Journal of Chromatography, 450 (1988) 47–69
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 1411

COLUMN HARDWARE IN PREPARATIVE LIQUID CHROMATOGRAPHY WITH AXIAL FLOW

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SUMMARY

Scale-up of liquid chromatography (LC) for preparative work poses a number of problems. Problems concerning the column as the carrier of the stationary phase are discussed. Only axial column flow and LC with elution are considered (not radial flow, thick-layer, centrifugal techniques, etc.). The choice, design and technology of columns have mechanical aspects, but they are also dictated by the choice of stationary phase, by sample size requirements and by other chromatographic requirements. A distinction is made between laboratory-size and production-size instrumentation. The design, packing and use of laboratory-size preparative LC columns is not so very different from usual analytical LC practice. Larger-size instrumentation requires different approaches. A bewildering variety of larger-sized column designs is already commercially available. Dry-packing, slurry-packing, axial and radial packing or a combination of the two, chromatography with or without compression all are advocated. About 50 manufacturers of preparative LC columns were asked to provide their latest documentation on larger-scale systems. An analysis and survey of current commercial production-scale LC columns, based on the replies received, is presented.

INTRODUCTION

The increased interest in preparative liquid chromatography has recently been manifested by several events. Dedicated symposia have been organized on both sides of the Atlantic[1,2], a specialized journal has been launched[3], several books have appeared[4–6] and the numbers of papers and reviews on the subject has risen sharply. The present contribution to preparative LC concentrates on some physical aspects of the column as a carrier of the stationary phase. Stationary phases will not be discussed, or only to the extent that they influence the column shape. Radial-flow chromatography, thick-layer and centrifugal techniques, etc., will also not be considered.

Scale-up of LC for preparative purposes involves a number of choices concerning the column shape. The size, design and technology of columns have mechanical aspects, but they are also dictated by the choice of the stationary phase, by sample size requirements and, of course, by general chromatographic requirements. In particular, the column entrance and exit design must avoid peak broadening by unequal flow patterns.

0021-9673/88/$03.50

A clear distinction should be made between research and development preparative LC and process and production preparative LC. The first belongs in the laboratory, and the amount of compounds to be separated will generally be in the range μg to g. Often, the separated compounds are used for structural investigations by spectrometric techniques. The economics and engineering aspects of this kind of preparative LC are of secondary importance and any instrumentation which can accomplish the desired result is acceptable. Such preparative LC has been around for a long time. It has evolved very dramatically in the last few years. The second variety, or process and production preparative LC for commercial purposes, is a new development. This may cover much larger sample sizes (in the range kg to ton), and the economics are all-important. The difference between these two forms of preparative LC is reflected in the column design.

COLUMN DESIGN AND SAMPLE SIZE

For a long time, organic chemists have performed preparative chromatographic separations in glass tubes with gravity percolation of the solvents. The rule-of-thumb was to use 30 g of silica gel per gram of the mixture to be chromatographed[4]. The size of the column was adapted to the available sample size. With the advent of modern LC and the higher cost of the metal columns and packing materials currently used, the sample size today is adapted to the size of the available columns and instrumentation.

Separation is the goal. This is determined by the resolution, R_s

$$R_s = \frac{1}{4}\sqrt{N} \cdot \frac{\alpha - 1}{\alpha} \cdot \frac{k'}{k' + 1}$$

where N is the plate number of the column, α is the separation factor or the ratio of the capacity factors of the pair to be separated and k' is the capacity factor of the compound with the longest retention. While N is strongly influenced by the sample size, α and k' are practically not influenced at all. For a given separation problem the sample size is therefore mainly dictated by the efficiency/sample size dependence of the column. It should be stressed that this is for given conditions (fixed α) since, of course, other conditions (other α) may give very different sample size possibilities.

The usual ranges for the parameters of the resolution equation in analytical LC are: for N, 5000–25 000; for α, 1.01–2.0; and for k', 1–10. In this range, an increase of only 10% in α dramatically increases the resolution, while a 10% increase in the other factors has little or no influence. Since the value of α thus has the greatest impact on the resolution, it is essential to optimize α. If α can be increased above 2 or even higher, the separation becomes easy and sample sizes can be very large. This is not necessarily a desirable situation in analytical LC, because very large α may mean longer analysis times for the same column length. In analytical LC there is no need to aim for α values as large as 10–20, but in preparative LC these are very desirable. In preparative LC the α value is very important and should be as high as possible.

For a given chromatographic system, α, and also k' are in principle independent on the sample size. The plate number is the only other important parameter. Curves relating efficiency to the sample size have been discussed repeatedly in the literature. About twenty papers are cited in ref. 1. A knowledge of this relationship is important

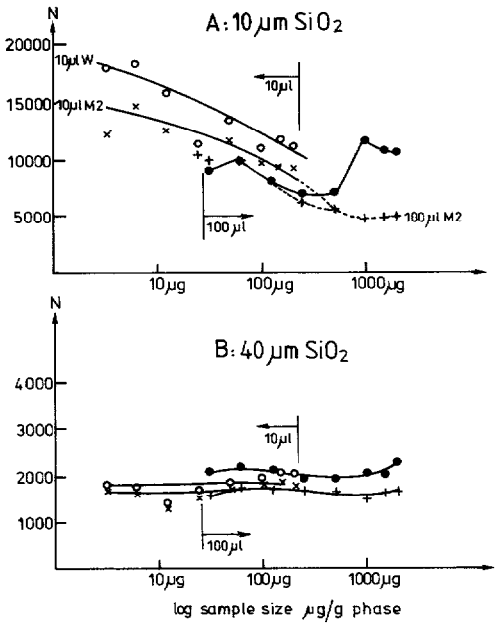

Fig.1. Plate number per metre against log of sample size per gram of silica gel for normal-phase LC of 4-nitrophenol with 15% propanol in hexane. Sample injected in 10 or 100 μl. Circles: plate number calculated from the peak width. Crosses: plate number calculated from the second moment. Packings: (A) 10-μm RSil; (B) 40-μm RSil (RSL-Alltech Europe, Eke, Belgium). ○ = W (10 μl); ● = W (100 μl); × = M2 (10 μl); + = M2 (100 μl).

for the optimization of large-scale preparative LC. However, establishing such curves experimentally presents a number of problems. There is, *e.g.*, no consensus on whether to express the relationship on the basis of plate number or plate height. Some authors use a logarithmic scale for the sample size (sometimes expressed per gram of stationary phase) and/or for the plate number. How to measure the plate number or plate height is also a matter of controversy. From the purely practical standpoint, solubility and detection difficulties often make it impossible to cover the desired range of sample sizes.

Fig.1 illustrates some of these difficulties. The difference between measuring the plate number by peak width or by the second-moment method is evident from Fig.1A. This is not always so. In the present case, the non-ideal curve shape is attributed to anomalies in the adsorption isotherm of 4-nitrophenol. With small particles (10 μm), a wide range of efficiencies is available by simply changing the sample size. For larger particles (40 μm) the situation (Fig.1B) is much less critical, but the efficiency is always poor. For μg sample per gram stationary phase (Fig.1A), analytical efficiency may be achieved. With up to about 1 mg sample per gram stationary phase, acceptable chromatography is possible, with low-efficiency broadened peaks, but still with chromatograms resembling those in analytical LC. Whether the desired separation will still be possible at this sample load depends entirely on the problem (α value). However, in practice, much higher loads are often used. Organic chemists will not

hesitate to apply 10–100 mg of sample per gram of silica gel stationary phase. The peaks are so completely distorted then that they no longer appear as such on the chromatogram, which often can no longer be used as a guide in the separation. Displacement effects become important in this concentration range. Even larger loads are used in industrial applications. This then approaches selective filtration, rather than chromatography, but the hardware and technical approach are those of chromatography. Displacement chromatography can be aimed for deliberately, especially for dedicated production-scale applications. The column technology remains the same.

Resolution parameters are ultimately determined by the nature of the stationary and mobile phases. The sample size that can be separated in one experiment depends on the column volume. In modern laboratory preparative LC, with pressurized metal columns, most columns have an internal diameter (I.D.) of 7–10 mm and length 30–50 cm. Such columns will accommodate *ca.* 10–20 g of silica gel and can handle sample loads between 1 and 1000 mg, depending on the α value. A larger, much-used laboratory column is 25 cm × 2.2 cm I.D. and holds *ca.* 65 g silica gel or silica gel-based packing material. This size has to do with the fact that it is about the limit that can be: (i) handled by analytical LC instrumentation (flow-rate 10 ml/min is the optimum for such a column), (ii) packed efficiently with small particles by conventional methods, (iii) operated for a longer time without continuous column compression, and (iv) constructed with conventional ferrules and fittings.

In industrial or production preparative LC much larger columns are used. Sizes reported in the commercial literature range from 5 to 200 cm I.D. with variable bed lengths. These systems will accommodate, *e.g.*, 1–500 kg packing material, and the sample sizes that can be handled are in proportion. However, production preparative LC must not necessarily be linked to very big columns. Some compounds are used only in very small amounts, even in commercial applications, *e.g.*, biological preparations.

PACKING MATERIALS AND COLUMN DESIGN

Packing materials influence the column design not only through the α value which they determine, but also through their particle sizes. The particle size determines the packing procedure and the back pressure of the column. This again influences the physical aspect of the columns. With particles in the range 50–200 or even 200–500 μm, which are still used in some cases, packing can be achieved in the dry state by simply pouring the powdered packing material into the column. The back pressure of a column packed with such stationary phases is very small, and this considerably simplifies the requirements of column hardware. Any column tube material, even glass or plastic, can be used. With the recent tendency towards smaller particles, this has changed dramatically. The most popular particle size of packing materials is now in the range 15–20 μm. Currently, this seems to be the best compromise between cost, efficiency and instrument capabilities. Such particles cannot be packed in the dry state and require high-pressure slurry packing. Pressures of at least 10–50 bar are needed. For10-μm materials, which provide still greater efficiency, the packing pressure must be even higher. For analytical-sized columns (0.46 cm I.D.), the packing pressure with a 10-μm material is usually 400–600 bar. Naturally, the same pressure should also be applied to columns of larger I.D. if 10-μm particle-size packing materials are used. This

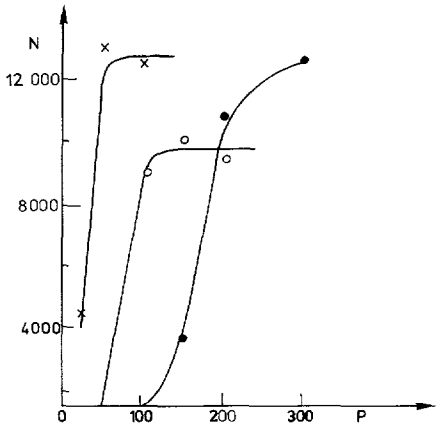

Fig. 2. Plate number for a 25 cm × 22 mm I.D. column packed with 10-μm reversed-phase silica gel (10-μm RSil-C18-HL-D) as a function of packing pressure, P, and slurry solvent viscosity, η. Solvents: ● = methanol (η = 0.60 cP); ○ = diethyl ether (η = 0.23 cP); × = pentane (η = 0.23 cP).

is not so easily accomplished. A stainless-steel cylinder of 20 cm I.D. with a wall thickness of 1 cm can withstand 100 bar, but not much more. With low-viscosity solvents, which increase the packing speed and the impact speed of the particles on the bed, slightly lower pressure may be adequate[7]. This is shown in Fig. 2 for 2.2 cm I.D. columns. While a 10-μm reversed-phase derivatized silica gel would be packed in analytical-sized columns, at 400–600 bar in, *e.g.*, a methanol or carbon tetrachloride slurry, a pressure of 50 bar is sufficient for low-viscosity pentane as the slurry solvent. A word of warning is necessary. Slurry packing of columns of larger I.D. is still an arcane art. The results presented in Fig. 2 may not be obtainable with another/different packing material.

A fused-silica capillary with an I.D. of 320 μm as used in micro LC[8] can withstand up to 500–800 bar of slurry-packing pressure. At 1000 bar, the fused-silica

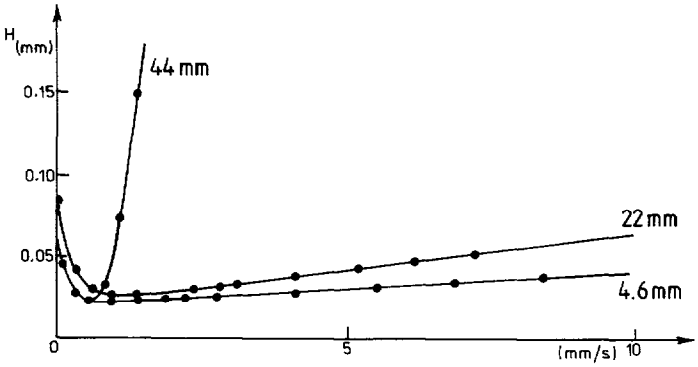

Fig. 3. Influence of column I.D. on the H/u curve (u in mm/s) for small packing particles. Stainless-steel columns of 25 cm × 4.6, 22 and 44 mm I.D. Stationary phase: reversed-phase silica gel (10-μm RSil-C18-HL-D). Mobile phase: water–acetonitrile (25:75) with pyrene (k' = 6) as sample.

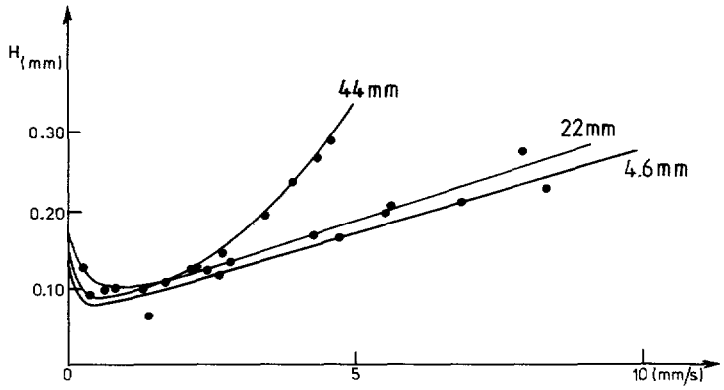

Fig. 4. As in Fig. 3, but with coarser particles (20-μm RSil-C18-HL-D).

column may explode. Stainless-steel analytical LC columns with an I.D. of 0.46 cm are frequently packed at 600–800 bar. They have a wall thickness of about 1 mm. With columns of much larger I.D., in the range 20–50 cm, the wall thickness has to be much larger, if not for packing, then for just operating the columns, which may also require rather large pressures. While the wall strength is not so important for analytical LC, this is not true of preparative LC. Large-scale preparative LC columns must be very heavily walled if they are to be used with small-sized particles. The particle size of the packing material determines the working pressures of the columns and this, in turn, influences the mechanical aspects of the columns. Safety considerations are most important. Rupturing an analytical LC column may not be very dangerous, but this is certainly not true for larger systems. With the modern tendency towards smaller-particle packings and therefore higher pressures, this point should not be ignored.

The particle size of the packing material is also important for the frictional heat generated in the column when the solvent is forced through it. The frictional heat depends not only on the particle size but also on the viscosity of the solvents. When viscous mixtures of methanol–water or acetonitrile–water are used in reversed-phase systems, the frictional heat is more important than in normal-phase LC systems where

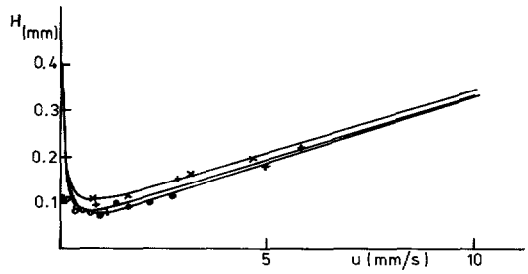

Fig. 5. *H*/*u* curves for columns of various I.D., packed with normal-phase silica gel (Prep-RSil of average particle size *ca.* 20 μm). The reduced plate height is about 3–4 for all systems. No deviations are observed, also not for the "slurry"-packed 4.1 cm I.D. column. Floating-piston-packed and compressed columns do slightly better. The I.D. of 4.1 cm is still modest. Columns: × = 0.46 cm I.D.; + = 4.1 cm I.D., slurry packed; ● = 4.1 cm I.D., floating piston; ○ = 10 cm I.D., floating piston.

low-viscosity solvents are used. The frictional heat would also be more important in columns of large I.D. where heat dissipation is more difficult. These remarks are illustrated experimentally by the curves of plate height (H) versus linear velocity (u) of Figs. 3–5.

Guiochon and Colin[9] state that the shape of the H/u curve is not affected by the I.D. in the range of 1–10 mm. This can be extended to 2.2 cm I.D. columns, even for 10-μm particle and reversed-phase systems. However, in columns of larger I.D. deviations from the H/u curve can occur, as shown in Fig. 3. With 20-μm particles, the deviations are less pronounced, as shown in Fig. 4, but they are still noticeable. The H/u curves in Figs. 3 and 4 can be reproduced repeatedly on the same column, but for the columns of larger I.D. this is true only for a few cycles. The deviations are ascribed to frictional heat, which acts in two ways. First, the flow profile is deformed immediately, because the solvent viscosity is not the same throughout the whole column cross-section. Secondly, the quality of the packing is destroyed, not immediately, but still fairly rapidly. The stainless-steel mantle and the packed bed do not have the same thermal expansion, and this leads to voids and gaps in the packing, specifically at the column wall. With columns of still larger I.D., *e.g.*, 20 cm, this defect can even be visually demonstrated. A freshly packed column has no visible gap between the packed bed and the wall, while such a gap may appear in a column which has been used under conditions where frictional heat effects can be expected. In normal-phase systems, the frictional heat should be less important, because of the lower viscosity of the solvents, resulting in a lower working pressure. This is shown in Fig. 5, where H/u curves for such chromatographic conditions are seen to be without deviation for a 4.1 cm I.D. column. With a 10 cm I.D. column, abnormal H/u curves are obtained, even in normal-phase LC. Columns of larger I.D. are thus unstable, if they are packed with 20-μm and finer material. The line between normal and abnormal H/u curves is not clear-cut. This is obvious, considering the above remarks, and explains controversial opinions about column stability in the field of preparative LC. It is possible to pack even 20 cm I.D. columns of conventional design efficiently with smaller particles, but the columns are not stable. We have repeatedly packed such columns. Even under favourable conditions, *e.g.*, with 20-μm silica gel and hexane as the solvent at a moderate flow-rate, the system, which originally had a good reduced plate height, could hardly withstand more than a few hours of use.

Thus, it is a fact that columns of large I.D. of the conventional type are less stable than analytical-sized columns. Frictional heat is one of the reasons. Through repeated thermal expansion and contraction the packed bed changes, and a void is produced at the top and along the walls of the column. If the packing pressure applied to such systems is too low, this also leads to instability. The pressure surges of the pump during chromatography are probably not compensated by pulse dampeners (as in analytical LC) and this too has a deleterious effect. At very low flow-rates, as used with soft packing materials, or with coarse packing materials, these problems are less important, but in modern high-speed small-particle LC they are. The way to solve this instability problem is to adapt the column volume continuously to the changing packed-bed volume. This is possible with judiciously applied continuous column compression during chromatography. An alternative is to compress the column bed intermittently between consecutive chromatographic experiments. The pumping of liquid through the column during chromatography also compresses it, but this would tend to

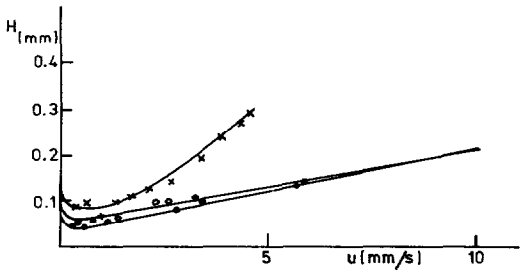

Fig. 6. As in Fig. 5, but with reversed-phase silica gel (Preparative RSil-C18-HL-D). With the higher-viscosity solvents, deviation occurs in 4.1 cm I.D. columns which are slurry-packed. The floating-piston-packed and compressed 4.1 cm I.D. column behaves normally. Columns: ● = 0.46 cm I.D.; × = 4.1 cm I.D., slurry packed; ○ = 4.1 cm I.D., floating piston.

destabilize the system, as mentioned, because it does not change the column volume. The compression should reduce the column volume or adapt the available column volume to the changing volume of the packing material. That this is effective is shown in Figs. 5 and 6 for 4.1-cm columns, even when packed with reversed-phase 20-μm material. While these conditions lead to abnormal H/u curves without compression (see Figs. 4–6) they do not when the column is compressed. Column compression is therefore essential for large-scale LC with relatively small-sized particles.

The reduced plate heights in Fig. 5 are about 3 for all systems. The results are slightly better with the floating-piston compression system. For the columns of larger I.D., the very high flow-rates, which are desirable for establishing the H/u curve over its entire range, could not be achieved with the available pumps. With the reversed-phase columns in Fig. 6 the reduced plate heights attainable were even slightly better and close to 2, although the packing material showed quite a spread in particle size.

Whether a column will be compressible or not affects its design considerably. Since column compression can be avoided by using coarser particles, normal-phase

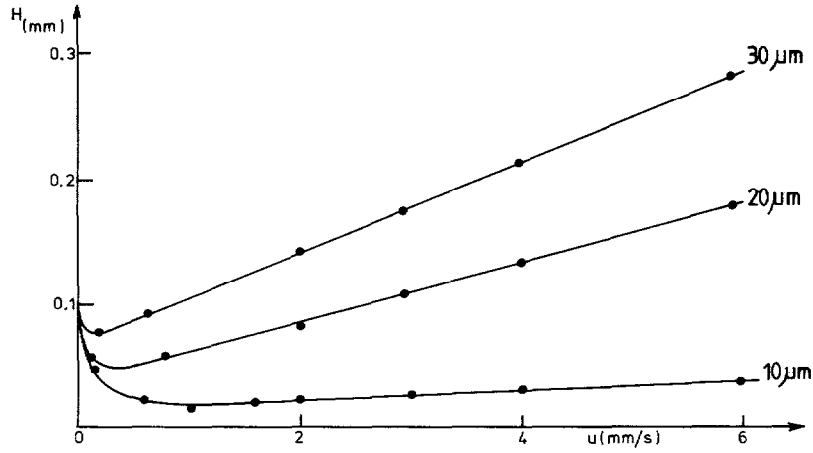

Fig. 7. H/u curves and particle size. 25 cm × 4.6 mm columns, packed with 10, 20 and 30 μm RSil-C18-HL-D reversed-phase silica gel.

solvents and slow eluent speeds, why go to the trouble of working with much costlier compressed systems? Fig. 7 shows why relative smaller particles should be chosen, if possible, as a function of cost and instrument capabilities. Such curves are well known in both gas chromatography (GC) and LC. The steepness of the curve with increasing flow-rates increases dramatically with larger particles. This is even more pronounced for still larger sizes than those shown in Fig. 7. At the optimum flow-rate the 30-μm material is only three times less efficient than the 10-μm column, and this would seem to be acceptable. However, the flow-rate at the optimum for the 30 μm material is very low. At the more usual and practical flow velocities of 4–6 mm/s, the 30-μm material is much less efficient than the smaller-sized packings. Differences up to a factor of 10 are then observed. Since smaller particles are preferable, column bed compression in larger systems is thus of real importance. Compressed columns have been commercially available for a long time. The above arguments and discussion could therefore be deemed to be unnecessary. However, the recent literature shows that the importance of compression in preparative LC is still not generally recognized. Compression is considered to be necessary for packing the column, which is of course correct, but it is also necessary during chromatography or intermittently to adapt the column bed volume. Whether continuous compression is needed or intermittent compression sufficient is not known. This may well depend on the particular conditions. If really high efficiency is needed, smaller particles, longer columns and higher pressures will be required. Under these conditions, for packing and during chromatography, we believe compression is necessary.

COMPRESSION AND COLUMN DESIGN

For columns up to about 2.2 cm in I.D., the approach to column design, packing and handling can be the same as for the conventional columns, as already mentioned.

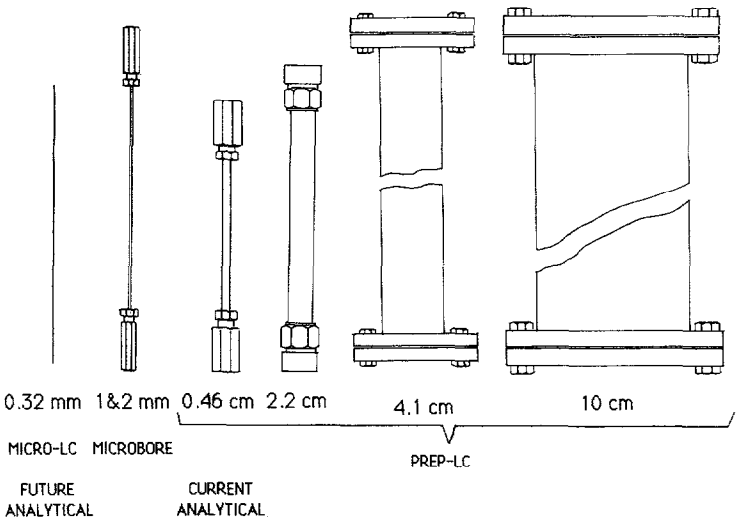

Fig. 8. Schematic drawing of columns of various I.D. (without compression). Up to 22 mm I.D. a nut-and-ferrule system can be used. Columns of larger I.D. must be flanged. Current analytical columns are the small preparative (Prep) LC columns of the future.

However, the ferrules, needed to keep the fittings in position, must be very carefully adapted or else they will slide off the column under the pumping pressure. This difficulty increases rapidly with increasing column diameter and 2.2 cm I.D. is about the limit. A drawing of such a column is shown in Fig. 8. Up to that I.D., column bed compression is not needed and reasonably stable columns can be made without compression. Still, such columns should be handled carefully and not be subjected to very high pressures. Above that I.D., the columns must be terminated and closed in a different manner (with flanges, as also shown in Fig. 8). They also require bed compression, at least for packing, if not during the chromatography to extend the column life expectancy.

Compression can be accomplished either axially or radially. Instruments based on both approaches have been patented and are commercially available from a number of manufacturers. Axial compression can be exerted from above with an adjustable column head (Axxial, Cedi, Merck, Rainin) or from the bottom with a fixed or floating piston (Axxial, Prochrom). A floating piston requires less head space and seems particularly attractive. Radial compression can be achieved with a gas or liquid between a flexible mantle and the column or with prongs (Waters). A combination of axial and radial compression is obtained by inserting a wedge or plunger in the centre of the column top, which is screwed down as the column conditions require (Separations Technology, SepTech). Another axial compression system involves prongs and autocompression from above (Cedi). In the Cedi approach, the plastic packing material cartridges are so heavily walled that the radial compression is thought only to hold these columns and not to compress them. These compression concepts are discussed further and illustrated in Figs. 9–19 with documentation obtained from the manufacturers. Some systems combine compression packing and chromatography under compression (Axxial, Cedi, Prochrom). Others use compression only for packing (Amicon, HT-Chemicals). Still others offer both possibilities (Merck, Varex, Waters). The Rainin and Separations Technology techniques achieve compression in quite a different way. Time will tell which technique is preferable. With really coarse particles no compression is needed, and very simple, not so sturdy instrumentation will be adequate. For intermediate situations, compression for packing only may be sufficient. It is also important how easily and rapidly a used column can be re-compressed. For small-particle, efficient, production-scale preparative LC with long column life expectancy, compression is needed, we believe, not only for packing, but also during chromatography.

PACKING COLUMNS OF LARGER I.D.

The packing technique for columns of larger I.D. depends on the particle size and the column hardware. Very large particles (30–50 μm and above) can be dry packed (the packing material being evenly poured into the column). For very wide 1–2 m I.D. columns, the packing material may be added through a sieve. Smaller-sized particles are either slurry-packed in the conventional way, as for analytical-sized columns, or they are compression-packed. Pumping a slurry at the required speed into a really large column (packing the column like an analytical-sized one) is very difficult. Few pumps have the required capacity. Special know-how must compensate for this (see Fig. 2). Slurry packing is, in fact, forced filtration which eventually results in

a packed bed. Compression packing physically squeezes the packing together. Compression packing a column is rapid and easy, especially with a cartridge system. It is always amazing to see how quickly the column packing is actually achieved with a piston compression instrument. Some manufacturers compression-pack the columns in a separate instrument (a packer) and then take the column over to a chromatographic unit which does not apply extra compression during chromatography (Amicon, HT-Chemicals). Other systems use compression for packing and continuously during chromatography. With other systems, compression is actuated intermittently as the column conditions require. Commercial publicity stresses that stationary phases can be reused many times in preparative LC. In this respect, the brittleness of the particles is important. All packing techniques, and certainly those in which physical compression is used, will crush particles. This may create so much "fines" that permeability problems arise. The hardness of the particles is therefore an important quality criterion. This hardness is indeed very different for different silica gels. Better means for determining the hardness of packing particles ought to be developed. Partly crushed materials can, in principle, be reclassified. For silica gel this can be accomplished without problems. For derivatized silica gel, the breaking of particles produces new surfaces with new silanol functions that are not derivatized. This changes the chromatographic characteristics and has to be avoided.

LARGER COMMERCIAL SYSTEMS

At the end of 1987 about fifty manufacturers of preparative LC columns were asked to communicate their latest developments in the field. Not all of them have replied nor are all specifically active in preparative LC column production. The following list is based on the replies received. Only systems with column I.D.s larger than 5 cm are mentioned. Many other manufacturers produce smaller columns for laboratory preparative LC.

Amicon (Upper Mill, Stonehouse, Gloucester, GL10 2BJ, U.K.)

Amicon offers a wide range of preparative LC columns: in glass up to 4.4 cm I.D., in high-pressure (135 bar)-resistant stainless steel up to 30 cm I.D. and, for lower pressure, production preparative LC on the largest scale, columns with adjustable bed length of 200 cm I.D. Amicon separates the packing procedure from the actual chromatography. The K Prime 3000 columns are thus not "compressed", but the adjoining "packer" (N Pack 3000) does use ram compression. This should facilitate column regeneration. Fig. 9 is a picture of the Amicon N Pack 3000 packing station, showing the ram piston just above the column.

Axxial (44, Rue Maurice de Broglie, F-92800 Aulnay-Sous-Bois, France)

Axxial is a new firm continuing the activity of Jobin-Yvon and Chromatofield. Their Modulprep has been commercially available for a long time. This is both a packing and a chromatography station, which has now been joined by a newer Pilotprep for columns up to 10.9 cm in I.D. An interesting feature is the compression of the column bed from both sides with movable pistons. The Pilotprep separates the packing and chromatography functions. The principle is illustrated in Fig. 10. The column is also axially compressed during chromatography. As a result of the long

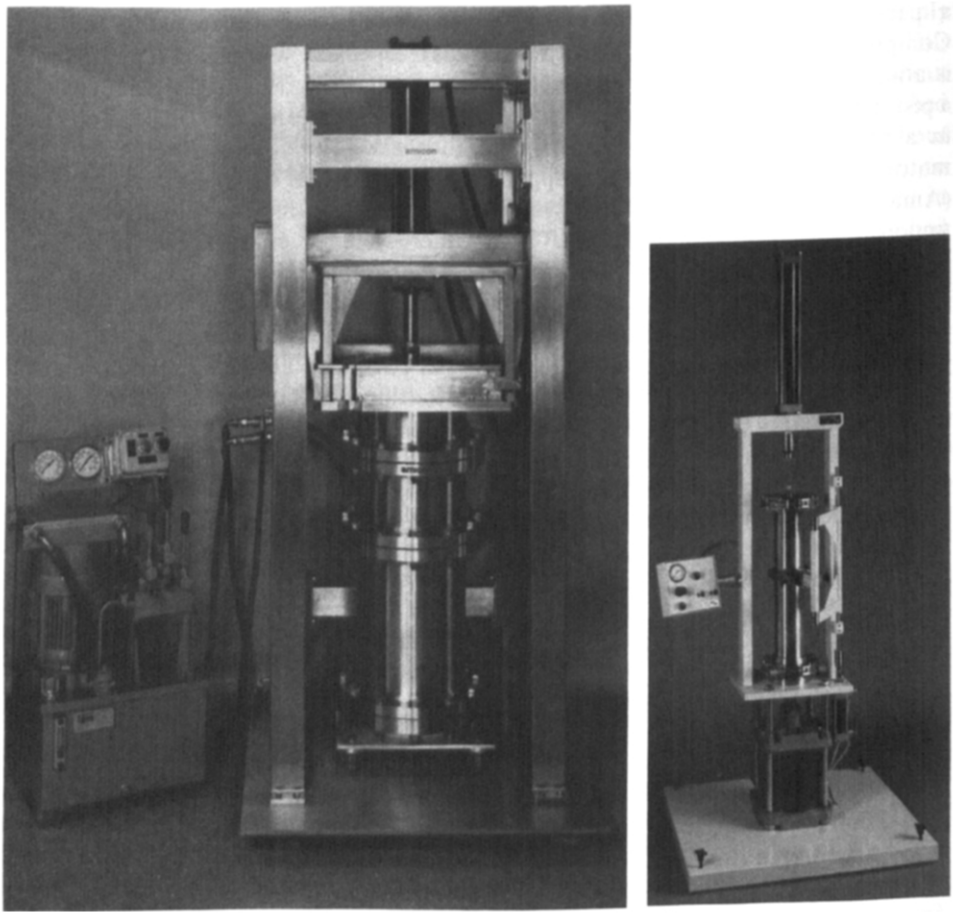

Fig. 9. The N Pack 3000 packing station of Amicon. This device is used for compression packing and for regeneration of columns.

Fig.10. The Axxial Pilotprep system, with which packing and chromatography are not carried out on the same column-holding hardware.

experience in the preparative LC field of Jobin-Yvon, a long list of applications is available.

Büchi (Meierseggstrasse, 40, CH-9230 Flawil, Switzerland)

Although the Büchi system involves glass columns, the approach is that of modern LC. The highest allowable pressure is given as 40 bar, but this seems high for glass and must not be without danger. So is one of the packing procedures described with pressurized gas. Büchi is, of course, aware of this and uses a plastic protective coating over the columns. The largest I.D. available is 10 cm. Normally, Büchi columns are not "compressed". However, a most recent version of the 7 cm I.D. type has a top piston for bed compaction. Glass has its advantages: its very smooth surface makes packing easier, and visibility and high chemical inertness are also desirable. In some cases, inertness may be a most important factor.

Cedi (Route des Usines, F-65300 Lannemezan, France)

This newcomer to the field uses top-applied axial (auto)compression on a piston, sliding in heavily walled plastic cartridges. The cartridges are held in a metal device, which closes with "prongs", as in a radial compression instrument. A specially designed inlet device diverts the eluent in such a way that it pressurizes the column-top piston, hence the name "autocompression". The system can be used with either one or two cartridges. The first cartridge can function as a guard column or can contain a different stationary phase to increase the separation specificity. The double-cartridge approach also allows recirculation without the need for the sample to go through the pump. Recirculation is a most attractive feature, deserving of more attention. Figs. 11 and 12 illustrate the Cedi technique. Columns up to 10 cm in I.D. are available.

Dorr-Oliver (77 Havemeyer Lane, Stamford, CT 06904, U.S.A.)

Dorr-Oliver supplies proprietary-design hardware for columns up to 1 m in I.D. A range of packing materials is available, and the packing technique and know-how for columns of large I.D. is transferred to customers. Compression is not mentioned. Dorr-Oliver states that each column I.D., and each particle size, requires a different packing method and that therefore optimum results can be achieved only in close cooperation between the customer and manufacturer: "We have chosen not to supply column packing systems to the market, because aspects of our technique ... require considerable know-how and experience". The smaller-sized columns are offered with a guaranteed plate number, *e.g.*, 22 000 for 1 m × 7.5 cm I.D. column packed with 10/20 μm octadecylated silica gel.

HT-Chemicals (4221 Forest Park Bvd, St. Louis, MO 63108, U.S.A.)

This firm has a range of stainless-steel columns up to 10.2 cm in I.D. Larger systems can be provided on request. A particularity of HT-Chemicals is their use of a SCRAM (slurry compression ram) for packing the columns. In actual LC, the columns are used without compression. A packing station is available. This can probably also be used for chromatography.

Merck (Frankfurterstrasse 250, D-6100 Darmstadt, F.R.G.)

The new, large Merck columns (Prepbar) are used with axial compression, applied at the top. This "compression" is continuous when the packed column is used in the Merck preparative chromatograph, but the packed columns can also be used in other instruments. In this case, the packing can be recompressed intermittently whenever necessary or at regular intervals, by tightening the bolts at the column top, thus adapting the column volume to the packed-bed volume. A range of Merck columns is shown in Fig. 13. The largest columns in this series have an I.D. of 10 and 20 cm. Merck advocates the use of cartridges and offers a wide range of packing materials. Cartridges somewhat restrict the free choice of the operator (this should in our opinion not be overestimated), since they determine the column length, but they have the advantage that the large amounts of silica gel can be cleanly handled. Fig. 13 also shows some large columns without a compression head device. In addition to these higher-pressure systems, Merck still advocates their Lobar non-compressed low-pressure glass columns. A recent review illustrates that, for laboratory applications, they can still be useful[10].

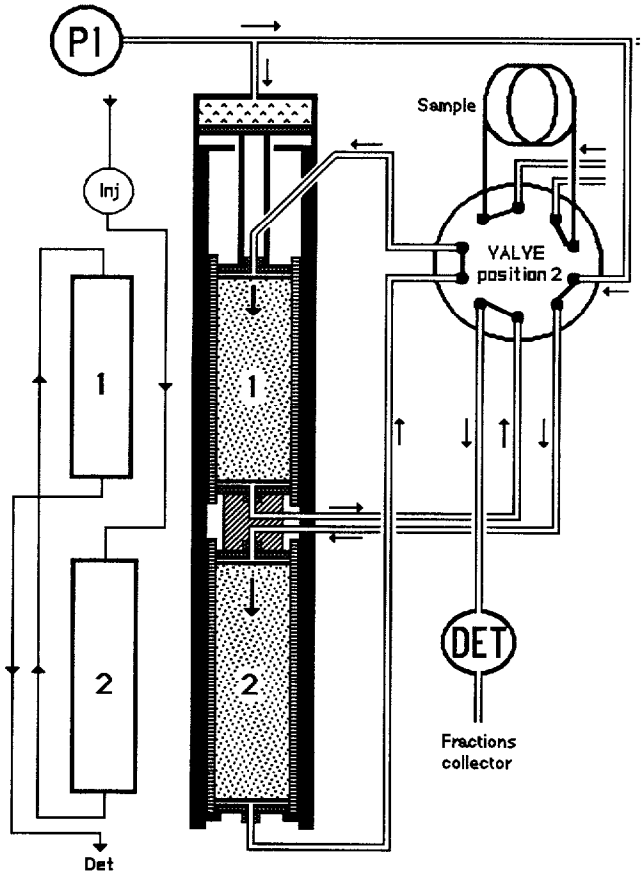

Fig. 11. The Cedi system. Two cartridges can be used either in series or in bypass. The Autocompression system is also shown. PI = Pressurized inlet; DET = detector; 1 and 2 = cartridges.

Pharmacia (Björkgatan 30, S-75182 Uppsala, Sweden)

The latest product from Pharmacia is the BioPilot concept. This preparative liquid chromatograph is quite different from the others in the sense that it involves glass and plastic columns, only works at a maximum pressure of 20 bar and strongly emphasizes biocompatibility. The size of the packing materials, like Sepharose and Mono Q beads, is relatively large and they are sterilized with strong sodium hydroxide solutions (something which is not possible or mentioned for other systems). Preparative LC is carried out in 6 cm I.D. columns and thus has the large capacity of production-scale preparative LC.

PPG Industries (P.O. Box 2844, Pittsburgh, PA 15230-2844, U.S.A.)

The latest in column technology comes from the Fiber Glass Research Center of PPG. They state their case as follows: "Column packing of aligned fibrous-cylindrical silica gel overcomes the limitations of particle-packed columns. These columns

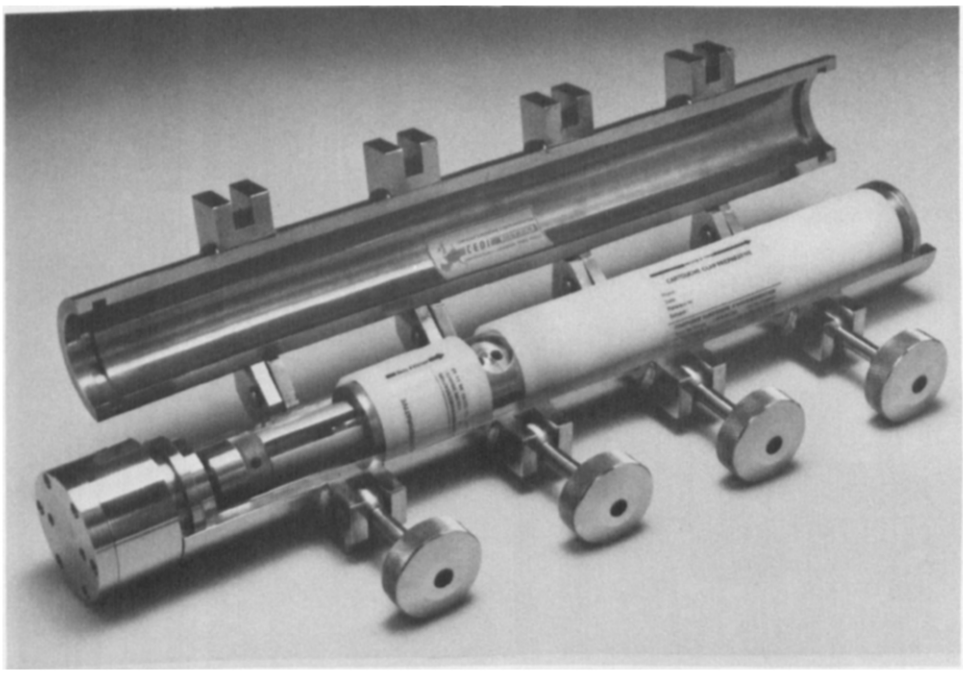

Fig.12. The metal holder of the plastic cartridges of the Cedi system.

combine high packing density with very good permeability. Techniques have been developed for reducing fiber variation and packing inconsistencies". PPG announces columns with I.D. of 7.5 cm. Such columns would not need "compression" techniques. It will be interesting to see how this approach develops.

Prochrom (Chemin des Blanches-Terres, BP 9, F-54250 Champigneulles, France)

Prochrom (formerly Chromatelf) offers 20, 30 and even 60 cm I.D. axially compressed columns with all necessary ancillary equipment. The advantages of this system are that the user packs his own columns and that various column lengths are thus available. Packing materials can be reused many times. The choice of packing material is also freer than with other systems involving cartridges or pre-packed columns. Prochrom obviously also has much experience with these largest I.D., commercially available, compressed production preparative LC columns. Fig. 14 is the by now well known illustration accompanying Prochrom advertisements. The same set-up is used for packing and for chromatography, which is thus carried out under continuous compression. Note that the Prochrom systems require hall space for their location. In these Prochrom columns of large I.D., the piston actually moves slightly up and down during chromatography. This is accompanied by a characteristic noise and is a clear indication that the packing is "alive" and that continuous bed volume adaptation or "compression" is needed

Fig. 13. Merck columns, which are probably packed at very high pressure. The column top can be screwed in to actuate column compression.

Rainin (Woburn, MA 01801, U.S.A.)

The *dynam*ic *ax*ially compressed or Dynamax system of Rainin is based on axial compression by screwing down the whole column top (Figs. 15 and 16). The aim is to eliminate the voids that could develop at the column top. As the Rainin literature puts it: "As large volumes of mobile phase pass through, the cumulative effects of infinitesimal dissolution of packing material and minute pressure pulsations, no matter how small, eventually take their toll. The ideal bed structure disappears. Small

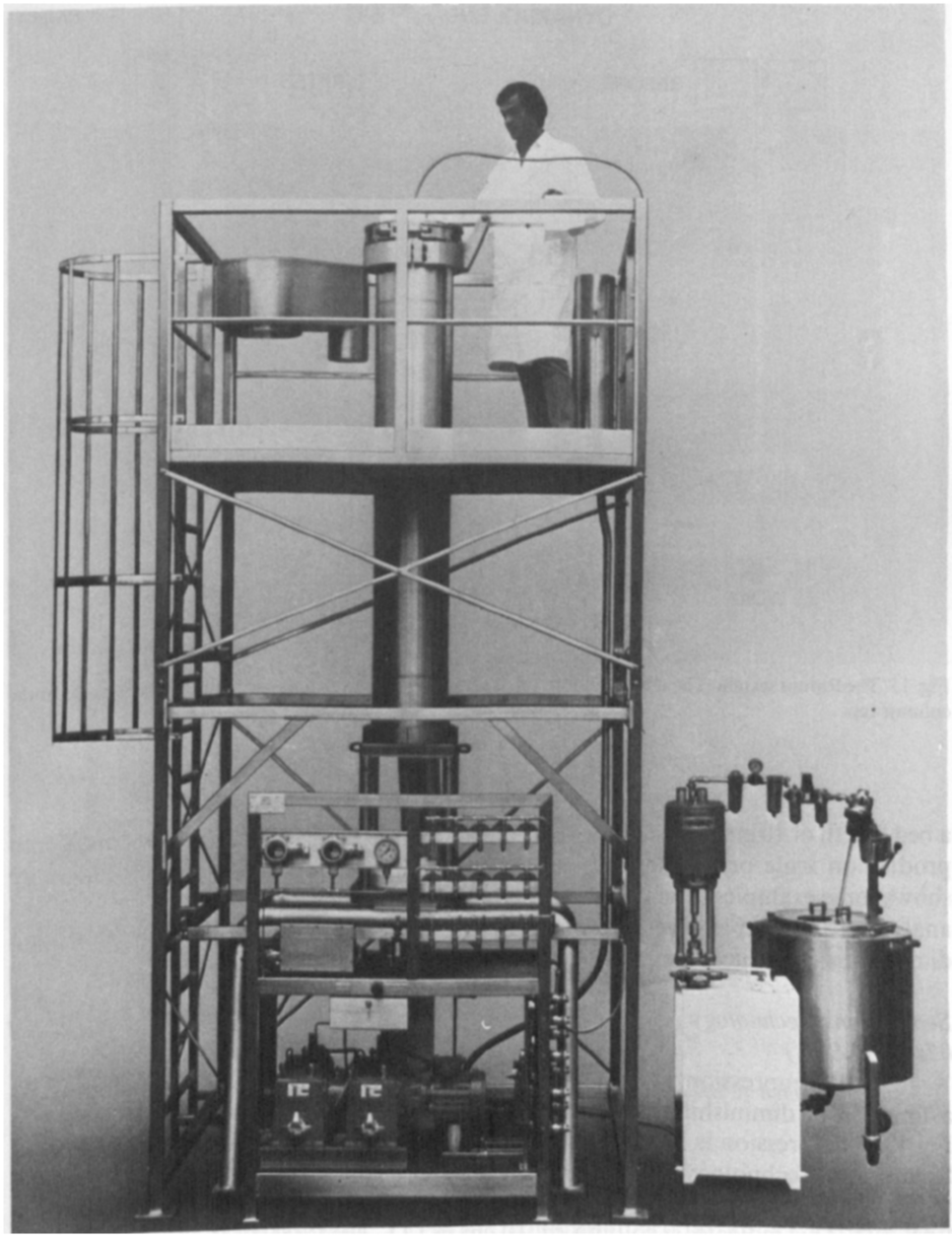

Fig. 14. The set-up for a 30 cm I.D. Prochrom axially compressed system. The required height for this instrument must be considered.

voids form at the head of the column. Efficiency and symmetry decline, and the column becomes unusable". Rainin applies its technique to analytical as well as to larger column sizes. The most recent Rainin preparative LC column has an I.D. of 10 cm with

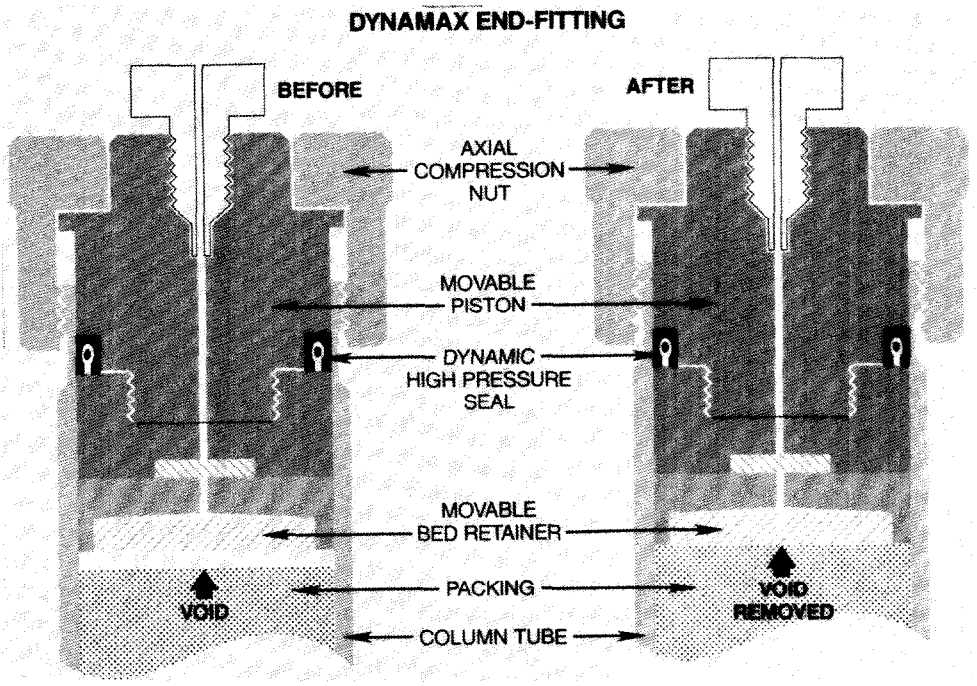

Fig. 15. The Rainin system. The whole column top is screwed down to reduce voids which may appear at the column top.

a bed length of 10 cm. This handles multigram sample sizes. Whether this is considered production scale or not depends on the problem, of course. The Rainin literature shows some examples of scaling-up experiments, for which the absolute identity of the analytical and preparative LC tracings is remarkable. Usually, changes in column dimensions introduce unavoidable chromatographic pattern changes.

Separations Technology (SepTech) (P.O. Box 63, 2 Columbia Street, Wakefield, RI 02879, U.S.A.)

This compression system inserts a wedge or plunger into the column. As a function of diminishing performance, this plunger is screwed down, as shown in Fig.17. Compression is exerted at the same time in both axial and radial directions. Separations Technology introduced its Macrobore Annular Expansion methodology at the 1987 Pittsburgh Conference. Good chromatograms were shown at 200 ml/min flow-rate. This indicates a column *ca.* 10 cm in I.D. The largest column is claimed to hold 40 kg of packing material. With the apparent density of silica gel (about 0.5) this corresponds to a column of 70–100 cm × about 30–40 cm I.D. Documentation and/or a prototype of such a large SepTech Macrobore A/E instrument is not yet available.

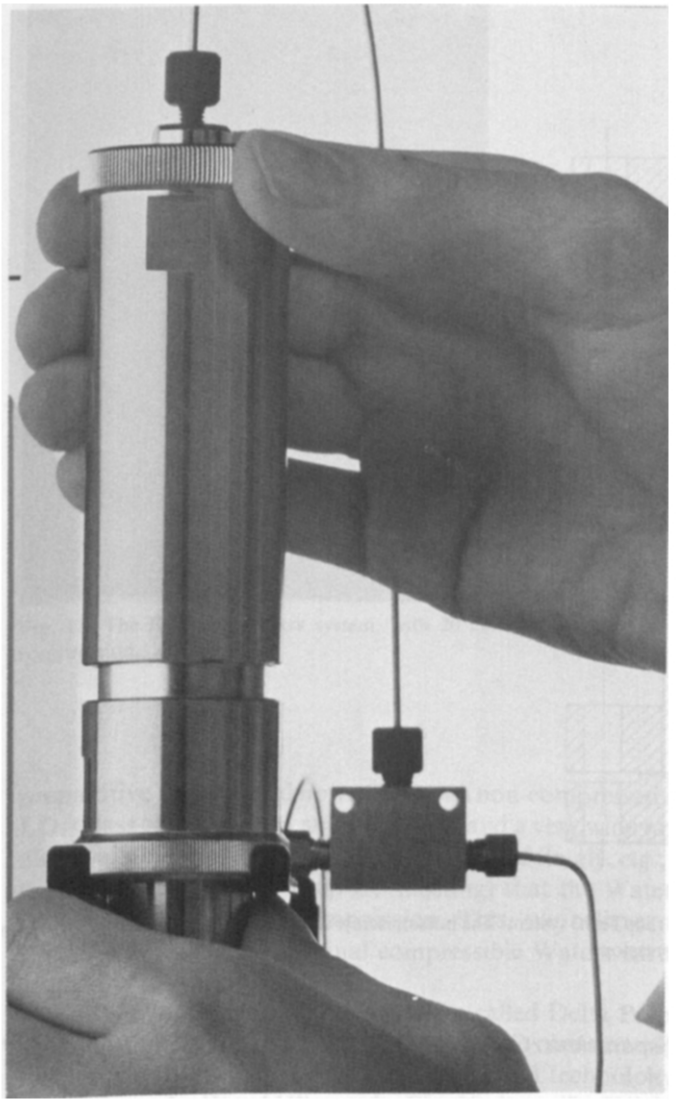

Fig. 16. Compression of the column top in the Rainin system can be achieved by hand tightening.

Varex (12221 Parklawn Drive, Rockville, MD 20852, U.S.A.)

The Varex Versa series covers a wide range of preparative LC columns, from the laboratory-range preparative LC 2.5 cm I.D. columns to production-LC systems with 30 cm I.D. columns. These are non-compressed systems. Varex offers a very wide range of pumps, detectors, injectors, collectors, gradient, recycling facilities, etc. High-performance preparative liquid chromatography at Varex has become HP^2LC which reminds us of the $(GC)^2$ of some years ago.

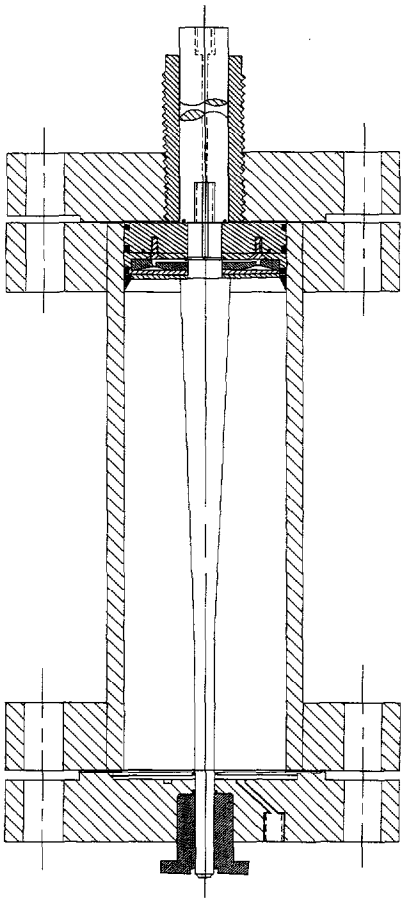

Fig. 17. Separations Technology (SepTech) system. The bottom screw can draw in the tapered plunger, thus achieving axial and radial compression.

Vydac. Division of the Separations Group (17434 Mojave Street, P.O. Box 867, Hesperia, CA 92345 U.S.A.)

Although this firm has no literature presently available on columns of larger I.D., it has started to offer 5 cm I.D. columns, pre-packed with its materials. Vydac specializes in the separation of polypeptides and other biomolecules. This particular field is bound to become more important with the development of biotechnologies. The success of recombinant DNA techniques will depend on good preparative LC. We feel that this particular point has so far not received the attention it deserves.

Waters (34 Maple Street, Milford, MA 01757, U.S.A.)

Waters, which is now a division of Millipore, is well known for its radial compression systems. Its earlier Prep LC/System 500 is probably the most popular preparative chromatographic system at this time. It is based on rather coarse packing materials, even in the 100-μm range. However, Waters has greatly diversified in the

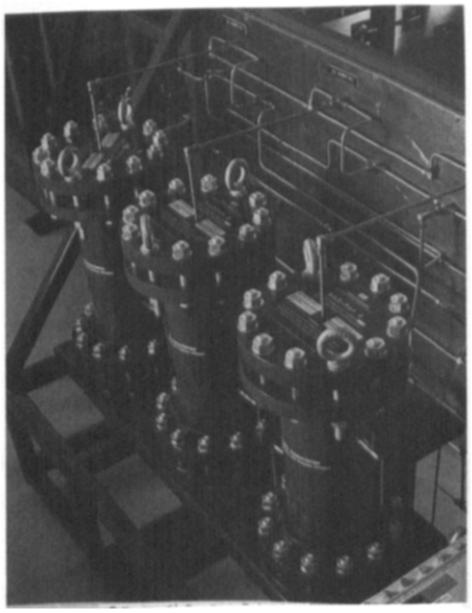

Fig. 18. The Kiloprep Waters system, with 20 cm I.D. columns, which involves cartridges and axial compression.

preparative LC field and now offers even non-compressed metal columns, up to 5 cm in I.D. Glass columns, very small particles and a very wide range of packing materials are also available. Chromatographers have stated (lately, *e.g.*, at the discussion sessions of the Baden-Baden 1988 Prep-LC meeting) that the Waters columns perform as well with as without radial compression. This, we believe, is true only for very large particles, but then the original compressible Waters cartridges were filled with very large, about 70-μm, particles.

The successors of Waters 500 are called Delta Prep and Kiloprep. The largest Waters columns have an I.D. of 20 cm and involve cartridges and radial compression. Precise details on the column dimensions and technology for the larger systems are absent from the Waters literature. Fig. 18 shows the Kiloprep (or largest) system with 20 cm I.D. columns.

Whatman (9 Bridewell Place, Clifton, NJ 07014, U.S.A.)

Whatman offers a stainless-steel Magnum 70 column, 100 cm × 7 cm I.D. This column is intended for packing with Partisil 40. It is not a compressed column, although Whatman has a compression technology (WCS or Whatman Compression Screw). Whatman also offers a glass-reinforced, polymeric Prep-25 column for cellulosic media, with an I.D. of 45 cm and a bed 16 cm deep. This "pancake or waffle"-type column is something we will probably see much more of in the future (Fig.19). The logical endpoint of the quest for high efficiency and large capacity is indeed the use of small particles in a wide and short column. Another reason for

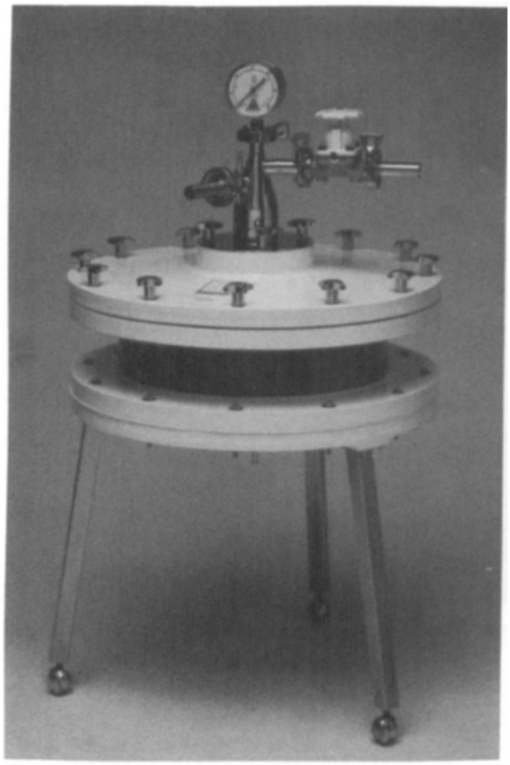

Fig. 19. "Biocompatible" short and wide Whatman column for cellulosic and other "soft" media.

choosing this column type may be that the particles needed can not withstand high pressure and can therefore be used only in short beds. Higher capacity can be obtained only by increasing the column I.D. which again leads to the "pancake waffle type".

CONCLUSION

Recent years or even months have seen spectacular new developments in preparative LC column technology. The field is growing very rapidly. The array of larger systems for process-production preparative LC is already impressive and is bound to increase.

Columns are dry-, slurry- or compression-packed. Some systems involving compression packing do not use compression during the actual chromatography. Whether this is needed or not depends on the difficulty of the problem. For easy separations which can be accomplished in the normal-phase mode, and with relatively large particles, compression may not be needed, or only intermittently. A compressed system can, of course, accomplish both easy and difficult separations, but its cost is much higher.

Considering the large number of manufacturers who all use more or less the same principles, the patent problem is serious. This too could have an influence on the future of some systems.

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