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# A novel technology for packing and unpacking pilot and production scale columns

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## Abstract

A new method of packing and unpacking large scale chromatography columns is described. This involves use of a 3-position valve that can inject chromatography media into a closed column thereby packing it. This same valve in another state is then used to unpack the column. Heights equivalent to a theoretical plate and asymmetry factors of packs on columns from 280 to 800 mm diameter are discussed. © 1998 Elsevier Science B.V.

*Keywords:* Preparative chromatography; Packing of columns

## 1. Introduction

Traditionally large scale chromatography columns (those with diameters greater than 100 mm) are packed with media by removing the piston and pouring in a slurry [1–3]. In the case of soft or semi-rigid gels (those based on agarose or cellulose) this slurry normally consists of about 50% volume of settled wet media and 50% slurry buffer [4,5]. In the case of silica-type media the dry medium is generally slurried up to 1.5 times its packed bed volume [6].

Once the slurry has been poured into the open column, the piston is replaced. In the case of soft or semi-rigid media, the bed is percolated at 1.5 times the process flow rate and allowed to compress under the resultant flow and pressure [7]. A mark is placed on the column where the bed is compressed to. The piston is then adjusted to that mark. For silica-type media the slurry is compressed using the piston, which is often driven using hydraulic cylinders. The excess slurry solvent (often propan-2-ol) exits via the opposite mobile phase port to the piston [6].

Another method, whereby the medium is compressed on large ion-exchange columns using suction is described by Barry and Chojnacki [8]. In this method the medium is pulled down by suction in an open column and at the appropriate point, the column lid is fitted.

This article describes a new method of packing columns whereby the column is not dismantled; the lid is not removed during packing or unpacking. A valve positioned at the top and bottom mobile phase ports is used to access the inside of the column. The valve has three ports and three positions. The ports carry mobile phase, slurry to be packed and waste unpacked slurry. The three positions of the valve are, running, packing and unpacking.

## 2. Experimental

### 2.1. Materials and methods

All reagents were of analytical or spectroscopic grade from Fisher (Loughborough, UK) or Sigma (Poole, UK). Water was filtered and deionised. Graco

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Husky pumps (Smethwick, Warley, UK), Model 307, 715 and 1040 were used to pump the slurry and cleaning solutions.

## 2.2. Column liquid chromatography

Columns used were an 800 mm diameter, 200 to 400 mm length Chromaflow variable column; a 400 mm diameter, 200 to 400 mm length Chromaflow variable column; a 280 mm diameter, 200 to 400 mm length Chromaflow variable column and a 400 mm diameter, 150 mm length Chromaflow fixed column. These columns were obtained from Pharmacia Biotech (Uppsala, Sweden). Media, Sepharose 4 Fast Flow, Q Sepharose 6 Fast Flow and Q Sepharose Big Beads were obtained from Pharmacia Biotech.

Fig. 1 shows a fixed column and Fig. 2 a variable column with their accompanying valves. Figs. 3–7 show how the column is used.

## 2.3. Column packing

The column and all lines were firstly primed with water. Slurry was recycled through the valve, with the valve in the clean in place (running) position, as shown in Fig. 5. The pack was started by inserting the tube carrying the slurry into the column (as shown in Fig. 3 or Fig. 4) and then opening the opposite end mobile phase. The column can be

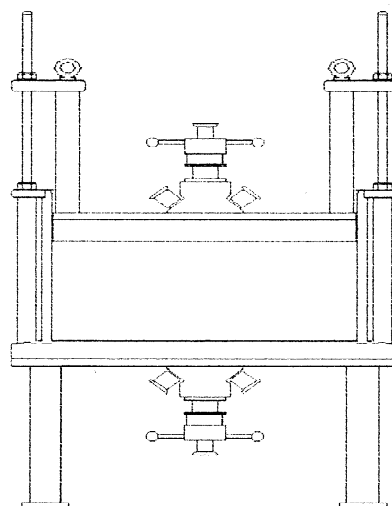


Fig. 2. A variable bed length column.

packed by pumping slurry into the top nozzle with excess liquid leaving via the bottom mobile phase. As was done with Sepharose 4 Fast Flow in the 280 mm diameter column, or upwards, as was done with the Q Sepharose 6 Fast Flow in the 800 mm diameter column. Different packing directions suit different media. Figs. 3 and 4 show upward and downward packing methods.

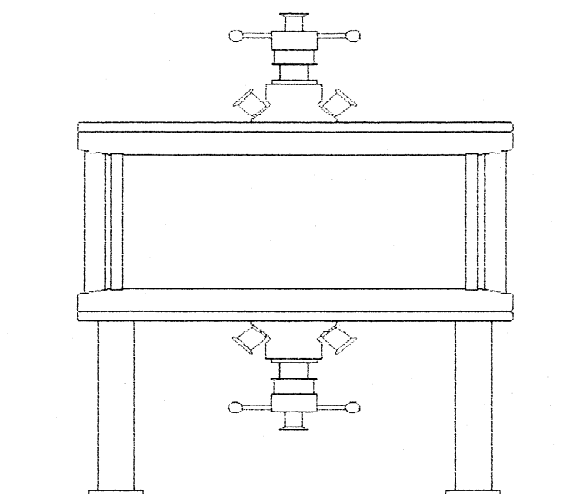


Fig. 1. A fixed bed length column.

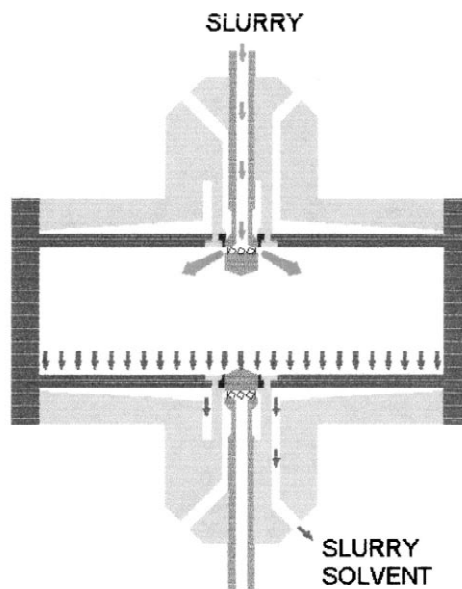


Fig. 3. The column being packed downwards.

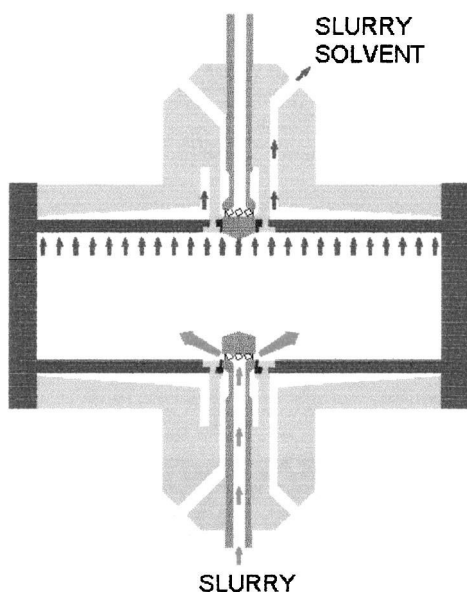


Fig. 4. The column being packed upwards.

Excess slurring liquid left via the mobile phase port and packed bed built up on the end of the column. The packing flow rate was always initiated at above 100 cm/h and below 500 cm/h, in general

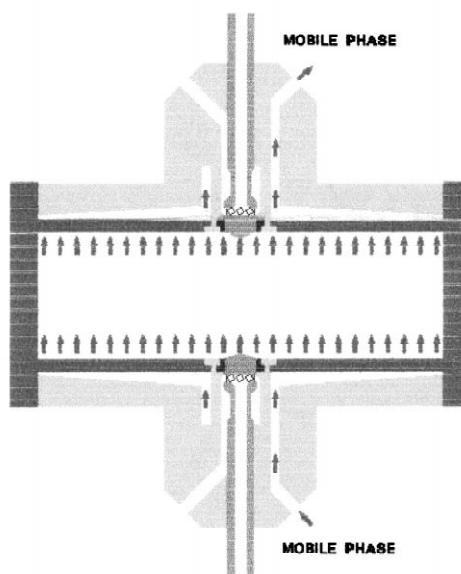


Fig. 6. The column being run in process.

300 cm/h was used. The slurry solvent was always water and the concentration was 50% settled bed volume. The flow was allowed to drop as the back pressure increased.

Once the column was fully packed, and the slurry tubes themselves packed, the pump stalled and

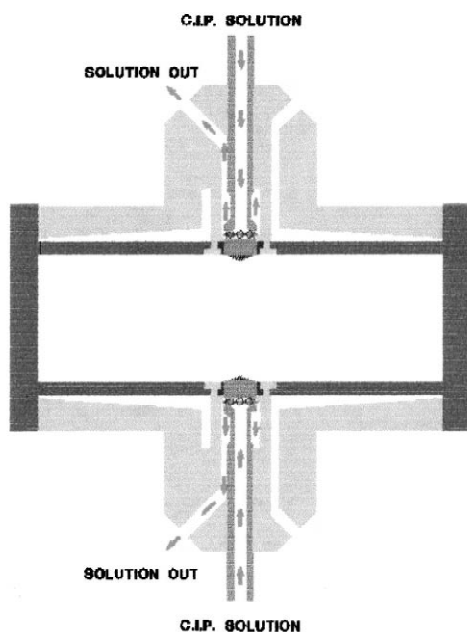


Fig. 5. The valve being cleaned in place.

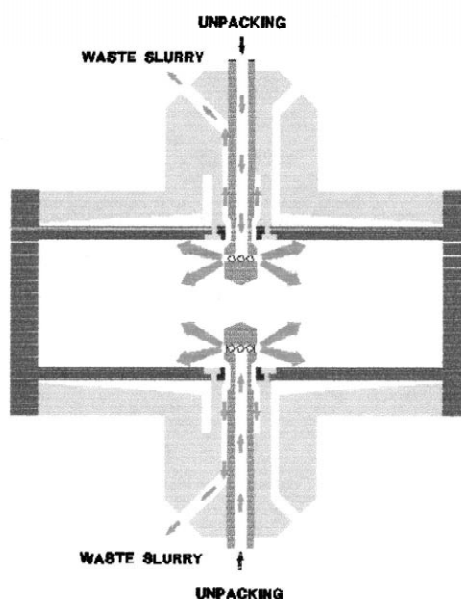


Fig. 7. The column being unpacked.

stopped pumping. This marked the end of the pack. The valve was then changed to the clean in place (running) position, in this state the slurry lines were completely isolated from the column bed and mobile phase, see Fig. 5. Cleaning solution was pumped through the valve clearing the lines of slurry.

The column was ran as shown in Fig. 6.

#### 2.4. Test probe preparation

A 1% volume for volume solution of acetone in water was prepared. Acetone acts as a void volume marker.

#### 2.5. Chromatography

Chromatography was performed on a Pharmacia (Uppsala, Sweden) Biopilot or Bioprocess Chromatograph. The test linear flow rate was 20 to 30 cm/h. An injection of 1% column bed volume of the test probe solution was applied to the column. The test mobile phase was always water. The conditions and calculations applied were as follows [9]: sample used: 1% acetone in water; injection volume: 1% of column volume; detection wavelength: 280 nm UV absorption; detector sensitivity: at path length of 5 mm, 0.1 AUFS; response: about 50% full scale deflection obtained; flow rate: 20–30 cm/h. Number of theoretical plates active in the column length:  $N = (V_e/W_{1/2})^2 \cdot 5.54$ , where  $V_e$  = peak elution volume or mm of chart,  $W_{1/2}$  = peak width at 1/2 height in volume units or mm of chart. HETP was calculated according to the formula:  $HETP = L/N$  where HETP = height of a theoretical plate (in units used for  $L$ ),  $N$  = number of theoretical plates active in the column length used,  $L$  = bed height.

Reduced plate height,  $h = HETP/\text{particle diameter}$ , where HETP and particle diameter are in the same units of length, e.g., cm. Asymmetry factor ( $A_f$ ) was obtained from the equation

$$A_f = b/a$$

which was obtained as follows: from apex to baseline drop a perpendicular line, then draw a horizontal line 10% above baseline.  $a$  in this formula indicates the width of the first half of the peak at

10% above the baseline,  $b$  equals the width of the second half of the peak at 10% above the baseline

#### 2.6. Column unpacking

Fig. 7. shows the valve in its unpacking state. In this position the columns were unpacked. Water was pumped at between 500 and 1000 cm/h into both top and bottom slurry lines. This sprayed into the packed bed thereby breaking it up. In the unpacking state a third port, an annular exit around the slurry line, is open to the column interior. A second pump was put in line on this port and waste media in the form of a slurry was pumped out via this port and collected in a vessel. Air was allowed to enter via the top waste port. Once all the media had been removed, the column was sprayed with 0.5 M NaOH as a clean in place routine. During the whole process the columns were never dismantled.

### 3. Results and discussion

Fig. 8 shows repeat packs of 200  $\mu\text{m}$  agarose beads into a 150 mm long 400 mm diameter steel column. The reduced plate height was reproducible, between 1.9 and 2.3, while the asymmetry varied between 0.9 and 1.5. This was within this laboratory's acceptance criteria and the packing method was considered reproducible. This laboratory's acceptance criteria were as follows: reduced plate height less than 3.5 and asymmetry 0.8 to 1.8.

Fig. 9 shows 6 repeat packs with Sepharose 4 Fast Flow into a 400 mm diameter by 300 mm bed length acrylic column. In this group of repeat packs the variation in efficiency is higher than with the Big Beads. The reduced plate height varied between 2.2 and 3.3. This is still an acceptable efficiency, however not as efficient as the Big Beads packs. The asymmetry results were all below 1.5 and above 1, this was similar to the results with the Big Beads. It is interesting to note that the shorter bed gave better reduced plate heights. It suggests that the spraying of the media into the column does not disrupt the bed at the end of the pack, otherwise the shorter bed would have lower efficiency/asymmetry than the longer 300 mm bed. Also, and perhaps more importantly, the Big Beads were packed into a fixed bed length

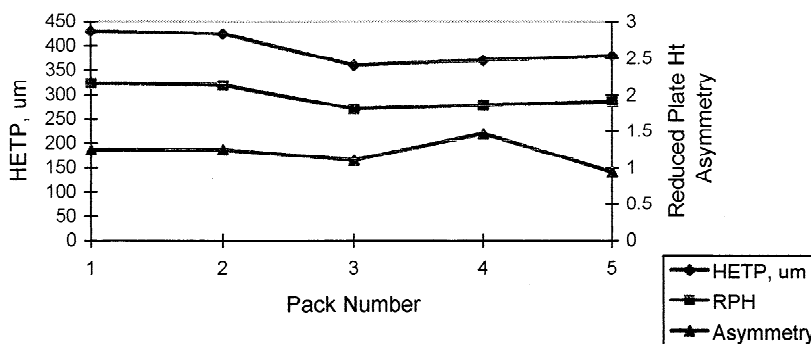


Fig. 8. Efficiency and asymmetry of 5 repeat packs of Q Sepharose Big Beads into a 400 mm diameter by 150 mm long fixed steel column.

column (see Fig. 1). The fixed bed column has no piston seals, it consists of a tube with two end plates. These plates are sealed with an o-ring that allows no dead space between tube and plate. The variable column has a piston (See Fig. 2) which requires a seal that is slightly indented away from the column bed. This gives rise to a dead space, albeit small. It is possible that this dead space is the cause of the slightly reduced efficiency—greater band spreading—in the results obtained with the variable column (see Fig. 9) compared to the results from the fixed column (see Fig. 8).

Fig. 10 shows the results from a scale-up study with Q Sepharose 6 Fast Flow. Three packs were carried out in a 280 mm diameter column and three in an 800 mm diameter column. The bed lengths were 300 and 280 mm, respectively. The graph shows how the reduced plate heights obtained in the 280 mm diameter column were marginally better

than with the 800 mm diameter column. However, both columns' comparable efficiencies were between 2 and 2.7 reduced plate height. Asymmetries obtained with both columns on all three packs were 1 to 1.4.

The 800 mm diameter column was fitted with a larger pack/unpack valve in comparison to the 280 mm diameter column. The 800 mm diameter column was packed using a valve with pipework of 35 mm outer diameter and 22.1 mm internal diameter. The internal diameter of the tube which carried the slurry into the column was 22.1 mm. In comparison, the 280 mm diameter column slurry tube was 6.5 mm internal diameter; the pipework was 16 mm outer diameter with 10 mm internal diameter. These internal diameters were chosen such that the linear flow velocity through the pipe was between 1 and 2 m/s during general operation. Both these valve sizes packed columns with acceptable efficiency and

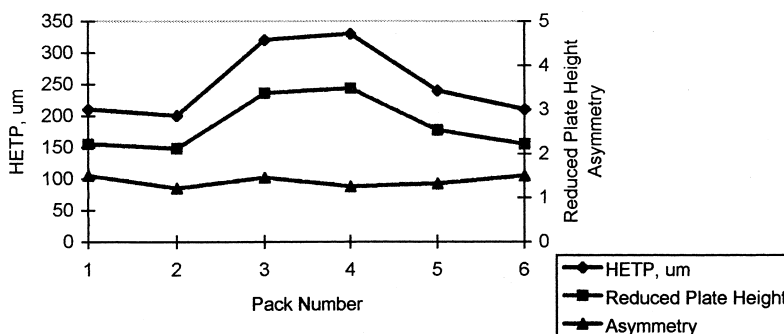


Fig. 9. Results from 6 repeat packs of Sepharose 4 Fast Flow into a 400 mm Diameter by 300 mm long variable column.

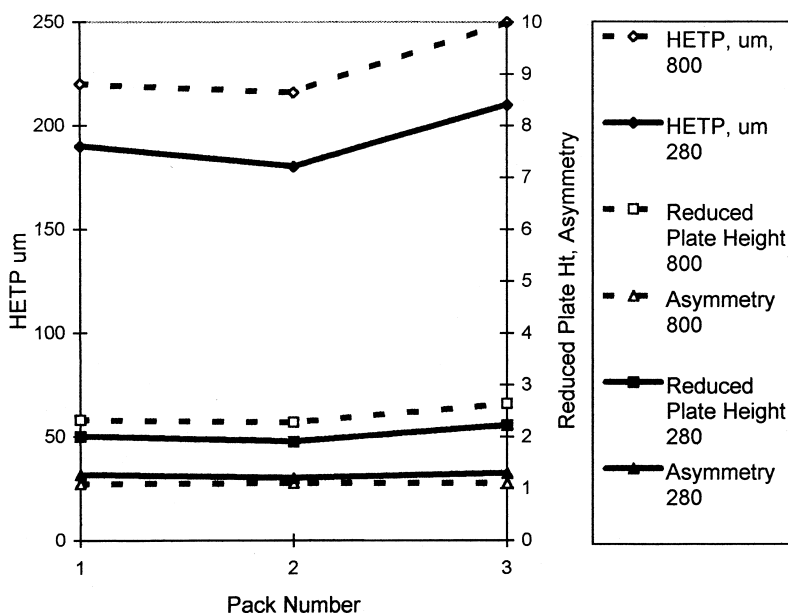


Fig. 10. Q Sepharose 6 Fast Flow packed into an 800 mm diameter variable column (280 mm long) and into a 280 mm diameter variable column (300 mm long).

symmetry. This experiment thus showed that both the method and the pack/unpack valve itself are scalable.

#### 4. Conclusion

These experiments showed that using a valve to inject media into a large scale liquid chromatography column, thereby packing it, can yield well packed columns with good to excellent efficiencies and asymmetries, repeatedly. The valve allowed the columns to be packed and unpacked without removing the top plate or piston. It was shown that such a valve can be scaled up in size and give comparable results of efficiency and asymmetry which were well within this laboratory's acceptance criteria.

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