GE Healthcare
Life Sciences

Constant Flow Packing Method

Method Description

Constant flow packing is a two step method that is simple and applicable to all types of media. Assuming a suitable liquid delivery system is available, the main requirements are that the column must have a moveable adapter and a pressure rating which allows the optimal packing flow rate to be obtained. In the first step, a low flow rate is used to form a loosely packed bed from the slurry. This allows the adapter to be lowered the majority of the distance towards its final packed position with little or no expansion of the bed during travel time. In the second step, the flow rate is increased to a predetermined optimal value to obtain the desired packing density. When flow is stopped, the proximity of the adapter to the bed minimizes the time necessary to successfully reposition the adapter to stabilize the packed bed.

Practical Example

A BPG™ 200/500 column will be packed to a bed height of 20 cm with Q Sepharose™ FF using the two step flow packing technique. A well-packed column of this type when tested appropriately should give ~5000 ppm with an As of 0.8-1.5.

Materials and Methods

Bioprocess Glass (BPG) 200/500 column with 23 micron nets

Q Sepharose FF, ~ 50% slurry in water

Tank with dist. water for packing (~50 liters)

4-port 4-way valve, 6 mm i.d.

4-port 2-way valve, 6 mm i.d.

Pressure gauge (45 psi)

Hosing, 6-10 mm i.d. (4 pieces, £1 m length)

Triclamps and 6 mm gaskets

Diaphragm pump on 10 mm Skid with flow monitoring capability

Level, wrench, stirring paddle, squirt bottle, 20% ethanol

Setup Description

Hardware Setup - The column is fitted with a 4-port 4-way valve on the adapter outlet and a 4-port 2-way valve on the bottom outlet. The valves, hosing and gaskets used should have the same i.d. as the column outlets or slightly larger. One port on the top valve perpendicular to the adapter has a pressure gauge followed by hosing connected to the pump and water tank. The other ports of the top valve have hosing directed to the waste for purging the pump and column. The bottom valve is equipped with hosing on a port perpendicular to the column outlet that will be directing flow to waste or recycling water back to the tank. The hosing on the bottom of the column is connected to the packing skid so the flow monitor can be used to accurately control the flow rate. A torque wrench may be necessary to secure the adapter plate to the column.

Slurry Calculations and Preparation

The slurry is prepared in packing buffer to a final volume which must fit into the column with the adapter in place at its highest position. The slurry should be temperature equilibrated under packing conditions. A stirring paddle or stick will be needed to stir the slurry in the column.

Column Diameter = 20 cm

Ac = 0.314 L/cm

Vc packed bed = 20 cm x 0.314 L/cm = 6.28 L

Compression Factor, CF = 1.15

Volume Gravity Settled Gel Required , Vgs = 6.28 L x 1.15 = 7.2 L

Slurry Volume at 50% Slurry = 7.2 L/ 0.5 = 14.4 L

Column Preparation

Operation of the adapter, integrity of the adapter O-ring and porosity of the support nets should be checked before starting. The column tube and all internal surfaces must be clean. The column nets are wetted with 20% ethanol and 2-3 centimeters of packing buffer are poured into the column with the bottom valve closed. Air is removed from under the bottom net using suction and about 1 cm buffer left in the column. The column is leveled in position for packing and the wheels locked if applicable.

Column Packing

First Step

The first step of constant flow packing is to form a loosely packed bed at a low flow rate (20 – 30 cm/hr = 6 – 10 L/h) and position the adapter near the final bed height. To accomplish this, the slurry is stirred in the column until homogeneous with care taken not to damage the bottom net with the stirring paddle. The adapter is quickly bolted in place and lowered until the slurry passes just above the adapter O-ring. A quick jiggle of the adaptor will help clear air from the under side. The O-ring is tighten such that slurry will no longer pass above it, and the top valve placed in position to allow buffer to exit from the column up through the adapter to waste. The adapter is again lowered until air under the net and in the adapter shaft has been purged. The pump is started and the hosing to the column also purged of air without changing the valve position. Flow should be quickly adjusted to approximately that desired for the first step and then the top valve turned to place the column inline with the pump. The bottom valve is immediately opened to waste or recycle, and the flow rate adjusted as needed. After the bed height of the media stabilizes in 1-2 CVs, the bottom valve is closed and the top valve turned to take the pump out of line with the column. The adapter O-ring is loosened so buffer will pass above it, and the adapter lowered to 0.5-1 cm above the top of the bed. The O-ring is retightened and the adapter lowered to 1-2 mm above the bed with buffer passing out the top valve to waste. The top valve should be turned to place the pump in line with the column again and the bottom valve opened. The operator should proceed immediately to the second packing step.

Second Step

The second step of flow packing is to compress the bed using a flow rate predetermined from the pressure flow curve data (400 – 450 cm/h = 125 – 140 L/h), and rapidly set the adapter position to maintain the packed bed density. The pump is adjusted quickly to the desired flow rate to obtain the final bed height, and the integrity of the adapter Oring seal checked and further tightened if necessary. When the bed has stabilized in ~ 1 CV, the bed height is marked on the column tube. The top valve is turned to place the pump out of line with the column at the same time as the bottom column valve is closed.

The adapter must be rapidly lowered into place with minimal disturbance of the packed bed. This should be done with the adapter O-ring just loose enough that buffer exits out of the top valve and not around the O-ring. Once the adapter is in place, the O-ring should be retightened immediately. If the packed bed decompresses significantly above the marked bed height before the adapter can be lowered in place, this second packing step can be repeated once. Wait at least 5 minutes for the bed to cure before moving the column.

Test Method

The column was tested at 20 cm/h (6.2 L/h) with water as mobile phase and 1% acetone as the sample. The sample volume was 625 ml (1% Vc).

Helpful Hints and Potential Pitfalls

Media and Slurry Handling

Settled media in a closed plastic container can be rapidly reslurried by inverting the container and sharply tapping the bottom with a rubber mallet.

A squirt bottle with water or packing buffer facilitates rinsing the container for

quantitative media transfer and the column walls to assure a good adapter seal.

Avoiding air in the column before packing

When the slurry passes above the adapter O-ring, a gently "jiggle" or twist of the threaded shaft helps remove bubbles around the O-ring. Elevate the waste hosing on the top valve that is used when purging air from the top net and adapter. This facilitates purging and prevents siphoning. Purge the pump and attached hosing lines at high flow before starting and make sure there is flow when switching the top valve to put the column in line with the pump.

Lowering the adapter

The adapter must be lowered as quickly as possible in the second step of flow packing. However, excessive rocking of the column, media loss from the bed around the O-ring, and excessive mechanical compression of the bed with the adapter can compromise your pack. A second person can be helpful to hold the column in place and to call the pause for O-ring tightening before the bed is disturbed. If after the second flow packing, the adapter can not be lowered before the bed decompresses more than 0.5, the step should be repeated rather than trying to compress the bed with the adapter.

Storage

Both outlets of the column should be sealed after equilibrating the column in storage buffer. The column must be stored in a temperature-controlled environment with water or 20% ethanol above the adapter O-ring to prevent it from drying out and cracking. Covering the column with a clean plastic trash bag helps prevent dust and debris from accumulating on exterior surfaces.

Unpacking

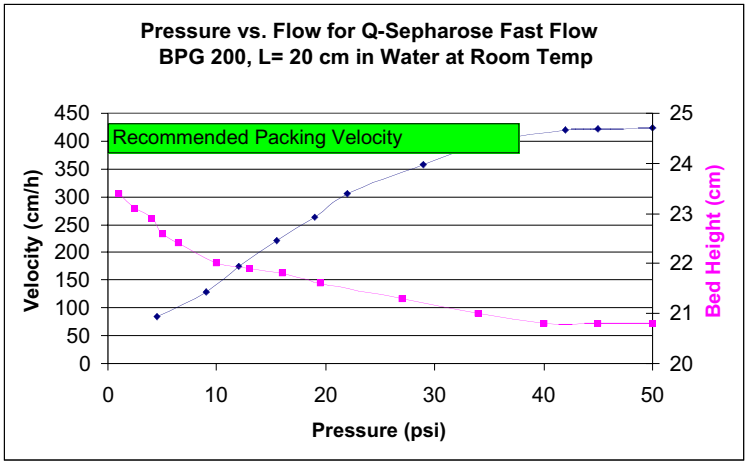
Place several centimeters of buffer or water above the adapter o-ring of the BPG column and fully loosen the wing nut which seals the adapter O-ring. Make sure the adapter outlet is open to waste. If the adapter is not easily raised, try slightly

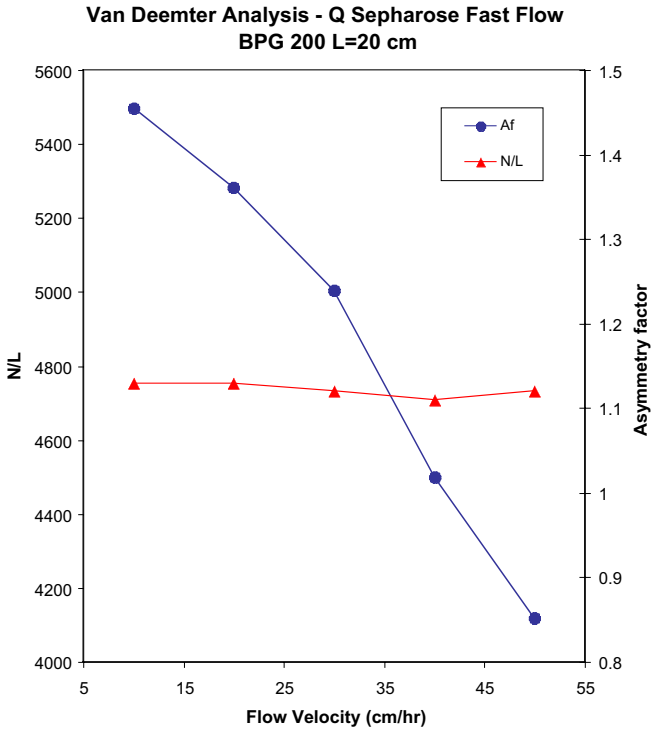
lowering it to break the O-ring seal on the glass tube. If the O-ring rolls, tighten the wing nut a bit to prevent this during adapter ascent. A firm "jiggle" or twist on the threaded adapter shaft is also useful when attempting to unseal the O-ring. Raise and remove the adapter. If the packed bed is difficult to reslurry, pumping buffer up through the bottom valve will help to lift and disperse the media more rapidly and clear the bottom bed support.

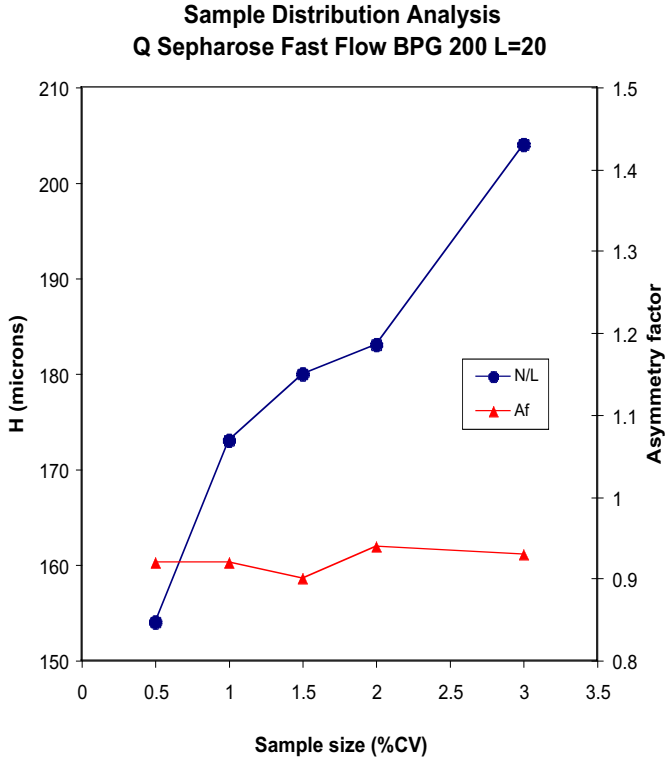
Scaleup

When a packing reservoir is used, an extra valve for the lid is recommended and a cart will be useful if the reservoir is large. A hoist should be used to raise and lower a heavy adapter. A large wrench will be necessary to adjust the height of the

legs. Remember for large scale that using a paddle and diaphragm pump to mix and transfer a 60-liter carboy of media is much easier then shaking and pouring twenty 5-liter containers.







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29-0017-95 AB 10/2011