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Note

Packing of Toyopearl columns for gel filtration

II. Dependence of optimal packing velocity on column size

YOSHIO KATO*, KATSUO KOMIYA, TOSHINAO IWAEDA, HIROO SASAKI and TSUTOMU HASHIMOTO

Central Research Laboratory, Toyo Soda Mfg. Co., Ltd., Tonda, Shinnanyo, Yamaguchi (Japan)

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It is well known in high-performance liquid chromatography that the column packing technique greatly influences the performance of the columns obtained. However, the importance of the packing technique has not been recognized for soft gels such as Sephadex, as pointed out by Sosa[1]. Only a few studies have been reported on packings of soft gels[2,3] and efficient and reproducible packing procedures still seem to be lacking. 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), which is a hydrophilic porous polymer packing material for gel filtration, resistant to pressures up to several atmospheres. The dependence of column performance on packing velocity for constant-velocity packings has already been reported[4]. The influence of column size on the dependence of column performance on packing velocity is described in this note.

Toyopearl HW55S (Lot No. 55009-16M) of particle size 20–40 µm was used. This is the same material as Fractogel TSK HW55 (0.025–0.037 mm) available from E. Merck (Darmstadt, G.F.R.). This gel was packed into commercial glass columns (Amicon, Lexington, MA, U.S.A.) of several different sizes (Table I) by the constant-velocity method and the performance of the packed columns was tested with a mixture of bovine serum albumin and myoglobin as described previously[4], with two exceptions. A slurry with a 40% gel concentration was employed in the packing of

TABLE I

SIZES OF CHROMATOGRAPHIC COLUMNS AND SLURRY RESERVOIRS

Chromatographic column (cm)	*Slurry reservoir (cm)*
30 × 2.2 I.D.	45 × 2.2 I.D.
45 × 2.2 I.D.	60 × 2.2 I.D.
60 × 2.2 I.D.	90 × 2.2 I.D.
90 × 2.2 I.D.	120 × 2.2 I.D.
60 × 1.0 I.D.	120 × 1.0 I.D.
60 × 1.6 I.D.	90 × 1.6 I.D.
60 × 3.2 I.D.	90 × 3.2 I.D.
60 × 4.4 I.D.	90 × 4.4 I.D.

60 × 1.0 cm I.D. columns because of inferior packing reproducibility with a slurry concentration of 45% for this size of column. The protein concentrations of the solutions injected were varied in proportion to the column capacities.

The resolution factor for bovine serum albumin and myoglobin, *R*(BSA, myoglobin), was calculated by using eqn. 1 in ref. 4 as a measure of column performance. The dependences of *R*(BSA, myoglobin) and the final packing pressure on packing velocity are shown in Figs. 1 and 2. Very similar tendencies were observed for all columns (except the 90 × 2.2 cm I.D. column), *viz.*, *R*(BSA, myoglobin) was constant above some critical packing velocity and decreased with packing velocity below the critical point. On the other hand, the final packing pressures increased approximately linearly with increasing packing velocity at low packing velocities but began to rise more rapidly at higher packing velocities. Although these two kinds of critical packing velocities differed according to column size, the final packing pressures corresponding to them were independent of column size and were approximately 0.6 and 1.0 atm, respectively. Accordingly, we defined the packing velocities between the final packing pressures of 0.6 and 1.0 atm as optimum, and these optimal packing velocities are summarized in Table II and are plotted against column length and

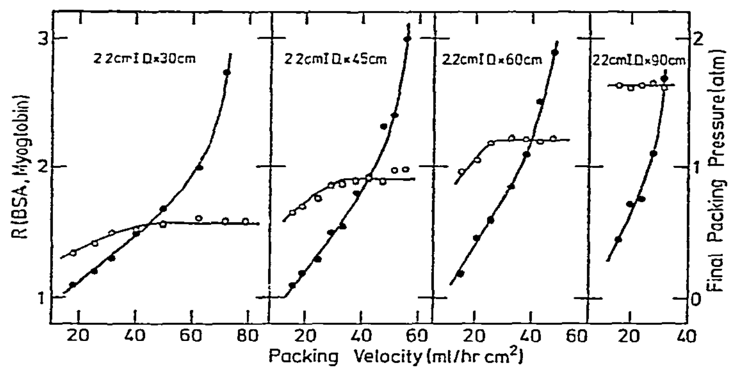


Fig. 1. Dependences of *R* (BSA, myoglobin) (○) and final packing pressure (●) on packing velocity in constant-velocity packings of Toyopearl HW55S in columns of 2.2 cm I.D. and various lengths.

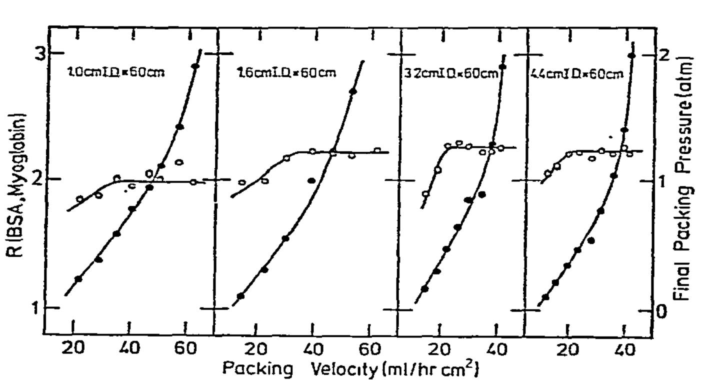


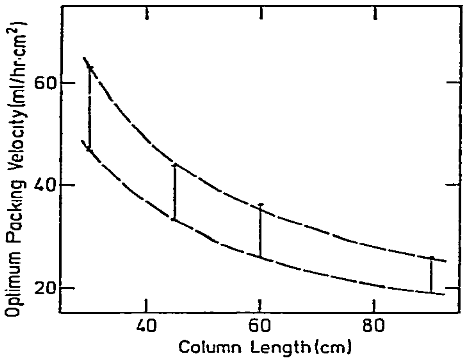
Fig. 2. Dependences of *R*(BSA, myoglobin) (○) and final packing pressure (●) on packing velocity in constant-velocity packings of Toyopearl HW55S in columns of various inner diameters and 60 cm length.

TABLE II

OPTIMAL PACKING VELOCITIES AND *R*(BSA, MYOGLOBIN) FOR COLUMNS PACKED AT OPTIMAL VELOCITIES FOR TOYOPEARL HW55S

Column dimensions (cm)	*Optimal packing velocity ($ml/h \cdot cm^2$)*	*R(BSA, myoglobin)*
30 × 2.2 I.D.	47–63	1.57
45 × 2.2 I.D.	33–44	1.90
60 × 2.2 I.D.	26–36	2.22
90 × 2.2 I.D.	19–26	2.65
60 × 1.0 I.D.	36–47	1.98
60 × 1.6 I.D.	31–42	2.21
60 × 3.2 I.D.	23–32	2.25
60 × 4.4 I.D.	22–32	2.23

column inner diameter in Figs. 3 and 4, respectively. These results indicate that the optimal packing velocities vary with both column length and column inner diameter. Therefore, the optimal packing velocity must be known for each column to be packed. However, it cannot be established exactly from the above results, but an approximate estimate may be possible by assuming that the optimal packing velocities are inversely proportional to column length, as shown in Fig. 5 for columns of 2.2 cm I.D. First, the optimal packing velocity for the 60 cm long column of the same inner diameter as the column to be packed is known from Fig. 4. Then, the optimal packing velocity is corrected for the column length by utilizing the assumption of the above inversely proportional relationship.



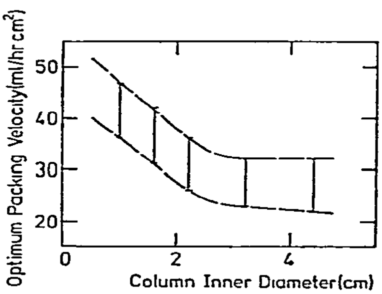
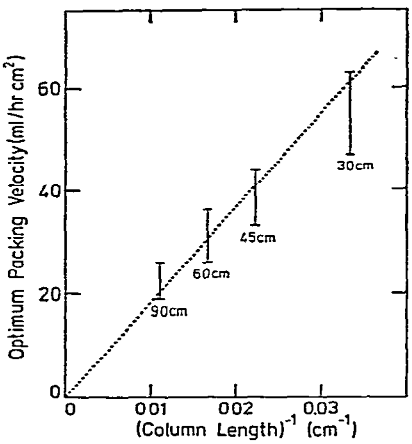


Fig. 3. Dependence of optimal packing velocity on column length in constant-velocity packings of Toyopearl HW55S in 2.2 cm I.D. columns.

Fig. 4. Dependence of optimal packing velocity on column inner diameter in constant-velocity packings of Toyopearl HW55S in 60 cm long columns.

Table II or Fig. 3 shows that the optimal packing velocity decreases with increasing the column length. This means that the time required to pack longer column increases more than proportionately to the column length. Moreover, as the maximal operating velocities of packed columns are lower than the packing velocities



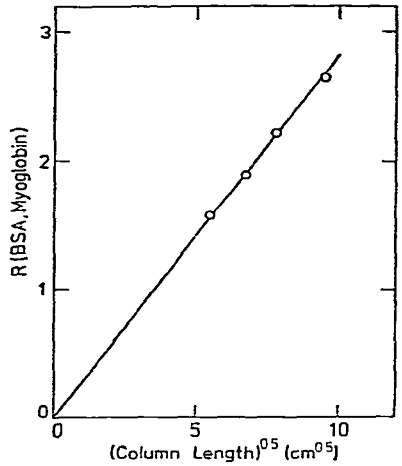


Fig. 5. Plots of optimal packing velocity against reciprocal of column length in constant-velocity packings of Toyopearl HW55S in 2.2 cm I.D. columns.

Fig. 6. Dependence of *R*(BSA, myoglobin) on column length for 2.2 cm I.D. columns packed with Toyopearl HW55S by the constant-velocity method at optimal velocities.

used initially, it is more convenient to use sever alshort columns in series when a longer column is required. Table II or Fig. 4 also shows that the optimal packing velocity increases with decreasing column inner diameter, especially with columns of small inner diameter. However, although almost the same resolutions were obtained with columns with inner diameters larger than or equal to 1.6 cm, a considerable decrease in resolution was observed with columns of 1.0 cm I.D., as shown in Table II. Therefore, very narrow columns are not desirable when there is no limitation to the column size. Columns of about 2 cm I.D. seem to be best in such instances.

Fig. 6 shows the dependence of *R*(BSA, myoglobin) on column length for 2.2 cm I.D. columns. *R*(BSA, myoglobin) was proportional to the square root of column length, as theoretically expected, indicating that longer columns were packed as well as shorter columns.

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