Journal of Chromatography, 208 (1981) 71–73
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,569

Note

Packing of Toyopearl columns for gel filtration

III. Semi-constant-pressure packing

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

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

(Received December 2nd, 1980)

We have been investigating packings of Toyopearl (Toyo Soda, Tokyo, Japan), a hydrophilic porous polymer packing material for gel filtration, which is resistant to pressures up to several atmospheres, and have already reported on constant-velocity packings[1,2]. This note reports on semi-constant-pressure packing in which packing velocities initially set to high levels gradually decrease and pressure drops approach the pre-set maximum output pressures of the pump during the packing processes. This type of packing can be conveniently performed with a peristaltic pump since the maximum output pressure can be optionally and easily adjusted by controlling the degree of tightening of the rubber tube of the pump.

Packings of columns and tests of the packed columns were carried out as described previously[1,2], except that maximum output pressures of the peristaltic pump were adjusted before the start of packing. Toyopearl HW55S and HW55F (Lot Nos. 55009-16M and 55108M) were used. Particle sizes of these gels are, respectively, 20–40 μm and 30–60 μm. They are the same materials as Fractogel TSK HW55: 0.025–0.037 mm and 0.037–0.064 mm available from E. Merck (Darmstadt, G.F.R.).

Table I shows the results of packing HW55S (Lot No. 55009-16M) into 60 × 1.6 cm I.D. columns. In these packing procedures maximum output pressures of the pump were set to *ca.* 1, 2 or 3 atm and initial packing velocities were set to *ca.* 75 or

TABLE I
RESULTS OF SEMI-CONSTANT-PRESSURE PACKINGS OF TOYOPEARL HW55S INTO 60 × 1.6 cm I.D. COLUMNS

Expt. No.	*Packing velocity (ml/h · cm²)*		*Final packing pressure (atm)*	*R_s (BSA, Myo)*
	Initial	*Final*		
1	74	37	0.94	2.26
2	80	48	1.70	2.29
3	72	51	2.82	2.23
4	158	37	1.10	2.04
5	168	44	1.88	1.95

0021-9673/81/0000–0000/$02.50

160 ml/h·cm^2. Figs. 1–4 show the profiles of velocities, pressure drops and total effluent volumes as functions of time after the start of packing in experiments 1–4. The profile in experiment 5 was very similar to that in experiment 4, and these two experiments were almost constant-pressure packing procedures. Ratios of initial and final packing velocities ranged from 5:7 to 1:2 in experiments 1–3 and *ca.* 1:4 in experiments 4 and 5. Resolution factors for bovine serum albumin and myoglobin, R_s

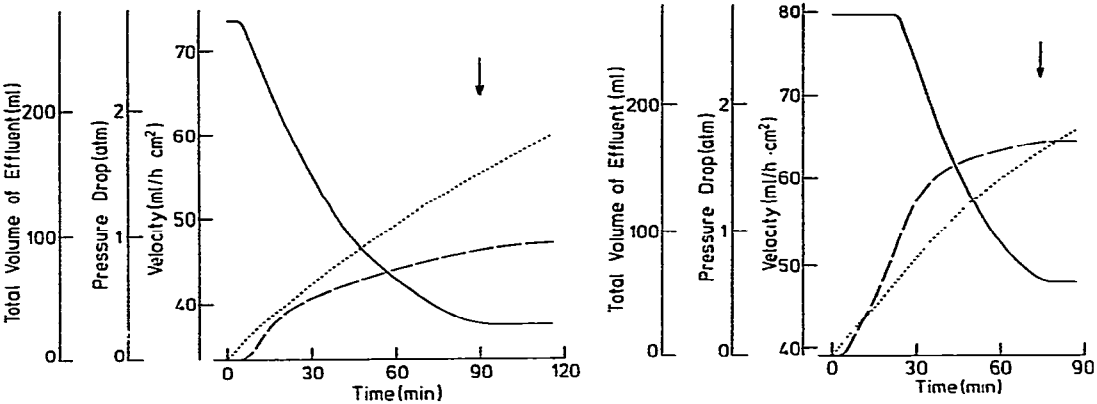

Fig. 1. Velocity (———), pressure drop (– – –) and total volume of effluent (·····) as functions of time after the start of packing in a semi-constant-pressure packing (experiment 1). The arrow indicates the time when the formation of gel bed has just completed.

Fig. 2. Velocity (———), pressure drop (– – –) and total volume of effluent (·····) as functions of time after the start of packing in a semi-constant-pressure packing (experiment 2). The arrow indicates the time when the formation of gel bed has just completed.

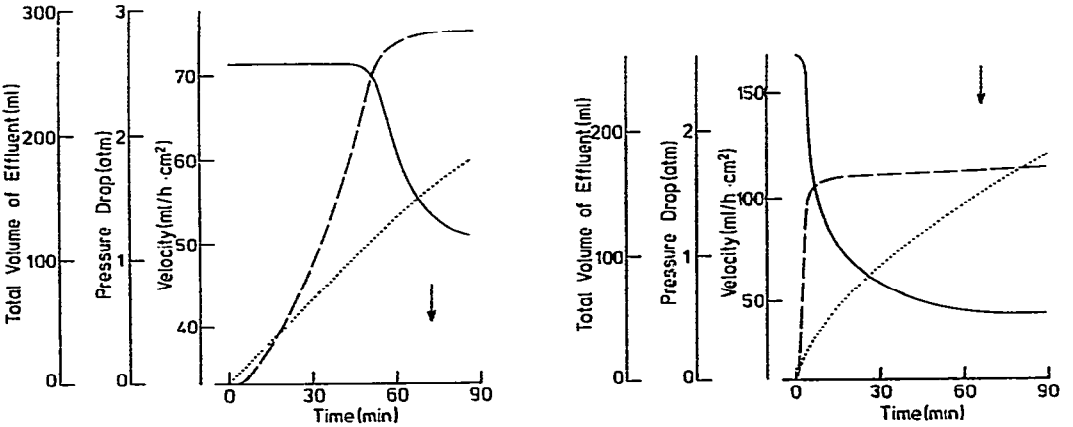

Fig. 3. Velocity (———), pressure drop (– – –) and total volume of effluent (·····) as functions of time after the start of packing in a semi-constant-pressure packing (experiment 3). The arrow indicates the time when the formation of gel bed has just completed.

Fig. 4. Velocity (———), pressure drop (– – –) and total volume of effluent (·····) as functions of time after the start of packing in a semi-constant-pressure packing (experiment 4). The arrow indicates the time when the formation of gel bed has just completed.

TABLE II
REPRODUCIBILITY OF SEMI-CONSTANT-PRESSURE PACKINGS OF TOYOPEARL HW55S AND HW55F INTO 60 × 2.2 cm I.D. COLUMNS

Gel	*Packing velocity (ml/h · cm²)*		*Final packing pressure (atm)*	R_s *(BSA, Myo)*
	Initial	*Final*		
HW55S	57	36	1.00	2.18
	59	39	1.13	2.16
	59	40	1.08	2.20
	61	42	1.20	2.16
	62	42	1.12	2.20
HW55F	123	62	1.25	1.69
	123	66	1.23	1.67
	123	60	1.08	1.71
	123	64	1.20	1.71
	123	69	1.20	1.72

(BSA, Myo) obtained in experiments 1–3 were almost the same as those obtained by constant-velocity packing procedures at optimum velocities (2.21 on the average[2]), whereas slightly lower values of R_s (BSA, Myo) were obtained in experiments 4 and 5. Therefore, constant-velocity packing procedures may be better for soft or semi-soft gels than the constant-pressure procedures widely applied to rigid packing materials[3–5]. However, semi-constant-pressure packing procedures in which decreases in velocities during the processes are less than 50% can be considered comparable with constant-velocity procedures with regard to resolutions of packed columns. On the other hand, a semi-constant-pressure procedure can be completed in a shorter time than the corresponding constant-velocity procedure at the same final packing pressure. Furthermore, reproducibility of semi-constant-pressure packing procedures investigated by packing Toyopearl HW55S and HW55F (Lot No. 55108M) into 60 × 2.2 cm I.D. columns five times under similar conditions was excellent, as indicated in Table II. In addition, the semi-constant-pressure procedures would be very convenient in practice, especially in glass columns resistant only to low pressures, since the final packing pressures can be optionally controlled.

REFERENCES

1 Y. Kato, K. Komiya, T. Iwaeda, H. Sasaki and T. Hashimoto, *J. Chromatogr.*, 205 (1981) 185.
2 Y. Kato, K. Komiya, T. Iwaeda, H. Sasaki and T. Hashimoto, *J. Chromatogr.*, 206 (1981) 135.
3 R. E. Majors, *Anal. Chem.*, 44 (1972) 1722.
4 J. J. Kirkland, *J. Chromatogr. Sci.*, 10 (1972) 593.
5 R. M. Cassidy, D. S. LeGay and R. W. Frei, *Anal. Chem.*, 46 (1974) 340.