Column Packing of Cellufine Sulfate

Cellufine Sulfate is an affinity chromatography resin designed for the concentration, purification and depyrogenation of virus, viral coat, virus like particles (VLP) and microbial antigens and specific proteins. This resin is based on spherical non-cross-linked cellulose beads functionalized with a low surface density of sulfate esters. This confers on the gel unique chromatographic selectivity that, in some cases, is similar to immobilized heparin. This resin shows good flow properties and has been packed in column sizes up to 2-meter diameter.

Flow Packing Procedure (for columns up to 45cm in diameter) with flow adapters

1) ***For column volumes < 1 l***; transfer sufficient slurry for the target column volume (CV) into a filter funnel (glass fitted) and wash with at least 5 volumes of water for a total of 3 x to remove the storage solution. If necessary, repeat with packing buffer if different from water.

2**)** ***For column volumes > 1 L;*** decant the storage buffer from above the settled resin in the shipping container and replace with water. Then re-suspend the resin and allow to settle again to wash away the storage buffer. Repeat 2-3x or consider packing in the storage buffer and washing the column on-line.

3) After final wash add sufficient packing buffer to suspend the washed resin into a 50-60% slurry.

4) Transfer some of the slurry into a 50 mL measuring cylinder and allow to settle overnight or a minimum of 4h. Measure bed height of a gravity settled bed and calculate the slurry% from;

% = Gravity settled bed height/Total slurry volume

5) Adjust to a 50 % slurry concentration of resin.

6) Calculate the volume of slurry required to pack the column using the following equation;

Volume 50% slurry = (Target CV x 2) x (Cf)

Cf is the resin compression factor derived from:

Cf = gravity settled /flow packed bed heights

For example, for a 100 ml CV you will need (100 x 2) x 1.15 = 230 mL, for a resin compression factor of 1.15.

7) Assemble the column hardware with the bottom flow adapter in place. Prime the bottom frit assembly to remove air with packing buffer from a syringe or pump for a large diameter column. Leave about 1 cm in the bottom of the column.

8) If necessary add a bed height adapter to the top of the column to accommodate the full volume of the slurry. Note: the full volume of slurry will be poured into the column in one step to ensure a uniform packed bed.

9) Close the bottom outlet of the column.

10) Pour the volume of slurry into column in one operation and avoid trapping air in the resin slurry.

11) Open a bottom outlet and allow the bed to start to settle until 2-3 cm of clear liquid is seen above the resin bed.

12) Stop the outlet flow and carefully fill the column with packing buffer up to the top without disturbing the settling resin bed.

13) Prime the upper flow adapter as described in step 6 above.

14) Assemble the top flow adapter on to the column minimizing any trapped air bubbles in the

head of the column.

15) Initiate flow with the packing buffer at 200 cm/h for 30 to 60 min flow pack the resin bed. Note: the column back pressure* should be in the range *0.15 – 0.25 MPa* at this flow rate. This is a higher flow rate than normal operation of the column to ensure a stable bed packing.

* This is the pressure drop across the column when the column is filled with resin. Allowance should be made for the system back pressure where an empty buffer filled column of the same size is placed in-line. Backpressure is best measured with a gauge on the inlet side of the column.

This is a higher flow rate than normal operation of the column to ensure a stable bed packing.

16) After the bed height has stabilized, close the outlet and open up flow from the top of the column (DO not remove the flow adapter) and slowly move the top flow adapter down displacing packing buffer from the top of the column. Bring the top adapter down to contact the settle resin bed.

17) Reconnect the upper flow adapter, open the outlet and re-start flow at 200 cm/h. If the bed settles and shrinks away from the top adapter, adjust the top adapter down to accommodate the new bed height.

18) At the final bed height, calculate the column volume. If the bed volume is higher than expected, axial compression can be applied by lowering the top adapter. The final column volume should be close to the target. If the volume is lower than expected, the original volume of slurry may have been lower or the resin may have packed down more on flow since its compression factor may have been higher than 1.15

19) Check and evaluate the status of packing by measuring HETP and peak symmetry(As) by injection of a small volume (1% of column volume) of an un-retained material (1-2% acetone or 2 M NaCL) at a 150 cm/h flow rate. Calculate As, N (# of theoretical plates / M column length) from the resulting peak monitoring at 280 nM or by conductivity for NaCl.

Example of flow packing a 3.2 cm I.D column

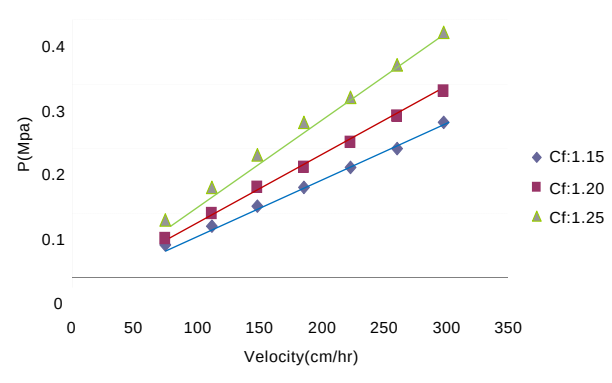
- Column: 3.2 cm ID x 25 cm L (EMD Millipore Vantage)
- Packing buffer: Pure water (25℃)
- Packing condition: 50 % slurry and flow packed at 40 ml/min (298 cm/h)
- Axial Compression; Manual-packing till bed height of 20 cm (target bed volume = 160 mL) was achieved.

The packing and pressure/flow properties of Cellufine sulfate in a 3.2 cm diameter column with different compression factors are summarized in Table 1 and Figure 1

Table 1, Summary of peak properties at a range of resin compression factors

Cf	N(m^{-1})	A_s
1.15	5900	1.13
1.20	6400	0.96
1.25	5600	1.04

Figure 1, Pressure/Flow in a 3.2 cm ID column



In this column format Cellufine sulfate at a range of

packing compression factors from 1.015 to 1.025 showed good flow and peak performance up to 250 cm/h while keep under 0.3 MPa backpressure.

Example of flow packing a 30 cm I.D column

- Column: 30 cm ID x 22.9 cm L (BPG column)
- Packing Process;
  1. Prime bottom outlet with packing buffer to remove trapped air bubbles. Leave 5 cm of buffer in the column.
  2. Pour the re-suspended resin slurry into the column and then open the bottom outlet.
  3. After gravity settling (22.9 cm bed height), run flow with pure water for 30 min over a pressure range from or 0.15 to 0.25 MPa.
  4. Finally, axially compressed the resin with the top flow adapter for each compression factor (Cf) listed in Table 2
- Evaluation of column packing:
  1. Equilibrate the column at 75 cm/hr (0.83 L /min) for 1CV.
  2. Inject 16 mL (1 % of CV) of 2 % acetone (Mobile Phase: pure water) at a flow rate of 30 cm/h (0.35 L/min),
  3. Elute at the same flow rate for 1.3 CV.

The results of this study are summarized in Table 2 below.

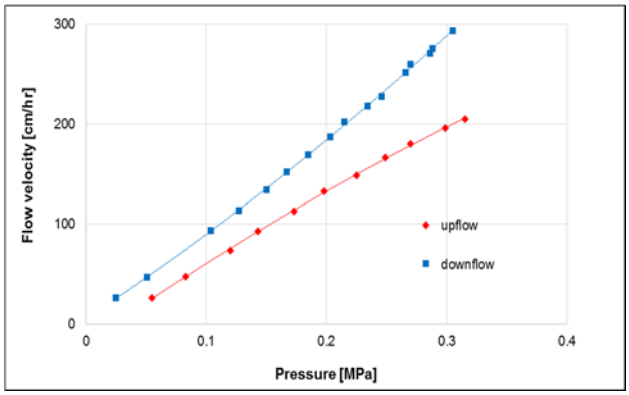
Table 2, Packing properties for a 30 cm ID column

Pressure (MPa)	Cf	N(m^{-1})	A_s	RPH*	
0.25	1.20	3200	1.15	4.47	
0.20	1.20	3600	1.29	3.93	
0.15	1.20	4000	1.16	3.57	
0.15	1.18	4300	1.15	3.31	
0.15	1.15	2100	1.07	6.91	

* Reduction of theoretical plate height

This data suggests that an optimal compression factor for packing Cellufine Sulfate in a 30 cm ID column was 1.18 to 1.20. In Figure 2 below, a pressure- flow velocity curve of Cellufine Sulfate packed in a 30 cm I.D. column is shown with flow in both directions.

Figure 2, Pressure/Flow properties for a flow packed 30 cm ID column



Column was packed from a 50 % slurry in water at 0.15 MPa for 30 min to a final bed height of 19.1 cm with a compression factor (Cf) of 1.20. The above data shows that a 30 cm ID column can be packed with Cellufine sulfate (particle size: 70 μm) at an optimal compression factor of 1.02 and operated at 275 cm/h (3.23 L/min flow rate) at under 0.3MPa back pressure in down flow configuration. Slightly higher back pressure is seen with up flow as you might expect for a column initially packed in a down flow direction.

Example of flow packing a 45 cm I.D. column Experimental Condition's

- Column: 45 cm ID x 23.0 cm settled bed height (column of 36.6 L volume)
- Packing mode: Gravity settled from a 50 % slurry
- Process;
  1. Prime bottom outlet with packing buffer to remove trapped air bubbles.

2. Pour the re-suspended resin slurry into the column and then open the bottom outlet.
3. After gravity settling (23 cm bed height), run flow with pure water for 30 min over a pressure range from or 0.10 to 0.20 MPa.
4. Finally, axially compress the resin with the top flow adapter for each compression factor (Cf) in Table 3

➢ Column packing evaluation:

1. Equilibrate the column at 75 cm/hr (1.9 L/min) for 1CV.
2. Inject 37 mL (1 % of CV) of 2 % acetone with a mobile phase of pure water at a flow rate of 30 cm/h (1.03 L/min),
3. Continue elution at the same flow rate for 1.3 CV.

The results of this study are summarized in Table 3 and an overlay of the column qualification chromatograms is shown in Figure 3.

Table 3, Packing properties for a 45 cm ID column

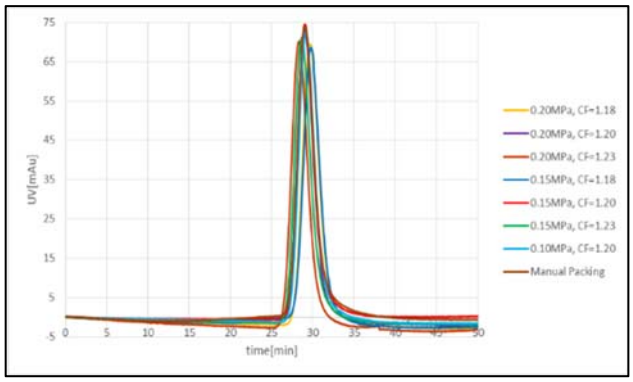
Pressure (MPa)	Cf	N [m^{-1}]	As	RPH*
0.20	1.18	4900	1.19	2.91
0.20	1.20	5000	1.18	2.85
0.20	1.23	5000	1.21	2.88
0.15	1.18	5200	1.16	2.75
0.15	1.20	5300	1.25	2.67
0.15	1.23	5100	1.30	2.82
0.10	1.20	5300	1.25	2.69
Manual*	1.20	5000	1.24	2.88

* Manual packing with no axial compression.

** Reduction of theoretical plate height

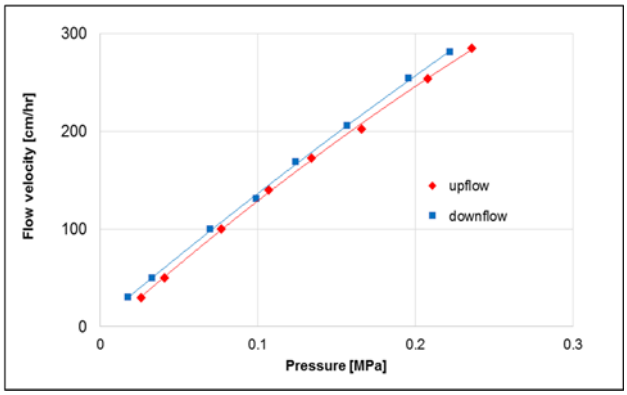
Optimal packing conditions for this 45 cm ID column are flow packing to 0.15 MPa pressure with a compression factor of 1.02 giving the highest # of theoretical plates

Figure 3,
Overlay of Column efficiency curves for data summarized in Table 3



In Figure 4 below, a pressure- flow velocity curve of Cellufine Sulfate packed in a 45 cm I.D. column is shown with flow in both directions.

Figure 4, Pressure/Flow properties for a flow packed 30 cm ID x 19.1 cm bed height column



Column was packed from a 50 % slurry in water at 0.15 MPa for 30 min to a final bed height of 19.2 cm with a compression factor (Cf) of 1.20. The above data shows that a 45 cm ID column can be packed with Cellufine sulfate (particle size: 70 µm) at an optimal compression factor of 1.02 and operated at flow rates up 300 cm/h (7.9 L/min flow rate) under 0.3MPa back pressure in both flow directions.

Conclusion

This Technical Note describes an optimal Methodology to successfully flow pack Cellufine sulfate affinity resin into columns up to 45 cm in diameter with 20 cm bed heights. The resulting columns showed good peak symmetry with As in the range 0.96 to 1.29 applying resin compression factors Cf in the range 1.15 to 1.25 to axially compress the packed bed. Pressure/flow curves showed that flow rates at 300 cm/h in Cellufine sulfate resin columns up to 45 cm in diameter gave back pressures < 0.3 MPa. In all examples described in this Technical Note, water was used as the packing solution simplifying the process of pouring highly efficient chromatography beds. The new design of this cellulose based Cellufine spherical bead offers superior mechanical stability and can be easily flow packed into small to large diameter Vantage or BPG bio-production columns. The packing process described in this Technical Note is scalable and shows that Cellufine family of resins are amenable to manual flow packing as well as axial compression in hardware with moveable flow adaptors.

Ordering Information

Description	Quantity	Catalogue No.
Cellufine Sulfate	5 x 1 mL (mini-column)	19845-51
	1 x 5 mL (mini-column)	19845-15
	10 mL (sample)	676 943 324
	50 mL	19845
	500 mL	19846
	5 L	19847
	10 L	19849

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