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Note

Packing of Toyopearl column for gel filtration

I. Influence of packing velocity on column performance

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The performance of analytical liquid chromatographic columns has been much improved in the last 10 years by studying packing techniques[1–4] and by developing rigid microparticulate packing materials. On the other hand, although soft gels such as Sephadex with large particle sizes have been widely used for preparative purpose, the importance of packing techniques has not been recognized[5]. Even today they are usually packed into columns by gravitational methods proposed about 20 years ago[6,7]. Although some problems, such as inferior reproducibility and the long time required to pack a column, have been pointed out for these packing methods[8], little effort has been devoted to the study of packing techniques for soft gels.

We have been investigating the influence of various packing conditions on the column performance for semi-soft gels by using Toyopearl (Toyo Soda, Tokyo, Japan), a hydrophilic porous polymer packing material for gel filtration, resistant to pressures up to several atmospheres. The influence of packing velocity in constant-velocity packings is described here.

EXPERIMENTAL

Toyopearl HW55S and HW55F (Lot No. 55108), with particle sizes of 20–40 and 30–60 μm respectively were used. They are the same materials as Fractogel TSK HW55, 0.025–0.037 and 0.037–0.064 mm, available from E. Merck (Darmstadt, G.F.R.).

A schematic diagram of the packing set-up is shown in Fig. 1. The chromatographic column and the slurry reservoir were commercial glass columns of 60 × 2.2 cm I.D. and 90 × 2.2 cm I.D., respectively (Amicon, Lexington, MA, U.S.A.). These columns can be operated at pressures up to several atmospheres and the end-fittings are designed so that dead-space volumes are minimal. The chromatographic column and the slurry reservoir were connected with a coupling ring. A peristaltic pump, Model SJ-1211H (Atto, Tokyo, Japan), was used to supply solvent at constant velocity. The maximal operating pressure of the pump is 2.1 atm according to the manufacturer's specification. Packing was carried out by the following procedure.

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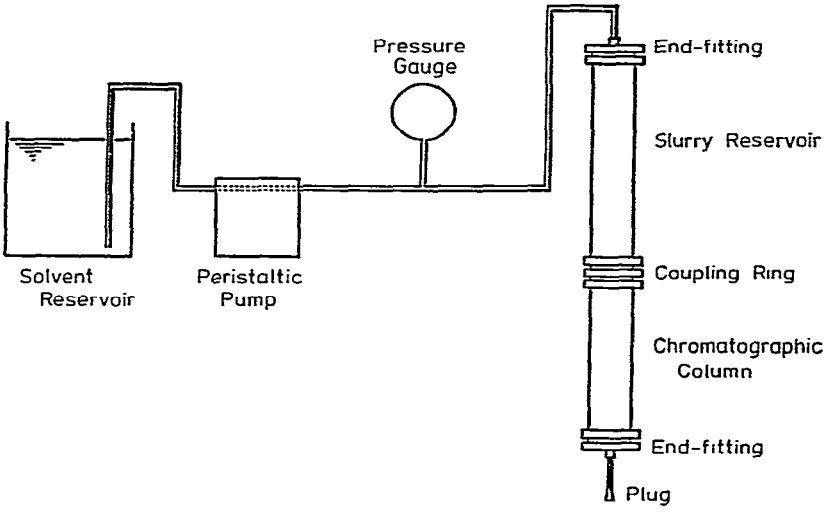

Fig. 1. Schematic diagram of the packing set-up.

About 300 ml of pre-swollen Toyopearl HW55S or HW55F were suspended in about 2 l of distilled water and allowed to stand. After most particles had settled, the fines remaining in the supernatant were removed by decantation. This procedure was repeated several times until the supernatant became clear. The Toyopearl was washed three times in a glass filter with about 600 ml of packing solvent (0.1 *M* phosphate buffer of pH 7 containing 0.1 *M* potassium chloride) by suspension and aspiration. A slurry of 45% Toyopearl by volume was prepared in the packing solvent. The resultant slurry was poured into the slurry reservoir and the chromatographic column mounted vertically. Then the top of the slurry reservoir was connected with the end-fitting, taking care not to include air bubbles as far as possible. The pump was started so as to deliver solvent at a pre-set constant velocity and the bottom outlet from the column was opened. After passing 350 ml of solvent (equivalent to about 1.6 column volumes) pumping was stopped and the bottom outlet from the column was again plugged. The column was disconnected from the slurry reservoir and another end-fitting was attached to the top of the column, taking care not to include air bubbles.

The performance of the packed column was tested with the set-up shown in Fig. 2. A peristaltic pump (Model SJ-1211H, Atto), a valve injector (Model 7010, Rheodyne, Berkeley, CA, U.S.A.) and a UV detector (Model SF-770, Schoeffel, Westwood, NJ, U.S.A.) were used. The UV detector was operated at a wavelength of 220 nm. The same solvent as for the packing was used and the flow-rate was 13 ml/h·cm^2. A mixture of bovine serum albumin and myoglobin was used as a test sample and 0.1 ml of a solution of 0.25% of each protein was injected. Bovine serum albumin and myoglobin were purchased from Wako (Osaka, Japan) and Sigma (St. Louis, MO, U.S.A.), respectively.

All experiments were carried out at 25 $\pm$ 2°C.

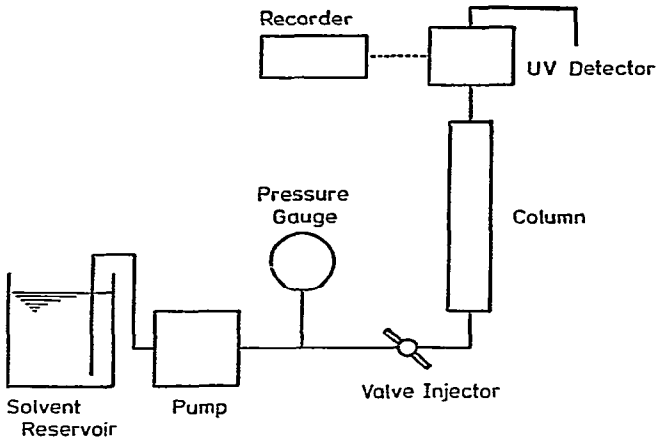

Fig. 2. Schematic diagram of the set-up for the measurements of column performance.

RESULTS AND DISCUSSION

The resolution factor for bovine serum albumin and myoglobin, R (BSA, myoglobin), was calculated from chromatograms of test samples as a measure of column performance, using the equation

$$R\,(\text{BSA, myoglobin}) = 2(V_2 - V_1)/(W_1 + W_2) \qquad (1)$$

where V and W represent elution volumes and peak widths at the base, respectively, of two components. The dependence of R (BSA, myoglobin) on the packing velocity is shown in Fig. 3. The same tendency was observed for both HW55S and HW55F, *i.e.*, R (BSA, myoglobin) was constant at packing velocities above certain critical values and decreased with packing velocity below these critical values. Therefore, the

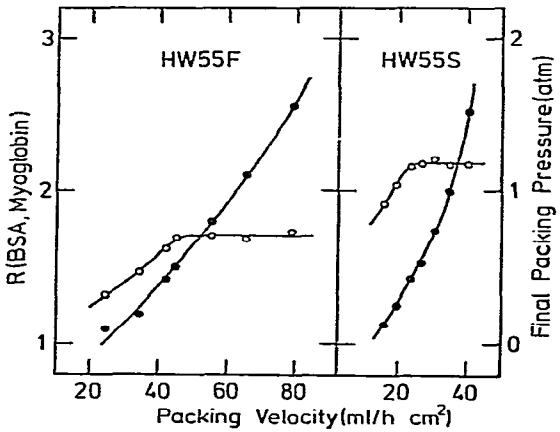

Fig. 3. Dependences of R (BSA, myoglobin) (○) and final packing pressure (●) on the packing velocity for Toyopearl HW55S and HW55F.

packing velocity should be high in order to obtain high-efficiency columns. However, extremely high packing velocities, resulting in high pressure drops, are not necessarily required.

The high column efficiency was probably obtained at high packing velocities partly because of the resultant dense packing, as predicted by Altgelt[9]. However, the high packing velocity seems to facilitate not only dense packing but also even packing. Fig. 4 shows chromatograms of the test sample obtained on columns packed at velocities of 25 ml/h·cm² (lower than optimum) and 79 ml/h·cm² (optimum). The peaks of two proteins were almost symmetrical with the column packed at 79 ml/h·cm², indicating that the bed was evenly packed. However, tailing peaks, which is a sign of an unevenly packed bed, were observed on the column packed at 25 ml/h·cm². All peaks showed tailing when a poor performance was observed on the columns packed at low velocities.

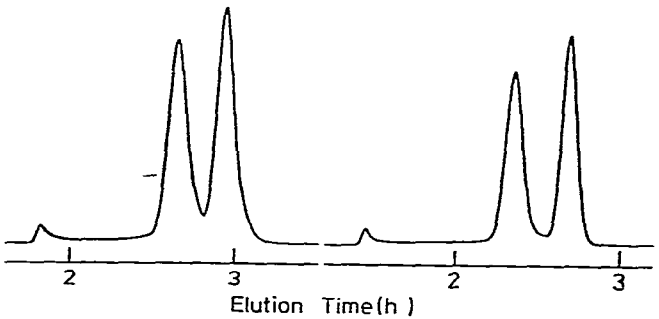

Fig. 4. Chromatograms of the test sample obtained on columns packed at velocities of 25 ml/h·cm² (left) and 79 ml/h·cm² (right).

It is interesting that although the critical packing velocities were approximately 25 and 45 ml/h·cm² for HW55S and HW55F, respectively, both of them corresponded to a final packing pressure of about 0.5 atm, as shown in Fig. 3. Further studies of this aspect are under investigation.

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