UPWARD SLURRY PACKING OF LIQUID CHROMATOGRAPHY COLUMNS

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SUMMARY

Some factors affecting the efficiency of packing microparticulate liquid chromatography columns are discussed with some experimental evidence on the most critical factors. Upward packing of a dilute slurry is considered to have some advantages.

INTRODUCTION

Slurry packing has become the accepted method to prepare columns with particles below about 30 μm (after a period of being ignored despite earlier work demonstrating its advantages for large particles[1] and for small ion-exchange resins[2]). It is clear from our experiments, and the work of others, too many to detail, that many techniques can give satisfactory results.

Laird et al.[3] have already drawn attention to the looser packing produced by slurry packing of Spherisorb S10W spherical silica compared to a less permeable (and presumably denser) dry vibration pack. For the user a more permeable column is an advantage provided that the lower weight per length[4,5] is negligible and band broadening does not increase. This process can be carried too far when the packing structure becomes unstable. As the pressure is applied and relieved in normal use, the column may collapse and bed down: peaks become much broader and tail badly. We have observed this phenomenon most frequently on closely sized spherical packings. Since these packings have desirable low separation impedance[6], $h^2 \varnothing'$ (where h is the reduced plate height and $\varnothing'$ the column resistance factor), we felt it vital to discover a reliable method of packing, loose enough to be highly permeable and yet stable in use.

Recipes for slurry packing seem to differ in the type of liquid. One school selects non-polar balanced density liquids, of high viscosity[7–9]. Since the particles do not fall, proponents imply that there is almost no limit to the time available to pack and that the viscosity is unimportant. Because these liquids do not disperse polar silica particles well, leading to agglomeration, apparent as curdling of the slurry, others favour polar and low-viscosity liquids such as water[10], methanol, or acetone. The

particles sink rapidly but also pack rapidly. To overcome agglomeration, a factor commonly acknowledged as deleterious, polar dispersing agents are often added to balanced density liquids[7]. Another disadvantage of UV-opaque halogenated hydrocarbons used for their high density is that they are troublesome to remove if short wavelengths below 230 nm are being used.

Our experience with short (100-mm) columns was that satisfactory results (5000–10 000 plates for 1-μl on-column injections of a test mixture at near optimum reduced velocity) were obtained for spherical and granular materials. We could not discern that other variables such as slurry liquid, slurry concentration (1–50% w/v), and packing pressure and flow had any effect. Longer columns often gave initially satisfactory results with almost the theoretically predicted increase in plate number, but many columns bedded down immediately, or in later use.

Increasing the packing pressure seemed a simple solution but seemed to have little effect (2000 p.s.i. using a coil pump to 5000 p.s.i. with a pneumatic amplifier pump).

Observation of the packing process proved possible at 2000 p.s.i. in some glass columns (behind a safety screen). The first 50 mm packed with a perfectly flat surface; later a pile built up in the centre, and then collapsed to the side. Clearly, the packing liquid velocity had fallen to near that of the sedimentation velocity. Intuitively, an even and level pack seems likely to produce a denser and more efficient column.

A tentative first hypothesis was that a thicker slurry should pack more quickly, and thus there will be less sedimentation packing. However, there will be a limit to the length for which this tactic may be expected to succeed for any available pressure. Intuition also pointed in a different direction to a more dense and therefore stable pack coming from a dilute slurry because each particle can pack nearly independently and have more chance to reorientate slightly before being wedged in by the bed building up above. To eliminate sedimentation the upward packing concept was added.

Observation and calculation (which could be only very approximate for porous spherical particles subject to Brownian motion) suggested fall velocities of approx. 0.05 mm/sec for 5-μm particles. If the upward velocity is greater, the column should pack, usually with an impact velocity above the fall velocity. Observation of a glass column confirmed that the surface was perfectly flat all the time the column packed. The bed did not collapse when the flow was switched off.

In order to hold the dilute slurry (usually 1–5% w/v), a sample cylinder of much larger diameter than the column was convenient.

EXPERIMENTAL

Reagent-grade methanol was used without further purification. Hexane and acetonitrile were LC grade from Rathburn Chemicals (Walkerburn, Peebleshire, Great Britain).

The packing apparatus consisted of a CE 210 coil pump (Cecil, Cambridge, Great Britain) connected via a supplementary valve, Type SS-OKS2 (Crawford Fitting, Salon, Ohio, U.S.A.) for convenience to the bottom of a sample cylinder Type 304-HDF4-75 or -150, internal diameter 34 mm, and with a volume many times the column volume.

The pump was charged with methanol and pressurized with all available cylinder pressure, usually above 2000 p.s.i., with the supplementary valve shut. A slurry of silica or alumina in the sample cylinder volume (75 or 150 ml) of methanol was shaken (and sometimes dispersed using an ultrasonic bath) and poured in with a funnel. The column was quickly attached pointing up and flow started by opening the valve. The column was judged packed when the flow-rate became constant, usually before a few times the cylinder volume had passed. Vibration at 50 Hz up and down was applied to some columns but seemed to make no difference.

Columns were tested using 1 % acetonitrile in hexane. Column parameters were calculated from raw experimental data using program COLUMN in the language BASIC on a time-shared computer service. (Listing and tapes are available from P.A.B.)

A pneumatic amplifier Type MCP-110 made by Haskel (Burbank, Calif., U.S.A.) was used to provide higher pressures (using 1/2 in. O.D. tube as reservoir) above the working pressure of the sample cylinder.

Spherisorb silicas and aluminas were obtained from Atomic Energy Research Establishment (Harwell, Great Britain) or Phase Separations (Queensferry, Great Britain), who market those types commercially available. Partisil silicas were from Whatman (Maidstone, Great Britain).

Injection volumes were generally low (about 1 μl) to focus attention on packing characteristics. The injection systems were selected to maintain the infinite diameter column effect and minimise spurious results from wall effects. Practical chromatography might not normally achieve these values.

RESULTS

Table I shows the packing density achieved in dry packing experiments. Low-density results, for example with 5-μm silicas, correlate with unsatisfactory column packing experience. Table II compares dry and wet packing and shows a denser pack from higher pressure and flow-rate.

TABLE I

PACKING DENSITY OF VARIOUS MATERIALS AFTER VIBRATION AT 50 Hz

Type	Density (g/ml)	Particle size (μm) (nominal)
Partisil 5	~0.3	5
20	0.56	20
40	0.64	40
Spherisorb silica S5W	0.51	5
S10-20W	0.61	10–20
S20W	0.58	20
Spherisorb alumina A5Y	0.98	5
A20Y	1.02	20
AYP(7-30)	1.03	7–30

TABLE II

PACKING DENSITY ACHIEVED BY DIFFERENT METHODS USING SPHERISORB SILICA S10-20W

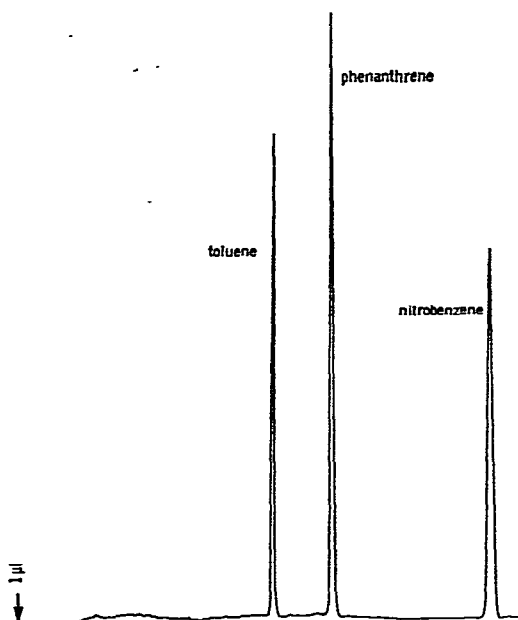| Method	Pressure (p.s.i.)	Density (g/ml) (± 0.01)
Least dense pack by dry inversion	—	~0.4
Settled slurry	zero (gravity)	0.5
Slurry pack	40	0.51
Slurry pack	850	0.58
Slurry pack	1800	0.58
Vibration dry (slow addition)	—	0.61

Fig. 1. Test chromatogram. Data calculated below on phenanthrene peak. Conditions, as in text and printout below.

DISCUSSION

Results are shown in Fig. 1 and typical vital column statistics are shown in the first section of the printout below.

The results show that efficient columns can be obtained by upward packing. Because of the difficulties of making valid comparisons between packing methods without extremely tight control of all other variables, including the use of a single batch of material, and especially of the injection* and column system, we do not claim the method to be superior to others.

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* It is clear that the results of many experiments cannot be compared with each other due to the varying effect of the wall. Because of the disturbances produced by injection from a microlitre syringe needle we believe the formula for calculation if the infinite diameter effect applies is not conservative enough for the size of injection used in practice. The formula $w_r = 32D_m t_0 + 2.4 L d_p = w_i^2$ has been proposed by Knox[11], where w_r = radial dispersion, D_m = diffusivity, t_0 = solvent time, L = length, d_p = particle diameter, w_i = initial sample width.

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COLUMN PARAMETERS          VER 1.5      TYPE 53W

LENGTH   995 MM   INTERVAL DIA   8.0 MM   PARTICLE DIA   3.0 UM
COLUMN TUBE VOLUME  49.99 ML      AREA   50 MM2
KNOX-PARCHER RATIO    21.4     INFINITE DIAMETER MODE POSSIBLE

DISPERSION RATIO 139.6  PLATE NO 108026  PLATE HT    9.2 UM
PEAK DISPERSION  AXIAL   12.1 MM  RADIAL   4.6 MM
ELUENT TIME 1060 SEC  PEAK TIME 1312 SEC  WIDTH   16.0 SEC
ELUENT VOL   44.17 ML  ELUENT PEAK VOL   537 UL  PEAK VOL    665 UL
FLOWRATE   41 UL/SEC     VELOCITY  0.94 MM/SEC    PRESSURE 150 BAR

REDUCED H   3.1  REDUCED VELOCITY   0.7  REDUCED LENGTH   331000
FLOW RESISTANCE 0'   443      PERMEABILITY 2.01E-14 M2
SEPARATION IMPEDANCE  4226      POROSITY .884

PEAK K'  0.24    RETENTION RATIO R  .808
SEPARATION NO   3985      EFFECTIVE PLATES/SEC   3 FOR PEAK
PEAK CAPACITY  206 FOR K' = 10



COLUMN PARAMETERS          VER 1.5      TYPE PARTISIL 5 BX A294

LENGTH    95 MM  INTERNAL DIA   5.0 MM   PARTICLE DIA   6.2 UM
COLUMN TUBE VOLUME   1.86 ML      AREA   19 MM2
KNOX-PARCHER RATIO    42.4     INFINITE DIAMETER MODE POSSIBLE

DISPERSION RATIO   31.4  PLATE NO    5477  PLATE HT    17.3 UM
PEAK DISPERSION  AXIAL    5.1 MM  RADIAL   1.1 MM
ELUENT TIME    47 SEC  PEAK TIME   110 SEC  WIDTH    5.9 SEC
ELUENT VOL    1.50 ML  ELUENT PEAK VOL    81 UL  PEAK VOL    190 UL
FLOWRATE   32 UL/SEC     VELOCITY  2.02 MM/SEC    PRESSURE   33 BAR

REDUCED H   2.8  REDUCED VELOCITY    3.3  REDUCED LENGTH    15000
FLOW RESISTANCE 0'  2053      PERMEABILITY 1.87E-14 M2
SEPARATION IMPEDANCE 16070      POROSITY .807

PEAK K'   1.34    RETENTION RATIO R  .427
SEPARATION NO   1796      EFFECTIVE PLATES/SEC  16 FOR PEAK
PEAK CAPACITY   47 FOR K' = 10



COLUMN PARAMETERS          VER 1.5      TYPE S5W

LENGTH    95 MM  INTERNAL DIA   5.0 MM   PARTICLE DIA   5.9 UM
COLUMN TUBE VOLUME   1.86 ML      AREA   19 MM2
KNOX-PARCHER RATIO    44.6      INFINITE DIAMETER MODE POSSIBLE

DISPERSION RATIO   45.8  PLATE NO   11648  PLATE HT    8.2 UM
PEAK DISPERSION  AXIAL    3.5 MM  RADIAL   1.1 MM
ELUENT TIME    44 SEC  PEAK TIME   110 SEC  WIDTH    4.1 SEC
ELUENT VOL    1.61 ML  ELUENT PEAK VOL    59 UL  PEAK VOL    149 UL
FLOWRATE   36 UL/SEC     VELOCITY  2.16 MM/SEC    PRESSURE    8 BAR

REDUCED H   1.4  REDUCED VELOCITY    3.4  REDUCED LENGTH    16000
FLOW RESISTANCE 0'   466      PERMEABILITY 7.46E-14 M2
SEPARATION IMPEDANCE   891      POROSITY .865

PEAK K'   1.50    RETENTION RATIO R  .400
SEPARATION NO   4193      EFFECTIVE PLATES/SEC  38 FOR PEAK
PEAK CAPACITY   68 FOR K' = 10
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Calculated data from chromatogram above (Fig. 1) and other typical columns.

Rationalisation of our observations and results leads us to propose tentatively the following factors in successful slurry packing of liquid chromatography columns, though it is clear that the subject is very complex and merits further detailed study.

The slurry liquid must reduce interparticle forces so that each particle is free to pack randomly uninfluenced by other particles. Agglomeration must be prevented: its presence can be seen under a microscope.

Dilute slurries are likely to give best dispersion: We are unclear how concentrated the slurry may be. Vigorous or ultrasonic agitation and degassing improve dispersion.

Polar liquids disperse aluminas and silicas well. Aqueous 0.001 M NH_4OH[12], methanol, and acetone have all been used successfully. We have chosen methanol for its low viscosity.

The velocity of the particles striking the bed must be sufficiently high and in upward packing that of the liquid high enough to prevent particles from falling back from the bed.

Vibration might be expected to aid dense, stable and efficient packing but the experimental evidence for denser packing is inconclusive.

Denser packing may give marginally less band broadening. Theoretical predictions[13] are not very positive, but it is difficult to see how a large number of bridged regions can but add to dispersion because of interchannel velocity differences. Unless dramatic improvements to the tantalizing but quite useless efficiencies reported by Knox and Parcher[14] for 0.48-mm solid glass beads can be achieved, these factors are secondary to the reduction of separation impedance $h^2 \varnothing'$. (A 20-μm alumina column, dry vibration packed, and thus quite dense, with a reduced plate height of 1.36 is our closest approach to this result[15].)

Particle size variations across the column cross-section are undesirable but differences along the column, such as would occur if sedimentation occurs during upward packing so that larger particles are packed last, are unlikely to have any marked effect unless the range of size is very great and the up velocity close to the fall velocity of the largest particles.

Upward packing discriminates against agglomerates (some often seem to persist despite all dispersal attempts) because these sink too fast in the low upward velocity zone in the reservoir.

Fall velocities of porous micrometer diameter particles are sufficiently low to allow 3-μm silica columns of at least 1 m to be packed at 2000 p.s.i. with an impact velocity many times the sedimentation velocity.

Packing pressures exceeding the intended-use pressures are probably unnecessary. If conditions allow re-ordering at the surface layer, it is plausible to suppose that pressure per se has little effect and that it is the particle impact velocity that counts.

Slow packing will also offer most opportunity for surface re-ordering to give a more densely packed surface. Dilute slurries are to be preferred on this hypothesis. Experimental information will be required to determine the concentration at which this factor becomes important. Upward packing gives an additional dilution because particles are falling into the fresh liquid below until they are swept up into the column itself.

The viscosity of the liquid controls the time taken to pack a given column and the impact velocity but not the maximum length that can be packed upward.

The particle size does not affect the length that can be packed (rather than sedimented) for a given pressure.

Increasing pressure capability increases the feasible length before the final upward flow is too small to support packing; or for downward packing, when sedimentation will become the main packing mechanisation.

Density of both packing and slurrying liquid should be more closely matched to make longer columns feasible, except that this must not lead to agglomeration and if the viscosity is increased too much the impact velocity will fall. Halomethanes alone meet the low viscosity requirement but most are expensive.

If impact velocity is important in packing then constant flow pumps should give most consistent packing along the column. That many workers achieve good results with constant pressure pumps and a concomitant wide variation in impact velocity suggests that above a minimum value this factor is not too important.

A flat packing surface reduces transcolumn non-uniformities: thus free settling is to be avoided and this places a lower limit on impact velocity.

Selection of the reservoir diameter ensures that the velocity in the column exceeds the fall velocity by some margin. An arbitrary minimum factor of ten in area and velocity or three in diameter seems to be practical and reasonable.

In addition to the impact velocity it is conceivable that the continued viscous drag forces are significant in controlling packing density. Thus a particle on the bed surface is subjected to a force determined by the viscosity of the slurry liquid but not controlled by the impact velocity. Thus a viscous liquid slurry[16] will pack with a low impact velocity, even if extreme pressures are available, but press particles very firmly onto the bed even at low liquid velocity. That higher pressures are reported by those using high-viscosity liquids, mainly ethylene glycol and haloethanes, may be only coincidence, but does suggest that this pressing factor is not one leading to denser packing.

ACKNOWLEDGEMENTS

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