Methods for packing MabSelect Xtra in production scale columns

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Introduction

A well-packed column that performs reliably and robustly is essential in the large-scale manufacture of modern biopharmaceuticals such as monoclonal antibodies. In contrast, a poorly-packed column will lead to costly process disruptions and loss of valuable product. If the bed is not well-packed, it may crack and cause channeling, or be further compressed causing a liquid gap where mixing can occur. Long-term stability issues can also result from a poorly packed bed. Proper packing and testing eliminates such concerns. The resulting bed is stable and performs according to expectations over many processing cycles.

MabSelect Xtra™ is used in the commercial manufacture of monoclonal antibodies (MAbs). The very high dynamic binding capacity of MabSelect Xtra is especially well-suited for the cost-effective capture of antibodies from high-expression feedstocks.

MabSelect Xtra packed in large-scale columns is thus an attractive combination for MAb manufacturers seeking to lower their antibody production costs. This Application Note describes methods for packing three types of large-scale columns suitable for GMP manufacture: Chromaflow™, BPG™, and BioProcess™ LPLC. It also summarizes key test data obtained from the packed beds.

Product characteristics

MabSelect Xtra

MabSelect Xtra is a member of the MabSelect™ family of recombinant protein A-based affinity media for capturing monoclonal antibodies. Like MabSelect, MabSelect Xtra is based on an innovative, high-flow agarose base matrix, but has larger pores, a smaller mean particle size and a higher ligand density. This results in greater capacity; MabSelect Xtra has 30% higher dynamic binding capacity for polyclonal human IgG than conventional protein A-based media. The maximum operating nominal velocity of MabSelect Xtra is high, about 300 cm/h at a bed height of 20 cm.

Fig 1. Chromaflow columns can be packed, unpacked, and cleaned with the lid in place.

MabSelect Xtra shares the same recombinant Protein A ligand as MabSelect. Both MabSelect and MabSelect Xtra contain a C-terminal cysteine residue. Similarly, coupling conditions, designed to favor single-point attachment of the ligand, are the same.

Chromaflow columns

Chromaflow columns (Fig 1) are process-scale columns for GMP manufacture. A patented three-position nozzle in the center of the top and bottom bed supports allows packing, unpacking, and cleaning with the lid in place. The standard range offers inner diameters from 400 mm to 1000 mm. Custom columns can be ordered up to 2000 mm. All are pressure-rated for operation at 3 bar.

Chromaflow columns simplify chromatographic procedures (especially packing), thereby reducing labor costs and increasing convenience. Manual operation is minimized, helping achieve reproducible packing and results. Because all column operations are performed in a 'closed' environment, overall safety and hygiene are improved.

Fig 2. BPG columns feature a single-screw adapter for easy, efficient packing and running.

Fig 3. BioProcess LPLC columns are quickly and efficiently packed using axial compression.

BPG

BPG columns (Fig 2) are glass chromatography columns for process development and biopharmaceutical manufacture. Component materials include calibrated precision glass and high-grade electropolished stainless steel. In use, the single-screw adapter allows easy, efficient packing and running.

BioProcess LPLC columns

BioProcess LPLC columns (Fig 3) comprise a family of stainless steel chromatography columns with a wide range of tube lengths and inner diameters. Their well-proven design simplifies packing and ensures speedy, reliable, and convenient process-scale purification for biopharmaceutical manufacturing.

The columns feature dynamic axial compression that uses liquid or air as the compression medium. They are easy to fill with medium and simple to pack.

Packing and test methods

The lot of MabSelect Xtra used in the packing studies was a standard production batch, specifically tested to ensure that it was representative of routine manufacture. Similarly, the three columns and their related equipment (pumps, tanks, etc.) were standard products.

Evaluation of packing

If large-scale columns packed with MabSelect Xtra are to live up to the demands of commercial manufacture, they must perform with a high degree of efficiency over many processing cycles, i.e. display very high stability.

A number of tests can be applied to check the efficiency of the packed bed and, if repeated at intervals, monitor the state of the bed during the working life of the column.

Tests used to evaluate the packing of MabSelect Xtra are summarized below. Results are given in the sections outlining the packing methods.

Height equivalent to a theoretical plate and asymmetry factor

A good method of expressing the efficiency of a packed column is in terms of height equivalent to a theoretical plate (HETP) and asymmetry factor (A_s). These values are easily determined by applying a sample such as 1% acetone solution to the column using water as eluent. Sodium chloride can also be used as a test substance. (Use 0.8 M NaCl in water as sample with 0.4 M NaCl in water as eluent.)

The calculated HETP will vary depending on the test conditions and should therefore be used as a reference value only. Keep conditions and equipment constant so that

results are comparable. Changes in sample composition, sample volume, eluent, nominal fluid velocity (cm/h), liquid pathway, temperature, etc., will influence the results. If an acceptance limit is defined in relation to column performance, the column HETP can be used as part of the acceptance criteria for column use.

Method for measuring HETP and A_s

To avoid diluting the sample, apply it as close to the column inlet as possible. Place the UV or conductivity monitor as close to the column outlet as possible.

Conditions

Sample volume:	1.0% of the bed volume
Sample conc.:	1.0% v/v acetone
Flow velocity:	20 cm/h
UV:	280 nm, 1 cm, 0.1 AU

Calculate HETP and A_s from the UV curve (or conductivity curve if NaCl is used as sample) as follows:

$N = 5.54(V_e / W_h)^2$

$HETP = L/N$

where

L = Bed height (cm)

N = Number of theoretical plates

V_e = Peak elution distance

W_h = Peak width at half peak height

V_e and W_h are in the same units

To facilitate the comparison of the performance of columns packed with media of different particle diameters, the dimensionless concept of reduced plate height is often used. Reduced plate height is calculated as:

$h = HETP/d_{50v}$

where

d_{50v} is the median diameter of the bead, using the same unit as for HETP.

As a guideline, a value of $h < 3$ is normally very good if the optimal test conditions outlined above are used. Any other test velocity or sample volume will alter the test results.

The peak should be symmetrical and the asymmetry factor as close as possible to 1 (values between 0.8 and 1.5 are usually acceptable). A change in the shape of the peak is usually the first indication of bed deterioration due to use.

Peak asymmetry factor is calculated as:

$As = b/a$

where

a = 1st half peak width at 10% of peak height

b = 2nd half peak width at 10% of peak height

As a guideline, an asymmetry value between 0.8 and 1.6 is acceptable using the optimal test conditions outlined above.

Van Deemter analysis, sample volume analysis, and pressure/flow rate

A number of other tests can be applied to complement single HETP and A_s measurements. The so-called Van Deemter analysis shows if a bed is well-packed and whether the column distribution system is functioning optimally by testing at different velocities. The sample volume test examines the same features, this time under increasing sample loading. If distribution is good, bed efficiency should decrease with higher test velocity and sample volume. Asymmetry should remain unchanged. These two tests also show the relative effect of using test velocities and sample volumes other than the ones outlined above for testing the bed efficiency on a regular basis.

Finally, pressure/flow curves provide a simple yet effective illustration of column performance in terms of the maximum velocity at which the process can be run. Running a pressure/flow curve can also provide information on whether or not liquid gap formation will occur.

Packing MabSelect Xtra in Chromaflow 600

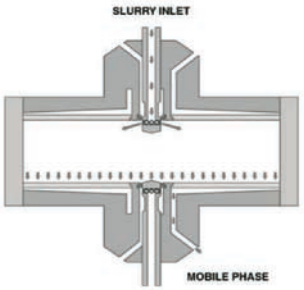
The method used to pack Chromaflow 600 is based on a standard Chromaflow procedure, i.e. using nozzles in the top and bottom end-pieces to fill, pack, and unpack the medium without removing the lid or adapter. The method makes use of the optional Chromaflow Packing Station, which includes a control panel with pneumatically-actuated diaphragm pumps and valves, plus a tank to hold the slurry.

Table 1 lists key items of equipment. Figure 4 shows the basic Chromaflow column top and bottom end-piece designs and identifies the main valves, inlets/outlets and connections, etc. referred to below.

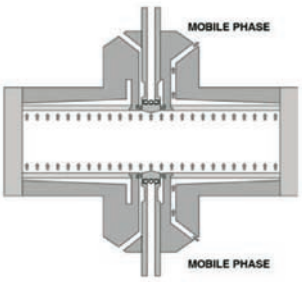
Table 1. Key equipment used to pack and test MabSelect Xtra in Chromaflow 600

Column:	Chromaflow 600
Chromatography system:	10-mm BioProcess
System	
Packing station:	Pack 50
Pressure-flow test pump:	2-inch rotary lobe

Part 1: pack and run



Part 2: run and run



Part 3: unpack and unpack

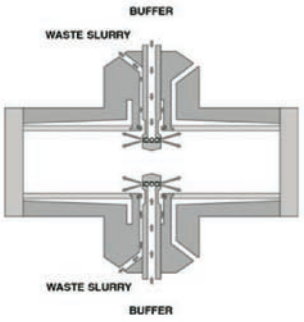


Fig 4. The three positions of the Chromaflow nozzle showing packing from the top.

Medium preparation

For a bed height of 25 cm, prepare a known concentration of slurry (here 61%) from 85 l of MabSelect Xtra to give a final slurry volume of 140 l. For best results, decant the 20% ethanol in which the medium is delivered and replace with water. A calculation example is shown below:

Column diameter:	60 cm
Bed height:	25 cm
Column volume:	70.7 l
Required medium volume:	84.8 l
Required medium volume in column:	81.3 l (Target compression factor = 1.15*)

$$\text{* Compression factor} = \frac{\text{Gravity settled medium volume}}{\text{Packed bed volume}}$$

Add all the medium (approximately 85 l) to a previously volume-calibrated slurry tank. Measure the slurry volume carefully.

Stir the slurry and remove a 1 l slurry sample in a graduated cylinder. Let the sample stand overnight and measure the slurry concentration. Add the sample to the tank. Calculate the medium volume in the tank according to:

Medium volume = slurry volume × slurry concentration

Add packing buffer to make a 50–60% slurry. Note the volume of buffer added. Calculate the slurry concentration in the tank once more.

The end point of the packing will be when 81.3 l medium has been pumped into the column. Using the new slurry concentration, calculate how much slurry has to be transferred to the column and the amount of slurry to remain in the tank and hoses at the end of the packing.

Column preparation

1. Level the column with a spirit level and set the adapter to give a packed bed height of 25 cm. Connect the column and packing station via a 2-m long, 1-inch i.d. tubing. Mount a flow meter on the tubing leading to the top slurry inlet.
2. Fit a pressure gauge (to record column pressure during packing) on the top mobile phase outlet. Mount 25-mm, 3-port, 2-way valves on the top (on the pressure gauge) and bottom mobile phase inlets/outlets and fit reducers to 10-mm tubing on both sides of the top valve and on one side of the bottom valve. The 10-mm tubing fitted to the top valve should lead in two directions; one side to the BioProcess System (skid) and the other to waste for purging the tubing. On the bottom valve, one 1-inch tubing should lead to waste (for packing) and the 10-mm reducer with 10-mm tubing to the skid.
3. Using the packing station, fill the column with distilled water pumped at 20–30 l/min through the bottom slurry inlet with the bottom nozzle in the *pack* position and the top nozzle in the *unpack* position. Stop the pump when the column is completely filled, all air is removed, and the top slurry outlet fully primed with water.
4. Move the top nozzle to its *run* position. With the top mobile phase (MPT) valve open to waste, start the packing pump (set at a lower flow rate, e.g. about 10 l/min) to prime the top net and distribution system.
5. Close the MPT valve and shut off the pump. Repeat the procedure with the MPB valve to prime the bottom net and distribution system. Make sure the MPB valve is closed after this procedure.
6. Drain the column by moving both nozzles to the *unpack* position and using the Packing station unpack pump to empty the column. Move the bottom nozzle to the *pack* position.

Packing

1. Lead the top slurry outlet tubing back to the slurry tank and secure it. With slurry being stirred to keep it homogeneous, open the slurry tank and pump slurry into the column via the bottom nozzle until it exits the top slurry outlet port. Stop the pump and move both nozzles to the *run* position. Do not let medium settle, but continue with step 2.
2. Start the packing pump and pump slurry through the top slurry inlet and back into the slurry tank via the top nozzle. As the aim of this procedure is to prime the tubing and allow pump speed to be set, the column is by-passed by having the nozzle in the *Run* position
3. When the pump gives an initial packing flow rate of about 800 cm/h (here about 35 l/min), open the bottom mobile phase valve to waste and move the top nozzle down into the *pack* position. The pump momentarily stalls, but resumes as soon as the pack position is reached. The column fills with slurry and the bed builds up from the bottom as excess liquid exits via MPB.
4. Switch off the packing pump by pressing the emergency stop button when all the required media has been transferred to the column. The packing flow rate has been chosen so that this will occur when the bed is approximately 0.5 cm from the top net. Quickly move the top nozzle back up to the *run* position and close the bottom mobile phase valve. The bed then expands the final 0.5 cm up to the top net, thus completing the packing.
5. Use packing buffer to rinse medium from the hoses and nozzles. Pump packing buffer through the top nozzle back into the tank and then repeat through the bottom nozzle.
6. To remove any residues of air in the top screen and distribution system, run the column with upflow at 150 cm/h until no air exits the column.

Since the packing flow rate was very high, the column is packed in about three minutes. The whole procedure took no more than 30 min and required just one operator.

Test results

Column efficiency and asymmetry were measured for three idential packings. For one of the packings, a more extensive analysis was carried out. This involved Van Deemter analysis, sample volume analysis, and pressure/flow measurements. Results are shown in Tables 2 and 3.

General bed efficiency

Table 2 shows column efficiency data for the three column packings. Plate numbers were very good and the asymmetry values were close to 1.0 in all cases.

A stability test was run on packing no. 3. The column was run with upward flow for 16 h at 250 cm/h. Plate numbers changed only 1% (upward flow) and 5% (downward flow) compared to the original test on packing no. 3. Asymmetry changed only 6% and 3%, respectively. It can therefore be concluded that the bed will be stable for many processing cycles.

Table 2. Efficiency data tested at a fluid velocity of 40 cm/h. These figures show that the method results in highly efficient packed beds

Pack no.	Plates/m*	Reduced plate height, h†	Asymmetry*
1	↑: 4460	2.82	↑: 1.18
	↓: 5180	2.43	↓: 1.08
2	↑: 5410	2.32	↑: 1.13
	↓: 6080	2.07	↓: 1.14
3	↑: 4990	2.52	↑: 1.20
	↓: 5170	2.43	↓: 1.16

* Arrows indicate test direction, i.e. upward or downward flow.
† Reduced plate height, h = 1/(plates/m × particle size).

Detailed analysis

Table 3 shows the outcome of a Van Deemter analysis of packing no. 2. The results were exactly as expected, with plate numbers decreasing somewhat, i.e. the reduced plate height values increased as fluid velocity was increased from 20 to 120 cm/h. Asymmetry values were essentially unchanged. This effect on plate numbers is in line with Van Deemter analysis theory, and the minor effect on asymmetry is what is expected from a well-functioning distribution system. Figure 5 shows these results graphically.

Table 3. Van Deemter test results confirm the efficient nature of the packed bed.

Test velocity	Plates/m	Reduced plate*	Asymmetry
20	6390	1.97	1.06
40	5800	2.17	1.06
60	5230	2.40	1.07
80	4700	2.67	1.12
120	3970	3.16	1.09

* Reduced plate height, h = 1/(plates/m × particle size).

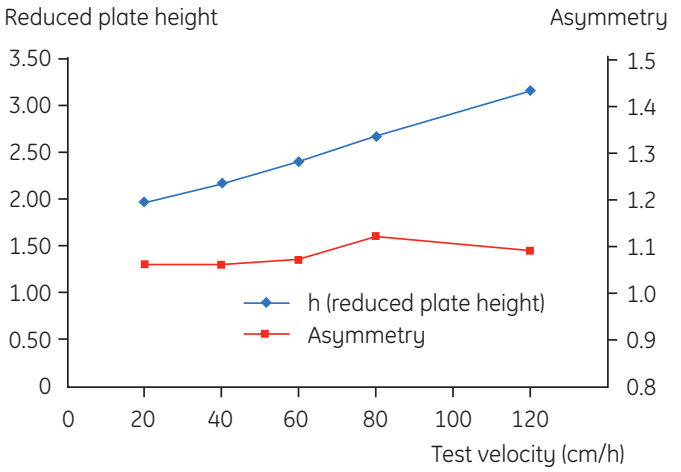


Fig 5. The reduced plate height curve and asymmetry values show that the bed is well-packed and that the distribution system functions well.

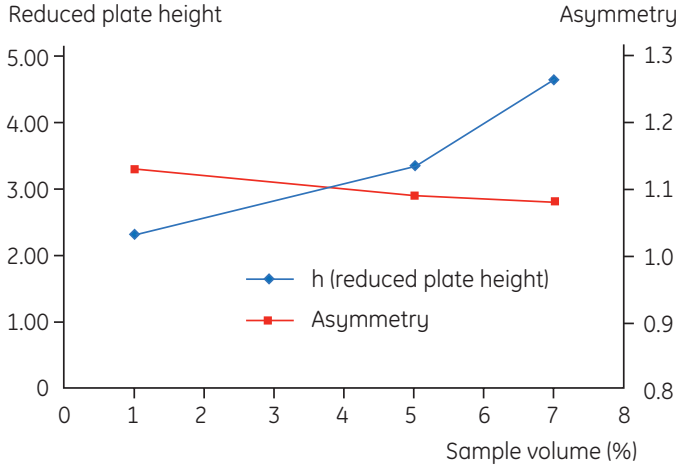


Fig 6. The sample volume test, run on column packing no. 2, confirms an efficient bed and a well-functioning distribution system.

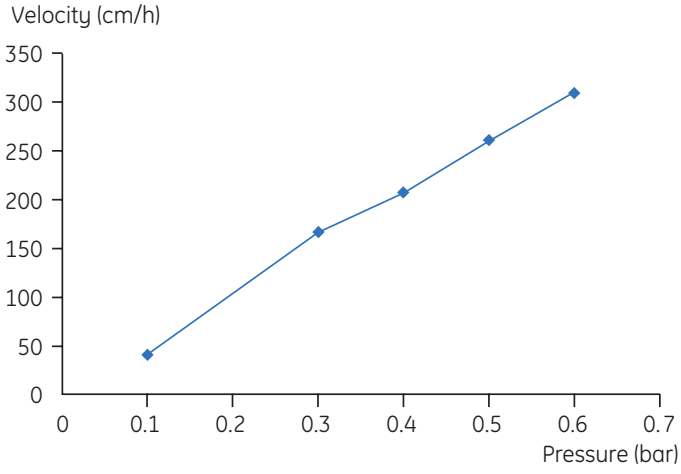


Fig 7. Pressure/flow curve of the packed bed shows linear behavior. The bed is stable and the pressure drop at 300 cm/h is below 1 bar.

Figure 6 shows the results from the sample volume test where the effect of increasing sample volume from 1 to 7% on reduced plate height and asymmetry was measured at a fluid velocity of 40 cm/h. The result is very similar to that seen in Figure 5, as is the conclusion, i.e. the described method of packing MabSelect Xtra in Chromaflow 600 is efficient and gives well-packed beds.

Pressure/flow measurements

For the pressure/flow test, a rotary lobe pump was connected between a water tank and the packed column using 1-inch tubing. Flow direction was downwards. A flow meter was positioned at the pump outlet and a pressure gauge at the column MPT inlet valve. A 1-inch valve with 1-inch tubing was connected on the outlet side of the column.

The resulting pressure/flow curve (Fig 7) shows predictable linear behavior without any sign of liquid gap formation. The pressure drop at 300 cm/h is below 1 bar.

Packing MabSelect Xtra in BPG 300

MabSelect Xtra is packed in BPG columns with conventional flow packing by pumping packing buffer through the chromatographic bed at constant flow rate.

Medium preparation

MabSelect Xtra is supplied in a buffer containing 20% ethanol. Packing using this buffer is not recommended. Instead, decant the ethanol solution from the containers and replace with water. Resuspend the slurry and let the medium settle by gravity. For optimal results, carefully remove the supernatant and replace with water.

Column preparation

The packing pump should be as pulsation-free as possible. Excenter screw or rotary lobe pumps are normally the best. Level the column with a spirit level. A pressure relief valve could be used for safety reasons, especially against pressure spikes. Position this valve on the pump outlet and a pressure gauge on the column inlet. Mount a 10-mm 4-port-2-way valve on top of the pressure gauge.

Packing

MabSelect Xtra is compressible. To pack a 20-cm bed, the settled gel height in the column should be 23 cm. Optimal packing velocity is best measured by running an open bed pressure-flow curve prior to packing the column. Settle the slurry at 200 cm/h. When the bed is formed, measure the bed height. This height is taken as the initial bed height.

Increase velocity in 50 cm/h steps until the compression factor, i.e. the ratio of initial bed height to packed bed height is 1.12. This is the packing velocity to use in step 5 below.

1. Make sure that no air is trapped under the bottom bed support by carefully pumping packing buffer through it from below. Leave about 2 cm of buffer in the column. Excess buffer and remaining air can be removed by running a hose connected to the suction side of a pump over the bed support if needed.
2. Mix the packing buffer and medium to form a homogeneous slurry. The slurry concentration should not exceed 60% (settled bed volume/slurry volume = 0.6). Note that the available height in a 500-mm long column is only 40 cm. For packing beds higher than 20 cm, use a longer column tube; 750 and 950 mm tubes are available.
3. Pour the slurry into the column up to a height of 40 cm. Wait until there is about 1 cm clear liquid on top of the slurry. Insert the adapter and lower it to the surface of the slurry. Make sure no air is trapped in the column. Allow some liquid to pass the O-ring.
4. Seal the adapter O-ring and lower the adapter a little into the slurry, enough to fill the adapter inlet with packing buffer and to remove air trapped in the adapter net. Make sure the top valve is completely primed with buffer. Connect the pump outlet hose to the valve and a hose from the valve to waste.
5. Start pumping at the optimal packing velocity measured in the open bed pressure/flow test and fill the tubing with liquid. A guideline value for packing a 20-cm bed in a BPG 300 is 425 l/h, which corresponds to 600 cm/h.

   Other bed heights will require different packing velocities. Once the tubing is primed, shift the top valve in order to direct flow to the column and **immediately** open the bottom valve. Run until the bed is fully packed and then at least 15 more minutes. Mark the compressed bed height on the column tube.
6. Stop the pump and close the bottom valve. The bed will start to expand.
7. Immediately after shutting off the pump, shift the top valve to waste. Loosen the O-ring and lower the adapter down to 3 cm above the bed. Seal the adapter O-ring and lower the adapter firmly but gently down to approximately 3 mm below the mark on the column tube. The packing buffer will flush the adapter inlet.
8. Remove any trapped air by pumping liquid from the bottom (after the inlet tubing and the bottom valve have been properly filled).

Test results

Column efficiency and asymmetry were measured for three column packings. A more extensive analysis, involving Van Deemter analysis, sample volume analysis, and pressure/flow measurements, was carried out for one of them. Results are given below.

General bed efficiency

Table 5 shows column efficiency data for the three packings. Both plate numbers and asymmetry values were very good in all cases.

Table 5. Efficiency data tested at a fluid velocity of 30 cm/h. These figures show that the method results in highly efficient packed beds

Pack no.	Plates/m*	Reduced plate height, h†	Asymmetry*
1	↑: 5450	2.37	↑: 1.23
2	↑: 5470	2.36	↑: 1.24
	↓: 5180	2.49	↓: 1.27
3	↑: 5320	2.43	↑: 1.32

* Arrows indicate test direction, i.e. upward or downward flow.
† Reduced plate height, h = 1/(plates/m × particle size).

A stability test was run on packing no. 2. The column is then run with upward flow for 16 h at 415 cm/h. The plate numbers changed only 5% and the asymmetry changed only 9% compared to the original test on packing no. 2.

Detailed analysis

Table 6 shows the outcome of a Van Deemter analysis of packing no. 2, where the test velocity was increased from 30 to 125 cm/h. Figure 8 shows these results graphically.

Table 6. Van Deemter test results show that the packed bed behaves as expected

Test velocity (cm/h)	Plates/m	Reduced plate height*	Asymmetry
30	6050	2.14	1.18
50	5500	2.35	1.15
75	4830	2.67	1.14
100	4310	3.00	1.16
125	3920	3.30	1.14

* Reduced plate height, h = 1/(plates/m × particle size).

Figure 9 shows the results from the sample volume test where the effect of increasing sample volume from 1 to 8% on reduced plate height and asymmetry was measured at a fluid velocity of 50 cm/h. The result is very similar to that seen in Figure 8. The described method of packing MabSelect Xtra in BPG 300 is efficient and gives well-packed beds.

Pressure/flow measurements

For the pressure/flow test, an excenter screw pump was connected between a water tank and the packed column using 10-mm tubing. Flow direction was downwards. A flow meter was positioned at the pump outlet and a pressure gauge at the column MPT inlet valve.

The resulting pressure/flow curve (Fig 10) shows predictable behavior without any sign of liquid gap formation. The pressure drop at 400 cm/h is below 1 bar.

Packing MabSelect Xtra in BioProcess LPLC 300/700

The method described below is based on axial mechanical compression using liquid as the compression medium. This quick and simple method generates efficient beds. Table 7 lists the key items of equipment.

Table 7. Key equipment used to pack and test MabSelect Xtra in BioProcess LPLC 300/700

Column:	BioProcess LPLC 300/700 (3 bar). Distribution system designed for optimal performance at 300 l/h
System:	10-mm BioProcess System
Suction pump:	Tapflo membrane pump
Packing pump:	10-mm excenter screw

Medium preparation

For best results, decant the 20% ethanol in which the medium is delivered and replace with water. Some key figures for this packing are presented below:

Column diameter:	30 cm
Bed height:	20 cm
Column volume:	14.2 l
Required medium volume:	17 l
Required medium volume in column:	16.3 l (target is compression factor = 1.15)

Add approximately 17 l of medium to a volume-calibrated tank. Carefully measure the exact total volume of slurry and stir until it is homogenous. Remove a sample (at least 1 l) in a graduated cylinder. Let the sample settle overnight and measure the concentration. Add the sample to the tank and perform the following calculation:

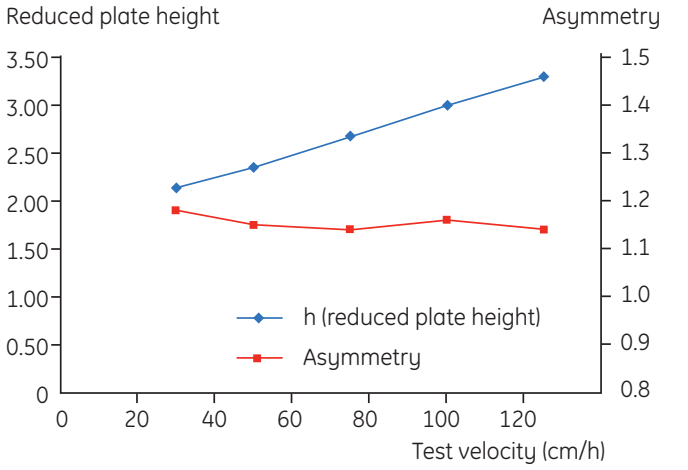


Fig 8. The reduced plate height and asymmetry curves show that the bed is well-packed and that the distribution system is efficient.

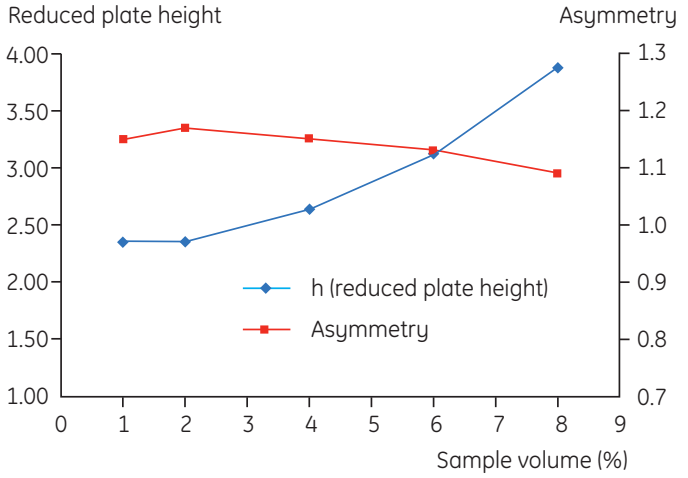


Fig 9. The sample volume test, run on column packing no. 2, confirms an efficient bed and a well-functioning distribution system.

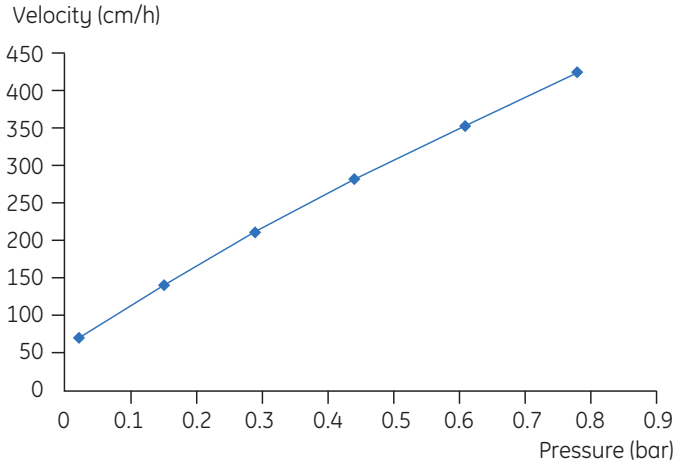


Fig 10. Pressure/flow properties of the packed bed are linear. The bed is stable and the pressure drop at 400 cm/h is below 1 bar.

Medium volume = slurry volume × slurry concentration

Add packing buffer to make a 50–60% slurry. Note the volume of buffer added. Calculate the slurry concentration in the tank once more.

The end point of the filling will be when 16.3 l medium has been pumped into the column. Using the slurry concentration, calculate how much slurry has to be transferred to the column. Then calculate the amount of slurry to remain in the tank and hoses when the column is filled.

Column preparation

Figure 11 shows a schematic representation of the column, Figure 12 the hydraulic inlet/outlet assembly (position E in Fig 11). Notations used in the packing description are as follows:

MPT = Mobile phase top (A)
MPB = Mobile phase bottom (B)
HI = Hydraulic chamber inlet (E)
TSP = Top side port (C)
MV = Media valve (D)
HB = Hydraulic chamber bleed (E)
MPTB = MPT bleed (A)
MPBB = MPB bleed (A)

1. Connect pressurized air to the control box on the side of the column.
2. Level the column using a spirit level.
3. Connect 6-mm tubing on the MPTB and MPBB to waste.
4. Connect the HI and HB valves to the hydraulic chamber. Connect a 10-mm hose on the HB to waste.
5. Connect tubing between the TSP and the skid.
6. Replace the safety valve and the pressure gauge on top of the hydraulic chamber with tubing leading to waste. Start pumping 20% ethanol or 2% benzyl alcohol from the skid to the TSP to completely fill the hydraulic chamber with liquid. Continue until liquid starts exiting the waste tubing on the connection to the safety valve and pressure gauge. Shut off the pump and close the TSP. Re-position the pressure gauge and safety valve.
7. Place a 10-mm hose between the BioProcess system or the packing pump and the hydraulic inlet (HI).
8. Run the adapter down all the way to the bottom position by pumping liquid into the hydraulic chamber via the HI. Make sure the MPTB valve is open. It is very important to open the HB when the adapter comes to rest at its lowest position to avoid damaging the adapter rod. Stop the pump and close the HI.
9. Fill the column properly by pumping water from the MPB at 200 cm/h. Run at least 1.5 CV to fill it completely.
10. When the adapter is in its lowest position, it is not flush with the bottom screen. The distance at the lowest point is to allow for the media valve to be inserted into the column. This distance should be measured. Drain the column completely to calibrate the volume of the column at the lowest position of the adapter as follows: Connect a hose to the MPB and an air hose to the MPT. Apply slight air pressure and collect the liquid in a graduated container. The drained liquid volume using this specific column was 2.83 l, equivalent to 4.0 cm. The bed height is measured on the adapter rod. If, for example, this is moved up 10 cm from the bottom position, the height inside the column is 14 cm.

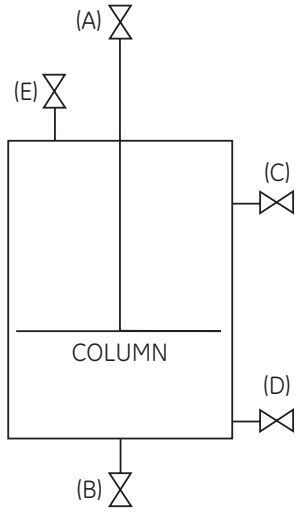


Fig 11. Schematic representation of the BioProcess LPLC 300/700.

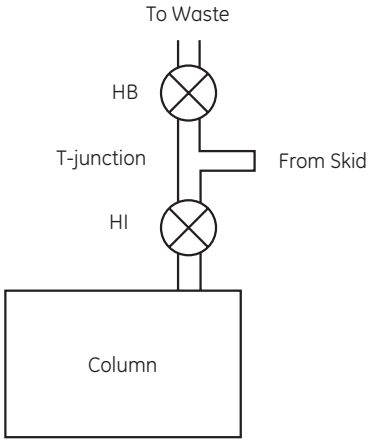


Fig 12. The hydraulic inlet/outlet assembly (E in Fig 11).

Priming the column, suction pump and slurry hose

1. Connect the suction side of the membrane pump to the TSP. Connect the pump outlet hose to a tank or to waste. Make sure the hoses between the TSP and tank are primed with hydraulic liquid before they are connected.
2. Connect the slurry tank to the media valve.
3. Prime the column by pumping liquid into the MPB with upward flow through the column and out through the MPT. Keep the HI closed during this operation. After 1 CV, remove the air present in the slurry tank hose by inserting the media valve into the column (*open* on control box) for three seconds. Make sure the slurry tank is open. Close the MV and continue running for at least 2–3 CV or until no air bubbles can be seen exiting from the MPT.

Filling column with slurry

Use a stirrer to prevent vortex formation in the tank. If a small amount of air enter the column, this can be removed before the column is packed.

1. Make sure that the MPT, MPB, and HI valves are closed.
2. Stir the slurry and make sure the tank is open.
3. Start the suction pump and open the media valve followed by the TSP. Increase pump speed until the adapter starts moving at about 3 cm/min.
4. Lower the pump speed when most of the slurry has been pumped into the column to prevent air entering.
5. When all the required slurry, based on previous calculations, is transferred into the column, close the MV and TSP and shut off the pump.

Packing

1. Start the pump at 150 cm/h and prime the MPB tubing. Shift the MPB valve to column and immediately open the MPT to remove any residues of air in the column. HI and HB must be closed. Run for 30–60 s before pump is stopped.
2. Start the pump at 200 cm/h leading into the MPT. Run the column for at least 1 CV, based on the slurry volume in the column. The pressure will increase linearly until the bed is fully settled. Before stopping the pump, read steps 3 and 4.
3. There are two possible ways to move the adapter down at this point. One uses the same pump as for settling the bed (A), the other a separate packing pump (B). Option B is recommended.
4. (A) Stop the pump and shift the pump inlet from packing buffer to hydraulic liquid. Close MPT and start the pump at 200 cm/h into the HI. Initially the HB is open to prime the hose, but when this is closed and HI opened, the adapter starts moving down, expelling liquid though the MPB.

   (B) Before the pump is stopped in step 2, start the second packing pump at 200 cm/h, connected to the HI. Recirculate hydraulic liquid through the open HB valve until step 2 is finished. At that point, stop the first settling pump and close MPT. Very soon thereafter, close HB and open HI to move the adapter down to pack the bed.
5. Continue until the desired bed height (20 cm) is reached. When the adapter meets with the bed, there will be a significant increase in column pressure.
6. To complete the packing, shut off the pump, immediately close the HI and the MPB valve.
7. After packing, it is recommended to flow condition the bed starting with upward flow at 350 cm/h for 3 CV. Slowly decrease the flow and shift the flow direction. Then, run downward flow through the column at 350 cm/h for 3 CV and slowly decrease flow down. The flow conditioning may improve bed homogeneity and thus bed stability. After applying this procedure, the bed will be stable for a long period of time.
8. Before testing the packing, make sure all tubing leading to the column is connected to the skid and properly primed.

Unpacking

1. Open the HI and HB valve to allow liquid to escape from the hydraulic chamber.
2. Pump water upwards through the MPB at 150 cm/h to lift the bed in the column. Run the adapter up about one quarter of the original bed height.
3. Close the MPB and open the MPT.
4. Pump packing buffer downwards through the MPT valve. Run the adapter up about one quarter of the original bed height. Shut off the pump and close the MPT and MPB. Let the column stand for 5 min to allow the bed to break up.
5. Start pumping hydraulic liquid at 150 cm/h to the HI to force the adapter down. Open the media valve connected to the slurry tank to force slurry out from the column. If pressure increases rapidly during this operation, stop the pump, close the HI, and pump 5 l packing buffer through the MPB and out into the slurry tank. Then begin once more with step 5.
6. Open the HB when the adapter reaches its bottom position. Stop the pump. Start pumping upwards flow through the MPB. (All other valves except the media valve should be closed.) Vary the flow rate from very low to very high to empty the column of medium. When no medium is seen in the liquid from the column, the column can be considered unpacked.

Test results

Column efficiency and asymmetry were measured for three column packings. In addition, Van Deemter analysis, sample volume analysis, and pressure/flow measurements were carried out. Results are given below.

General bed efficiency

Table 5 shows column efficiency data for the three packings. Both plate numbers and asymmetry values were very good in all cases.

Table 5. Efficiency data tested at a fluid velocity of 30 cm/h. These figures show that the method results in highly efficient packed beds

Pack no.	Plates/m*	Reduced plate height, h†	Asymmetry*
1	↓: 5950	2.17	↓: 1.31
2	↓: 5650	2.29	↓: 1.34
3	↓: 4730	2.73	↓: 1.33

* Arrows indicate test direction, i.e. upward or downward flow.
† Reduced plate height, h = 1/(plates/mm × particle size).

A stability test was run on packing no. 1. The column was run with upward flow for 16 h at 300 cm/h. The plate numbers changed only 6% and the asymmetry changed only 2% compared to the original test on packing no. 1.

Detailed analysis

Table 6 shows the outcome of a Van Deemter analysis of packing no. 1, where the fluid velocity was increased from 30 to 120 cm/h. Figure 13 shows these results graphically.

Table 6. Van Deemter test results

Test velocity (cm/h)	Plates/m	Reduced plate height*	Asymmetry
30	5950	2.17	1.31
60	4760	2.82	1.23
90	4070	3.17	1.17
120	3600	3.59	1.13

* Reduced plate height, h = 1/(plates/m × particle size).

The asymmetry value decreases slightly with increasing test velocity owing to this particular distribution system, having optimal performance at 300 l/h. The decrease is not large, however. The asymmetry at 30 cm/h is 1.31 and at 120 cm/h the asymmetry is 1.13, both values being well within standard acceptance criteria.

Figure 14 shows the results from the sample volume test where the effect of increasing sample volume from 1 to 7% on reduced plate height and asymmetry was measured at a fluid velocity of 30 cm/h. The result is similar to that seen in Figure 13.

Also in this case, the asymmetry decreases slightly with increasing sample volume when the column is tested at 30 cm/h. This is also due to the design of the distribution system, where a large sample volume has the same effect on the asymmetry as a higher velocity has.

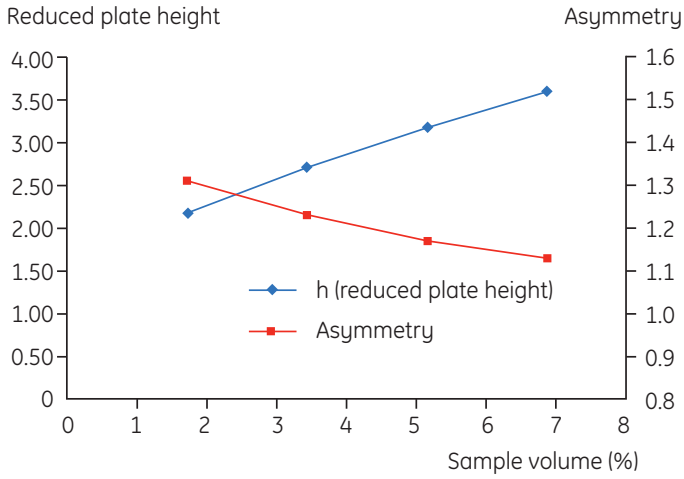


Fig 13. The reduced plate height changes with velocity as expected from the Van Deemter theory. Asymmetry values decrease slightly as velocity is increased since the distribution system used is specifically designed to have optimal distribution at 300 cm/h.

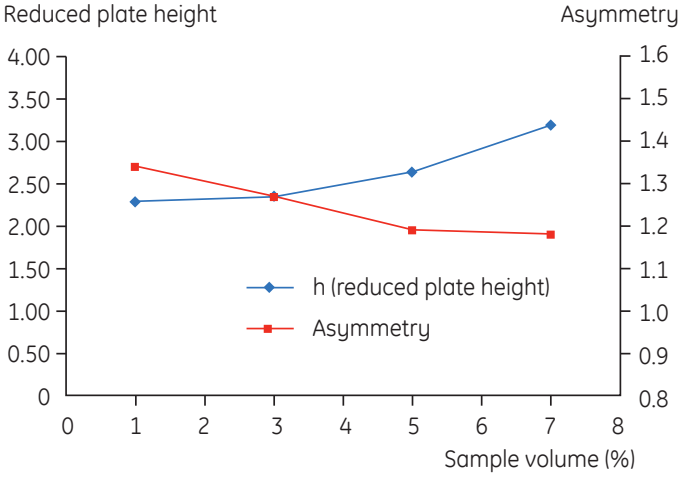


Fig 14. The sample volume test, run on column packing no. 2, confirms the findings from the Van Deemter test.

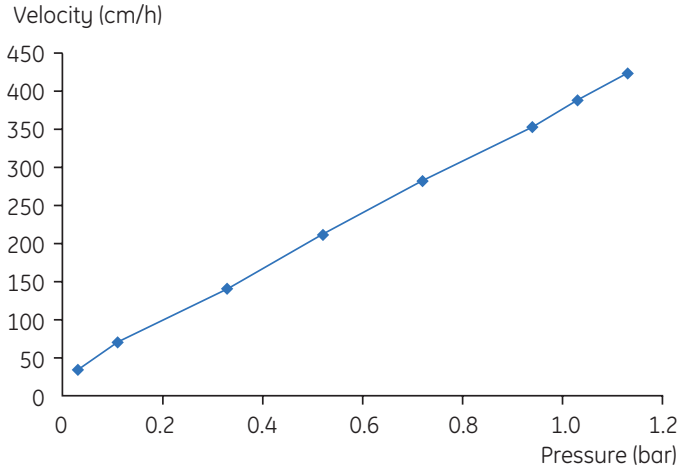


Fig 15. The Pressure/flow curve in of the packed bed is linear. The bed is stable and the pressure drop at 400 cm/h is close to 1 bar.

Pressure/flow measurements

For the pressure/flow test, the 10-mm BioProcess system was used, including a flow meter. A pressure gauge was placed at the column MPT inlet valve to record the pressure over the column only.

The resulting pressure/flow curve (Fig 15) shows predictable behavior without any sign of liquid gap formation. The pressure drop at 400 cm/h is approximately 1 bar.

Conclusions

Packing MabSelect Xtra in Chromaflow, BPG and BioProcess columns with the above methods using a compression factor of 1.15 is fast, simple and successful. The efficiency of the packed bed is very good in all cases and stability data indicate that all columns would be robust and reproducible in use.

All methods are fast and simple and the packing can be performed in less than 1–2 h by a single operator. The packing method and its outcome should apply equally successful to larger columns in each respective column family.

Related literature

Data files	
MabSelect Xtra	11-0011-57
Chromaflow columns	18-1138-92
BioProcess columns	18-1167-76
BPG columns	18-1115-23

For contact information for your local office, please visit, www.gelifesciences.com/contact

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