Column Packing of Cellufine MAX HIC

Cellufine MAX HIC is a new generation of Cellufine resin, in which a new patented cross-linking technique has been applied to spherical cellulose particles. This process confers rigidity on the bead structure allowing operation at high linear velocity flow rates as required for production scale applications. JNC offers Cellufine MAX Butyl and Cellufine MAX Phenyl / Phenyl LS (low substitute) chemistries. This HIC family can be utilized for a range of hydrophobic interaction applications, such as antibody polishing post Protein A capture and removal of protein aggregates and is based on a highly stable cellulose bead structure amenable to use in small scale, pilot and full production scale protein purification workflows.

Flow packing procedure (for columns up to 30cm 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)
   Where Cf is the resin compression factor:
   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 *0.25 – 0.30 MPa* at this flow rate.

* 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 ramping up from 30 to 800 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 (2% acetone or 2 M NaCl) at a 30 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. Column packing is acceptable in the ranges 0.8 to 1.4 for As and 2-3 for RPH.

20) Packed resin beds can be flow conditioned post column packing. For example, run in up flow at a pressure of 0.25 – 0.3 MPa for 30 – 60 min and then return to down flow at a pressure of 0.25- 0.3 MPa for 30 – 60 min. This process can lead to a more uniform resin packing or may be applied during column cleaning or base CIP sanitization to remove any material that may be accumulating on the head of the column. This is optional on a new column, but recommended for a packed column after 5-10 cycles of re-use.

Example of flow packing a 10 cm ID column with Cellufine MAX HIC-Butyl chemistry

- Column: 10 cm ID x 50 cm L
- Packing Bed Height: 23 cm
- Packing condition: 50 % slurry flow packed at up to 0.3 MPa back pressure* till the bed height stabilizes.
- Evaluate resin compression factors Cf of

1.15, 1.18,1.20,1.22 and 1.25in

- Mobile phase for column qualification: 0.5M NaCL
- Injection: 1 M NaCl (1% of CV)

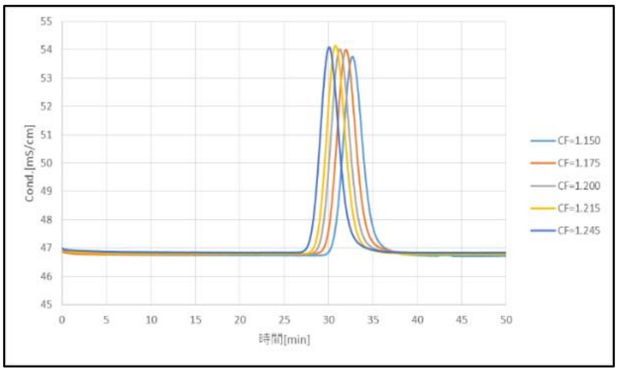
The impact of pressure on column packing performance was measured and is summarized in Table 1. Overlays of the elution peaks are shown in Figure 1, below.

Table 1, Column packing efficiency at a range of pressures with Cellufine MAX-Butyl

Cf	N [m^{-1}]	As	RPH
1.150	5100	1.28	2.17
1.175	5200	1.22	2.15
1.200	5100	1.23	2.17
1.215	5100	1.24	2.17
1.245	5000	1.25	2.21

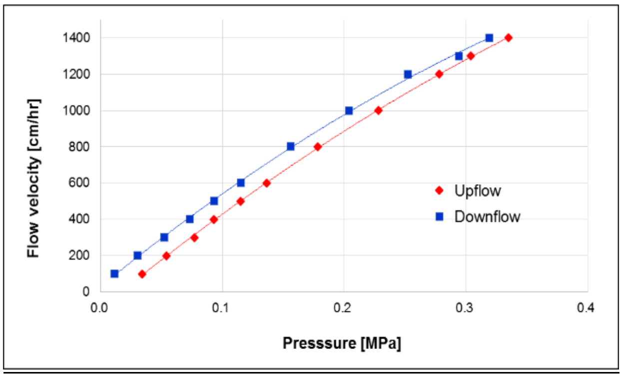
Particle size: 90 μm

Figure 1, Overlay of elution peaks of column



In Figure 2, below the pressure/flow properties are shown for up and down flow of a packed bed of Cellufine MAX HIC-Butyl

Figure 2, Pressure/Flow curves for Cellufine MAX HIC-Butyl flow packed in a 10 cm ID column*



* 19.8 cm bed height

Cellufine MAX HIC-Butyl can be packed in a 10 cm ID column, bed height 23 cm to yield elution peaks of As in the range 1.22 to 1.28. This packed bed can be flowed at up to 1200 cm/h in both directions without exceeding the 0.3 MPa pressure limit.

Example of flow packing a 10 cm ID column with Cellufine MAX HIC-Phenyl and Phenyl LS (low substitution) chemistries

- Column: 10 cm ID x 50 cm L
- Packing Bed Height: 19.8 and 21.9 cm
- Packing condition: 50 % slurry flow packed at 0.20, 0.25 and 0.30 MPa back pressure* for 30 min till the bed height stabilizes.
- Final resin compression factors Cf of 1.10
- Mobile phase for column qualification: 0.5M NaCL
- Injection: 1 M NaCl (1% of CV)

The impact of pressure on column packing performance was measured and is summarized in Table 2 below

Table 2, Column packing efficiency at a range of pressures with Cellufine MAX-Phenyl

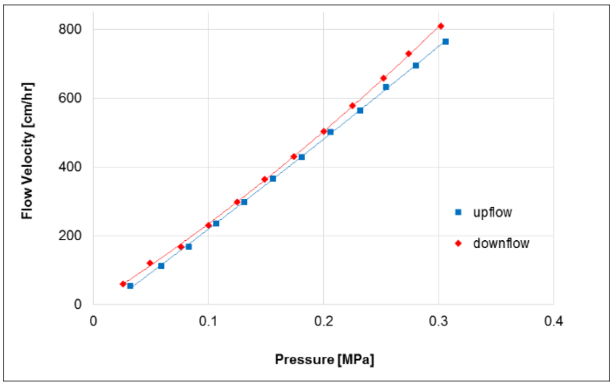
Pressure [MPa]	N [m^{-1}]	As	RPH
0.30	4200	1.25	2.65
0.25	4200	1.31	2.62
0.20	4100	1.35	2.72

Particle size: 90 μm

Figure 3, below showed pressure/flow curves of Cellufine MAX Phenyl (Panel A) and MAX Phenyl LS (Panel B) in 10 cm I.D. column packed at 0.3 MPa for 30 min with a final resin compression. Cf of 1.10.

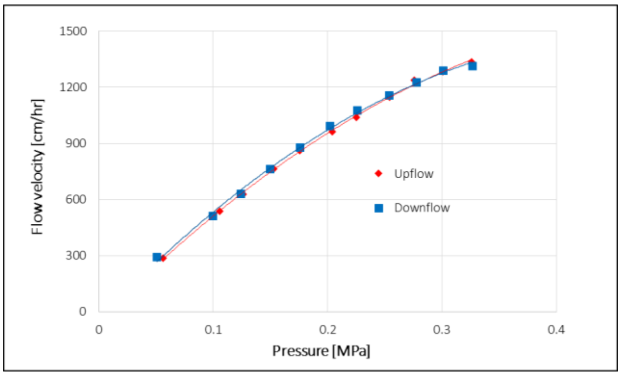
Figure 3, Pressure/Flow curves for Cellufine MAX HIC flow packed in a 10 cm ID column

Panel A, Column** packed with Cellufine MAX-Phenyl



** Bed height 19.8 cm (Cf 1.10)

Panel B, Column*** packed with Cellufine MAX Phenyl (LS)



*** Bed height: 21.9 cm (Cf: 1.05)

Cellufine MAX HIC-Phenyl and can be packed in a 10 cm ID column, bed height 19.8 cm to yield elution peaks of As in the range 1.25 to 1.35 over a range of packing pressure up to 0.3 MPa. These packed beds can be flowed at up to 800 (Phenyl) or 1200 cm/h (Phenyl LS) in both directions without exceeding the 0.3 MPa pressure limit.

Conclusion

This Technical Note describes an optimal Methodology to successfully flow pack Cellufine MAX HIC resin into columns up to 10 cm in diameter with 20 cm bed heights. The resulting columns showed slightly tailing peak asymmetry with As in the range 1.22 to 1.35 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 600 cm/h in MAX Phenyl and 1200 cm/h in MAX Phenyl (LS) columns up to 10 cm in diameter giving back pressures < 0.3 MPa at these flow rates. 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 MAX Butyl	1 mL x 5 (mini-columns)	21100-51
	5 mL x 1 (mini-columns)	21100-55
	100 mL	21100
	500 mL	21101
	5 L	21102
	10 L	21103
Cellufine MAX Phenyl	1 mL x 5 (mini-columns)	20700-51
	5 mL x 1 (mini-columns)	20700-55
	100 mL	20700
	500 mL	20701
	5 L	20702
	10 L	20703
Cellufine MAX Phenyl LS (low substitution)	1 mL x 5 (mini-columns)	20800-51
	5 mL x 1 (mini-columns)	20800-55
	100 mL	20800
	500 mL	20801
	5 L	20802
	10 L	20803

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