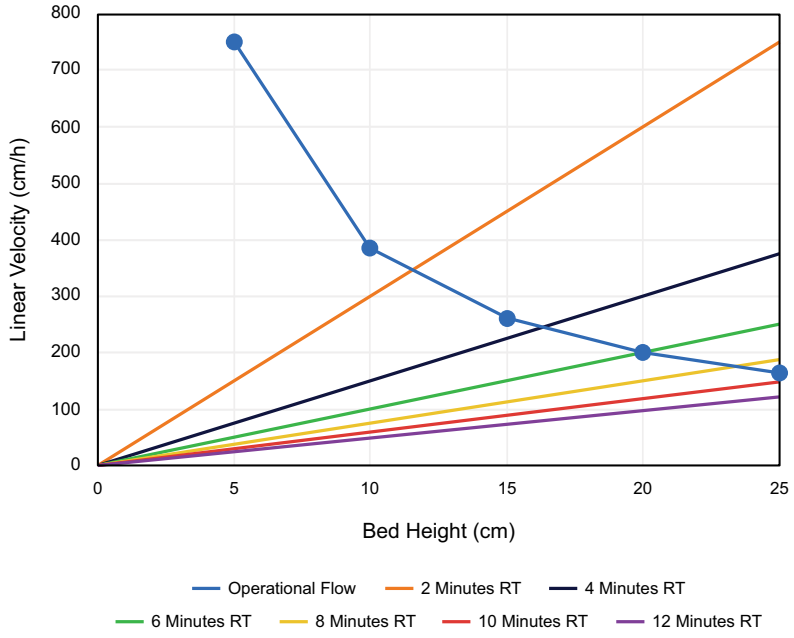


FIGURE 2

Flow expectation on extrapolation of column diameter at a 20 cm bed height in a solution of viscosity of 1 cp.

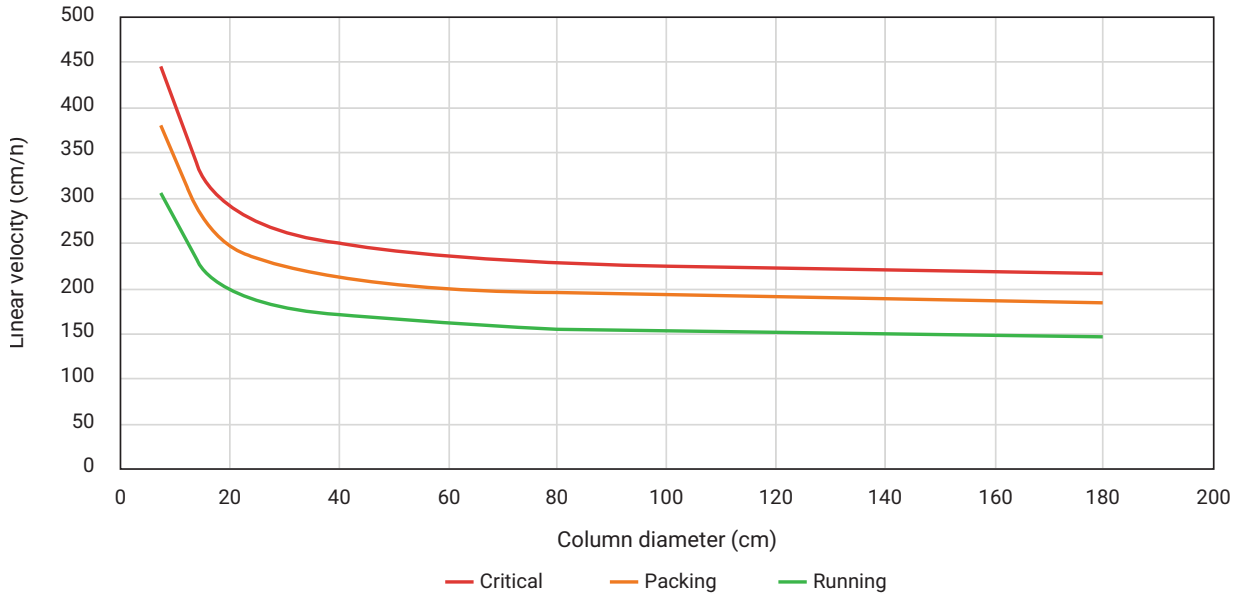


FIGURE 3

Pressure expectation on extrapolation of column diameter at a 20 cm bed height in a solution of viscosity of 1 cp.

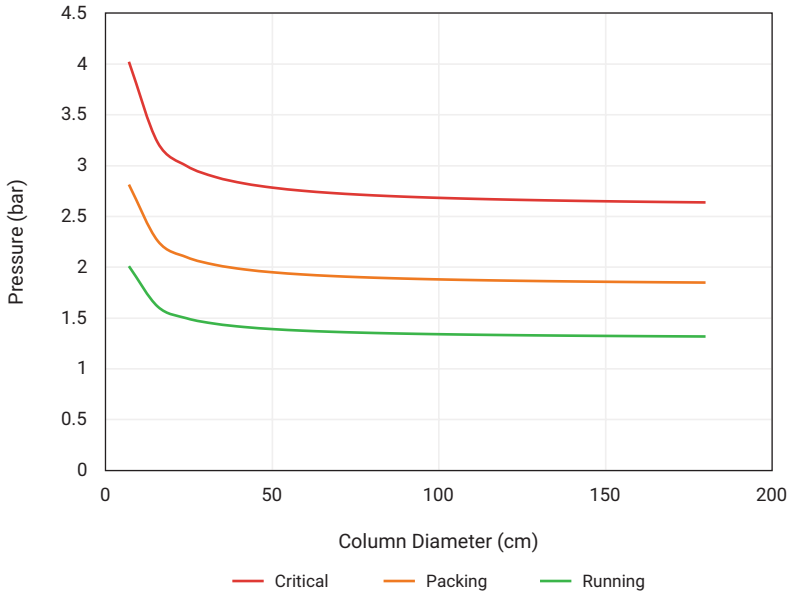


TABLE 1 Pressure expectation on extrapolation of column diameter at a 20 cm bed height in a solution of viscosity of 1 cp.

Column Diameter (cm)	Recommended Packing Factor	Recommended Compression Factor
7	1.20	1.17
14	1.21	1.18
30	1.23	1.20
160	1.25	1.22

Pressure Flow — Pressure and Manual Compression Packing

FIGURE 4

Pressure versus flow for DurA Cycle A50 at 30 cm inner diameter with bed heights of 10, 15, 20 & 25 cm. Packed by pressure and manual compression in a BPG 300 column.

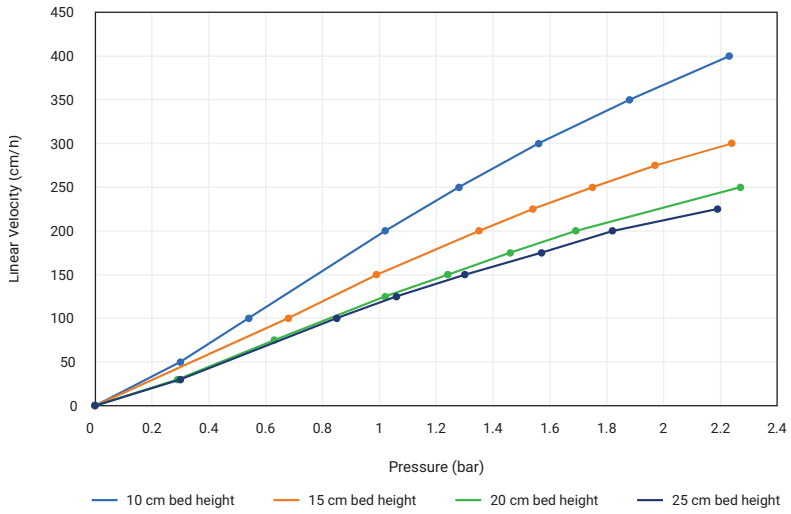


TABLE 2 **Pressure versus flow data for DurA Cycle A50 at 30 cm inner diameter with bed heights of 10, 15, 20 & 25 cm. Packed by pressure and manual compression in a BPG 300 column.**

Bed Height (cm)	Maximum Linear Velocity (cm/h)	Pressure (bar)	Compression Factor
10	340	1.8	1.22
15	250	1.8	1.22
20	200	1.8	1.20
25	180	1.8	1.20

Pressure flow data generated using pressure packing and manual compression. Axial compression offers the user a 'looser' pack resulting in higher achievable flows.

FIGURE 5

Pressure versus flow for DurA Cycle A50 at 60 cm inner diameter with bed heights of 12, 15 & 20 cm. Packed by axial compression in an AxiChrom 600 column.

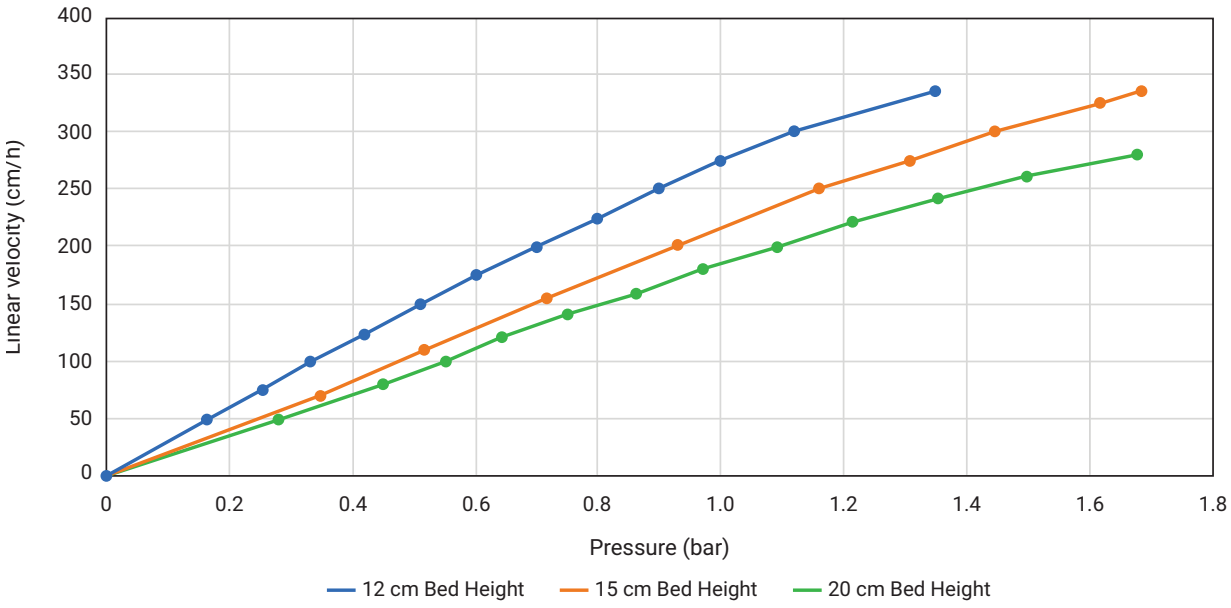


TABLE 3 Pressure versus flow data for DurA Cycle A50 at 60 cm inner diameter with bed heights of 12, 15 & 20 cm. Packed by axial compression in an AxiChrom 600 column.

Bed Height (cm)	Maximum Linear Velocity (cm/h)	Pressure (bar)	Compression Factor
12	340	1.3*	1.22
15	325	1.6	1.22
20	275	1.6	1.20

*Reached maximum pump during test.

It is recommended to pack DurA Cycle A50 above 30 cm inner diameter in axial compression columns for optimal flow properties.

Slurry Determination

The percentage slurry is needed to calculate the required volume to be added to achieve a desired bed height.

There are several techniques employed to determine slurry percentage which include centrifugation, gravity settling and a small-scale column using syringe drip force (Cytiva slurry concentration kit).

The accuracy of the slurry percentage measurement is an important parameter and robustness in the measurement is key whichever technique is to be employed.

If using gravitational settling, 48 hours using a 1 L volumetric measuring cylinder is recommended.

Suggested Materials and Equipment

- DurA Cycle A50
- Demineralised water or 100 mM NaCl solution (packing solution)

Sample and Column Preparation

- Assemble the column as per the manufacturer's instructions.
- Prime the column and system selected with the appropriate packing solution prior to column packing.
- Recommended slurry percentage = 40–70%.
- Determine the slurry percentage.
- Calculate the required slurry to add to the column using the following equation:

$$\text{Volume (mL)} = \frac{\text{Radius}^2\ \text{(cm)} \times \pi \times \text{Bed Height (cm)} \times \text{Compression Factor}}{\left(\frac{\text{Slurry (\%)}}{100}\right)}$$

- Remove storage solution by means of column washing or decant off the liquid level after settling the resin in the selected column.
- Add packing solution and resuspend the resin ready for the packing procedure.
- Allow the slurry to settle (at least 2 cm from top, it may require up to 30 minutes to settle) before inserting the adaptor.
- For AxiChrom columns greater than 20 cm in diameter, use an appropriate slurry vessel for transfer to the column.

BPG (Cytiva™)

10–45 cm I.D

Packing by Consolidation and Manual Compression

Target bed height of 20 cm as an example.

Step 1: Prime the column prior to operation, by running a flow in upward directions through the bottom net, displacing any air, leaving a liquid level at approximately 2 cm in bed height.

Step 2: Determine the appropriate slurry volume to add to the column based on bed height and compression factor (choice of packing method determines which factor to use).

Step 3: Add slurry volume to the column, top up to 40 cm with packing solution if needed. Recommended slurry percentage = 50–70%.

Step 4: Allow resin to settle approximately 5 cm before adding top adaptor.

Step 5: Loosen the O-ring before manually inserting the top adaptor.

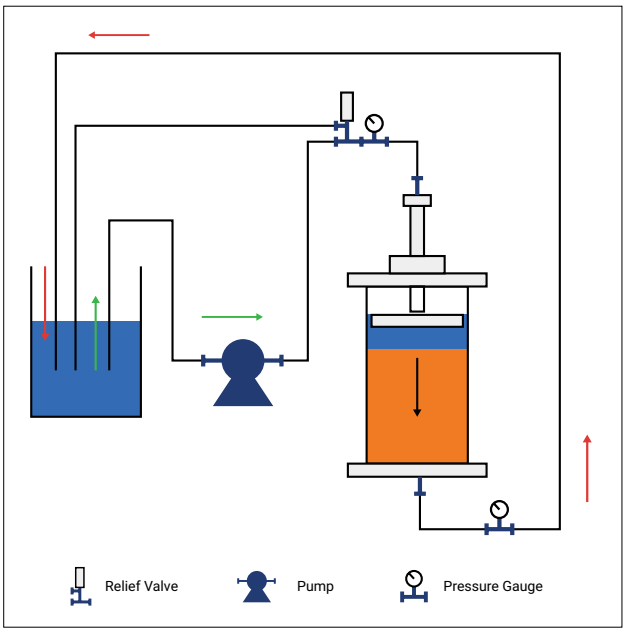
Step 6: Allow excess buffer to pass around the column head, displaying any large air bubbles.

Step 7: Lock the adaptor in place by attaching the supplied bolts and tighten the O-ring.

Step 8: Manually lower the adaptor a few centimetres, expelling liquid through the top of the column.

Step 9: Start a consolidation flow at 30 cm/h.

Steps 1–9



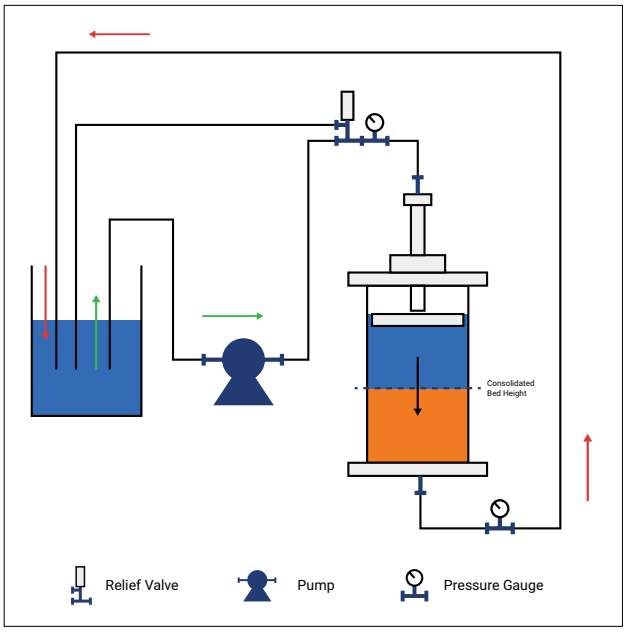
Steps 10–11

Step 10: After sufficient consolidation (minimum 30 minutes after final bed formation), measure the bed height.

Step 11: Use the measured bed height to calculate the target bed height based on packing factor.

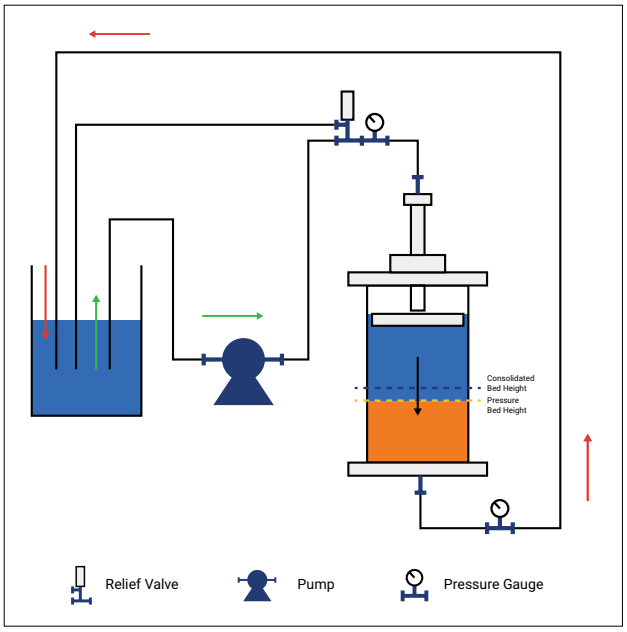
Example: Settled bed height (cm)/Packing Factor (P.F) = Desired bed height (cm)

Example for a 24.0 cm settled bed height; 24.0 cm (Settled bed height)/1.20 (P.F) = 20 cm



Step 12

Step 12: Increase the flow to a moderate pressure 1–1.5 bar to aid in the final compression of the resin.

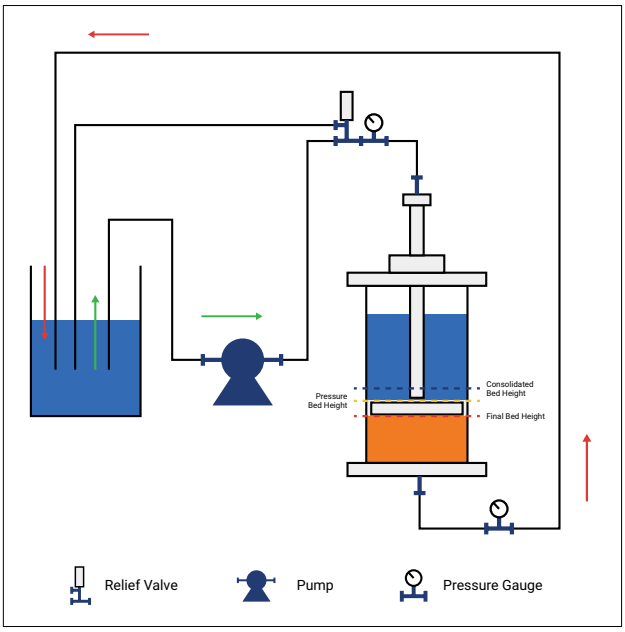


Step 13: Stop the flow, disconnect the top tubing, or direct to a waste tube via inline valve.

Step 14: Manually compress the resin to the calculated final bed height.

Step 15: Column is ready for column efficiency testing.

Steps 13–15



Packing by Pressure

Step 1: Prime the column prior to operation, by running a flow in upward directions through the bottom net, displacing any air, leaving a liquid level at approximately 2 cm in bed height.

Step 2: Determine the appropriate slurry volume to add to the column based on bed height and compression factor (choice of packing method determines which factor to use).

Step 3: Add slurry volume to the column, top up to 40 cm with packing solution if needed. Recommended slurry percentage = 50–70%.

Step 4: Allow resin to settle approximately 5 cm before adding top adaptor.

Step 5: Loosen the O-ring before manually inserting the top adaptor.

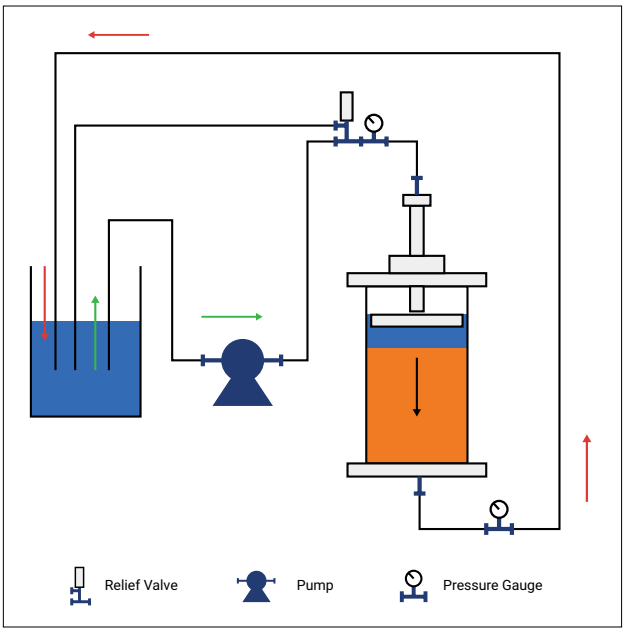
Step 6: Allow excess buffer to pass around the column head, displaying any large air bubbles.

Step 7: Lock the adaptor in place by attaching the supplied bolts and tighten the O-ring.

Step 8: Manually lower the adaptor a few centimetres, expelling liquid through the top of the column.

Step 9: Connect the system or pump to be used using a low flow (30 cm/h).

Steps 1–9



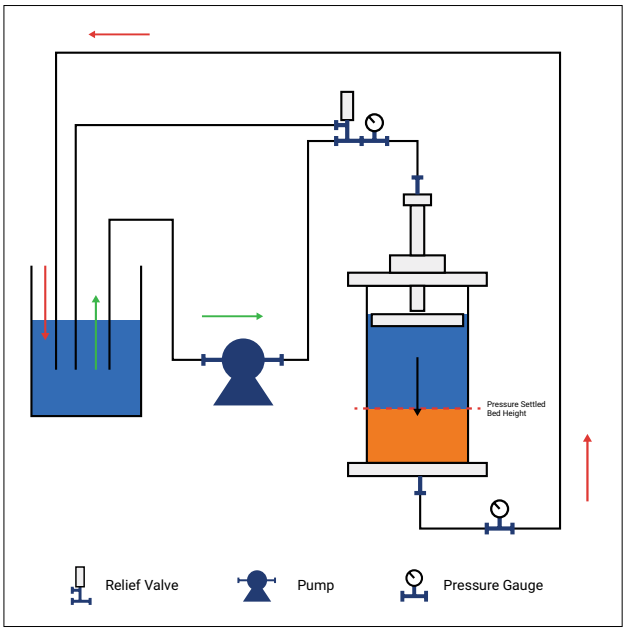
Steps 10–12

Step 10: Once stabilized, increasing the flow to obtain a stable pressure (Refer to resin information for selected packing pressure, set relief valve at packing pressure)*.

Step 11: Adjust flow according if packing solution being displaced during packing process, to maintain a stable pressure. Best practice is to buffer exchange prior to packing.

Care needs to be taken when increasing the flow if a pressure relief valve is not in place to avoid over compression.

Step 12: Allow the packing to stabilise at packing pressure for 20–30 minutes. Mark the settled bed height.

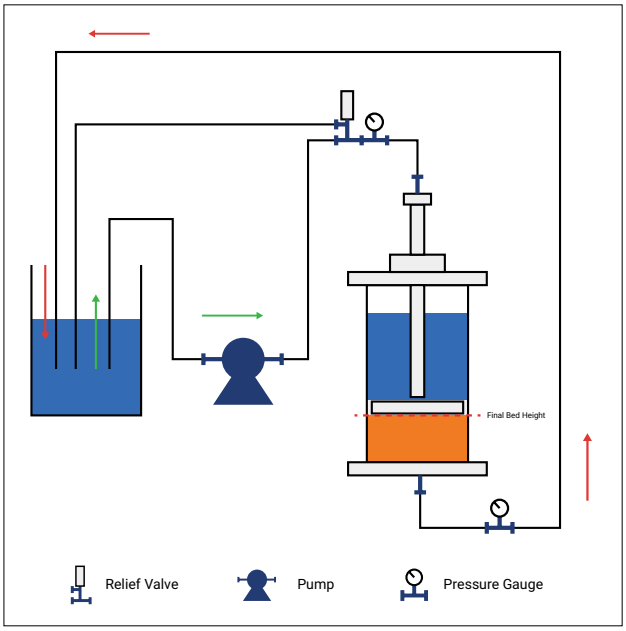


Steps 13–16

Step 13: Stop the flow, disconnect the top tubing, or direct to a waste tube via inline valve.

Step 14: Manually compress the resin to the 2 mm past the marked bed height. This process needs to be performed as quickly as safely possible to do so, to prevent too much back spring from the compressed bed.

Step 15: Column is ready for column efficiency testing.



AxiChrom (Cytiva™)

7–20 cm I.D

Recommend Using 'Intelligent Packing'
Provided by Cytiva on AKTA Pilot and AKTA Process Systems.

Step 1: Connect the selected column to the AKTA system.

Step 2: Make a packing program in Unicorn using the Wizard for packing Axichrom Columns.

Step 3: Use following Instructions.

- Column Size — Axichrom 70–200
- Mesh Size — 10 µm
- Media — Custom
- Packing Factor (7 cm ID column) - 1.20
- Adaptor Flow velocity — 30 cm/hr
- Conditioning Flow velocity — 200 cm/hr
- Sample Volume — 1.5 %
- Equilibration Volume — 3 CV
- Elution Volume — 1.4 CV
- HETP Testing Velocity — 30 cm/hr
- HETP — Testing downflow

Step 4: Prepare packing and testing solutions as follows:

- A1/A2 — 100 mM — 500 mM NaCl
- Sample Inlet S1 — 1M NaCl

Step 5: Run Packing and HETP testing Method from Unicorn in AKTA System and follow instruction to pack column.

Step 6: Evaluate Result using HETP Analysis to get value of A_s, HETP, h etc.

AxiChrom (Cytiva™)

30–200 cm I.D

Conditions for using 'Intelligent Packing'
Provided by Cytiva on AKTA Process Systems.

Step 1: Using an appropriate slurry vessel, ensure the slurry is homogeneous and determine the slurry percentage.

Step 2: Using the intelligent packing protocol in Unicorn software or an AxiChrom™ master, use the following packing parameters.

- Column: 600/300 (10/20) µm
- Media: Other
- Target Bed: 20 cm
- Max: 21 cm
- Min: 19 cm
- Packing Factor: 1.23
- Filling Speed: 300 cm/hr
- Packing Speed: 30 cm/hr
- Conditioning flow velocity: 200 cm/hr

Step 3: Follow the prompted instructions during the packing process.

Step 4: Evaluate the packing using HETP analysis to get values for A_s, HETP, h etc.

Manual Operation

Guidance on how to handle AxiChrom columns with regards to adaptor movements, flows and flow direction.

The valves used in the following pages are an example of experimental set up. Depending on facility fit, this set up may differ. It is important to note how the valves depicted direct flow throughout the guide.

Caution — When using PE sintered bed support special need for caution is essential as air does not go through the bed support and rupture of bed support may occur.

Labeling Guidelines

- B is always open.
- A and C are always connected within the valve.
- When the slurry vessel valve is open, liquid can go in or out of the column through one or both A and C.

A = Slurry vessel port

B = Mobile phase lower port (column bottom)

C = Rinse port

D = Mobile phase upper port (column top)

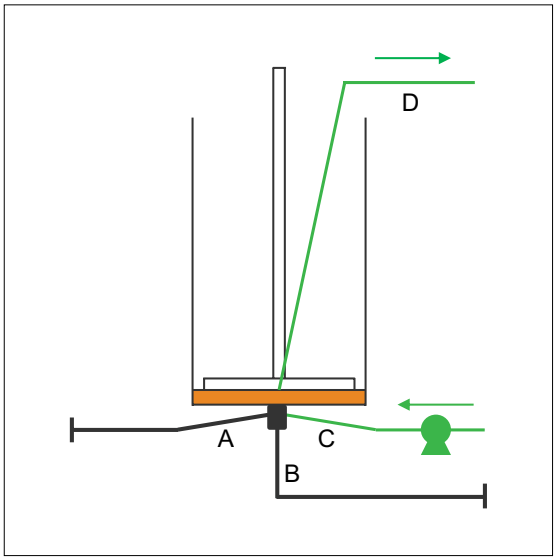
Priming the Column

The column must be primed with liquid and air should be removed both inside the column, underneath the bottom net and above the top net.

Using 20% v/v Ethanol instead of water will reduce the capillary forces and thus enhance this procedure.

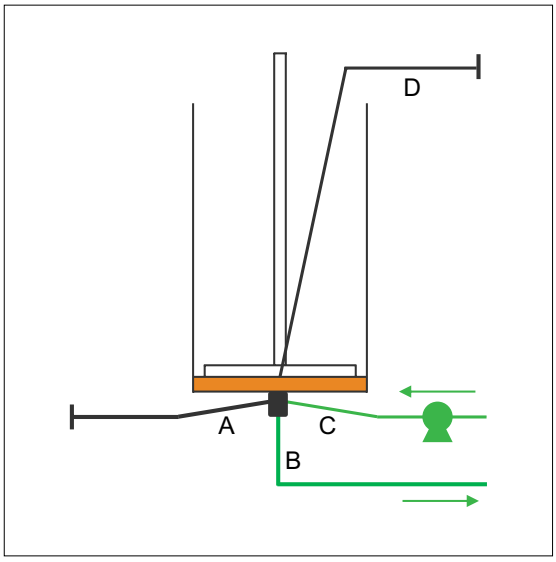
Stage 1

- Open the slurry vessel valve on the column.
- Open D.
- Close A and B.
- Pump in packing solution through C.
- Air in-between the sinters and above the adaptor leaves the column through D. Perform until no more air leaves the column.



Stage 2

- Open the slurry vessel valve on the column.
- Close A and D.
- Open B.
- Pump in packing solution through C.
- Most air under the bottom net leaves the column through B. Perform till no air leaves the column through B.

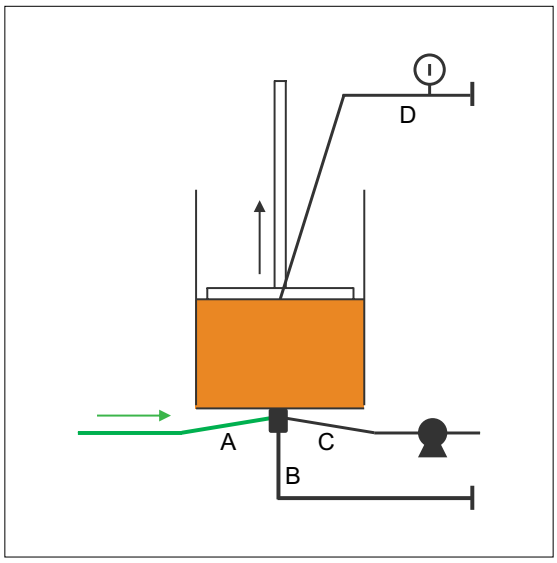


Packing the Column

Stage 1

Prime Slurry Vessel Tubing

- Prime the tubing from the slurry vessel to the column just prior to packing or calculate additional volume to be added to the column.



Stage 2

Fill the column with resin slurry from the slurry vessel.

(This process is performed by the AxiChrom master when using intelligent packing protocol).

- Open the slurry vessel valve on the column.
- Close B and D.
- The AxiChrom Master's pressure sensor is mounted inline from the column top at port D.
- The adaptor is raised until the correct volume of resin slurry has been added.
- Close the slurry vessel valve on the column.
- Rinse the slurry out from the valve and tubing on the bottom of the column by pumping from C to A.

Stage 3

Packing the resin within the column.

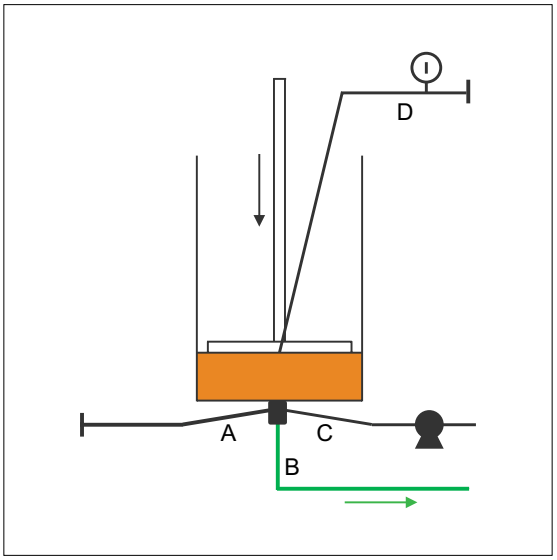
(This process is performed by the AxiChrom master when using intelligent packing protocol).

- Close the slurry vessel valve on the column.
- Close A and D.
- The AxiChrom Master's pressure sensor is mounted inline from the column top at port D.
- The adaptor is lowered until the target bed height, excess packing solution is expelled through B.

Stage 4

Finish packing.

- Close both ports B and D, then remove the pressure sensor.
- The column is now ready to be connected to specified system for column efficiency testing.



Chromaflow/Resolute Packing

This packing guidance is to advise on how to operate 'pack in place' columns in a non-automatic mode as best practice when using modern high flow compressible resins such as the Praesto range of chromatography resins.

The 'pack in place' columns were originally designed for soft resins with relatively large bead sizes.

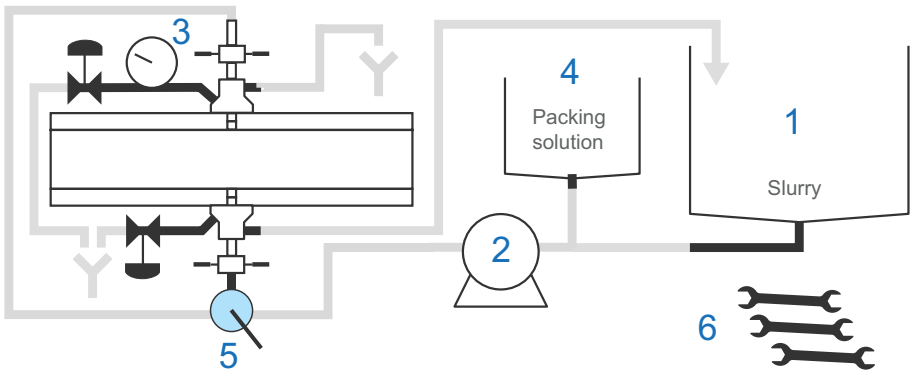
When packing smaller and more rigid beads the stall packing technology may result in portions of hard packed resin that will build up in the packing entrance of the column. This negatively impacts the accuracy of determining the stall point, resulting in poor HETP, asymmetry and packed bed lifetime.

The methodology described in this guidance document is a more manual process with a higher packing success rate and bed longevity expected.

Column packing, following this methodology, is more in keeping with methodologies used with BPG and AxiChrom columns.

Pre-requisites

- Surplus slurry is required for this packing methodology. Surplus resin will reside in the tubing after packing.
- A vortex preventing device should be mounted in the bottom of the slurry vessel.
- A packing station appropriate for the size of the column to be pack. A membrane pump can also be used for this task.
- A pressure gauge with pass through flow path of the same inner diameter as the mobile phase line.
- A valve to direct slurry flow to upper or lower nozzle, referred to as slurry valve.
- Three solid wrench tools for manual movement of the adaptor.



Abbreviations

Packing Height = PH

Final Bed Height = FBH

Packing Factor = PF

Nozzle Length Inside During Packing = NLI

PH > FBH * PF + NLI

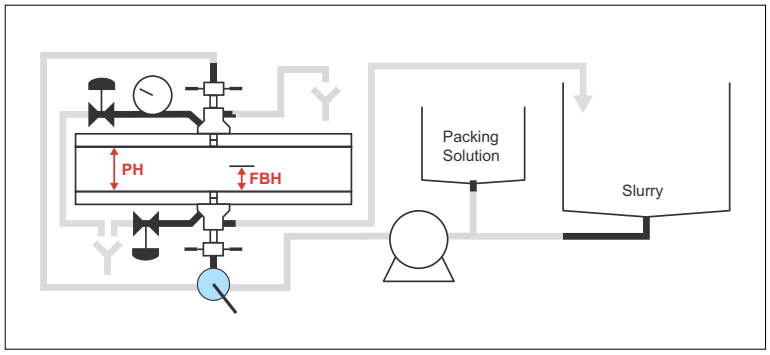
Example: FBH = 20 cm, PF = 1.20, NLI = 3 cm → 20 * 1.20 + 3 = 27 cm

Packing

Stage 1

Position the adaptor at a height so that the packing nozzle never touches the bed:

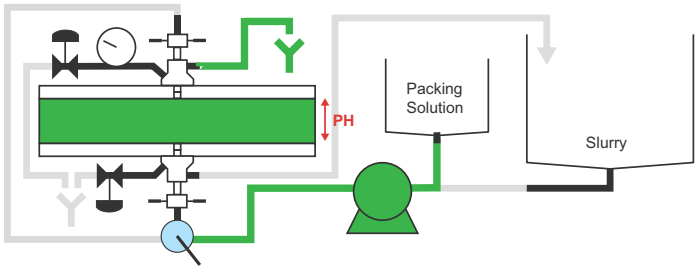
- Example: FBH = 20 cm, PF = 1.20, NLI = 3 cm → 20 * 1.20 + 3 = 27 cm



Stage 2

Priming column with packing solution

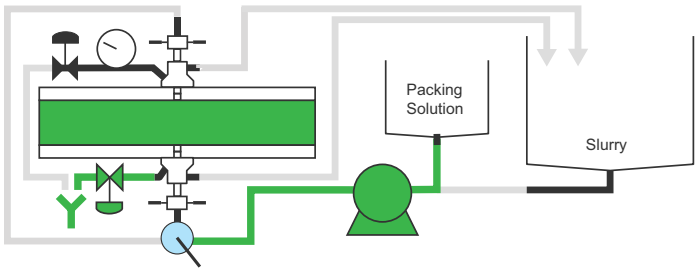
1. Top nozzle in emptying position (fully inserted).
2. Slurry valve open towards bottom valve.
3. Bottom nozzle in filling position (half inserted).
4. Priming of column with packing solution.
5. Stop the pump when no air is leaving the column.



Stage 3

Priming of bottom mobile phase

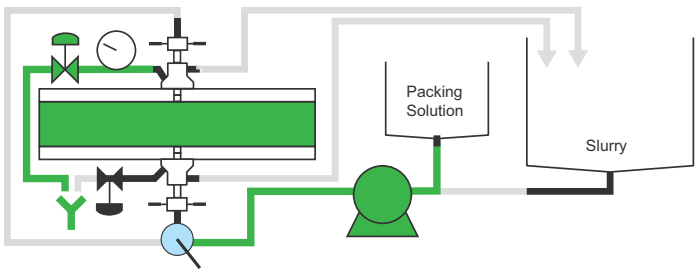
1. Move resin outlet tube to the resin tank (A).
2. Retract upper nozzle fully (only mobile phase open).
3. Open the lower mobile phase while pump is active at low flow.
4. Prime till no air is coming out.
5. Close the lower mobile phase while pump is active at low flow.



Stage 4

Priming of top mobile phase

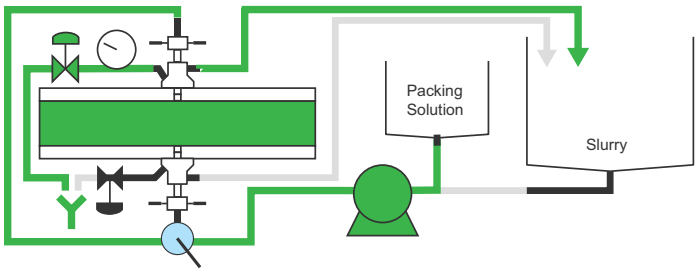
1. Open the upper mobile phase while pump is active at low flow.
2. Prime till no air is coming out.
3. Close the upper mobile phase while pump is active at low flow.
4. Stop the pump.



Stage 5

Priming of packing line with slurry

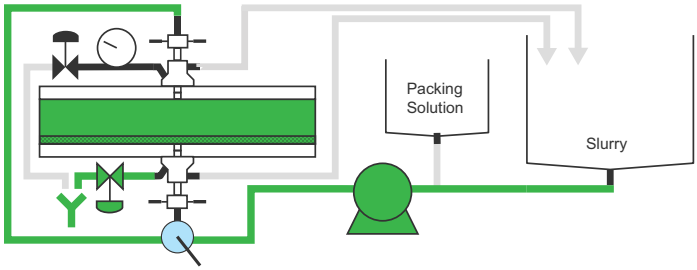
1. Re-tract the bottom nuzzle fully.
2. Slurry valve changed towards upper nozzle.
3. Pump at packing speed.
4. Stop the pump.



Stage 6

Packing the column

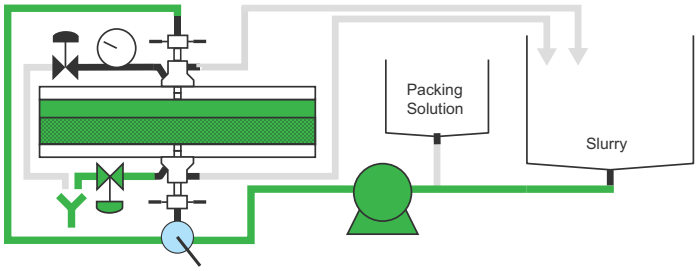
1. Insert the upper nozzle to packing position.
2. Start the pump.
3. Open the valve on the mobile phase outlet at the bottom.
4. Packing is started.



Stage 7

End of packing

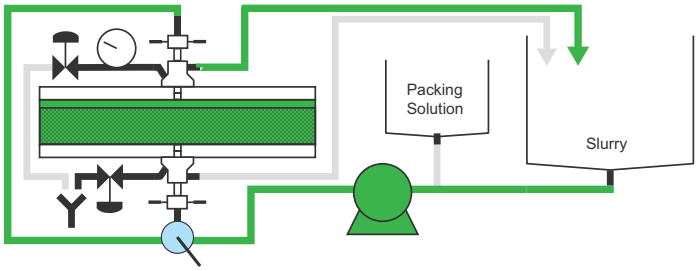
1. At this stage the bed height should be in-between packing height and sedimented height.
2. If possible move the mobile face to the slurry tank when getting close to the end of the packing to utilize as much as possible of the resin. If so there is no immediate action to take finalizing the packing as liquid is just re-circulating. Having a vortex prevention device at the bottom of the tank will ensure that air is not entering the bed.



Stage 8

Finalizing the packing

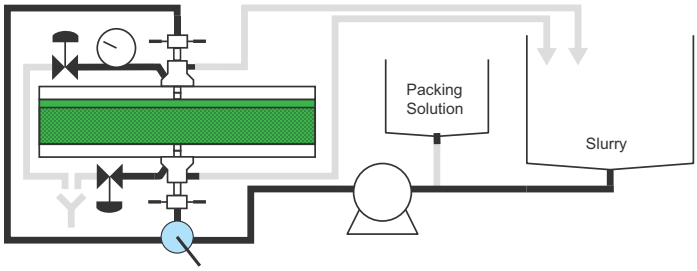
1. Stop the pump, close the bottom mobile phase and re-tract the upper nozzle to mobile face only.



Stage 9

Preparing for manual compression

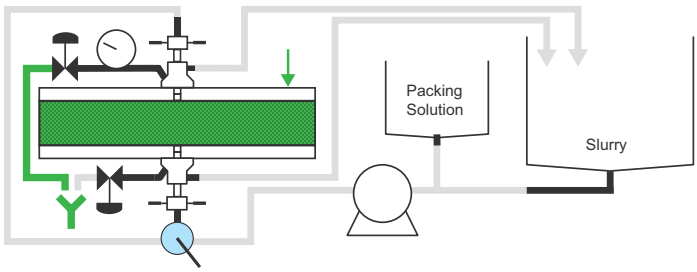
1. Close Slurry valve.
2. Resin will raise towards same as sedimented height but this is normal. No issue as long as the column is not moving around.



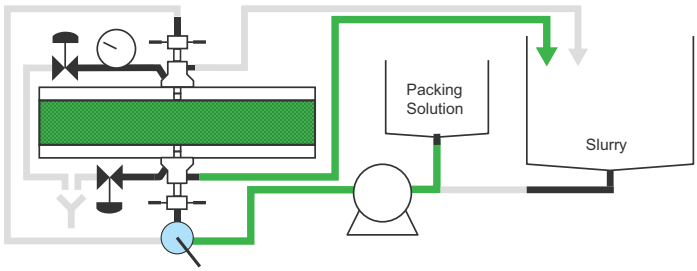
Stage 10

1. Loosen all the nuts under the adaptor to below the expected bed height position. The mobile phase shall be going out through the upper valve else the force needed to lower the adaptor will take too much strength. Using three solid wrench tools, as described below, but only turning three nuts simultaneously will make the lowering relatively easy and safe. From time to time also adjust the three other nuts by hand. During this procedure, frequently check and if needed correct so that all nuts are pushing the adaptor down equally and in level. Lubricate the nuts with either Ethanol solution or silicon grease continuously to avoid share.

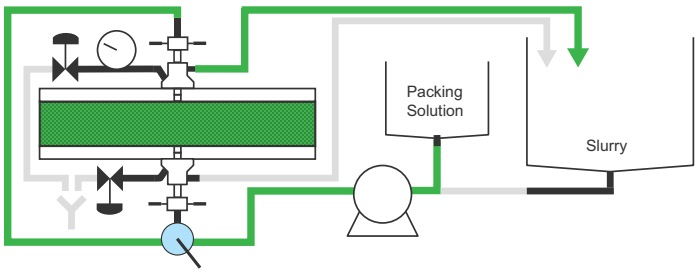
Comments: use three solid wrench tools.



2. Clear the bottom slurry line.
3. Open the slurry valve towards the bottom valve.



4. Start the pump.
5. Clear the top slurry line.
6. Open the slurry valve towards the upper valve.
7. Stop the pump when all lines are cleared.
8. Disconnect the tubes for slurry in and out and put blinds on all open ports.
9. Remove the pressure gauge and connect upper and lower mobile phase tubings to each other without getting are in.
10. The column is now ready for transport and further use.



Column Efficiency Testing

The column efficiency should be tested immediately after packing and at regular intervals during use to monitor any deterioration.

The preferred method for determining the efficiency of a packed column is using the height equivalent to a theoretical plate (HETP) and the asymmetry factor (A_s). The HETP and A_s values are determined by applying a sample such as 2% acetone or 1 M NaCl to the packed column.

A sample volume of approximately 1.5 % of the column volume and a flow velocity of 60 cm/h will give the optimal results.

Calculating HETP and A_s

Below is the calculation by which HETP and A_S are determined. This is done by using the UV or conductivity curve.

$$HETP = \frac{L}{N}$$

L = bed height (cm)
N = number of theoretical plates.

$$N = 5.54 \times \left(\frac{V_R}{W_h}\right)$$

V_R = volume eluted from the start of the sample application to the peak maximum.

W_h = The width of the recorded peak at half of the peak height.

V_R and W_h have the same units.

The reduced plate height is calculated by the following equation;

$$h = \frac{HETP}{d_{50v}}$$

d_{50v} = mean particle size (cm).

The reduced plate is often taken into consideration when evaluating column packing efficiency. As a guide a value of < 4 can indicate a well packed column.

The peak corresponding to the acetone or NaCl sample should be symmetrical with an asymmetry factor as close to 1 as possible.

An acceptable limit is $0.8 < A_S < 2.0$

$$A_s = \frac{b}{a}$$

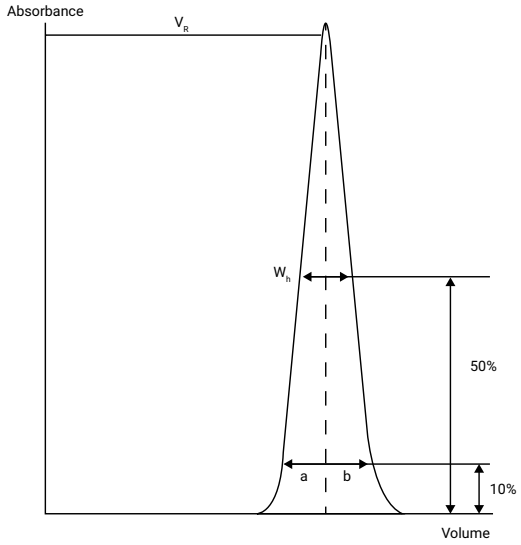
a = ascending part of the peak width at 10% peak height.

b = descending part of the peak width at 10% of peak height.

A change in the shape of the peak is usually the first indication of bed deterioration.

FIGURE 3

An example an HETP chromatogram



The calculated plate number will vary according to the test conditions, and it should only be used as a reference value. It is important that test conditions and equipment are kept constant so that results are comparable. Changes of solute, solvent, eluent, sample volume, flow velocity, temperature will all affect the results.

Acknowledgments

Bo Forsberg, Forsberg Biotech / NIPU Konsult HB

Innovative Solutions for Bioprocessing

In partnership with Repligen, Purolite™ develops and supplies innovative solutions for the bioprocessing industry, working with many of the top pharmaceutical companies to deliver the next-generation of healthcare. Our resins are used across the globe to deliver lifesaving medicines.

Global Support Network

No matter the location, our expert field application team members are positioned to help you solve your technical and downstream purification challenges, together. We provide the guidance necessary to develop robust, scalable, high productivity purification processes for mAbs and recombinant processes using Praesto™ Jetted chromatography resins. For wherever you are in your biomanufacturing journey, we are here to help.

Purolite Affinity Resin Toolbox

Purolite's diverse toolbox offers Protein A resins, Praesto Jetted A50 and Praesto AP+80, designed for high performance and increased sustainability, as well as novel resins, Praesto Jetted A50 HipH and Praesto 70 CH1, designed to enable cost-effective and reliable purification of bispecifics and Fc fusion proteins.

Purolite Ion Exchange Toolbox

Purolite's ion exchange toolbox consists of Praesto SP and Praesto Q resins in four particle sizes to ensure predictable selectivity across particle sizes, allowing for rapid performance screening.

Notes

Notes

Ecolab is a global developer, manufacturer, and supplier of Purolite™ Resins including ion exchange, catalyst adsorbent and advanced polymers that make the world cleaner and healthier.

www.puroliteresins.com

We're ready to solve your process challenges.

For further information on Purolite products and services,
visit www.puroliteresins.com or contact us at the addresses below.

Americas
americas@ecolab.com

Asia Pacific
asiapacific@ecolab.com

EMEA
emea@ecolab.com

P-000144-100PP-52024-ENG-R1-BP