GE Healthcare
Life Sciences

Axial Compression Packing Method

Method Description

The axial compression packing method is quite easy and requires a minimal amount of equipment. It is useful for situations where rapid, repetitive, and highly reproducible packing is important. It has been used successfully with many types of media such as Superdex and Sephacryl, and gives especially good results with relatively noncompressible media types such as Source. In this method, hydraulic pressure is used to drive the column adapter into the column containing the media slurry. The media builds up on the bottom bed support within the column as the packing buffer exits through the bottom valve. The rate of adapter descent controls the density of the bed as it forms. As the adapter touches the bed, mechanically compression of the media establishes the final packed bed configuration.

Practical example

We will pack Source 15Q in a Fineline 100 column to a bed height of approximately 10 cm using the axial compression packing method. Typical numbers obtained by this method using the recommended test methods are N/L of >20,000 ppm with an As of 0.8 to 1.3.

Materials and Methods

Fineline 100 column with locking pins

Source 15Q – 816 ml in a £ 50% slurry in packing buffer

Packing Buffer – 50 mM NaCl containing 20% ethanol (3 liters)

Pressure vessel with regulator and 60 psi pressure gauge filled with 5 liters of water

Two 4-port 2-way valves, 6 mm i.d.

Three shutoff valves, 6 mm i.d.

Three pieces hosing, 6 mm i.d., ~ 1 meter length

One piece clear hosing 1.9 – 6 mm i.d. ~ 1 meter length

Pressure gauge 60 psi, 6 mm i.d.

Triclamps and 6 mm gaskets

Level, 13 mm socket wrench, stirring rod, waste bucket, tape measure

Setup Description

Hardware Setup

The column outlets should be equipped with 4-port 2-way valves and hosing with the same i.d. as the column. Hosing to waste is connected to a port on the bottom valve perpendicular to the column outlet using triclamps with matching i.d. gaskets. Both hydraulic chamber outlets are equipped with shutoff valves followed by hosing to waste on one outlet and to a pressure vessel on the other. The pressure gauge is positioned on a top valve port perpendicular to the adapter outlet, followed by clear hosing to observe air removal and a shutoff valve to a waste container. The pressure vessel is filled with enough water or 20% ethanol to completely purge air from the hosing and column, as well as fill the hydraulic chamber during packing. A pressure regulator and gauge is fitted on the vessel and hooked to an adequate source of compressed air. A shutoff valve on the vessel makes releasing the pressure after packing more convenient.

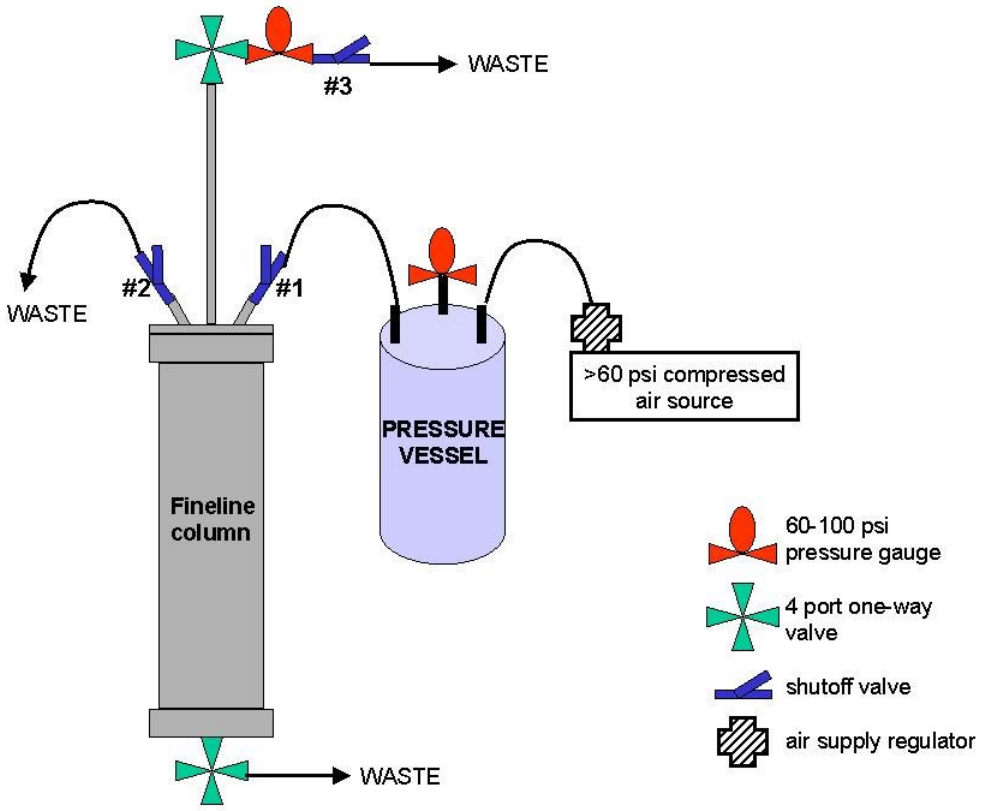


Figure 1. Hardware

Slurry Preparation

The slurry is prepared in packing buffer to a final volume to fit into the column at or below the bevel at which the adapter will be seated. The slurry should be temperature equilibrated under packing conditions. The slurry must be thoroughly mixed just prior to pouring it into column.

Column Preparation

Position and level the column for packing. Assemble the column such that all bolts thread easily in place. Mark the flange of the column and lid to facilitate reassembly in the same relative position after slurry is added to the column. Remove the adapter and pour 2-3 cm of packing buffer into the column. Open the bottom valve and briefly allow buffer to purge air from the bed support leaving about 1 cm of buffer in the column.

Column Packing

Purging air from the column

Make sure the shutoff valve on the column lid is closed to the pressure vessel and pressurize the tank to 60-63 psi. Mix the slurry thoroughly and immediately pour it into the column. Use a long plastic rod to stir the media for ~10 seconds and avoid scraping the bottom screen. Bolt the adapter and lid with o-ring loosely in place, thread one of the locking pins (with washer) just through the column flange and tighten the bolts on the lid with a wrench. Push down gently on the adapter rod to seal the adapter into the column tube and make sure the top adapter valve is open from the column to the pressure gauge. The shutoff valve following the top pressure gauge is closed and the shutoff valve on the column lid that goes to waste is opened. Partially open the shutoff valve to the pressure vessel and allow the hydraulic chamber to fill. When water exits to waste from the opposite shutoff valve, the chamber is full. Close the valve to waste which results in pressurizing the hydraulic chamber. Open the shutoff valve after the clear tubing on the adapter and allow the adapter to descend just until the majority of air has been cleared from above the slurry and in the adapter shaft. Close this top shutoff valve and fully open the shutoff valve to the pressure vessel.

Packing the column

Open the bottom valve of the column to waste. Allow the adapter to descend unhindered while monitoring the bed pressure on the gauge at the top valve. It should increase and stabilize around 50 psi, then begin to drop as the flow rate exiting the column decreases and the bed is mechanically compressed. When the pressure drops to 5 ± 1 psi, the bottom valve on the column is closed. The locking bar is rapidly placed over the adapter rod and secured in place with both locking pins and washers. Tighten the pins as snugly as possible by hand only! Release the pressure in the pressure vessel and disconnect it from the column. Gradually release the pressure on the column bed using the shutoff valve after the top pressure gauge.

Curing the column

Remaining air in the adapter is removed and the bed cured by running 1-2 CVs of packing or testing buffer upflow at 30-50 cm/hr. Best results will be obtained if the column is then allowed to sit undisturbed overnight before testing.

Helpful Hints and Potential Pitfalls

Media quantitation

Source settles as a very firm bed allow storage buffer to be completely decanted without loss of media. If a known volume of packing buffer is then added and the resulting slurry volume measured, the settled volume of media can be determined directly from the difference in these volumes.

Slurry preparation

Source media has a very hydrophobic matrix that causes the beads to clump unless 20% ethanol is in the packing buffer. It is also recommended to keep slurry concentration between 30-35% to further minimize clumping. Even with these adjustments, extra stirring is usually necessary with this media to disperse the beads thoroughly before setting the adapter. Do not use a magnetic stir bar, as this will damage the media.

Purging Air from the Column

Care should be taken to perform these steps smoothly at a controlled rate. Lowering the adapter too far or quickly during the column purge will result in media buildup on the underside of the adapter screen and be detrimental to the pack. Always run the column upflow after packing to remove residual air from the adapter and prevent drying out and damage to the bed.

Testing

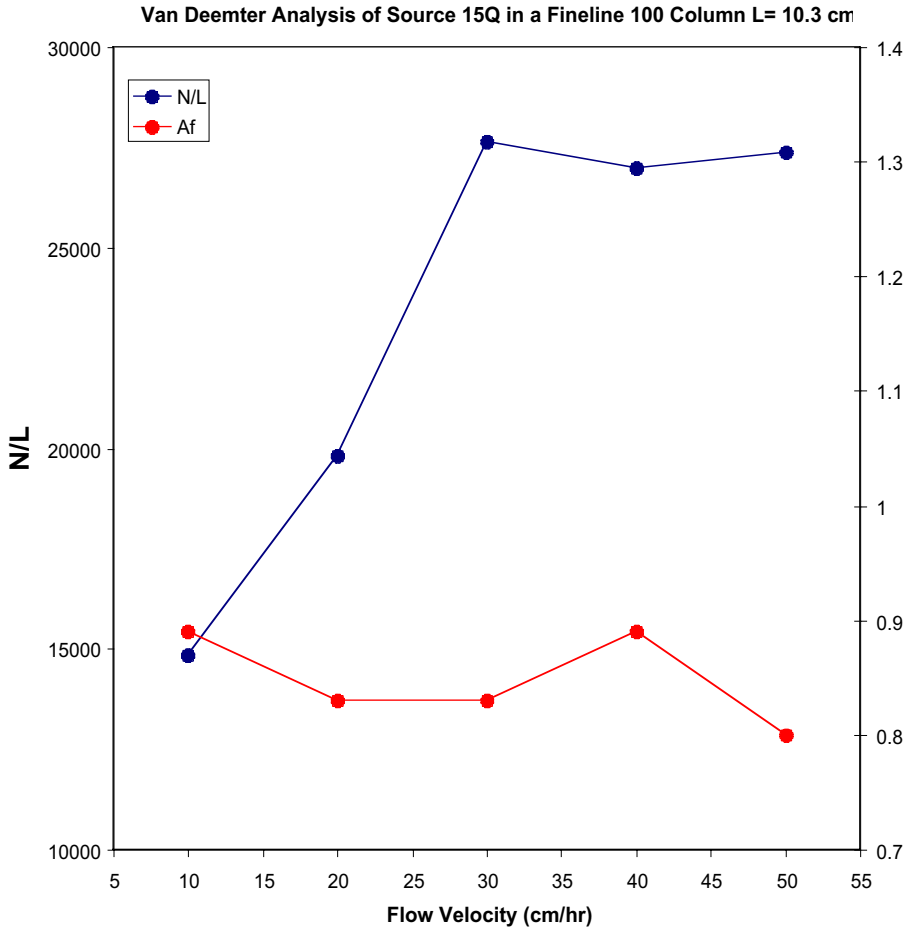
One recommended procedure uses packing buffer as the mobile phase at rate of 20-30 cm/hr. The sample is 150 mM NaCl containing 20% ethanol and totals ~1% of the packed column volume. Acetone interacts with the Source matrix and should not be used.

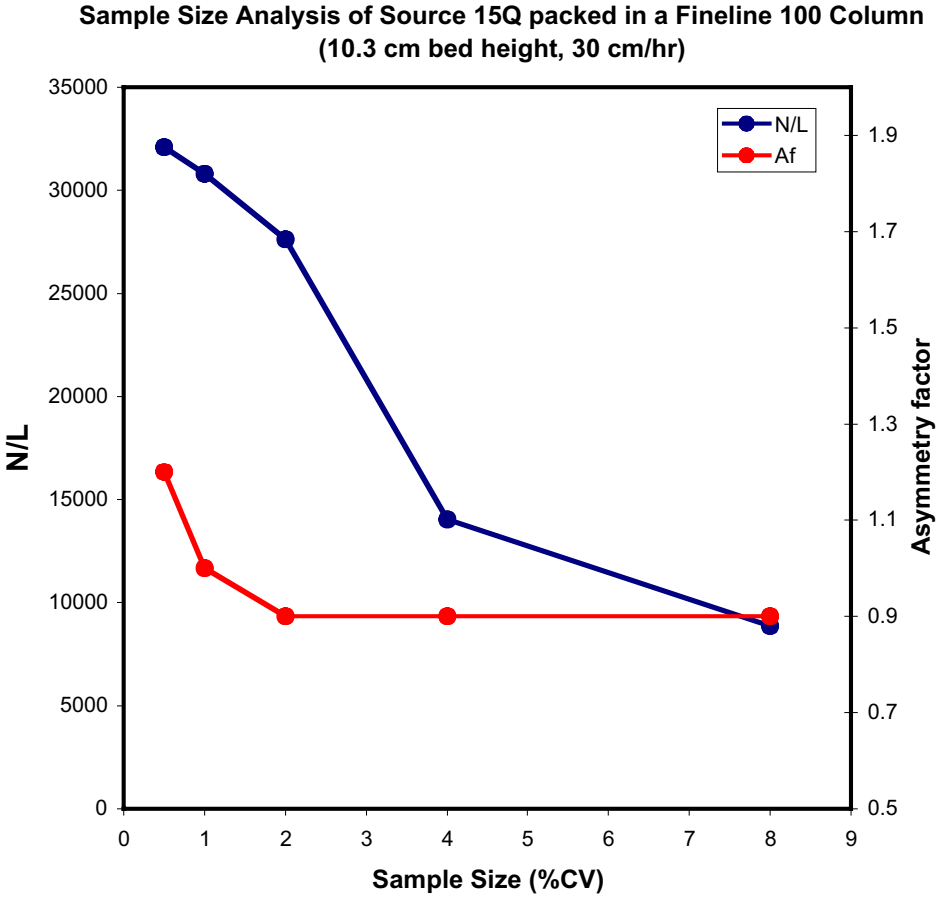
Storage

Once the locking bar is in place, packing buffer inside the hydraulic chamber can be removed or changed. It is recommended that liquid be kept in this chamber to lubricate the O-ring around the adapter. This storage solution should contain a bacteriostatic agent if not replaced regularly, and avoid salts that can damage the steel surfaces over time.

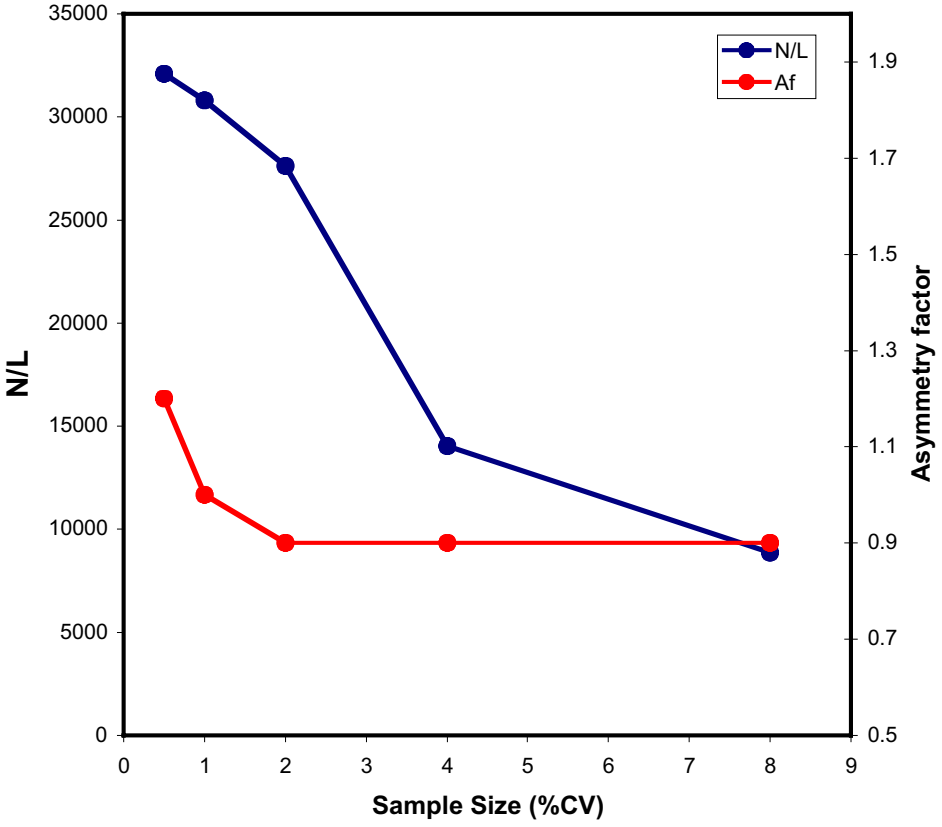
Unpacking

An easy way to unpack the Fineline column is to flow packing buffer from the pressure vessel up through the bottom valve and push the adapter and bed away from the bottom screen. One outlet of the hydraulic chamber must be open to waste and the top valve closed. When the adapter has risen about 10 cm, close the bottom valve and flow buffer into the top valve to raise the adapter off the bed and pass the bevel for easy removal. This prevents loss of media when the adapter is removed and loosens the bed for easy dispersion in the unpacking buffer.

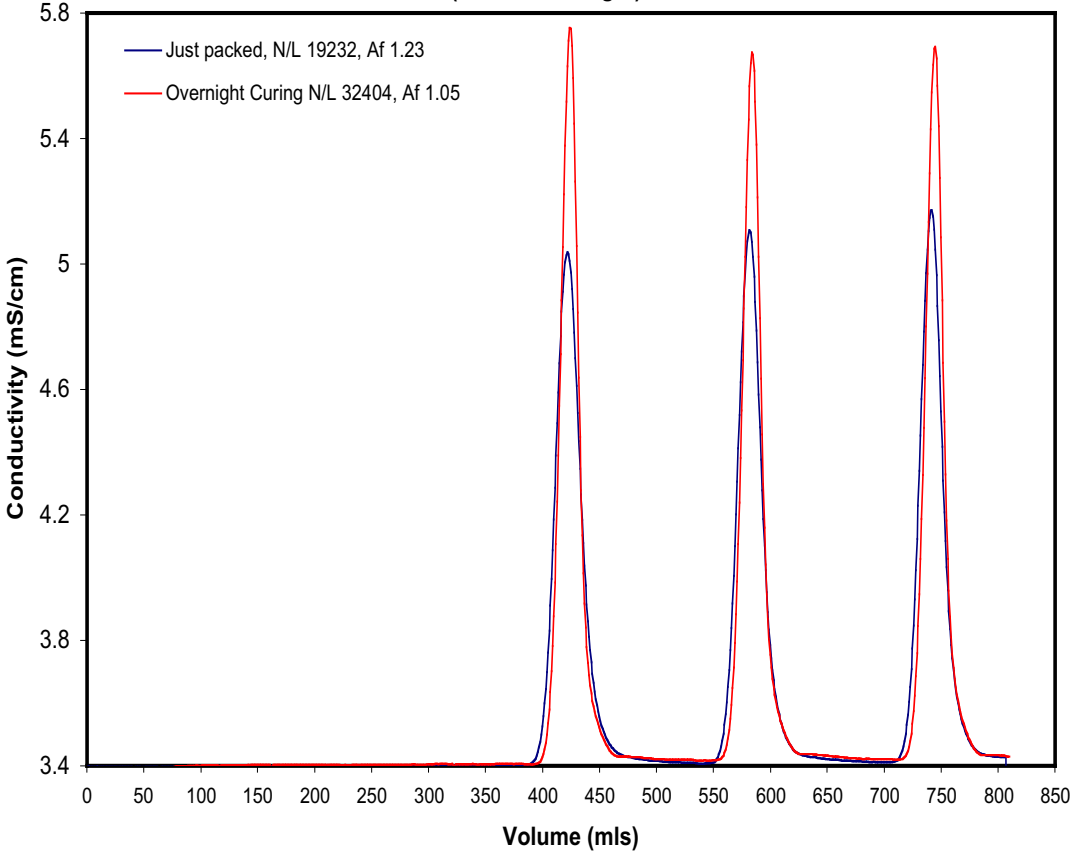

Van Deemter Analysis of Source 15Q in a Fineline 100 Column L= 10.3 cm
N/L
Af
N/L
Flow Velocity (cm/hr)
30000
25000
20000
15000
10000
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
5
10
15
20
25
30
35
40
45
50
55


Sample Size Analysis of Source 15Q packed in a Fineline 100 Column
(10.3 cm bed height, 30 cm/hr)
N/L
Af
N/L
Asymmetry factor
Sample Size (%CV)
35000
30000
25000
20000
15000
10000
5000
0
1.9
1.7
1.5
1.3
1.1
0.9
0.7
0.5
0
1
2
3
4
5
6
7
8
9

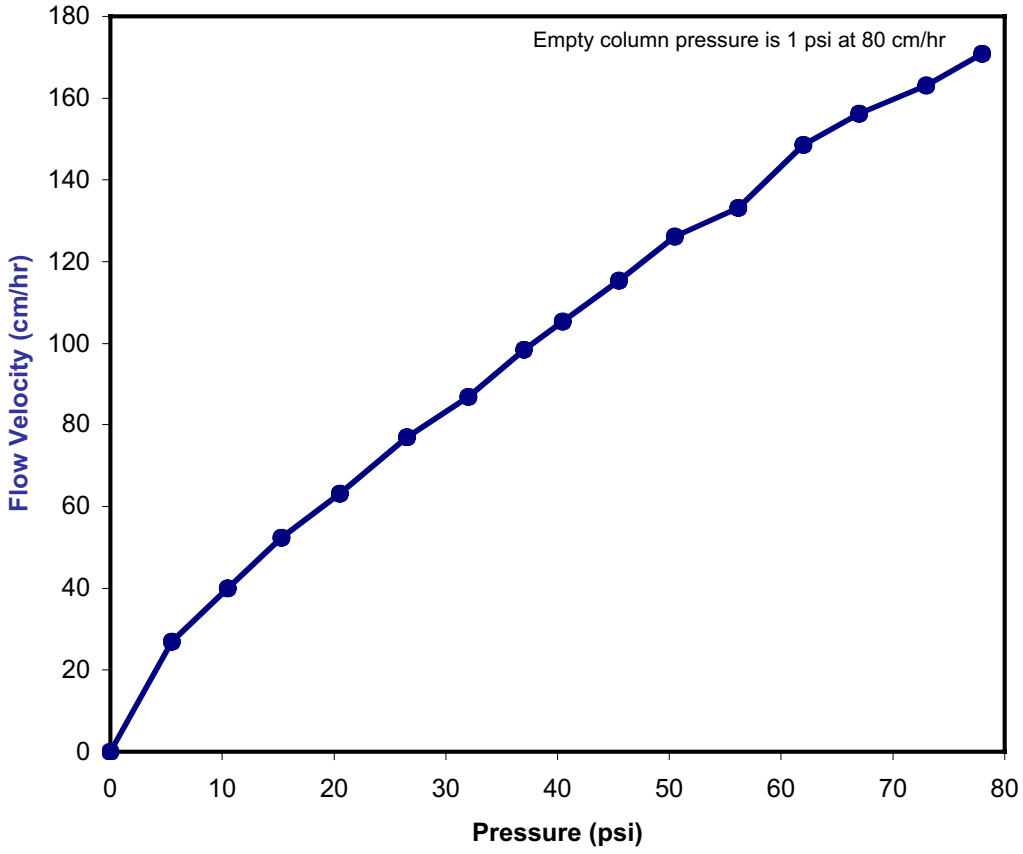
Sample Size Analysis of Source 15Q packed in a Fineline 100 Column
(10.3 cm bed height, 30 cm/hr)



Curing Source 15Q beds packed in a Fineline 100 Column
(10 cm bed height)



Pressure Flow Curve of Source 15Q in a Fineline 100 Column



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