

7. PACKING INSTRUCTIONS

Packing the column with soft gels

Only degassed and filtered solvents or buffers may be used when packing chromatography columns.

The lower frit is dampened and covered with approx. 1cm solvent. Next the slurry is introduced carefully and quickly, ensuring that no air bubbles occur. The column outlet should be open while the column is being filled; the solvent can also be sucked from the column outlet with a peristaltic pump at the same time. When the slurry has all been poured in, the gel must be allowed to settle and the solvent sink to approx. 0.5 – 1cm above the packing level in the gel bed. The gel bed must not be allowed to run dry. The column outlet is closed or the peristaltic pump stopped. Next the variable piston is inserted, without allowing particles to come between the seal and the column body. By turning the lock slowly, the piston can be moved towards the gel bed. At the same time, all the air above the gel bed should be forced out of the column inlet. It is essential that the gel bed is not compressed when moving the piston towards it. Now the column can be equilibrated with the appropriate buffer or solvent.

Dead volume can occur between the gel bed and the piston during normal use, but this can be removed by moving the piston inwards.

Quality control

We recommend that you determine plate count and peak symmetry with a suitable (non-adsorbent) test substance after packing the column. By repeating this test frequently, the quality and durability of the packing material can be recorded efficiently.

Amount of theoretical plates (N):

$$N = 5.54 \times (T_r / W_{1/2})^2$$

T_r : retention time (sec)
 $W_{1/2}$: peak width (sec) at half peak height

$$HETP = L / N$$

L : column length in mm

Peak symmetry (S):

$$S = W_{1/2,r} / W_{1/2,l}$$

$W_{1/2,r}$: peak width to the right of the peak median
 $W_{1/2,l}$: peak width to the left of the peak median

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