Journal of Chromatography, 149 (1978) 13–28

CHROM. 10,841

PACKING, PERFORMANCE AND PERMEABILITY OF LARGER AND WIDER LIQUID CHROMATOGRAPHY COLUMNS AND THEIR USE IN PREPARING SAMPLES FOR IDENTIFICATION

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SUMMARY

Liquid chromatographic columns, which are longer, and perhaps wider, than those commonly used for analytical work, have advantanges in small-scale preparative work, in size-exclusion chromatography and in giving a very high peak capacity without changing the eluent composition during the chromatography.

Packing 8-mm bore columns up to 500 mm long from a deflocculated methanol slurry is simple and reliable even for materials with a wide range of particle sizes, but it has only proved possible to achieve the expected performance for 1-m columns with closely sized spherical materials. The performance of Spherisorb silica microspheres prepared or mixed to give a wider range of particle size, judged by the separation impedance, is nearly equivalent to that of closely sized materials provided that the ratio of the smallest to largest particle sizes does not exceed about 4.

INTRODUCTION

The packing and use of short liquid chromatographic (LC) microparticle (usually <250 mm) columns is now too well studied and established to need further discussion, except that it is perhaps worth observing that the large number of methods, recently reviewed by Martin and Guiochon[1], presented as optimal by many authors provide contradictory evidence about the actual processes involved, and despite all the work done our understanding of the packing process is far from complete. There is agreement that microparticle packing materials should be filtered on to the growing column bed from a slurry, but little else. A wide range of liquids and mixtures have been declared to be optimal, or at least preferred. Variations in testing methods and reporting results makes it difficult to draw many clear conclusions from all of these results except to conclude that the choice of slurry liquid and packing pressure is not very important in practice. (To be significantly different, two columns need to have plate numbers about 30% different because the plate concept squares the real resolving power or efficiency[2]).

For nearly all analytical problems, there is little point in trying to increase the resolving power by increasing the column length because each doubling of length

gives only a 50% increase in resolving power, but also doubles the analysis time and solvent consumption per analysis. For many problems, columns 100 mm long giving 5000 plates are a good compromise and only prejudice and the advantages of standardization prevent the use of even shorter columns. Nevertheless, as with gas chromatography, there is always a need felt, if small, for ever higher efficiency. Kraak *et al.*[3], Martin *et al.*[4] and Godbille and Devaux[5] have shown what separations are feasible with 50,000-plate columns.

Two other areas sensibly need columns with large plate numbers. One is exclusion chromatography (EC)[6–10], also known as gel permeation chromatography. With this technique, all peaks are eluted before or at the "solvent front" of conventional LC (molecules must permeate to be retained). No separation can be achieved by increasing the retention parameter k' (column capacity ratio). A high plate number and highly porous particles are needed for high-efficiency separation, and small particles for high-speed separations.

The second application area is in small-scale preparative LC where the objective is to collect small amounts of sample for identification by other methods, such as NMR and IR spectroscopy. In order to compensate for the decrease in separation caused by mass and/or injection volume overloading, it is necessary to use either a longer column or to recycle. Of these alternatives, recycling is probably more powerful, but it is more complicated and has certain limitations[11]. Longer columns are a potentially simpler alternative, especially as analytical materials can be used and separations scaled up easily. In order to retain the plate number of an analytical column, typically several thousand, it is useful to increase the length by 5–10-fold and perhaps to increase the diameter of the column also, thus further increasing the weight of silica in the column.

For EC it is also useful to use a larger column diameter because the actual peak volumes are so small (for completely excluded compounds roughly half of the peak volume of an unretained compound in adsorption chromatography). For short (100-mm) high-efficiency columns (5-μm particles), peak volumes can therefore decrease from 50 to 25 μl for completely excluded molecules, when peak broadening by 8-μl detector flow cells will be very significant. As separation is difficult to achieve, it is advisable to ensure that losses caused by the detector are as small as possible. Larger diameter columns also reduce the fraction of material near to the wall and thus reduce wall effects.

These requirements led to this study of packing columns of 8-mm I.D. ($^3/_8$-in. O.D.) with lengths of 500 mm and 1 m and, for comparison, shorter columns (100 and 200 mm) as used analytically. For reasons of cost and convenience, and to avoid some inevitable lossess on coupling columns, the objective was to find ways of packing the whole column in one length. A subsidiary objective was to study the effect of increasing the range of particle sizes, as close sizing increases the cost of LC microparticle packings markedly, especially the interaction of this parameter and packing efficiency, and pressure drop. It was also hoped that some information on wall effects (the "infinite diameter effect") which, despite intense study by Knox and coworkers, leaves us in doubt about the interaction of wall effects, particle size distribution, slurry packing methods (all of the results obtained by Knox *et al.*[12] were with dry-packed columns) and injection slug distribution across the column area.

THEORETICAL

The theoretical limits to what can be achieved are now being shown to be practical limits also[13,14]. The early calculations by Giddings[14] have been extended by Halász *et al.*[15]; as usual, the calculations are more simply expressed in reduced terms, and this presentation was used recently by Bristow[18]. Scott and Kucera[17], have confirmed the paradoxical prediction of Giddings that larger particles (20 μm) are necessary for columns with the highest plate numbers but also that very long columns (10 m for 250,000 plates) imply long elution times (*ca.* 4 h) for a chromatogram if a modest pressure (calculated <100 bar) is used. In contrast, the 1-m column prepared in this work should be capable of giving 100,000 plates in <10 min, but needs a pressure of *ca.* 500 bar.

General equations[18] for calculating the time (t_0) for elution of an unretained (but not excluded) solute, and the pressure (p) required for a reduced velocity $\nu \approx 5$, the speed giving the best resolution, at least for reasonably small molecules, are

$$t_0 \approx L(\text{cm}) \cdot d_p(\mu\text{m})$$

and

$$P(\text{bar}) \approx 60\, L(\text{mm})/d_p^3(\mu\text{m})$$

where L is the column length and d_p the particle diameter. These two practical examples illustrate that further increases in plate number can be achieved only with a rapidly increasing column length, elution time and pressure. To double the effective resolving power, or peak capacity, a four-fold increase in any of these parameters is necessary.

EXPERIMENTAL

A Cecil Instruments (Cambridge, Great Britain) Type CE 210 coil pump (direct gas pressure driven) was used both for packing and for chromatography. In later experiments, a Haskel MCP-110 pneumatic amplifier piston pump was used for packing columns. It was established that a reciprocating pump gave identical results to a pulse-free constant pressure pump. At high pressure the volume of the sample cylinder (150 ml) provided considerable pulse damping because of the compressibility of the liquid (about 7.5 ml at 500 bar).

Column components and slurry packing apparatus were obtained from hetp (Macclesfield, Great Britain). The injector was a septum type, Part No. 301, giving a central on-column injection to reduce wall effects. Column tubes were $^3/_8$-in. O.D., 8-mm I.D. cold-drawn seamless stainless-steel (316) tubing to ASTM A269 specification, but not surface polished in any way apart from cleaning with trichloroethylene carried out by the manufacturers. All tubes were from a single batch of tubing.

The top of the column was prepared in a now well tried method for the injector by removing 4 mm of packing, pressing in an 8-mm disc of stainless-steel mesh with a nylon tool of 7.5 mm diameter, adding 150–200-μm glass beads and a plug of sintered PTFE. The function of this arrangement is to prevent packing material from entering the needle, and to provide a consistent injection position and depth into a zone which can easily be replaced in order to remove sample and septum debris.

The upward slurry packing method has been described previously[16]. The A kit of parts from hetp (Part No. 366) including a 150-ml sample cylinder made by Hoke International (New Barnet, Great Britain), Part No. 4HD-150 which acts as a reservoir to hold the slurry. (In order to pack long columns of 8-mm I.D., a sample cylinder larger than the normal 75 ml is used in order to keep the slurry concentration low enough, 35 g/150 ml.)

In one experiment, a reservoir consisting of a *ca.* 20-m length of 3/8-in. O.D. tube placed vertically was used. As with the sample cylinder, the column was placed vertically on the top, pointing upwards.

The reservoir was stirred magnetically in a few experiments by placing a high-power U-magnet in the chuck of an air-motor and an egg-shaped PTFE-coated follower inside the cylinder. The axis of the motor was perpendicular to the axis of the cylinder and column.

Particle size determinations were carried out on a series of single homogeneous batches of each grade of Spherisorb by M. J. Holdoway at AERE (Harwell, Great Britain) using a Micromeritics (Norcross, Ga., U.S.A.) Sedigraph after ultrasonic dispersion in Sediperse WO8 at *ca.* 30°. Used samples were also remeasured.

Solvents were obtained from Rathburn Chemicals (Walkerburn, Great Britain), as follows: HPLC grades of *n*-hexane, dichloromethane, tetrahydrofuran (unstabilized) and acetonitrile.

The detector was a Cecil Instruments CE 212 with a 0.3-mm I.D. connecting tube (hetp, Part No. 323) instead of the 0.15-mm O.D. tube supplied. At high flow-rates, the narrow-bore tubing, ideal for smaller analytical columns, gave sufficient resistance to cause some errors in assessing pressure drop. In normal use, of course, this would be negligible, but it does illustrate the type of errors that were made during our initial studies and were detected from inconsistent results obtained.

Chromatograms were recorded at 1 mm/sec or faster chart speed with a foot switch to give an accurate marker of the time of injection. At high pressures, some stopped-flow injections were more convenient; for these the time at which the flow was turned on was marked. With a constant-pressure pump there is no delay in pressure building up as the liquid is compressed. Injections with flow on and off produced identical times for elution of pentane, the unretained solute marker.

Pressure was measured with a 0–100 bar precision gauge, precise to 0.1 bar and calibrated to 0.2 bar (Budenburg Ltd., Altringham, Great Britain). The pressure was measured at the start, middle and end of the chromatogram and the mean calculated.

Flow was measured with a modified pipette (class B) to a precision of better than 1 %, timing for at least 60 sec and taking the mean of two determinations.

Packing at *ca.* 100° was achieved by steam-jacketing a pre-column, the sample cylinder and the column itself (hetp, Part No. 357). As the methanol from the column would boil off, the waste tube was discharged under water. At 100° the viscosity of methanol is *ca.* 0.27 mN·sec/m^2.

The packing method used consists of the following steps:

(1) Assemble the column end-fitting and swage nuts on the column.

(2) Charge the pump with reagent-grade methanol.

(3) Weight the silica (3.5 g per 100 mm of column length for an I.D. of 8 mm).

(4) Slurry the silica with 150 ml of methanol and disperse the mixture ultrasonically for *ca.* 5 min.

(5) Pour the well shaken slurry into the sample cylinder from the top using a suitable funnel (the feed from pump is attached to the bottom via an on–off tap).

(6) Quickly attach the column and a PTFE tube to take methanol to waste.

(7) Start pumping and pass >800 ml of methanol.

(8) Invert the column to point downwards and turn off the flow. Wait at least 1 min to allow all flow to stop. This pause is vital. Remove the column and finish the top with mesh, glass beads and PTFE sinter, or materials to suit the injector in use.

At the column ends (hetp, Part No. 201) a very thin (*ca.* 100 μm) double-woven stainless-steel mesh disc is used to retain the packing (down to *ca.* 1 μm, the size of the smallest particles in Spherisorb grade S3W). This was shown to have about one tenth of the impedance to flow of the sintered stainless-steel frit used in other types. The impedance of these types would have caused errors in impedance measurements without some correction.

ASSESSMENT OF COLUMN PERFORMANCE

The performance of columns was assessed by following the recommendations of Bristow and Knox[19]. The actual computer program used was that given by Bristow[18], but it gives precisely the same results as that given in ref. 19; only the format of input and output is different. Calculations were based on nitrobenzene as test solute with $k' \approx 1$.

A 1% (v/v) solution of acetonitrile in *n*-hexane was used as the eluent. At least 10 column volumes were needed to elute residual methanol and even after 20 column volumes some slight drift of retention for nitrobenzene was noted, but this was not sufficient to alter the observed efficiencies.

The test solution contained nitrobenzene, *m*-xylene and pentane (*ca.* 20%). Pentane was detected by a derivative refractive index change in the UV detector. If there was any doubt about the position of this peak, it was calculated from known k' values for xylene and nitrobenzene, using the following equation:

$$\text{pentane peak time} = (\text{xylene time} - 0.102 \cdot \text{nitrobenzene time})/0.898$$

It is now generally accepted that the performance of LC columns packed with different materials is best described in terms of reduced parameters[18–20]. For "well-packed" columns, numerous studies have shown that the plate height *versus* velocity curve shows a minimum reduced plate height, h, of between 1.5 and 3 at a reduced velocity, v, in the range 2–8. (For a wide range of column lengths and particle sizes it is experimentally difficult and time consuming to determine enough of this h *versus* v curve to be worthwhile, so it was decided to concentrate on measuring only the reduced plate height at the minimal reduced velocity region. A few columns were tested more fully but with no sign of deviation from the well established curve.)

For materials with a wider range of particle size, one is less certain of which value of the particle diameter to take for calculating reduced parameters. One additional chromatographic parameter that has been proposed to avoid the need to make any assumptions about d_p is the separation impedance, defined as either H^2/K or h^2/φ, which give numerically identical results, where H is the plate height measured, K the experimental column permeability and φ the flow resistance parameter (an inverse reduced permeability requiring an assumption about the particle size, d_p).

The permeability K (m^2) is defined as $n L^2/(Pt_0)$ where n is the viscosity ($N \cdot sec/m^2$), L the column length (mm), P pressure drop (bar) and t_0 the time for an unretained solute (note that this definition of "chromatographic" permeability differs from the specific permeability, k^0, calculated only from the flow-rate, pressure and geometry of the column, by a factor equal to the total porosity, E_T). The resistance is defined by

$$\varphi = P\, t_0\, d_p^2/nL^2$$

In practice, the separation impedance was evaluated from the equation

$$E = 0.036 \cdot \frac{t_r^2\, P\,(\text{bar})\, t_0\,(\text{sec})}{w_{\frac{1}{2}\text{ht.}}\, n\,(\text{N}\cdot\text{sec/m}^2)}$$

where t_r is the retention time of a retained test solute (nitrobenzene) and $w_{\frac{1}{2}\text{ht.}}$ is the peak width at half-height. The last two parameters can be in any convenient units, for example chart distance in millimeters, provided that they are the same.

Obviously, if the speed of elution is increased eventually the plate height, H, will increase while the permeability will be constant. Hence there will be a minimal separation impedance and this value provides a useful basis for a comparison of columns of any particle size or size distribution.

When known, a value for the particle size can be used, but for a sample containing a wide range of sizes, particularly when discrete sizes are mixed, a reasonable way of assessing an equivalent size is to calculate an effective size from the permeability. This requires an assumption that the packing factor, φ, is a constant regardless of the particle size distribution. As it is known to be almost constant for the whole range of spherical silicas, this is not an unreasonable assumption even for mixtures. In fact, in view of the difficulties in obtaining sufficiently precise and accurate measurements of particle size, it may be more practical to assess the particle size of LC packings on the arbitrary assumption that $\varphi = 500$, at least for porous silicas with a total porosity of *ca.* 0.7. (Endele *et al.*[21] made a similar but not exactly equivalent suggestion).

The effective spherical diameter is defined by $\varphi\, n\, L^{2/(P\, t_0)}$

or

$$d_p^{\text{effective}} = \sqrt{\frac{5000\,\eta\,(\text{N}\cdot\text{sec/m}^2)\, L^2\,(\text{mm})}{P\,(\text{bar})\, t_0\,(\text{sec})}}$$

where η = viscosity, and this was used to calculate reduced plate heights from experimentally determinated plate heights using the equation

$$h = H/d_p^{\text{effective}}$$

RESULTS

Particle size distribution

The observed sizes and distributions of sizes of the Spherisorb materials used are shown in Table I. The size distributions of the SW 5,10,15 and 20 μm grades are very small. The experimental S3W grade shows a wider spread.

TABLE I

PARTICLE SIZE DATA

Material	*Size (μm) at which cumulative mass-% finer is*								
	Nominal	*1%*	*10%*	*Fraction* 10%/50%*	*50%*	*90%*	*Fraction* 90%/50%*	*99%*	*Fraction** 90–10%/50%*
Spherisorb SW	3	2.2	2.8	0.82	3.4	4.9	1.4	~10	0.62
	5	4.7	5.0	0.93	5.4	6.8	1.26	9	0.33
	10	8.0	8.5	0.91	9.3	11.4	1.22	15	0.31
	15	9.0	13.0	0.93	14.0	16.7	1.19	25	0.26
	20	15.0	17.5	0.91	19.3	23.0	1.19	33	0.29
Spherisorb WR	5	3	4.6	0.74	6.2	9.8	1.58	15	0.83
	7	4	6.2	0.89	7.0	8.8	1.25	11	0.37
	14	5	11	0.86	12.8	20	1.56	30	0.7
	2–20	2.5	5	0.60	8.5	12.5	1.47	20	0.88

* For monosized particles this ratio would be unity.

** For a mixture of particles with one size twice the other in equal proportions, this ratio would be 0.8.

The spread of sizes of the particles in the WR grades is wider, especially the S2-20 type. This size distribution is similar to those of many ground and sized materials such as Merck LiChrosorb and Whatman Partisil, for example, for which particle size analyses of some batches have been published[22,23].

A feature of the spherical particles is that the number of particles outside a narrow band, just beyond the 99 and 1 % figures given, observed on photomicrographs is actually zero, rather than very small as with a material with a gaussian distribution of size. For ground materials there are a few much larger particles and many much finer particles. The dramatic effect of a few very small particles can be seen for the mixture of 3- and 20-μm materials, and this explains why granular sized materials always seem to have a lower permeability.

TABLE II

SEPARATION IMPEDANCES FOR CLOSELY SIZED SPHERISORB SILICAS

Material	*Nominal size (μm)*	*Observed size (μm)*	*Effective size (μm)*	*E*	*N*	*h*
Spherisorb SW*	3	3.6	3.0	6000	19,000	3.5
	5	5.4	5.8	2000	17,000	2.3
	10	9.3	10.1	2200	9000	2.1
	15	14.0	16.1	1500	7000	1.9
	20	19.3	18.5	3800	3500	2.7

Material	*Nominal size (μm)*	*H (μm)*	*E*	*N*	*d_p effective (μm)*	*K (μm^2)*
Spherisorb WR**	5	14	5000	14,000	4.4	0.039
	7	12	1500	16,000	7.5	0.107
	2–20	15	2500	13,000	6.4	0.082
	14	28	2000	7000	13.5	0.36

* Column length 200 mm, packed at 200 bar in all instances.

** These grades are not commercially available at present.

TABLE III

VARIATION OF PERMEABILITY WITH SIZE

Spherisorb SW. Column length, 200 mm; I.D. 8 mm; packed at 200 bar.

Nominal	*Measured*	*Effective*	*Resistance*		*K°* (μm^2)	*K* (μm^2)	*Porosity*	*Weight of silica(g)*	*Diameter, measured/ effective*
			*Nominal**	*Observed***					
3	3.6	3.0	505	710	0.012	0.018	0.68	6.05	1.2
5	5.4	5.8	370	431	0.045	0.068	0.68	5.23	0.93
10	9.1	10.0	495	410	0.14	0.20	0.69	5.35	0.91
15	14.0	16.1	430	375	0.37	0.53	0.69	5.47	0.87
20	19.3	18.5	580	540	0.47	0.69	0.69	5.39	1.04

* Calculated using nominal particle size.

** Calculated using measured particle size.

TABLE IV

PACKING OF SYNTHETIC MIXTURES OF CLOSELY SIZED SPHERISORB SW SILICAS

Values of plate number N, plate height H, separation impedance E, reduced plate height h and velocity ν are minima observed and the particle diameter is that calculated assuming resistance $\varphi = 500$.

SW grades mixed	*Proportions (w/w)*	*Effective diameter (μm)*	*Plate No. (N)*	*h*	*ν*	*H (μm)*	*u (mm/sec)*	*E*
10 and 20	1:1	11.3	7000	2.5	4.3	28	1.4	3000
5 and 10	1:1	6.4	15000	2.1	2.8	13	1.7	2100
5 and 20	1:1	9.8	4000	4.8	4.8	47	1.8	11,000
5 and 20	1:9	10.8	4300	4.8	4.8	46	1.7	8000
3 and 20	1:9	6.1	650	49	1.9	301	1.2	Very large
3 and 20	1:99	2.5	5000	13	0.5	38	0.72	Very large

Separation impedances for columns packed with both closely sized and wide-range Spherisorb silicas are given in Table II. The permeabilities of the closely sized materials are listed for reference in Table III. The results in Table IV show the effect of mixing closely sized silicas. Tables VI and VII show the effects of varying the packing conditions (pressure and length) for Spherisorb S5W.

Effect of injected volume

The results in Table VIII show that the real decrease in resolution caused by increasing the volume injected is small. An injection of up to 0.5 ml still gives half the

TABLE V

SETTLING RATES IN METHANOL USING SPHERISORB SW

Nominal diameter (μm)	*Observed rate (mm/sec)*	*Calculated rate* (mm/sec)*
3	0.002	0.009
5	0.01	0.02
10	0.03	0.1
20	0.17	0.4

* Assuming the density of silica of porosity 0.4 full of methanol is 1.8 g/cm^3.

TABLE VI

VARIATION OF PACKING DENSITY WITH PRESSURE

Spherisorb S5W. Column length, 200 mm; I.D., 8 mm; actual particle diameter, 5.4 μm.

Packing pressure (bar)	*Resistance*	*Effective diameter (μm)*	*K° (μm^2)*	*K (μm^2)*	*Total porosity*	*Weight of silica (g)*
20	431	5.8	0.047	0.068	0.69	5.87
50	420	5.8	0.048	0.068	0.69	5.91
100	431	5.8	0.045	0.068	0.68	—
100	460	5.6	0.044	0.063	0.69	—
500	536	5.2	0.038	0.054	0.70	6.06

TABLE VII

VARIATION OF PACKING DENSITY WITH LENGTH

Spherisorb S5W. Actual particle diameter, 5.4 μm; packing pressure, 500 bar.

Column length (mm)	*Bed length (mm)*	*No. of columns packed*	*Effective diameter (μm)*	*Resistance*	*K° (μm^2)*	*K (μm^2)*	*Total porosity*	*Weight of silica (g)*	*Weight per unit length (g/m)*
100	96	1	5.0	490	0.034	0.051	0.68	2.94	29.4
200	196	4	5.2	460	0.038	0.054	0.70	6.06	30.9
500	496	1	5.5	420	0.042	0.060	0.70	—	—
1000	996	3	5.5	410	0.044	0.061	0.71	31.78	31.9

resolving power. For preparative work, the range 100–500 μl is most useful, but 1 ml is not an unreasonable volume, particularly on 0.5- or 1-m columns. In practice, because the volume variance (and that due to mass overload) becomes significant, the importance of other contributions is relatively smaller. Hence the high pressures needed for 5- and 10-μm particles are much less worthwhile and a particle size of

TABLE VIII

EFFECT OF INJECTED VOLUME

Packing material	*Volume injected (μl)*	*Dispersion ratio**	*Plate number (nitrobenzene)*
Spherisorb S5W	5	133	100,000
(length 1 m, I.D. 8 mm)	20	133	100,000
	100	120	80,000
	800	62	21,000
Spherisorb S10W	5	41	9300
(length 200 mm, I.D. 8 mm)	10	36	7200
	25	34	6400
	50	28	4300
	100	25	3500
	100 × 2	23	2900
	500	20	2200
	1000	13	900

* Ratio of peak elution time to width at peak half-height.

10–20 μm is a better compromise. The lower cost of granular materials may also dictate their use rather than the more expensive spherical materials used in this study.

Mass that can be separated

It is unfortunate that the separation efficiency of silica decreases steadily with increasing load, as demonstrated by Done[24]. The test results are obtained at "infinitely" small loads of sample mass. The greater the efficiency of the original column, the smaller is the load at which a variance due to the mass being separated can be discerned. At even higher loads, there is evidence that breakthrough of material is occurring so that collected samples contain some compounds which in theory are so well separated that their concentration should not be detectable.

Columns of I.D. 8 mm contain *ca.* 30 g/m of silica. A useful general rule is that not more than one thousandth of the weight of adsorbent in the column can be separated efficiently. For impurities present in a reasonable concentration (*ca.* 10%), these columns can give the few milligrams now needed for identification, but for concentrations below 1% success is less certain and a preliminary concentration step may be needed. It may be necessary to evaporate the solvent from initial fractions, preferably on a separate column, or it may be possible to re-inject directly, *i.e.*, effectively a manual recycling operation.

Results obtained so far do not suggest that there is a clearly defined optimum geometry for a column, provided it is reasonably well packed, say with a minimum reduced plate height less than five at the optimum reduced velocity. This in turn demands an effective injection system which does not cause too much peak broadening and tailing. Certain configurations such as very wide columns or centrifugal columns make it much more difficult to inject the very thin band required by efficient small particle columns, so these systems are normally used with higher loadings, larger particles, and effectively made longer by recycling procedures if the degree of separation is not sufficient.

DISCUSSION

The choice of methanol as the slurrying liquid has been discussed previously[16]. It is cheap, not too toxic, UV transparent, polar enough to disperse silica (but not non-polar alkylsilane bonded to silicas) and has a low viscosity.

Upward packing has two practical advantages. It is convenient to fill the sample cylinder this way up without the column attached, and when the column is fitted and the flow is started air is dispelled upwards before any slurry starts to pack. Any lumps or agglomerates will fall to the bottom of the reservoir and not be packed, whereas individual spheres of silica will be swept up into the column and packed against the growing bed. Table V gives some setting rates in methanol.

Stirring

A brief comparison of wide size range material packed with and without stirring was made. No differences were detected. Provided that the upward velocity is several times the settling velocity of the smallest particles, the amount of segregation would be expected to be negligible. There will also be some natural mixing in the cylinder (which is why it is necessary to pass several times the cylinder volume). Additional

stirring therefore seems to be unnecessary. In any case, there is no reason to suppose that size segregation along the length of the column will be anything but beneficial compared with a more homogeneous but mixed bed.

Effect of pressure

The effect of the pressure used when packing columns has been much discussed, although usually when considering short columns. The consensus view seems to be that about 200–500 bar is optimal although the results in Table VI show that it is possible to pack efficient columns at much lower pressures. It was found possible to use these columns at pressures 50% greater than the packing pressures without any settlement.

It is not clear why pressure is needed. Is it the actual force exerted on the bed, or the speed at which the particles hit the bed, or the flow-rate keeping the recently packed particles pressed against the remainder of the bed? Whichever hypothesis is chosen, it seems reasonable to suppose that if a short column can be packed with a given pressure, then a column twice as long can be packed with twice the pressure. While this seems to be so with closely sized SW materials, with a wider spread of particle sizes (in the case of the Spherisorb WR grades, and mixtures of closely sized SW silicas), attempts to pack lengths greater than 500 mm have failed to give stable and efficient columns. The most striking symptom of failure was peak asymmetry, with both fronting and tailing. That both types of asymmetry are observed is particularly puzzling.

Two further methods were tried in order to compensate for the increase in length and provide an equivalent environment for packing of the second 0.5 m of the column as the first. In one experiment, the viscosity of methanol was reduced by heating the whole packing reservoir and column to 100°. A 1 m × 8 mm I.D. column was packed at 200 bar pressure with WR 15 but settled after passing *n*-hexane eluent in order to equilibrate the column. This procedure gave the expected 3-fold increase in packing speed, but not a useful column. Finally, a more dilute slurry was produced, first by using two sample cylinders joined by a tee-piece connected to the pump. The upper cylinder was filled with slurry and the lower cylinder and the column (pointing downwards) with methanol. The silica fell very slowly into the lower reservoir and was finally packed into the column. Several litres of methanol were required in order to pack the column, which was highly permeable but inefficient chromatographically with marked fronting. In a final attempt, a very long tube (*ca.* 20 m) was used as a reservoir (at 500 bar) but even this did not give a column with satisfactory peak shapes with WR 15 material.

These results on wider size-range materials have recently been corroborated by success in packing 20-μm Partisil 20 and LiChrosorb in 500-mm columns, but failure for 1-m lengths, at least as judged by plate numbers. These failures and symptoms seem to indicate some interaction of wall effects and the particle size inhomogeneity across the column.

Packing longer columns

Although the gains to be made from using even longer columns become ever smaller, there is still a clear need and advantage in using columns longer than the 1 m and below which can be packed with current techniques. From these results, one is

tempted to conclude that some radical change in method is needed for lengths much greater than 1 m, without coupling columns which is desirable to avoid if possible. This is particularly true if we wish to use mechanically weaker packings such as polystyrene resins, and perhaps carbons, which have useful and controllable pore size and structure, and are naturally non-polar. One possible approach is to compress a slurry simultaneously along the length of the bed. This process is equivalent to packing many columns of which the length is only the diameter of the column. This method has already been used commercially to pack preparative columns*. For normal analytical columns, the mechanical complexity of a thin-walled inner column which can be deformed by fluid pressure seems superfluous as it is so easy to pack short columns, but for very long columns this expense may prove worthwhile. An added conveniende could be that coiled columns could become feasible again.

Packing other materials

The methanol slurry packing method has failed to give good columns with 50-μm glass beads and 20-μm alumina microspheres. This suggests that our understanding of the packing process is still far from complete because both of these materials dry-pack well. Perhaps it is significant that both materials are denser than silica. However, the predicted rates of fall are not so great that these materials do not pack upwards, but the packing density is too low for stability and the columns settle if they are re-pressurized.

Packing very small particles (<5 μm)

It is often asserted that these materials are or would be more difficult to pack. Table II does not provide much evidence to support this contention, bearing in mind some complicating factors. Firstly, the spread of particle size of S3W is much greater, much more like the WR grades. The reduced length of the columns is, of course, nearly twice as great as for S5W (*i.e.*; twice as many particles can fit end to end into the column). The peak volumes are also about half those which would cause an appreciable loss of resolution in most detectors with smaller diameter columns. Most important, the axial distance over which the injection can be spread without causing peak broadening is also reduced. As this is well known to be a critical factor, the most likely explanation is that the injection process is not controlled enough to prevent it causing some additional broadening.

The objections to using even smaller materials are thus the more practical ones of deficiencies in the engineering of injection and detection and the high pressures needed for columns of any length.

EXAMPLES OF USE

Some chromatograms showing use of columns 500 mm long and 8 mm I.D. containing about 15 g of silica are shown in Figs. 1–3. Small-scale preparative separations are illustrated in Figs. 1 and 2. Enough material for spectroscopic identification was collected in a single pass. A size separation is shown in Fig. 3. The last peak is toluene (and other small molecules) added as a non-excluded, non-absorbed component.

* Waters Assoc. Milford, Mass., U.S.A.

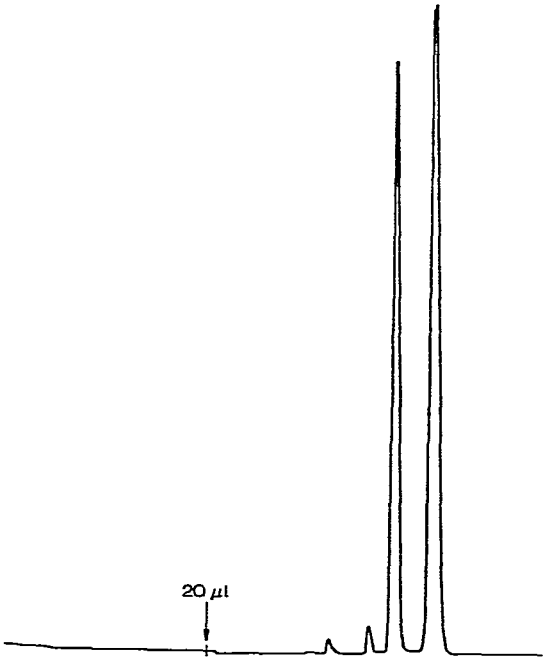

Fig. 1. Separation of 10 mg mixed *cis–trans* isomers (sample volume 20 µl). Column: 500 × 8 mm I.D.; packing: Spherisorb WR 14 silica; eluent: methylene chloride–*n*-hexane; detection: Cecil Instruments CE515 at 280 nm, 2 A.

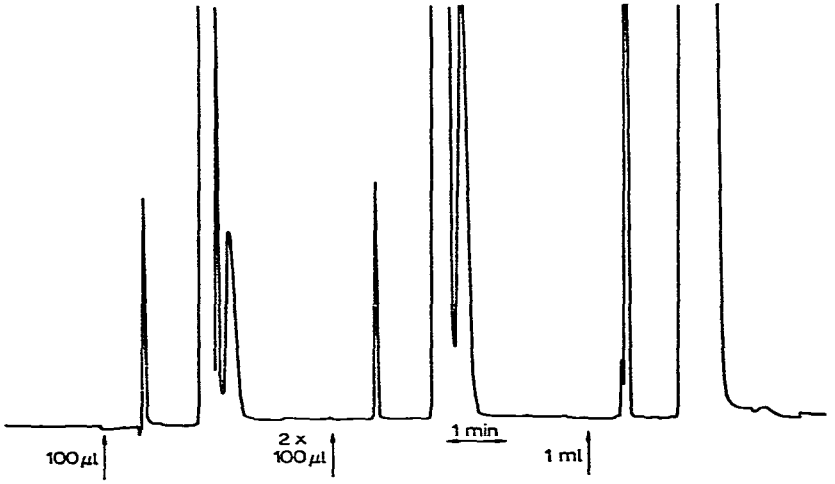

Fig. 2. Separation of diazepam crude, saturated solution in eluent (1%, w/v). Column: 500 × 8 mm I.D.; packing: Spherisorb WR 14 silica; eluent: ethanol–*n*-hexane (1:9) at 0.44 ml/sec; detection: Cecil Instruments CE515 at 220 nm, 2 A.

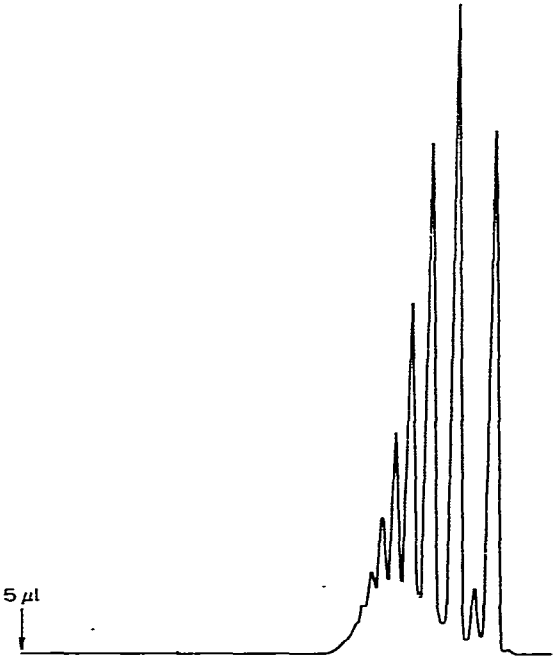

Fig. 3. Separation of diisocyanatophenylmethane–dianol 33 copolymer. Column: 500 × 8 mm I.D.; packing: Spherisorb S5W silica; eluent: unstabilized tetrahydrofuran, 0.035 ml/sec; detection: Cecil Instruments CE515 at 260 nm, 2 A; pressure: 40 bar. A non-retained, non-excluded marker was eluted at 18.0 ml (510 sec).

CONCLUSIONS

(1) Silicas and aluminas are dispersed in methanol and water, incompletely dispersed in acetone, and agglomerated in less polar liquids such as choroform and carbon tetrachloride, as judged by a comparison of the settling rates predicted by Stokes equation and those observed. Silicas with a non-polar (alkyl) surface agglomerate in water and methanol (for example see Table V) but are dispersed in acetone, chloroform or *n*-hexane.

(2) Spherisorb materials are not visibly changed or broken by being packed in, and unpacked from, a column at 500 bar.

(3) Sedimentation of a mixture of 3- and 20-μm Spherisorb SW particles produced little size segregation along the column length during the time it took to pack a column at 200 bar upwards from 75 ml of methanol slurry. This shows that a balanced-density slurry liquid is not needed in order to avoid size segregation.

(4) Columns (200 mm) of equal efficiency can be packed at pressures ranging from 20 to 500 bar. Only above 100 bar does the resistance increase slightly, and a little more material can be packed into the column tube. The advantages of a higher pressure are in speed of packing and the likelihood of a more stable bed rather than column efficiency.

(5) Materials with a wider particle size distribution can be packed into columns up to 500 mm long with equal efficiency for their permeability compared by measuring

the minimal separation impedance, provided that the ratio of the largest to the smallest particles does not exceed about 4.

(6) It was possible to pack longer (1-m) columns only with closely sized particles (S3W and S5W). The reasons why longer columns present problems are not clear in that decreasing the viscosity and increasing the pressure, and thus the flow-rate in order to compensate for the increased length, did not increase the length of column that could be packed effectively.

(7) A few small particles cause a dramatic decrease in permeability if the spaces between the large particles can be filled by these small particles. This starts to take place when the ratio of the particle sizes exceeds about five[25]. The separation impedance of a column of this type is extremely high and peak shapes are poor.

(8) There is some evidence[26] that thick slurries (>30%, w/v) pack more loosely and give a less stable bed.

(9) For upward packing, the upwards velocity in the reservoir (sample cylinder) should be >20 times the settling rate of the smallest particles. With this velocity, it is unnecessary to stir the reservoir (and only slows the packing process).

(10) There is only slight evidence to support the assertion that very small particles (<5 μm) are difficult to pack.

(11) The total porosity of all columns was identical at 0.70 (within an experimental error of $\pm$0.2). This value can be used with confidence for all Spherisorb SW and WR silicas (and for Partisil and LiChrosorb silicas[26]). The porosity does not change perceptably with packing method or size distribution.

ACKNOWLEDGEMENTS

Careful experimental work was carried out by Janice Hurst. M.J. Holdoway of AERE Ceramics Centre, Harwell, provided samples of Spherisorb materials, including some special batches of wide particle size range, together with particle size data on the particular batches used. A sample of S15W was given by Phase Separations, Queensferry (Great Britain).

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