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Note

Rapid method for packing microparticulate columns packed with a chemically bonded stationary phase for high-performance liquid chromatography

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A number of methods for packing microparticulate high-performance liquid chromatographic (HPLC) columns have been published, but the techniques are still under development. The recent extensive use of microparticulate columns for HPLC has led to the need for simple methods for preparing inexpensive and high-efficiency columns in the laboratory. Several workers[1–4] have discussed the advantages and disadvantages of various packing methods such as the balanced density[5–13], balanced viscosity[7,13–15] and mechanical stirring methods[16–19] and the use of the mobile phase[20], and proposed convenient methods that give good microparticulate columns for routine use. A technique that gives high-efficiency (15.6 μm HETP) silica gel microparticulate columns (*ca.* 3–5 μm) has been described[13]. However, many of the methods are not suitable for chemically bonded stationary phases but only for silica gel columns. So far, few methods that guarantee high-efficiency (HETP less than 20 μm) home-made microparticulate columns packed with a chemically bonded stationary phase have been reported.

In this paper, a rapid packing method that gives high-efficiency chemically bonded normal- and reversed-phase microparticulate columns (*ca.* 5 μm) for routine use is described.

EXPERIMENTAL

Solvents and materials

The solvents used were of special grade from Wako (Osaka, Japan). Methanol was filtered through 0.22-μm Fluoropore filters (Sumitomo Electric, Osaka, Japan) before use. Nonipole 40 (nonylphenyl polyethylene glycol, "4 moles" ether) was purchased from Sanyo Kasei Kogyo (Kyoto, Japan). The composition of the slurry solvent is shown in Table I. Polygosil and Nucleosil materials were obtained from Machery, Nagel & Co. (Düren, G.F.R.) and LiChrosorb RP-18 from E. Merck (Darmstadt, G.F.R.).

Apparatus

The slurry reservoir is shown in Fig. 1. The reservoir is made of 316 grade seamless stainless steel and holds 45 ml of slurry up to the top inlet. The packing

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TABLE I

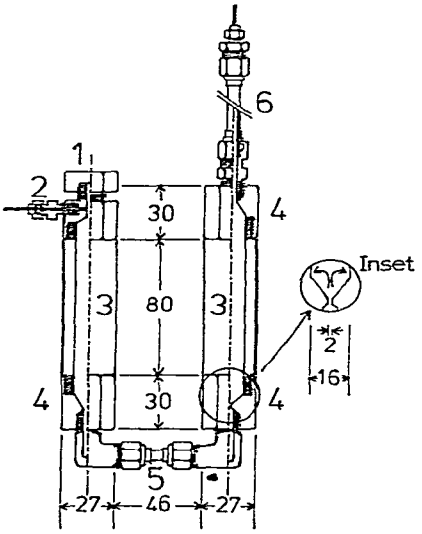
APPROPRIATE AMOUNTS OF PACKING MATERIALS AND COMPOSITION OF SLURRY SOLVENT

Packing material	*Appropriate amount of packing material*		*Solvent composition*	
	Amount (g)	*Column length and I.D. (mm)*	*Component*	*Concentration (%, v/v)*
LiChrosorb RP-18 (5 μm)	1.5	150 × 4.0	Methanol	10.0
Polygosil 60-5C_{18}	3.0	300 × 4.0	Isopropanol	5.0
Polygosil 60-5NO_2	2.0	150 × 4.6	Cyclohexanol	10.0
Nucleosil 5NO_2	2.5	200 × 4.6	Cyclohexane	4.0
Polygosil 60-5NH_2	3.2	250 × 4.6	1,1,1-Trichloroethane	70.0
Nucleosil 5NH_2			Nonipole 40	1.0

apparatus is shown in Fig. 2. A Coulter Electronics (Hialeah, FL, U.S.A.) TA II particle analyser was employed to determine the particle size of the slurried materials by using 4% lithium chloride in methanol[21,22].

Packing procedure

The appropriate amount of packing material for the column dimensions (see



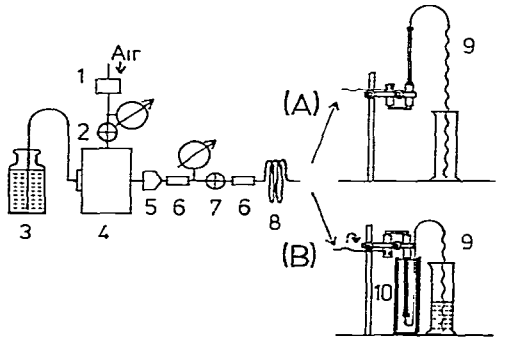


Fig. 1. Slurry reservoir. 1 = Top inlet and removal cap with a PTFE gasket; 2 = eluent inlet; 3 = cell (20 ml); 4 = union; 5 = Tylok (Euclid, OH, U.S.A.) 316 grade stainless-steel 1/4-in. standard connections; 6 = column. Dimensions in millimetres.

Fig. 2. Packing apparatus. 1 = Pressure regulator (0–10 kg/cm^2); 2 = on-off valve; 3 = eluent reservoir (methanol); 4 = Haskel (Burbank, CA, U.S.A.) MPC-110 pneumatic amplifier pump; 5 = damping unit (60 ml); 6 = 10 cm × 4.6 mm I.D. guard column packed with Polygosil 60-2540; 7 = high-pressure valve; 8 = 2 m × 1.7 mm O.D. 316 grade stainless-steel tube; 9 = 1.7 mm O.D. PTFE tube; 10 = heating-bath (60°C).

Table I) was mixed with 40 ml of the slurry solvent and the suspension was placed in an ultrasonic bath for 5 min. The dispersed slurry was poured into the slurry reservoir by using a syringe, and the empty part of the reservoir was filled with the slurry solvent. The packing was started with an upward flow (see Fig. 2A). The packing pressure on to the eluent (methanol) was programmed stepwise from 400 to 700 kg/cm^2 at 50 kg for every 5 cm of the packing. When 15 ml of the eluent had been pumped, the reservoir was inverted (see Fig. 2B), then the column was heated to 60°C. Once the packing was completed, the eluent was allowed to flow at room temperature for 10 min under the final packing pressure. The flow was then stopped and the column was removed from the reservoir and assembled with the frit and the reducing unit.

RESULTS AND DISCUSSION

A relatively non-toxic and very stable slurry solvent was prepared (see Table I). Methanol was used to increase the dispersibility of the solvent and isopropanol and cyclohexanol to control viscosity other than dispersibility. The use of the nonionic surfactant (Nonipole 40) was effective in preparing stable and well dispersed slurries of the packings (*ca.* 5 μm). The use of 0.5–1.5% (v/v) of surfactant minimized the rates of sedimentation and the sedimentation volumes in 24 h for both normal- and reversed-phase materials. The rates of sedimentation, defined as the ratio of height of sedimentation to standing time, were constant for 1 h in the range 0.07–0.11 mm/min for the packing materials used. The use of less or more of the surfactant was less effective in reducing aggregation or flocculation of the dispersed particles. The particle size analysis and direct microscopic observations showed that the slurries were stable and well dispersed.

The reservoir used resulted in minimal use of the packing materials (see Table I), with substantial savings of the packing materials over conventional methods. As slurries usually form aggregates at the bottom, the upward packing technique[1,17,18] may be useful in introducing a dispersed slurry into the column, especially in the initial packing stage. In the initial upward packing with the U-shaped reservoir, the nozzle at the bottom of the second cell (see Fig. 1, inset) was useful for producing mixing effects around the bottom so as to maintain well dispersed particles.

The pressure programming and the column heating were used to maintain constant high flow conditions. The flow-rates in preparing 20 cm × 4.6 mm I.D. columns became stable with 1 min, and were maintained nearly constant until the packing was completed. Typical flow-rates were 7–8 ml/min for LiChrosorb RP-18 and the Polygosil materials and 3–4 ml/min for the Nucleosil materials. Thus, the packing of the columns was completed in a much shorter time than by conventional methods. The high flow-rate may be useful in packing the dispersed slurry into the column before aggregation occurs.

A number of 20 cm × 4.6 mm I.D. microparticle columns were prepared at different times and their performances were examined. Few failures of the packings occurred. Fig. 3 shows typical chromatograms for the evaluation of the column performance. Table II indicates that high-efficiency columns are reproducibly prepared by the proposed method. Occasionally, superior columns with up to 18,000 theoretical plates (11.1 μm HETP) were obtained for LiChrosorb RP-18 and Poly-

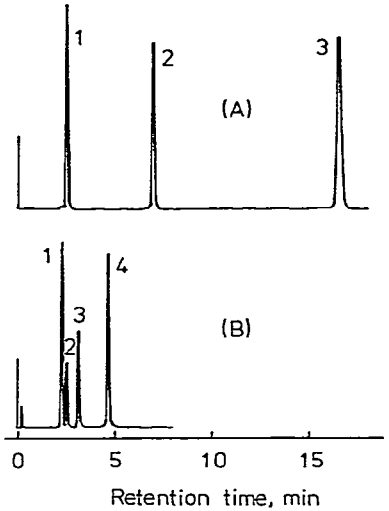


Fig. 3. Typical liquid chromatograms for evaluation of column performance. Columns, 20 cm × 4.6 mm I.D.; mobile phase, acetonitrile–water (60:40); flow-rate, 1.0 ml/min; amount of compound, 0.2–1.5 μg in a 2-μl injection; UV absorbance detector, 254 nm. (A) Polygosil 60-5C_{18}: 1 = uracil; 2 = benzene; 3 = acenaphthene. (B) Nucleosil 5NH_2: 1 = benzene; 2 = *o*-chlorophenol; 3 = uracil; 4 = 2,5-dichlorophenol.

TABLE II

PERFORMANCE OF MICROPARTICULATE COLUMNS PACKED WITH CHEMICALLY BONDED STATIONARY PHASES

Analytical conditions: see Fig. 3. The capacity factors (k') of LiChrosorb RP-18 and Polygosil 60-5C_{18} columns were obtained by using acenaphthene as a reference peak and uracil as an unretained peak, and the k' values of Nucleosil 5NH_2 columns were obtained by using 2,5-dichlorophenol as a reference peak and benzene as an unretained peak.

Packing material	*Column number*	*Column performance*			
		Theoretical plates	*HETP (μm)*	*Reduced HETP*	*k'*
LiChrosorb RP-18 (average size 5.7 μm)	1	10,700	18.7	3.3	8.64
	2	11,700	17.1	3.0	8.61
	3	10,900	18.3	3.2	8.75
	4	11,600	17.2	3.0	8.41
Polygosil 60-5C_{18} (average size 4.9 μm)	1	11,500	17.4	3.6	6.10
	2	12,500	16.0	3.3	6.02
	3	12,800	15.6	3.2	6.09
	4	11,400	17.5	3.6	5.97
Nucleosil 5NH_2 (average size 4.2 μm)	1	11,800	16.9	4.0	1.12
	2	12,400	16.1	3.8	1.09

gosil 60-5C_{18}. The number of theoretical plates obtained here are very similar to or greater than those for columns previously reported or commercially available.

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