Packing Procedure for Fractogel® EMD Resins in 5-20 cm i.d. Laboratory Columns

Calculation of percent compression and resin required

To achieve a stable packed bed, 25 to 30% compression is recommended for Fractogel® EMD (M) resins, whereas a minimum of 27% compression is necessary for Fractogel® EMD (S) resins. „Compression“ refers to the actual volume reduction of packed resin compared to the volume of the gravity settled resin prior to packing. Compression is achieved by removing part of the void volume from the interstitial space between resin beads by mechanical force. Deformation of the resin beads does not occur.

Given the following packing parameters:

- column inner diameter: i.d. = 20 cm
- desired packed gel bed height: h = 20 cm
- desired percent compression: 25
- percent compression is defined as (SBV - PBV)/SBV × 100, where SBV is the settled bed volume and PBV the packed bed volume

- Calculate the packed bed volume according to:

$$PBV = \pi \times (i.d./2)^2 \times h = 3.14 \times (20\ cm/2)^2 \times 20\ cm = 6283\ ml \approx 6.3\ L$$

- To calculate the amount of settled bed volume required at a given compression from the packed bed volume:

$$SBV = 100 \times PBV/(100 - \%\ compression)$$ $SBV = 100 \times 6.3/(100 – 25) = 8.4$ L

- Calculate the amount of original 70% gel suspension needed according to:

$$Original\ bulk\ suspension = SBV/0.70 = 8.4\ L\ /0.70 = 12\ L$$

The following table summarizes these relationships for various percent compressions:

Percent compression	PBV (L)	SBV (L)	Bulk suspension needed	Compression factor
20	6.3	7.9	11.3	1.25
25	6.3	8.4	12.0	1.33
27	6.3	8.6	12.3	1.37
30	6.3	9.0	12.8	1.43
35	6.3	9.7	13.8	1.54

where compression factor = SBV/PBV.
Compression factor = -1/[(percent compression/100)-1]

1. Column preparation

- Flush the empty column with sufficient deionized/distilled water prior to use to ensure that the column and the column frits are clean.
- Make sure the column is level.

2. Gel slurry preparation

Fractogel® EMD resins are usually supplied as 70% resin suspensions in 20% aqueous ethanol, containing 150 mM NaCl, pH 7.0. Prior to packing, the storage solution must be removed. Packing in the presence of ethanol will lead to increased column back pressure.

Ethanol present in the storage medium can be removed by washing the resin bed with 5-10 column volumes of deionized/distilled water

- Decant the storage solution (20% EtOH/150 mM NaCl) from the resin. Resuspend the resin in the bottle with water and stir until a homogeneous solution is achieved.
- Pour into the column or buchner funnel.
- Rinse down the walls of the column with deionized/distilled water, so that gel particles will not be caught between the top adjuster o-ring and the column wall.
- Attach the top adjuster, tighten the o-ring, lower the top adjuster (with the column inlet open) until it is below the surface of the liquid, allowing liquid to fill all of the tubing.
- Pump 5-10 column volumes of deionized/distilled water through the resin bed at a reasonable flow rate. Check the eluent to make sure there is no odor of ethanol. To remove excess water from the column, lower the plunger to the top of the settled resin bed.

- Draw an equal volume of 300 mM NaCl or 0.02 M NaOH into the column from the bottom (this makes reslurrying the resin easier). Reslurry the resin and let settle overnight
- If a buchner funnel is used, resuspend the resin with an equal volume of water and aspirate until dry. Repeat at least 5 times, then resuspend in an equal volume of 300 mM NaCl or 0.02 M NaOH, pour the suspension into the column, reslurry, and let settle overnight.
- It is better to prepare more slurry than needed.

3. Column packing

- Measure and record the settled bed height. Divide the SBH by 1.33 to determine the PBH. If necessary, remove or add resin to achieve the desired PBH.
- Reslurry the resin bed by stirring to achieve a homogeneous suspension. The conductivity of the supernatant prior to reslurrying should be between 15-20 mS/cm for NaCl, and 8-12 mS/cm for NaOH.
- Rinse down the walls of the column with the packing solution (150 mM NaCl or 0.01 N NaOH), so that gel particles will not be caught between the top adjuster o-ring and the column wall.
- Secure the column top, tighten the o-ring to obtain a seal, and lower the top adjuster to the surface of the liquid slurry, allowing excess liquid to escape through the top adjuster.
- Make sure the line is full of liquid before switching the column inlet to the pump.
- Open the column outlet and pack the column with 150 mM NaCl or 0.01 N NaOH at a starting flow rate of greater than 300 cm/hr (~1600 mL/min for a 20 cm id column) until the resin bed height no longer decreases, or 30 psi (= 2 bar) is obtained. To stay within the column back pressure limit, the flow rate may have to be decreased as the resin packs.
- Turn off the pump and close the column outlet simultaneously and switch the column inlet to waste. Lower the top adjuster, removing the liquid on top of the resin bed via the column inlet or outlet. Bring the adjuster to the desired packed bed height.
- Once the pump is turned off after flow packing the resin bed will rebound. Depending upon column design and how long it takes to lower the top adjuster, it may be necessary to lower the adjuster in stages to compensate for resin rebound. For example: flow pack until a stable bed is achieved, lower the adjuster to the resin bed. Repeat the previous steps until the difference between the desired bed height and the rebound bed height is < 1 cm, then lower the top adjuster to the desired bed height, even if it is below the resin bed.

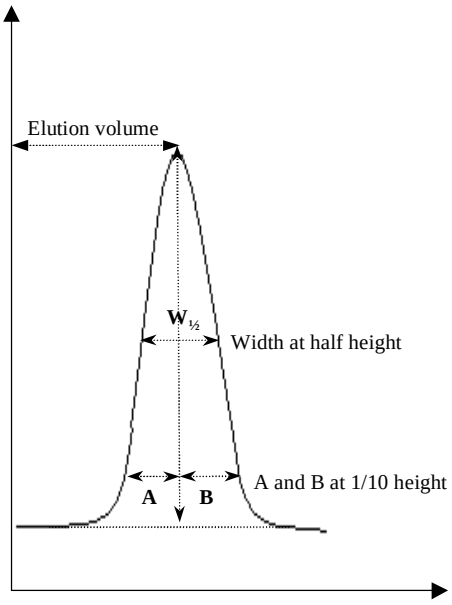
- Switch the column inlet to the pump and „condition“ the column at ≥300 cm/hr without exceeding the column back pressure limit for at least 15 min. in upflow mode followed by 15 min in downflow mode.
- Close the column outlet and stop the flow. Check the quality of the packed bed prior to running a pressure vs. flow rate (PvF) curve. A PvF curve will determine the maximum flow rate and back pressure the packed column will support before gap formation between the resin bed and top plunger occurs.

4. Quality of the packed bed

- The performance and some basic characteristics of the packed column can be checked by running the test chromatogram specified as follows:
- Run the column at a flow rate of ~100 cm/h or at the elution flow rate and inject 1-2% of a 1% acetone in water solution. Record the eluant absorption at 254 nm. Alternatively 1 M NaCl or water can be used to determine the HETP value using a conductivity meter as the detector.
- The elution volume of acetone is used to calculate the number of theoretical plates per meter of bed height (N_m), total plates (N), and asymmetry (A_s) according to:

$$N_m = 5.54\left(\frac{V_e}{W_{½}}\right)^2 \times \frac{100}{BH} \qquad N = 5.54\left(\frac{V_e}{W_{½}}\right)^2 \qquad A_s = \frac{B}{A}$$

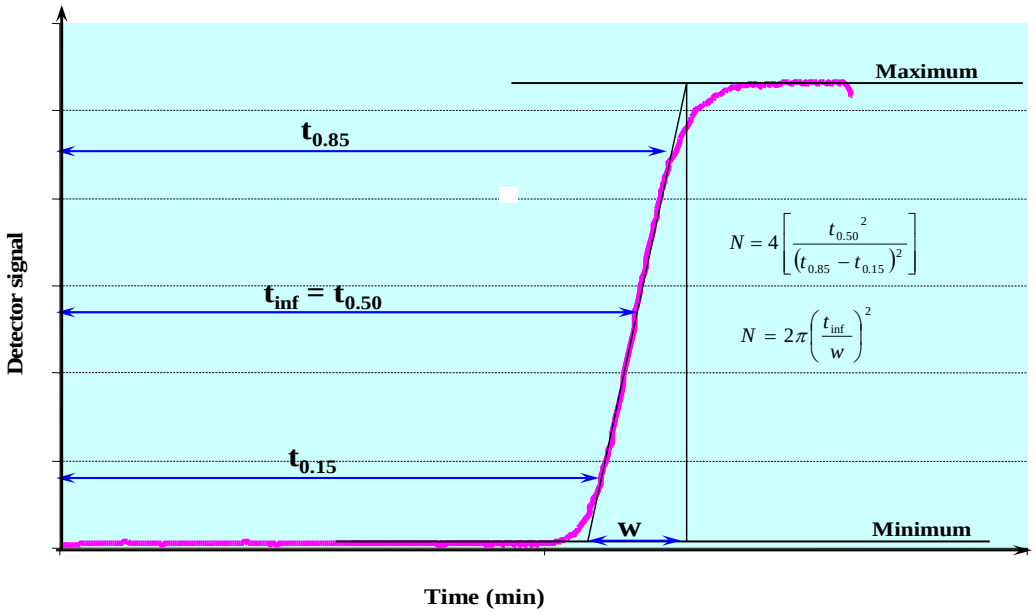
where V_e is the elution volume of acetone, $W_{1/2}$ the peak width at half height of the peak signal and BH is the column bed height in cm. A and B are the leading and tailing half of the peak width at 1/10th the peak height, respectively. The height equivalent to a theoretical plate, H = BH/total plates, and the reduced plate height, h = H/mean particle diameter are also used to describe the quality of the packed bed.



- Column efficiency can also be determined directly from breakthrough curves obtained from actual column runs during transitions from low to high salt and vice versa, or pH transitions during NaOH sanitation, using the following equations:

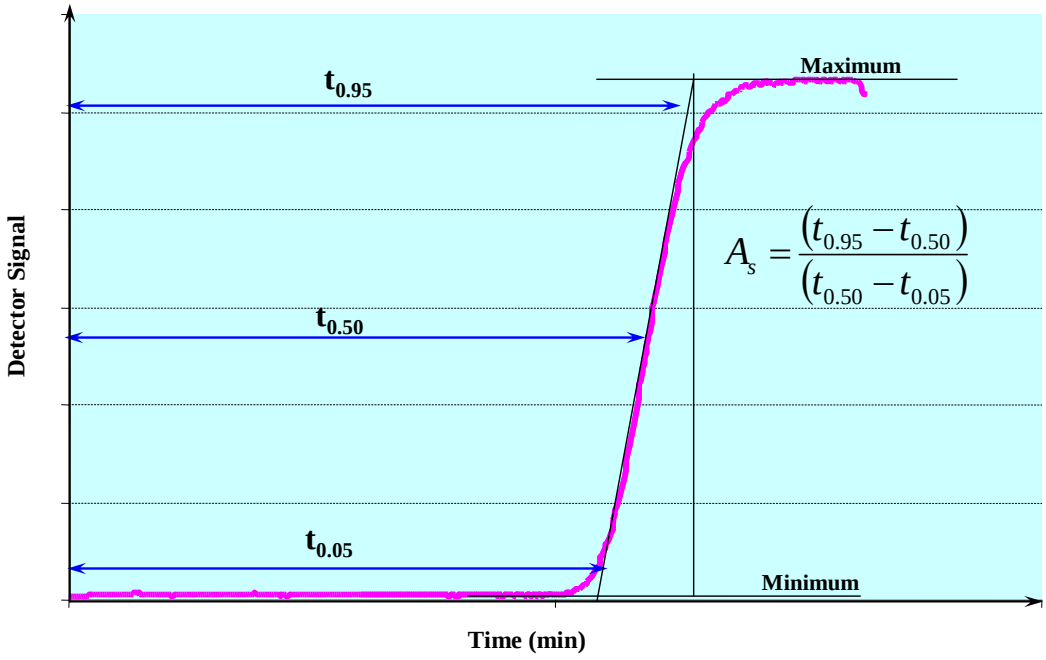
$$N = 4\left[\frac{{t_{0.50}}^2}{(t_{0.85} - t_{0.15})^2}\right] \quad \text{or} \quad N = 2\pi\left(\frac{t_{inf}}{w}\right)^2$$

where $t_{0.85}$,, t_{inf} = $t_{0.50}$, and $t_{0.15}$ is the volume/time after injection at 85, 50, and 15% of the total height of the breakthrough curve, respectivey; and w is the baseline width of the tangent triangle.



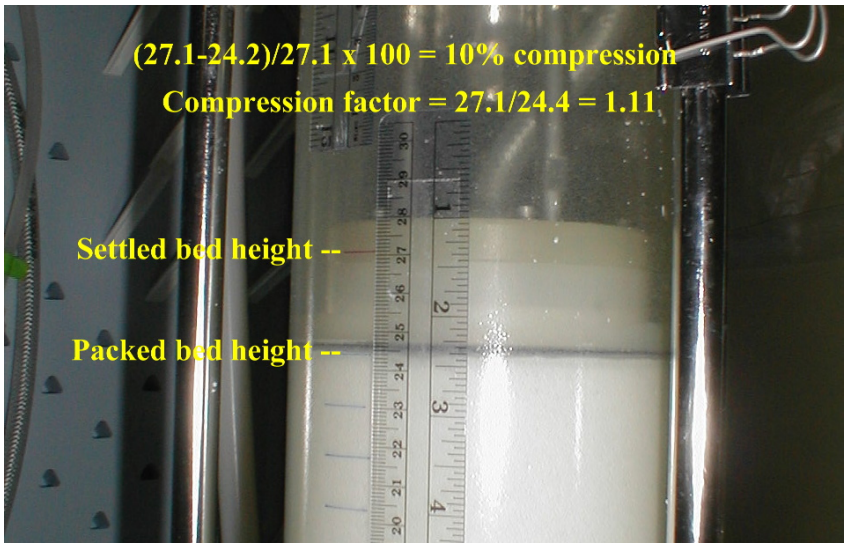
- In the same manner, asymmetry can be calculated using the equation where $t_{0.95}$,, $t_{0.50}$, and $t_{0.05}$ is the volume/time after injection at 95, 50, and 5% of the total height of the breakthrough curve, respectively.

$$A_s = \frac{(t_{0.95} - t_{0.50})}{(t_{0.50} - t_{0.05})}$$

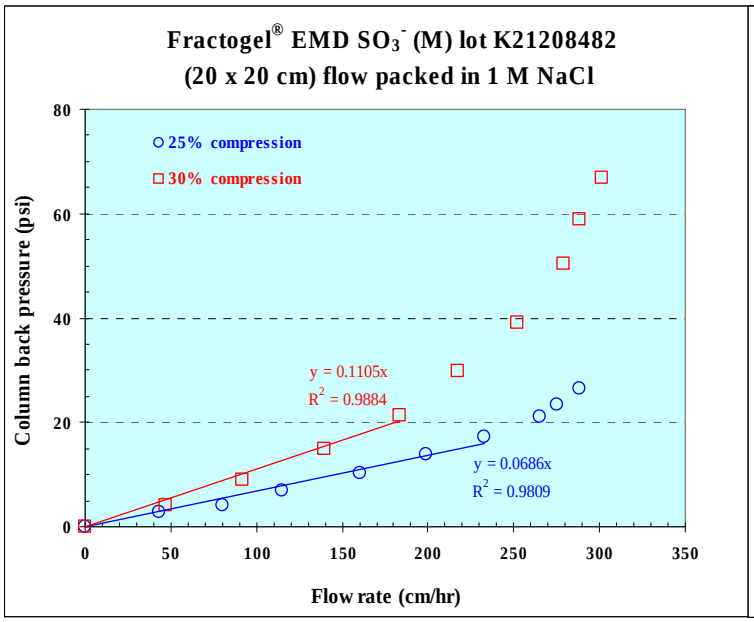


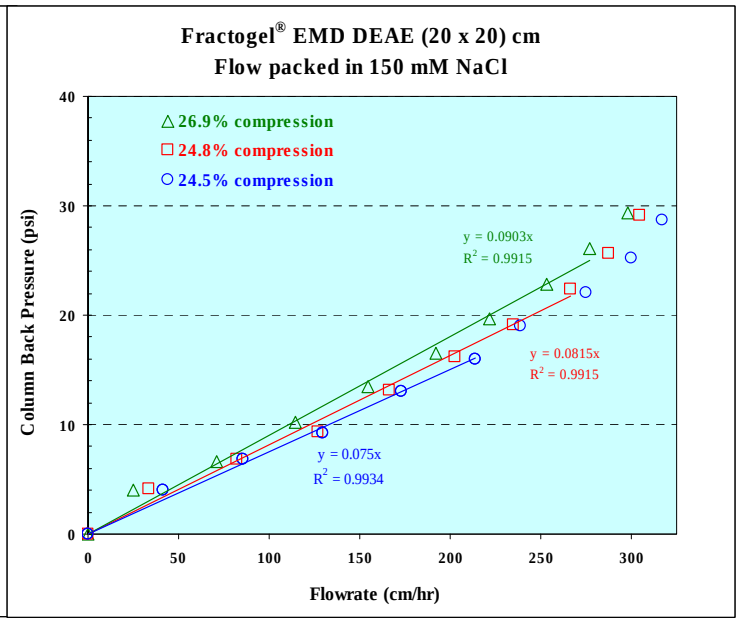
5. Appendix

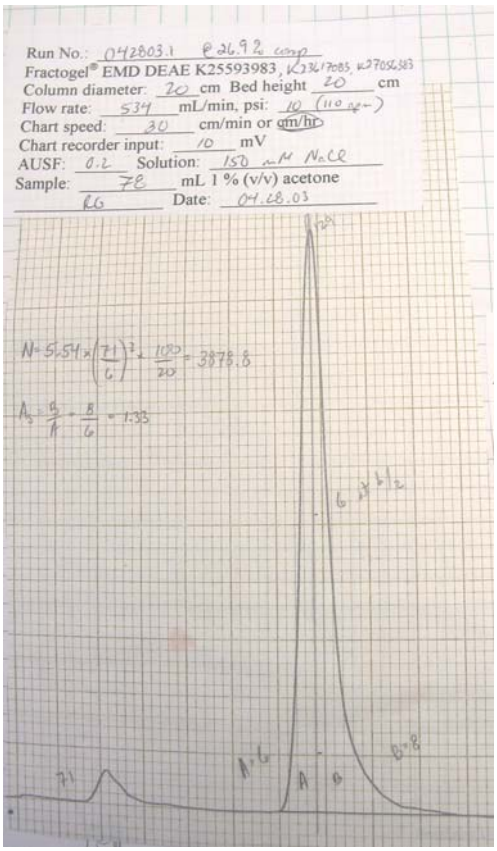
A photo of a packed column showing percent compression and compression factor calculations



Pressure vs. flow curves for (20 x 20 cm) Fractogel® EMD SO_3^- columns flow packed in 1 M NaCl at 25 and 30% compression and Fractogel® EMD DEAE columns flow packed in 150 mM NaCl to 24.5 and 27% compression are shown below:



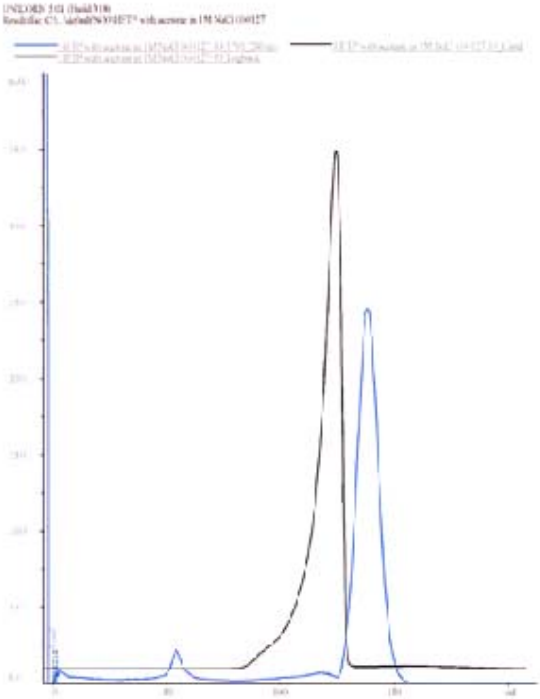


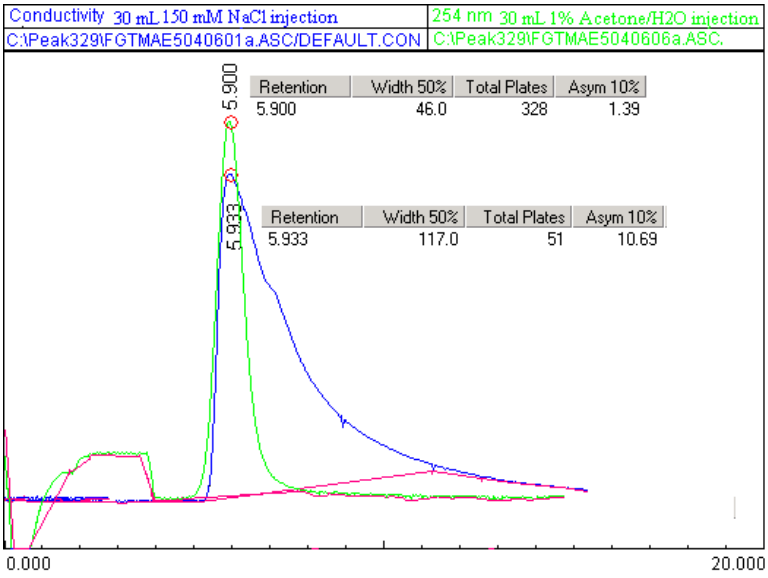


A representative HETP curve for (20 x 20) cm Fractogel® EMD DEAE at 26.9% compression run at 534 mL/min loading 78 mL 1% acetone is shown to the left. The calculated N/m was 3879, N equal to 776, with asymmetry of 1.3 and an H value of 0.026 cm.

HETP curves determined using 1 M NaCl as the probe in certain buffers may be subject to severe fronting due to an effect called viscous fingering. In those cases, switching to a H_2O pulse or 1% acetone in water instead of 1 M NaCl should alleviate the problem. An example of fronting caused by viscous fingering is shown below (courtesy of Andrew Griffiths, Cangene Corp) The sample, a 1% spike of 0.4% acetone in 1 M NaCl was injected onto a 160 mL Fractogel® EMD SO_3^- (2.6 x 31) cm column and both UV and conductivity detectors were used. The conductivity probe shows severe fronting whereas the UV detector doesn't. For the conductivity probe N = 1063, H = 0.029, A_s = 0.167; h = 4.5 beads; whererеas, N = 943, H = 0.033, A_s = 0.941, and h = 5.1 beads for the UV detector.

HETP curves determined using a 150 mM NaCl probe for a column packed in H_2O shows severe tailing due to interaction of NaCl with the ion exchange resin. Using 1% acetone in H_2O as the probe gives a peak without this effect. An example is shown below with Fractogel® EMD TMAE, (10 x 20) cm column packed in H_2O. For the conductivity probe, N = 51, H = 0.392, A_s = 10.69; h = 60 beads; whereas, N = 328, H = 0.061, A_s = 1.39, and h = 9.3 beads for the UV detector. Packing in water is not recommended. This column was packed solely to illustrate the effect interaction of a salt probe with Fractogel® EMD TMAE packed in H_2O has on HETP.





If you need any further information or support please do not hesitate to contact the specialists:

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