Packing Capto™ adhere and Capto MMC using verified methods

Good column packing is essential for any chromatographic process and plays a key role in the large-scale commercial manufacture of biopharmaceuticals. A bed packed too densely may crack, which can lead to channeling and early breakthrough. A bed packed too loosely can further compress, causing a liquid gap where mixing can occur. Either instance will lead to costly process disruptions and loss of valuable product. Proper packing eliminates such concerns and ensures a stable bed that performs according to expectations over many processing cycles. This procedure describes packing of Capto adhere and Capto MMC media in large-scale chromatography columns including AxiChrom™, BPG, and Chromaflow™ columns. The procedure includes robust and verified packing and testing methods that will minimize concerns and risks associated with poorly packed beds.

Fig 1. AxiChrom columns provide verified packing methods that utilize the Intelligent Packing concept.

Product characteristics

Capto adhere

Capto adhere is composed of a rigid, high-flow agarose matrix and a multimodal ligand. The strong multimodal anion exchange ligand gives a different selectivity compared to traditional ion exchangers. In a single step, Capto adhere can remove key contaminants such as DNA, host cell proteins (HCP), leached protein A, dimers and larger aggregates, and viruses. Together with a protein A capture step (e.g., using MabSelect SuRe™), Capto adhere allows the design of a two-step chromatographic process for MAbs.

Capto MMC

Capto MMC is based on rigid, high-flow agarose that allows high flow, which is an important factor for raising productivity in large-scale operations. Capto MMC combines agarose base matrix developments with innovative ligand chemistry, giving a different selectivity compared to traditional ion exchangers. It can be used to bind proteins at the conductivity of the feed material and to solve specific purification challenges at both high and low ionic strengths. Capto MMC can be designed with a wide range of bed heights and fluid velocities.

AxiChrom columns

AxiChrom columns are low-pressure, mechanical axial compression chromatography columns designed for process development and biopharmaceutical manufacturing environments. Mechanical axial compression enables accurate and reproducible control of the packing, even with large-diameter columns.

The columns are available in many different configurations and materials (see Data file 28929041 for more details). AxiChrom columns are designed to be scalable and give predictable results over the entire range of scales by enabling a uniform plug flow through the bed, irrespective of column size. The columns feature Intelligent Packing with preprogrammed methods that support all column sizes.

Intelligent Packing enables straightforward operation and very high packing success rates. The packing methods described here apply to bed heights up to 40 cm in AxiChrom columns up to 1600 mm in diameter.

BPG

BPG columns are glass columns for process development and manufacturing. The single-screw adapter allows easy, efficient packing and running. The columns have diameters from 100 to 450 mm. The packing methods described here apply to all BPG columns, except for BPG 450 (due to its lower pressure rating).

Chromaflow

Chromaflow columns are acrylic or stainless steel, pack-in-place columns for biopharmaceuticals manufacturing. The columns have diameters ranging from 400 to 2000 mm. The packing method described here applies to Chromaflow columns up to 800 mm. A short guideline for larger columns is also provided.

Packing

Definitions

The bed height of a gravity settled bed differs from the bed height of a bed settled at low flow (consolidated). Therefore, the compression factor (*CF*) has to be separated from the packing factor (*PF*). In water for example the *CF* is 1.10 and *PF*, when consolidating at 30-60 cm/h, is 1.15 for Capto MMC in BPG columns.

Equations to calculate *CF, PF,* and column volume (V_c) are shown below:

$$\text{Compression factor, } CF = \frac{L_{settled}}{L_{packed}}$$

$$\text{Packing factor, } PF = \frac{L_{cons}}{L_{packed}}$$

where

$L_{settled}$ = bed height measured after settling by gravity (cm)

L_{cons} = consolidated bed height, that is, bed height measured after settling the medium at a given flow velocity (cm)

L_{packed} = packed bed height (cm)

$$\text{Column volume, } V_C = L_{packed} \times A_C$$

where

A_C = cross-sectional area of the column (cm^2)

C_{slurry} = Concentration of the slurry

When packing BPG columns and axial compression columns *PF* is used in the packing procedure to calculate the packed bed height after the consolidation step. *CF* is used in the media preparation step to calculate the medium volume needed to pack a desired bed height. Since Chromaflow columns are pack-in-place columns they have no registered consolidated bed heights and the *CF* is used throughout the packing process.

Properties of the media in various packing solutions

Capto adhere and Capto MMC settle quickly in both water and in 20% ethanol. When using these solutions remember that tubing and nozzles must be rinsed directly after packing to prevent clogging of the flow path. Adding salt to packing solutions slows the settling of the medium beads and also allows them to settle less tightly. As a consequence, it is more difficult to measure slurry concentration in salt solutions than in water. However, using the Slurry Concentration Kit mentioned below allows quick and accurate determination of slurry concentration.

When the medium is settled at 30 to 60 cm/h the consolidated bed height will be 5% to 10% higher in salt solution compared to in water or 20% ethanol. The effect is almost the same for 1 mM NaCl as for 0.5 M NaCl and can be compensated for by using different packing factors. Table 1 shows packing factors for water, ethanol, and NaCl.

Table 1. Typical packing factors (*PF*) for Capto adhere and Capto MMC in different solutions in BPG columns. Packing factors were calculated for optimal bed performance where the bed is consolidated at 30 to 60 cm/h.

Solution	*PF*
Water	
Capto adhere	1.13
Capto MMC	1.15
20% ethanol	1.15
10 mM NaCl	1.18

Slurry preparation

Start by calculating the medium volume, *V*, needed to pack the desired bed height. In this step use the compression factor in water (*CF* = 1.10), since the slurry concentration determined by the method below corresponds to the gravity settled concentration in water.

$$V = \frac{A_C \times L_{packed} \times CF}{C_{slurry}}$$

Preparing media to form slurry can be done manually, mechanically, or by using the Media Wand™. Shaking gives good results, but is often not practical for large volumes. When stirring, it is best to use soft stirrers without sharp edges. The Media Wand suspends media directly in the containers and transfers the slurry to the slurry tank in one operation, making it suitable for large-scale packing.

Capto adhere and Capto MMC are supplied in 20% ethanol. Before packing, transfer the medium to the packing solution as described in the packing instructions for the relevant column.

Measuring slurry concentration

In order to achieve the correct amount of chromatography medium for packing the target bed height or compression, it is important to measure the slurry concentration correctly. Measuring slurry concentration can be performed with a Tricorn™ 10/100 column. GE Healthcare offers a Slurry Concentration Kit (see Ordering information) with all of the materials required for determination of slurry concentration.

Packing Capto adhere and Capto MMC in AxiChrom columns

When packing AxiChrom 50 to 200 columns for use with ÄKTA™ systems, Intelligent Packing control is managed by UNICORN™ control software. For AxiChrom 300 to 1600 columns, Intelligent Packing is performed by AxiChrom Master, a separate unit that comprises a touchscreen operated user interface, or from UNICORN control software on an ÄKTAprocess™ system.

Intelligent packing in AxiChrom columns—general considerations

Packing methods are created by entering values for the packing variables (e.g., column, medium, slurry concentration, target bed height) in the Intelligent Packing wizard. The packing factor given in the Intelligent Packing wizard is dependent on the entered packing variables and the packing solution. To pack the column, start the chosen method in UNICORN and follow the instructions.

When packing AxiChrom 50 to 200 columns, the slurry is introduced into the column by hand and adapter movement is driven by internal hydraulics. After the wizard method has been created and the medium has been equilibrated in packing solution, the column is primed and filled with slurry. The method controls the flow rate of hydraulic fluid to drive the adapter and packing of the bed (Fig 2).

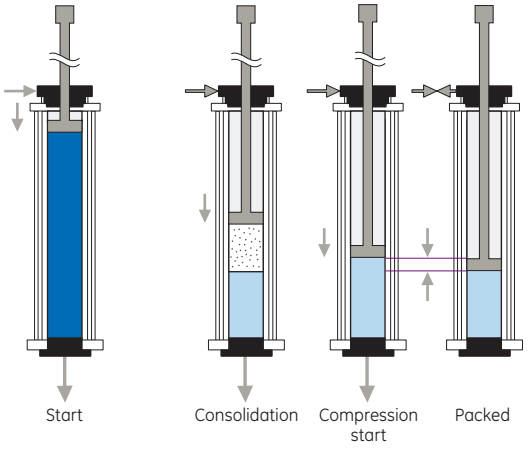

Fig 2. Intelligent Packing in small AxiChrom columns. The adapter is mounted to the column tube and the wizard is started (**Start**). The adapter moves down, forcing packing liquid out through the bottom bed support. The medium forms a consolidated bed (**Consolidation**). When the adapter comes into contact with the consolidated bed surface, the operator initiates bed compression in the UNICORN wizard (**Compression start**). Compression occurs according to a predetermined *PF*. The target bed height is attained (**Packed**).

In AxiChrom 300 to 1600 columns, slurry is introduced via a media valve in the center of the bottom bed support and the adapter is driven by an electric servomotor. The two-position media valve enables filling, packing, and unpacking without adjusting the assembled column.

After the column is primed, the adapter rises from its lowest position and the column fills with slurry via the media valve.

The slurry volume is calculated automatically from the target bed height, slurry concentration, and *PF*. Also the volume of the tubing connection between the column and slurry tank is taken into consideration. As an electric servomotor controls the movement of the adapter, its position is monitored with millimeter accuracy.

When the correct slurry volume has been drawn into the column, the adapter starts to lower and packing buffer is forced out through the bottom bed support and bed consolidation starts. The time to complete consolidation (i.e., when the adapter reaches the bed) is also automatically calculated (as for the AxiChrom 50 to 200 columns), allowing the operator to carry out other tasks in the meantime. As the adapter hits the consolidated bed, a very distinct dip is seen on the pressure curve, which is detected by Intelligent Packing wizard. When this occurs, the operator confirms that the adapter has hit the bed.

The compression of the medium starts and a graphical interface is shown on the control screen of UNICORN or AxiChrom Master. This graphical interface assists the operator in finishing the packing, giving a well-packed bed. When the adapter symbol is within the range of approved packing factors and bed height limits, the operator can end the packing.

If selected in the UNICORN wizard, Intelligent Packing will automatically run a packed bed evaluation test after the packing. For large AxiChrom columns, automatic methods for priming and unpacking can also be created with the Intelligent Packing wizard.

Packing Capto adhere and Capto MMC in BPG

Capto adhere and Capto MMC are packed with 10 mM NaCl in BPG 100 and with water in BPG 300. Because of the increased influence of the wall support, salt solution is required in columns with small diameters. The packing factor varies with the choice of packing solution (see Table 1) and therefore the packing factor will be 1.15 in water for BPG 300 (1.13 for Capto adhere) and 1.18 in 10 mM NaCl for BPG 100.

Medium preparation

Equilibration to the packing solution can be performed by using the BPG column as a "filter". Pour the medium into the column (amount calculated above), mount the adapter, tighten the adapter O-ring, move the adapter down, and compress the bed slightly. Connect the pump and wash the medium with the packing solution for at least 3 column volumes. Unpack and re-suspend the slurry and pack according to the method.

Column and system preparation

A detailed description of column preparation is available in the BPG instructions (18117070). The packing pump should be as pulsation free as possible. Screw or rotary lobe pumps are the most suitable for this task and multi-headed diaphragm pumps are satisfactory.

1. Place a new 23 µm net on both adapter and bottom end piece.
2. Level the column with the aid of a spirit level.
3. A pressure relief valve should be used for safety reasons, especially against pressure spikes. Position this valve on the pump outlet and add a pressure gauge on the adapter.
4. Mount one 4-port-2-way valve on bottom inlet and one on top of the pressure gauge, 10 mm inner diameter (i.d.) for BPG 300 and 6 mm i.d. for BPG 100 and 200.

Packing

1. Set the pressure alarm or pressure relief valve according to the pressure rating of the column/system. Purge the system and tubing of air.
2. Purge the end-piece net of trapped air by draining some packing solution through the column outlet. Leave about 2 cm of solution in the column and close the bottom valve. If air is still trapped under the end-piece net, add more packing solution and connect a tube to the suction side of a pump. Start the pump and place the tube on the bottom net and extract any remaining air.
3. Add the slurry to the column and, if needed, additional packing solution to about 40 cm. Mix the medium and the packing solution to homogeneous slurry

Note: The available height in a 50 cm column tube is only 40 cm to allow the adapter to be inserted. When packing beds higher than 20 cm, use a packing extension tube or a longer column tube; 75 cm and 95 cm tubes are available.

4. Rinse the wall from particles and let the medium settle until there is about 1 cm clear liquid on top of the slurry. This reduces the risk of particles sticking between the O-ring and the column wall, which can cause the column to leak.
5. Insert the adapter and secure it to the column top flange. Lower the adapter to the surface of the slurry and allow some clear liquid to pass the O-ring. Tighten the adapter O-ring.
6. Make sure the column top valve is open. Slowly move the adapter down until no air bubbles can be seen leaving the top valve.
7. Start the pump and adjust the settling velocity to 30 cm/h Shift the top valve into the column and immediately open the bottom valve and lead the liquid to waste.
8. Run the settling flow until the bed is completely consolidated. Note the consolidated bed height and calculate the packed bed height using *PF* = 1.18 for BPG 100 in 10 mM NaCl and *PF* = 1.15 or 1.13 for BPG 300 in water. The packed bed height is the ratio between the consolidated bed height and the packing factor. Use a marker pen to indicate the packed bed height on the column.
9. Stop the flow and close the bottom valve. Shift the top valve to waste. Loosen the O-ring and lower the adapter down to 1 cm above the settled bed. Seal the adapter O-ring.
10. Start the pump and adjust the flow velocity to 30 cm/h. Shift the top valve into the column and immediately open the bottom valve and lead the liquid to waste. Increase the flow to 800 cm/h. Run at this flow velocity for 5 min.
11. Stop the flow and close the bottom valve. Shift the top valve to waste. Use the adapter to mechanically compress the bed to the mark on the column (step 8). Excessive packing solution is removed through the adapter tube.

Note! Compressing Capto media in BPG columns, especially the larger BPG 300, is physically demanding. Do not use extension rods on the adapter height adjuster to compress the media.

12. Test the packing at the optimal test velocity, which for this medium is 20 to 30 cm/h.

Packing Capto adhere and Capto MMC in Chromaflow

The packing method developed for Capto adhere and Capto MMC in Chromaflow 600 is the standard method used for Sepharose™ Fast Flow or Sepharose High Performance media. To achieve the highest possible compression factor, the flow is increased to the maximized flow of the recommended packing station without exceeding the column pressure limit.

For Capto adhere and Capto MMC, beds of 20 cm and above work well in Chromaflow columns. The extreme flow rates needed to efficiently pack a shorter bed are difficult to achieve using standard equipment.

Note: The packing method described is for a Chromaflow 600 using a Pack 100 packing station. For higher operational flow velocities, the larger Pack 200 packing station is recommended. The Pack 100 flow capacity enables packing of these Capto media at 20 cm bed height compared with the AxiChrom columns where the full flow potential of Capto adhere and Capto MMC can be utilized at any available bed height and column size.

With larger column diameters, higher flows are required from the packing station to achieve sufficient bed compression. A Chromaflow 800 requires a Pack 200 or larger. A Chromaflow 1000 requires a Pack 400 and a specially designed column with a larger nozzle bore size to achieve the required packing flow rate. For Chromaflow columns larger than 1000, axial compression is needed, which requires a specially designed column. Contact GE Healthcare for advice.

Chromatography medium preparation

The recommendation for packing Chromaflow columns is to use the solution in which the medium is delivered or a decanted solution, as 10% to 20% ethanol in the slurry gives a good packing result. If the delivery solution is decanted, then replace it with water.

To avoid introducing air to the column when packing, extra slurry is required for the dead volumes in tank and tubing. Add the slurry to the slurry tank and stir the medium. Dilute the suspension to about 50% slurry concentration.

Column and system preparation

For a more detailed description about the column and packing station preparation, see Chromaflow columns Operating Instructions (28962232) and Chromaflow Packing Station Operating instruction (29046228). In this procedure, standard Chromaflow nomenclature is used for the connections on the column and the packing station and the three different modes of the Chromaflow nozzle are seen in Figure 3.

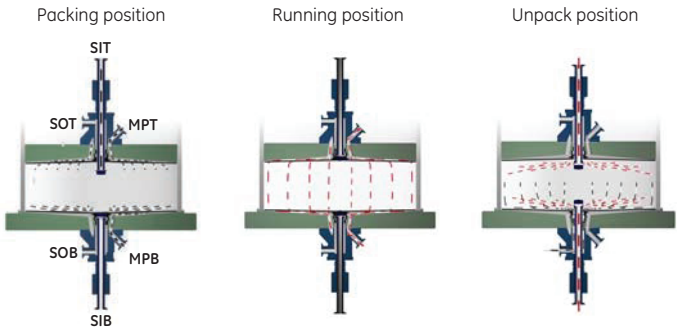

Fig 3. Chromaflow operating positions. Packing: The top nozzle is extended half-way into the column and the bottom nozzle is fully retracted. Slurry enters the column via the top nozzle and excess liquid exits via the mobile phase bottom. **Running**: After packing, the top nozzle is retracted to run position. The slurry lines are isolated from the mobile phase and can be cleaned independently. In this position mobile phase enters the column through the bed supports. **Unpacking**: Both top and bottom nozzles are fully extended into the column, thereby exposing a third passage through which medium leaves the column. Cleaning solution can be pumped through the nozzles and sprayed into the column without dismantling.

Note: It is important that the supply air flow rate follows the specification of the packing station (1000 L/min for Pack 100) and that the supply air pressure into the packing station is 6 to 7 bar.

1. Set up the column according to the Chromaflow columns instructions for use.
2. A pressure relief valve (adjusted to the operating pressure limit of the column) should be used for safety reasons. Position this on the slurry inlet top (SIT), with the waste tubing connected to the slurry tank. Place a pressure gauge on the mobile phase top (MPT) to record the pressure during packing. Mount one 3-port, 2-way valve on top of the pressure gauge and one on the mobile phase bottom (MPB). The top valve should lead in two directions: one side in to the system and one to waste for purging the tubing. On the bottom valve, one side leads to the system and a 1.5" to 2" tubing leads to waste (for packing). Part of the MPB waste tubing should be placed above the outlet valve to prevent air from entering through the MPB.
3. Connect appropriate tubing (i.d. 1" or 1.5") and tanks to the column and packing station. If a flow meter is used, place it between the SIT and the packing station.
4. Level the adapter to the desired bed height. Remember to loosen the nuts on the adapter rods to allow the adapter to be raised or lowered. Flush the adapter rods with 20% ethanol as lubrication.
5. Prime the column, packing station, and tubing with water according to the Chromaflow Operating Instructions.

Packing the column

Note: Packing Chromaflow columns is a rapid procedure compared with other packing procedures and it is therefore important to thoroughly read the packing instructions and go through the packing steps in advance of the packing.

1. Mount a flow meter on the tubing leading to the SIT.
2. Set both nozzles in run position to prime the tubing with slurry. Lead the Slurry Outlet top (SOT) tubing back to the slurry tank and secure it. Stir the slurry to keep it homogeneous, select slurry and SIT on the packing station, open the slurry tank and start the packing pump.
3. As the aim of this procedure is to prime the tubing and allow the pump speed to be set, the column is bypassed at this stage. Increase the pump flow to the packing flow rate according to Table 2.

Table 2. Packing parameters for Capto adhere and Capto MMC using a Packing Station Pack 100 and Chromaflow 600

	Packed bed height (cm)	
	20	30
Packing flow (L/min)	80	60
Pack station pressure (bar)	6 (max)	4.5

4. When the tubing is primed and the flow rate set, turn the SIT/slurry inlet bottom (SIB) switch to the position between SIT and SIB. This blocks the flow during Step 5 while maintaining the correct flow rate for the next step.
5. Move the top nozzle down into the Pack position.
6. Two operators should simultaneously open the bottom mobile phase valve to waste and turn the SIT/SIB valve to SIT on the packing station. The column then starts to fill with slurry and the bed builds up slowly from the bottom as excess liquid exits via the MPB.

Note: Column pressure must not exceed the operating pressure limit of the column (i.e., 3 bar). If this pressure is reached, gently decrease the packing flow so that the pressure remains just below 3 bar.

The final pressure reached in the column during the packing in this study was 2 to 3 bar depending on the viscosity of the liquid, diameter of column, bed height, etc.

Note: If pneumatic nozzles are used, stop the packing pump immediately with the packing pressure handle. This allows the top nozzle to move to the run position in the next step and is necessary since the emergency stop button kills the air supply.

Note: If a nontransparent column tube is used, stop the packing when the calculated volume of slurry is introduced into the column. Check the volume indicator in the slurry tank or use a volume totalizator.

7. Stop the packing pump when the bed is 2 to 3 mm from the top bed support by setting the SIT/SIB to the position between SIT and SIB, as described in step 4. Once the flow is stopped, the bed will expand to meet the adapter.
8. Immediately retract the top nozzle back to the run position.
9. Close the MPB valve when the pressure in the column is between 0.3 and 0.1 bar.
10. Use packing solution to rinse residual medium from the tubing and the top nozzle. Pump the packing solution through the top nozzle back into the slurry tank.

11. Close the slurry tank and empty the tubing between the tank and packing station.
12. Pump liquid upflow through the column until the air is expelled.

Testing the performance of the packed column

Process scale packed columns must perform with a high degree of efficiency over many processing cycles (i.e., display very high stability). The efficiency of a packed column can be expressed in terms of height equivalent to a theoretical plate (HETP) and asymmetry factor (A_s). This test should be repeated regularly to monitor the state of the bed throughout the working life of the column. If the test results are to be comparable over time, conditions such as fluid velocity (cm/h), liquid pathway, sample composition, and elution buffer should be kept constant. The requirements for the test have to be set in accordance with test conditions and the goal of the purification. This is further described in application note 28937207.

Test conditions used in this study

Test velocity:	20 to 30 cm/h
Eluent:	water
Sample volume:	1% of the column volume (V_C)
Sample:	2% v/v acetone

To compare the performance of columns packed with chromatography media of different particle diameters, the reduced plate height (h = HETP/average bead diameter [dp]) is typically used. As a guideline, a value of h < 3 is very good at the optimal test conditions.

Examples of results

The columns packed with the methods outlined above were tested for plate number, asymmetry, pressure-flow properties, stability, and van Deemter analysis.

AxiChrom columns

The efficiency and stability results for Capto MMC and Capto adhere in AxiChrom columns are shown in Table 3. The high efficiency (plates/m) and the A_s range close to 1 indicate well packed beds with good process performance.

Pressure/flow curves provide a simple, effective illustration of column performance in terms of maximum velocity at which the purification process can be run. The curves also show the magnitude of the backpressure in the system at a certain liquid velocity. Figure 4 shows the pressure/flow curves for Capto MMC and Capto adhere in AxiChrom columns.

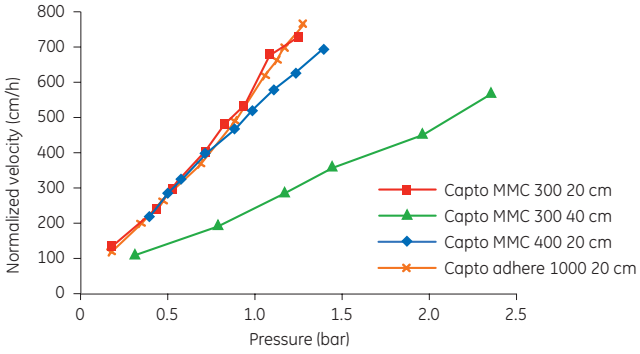

Fig 4. Pressure flow curves for Capto MMC and Capto adhere in AxiChrom columns. Column and system pressure are excluded.

Table 3. Column efficiency data for different packs of Capto MMC and Capto adhere in different AxiChrom columns

Medium	AxiChrom column	Bed height cm	Average N/m*	Reduced plates height (h) range*	Asymmetry factor (A_s) range*	Change after stability test (%)†	
						h	A_s
Capto MMC	50	20	7300	1.60–1.70	1.00–1.10	6	16
Capto MMC	70	20	7000	1.70–1.80	1.00–1.20	5	15
Capto MMC	100	40	9300	1.37–1.41	0.90–1.00	4	15
Capto MMC	140	40	7700	1.40–1.60	1.00–1.20	7	11
Capto MMC	200	20	8600	1.20–1.40	1.00–1.20	13	14
Capto MMC	300	20	9400	1.40–1.40	1.08–1.12	3	5
Capto MMC	300	40	9200	1.40–1.50	1.06–1.10	2	3
Capto MMC	400	20	8700	1.50–1.50	1.15–1.22	2	9
Capto MMC	1000	25	8200	1.40–1.70	1.10–1.30	9	2
Capto adhere	50	20	7300	1.60–1.70	1.00–1.20	5	13
Capto adhere	70	20	6800	1.50–2.00	1.20–1.40	10	17
Capto adhere	100	20	8200	1.40–1.50	1.00–1.10	9	9
Capto adhere	100	40	7200	1.40–2.30	1.00–1.10	10	7
Capto adhere	200	20	8700	1.30–1.40	1.00–1.10	1	7
Capto adhere	1000	20	8600	1.50–1.70	1.10–1.30	-3	-1

* Test performed at optimal test conditions. Average and ranges of upflow and downflow tests for at least three packs.
† Stability tests were run once for each bed height/medium/column combination in water for 16 h at least 600 cm/h at 20cm bed height and 300 cm/h at 40 cm bed height.

BPG

The efficiency and stability results for Capto MMC packed in BPG 100 and BPG 300 columns are shown in Table 4. The stability test showed that the bed was stable when running at least 600 cm/h for 16 h.

Figure 5 shows pressure/flow curves for Capto MMC and Capto adhere in BPG columns.

Table 4. Column efficiency data for different packs of Capto adhere and Capto MMC in BPG 100 and BPG 300 columns at 20 cm bed height, showing highly efficiently packed beds. The test was run at 30 cm/h. The data is an average of at least three packs, running tests upflow and downflow. Stability tests were run at least 600 cm/h during 16 h.

Medium	Average N/m	Average h	Range A_s	% change after Stability test h	% change after Stability test A_s
Capto adhere					
BPG 100	5800	2.2	1.10–1.30	-8	5
BPG 300	7000	1.9	1.00–1.20	1	-4
Capto MMC					
BPG 100	7300	1.8	1.07–1.24	3	8
BPG 300	7500	1.8	1.19–1.33	6	9

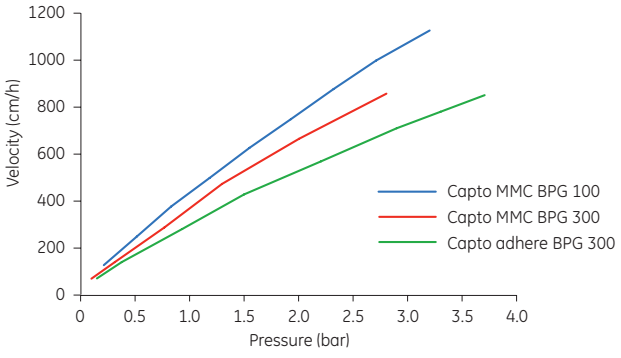

Fig 5. Pressure/flow curves at 20 cm bed height for Capto MMC and Capto adhere in water at 20°C in BPG columns.

Efficiency tests at different flow velocities

Efficiency tests were run at different velocities. Figure 6 shows that the curves follow the van Deemter theory, indicating a well-packed bed. The asymmetry factor is stable at the different fluid velocities. The reduced plate height increases with the velocity and the optimal result is achieved at 15 to 30 cm/h. When running at higher velocities the asymmetry factor and reduced plate height continue to behave linearly. This indicates that the efficiency test can be run at any velocity but that expectations of the results have to be changed compared to the optimal results. Similar behavior can be expected for packed beds in other columns such as Chromaflow and AxiChrom.

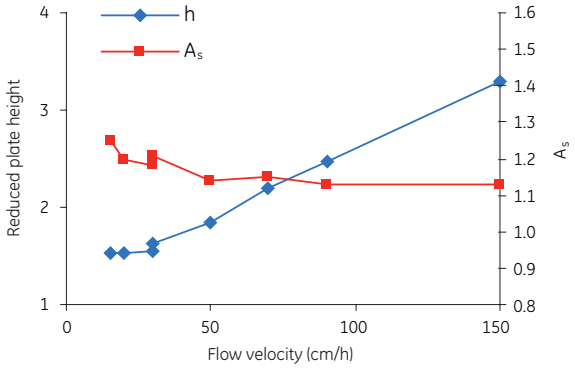

Fig 6. The reduced plate height and asymmetry values at different flow rates run on a 20 cm bed of Capto MMC in a BPG 300 column. The curves follow the van Deemter theory showing that the bed is well-packed.

Chromaflow 600

The efficiency results for Capto MMC and Capto adhere packed in Chromaflow 600 are shown in Table 5. Both media packed to 20 cm bed height give good plate numbers and asymmetry factors. In addition, the stability test shows that the bed is stable when running at 600 cm/ h for 16 h.

Table 5. Column efficiency data for different packs of Capto adhere and Capto MMC in Chromaflow columns at 20 cm bed height, showing efficiently packed beds. The test was run at 20 cm/h. The data are means of three packs, upflow and downflow test. Stability test was run at least 600 cm/h in water during 16 h.

Medium	Average N/m	Average h	Range A_s	% change after Stability test h	% change after Stability test A_s
Capto MMC	6400	2.0	0.93–1.05	3	-8
Capto adhere	7300	1.7	1.04–1.15	-3	1

The pressure/flow curves for Capto MMC and Capto adhere in Chromaflow columns are shown in Figure 7. The curves are parallel, which indicates that the velocity is a function of bed height. Since the optimal compression factor is difficult to achieve in pack-in-place columns, the maximum flow rate that can be run through the packed bed can be limited.

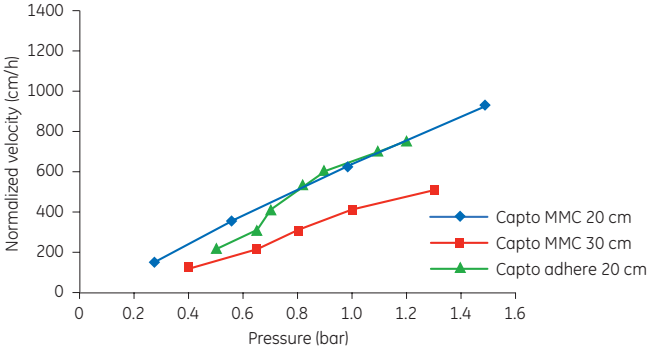

Fig 7. Pressure/flow curves for Capto MMC and Capto adhere in water at 20°C in Chromaflow columns.

Conclusions

This procedure describes packing of Capto adhere and Capto MMC in AxiChrom columns utilizing the easy-to-use and verified Intelligent Packing wizard. Methods for packing these media in BPG 100 and 300, and Chromaflow 600 columns are also described.

Capto adhere and Capto MMC media can be packed in AxiChrom columns to bed heights between 10 and 40 cm. The flexibility of column diameters and bed heights enables full utilization of the flow capacity of Capto adhere and Capto MMC, allowing processes with higher bed heights for improved resolution and increased residence time, or lower bed heights and larger diameters to decrease process time.

Each packing method described is related to a specific packing solution. Deviation from use of the packing solutions described can have significant impacts on the *PF* and subsequently on the packing result. To utilize the full flow potential of Capto adhere and Capto MMC medium, AxiChrom columns are recommended.

Ordering information

Products	Quantity	Product code
Capto adhere	25 mL	17544410
	100 mL	17544401
	1 L	17544403
	5 L	17544404
	10 L	17544405
	60 L	17544460
Capto MMC	25 mL	17531710
Capto MMC	100 mL	17531702
Capto MMC	5 L	17531704
Capto MMC	10 L	17531705
Capto MMC	60 L	17531760
Media Wand	1	28922767
Media Handling Unit	1	28922769

Related information

Products	Product code
Data file	
Capto adhere	28907888
Capto MMC	11003545
AxiChrom columns	28929041
BPG Columns 100, 140, 200, 300, and 450 series	18111523
Chromaflow columns	18113892
Media Wand	28923101
Instructions for use/Operating instructions/User manuals	
AxiChrom 50, 70, and 100 columns	28933108
AxiChrom 140 and 200 columns	28943123
AxiChrom 300–1600 columns	28956290
BPG columns	18117070
Chromaflow columns	28962232
Chromaflow Packing Station 50, 100, 200, and 400	29046228
Capto MMC	11003505
Capto adhere	28906405
Kits	
Slurry concentration kit	29096100

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