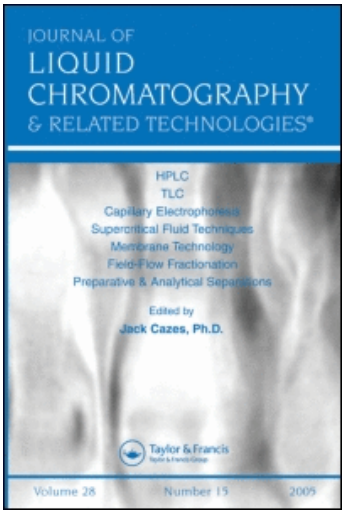

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An Easy Method to Pack Large Scale Stack Columns

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AN EASY METHOD TO PACK LARGE SCALE STACK COLUMNS

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ABSTRACT

A simple sucking method for the packing of large scale columns has been developed by the authors.

INTRODUCTION

The effectiveness of a chromatographic separation process depends on the number of theoretical plates (1) or on the height equivalent to a theoretical plate (HETP). One has to pack a column to achieve the smalest possible HETP, i. e. the bed has to be dense and evenly distributed in the whole column. There are a number of packing (2) technics. Most of them use pressure, by pumping water under pressure through the column until the surface of the bed sinks below the upper rim of the column. The last critical step is to place on

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the top lid. Other technics use organic solvent (alcohol) first to shrink the packings, then putting on the top lid and finaly swelling the bed by buffers.

We found the pressure technic too dangerous, for the column may explode in case of sudden high overpressure. Also, proper HETP sometimes requires many repackings. Thus, we sought a simple and safe sucking method for packing.

MATERIALS

The gels used in these experiments were the ion-exchanger DEAE-Sepharose FF and the gel filter Sephacryl S 200 (Pharmacia Uppsala).

All of the chemicals used were of analytical grade. After filling the DEAE-Sepharose FF was equilibrated with 3 column volume of 0,025 M Na-acetat buffer, pH 5,2 and the Sephacryl S 200 with 0,05 M NaCl.

The columns were K-370 stack columns (Pharmacia, Uppsala, Sweden). The water or the buffers were pumped by an R 411 LW membrane pump (Seyfert and Rehier, Immunhausen, Germany).

For the testing of the columns we used a PW 9505 conductivity meter (Philips, Eindhoven) coupled with REC 482 recorder (Pharmacia, Uppsala)

METHODS

The packing arrangement is seen on Fig. 1. The experiments have been carried out with a stack column and an applicator on it. At first the air bubbles had to be removed from the bottom sieve

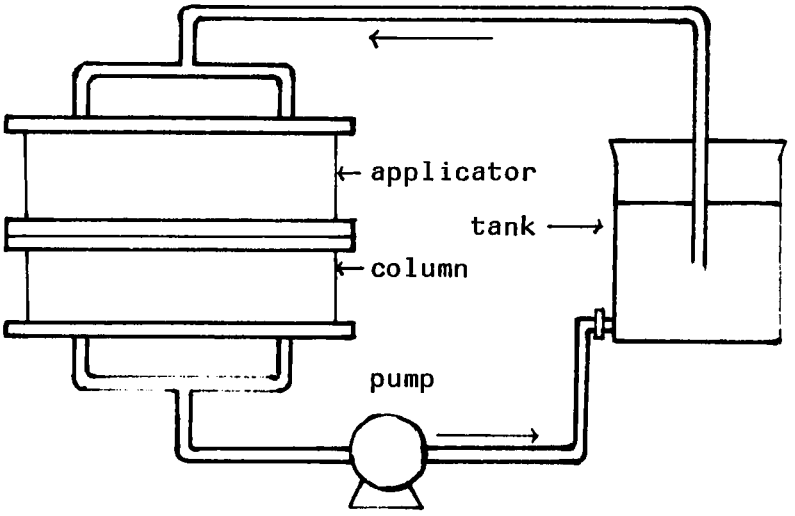

Fig 1. Scheme of the filling arrangement

and then the gel suspension was poured into the column. After putting the top lid on the applicator, the bottom was connected with the pump to suck the water out of the column. This pumped the water or buffer into a tank. The tank was connected to the top lid of the column. After starting the pump, the water began to flow from upward down in the column and the surface of the bed started sinking. The tank is very important because the pumping cannot develop an overpressure in the column and a continuous flow is maintained.

Starting flow rate was about 1050 ml/min. Pumping was continued until the gel sank below the upper rim of the stack column. The fluid was sucked from the applicator by disconnecting the tank and the applicator, then the pump was switched

off. When the applicator is empty in can be removed and the top lid put on the column easily, because the surface of the gel is bellow the rim. The packed column was equilibrated as before mentioned and tested by applying 400 ml, 0,25 M Na-acetat buffer to the gel filter column.

The conductivity of the eluted solution has also been recorded. HETP was calculated by the equations

$$N = 16 \left(\frac{V_e}{W}\right)^2$$

$$HETP = L/N$$

Elution volume (V_e) and the width of the peak (W) was obtained from the chromatogram; the height (L) was 15 cm.

RESULTS

The chromatogram of the S 200 is shown in Fig 2. As it can be seen, a very narrow peak with high symmetry can be obtained. The HETP in 10 experiments was always less than 0,03 if the quantity of the gel is optimal. The packing of a 16 liter stack column needs only 30-40 minutes at ionexchangers and 60 minutes at gel filters like Sephacryl S 200 superfine. With this method, one can also estimate the quantity of the gel easily. If the bed surface cannot be sinked below the rim of the column, there may be too much gel present. On the other hand, if the bed surface is under the rim then more gel is neaded.

DISCUSSION

A method has been developed for the packing of large scale stack columns by a continuous sucking

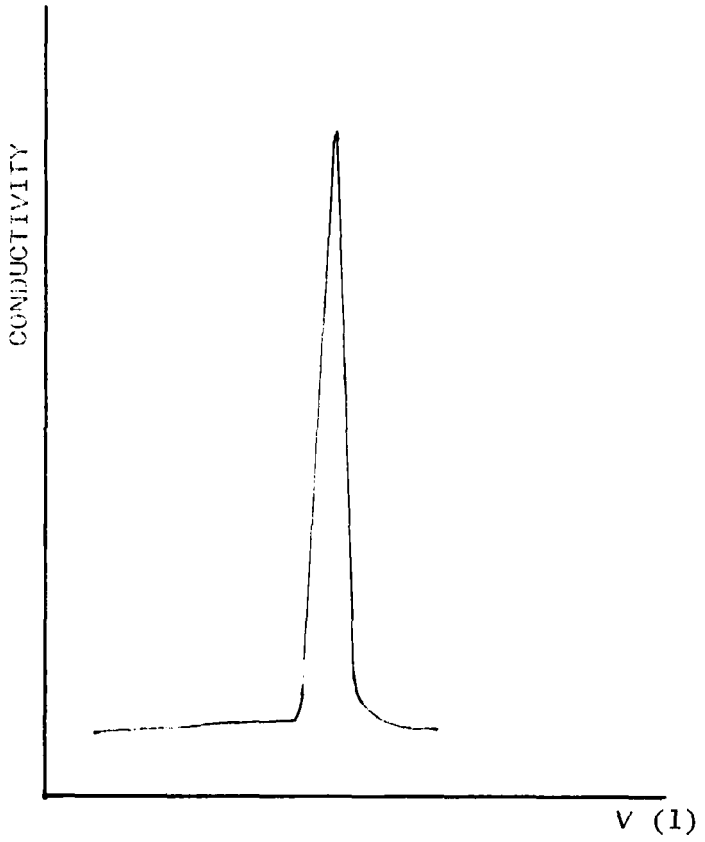

Fig 2. Chromatogram for the determination of HETP, K 370 column. DEAE Sepharose FF. Flow r. 420 ml/min. Buffer 0,025 M Na-acetat. Sample 400 ml 0,25 M Na-acetat V_e=15,4 l, W=2,61, L=15 cm, HETP=0,027

method. The packing can be carried out in a relativly short time without high overpressure, i. e. considerably reducing the risk of explosion. The HETPs obtained are better than by other methods used for the packing of large scale columns.

With the high pressure method used earlier HETP had been between 0,03-0,06. With this new method it is never greater than 0,03.

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