Packing Capto™ ImpRes media with verified packing methods

Capto ImpRes chromatography media (resins) are designed for high-throughput intermediate purification and polishing steps of a wide range of biomolecules. Capto ImpRes media are based on high-flow agarose and display excellent resolution. The ability to run at higher flow velocities and higher bed heights increases productivity and flexibility in process design. However, to achieve effective purification, it is important that the media are efficiently packed. Poorly packed columns can lead to costly disruptions and loss of valuable product. Robust and verified packing and testing methods can eliminate such concerns and risks. This application note describes packing methods resulting in columns with verified bed stability for Capto SP ImpRes, Capto Q ImpRes, Capto MMC ImpRes, and Capto adhere ImpRes media in large-scale chromatography columns.

Capto ImpRes media characteristics

Capto ImpRes media are based on highly rigid agarose that allows high flow, which is an important factor for raising productivity in large-scale operations. The small average bead size of 40 µm gives high resolution. Capto ImpRes media can be designed with a wide range of bed heights and flow velocities. The pressure-flow specification for Capto ImpRes media in large columns is 220 cm/h at less than 3 bar and at a 20 cm bed height (tested with water at 20°C in 1 m diameter column).

Large-scale columns for packing with Capto ImpRes media

AxiChrom™ columns

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

AxiChrom columns are available in many different configurations and materials (see data file 28-9290-41 for more details). AxiChrom columns are designed to be scalable and will give predictable results over the entire range of scales by enabling a uniform plug flow through the bed, irrespective of 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 800 mm in diameter and bed heights up to 20 cm for AxiChrom 1000.

BPG columns

BPG columns are glass columns for process development and manufacturing. The single-screw adapter allows easy and 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, which is not pressure rated for use with Capto ImpRes media.

Chromaflow™ columns

Chromaflow columns are acrylic or steel, pack-in-place columns for GMP manufacturing. The columns have diameters ranging from 400 to 2000 mm. The packing method described here applies to Chromaflow columns up to 600 mm having a standard configuration.

Packing

Definitions

The bed height of a gravity-settled bed differs from the bed height of a bed settled under low flow (consolidated). Therefore, the compression factor (*CF*) has to be separated from the packing factor (*PF*). For example in water, when consolidated at 30 to 60 cm/h, the *PF* is 1.15 and the *CF* is 1.12 for Capto ImpRes media.

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)

When packing BPG and AxiChrom columns, *PF* is used in the packing procedure to calculate the packed bed height after the consolidation step. *CF* is used in the chromatography medium preparation step to calculate the medium volume needed to pack a desired bed height. Because 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 Capto ImpRes media in various packing solutions

Capto ImpRes media settle quickly in both water and in 20% ethanol and when using these solutions it should be remembered 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 very difficult to measure slurry concentration by gravity with salt-based solutions. However, the slurry concentration method described below allows quick and accurate determination of slurry concentration.

When Capto ImpRes media are settled at 30 to 60 cm/h, the consolidated bed height will be 3% to 7% higher in salt solution compared to in water or 20% ethanol. The effect is almost the same for 10 mM NaCl as for 0.4 M NaCl and can be compensated for by using different *PF*. Table 1 shows *PF* for water and a variety of other packing solutions. However, for optimal performance of the packed bed, specific packing solutions are recommended for different column types and sizes.

Table 1. *PF* required for a 20 cm bed of Capto ImpRes media in different solutions for optimal bed performance where the bed is consolidated at 30 to 60 cm/h

Solution	Capto Q ImpRes and Capto adhere ImpRes	Capto SP ImpRes and Capto MMC ImpRes
Water	1.15	1.15
20% ethanol	1.15	1.15
10 mM NaCl	1.20	1.20
0.4 M NaCl	1.20	1.20
20% ethanol with 0.2 M sodium acetate	N/A	1.20

Slurry preparation

When preparing the slurry, start by calculating the chromatography medium volume (*V*) needed to pack the desired bed height. The slurry concentration can be determined in a number of ways, but to get an accurate slurry concentration determination of Capto ImpRes media in salt containing solutions, use the method described below.

When calculating the slurry volume (*V*) use the compression factor in water/20% ethanol (*CF* = 1.12).

Note: The slurry concentration determined by the method below corresponds to the gravity-settled concentration in water.

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

where

C_{slurry} = Concentration of the slurry

Preparing the medium to form a slurry can be performed manually or mechanically, for example by using Media Wand™ slurry mixing and transfer tool. Shaking gives good results, but is often not practical for larger volumes. When stirring, it is preferable to use soft stirrers without sharp edges. Media Wand suspends the medium directly in the container and transfers the slurry to the slurry tank in one operation which makes it suitable for large-scale packing.

Capto SP ImpRes and Capto MMC ImpRes is supplied in 20% ethanol with 0.2 M sodium acetate. Capto Q ImpRes and Capto adhere ImpRes is supplied in 20% ethanol. Before packing, transfer the media to the packing solution as described in the packing instructions for the relevant column.

Measuring slurry concentration

It is very important to measure the slurry concentration correctly. This is necessary in order to have the correct amount of chromatography medium in the slurry to pack to the target bed height at the correct level of compression. This method is performed using a Tricorn™ 10/100 column. A syringe is used in the method described below but a pump can also be used. Slurry is added to the column, then washed, resuspended, and allowed to settle for 30 min before the concentration is measured. The method is accurate for slurry concentrations below 60%. For higher concentrations,

dilute the slurry by adding exactly 4 cm of water to the Tricorn column before adding the medium in step 5 below. Calculate the concentration in the barrel or tank by dividing the measured concentration by the dilution factor 0.6.

Column filling

1. Mount the bottom end piece with filter in the Tricorn 10/100 column.
2. Carefully tape a transparent ruler on the side of the column, so that the zero point on the ruler coincides with the surface of the bottom filter.
3. Place a stop plug in the bottom outlet.
4. Place the column in an upright position.
5. Add thoroughly mixed slurry to the column with a pipette below the 10 cm mark to avoid medium on the column wall. Fill slurry to the 10 cm mark.
6. Add distilled water, until the column is filled.
7. Mount an end piece with filter on the top of the column.

Washing step

1. Mount a syringe (a 20 mL syringe is recommended although larger syringes can be used) filled with distilled water to the top of the column.
2. Remove the stop plug from the bottom outlet of the column.
3. Wash by pressing the syringe at an approximate flow of 6 to 10 mL/min.
4. Wash with a total of 50 to 60 mL distilled water and avoid pressing air into the column.
5. Close the bottom of the column using the stop plug.

Resuspension and settling

1. Remove the upper end piece.
2. Mix the chromatography medium in the column thoroughly by stirring with an appropriate tool.
3. Replace the upper end piece. Avoid introducing air into the column.
4. Mount a 20 mL syringe filled with distilled water to the upper end piece.
5. Remove the stop plug from the bottom outlet of the column.
6. Press the syringe at 6 to 10 mL/min until the liquid over the medium bed is clear.

Note: Keep the column vertical when settling the medium to get an accurate reading of bed height.

7. Stop the flow.
8. Close the bottom of the column using the stop plug.

Determination of the slurry concentration

1. Allow the bed to stabilize for 30 min without flow.
2. Read the bed height.

As 10 cm of slurry was measured up initially, the height after 30 min corresponds to the concentration of the slurry. For example, 4 cm bed height corresponds to 40% slurry concentration.

Packing Capto ImpRes media 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 1000 columns, Intelligent Packing is performed by AxiChrom Master, a separate unit that comprises a touch-screen operated user interface, or from UNICORN control software on ÄKTAprocess™ system.

Intelligent packing in AxiChrom columns – general considerations

Packing methods are created by entering values for the packing variables, for example, medium, slurry concentration, and target bed height, in the Intelligent Packing wizard.

When packing AxiChrom 50 to 200 columns, the slurry is introduced into the column by hand and adapter movement that is driven by internal hydraulics. After the wizard method has been created and the medium has been equilibrated to the packing solution, the column is primed and filled with slurry. The method controls the flow of hydraulic fluid to drive the adapter and pack the bed (Fig 1).

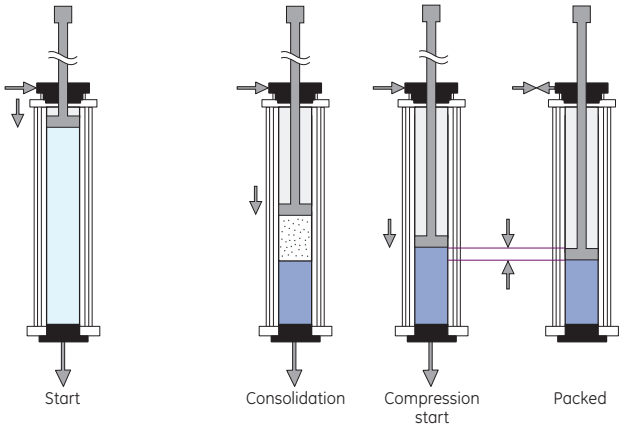

Fig 1. Intelligent Packing in AxiChrom 50 to 200 columns. The adapter is mounted to the column tube and the wizard is started **(Start)**. The adapter moves down, forcing packing liquid out through the bottom bed support. The medium forms a consolidated bed **(Consolidation)**. When the adapter comes in contact with the consolidated bed surface, the operator initiates bed compression in the UNICORN wizard **(Compression start)**. Compression occurs according to a predetermined *PF*. Finally, the target bed height is attained **(Packed)**.

In AxiChrom 300 to 1000 columns, slurry is introduced via a media valve in the center of the bottom bed support and the adapter is driven by an electric servo-motor. 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 servo-motor 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. When this occurs, the operator confirms that the adapter has hit the bed.

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

Specific considerations for packing Capto ImpRes media in AxiChrom columns

A general bed height interval for Capto ImpRes media in AxiChrom columns is 10 to 40 cm. When packing Capto ImpRes in AxiChrom, the Intelligent Packing wizard custom media option is used. When applying this option, manual input is needed regarding additional packing variables as *PF* and adapter velocity. Flow conditioning with desired flow velocity and time can be applied. Packing variables and recommended packing buffers are given in Table 2. The actual packing procedure using the custom media option works the same way as for media included in the Intelligent Packing wizard.

To achieve optimal bed performance, flow conditioning is recommended for AxiChrom 50 to 200 columns but is optional for AxiChrom 300. The flow velocities shown during flow conditioning are recommended for liquids with the same viscosity as water at 20°C.

Note: The maximum recommended bed height for AxiChrom 1000 is 20 cm using the recommended compression factor. Bed heights above 20 cm for AxiChrom 1000 column is possible at lower levels of compression and reduced flow performance. Confirmation studies for bed heights above 20 cm are ongoing.

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

Note: When unpacking AxiChrom 300 to 1000 columns, use the same Intelligent Unpacking method that is used for the corresponding Capto media (i.e., Capto S, Capto Q, Capto DEAE, Capto adhere, or Capto MMC).

Table 2. Packing variables for packing Capto ImpRes media in AxiChrom columns

Packing variable	AxiChrom 50 to 200		AxiChrom 300 to 1000
	Capto SP ImpRes, Capto MMC ImpRes, and Capto adhere ImpRes	Capto Q ImpRes	Capto ImpRes media
Packing solution	10 mM NaCl	0.4 M NaCl	Water
Packing speed/velocity	30 cm/h	30 cm/h	60 cm/h
Packing factor	Bed heights < 30 cm: 1.20 Bed heights ≥ 30 cm: 1.16	Bed heights < 30 cm: 1.20 Bed heights ≥ 30 cm: 1.16	1.13 to 1.15*
Flow conditioning	230 cm/h for 30 min	230 cm/h for 30 min	Optional for AxiChrom 300: 230 cm/h at 20 cm bed height, linearly decreasing to 150 cm/h at 40 cm bed height for 30 min

* When packing AxiChrom 300 to 1000 columns, it is important to stay within the *PF* interval of 1.13 to 1.15. Note this range is smaller then the range for verified packing methods of other media in AxiChrom process columns. Lower *PF* might result in instable bed with differences in bed efficiency and selectivity over time, especially for lower beds. Higher *PF* will result in high packing forces, especially for higher beds.

Table 3. Packing variables for packing Capto ImpRes media in BPG columns

Packing variable	BPG 100		BPG 300
	Capto SP ImpRes, Capto MMC ImpRes, and Capto adhere ImpRes	Capto Q ImpRes	Capto ImpRes media
Packing solution	10 mM NaCl	0.4 M NaCl	Water
Packing speed/velocity	60 cm/h	60 cm/h	60 cm/h
Packing factor	Bed heights < 30 cm: 1.20 Bed heights ≥ 30 cm: 1.16	Bed heights < 30 cm: 1.20 Bed heights ≥ 30 cm: 1.16	Bed heights ≤ 20 cm: 1.13 Bed heights > 30 cm: 1.10 Bed heights 20–30 cm, decreasing linearly from 1.13 to 1.10
Flow conditioning	3 bar for 5 CV	3 bar for 5 CV	3 bar for 5 CV

Packing Capto ImpRes media in BPG columns

Capto ImpRes media can be packed in BPG 100 to 300 columns. BPG 450 is, however, not recommended for Capto ImpRes media because of its low pressure limits. The bed height interval recommended for BPG 100 to 300 columns is 10 to 40 cm.

In BPG columns with small diameters (BPG 100), the increased influence of the wall support means that salt solution is required for packing. Capto SP ImpRes, Capto MMC ImpRes, and Capto adhere ImpRes medium is therefore packed with 10 mM NaCl and Capto Q ImpRes medium with 0.4 M NaCl in BPG 100. In BPG 300, all Capto ImpRes media are packed with water.

Chromatography medium preparation

Equilibration of the medium to the packing solution can be performed by using the BPG column as a "filter".

Pour the medium into the column (for calculation of amount see "Slurry preparation"), 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 at least three column volumes (CV) of the packing solution. Unpack and resuspend the slurry and pack according to the method below.

Note: Equilibration is critical for Capto SP ImpRes and Capto MMC ImpRes in BPG 300 as the delivery solution contains salt, and the recommended packing solution is water. Measure the conductivity of the flow through solution during equilibration and continue to equilibrate until the conductivity is zero.

Column and system preparation

A detailed description of column preparation is available in the BPG column instructions (18-1170-70). The packing pump should be as pulsation-free as possible. Screw and rotary lobe pumps are the most suitable for this task but multi-headed diaphragm pumps are also satisfactory.

Caution: Ensure that the column has no visible scratches in the glass tube and that the adapter moves smoothly in both upward and downward directions before packing. In addition, there should be no difficulty in tightening the adapter O-ring to the column inner wall.

1. Place a new 10 µm net on both the adapter and the bottom end pieces.
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 to the adapter.
4. Mount one 4-port, 2-way valve on the bottom inlet and one on top of the pressure gauge, i.d. 10 mm for BPG 300 and i.d. 6 mm for BPG 100.

Packing

1. Set the pressure alarm or pressure relief valve according to the pressure rating of the column. 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 tubing to the suction side of a pump. Start the pump and place the tubing 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 a homogeneous slurry.

Note: The available height to allow the adapter to be inserted into a 50 cm column tube (for filling slurry) is only 40 cm. Use a longer column tube when packing beds higher than 20 cm: 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 column leakage.
5. Insert the top 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 that 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 60 cm/h (4.7 L/h for BPG 100 and 42.4 L/h for BPG 300). 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. For BPG 100, *PF* = 1.20 in 10 mM or 0.4 M NaCl for bed heights up to 30 cm; for higher beds *PF* = 1.16. For BPG 300, *PF* = 1.13 in water for bed heights up to 20 cm, decreasing linearly to 1.10 for bed heights of 30 cm and higher. The packed bed height is the ratio between the consolidated bed height and the *PF*. Use a marker pen to indicate on the column the packed bed height.
9. Stop the flow and close the bottom valve. Loosen the O-ring and lower the adapter down to 1 cm above the settled bed and seal the adapter O-ring. Shift the top valve to waste and 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 media in BPG columns, especially the larger BPG 300 column, is physically demanding. Do not use extension rods on the adapter height adjuster to compress the media.

To increase the performance and stability of the bed, flow condition the column downwards with 5 CV of water or packing solution at 3 bar.

10. Connect the pump to the top of the column. Purge the system and tubing by running the mobile phase to waste by bypassing the column inlet with the 4-port valve. Start at a low flow velocity.
11. Shift the top valve to direct the flow into the column and immediately open the bottom mobile phase to waste or connect it to the buffer tank for recirculation.
12. Increase the flow until a pressure of 3 bar is reached, or to the maximum flow/pressure if 3 bar cannot be reached. Run the column at this pressure for 5 CV.
13. Slowly decrease the flow for 2 min to avoid disturbance of the conditioned bed.
14. Test the packing at the optimal test velocity (30 cm/h).

Packing Capto ImpRes media in Chromaflow columns

The largest recommended Chromaflow column for Capto ImpRes media is Chromaflow 600 with a recommended bed height interval of 20 to 40 cm when using the packing method described below. As the pump is stopped 5 mm from the adapter, as seen in the packing method, lower beds will not be compressed sufficiently. Moreover, the extreme velocities needed to efficiently pack a shorter bed cannot easily be achieved using standard equipment.

To pack Capto ImpRes media in Chromaflow 600, the slurry is introduced from the top nozzle by the use of Chromaflow Packing Station Pack 100.

Note: Operational flow velocity is reduced in Chromaflow columns compared to AxiChrom and BPG columns, from at least 220 cm/h to at least 150 cm/h at 20 cm bed height.

Note: The flow capacity of Chromaflow Packing Station Pack 100 is required for packing Capto ImpRes media in Chromaflow 600 for operational flow velocities of at least 150 cm/h.

Chromatography medium preparation

The recommendation is to use 10 mM NaCl as packing solution for Chromaflow columns and that this packing solution is used throughout the whole procedure.

Note: Water may potentially be used as packing solution but compression and flow velocities have not been verified by GE Healthcare.

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

Column and system preparation

For more detailed description about the column and packing station preparation, see Chromaflow Columns instructions for use (56-3193-25) and Chromaflow Packing stations instructions for use (56-3215-58). In this application note, standard Chromaflow equipment is used for the connections on the column and the packing station.

Note: It is important that the supply air flow rate follows the specification of Chromaflow Packing Station Pack 100 (1000 L/min) 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 (56-3193-25).
2. A pressure relief valve should be used for safety reasons. Position this on the slurry inlet top (SIT). Place a pressure gauge on the mobile phase top (MPT) to record the pressure during packing. Mount a 3-port, 2-way valve on top of the pressure gauge and the mobile phase bottom (MPB). The top valve should lead in two directions: one side into the system and one to the waste for purging the tubing. On the bottom valve, one side leads to the system and a 1.5" to 2" tubing leads to the waste (for packing). Part of the MPB waste tubing should be placed above the outlet valve to eliminate air 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 instructions for use.

Packing

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. 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.
2. The initial flow velocity should be at least 1700 cm/h, corresponding to an air pressure on the packing station of at least 3.5 bar for packed bed heights of 20 and 30 cm.
3. When the tubing is primed and the flow velocity set, set the SIT/slurry inlet bottom (SIB) to the position between SIT and SIB. This blocks the flow during step 4 while maintaining the correct flow rate for the next step.
4. Move the top nozzle down into the packing position.

5. 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 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. Typically, the final pressure in the column is 2 to 3 bar depending on the viscosity of the packing solution, column diameter, bed height, and so forth.

6. Stop the pump when the building bed is 5 mm from the top bed support by setting the SIT/SIB to the position between SIT and SIB, as described in 3. Once flow is stopped, the bed will expand to meet the adaptor.

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

7. Immediately move the top nozzle back to the run position.
8. Close the MPB valve when the pressure in the column is between 0.3 and 0.1 bar.
9. 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.
10. Close the slurry tank and empty the tubing between the tank and packing station.
11. Pump liquid upwards through the column until the air is expelled.

Figure 2 shows the increase in pressure and decrease in flow velocity when packing a 20 cm bed height in Chromaflow 600.

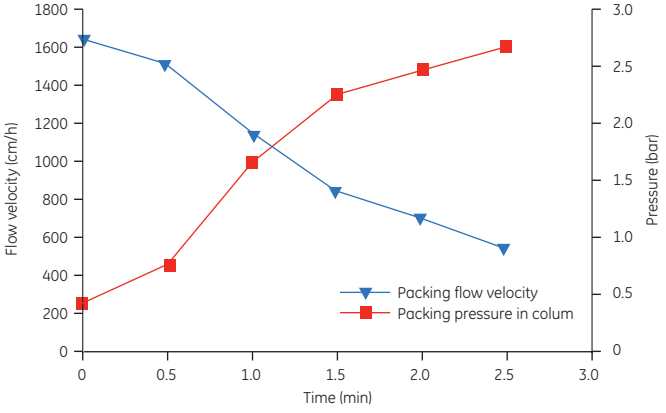

Fig 2. Column pressure and flow velocity during packing of a 20 cm bed height in Chromaflow 600 by the use of Chromaflow Packing Station Pack 100.

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 packed bed throughout the working life of the column. If the test results are to be comparable over time, conditions such as, flow 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 to the test conditions and the goal of the purification. This is further described in application note 28-9372-07.

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 optimal test conditions.

Test conditions used in this study

In this study optimal test conditions has been chosen. This also includes the use of recommended tubing for each column setup.

Sample:	2% v/v acetone 0.8 M NaCl for AxiChrom 50, 100, and 200 columns
Sample volume:	1% of the column volume (V_C)
Test velocity:	30 cm/h
Eluent:	Water 0.4 M NaCl for AxiChrom 50, 100, and 200 columns

Examples of results

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

AxiChrom columns

Examples of efficiency and stability results for Capto ImpRes media packed in AxiChrom 50, 100, and 200 columns can be seen in Table 4. Results for Capto ImpRes in AxiChrom 300 and 600 columns are shown in Table 5.

Capto SP ImpRes medium showed good and similar (in some cases identical) results from the different column sizes of AxiChrom 50, 300, and 600 (Table 4 and Table 5) and for different bed heights in AxiChrom 300 and 600 columns (Table 5). These result is also similar to the result for Capto Q ImpRes, Capto adhere ImpRes and Capto MMC ImpRes. Thus, the method developed, gave similar results for all four media independent of column size and bed height.

Table 4. Column efficiency data for AxiChrom 50, 100, and 200 (pilot-scale) columns packed with Capto ImpRes media

Medium	AxiChrom column	Bed height (cm)	Average plates/m*	Reduced plate height (h) range*	Asymmetry factor (A_s) range*	Flow velocity for stability test (cm/h)†	Change after stability test (%)†	
							h	A_s
Capto SP ImpRes	50	20	15 800	1.4 to 1.5	0.9 to 1.0	300	-4; -2‡	14; 2‡
Capto Q ImpRes	50	40	15 400	1.5	1.0	150	0	4
Capto Q ImpRes	100	10	16 500	1.4	1.1 to 1.2	500	-2	2
Capto Q ImpRes	200	30	13 900	1.6 to 1.7	1.2 to 1.4	200	3	-9
Capto MMC ImpRes	50	40	14 000	1.6 to 1.8	0.8 to 1.0	150	3; -1	16; 4
Capto MMC ImpRes	100	10	15 700	1.4 to 1.5	1.1 to 1.2	500	-1	5

* Test performed at optimal test conditions. Average and ranges of upflow and downflow tests for at least three packs, except for Capto Q ImpRes in AxiChrom 200 where only two packs were performed.
† Stability tests were run once for each bed height/media/column combination in water for 16 h at given velocity.
‡ Data from two consecutive stability tests.

Table 5. Column efficiency data for AxiChrom 300 and 600 (process-scale) columns packed with Capto ImpRes media

Medium	AxiChrom column	Bed height (cm)	Average plates/m*	Reduced plate height (h) range*	Asymmetry factor (A_s) range*	Flow velocity for stability test (cm/h)†	Change after stability test (%)†	
							h	A_s
Capto SP ImpRes	300	20	16 300	1.5 to 1.6	1.1 to 1.3	300	-7	7
Capto SP ImpRes	300	40	14 000	1.5 to 1.9	1.0 to 1.2	150	-14; 4‡	13; 3‡
Capto SP ImpRes	600	10	15 700	1.4 to 1.6	1.2 to 1.4	500	-8	1
Capto SP ImpRes	600	30	15 900	1.4 to 1.5	1.0 to 1.3	170	-10	5
Capto Q ImpRes	300	20	15 100	1.6 to 1.7	1.0 to 1.2	300	-13; 2‡	17; 0‡
Capto adhere ImpRes	300	20	14 200	1.6 to 1.8	1.2 to 1.4	300	-5	2

* 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/media/column combination in water for 16 h at given velocity.
‡ Data from two consecutive stability tests.

The stability test showed that the packed beds are stable when running in water at the velocities given in Table 4 and 5, and also that the packed beds stabilize with time as seen in the continued running of stability tests.

Pressure-flow curves provide a simple, effective illustration of column performance in terms of the maximum operating velocity at which the purification process can be run. They also show the magnitude of the backpressure in the system at a certain flow velocity. All AxiChrom columns can be utilized at the maximum flow velocity of Capto ImpRes (Figs 3 and 4).

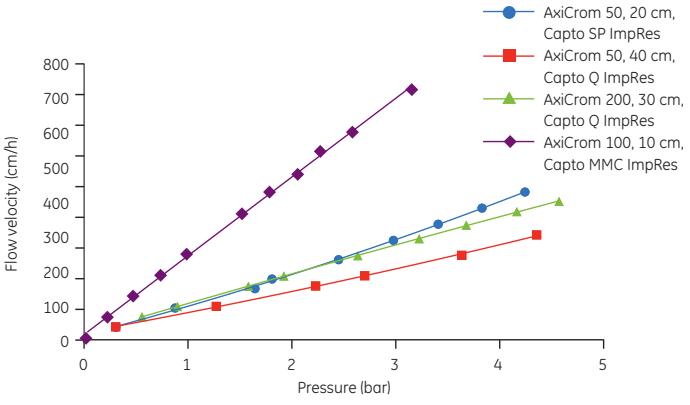

Fig 3. Pressure-flow curves for AxiChrom pilot-scale columns packed to different bed heights with Capto ImpRes media. Measurements were made with water at 20°C. The contribution of hardware pressure is excluded. Different chromatography media batches were used to cover a larger operational space.

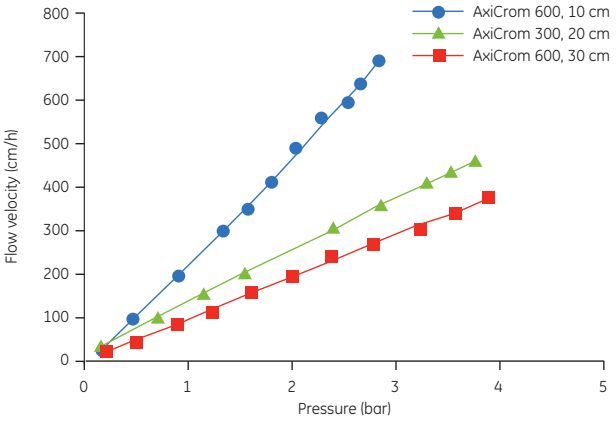

Fig 4. Pressure-flow curves for AxiChrom process-scale columns packed to different bed heights with Capto SP ImpRes medium. Measurements were made with water at 20°C. The contribution of hardware pressure is excluded. Different chromatography media batches were used to cover a larger operational space.

BPG columns

Examples of efficiency and stability results for Capto ImpRes packed in BPG 100 and BPG 300 columns are shown in Table 6. The results were very similar both for different column sizes and for bed heights. In general, the packing method works well for these media in BPG columns of different sizes and at different bed heights. The stability test showed that the packed bed was stable at the flow velocities given in the table.

The pressure-flow curves for BPG 100 and 300 columns, packed with Capto SP ImpRes medium to a bed height of 20 cm, are shown in Figure 5. For both column sizes, these curves show linear behavior. The pressure-flow profile for BPG 300 column shows that the medium can be run at 220 cm/h with less than 3 bar backpressure from the bed.

Chromaflow columns

Examples of efficiency results for Capto SP ImpRes packed in Chromaflow 600 column are shown in Table 7. Both 20 and 30 cm bed heights give good results.

The pressure-flow curve for Capto SP ImpRes in Chromaflow 600 column is shown in Figure 6. As the optimal *CF* is difficult to achieve in standard pack-in-place columns, the maximum flow velocity that can be run through the packed bed is limited. The highest operating velocity recommended for this type of column is 150 cm/h at a 20 cm bed height. Note that bed efficiency and bed stability are good, provided that the 150 cm/h guideline is met.

Pressure-flow curves for AxiChrom (Fig 4) and Chromaflow columns (Fig 6) showed higher backpressure in AxiChrom columns than in the Chromaflow 600 column at the same bed height and flow velocity. This results from the higher compression when packing AxiChrom columns compared to when packing Chromaflow columns. This higher compression is needed to utilize the full flow velocity of Capto ImpRes media. Still, the backpressure over AxiChrom columns at maximum flow velocity is far from the maximum operating range of the columns (4 bar).

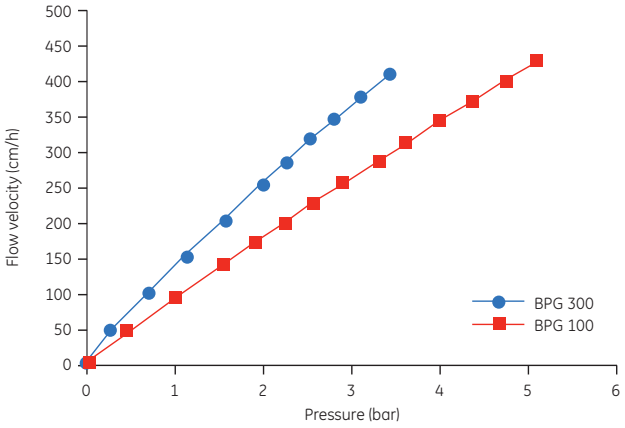

Fig 5. Pressure-flow curves for BPG columns packed to 20 cm bed height with Capto SP ImpRes medium. Measurements were made with water at 20°C. The contribution of hardware pressure is excluded.

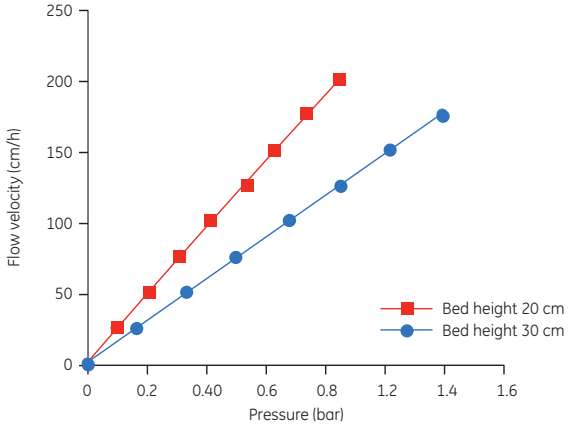

Fig 6. Pressure-flow curves Chromaflow 600 column packed to different bed heights with Capto SP ImpRes medium. Measurements were made with water at 20°C. The contribution of hardware pressure is excluded.

Table 6. Column efficiency data for BPG 100 and 300 columns packed with Capto ImpRes media

Medium	BPG column	Bed height (cm)	Average plates/m*	Reduced plate height (h) range*	Asymmetry factor (A_s) range*	Flow velocity for stability test (cm/h)†	Change after stability test (%)†	
							h	A_s
Capto SP ImpRes	100	20	14 800	1.5 to 1.6	1.0 to 1.1	300	4	1
Capto SP ImpRes	300	20	14 900	1.4 to 1.7	1.2	300	3	4
Capto SP ImpRes	300	30	13 900	1.5 to 2.0	1.2 to 1.3	190	7	7
Capto Q ImpRes	300	20	15 700	1.5 to 1.7	1.1 to 1.2	300	-2	-1

* Test performed at optimal test conditions. Average and ranges of upflow and downflow tests for at least three packs, except for Capto SP ImpRes medium in BPG 100 column where only two packs were performed.

† Stability tests were run once for each bed height/media/column combination in water for 16 h at given velocity.

Table 7. Column efficiency data for Chromaflow 600 column packed with Capto SP ImpRes medium

Medium	Chromaflow column	Bed height (cm)	Average plates/m*	Reduced plate height (h) range*	Asymmetry factor (A_s) range*	Flow velocity for stability test (cm/h)†	Change after stability test (%)†	
							h	A_s
Capto SP ImpRes	600	20	10 900	1.9 to 2.4	1.1 to 1.3	150	16	13
Capto SP ImpRes	600	30	11 400	1.9 to 2.3	1.2 to 1.5	100	10	2

* Test performed at optimal test conditions. Average and ranges of upflow and downflow tests for at three packs.

† Stability tests were run once for each bed height/media/column combination in water for 16 h at given velocity.

Efficiency tests at different flow velocities

Efficiency tests were run at different velocities. Figure 7 shows that the curves follow the van Deemter theory. The A_S is stable at the different flow velocities. The reduced plate height increases with the flow velocity and the optimal result is achieved at 20 to 40 cm/h. When running at higher flow velocities, the A_S and reduced plate height continue to behave linearly. This indicates that the efficiency test can be run at any flow velocity, but the expectation of the result has to be changed and compared to the optimal result.

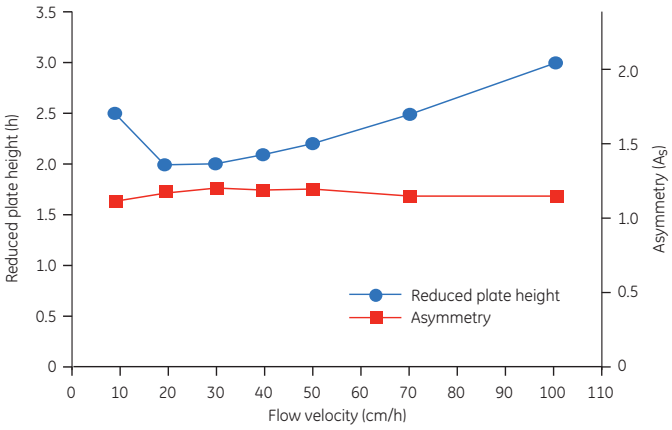

Fig 7. Reduced plate height and A_s values at different flow velocities run on a 20 cm bed of Capto SP ImpRes in a Chromaflow 600 column. This is an example but similar behavior can be expected for packed beds in other columns such as AxiChrom and BPG.

Conclusions

This application note describes packing of Capto SP ImpRes, Capto Q ImpRes, Capto MMC ImpRes, and Capto adhere ImpRes media in AxiChrom columns using the custom mode feature of Intelligent Packing, as well as packing of Capto ImpRes media in BPG and Chromaflow columns.

Capto ImpRes media can generally be packed in AxiChrom, BPG, and Chromaflow columns to bed heights between 10 and 40 cm see specific column type for details. The flexibility of column diameters and bed heights enables full utilization of Capto ImpRes media flow capacity, allowing for processes with increased bed heights if floor space is limited or at 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 may have significant impact on the *PF* and subsequently on the packing result.

To utilize the full flow potential of the Capto ImpRes media, AxiChrom columns are recommended.

Ordering information

Product	Quantity	Code number
Capto SP ImpRes	25 mL	17-5468-10
	100 mL	17-5468-02
	1 L	17-5468-03
	5 L	17-5468-04
	10 L	17-5468-05
Capto Q ImpRes	25 mL	17-5470-10
	100 mL	17-5470-02
	1 L	17-5470-03
	5 L	17-5470-04
	10 L	17-5470-05
Capto MMC ImpRes	25 mL	17-3716-01
	100 mL	17-3716-02
	1 L	17-3716-03
	5 L	17-3716-04
	10 L	17-3716-05
Capto adhere ImpRes	25 mL	17-3715-01
	100 mL	17-3715-02
	1 L	17-3715-03
	5 L	17-3715-04
	10 L	17-3715-05
Media Wand 50	1	28-9227-67
Media Wand Handling unit	1	28-9227-69

For more information about AxiChrom, BPG, and Chromaflow columns as well as Chromaflow Packing Stations, visit www.gelifesciences.com

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

www.gelifesciences.com/bioprocess

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