PREPARATION OF SHORT GLASS MICROBORE COLUMNS FOR LIQUID CHROMATOGRAPHY AND THEIR PROPERTIES

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Received April 21st, 1983

The preparation is described of thick-walled glass columns, inner diameter 0·5 mm and outer diameter 4·5 mm, packed with Separon SI C 18 (d_p = 6 and 10μm) from suspension under pressures of 20—35 MPa in the presence of an ionogenic detergent; the column length ranged from 5 to 30 cm. The minimum value of the height equivalent to a theoretical plate was $H_{min} = 2·8 - 3·3d_p$. The effect of the extra-column contributions to the dispersion on the slope of the linear part of the dependence of the height equivalent to a theoretical plate on the mobile phase velocity was examined and found to become significant at higher velocities, thereby imposing limitations on the shortening of the time of analysis.

A number of papers have appeared recently dealing with the application of small-bore packed columns (diameter below 1 mm) in liquid chromatography. Such columns feature distinct advantages such as savings of the expensive stationary and mobile phases, possibility of a direct LC-MS coupling, *etc.* On the other hand, the use of small-bore columns poses also some problems. High demands are placed on the miniaturization of the equipment incorporating the column for preventing efficiency deterioration arising from the extra-column contribution to the band broadening. Columns with inner diameters above 0·5 mm and lengths ranging from 25 cm to several metres[1-7] can often be coupled to adapted commercial equipment (pump, sample injector, detector). Columns with inner diameters of 0·12—0·5 mm and lengths of 3—100 cm (refs[8-15]), however, require a complex miniaturization of the entire apparatus. This applies, in particular, to short columns (not longer than 10 cm) with diameters below 0·25 mm.

The hitherto reported small-bore packed columns were made of Teflon, stainless steel, thin-walled glass, or fused silica capillaries. The effect of the column material on the efficiency has been examined; glass[9] and fused silica[10] capillaries have been found superior to those made of Teflon or stainless steel owing to the smoothness of their inner walls. This is the reason why glass was also chosen as the material for our columns. Since small-bore thin-walled columns require a similar operation technique as capillary columns and work with the brittle glass capillaries is rather troublesome, thick-walled glass capillaries, which are mechanically better resistant, were employed by us.

Little information is available as to the procedure of packing small-bore columns with the stationary phase. The sorbent is filled into the column from suspension using basically the same techniques as used for conventional packed columns. Acetonitrile[9], 2-propanol[7], 25% glycerol in methanol[1], and propanol–water 1 : 1 mixture[6] have been employed as solvents for the suspensions. The concentration of the suspension (if reported) was usually[7] about 10% (*m*/*m*), only Koroleva and co workers[6] used concentrations of 40–60% (*m*/*m*). Data about the pressures are also scarce. Scott and Kucera[1] applied a pressure of 170 MPa for filling 1 × 1 000 mm columns, Vadukul and Loscombe[7], 37 MPa for 1 × 500 mm columns, and Koroleva and co-workers[6], 10–12 MPa for 0·5 × 250 mm columns.

A negative factor in the preparation of suspensions of small particles (below 10 μm) is the particle agglomeration brought about by Coulomb forces. These attractive forces are supposed[16] to arise from interactions between the negative centres and the positive hydrogen of the acid silanol groups, which are present on the surface of silica gel as well as of the reverse phases, and can be eliminated by imparting a positive or negative charge to the surface of the particles; this can be achieved by adding aqueous ammonia[17], basic alkylamines[16], strong organic acids, aqueous buffer[18], or nonionogenic detergent[6] to the suspension. An ionogenic detergent, *viz.* sodium dodecyl sulphate, was employed by us for suppressing the reverse phase particle agglomeration.

An electrochemical detector with a cell volume of 20 nl and an injector valve with a loop volume of 0·2 μl, designed for operation with small-bore packed columns, were described by us previously[19,20]; the equipment was found applicable in conjunction with 0·5 × 100 mm columns, the extra-column contributions to the peak dispersion being negligible as compared with the contribution of the column itself. The present paper reports on the preparation of 5–30 cm long thick-walled columns with an inner diameter of 0·5 mm and outer diameter of 4·5 mm, and their packing with reverse phase particles 6 and 10 μm in diameter applying pressures of 20 to 35 MPa. A glycerol–ethanol mixture containing sodium dodecyl sulphate as detergent served as the medium for the suspension.

EXPERIMENTAL

Preparation of the packed microbore columns. A tube of soft PN glass (Jablonec Glass Works, Jablonec), i.d. 0·5 mm, o.d. 4·5 mm, was cut to the requisite length and the ends were ground perpendicularly; no additional treatment of the glass was applied. Metal fittings threaded for interfacing the column to the injector valve and to the detector were sealed to the column ends with an epoxy resin. Columns 5, 10, 20, and 30 cm long were packed with Separon SI C 18 (Laboratorní přístroje, Prague) the declared particle size of which was 5 and 10 μm. The material was filled from a glycerol–ethanol 1 : 1 mixture containing 0·1% (*V*/*V*) sodium dodecyl sulphate; methanol served as the pressure liquid. The sorbent content in the suspension was 15% (m/V) for the articles 10 μm in diameter, while for those 5 μm in diameter, better results were obtained

if their content in the suspension was 10% (m/V) only. The pressures applied were 20—35 MPa, according to the particle size and column length. After the packing procedure, the columns in the packing equipment were washed with methanol and water respectively.

Determination of the particle size distribution for Separon SI C 18. Electron microscope photographs (magnification 1 000×) were taken and evaluated for 150 particles selected at random. The distribution curves were plotted and the mean particle size was calculated.

Apparatus and procedure. The chromatograph employed was as described previously[19,20]. The solutes were injected by means of a valve with an inner loop of 0·2 μl; the column was screwed directly into the valve body without any coupling device. A two-electrode elctrochemical detector involving a polarizable platinum electrode, with a cell volume of 20 nl, was also attached directly to the column. The mobile phase contained 50% (V/V) acetonitrile, 1% (V/V)acetic acid, and sodium perchlorate (0·1 mol l^{-1}) in water. Injected was a test mixture of 1,3-dihydroxynaphthalene, β-naphthol, and 2-methyl-4-ethylphenol in the mobile phase.

RESULTS AND DISCUSSION

Thick-walled glass tubes proved to serve well as the bodies of small-bore columns; they could be run, with no preliminary treatment, under a pressure of 35 MPa without breakdown.

For determining the efficiency of the packed small-bore columns, the knowledge of the mean particle size of the stationary phase and of the particle size distribution was prerequisite. The particle size distribution curves obtained by evaluating the electron microscope photographs are plotted in Fig. 1. The data for the set of 150 particles led to the mean particle diameters of 6·1 and 10·4 μm, respectively.

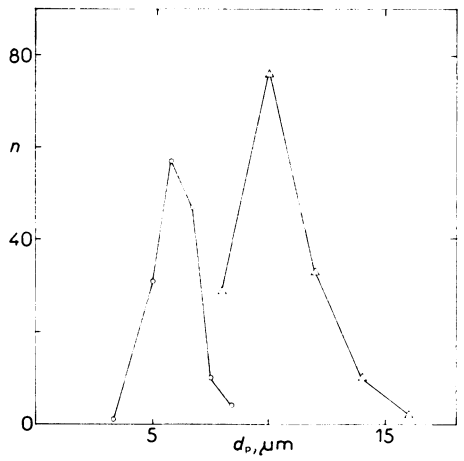

FIG. 1
Particle size distribution curves for Separon SI C 18, d_p = 6 and 10 μm

The height equivalent to a theoretical plate H was measured in dependence on the velocity of the mobile phase u for columns 5, 10, 20 and 30 cm long packed with particles 10 μm in diameter and for columns 5, 10, and 20 cm long packed with particles 6 μm in diameter. The results are plotted in Figs 2 and 3, repectively. The shape of the curves for $d_p = 10$ μm is virtually identical for all of the column lengths used. The height of a theoretical plate in the minimum of the curve is $H_{min} = 29-32$ μm for the columns 10–30 cm long and is independent of the capacity ratio of solute k. For the 5 cm column the values are somewhat higher, *viz.* $H_{min} = 36$, 35, and 34 μm for $k = 1{\cdot}1$, 2·8, and 4·4, respectively. The shape of the curves indicates that in accordance with work[20], the external contributions of the equipment to the curve dispersion are negligible.

This is not true of the short columns packed with sorbent of the grain size of 6 μm used at higher velocities of the mobile phase. Here the resulting height equivalent to a theoretical plate H is a sum of the contributions to the dispersion within the column, H_C, and in the external space, H_E,

$$H = H_C + H_E \,. \qquad (1)$$

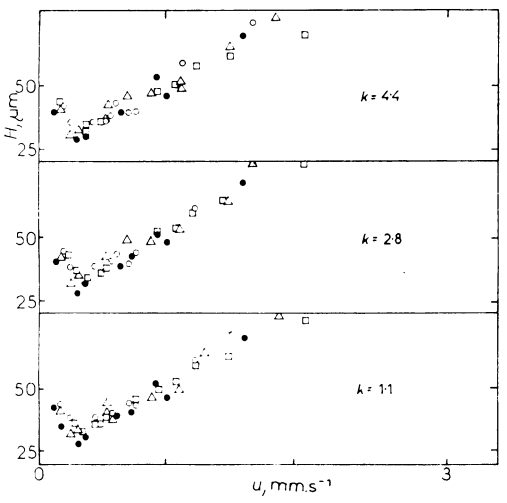

FIG. 2

Dependence of the height equivalent to a theoretical plate (H) on the linear velocity of the mobile phase (u) for columns packed with particles with $d_p = 10$ μm. Column length (cm): ○ 5, △ 10, □ 20, ● 30

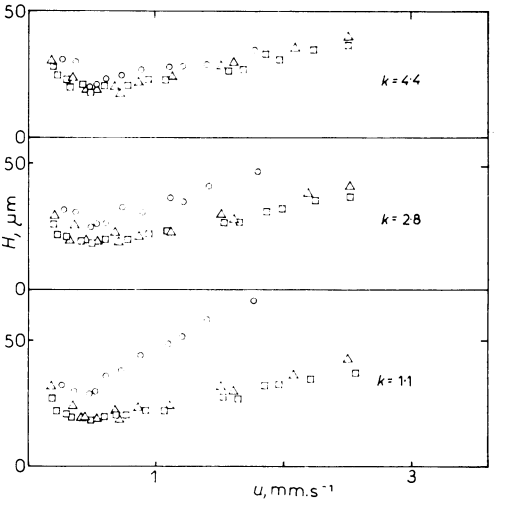

FIG. 3

Dependence of the height equivalent to a theoretical plate (H) on the linear velocity of the mobile phase (u) for columns packed with particles with $d_p = 6$ μm. Column length (cm): ○ 5, △ 10, □ 20

If the dependence of the height equivalent to a theoretical plate on the velocity of the mobile phase is followed up to velocities so high that $B/u \ll (A + Cu)$, so that the coefficient of the molecular diffusion in the mobile phase B can be neglected, the coefficient of the mass transfer between the phases C can be written as

$$C = (H_C + H_E - A)/u \,. \tag{2}$$

Assuming that for the 20 cm long column and solute with $k = 4{\cdot}4$ for all columns packed with the sorbent with $d_p = 6$ μm, H_C is much greater than H_E, then the experimental value (Table I) is

$$C_C = (H_C - A)/u = 0{\cdot}010 \pm 0{\cdot}001 \,. \tag{3}$$

Furthermore, rearranging Eq. (2) we have

$$C = (H_C - A)/u + H_E/u = C_C + C_E \tag{4}$$

and combining with Eq. (3),

$$C_E = C - C_C = C - 0{\cdot}010 \,. \tag{5}$$

The height equivalent to a theoretical plate corresponding to the solute dispersion within the column is

$$H_C = H - C_E u \,. \tag{6}$$

The experimental height equivalent to a theoretical plate and the calculated H_C values are given in Table II, together with the H'_C values calculated from Eq. (1) based on the experimental H_E values. The experiment was carried out so that the chromatographic column was replaced by a capillary 25 mm long with a diameter of 50 μm.

TABLE I

Experimental C values for columns packed with Separon SI C 18, grain size 6 μm

Solute	k	C (s) for the column length, cm		
		5	10	20
1,3-Dihydroxynaphthalene	1·1	0·025	0·012	0·010
2-Naphthol	2·8	0·017	0·014	0·010
2-Methyl-4-ethylphenol	4·4	0·009	0·011	0·010

The contribution of this capillary to the spreading is negligible against the external contribution. The H_C and H'_C values agree to within 18%.

TABLE II

Comparison of the H_C values calculated from Eq. (*6*) and the H'_c values calculated by virtue of the experimental H_E values for a 5 cm column packed with sorbent $d_p = 6$ μm

u mm s^{-1}	H mm	H_C mm	H'_C mm	H'_C/H_C[a]
		$k = 1{\cdot}1$, $C = 0{\cdot}025$ s, $C_E = 0{\cdot}015$ s		
0·88	0·044	0·031	0·037	1·19
1·10	0·048	0·032	0·038	1·19
1·20	0·051	0·033	0·041	1·24
1·39	0·058	0·037	0·041	1·11
1·77	0·065	0·040	0·047	1·20
		$k = 2{\cdot}8$, $C = 0{\cdot}017$ s, $C_E = 0{\cdot}007$ s		
0·88	0·031	0·025	0·029	1·16
1·10	0·037	0·029	0·034	1·17
1·20	0·035	0·027	0·032	1·18
1·39	0·042	0·032	0·038	1·19
1·77	0·047	0·035	0·042	1·20

[a] Average value: $1{\cdot}18 \pm 0{\cdot}04$.

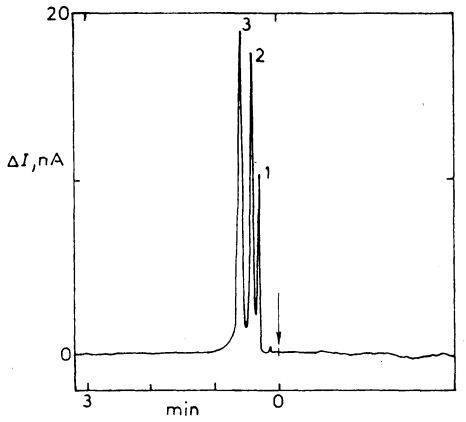

FIG. 4

Separation of aniline derivatives on a column 0·5 × 50 mm packed with Separon SI C 18, $d_p = 6$ μm. Mobile phase: 50% (V/V) acetonitrile, 1% (V/V) acetic acid, sodium perchlorate (0·1 mol l^{-1}) in water, $u = 3{\cdot}0$ mm . . s^{-1}. Solute: *1* aniline, *2* N-methylaniline, *3* N,N-dimethylaniline

It can be taken for proved that the extra-column contributions to the peak dispersion impose a serious constraint to the use of microbore columns in the region of higher velocities of the mobile phase, hence, in conditions necessary for shortening the time of analysis. This can be illustrated on the column 5 cm long packed with the sorbent with $d_p = 6$ μm. While in the range of minimum H, *i.e.*, at $u = 0{\cdot}5$ mm s^{-1}, the $(H_C + H_E)/H_C$ ratio for $k = 1{\cdot}1$ is about 1·3, it becomes as high as 1·6 if the mobile phase is allowed to flow at a velocity of $u = 1{\cdot}8$ mm s^{-1}.

The results obtained give evidence that columns 0·5 mm in diameter and 5 – 30 cm long can be packed as described, applying medium pressures (up to 35 MPa), their efficiency comparing well with that of conventional packed columns. Of importance is the presence of the ionogenic detergent in the suspension, suppressing the particle agglomeration. The efficiency of the columns was measured for suspensions with various concentrations of the detergent, and a concentration of 0·1% (m/m) was found sufficient. In contrast to ref.[7], where columns 1 mm in diameter and 50 cm long were packed at a pressure of 37 MPa for 5 hours, the packing of our 30 cm column with particles 10 μm in diameter under the pressure of 35 MPa was complete in approximately one and a half hours; the packing with the sorbent itself takes no more than half an hour, but the column must be then washed thoroughly with methanol to remove perfectly any trace of detergent.

The 5 cm column packed with particles 6 μm in diameter was employed for a rapid analysis of a mixture of substituted anilines (aniline, N-methylaniline, and N,N-dimethylaniline). The mobile phase was the same as used for the column testing. The corresponding chromatogram is shown in Fig. 4 at a linear velocity of the mobile phase of 3 mm s^{-1}, the last component eluted from the column within 50 s.

Thus, being mechanically better resistant than thin-walled glass capillaries and withstanding pressures in excess of 35 MPa, thick-walled glass capillaries proved to suit well as the bodies of small-bore packed columns. The packing technique, used under medium pressures (up to 35 MPa) in the presence of detergent, is usable for columns 5 – 30 cm long and 0·5 mm in diameter, which then satisfy well all the demands imposed on efficient packed columns. The relative contribution of the external factors to the band dispersion increases not only with the decreasing retention of solute and reduction in the column volume, but also with increasing flow rate of the mobile phase, which thus becomes a constraint to efforts made to shorten the time of analysis.

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Translated by P. Adámek.