Packing Procedure for Eshmuno™ S and Eshmuno™ Q resin in 5-20 cm i.d. lab columns

Outline

1. **Calculation of amount of resin required to pack the column**
2. **Column preparation**
3. **Gel slurry preparation**
4. **Column packing**
5. **Column testing**
6. **Appendix**

1. Calculation of percent compression and amount of resin required

To achieve a stable packed bed, 8 to 10% compression is recommended for Eshmuno™ . "Compression" refers to the actual volume reduction which occurs when settled resin is placed under packing pressure (or flow) resulting in a packed bed. Compression occurs by removing part of the liquid void volume from the interstitial space between resin beads. At typical packing pressures, deformation of individual resin beads does not occur.

Assuming the following column parameters:

Column inner diameter: i.d. = 10 cm
Packed bed height: h = 20 cm
% compression*: 10.6

* % compression is defined as (SBV - PBV)/SBV × 100. Alternatively, a compression factor can be calculated as SBV/PBV where SBV is the settled bed volume and PBV the packed bed volume.

a) Calculate the desired packed bed volume according to:
$PBV = \pi \times (i.d./2)^2 \times h = 3.14 \times (10\ cm/2)^2 \times 20\ cm = 1571\ ml \approx$ **1.6 L**

b) To calculate the amount of settled bed volume required at a given % compression:
SBV = 100 × PBV/(100 - % compression) SBV = 100 × 1.6/(100 – 10.6) = **1.8 L**

Or,

c) To calculate the amount of settled bed volume required at a given compression factor from the packed bed volume:
SBV = PBV x compression factor SBV = 1.6 x 1.119 = **1.8 L**

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

Original bulk suspension = SBV/0.70 = 1.8 L /0.70 = **2.6 L**

This is the amount of slurry which should be placed into the column.
It is critical to know how much slurry to place into the column. An error here will result in either a too tightly or too loosely packed bed – giving rise to high or low operating pressures and possibly poor HETP/A_s values.

The following table gives this relationship for various % compression/compression factors:

Table I. Bulk suspension needed for (10 x 20) cm column

Percent compression (%)	SBV (mL)	PBV (mL)	Bulk suspension @70% slurry (mL)	Compression factor
5	1653	1570	2362	1.053
6	1670	1570	2386	1.064
7	1688	1570	2411	1.075
8	1707	1570	2438	1.087
9	1725	1570	2465	1.099
10	1744	1570	2492	1.111
11	2245	1570	3207	1.124

2. 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 and fully hydrated (no dry spots).
- Make sure the column is level.
- Calculate the approximate amount of resin needed for the desired percent compression and packed bed volume as described in the previous section.

3. Gel Slurry Preparation/Slurry % Determination

Eshmuno™ is usually supplied as a nominal 70% resin suspension in 20% aqueous ethanol, containing 150 mM NaCl, pH 7.0. Prior to packing, ethanol in the storage solution should be removed and disposed of according to local regulations.

- After allowing resin to settle in the shipping container, decant the storage solution (20% EtOH/150 mM NaCl) once. Re-suspend the resin using diH_2O.
- Pour the desired amount of resin into the column or another appropriate container.
- Allow the resin to settle in the column or in a 1 L graduated cylinder for at least 3 hours or overnight to determine the settled bed height/volume (settling for less than 3 hrs will result in an over-estimation of the amount of resin available for packing).

- It is now possible to calculate the slurry % by the following equation:

$$(SBV/V_t) \times 100 \text{ where } V_t = \text{total slurry volume}$$

- Alternatively, a short centrifugation method may be used to calculate the amount of resin slurry needed to pack the column. See “Procedure to determine the amount of Exhmuno™ S resin needed to pack a column based on packed bed density” for details and worksheets.

4. Column Packing

- Different column designs can have slightly different packing options. Please consult the manual for your specific design.
- Add the appropriate amount of resin slurry to achieve the desired packed bed height at the recommended compression.
- Re-slurry the resin bed by stirring to achieve a homogeneous suspension.
- Rinse down the walls of the column with water, 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 inlet tubing.
- Make sure the column inlet line is full of liquid before switching the column inlet to the pump.
- Open the column outlet and pack the column with the appropriate packing solution (0.01 N NaOH is used in our labs – among suitable alternates are 150 mM NaCl or diH_20) at a starting flow rate greater than 300 cm/hr until the packed bed height no longer decreases, or 2 barg is reached. Eshmuno™ columns generate low column back pressures, so flow rates in excess of 1000 cm/hr can be achieved before reaching 2 barg. Turn off the pump.
- Lower the top adjuster to the desired packed bed height (this will generally be below the bed height achieved during packing). This can be done by opening the inlet and/or outlet line, enabling liquid to exit the column through the inlet and/or outlet lines.
- Turn on the pump to an appropriate flow rate for column testing.

5. Column Testing

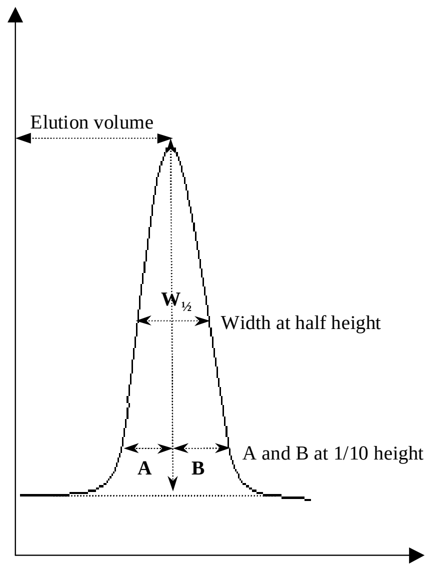
The performance and some basic characteristics of the packed column can be checked by running the test chromatogram specified as follows:

- Run the column at a flow rate of ~150 cm/h and inject 1-2% of the packed bed volume of a 1% acetone in water solution. Monitor the UV absorption of the column eluant at 254 or 280 nm. Assuming the column is equilibrated with salt or base, as an alternate, water can be used to determine the HETP value using a conductivity meter as the detector.

- The elution volume of the acetone peak (see below) 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:

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

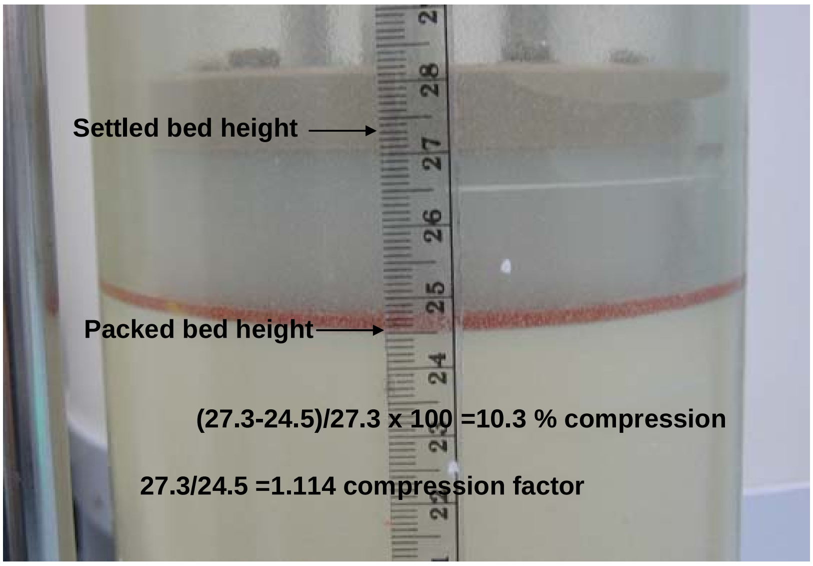
where V_e is the elution volume of acetone, $W_{1/2}$ the peak width at half height of the peak signal and BH is the column bed height in cm. A and B are the leading and tailing half of the peak width at 1/10th the peak height, respectively.



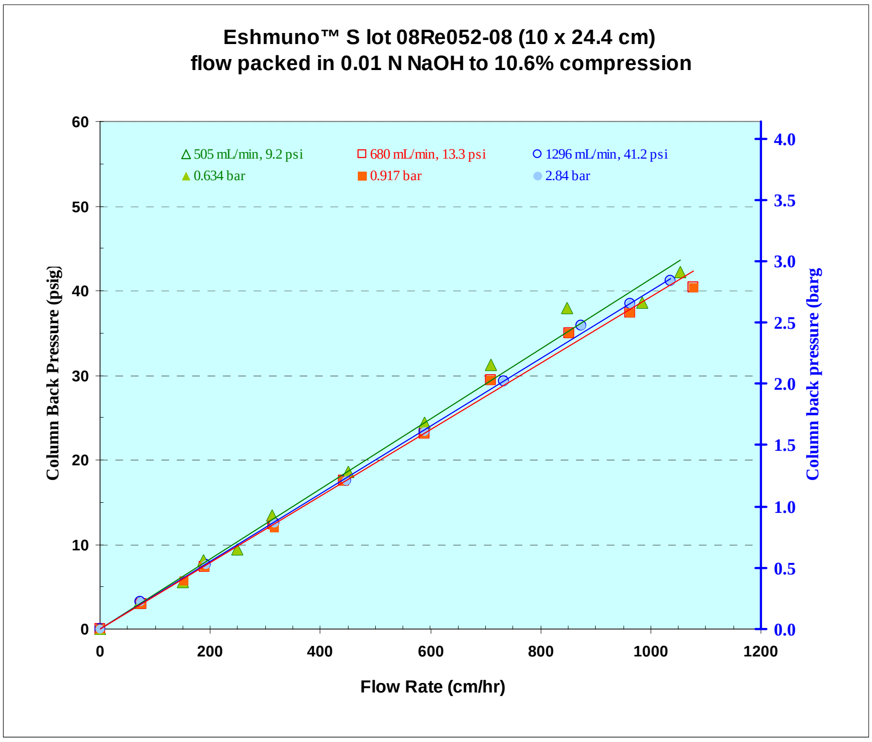
The height equivalent to a theoretical plate, H = BH/total plates, and the reduced plate height, h = H/mean particle diameter can also be calculated and are oftentimes used to describe the quality of the packed
bed.
A range of 0.8 to 1.9 for A_s and N_m values of > 1800/m (at ≈75 cm/h) will qualify acceptable column packings.

6. Appendix

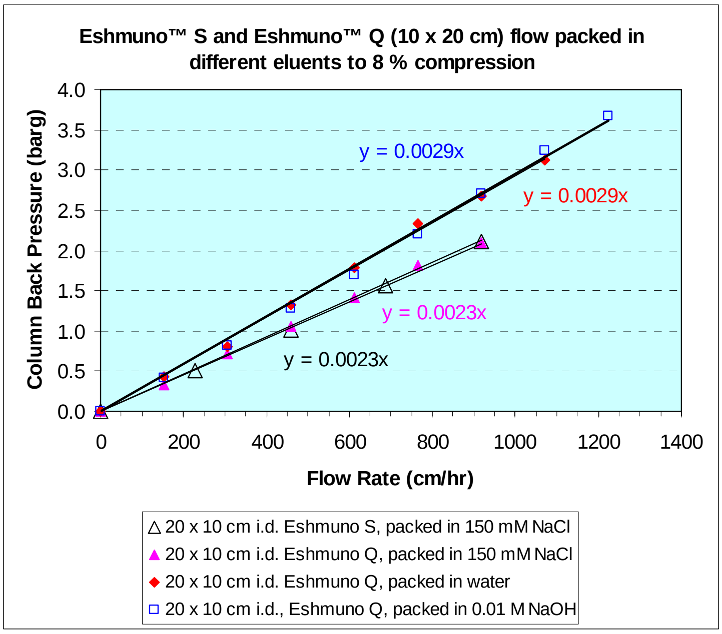
a) A photo of a packed column illustrating percent compression and compression factor calculations is shown to the right:



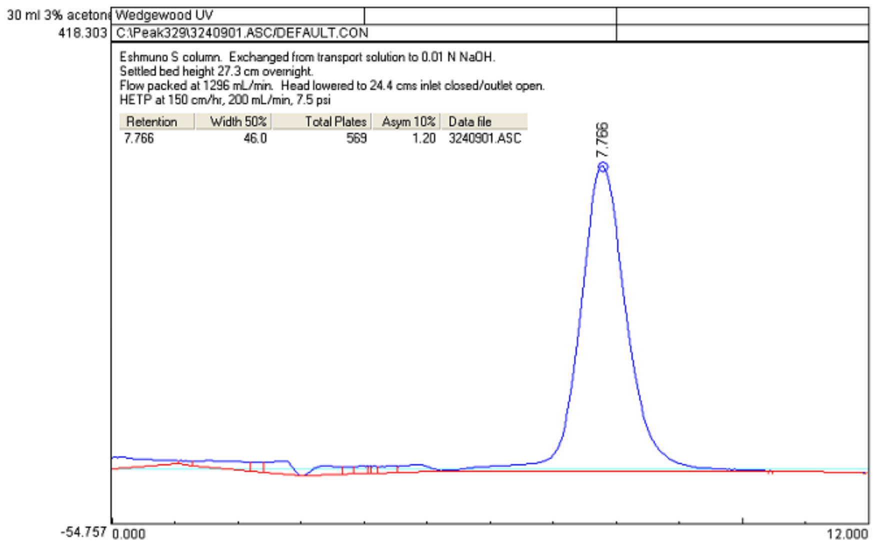
b) Representative pressure vs. flow curves for 10 cm id x 24.4 cm bed height Eshmuno™ S columns packed to 10.6 % compression at three different initial packing flow rates in 0.01 M NaOH are shown below (for all pressure vs. flow curves the system pressure was subtracted):



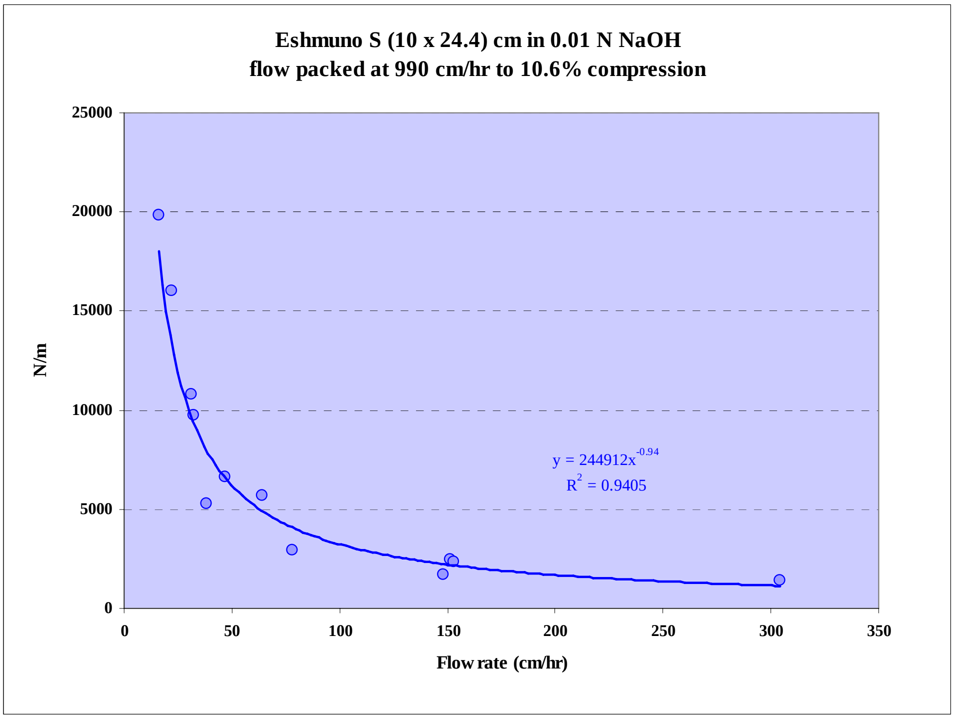
c) Representative pressure vs. flow curves for 10 cm id x 20 cm bed height Eshmuno™ S and Eshmuno™ S columns packed in different eluents to 8 % compression at three different initial packing flow rates in 0.01 M NaOH are shown below



d) A typical HETP curve for an Eshmuno™ column (10 x 24.4 cm) at 10.6 % compression run at 200 mL/min (150 cm/hr) loading 30 mL 3% acetone in water is shown below. The calculated N/m was 569 x 100/24.4 = 2332, N = 569, with asymmetry = 1.2 and an H value of 0.043 cm. Assuming a mean particle size distribution of 0.0085, h = 5 bead widths, which is indicative of a well packed column.



e) Below is the van Deemter plot showing the impact of testing flow rate on achieved HETP values for the same column. As per theory, with increasing flow rate one gets decreasing plate count in the same column.



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

processing@merck.de