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Note

Packing of Toyopearl columns for gel filtration

IV. Gravitational packing and influence of slurry reservoir size

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We have been investigating packings of Toyopearl (Toyo Soda, Tokyo, Japan), a hydrophilic porous polymer packing material for gel filtration, resistant to pressures up to several atmospheres, and have already reported some results[1–3]. Whereas all columns were packed with a peristaltic pump by a constant-velocity or a semi-constant-pressure method in the previous experiments, this paper reports the results of investigations on the gravitational packing method, which is commonly used with packings of soft gels such as Sephadex[4,5]. In the previous experiments, all columns were also packed using slurry reservoirs consisting of long columns with the same diameters as the chromatographic columns. However, as commercial slurry reservoirs are generally short and have larger diameters than chromatographic columns, the influence of the slurry reservoir size on the performance of packed columns was also investigated.

GRAVITATIONAL PACKING

A schematic diagram of the packing arrangement is shown in Fig. 1.

Toyopearl HW55S (Lot No. 55009-16M) of diameter 20–40 μm, the same material as Fractogel TSK HW55 (0.025–0.037 mm) available from E. Merck (Darmstadt, G.F.R.), was packed gravitationally into various sizes of glass columns as described previously[2]. However, a hydrostatic pressure of 250 cmH_2O was utilized instead of a peristaltic pump to supply the solvent and each packing operation was continued overnight. The packed columns were tested for performance with a mixture of bovine serum albumin and myoglobin as described previously[2].

The results are summarized in Table I and compared with those for constant-velocity packings. The resolution factors for bovine serum albumin and myoglobin, *R*-(BSA, myoglobin), obtained with gravitational packings were lower than those obtained with constant-velocity packings at optimal velocities. However, the differences were within 10% and were only 5% on average. Furthermore, higher resolutions can be expected with gravitational packings under higher hydrostatic pressures because the resolution increased with increasing final packing pressure up to 0.6 atm with constant-velocity packings. Nevertheless, as a hydrostatic pressure of 250 cmH_2O

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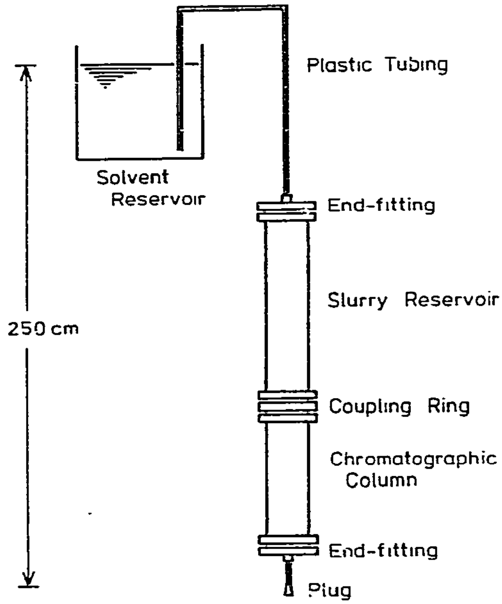

Fig. 1. Schematic diagram of arrangement for gravitational packing.

TABLE I

RESULTS FOR GRAVITATIONAL PACKINGS OF TOYOPEARL HW55S UNDER A HYDROSTATIC PRESSURE OF 250 cmH_2O

Column dimensions (length × I.D., cm)	*R(BSA, myoglobin)*	*R(BSA, myoglobin)**	*Final packing velocity (ml/h · cm²)*
30 × 2.2	1.49 (5%)**	1.57	20.5
	1.43 (9%)		19.8
45 × 2.2	1.78 (6%)	1.90	14.1
	1.89 (1%)		15.8
60 × 2.2	2.03 (9%)	2.22	9.8
	2.10 (5%)		11.9
90 × 2.2	2.63 (1%)	2.65	8.1
	2.53 (5%)		8.7
60 × 1.0	1.94 (2%)	1.98	13.6
	1.96 (1%)		15.5
60 × 1.6	2.12 (4%)	2.21	13.8
	2.06 (7%)		14.1
60 × 3.2	2.08 (8%)	2.25	11.3
	2.19 (3%)		9.4
60 × 4.4	2.11 (5%)	2.23	10.0
	2.07 (7%)		10.1

* Obtained by constant-velocity packings at optimal velocities[2].
** Values in parentheses are differences from *R*(BSA, myoglobin) for constant-velocity packings.

seems to be almost the maximum attainable in most laboratories, it may be concluded that constant-velocity packings with a pump are better than gravitational packings with respect to the resolution of packed columns for semi-soft gels such as Toyopearl. Moreover, the final packing velocities with gravitational packings were half to one third of the optimal packing velocities with constant-velocity packings. This means that columns obtained with gravitational packings must be operated at correspondingly lower velocities. Therefore, it is preferable to use pumps in the packing of semi-soft gels in order to take advantage of hardness of those gels.

INFLUENCE OF SLURRY RESERVOIR SIZE

Toyopearl HW55S (Lot No. 55009-16M) was packed into glass columns of various sizes using commercial slurry reservoirs of the shapes illustrated in Fig. 2. Both the chromatographic columns and the slurry reservoirs were purchased from Amicon (Lexington, MA, U.S.A.) and their sizes are given in Table II. Packings were carried out by constant-velocity or semi-constant-pressure methods.

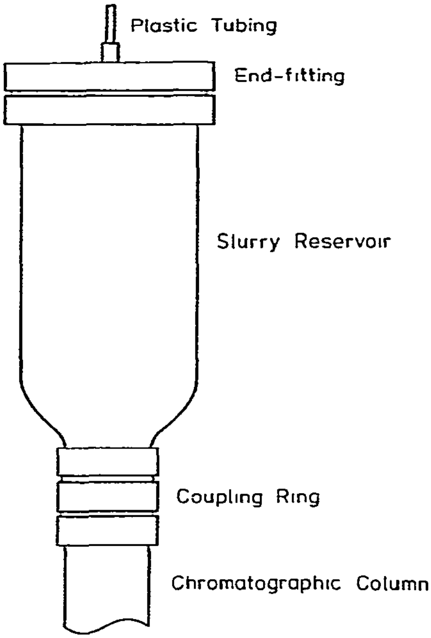

Fig. 2. Commercially available slurry reservoir.

R(BSA, myoglobin) values obtained with constant-velocity packings at various velocities are given in Table II. As *R*(BSA, myoglobin) was constant at final packing pressures above 0.6 atm with the previously examined constant-velocity packings using slurry reservoirs consisting of long columns of the same diameter as the chromatographic columns, packing was performed under such conditions in the present experiments. However, packings with commercial slurry reservoirs provided slightly lower values of *R*(BSA, myoglobin) than the previous packings, although the differences were only 2–3% and the *R*(BSA, myoglobin) values were independent of

TABLE II

RESULTS FOR CONSTANT-VELOCITY PACKINGS OF TOYOPEARL HW55S USING COMMERCIAL SLURRY RESERVOIRS

Column dimensions (length × I.D., cm)	*Slurry reservoir*		*Inner diameter ratio of slurry reservoir and column*	*Packing velocity (ml/h · cm²)*	*Final packing pressure (atm)*	*R(BSA, myoglobin)*
	I.D. (cm)	*Capacity (ml)*				
60 × 1.0	4.4	280	4.4	78.5	1.80	1.92
				73.0	1.65	1.86
				63.7	1.20	1.77
				54.4	0.95	1.66
60 × 1.6	4.4	290	2.8	55.5	1.55	2.04
				51.9	1.05	2.02
				45.6	1.00	1.97
				39.0	0.60	1.85
60 × 2.2	4.4	570	2.0	52.9	2.00	2.16
				48.5	1.42	2.17
				44.6	1.15	2.15
				39.5	0.98	2.20
				34.8	0.80	2.16
				30.0	0.62	2.15
60 × 3.2	6.1	1110	1.9	38.3	0.98	2.16
				34.1	0.75	2.14
				30.0	0.56	2.07
60 × 4.4	7.1	1470	1.6	31.7	1.05	2.16
				27.9	0.76	2.10
				24.1	0.52	2.15

packing velocity for columns of I.D. 2.2, 3.2 and 4.4 cm. With the I.D. 1.0 and 1.6 cm columns, however, the *R*(BSA, myoglobin) values increased with increasing packing velocity and even at final packing pressures of *ca.* 1.5 atm they were still 5–10% lower than those obtained previously. On the other hand, semi-constant-pressure packings at higher velocities provided improved column performances even in the columns of I.D. 1.6 cm, as shown in Table III.

TABLE III

RESULTS FOR SEMI-CONSTANT-PRESSURE PACKINGS OF TOYOPEARL HW55S INTO 60 × 1.6 cm I.D. COLUMNS USING COMMERCIAL SLURRY RESERVOIRS

Packing velocity (ml/h · cm²)		*Final packing pressure (atm)*	*R(BSA, myoglobin)*
Initial	*Final*		
85.5	51.9	1.70	2.06
85.5	60.0	1.60	2.15
85.5	46.5	1.48	2.20
85.5	61.5	1.52	2.16
85.5	51.6	1.50	2.08

It can be concluded that slurry reservoirs with the same inner diameter as chromatographic columns are best. With slurry reservoirs with inner diameters less than double the inner diameters of the chromatographic columns, the decrease in resolution of packed columns is only slight. With slurry reservoirs with inner diameters greater than double the inner diameters of the chromatographic columns, the packing velocity must be high in order to obtain reasonably high resolution columns.

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