Column Packing of Cellufine MAX IEX

Cellufine MAX IEX 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 a range of Cellufine MAX IEX beads with a range of chemistries such as Cellufine MAX S-h, Q-h, DEAE and CM based on surface modifications with dextran polymers or Cellufine MAX GS where the chemistry is via graft homo-polymer modification. This IEX family can be utilized for a range of IEX applications, such as antibody polishing post Protein A capture and removal of dsDNA on a rigid and 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%

% = Gravity settled bed height/Total slurry volume

5) Adjust to a 50 % slurry concentration.

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 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. 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.

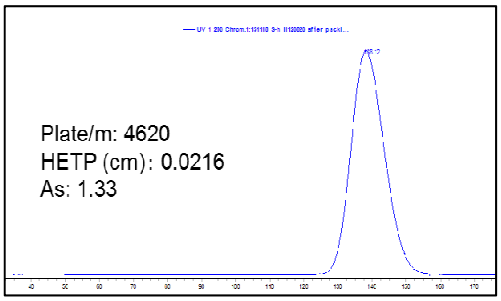
Example of flow packing and conditioning a 3.2 cm ID column with Cellufine MAX S-h

- Column: EMD Millipore Vantage Column ID 3.2 cm
- Packing Bed Height: 19.8 cm
- Packing buffer: Pure water
- Packing condition: 50% slurry flow packed at 0.25 to 0.30 MPa back pressure
- Column conditioning: Run up-flow for 30 min and then down flow for 30 min at 0.3 MPa back pressure
- Compression factor: 1.20

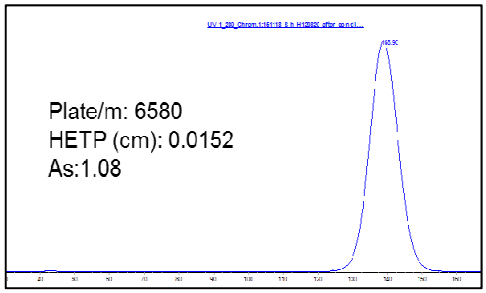
The impact of post packing column flow conditioning was measured and the resulting elution peaks are shown in Figure 1, below.

Figure 1, Impact of post packing flow conditioning

Panel A; before column conditioning



Panel B: after column conditioning



Post packing flow conditioning the 3.2 cm ID column did lead to a significant improvement in the number of theoretical plates/m and a very acceptable peak As of 1.08.

Example of flow packing a 30 cm ID column with Cellufine MAX S-h

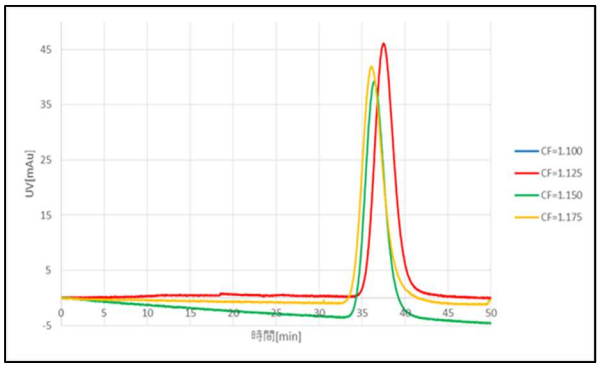
- Column: 30 cm ID (BPG 300)
- Bed height: 20 cm (Cf = 1.15)
- Packing buffer: Pure water (25℃)
- Packing condition: 50% slurry flow packed at 0.25 to 0.30 MPa back pressure*
- Injection: 2 % Acetone (30 cm/hr)

Table 1 and Figure 2 below, summarize column efficacy data, such as number of theoretical plate (N) and asymmetry (As) in 30 cm I.D. column when packed under various compression factors (Cf).

Table 1, Column efficiency at various resin* compression factors (As)

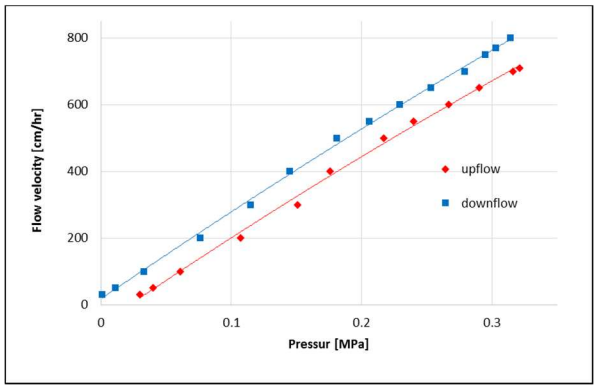
Cf	N [m^{-1}]	As	RPH
1.10	6,000	1.15	1.85
1.13	6,000	1.17	1.87
1.15	6,200	1.14	1.79
1.18	6,000	1.13	1.87

Figure 2, Overlay of column efficiency curves of data from Table 1



In Figure 3, below the pressure/flow properties are shown for up and down flow of a packed bed of Cellufine MAX S-h

Figure 3, Pressure/Flow curves for Cellufine MAX S-h flow packed resin



Example of flow packing a 30 cm ID column with Cellufine MAX S-r

- Column: 30 cm ID (BPG 300)
- Bed height: 19.3 cm (Cf = 1.13)
- Packing buffer: Pure water (25℃)
- Packing condition: 50% slurry flow packed at 0.25 to 0.30 MPa back pressure
- Injection: 2 % Acetone (30 cm/hr)

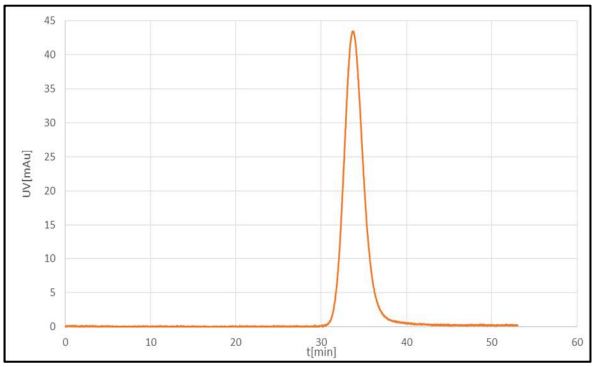
Figure 4 below, summarizes column efficacy data, such as number of theoretical plate (N) and asymmetry (As) in 30 cm I.D. column when packed with a compression factors (Cf) of 1.13.

Figure 4, Column efficiency data

Panel A, Data summary

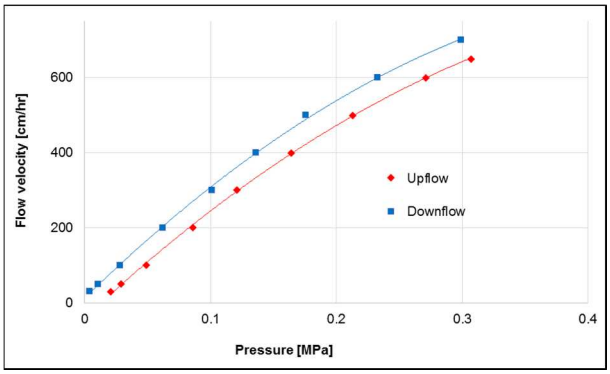
N [m^{-1}]	As	RPH
5700	1.13	1.95

Panel B, Elution curve data



In Figure 5 below, the pressure/flow properties are shown for up and down flow directions of a packed bed of Cellufine MAX S-r are summarized.

Figure 5, Pressure/Flow curves for Cellufine MAX S-r in a flow packed 30 cm ID column



Example of flow packing a 30 cm ID column with Cellufine MAX Q-h

- Column: 30 cm ID (BPG 300)
- Bed height: 20.4 cm (Cf = 1.13)
- Packing buffer: Pure water (25℃)
- Packing condition: 50% slurry flow packed at 0.25 to 0.30 MPa back pressure*
- Injection: 2 % Acetone (30 cm/hr)

Table 2, below, summarizes column efficacy data, for a 30 cm I.D. column when packed with a compression factors (Cf) of 1.13.

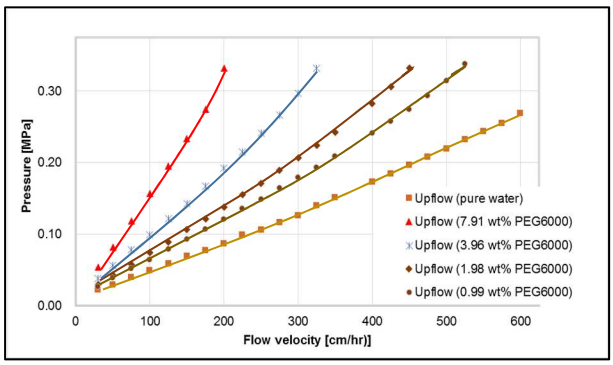
Table 2, Column packing efficiency data

N [m^{-1}]	As	RPH
5200	1.07	2.14

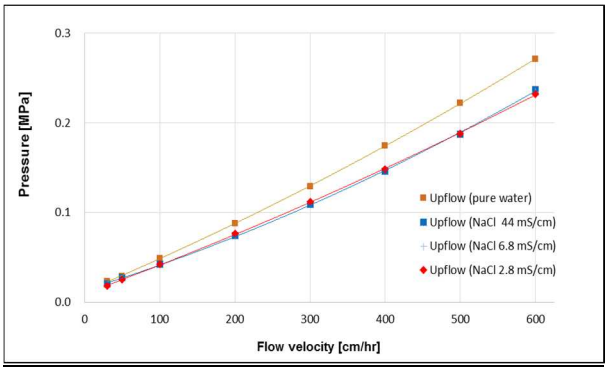
In Figure 6, below are a series of pressure-flow velocity curves of Cellufine MAX Q-h with various viscosity solutions (Panel A, PEG6000 and Panel B NaCl) in 30 cm I.D. flow packed column qualified as reported in Table 2.

Figure 6, Impact of sample viscosity on Pressure/Flow curves for a 30 cm ID column

Panel A, PEG6000 added in range 0.99 to 7.91%



Panel B, NaCl in conductivity range 2.8 to 44 mS/cm



The above data shows that a 30 cm ID column of Cellufine MAX Q-h can be successfully flow packed with a compression factor of 1.13 to yield a qualification peak with an As of 1.07. Flow rates in the presence of salt up to a conductivity of 44 mS/cm (~ 0.5M NaCl) showed back pressure < 0.3 MPa at up 600 cm/h. As expected, increasing buffer viscosity by adding PEG 6000 led to an increase in the slope of the pressure/flow curve. At the highest viscosity tested it was still possible to operate the column at < 0.3 MPa backpressure at a flow rate of 175 cm/h.

Example of flow packing a 30 cm ID column with Cellufine MAX Q-r

- Column: 30 cm ID (BPG 300)
- Bed height: 20 cm (Cf = 1.15)
- Packing buffer: Pure water (25℃)
- Packing condition: 50 % slurry flow packed at 0.25, 0.30 or 0.35 MPa for 60 min. Then axially compress to a Cf of 1.15
- Injection: 2% acetone (30 cm/hr)

Figure 7 and Table 3 below, summarizes column efficacy data, such as number of theoretical plate (N) and asymmetry (As) in 30 cm I.D. column when packed with a compression factors (Cf) of 1.13.

➢ Figure 7, Elution peak data

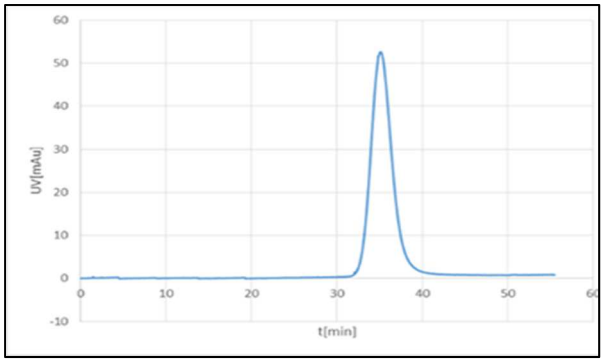
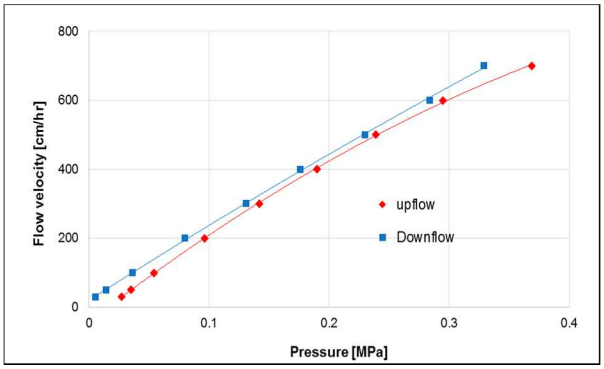


Table 3, Column packing efficiency data

Packing Pressure[MPa]	Cf	N [m^{-1}]	As	RPH
0.25	1.12	4,000	1.09	2.76
0.30	1.14	4,900	1.12	2.26
0.35	1.14	4,100	1.14	2.73

In Figure 8, below are a series of pressure-flow velocity curves of Cellufine MAX Q-r in a 30 cm I.D. column packed at 0.35 MPa for 60 min with a final a compression factor Cf of 1.15

Figure 8, Pressure/Flow curves for Cellufine MAX Q-r in a 30 cm ID flow packed column



Example of flow packing a 30 cm ID column with Cellufine MAX DEAE

- Column: 30 cm ID (BPG 300)
- Bed height: 20 cm (Cf = 1.15)
- Packing buffer: Pure water (25℃)
- Packing condition: 50 % slurry flow packed at 0.15 MPa for 30 min. Then axially compressed to a Cf of 1.10,1.13, 1.15 and 1.18
- Injection: 2% acetone (30 cm/hr)

Figure 9 and Table 4 below, summarizes column efficacy data, in a 30 cm I.D. column when packed with compression factors (Cf) of 1.10, 1,13, 1,15 and 1.18.

Figure 9, Elution peak data over a range of packing pressures

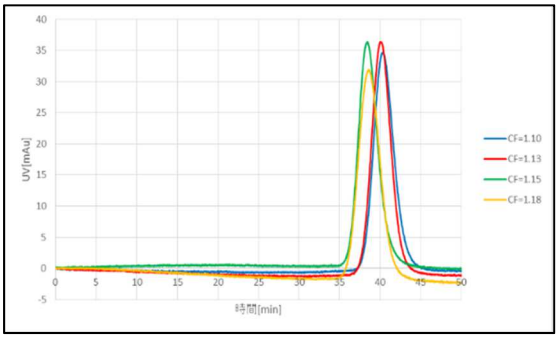


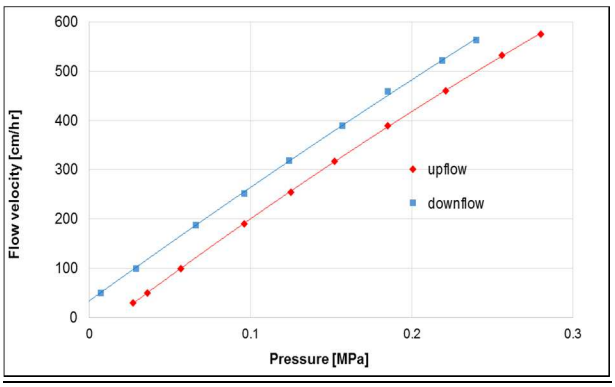
Table 4, Column packing efficiency data over a range of packing pressures

Packing Pressure [MPa]	Time [min]	Cf	N [m^{-1}]	As	RPH
0.15	30	1.10	5566.2	1.15	2.00
0.15	30	1.13	6135.8	1.09	1.81
0.15	30	1.15	5890.2	1.18	1.89
0.15	30	1.18	4727.2	1.08	2.35

Particle size; 90 μ m

In Figure 10, below are a series of pressure-flow velocity curves of Cellufine MAX DEAE in a 30 cm I.D. column packed at 0.35 MPa for 60 min with a final a compression factor Cf of 1.15

Figure 10, Pressure/Flow curves for Cellufine MAX DEAE in a 30 cm ID flow packed column



The above data shows that a 30 cm ID column of Cellufine MAX DEAE can be successfully flow packed with a compression factor in the range of 1.10 to 1.18 to yield a qualification peaks with a narrow As range of 1.09 to 1.18.

Conclusion

This Technical Note describes an optimal Methodology to successfully flow pack Cellufine MAX IEX resins into columns up to 30 cm in diameter with 20 cm bed heights. The resulting columns showed good peak symmetry with As in the range 1.03 to 1.18 applying resin compression factors Cf in the range 1.09 to 1.15 to axially compress the packed bed. Pressure/flow curves showed that flow rates at 600 cm/h in MAX IEX resin columns up to 30 cm in diameter gave back pressures < 0.3 MPa at this 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.	Description	Quantity	Catalogue No.
Cellufine MAX S-r	1 mL x 5*	20300-51	Cellufine MAX Q-r	1 mL x 5*	20500-51
	5 mL x 1*	20300-55		5 mL x 1*	20500-66
	100 mL	20300		100 mL	20500
	500 mL	20301		500 mL	20501
	5 L	20302		5 L	20502
	10 L	20303		10 L	20503
Cellufine MAX S-h	1 mL x 5*	20400-51	Cellufine MAX Q-h	1 mL x 5*	20600-51
	5 mL x 1*	20400-55		5 mL x 1*	20600-55
	100 mL	20400		100 mL	20600
	500 mL	20401		500 mL	20601
	5 L	20402		5 L	20602
	10 L	20403		10 L	20603
Cellufine MAX CM	1 mL x 5*	20900-51	Cellufine MAX DEAE	1 mL x 5*	21000-51
	5 mL x 1*	20900-55		5 mL x 1*	21000-55
	100 mL	20900		100 mL	21000
	500 mL	20901		500 mL	21001
	5 L	20902		5 L	21002
	10 L	20903		10 L	21003

*** Pre-packed mini-columns**

Purchase/Technical Support

(North America & Europe)
JNC America, Inc.
555 Theodore Fremd Ave,
Rye, NY 10580
Tel: 914-921-5400
Fax: 914-921-8822
Email: cellufine@jncamericany.com

(Asia & Others)
JNC Corporation
Chemical Division Life Chemicals Department
2-1, Otemachi 2-Chome, Chiyoda-ku
Tokyo 100-8105 Japan
Tel: +81 3 3243 6150
Fax: +81 3 3243 6219