Packing Capto™ S, Capto Q, and Capto DEAE in production-scale columns

Capto S, Capto Q, and Capto DEAE are, respectively, strong cation, strong anion, and weak anion exchange chromarography media (resins) designed for capture at high capacity. Capto media have outstanding pressure-flow properties allowing both high bed heights and flow velocities at large scales. Capto media are well-suited for processing large feed volumes in a fast and effective way in order to optimize the productivity and overall process economy. 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. Generic and robust packing and testing methods can eliminate such concerns and risks. The AxiChrom™ column platform was developed with this in mind and verified packing methods are available from pilot to process scale via the Intelligent Packing concept.

GE Healthcare Life Sciences has also developed packing methods for BPG and Chromaflow™ columns. These methods are presented in this application note. Test data obtained from packed beds in AxiChrom, BPG, and Chromaflow are also summarized and discussed.

Product characteristics

Capto S, Capto Q, and Capto DEAE

Capto media are based on highly rigid agarose that allows high flow, which is an important factor for raising productivity in large scale operations. The pressure-flow specifications for Capto S, Capto Q and Capto DEAE in large columns is 700 cm/h with less than 3 bar over a 20 cm bed height, tested with water at 20°C. Capto media can be designed with a wide range of bed heights and flow velocities.

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

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.

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 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 1000 mm in diameter.

BPG columns

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, which is not pressure rated for use with Capto media.

Chromaflow

Chromaflow columns are acrylic or 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, where the consolidated bed height (at 30–60 cm/h) is higher than the gravity settled bed height, the *CF* is 1.07 and *PF*, when consolidating at 30–60 cm/h, is 1.10 for Capto S, Capto Q, and Capto DEAE.

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 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 ion exchange (IEX) media in various packing solutions

Capto S, Capto Q, and Capto DEAE 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 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 S, Capto Q, and Capto DEAE media are settled at 30–60 cm/h the consolidated bed height will be 5% to 15% higher in salt solution than 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 and a variety of other packing solutions.

Table 1. Typical packing factors for Capto S, Capto Q, and Capto DEAE in different solutions for optimal bed performance where the bed is consolidated at 30–60 cm/h

Solution	Capto Q	Capto S	Capto DEAE
Water	1.10	1.10	1.10
20% ethanol	1.10	1.10	1.10
10 mM NaCl	1.17	1.17	1.17
20% ethanol with 0.2 M sodium acetate	N/A	1.20	N/A

Slurry preparation

When preparing the slurry, start by calculating the chromatography medium volume 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 of Capto S in its storage solution or Capto IEX 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* = 1.07).

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

Capto Q and Capto DEAE are supplied in 20% ethanol and Capto S in 20% ethanol with 0.2 M sodium acetate. Before packing, transfer these 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 to have the correct amount of chromatography medium in the slurry to pack to the target bed height at the correct level of compression. Measuring slurry concentration can be performed with 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, washed, resuspended, and allowed to settle for 30 min before the concentration is measured. This 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 a filter on 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 stopper in the bottom outlet.
4. Place the column in an upright position.
5. Add thoroughly mixed slurry to the column with a pipette up to the 10 cm mark, Keep the mouth of the pipette below the 10 cm mark to avoid leaving medium on the column wall.
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. Refit the upper end piece. Avoid introducing air into the column.
4. Mount the 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 media bed is clear.
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.

Note: The measured slurry concentration corresponds to the gravity-settled concentration in water.

Packing Capto S, Capto Q, and Capto DEAE 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 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).

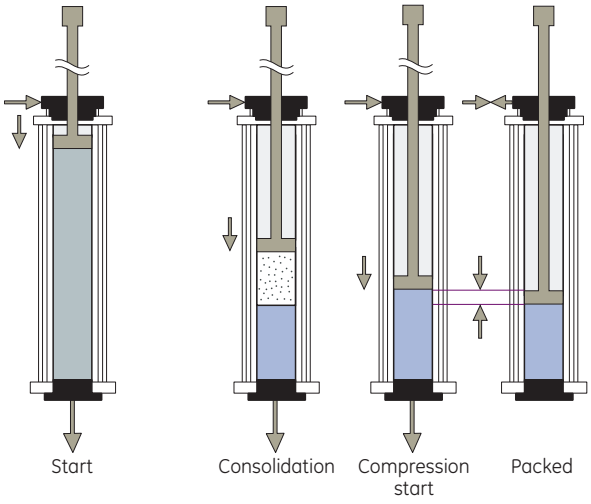


Fig 2. Intelligent Packing in AxiChrom 50 to 200 columns. The adaptor is mounted to the column tube and the wizard is started (Start). The adapter moves down, forcing packing liquid out through the bottom bed support. The medium forms a consolidated bed (Consolidation). When the adapter comes into contact 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 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 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 S, Capto Q, and Capto DEAE in BPG

Capto S, Capto Q and Capto DEAE can be packed in BPG 100 to 300 columns. BPG 450 is , however, not recommended for Capto IEX media because of its low pressure limit. Capto S, Capto Q, and Capto DEAE 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 above) and therefore the packing factor will be 1.10 in water for BPG 300 and 1.17 in 10 mM NaCl for BPG 100.

Chromatography medium preparation

Equilibration of the medium in 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 packing solution. Unpack and resuspend the slurry and pack according to the method below.

Note! Equilibration is critical for Capto S in BPG 300 as the delivery solution contains salt, and the recommended packing solution is water. Measure the conductivity of the solution leaving the column during the equilibration and equilibrate until the conductivity is zero.

Column and system preparation

A detailed description of column preparation is available in the BPG instructions for use (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 can also be used.

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 protection, especially against pressure spikes. Position this valve on the pump outlet and mount a pressure gauge on 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 trapped under the end-piece net, add more packing solution and connect a 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 (if a 20 cm bed is packed). Mix the medium and the packing solution to a homogeneous slurry.

Note! The available height for the adapter to be inserted into a 50 cm column tube (for filling slurry) is only 40 cm. Use a longer column tube or packing extension tube when packing beds higher than 20 cm: 75 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 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 (2.3 L/h for BPG 100 and 21 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 using *PF* = 1.17 in 10 mM NaCl for BPG 100 and *PF* = 1.10 in water for BPG 300. The packed bed height is the ratio between the consolidated bed height and the *PF*. Use a marker pen to indicate the packed bed height on the column.
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 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.

To increase the performance and stability of the bed, flow condition the column downwards for 5 CV and upward for 5 CV at 3 bar, or at the maximum flow if 3 bar cannot be reached. This can be performed with water or packing solution.

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 (approximately 100 cm/h).
11. Shift the top valve to direct the flow to 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. Exchange the mobile phase connections. Connect the tubing from the pump to the bottom valve and open the top valve to waste or to the buffer tank for recirculation.
15 Repeat steps 10 through 13 with upward flow to complete the conditioning procedure.
16. Test the packing at the optimal test velocity (20 to 30 cm/h).

Packing Capto S, Capto Q, and Capto DEAE in Chromaflow columns

The packing method for Capto S, Capto Q, and Capto DEAE in Chromaflow columns is a modified version of the procedure used for Sepharose™ Fast Flow or Sepharose High Performance media. To achieve the highest compression factor, a low initial packing flow rate is used, as the particles settle more tightly at a lower flow rate. When the bed is a few cm from the adapter screen, the packing flow rate is increased to the maximum flow the recommended packing station can give without reaching the column pressure limit.

For Capto S, Capto Q, and Capto DEAE, beds of 20 cm and above work well in Chromaflow columns. The extreme flow rates needed to efficiently pack a shorter bed cannot easily be achieved using standard equipment.

Note! The packing method described is for a Chromaflow 600 using a Pack 100 packing station. The Pack 100 flow capacity enables packing of Capto media as described for operational flow velocities of up to 500 cm/h compared with when packing an AxiChrom column where 700 cm/h is received. For higher operational flow velocities, the larger Pack 200 packing station is required.

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 require 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, additional slurry is required for the extra 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 more detailed description about the column and pack 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 nomenclature is used for connections on the column and packing station.

Note! It is important that the supply air flow rate follows the specification of the Chromaflow 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 (56-3193-25).
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 throught 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 instruction.

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 rate should be as low as possible while maintaining an even stroke rate. The pressure on the packing station should be 1.5 to 2 bar, which corresponds to the flow shown in Table 2.
3. When the tubing is primed and the flow rate set, set the SIT/slurry inlet bottom (SIB) to the position between SIT and SIB to block 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 then starts to fill with slurry and the bed builds up slowly from the bottom as excess liquid exits via the MPB.

6. When the bed has reached the L_x (Table 2), increase the packing flow quickly to the compression step pressure (on packing station).

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 2–3 bar depending on the viscosity of the packing solution, column diameter, bed height, and so forth.

Table 2. Parameters for packing of Capto S, Capto Q, and Capto DEAE in Chromaflow 600 using Pack 100

	Packed Bed height (cm)	
	20	30
Initial step		
Flow (L/min)	20	20
Pack station pressure (bar)	1.5 to 2	1.5 to 2
L_x[1] (cm)	16	25
Compression step		
Flow (L/min)	70	50
Pack station pressure (bar)	6 (max)	4.5

[1] Bed height at which the flow should be increased

Note! If a nontransparent column tube is used, increase the packing flow when about 75% of the calculated volume of slurry has been introduced and stop the packing when all calculated volume of slurry is introduced into the column. Check the volume in the slurry tank or use a volume totalizator.

7. Stop the packing pump when the bed is 1 to 2 mm from the top bed support by setting the SIT/SIB to the position between SIT and SIB, as described in step 3. Once the flow is stopped, the bed will expand to meet the adapter.
8. Immediately move 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 of 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 28-9372-07.

Test conditions used in this study

Sample: 2% v/v acetone or 0.8 M NaCl (AxiChrom 50 to 200)

Sample volume: 1% of the bed volume (V_C)

Test velocity: 30 cm/h for AxiChrom and BPG
20 cm/h for Chromaflow

Eluent: water or 0.4 M NaCl (AxiChrom 50 to 200)

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, and stability.

AxiChrom columns

Examples of efficiency and stability results for Capto S, Capto Q, and Capto DEAE packed in AxiChrom columns can be seen in Table 3. The results for Capto S in column sizes ranging from 50 to 1000 mm were very similar. The same results were also seen for Capto Q and Capto DEAE in AxiChrom 400 and 600 columns. Different bed heights also gave the same result (see Capto Q in Table 3).

This shows that the verified packing methods available in Intelligent Packing in the UNICORN Master wizard give the same result, independent of column size and bed height. The stability test showed that the beds were stable when running in water at velocities given in Table 3.

Table 3. Column efficiency data for different packs of Capto S, Capto Q, and Capto DEAE in different AxiChrom columns

Medium	AxiChrom column	Bed height (cm)	Average plates/m*	Reduced plates height (h) range*	Asymmetry factor (A_s) range*	Flow velocity for stability test (cm/h)†	Change after stability test (%)†	
							h	A_s
Capto S	50	20	6900	1.4–1.5	1.1–1.2	700	1	2
Capto S	400	20	6600	1.4–1.5	1.1–1.2	700	5	2
Capto S	1000	15	7400	1.3–1.5	1.2–1.3	950	2	1
Capto Q	400	20	7400	1.4–1.6	1.1–1.2	700	2	6
Capto Q	400	40	7300	1.4–1.6	1.1–1.2	450	5	2
Capto DEAE	600	20	7200	1.4–1.7	1.1–1.2	700	4	4

* Test performed at optimal test conditions. Average and ranges of up-flow and down-flow 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.

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. These curves also show the magnitude of the back pressure in the system at a certain liquid velocity. AxiChrom columns can utilize the full liquid velocity of Capto, even at large column diameters (Fig 3).

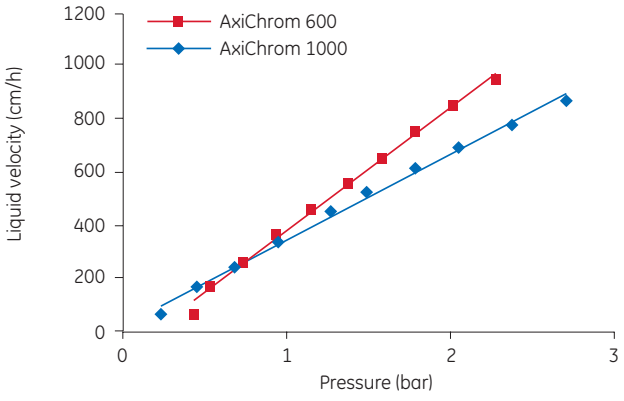


Fig 3. Pressure-flow curve in water at 20°C for Capto S, 20 cm packed bed height, in AxiChrom 600 and AxiChrom 1000 equipped with plastic bed supports. Capto can be run at 700 cm/h with a back pressure of less than 3 bar. System/tubing pressure is excluded.

BPG columns

Examples of efficiency and stability results for Capto S, Capto Q, and Capto DEAE packed in BPG 100 and BPG 300 columns are shown in Table 4. The results for Capto S, Capto Q, and Capto DEAE in BPG 300 at 20 cm bed height are very similar.

In general, the packing method works well for the three media in different sizes of BPG columns at different bed heights. The stability test showed that the bed was stable in water at velocities given in Table 4.

The pressure-flow curves for 20 cm bed height in BPG 100 are shown in Figure 4. These curves show linear behavior and the three media have the same pressure-flow properties.

The pressure flow profile for BPG 300 shows that the medium can be run at 700 cm/h with a back pressure of less than 3 bar (Fig 5).

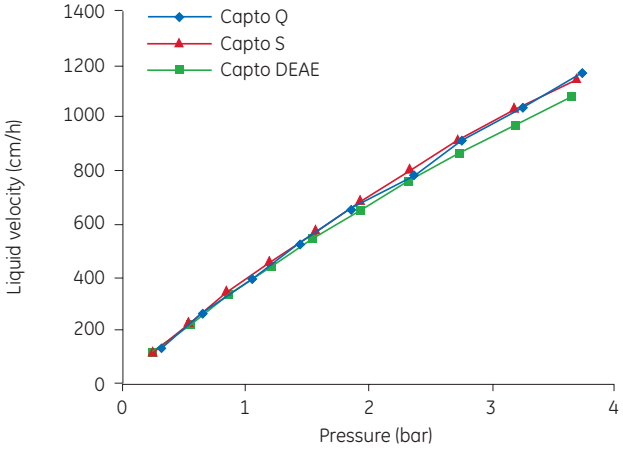


Fig 4. Pressure-flow curve in water at 20°C for Capto Q, Capto S, and Capto DEAE, 20 cm packed bed height, in BPG 100. The three media have nearly the same pressure-flow properties. System/tubing pressure is excluded.

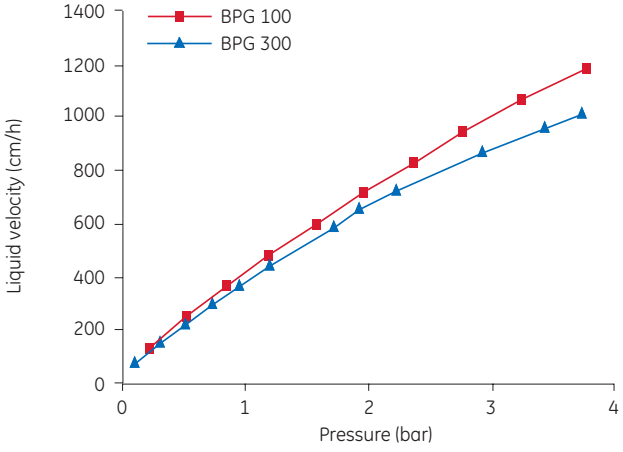


Fig 5. Pressure-flow curve in water at 20°C for Capto DEAE, 20 cm packed bed height in BPG 100 and BPG 300, showing that the medium can be run at 700 cm/h with a back pressure of less than 3 bar. System/tubing pressure is excluded.

Table 4. Column efficiency data for different packs of Capto S, Capto Q, and Capto DEAE in BPG 100 and BPG 300

Medium	BPG column	Bed height (cm)	Average plates/m*	Reduced plates height (h) range*	Asymmetry factor (A_s) range*	Flow velocity for stability test (cm/h)†	Change after stability test (%)†	
							h	A_s
Capto S	100	20	5300	1.6–2.5	1.2–1.4	700	3	6
Capto S	300	20	6000	1.6–1.9	1.1–1.2	700	6	1
Capto S	300	30	6000	1.4–2.4	1.1–1.5	500	3	6
Capto Q	300	20	7000	1.4–1.6	1.1–1.1	700	2	1
Capto DEAE	300	20	6100	1.5–2.0	1.1–1.2	700	5	4

* Test performed at optimal test conditions. Average and ranges of up-flow and down-flow 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.

Table 5. Column efficiency data for different packs of Capto S, Capto Q, and Capto DEAE in Chromaflow columns

Medium	Chromaflow column	Bed height (cm)	Average plates/m*	Reduced plates height (h) range*	Asymmetry factor (A_s) range*	Flow velocity for stability test (cm/h)†	Change after stability test (%)†	
							h	A_s
Capto S	600	30	4600	2.0–2.4	1.0–1.3	500	1	5
Capto Q	600	20	6800	1.4–1.9	1.1–1.4	500	1	9
Capto DEAE	600	20	5600	1.3–2.6	1.0–1.5	500	6	5

* Test performed at optimal test conditions. Average and ranges of up-flow and down-flow 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.

Chromaflow 600 column

The efficiency results for Capto S, Capto Q, and Capto DEAE packed in Chromaflow 600 are shown in Table 5. Both 20 and 30 cm bed heights give good plate numbers and asymmetry factors. In addition, the stability test shows that the bed is stable when running in water at the velocities given in Table 5.

The pressure-flow curve is shown in Figure 6. As the optimal compression factor 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 500 cm/h. Note that bed efficiency and bed stability are very good, provided this 500 cm/h guideline is met.

Comparative data from Chromaflow (Fig 6) and AxiChrom (Fig 3) columns show a slightly higher back pressure at the same velocity for AxiChrom columns, resulting from the higher compression when packing AxiChrom columns compared with when packing Chromaflow columns. This higher compression is needed to utilize the full liquid velocity of Capto IEX media. Still, the back pressure over the column at full flow velocity is far from the maximum operating range of AxiChrom columns (4 bar).

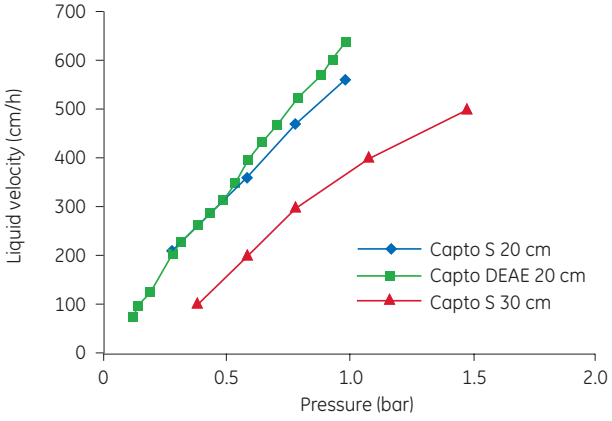


Fig 6. Pressure-flow curve in water at 20°C for Capto S and Capto DEAE, 20 cm packed bed height and Capto S 30 cm packed bed height in Chromaflow 600. As the optimal compression factor is difficult to achieve in standard pack-in-place columns, the maximum flow velocity that can be run through the packed bed is limited. The highest operating velocity recommended for this type of column is 500 cm/h compared with in AxiChrom columns where 700 cm/h could be used for a 20 cm bed.

Efficiency tests at different flow velocities

Efficiency tests were run at different test velocities. Figure 7 shows that the curves follow the van Deemter theory. The asymmetry factor is stable at the different flow velocities. The reduced plate height increases with the velocity and the optimal result is achieved at 10 to 40 cm/h. When running at higher liquid velocities, the asymmetry factor and reduced plate height continue to behave linearly, indicating that the efficiency test can be run at any flow velocity. However, the expectation of the result has to be adjusted based on the test velocity used.

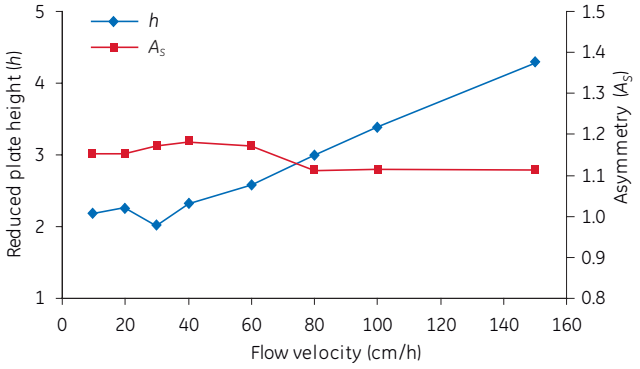


Fig 7. The reduced plate height and asymmetry values at different liquid velocities run on a 20 cm bed of Capto S in a BPG 100 column. This is an example, but similar behavior can be expected for packed beds in other columns such as AxiChrom and Chromaflow columns.

Conclusions

This application note describes packing of Capto S, Capto Q, and Capto DEAE media in AxiChrom columns utilizing the easy-to-use and verified Intelligent Packing wizard. Methods for packing of these media in BPG and Chromaflow columns are also included.

AxiChrom enables full utilization of Capto S, Capto Q, and Capto DEAE flow capacity and offers high flexibility in column diameters and bed heights.

This means processes can be run with higher bed heights if floor space is limited, or at lower bed heights and larger diameters to decrease process time. Capto Q, Capto S, and Capto DEAE media have much in common with previous media such as Q Sepharose Fast Flow and SP Sepharose Fast Flow. However, considering the design of these high-flow agarose Capto base matrices, there was also a need to develop new packing methods for existing columns in order to use the potential of these modern media and achieve competitive packing results. It is important to remember that each packing method is related to a specific packing solution. Changes in packing solution may have a significant impact on the *PF* and subsequently on the packing results. To utilize the full flow potential of Capto IEX media AxiChrom columns are recommended.

Ordering information

Product	Quantity	Code number
Capto Q	25 mL	17-5316-10
Capto Q	100 mL	17-5316-02
Capto Q	1 L	17-5316-03
Capto Q	5 L	17-5316-04
Capto Q	10 L	17-5316-05
Capto Q	60 L	17-5316-60
Capto S	25 mL	17-5441-10
Capto S	100 mL	17-5441-02
Capto S	1 L	17-5441-03
Capto S	5 L	17-5441-04
Capto S	10 L	17-5441-05
Capto S	60 L	17-5441-60
Capto DEAE	25 mL	17-5443-10
Capto DEAE	100 mL	17-5443-02
Capto DEAE	1 L	17-5443-03
Capto DEAE	5 L	17-5443-04
Capto DEAE	10 L	17-5443-05
Capto DEAE	60 L	17-5443-60
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

Related literature

Data files	Code number
Capto S, Capto Q, and Capto DEAE	11-0025-76
AxiChrom columns	28-9290-41
BPG Columns 100, 140, 200, 300 and 450 series	18-1115-23
Chromaflow columns	18-1138-92
Media Wand	28-9231-01

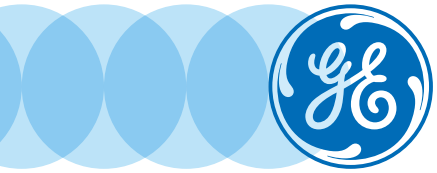
Application notes	
Column efficiency testing	28-9372-07

Instructions for use	
BPG column	18-1170-70
Chromaflow	56-3193-25
Chromaflow Packing Stations	56-3215-58

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

www.gelifesciences.com/bioprocess

GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden



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GE Healthcare UK Limited
Amersham Place
Little Chalfont
Buckinghamshire, HP7 9NA
UK

GE Healthcare Europe, GmbH
Munzinger Strasse 5
D-79111 Freiburg
Germany

GE Healthcare Bio-Sciences Corp.
800 Centennial Avenue, P.O. Box 1327
Piscataway, NJ 08855-1327
USA

GE Healthcare Japan Corporation
Sanken Bldg., 3-25-1, Hyakunincho
Shinjuku-ku, Tokyo 169-0073
Japan

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