GE Healthcare
Life Sciences

Pack-in-place packing procedure

Method Description

The pack-in-place technology is a novel packing technique used to pack columns where the stationary phase (chromatography medium) is introduced and packed in the column via special nozzles, thus eliminating the need to remove and replace the adaptor every time the column is packed and unpacked. This method is only applicable to chromaflow columns. The same procedure could be used to pack a variety of chromatography media in chromaflow columns.

Practical example

The procedure described below is a robust method for the quick and efficient packing of Q-Sepharose Big Beads in a Chromaflow column with internal diameter of 40 cm at a bed height of 30 cm. A trained operator could carry out the packing procedure alone.

Materials and equipment

Chromaflow CFV400, 40 cm. internal diameter, equipped with 50 micron

stainless steel screens.

Packing station PS-2

Stainless steel tank for the slurry

Stainless steel tank for water

10 mm standard Bioprocess System (skid)

Two 4-port 4-way valves (10 mm i.d.)

Pressure gauge 0-60 psi

Minimal 14 mm i.d tubing for all connections used in packing and unpacking

10 mm i.d. tubing for all other connections

Tri-clamp connectors and gaskets

Q-Sepharose Big Beads at 50-60% slurry in water

Water

2% Acetone, 0.5M NaCl in water

Setup Description

Hardware Setup

Level the column. The level should be placed on the tube's top flange and not on top of the adapter flange.

Connect a 4-port 4-way valve both to the top and bottom mobile phase outlets. Mount a pressure gauge to the top valve followed by the shortest possible tubing connected to the skid. The other ports of the top valve have hosing directed to waste for purging. Connect the bottom mobile phase valve via the shortest possible tubing to the skid; the other two ports should be connected to waste. Then connect the packing station to the column and to the water and slurry tanks according to the diagram provided on the packing station's control panel. Connect the packing station and the skid to a compressed-air source.

Close both mobile phases, place the bottom nozzle in PACK position and the top nozzle in **Unpack** position. Using the packing station, fill the column with water until all the air has been completely purged. Retract the top nozzle. Loosen the nuts holding the adapter in place. Lower or raise the adapter to the desired bed height. To raise the adapter, pump water into the column using the packing pump on the packing station. To lower the adapter, place the bottom nozzle in **Unpack** position and the top nozzle in **Run** position, and using the suction pump on the packing station suck the adapter down. Secure the adapter in its final position. Measure the distance between the tube's top flange and the adapter's flange and ensure it is exactly the same throughout. Adjust, if necessary. Drain the column completely via the "waste slurry" port on the bottom nozzle, collect and measure the volume of the drained water. This is the column volume.

Slurry Preparation

Determine the % slurry of the media and adjust to approximately 50% with water if necessary. Record the exact slurry concentration. Determine the amount of slurry needed for packing at the desired bed height (in this case, 30 cm). It is better to use the actual column volume determined above. Alternatively, multiply the bed height (30 cm) by the column's cross-sectional area (1,256 sq. cm for Chromaflow CFV400). Multiply the result by the compression factor recommended for the particular chromatography medium (in this example, the compression factor for Q-Sepharose Big Beads is 1.15). Divide the result by the slurry concentration. In this case, (30 × 1, 256 × 1.15)/0.5 = 87 L. This is the volume of slurry needed for packing.

Determine the volume of the following components (External Components Volume): - tubing from slurry tank to packing station, packing station's packing pump, tubing from packing station to top nozzle's inlet, tubing from top nozzle's outlet to packing station, tubing form packing station to slurry tank. This could be done either by calculation, or empirically by filling with water and using a graduated cylinder to measure the water's volume. Normally, the total external components volume (ECV) should not exceed 2 L, although this is not critical. Add an amount of slurry equal to the ECV to the slurry tank. Then add the amount of slurry needed for packing.

Column Packing Procedure

Prime the bottom nozzle and the packing lines with slurry. Stop the packing pump and insert the bottom nozzle into the empty column in PACK position. Insert the top nozzle in **Unpack** position. Both the top and bottom mobile phase outlets should be closed. Using the knob regulating the air feed pressure to the packing pump, dial up the pressure to the point where the pump just starts. As soon as enough slurry is introduced into the column to avoid formation of air bubbles, which otherwise would occur if the pump is spraying slurry into the column at great speed, adjust the air feed pressure to 3.5 bar and fill the column with slurry.

Immediately, retract both nozzles. Purge the packing station, bottom nozzle and packing lines with water using the packing station, and collect the purged slurry in the slurry tank. Purge the top nozzle and packing lines with slurry. Stop the pump and insert the top nozzle into the column in **Pack** position. Open the bottom MP to waste, or floor drain, so that the evacuating water can go to the drain. Start the packing pump and, if necessary, adjust the air feed to the packing pump to 3.5 bar. Since the pressure gauge's needle fluctuates with every stroke of the pump, the pressure should be dialed up until the needle fluctuates in the range of 3.2-3.6 bar.

During the packing the slurry should be constantly agitated using the stainless steel paddle in order to avoid settling of the gel in the slurry tank. Also, ensure that no vortex is formed in the slurry tank as this will cause air to be pumped into the column.

As soon as all slurry has evacuated the slurry tank, stop the packing pump and immediately retract the top nozzle, and close the bottom mobile phase outlet. Purge the top nozzle with water using the packing station.

Using the skid, pump 2-5 L of MP through the column at 100 L/h in UPFLOW direction in order to purge the top screen and the gel bed from any air bubbles that may have been trapped at the top during the packing Reverse the flow direction and pump 10-15 L of MP in downflow direction at the same flow rate. The column is now ready for testing.

Testing

The column is tested using the skid for delivery of both the MP and the sample. A test method could employ the following concepts:

1. Run 1L of MP through the system with the airtrap in line and with the column bypassed in order for the skid's pump to be brought up to speed.
2. With both the column and airtrap bypassed, the skid is purged with 2 L of sample at which point the column could be brought inline in a **Downflow** mode.
3. After the desired amount of sample is applied, the column is taken out of line and the skid is purged with 5 L of mobile phase.
4. After the purging, both the column and airtrap are brought inline and the testing continues until 75-80 L of MP have passed through the column.

Unpacking

Insert both nozzles in **Unpack** position. Using the packing pump, pump 60-70L of water through the column and collect the evacuating slurry in the slurry tank. Shut off the water feed and open the slurry feed. Let the packing pump continue uninterrupted until all of the gel bed is broken up, i.e. the column is now filled with slurry. Stop the packing pump. Turn on the suction pump and pump the slurry out of the column and into the slurry tank. Rinse the column with water using the packing station.

Helpful tips and Potential Pitfalls

Remember to keep the adapter's O-rings lubricated with water or 20% ethanol. Avoid creating vacuum in the column when suctioning slurry or raising the adapter.

When filling the column with water or slurry, keep the top MP closed and let the air exit via the top nozzle.

Some small air bubbles may remain in the column after even the most careful filling/purging. This is OK and it will not adversely affect the quality of the pack. When unpacking the column, some slurry may remain adhered to the top screen. It could be rinsed off by placing the top nozzle in PACK position and pumping some water using the packing pump.

It is a good idea to run the column upflow after packing to get rid off air bubbles, which may have become trapped during packing and to ensure that the top screen is properly wetted before testing.

Never allow any slurry to remain and settle in the packing station, the packing lines, and especially in the nozzles. If this happens, it may be necessary to remove and disassemble any components clogged with settled gel.

Slurry tanks with side openings do not normally allow vortexing during packing as the slurry is sucked out of the tank. If the slurry tank has a conical bottom with an opening in the center, ensure that no vortex is created during packing as this will cause air to enter the column in which case the column has to be unpacked and repacked.

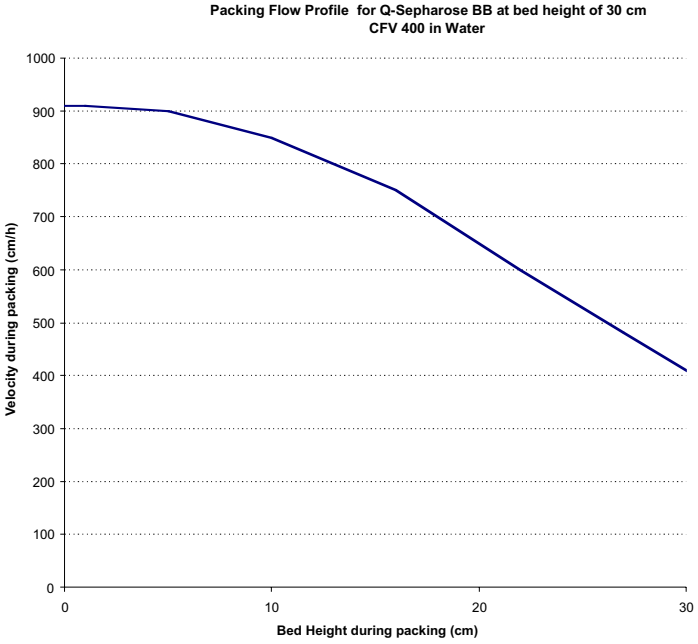
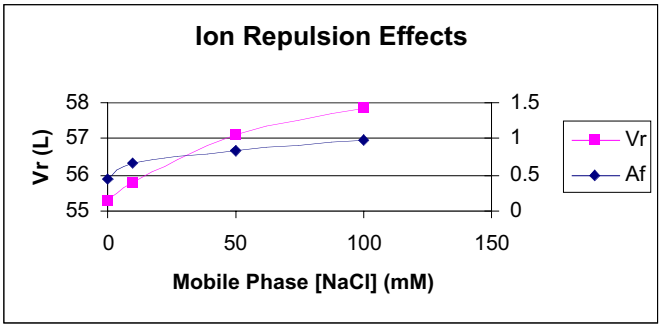


Figure 1.



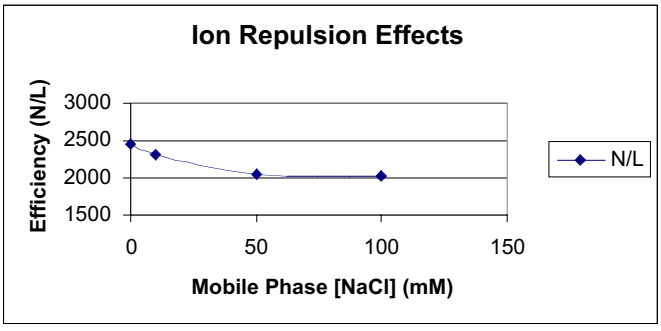


Figure 2. The effect of mobile phase ionic strength on the retention volume, efficiency and asymmetry as determined with Vs = 1% Vt of 1M NaCl .

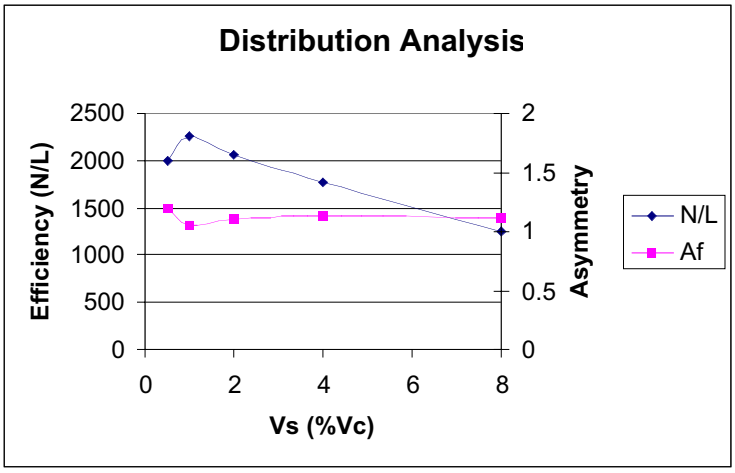


Figure 3. Distribution analysis of Q Sepharose Big Beads in a CFV 400 Mark II with 50 mm Bopp screens with L = 30 cm.

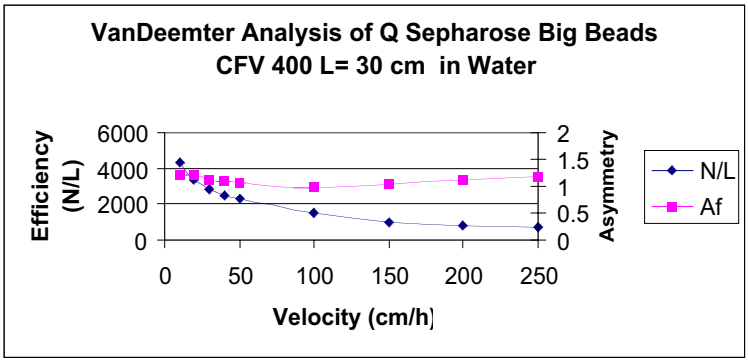


Figure 4. Efficiency and asymmetry as a function of mobile phase velocity for Q Sepharose Big Beads in water with Vs = 1% Vc. Sample was 1% Acetone in water.

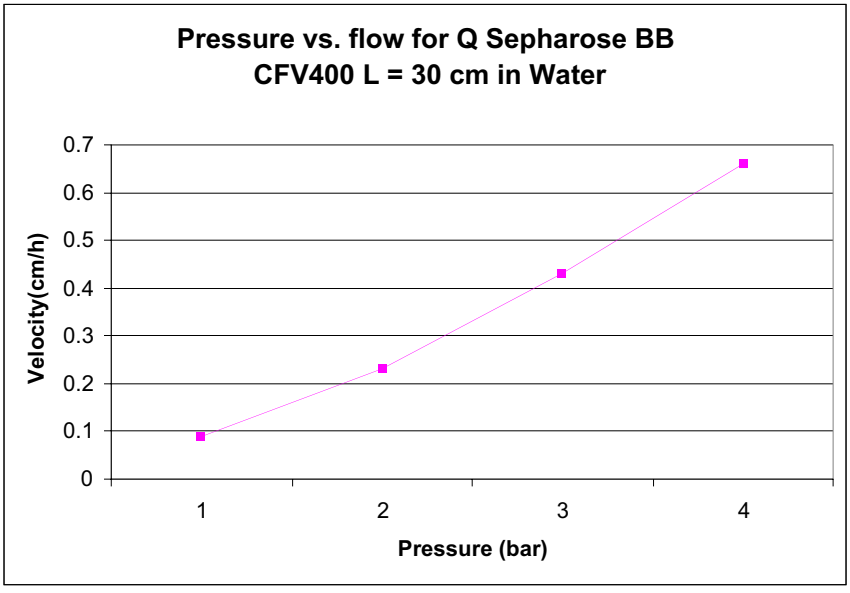


Figure 5. Pressure Flow Curve for Q Sepharose Big Beads in a CFV 400 L= 30 cm CF = 1.15

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