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Study of the packing behavior of axial compression columns for preparative chromatography

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Abstract

The behavior of the packing of axial compression columns (5 cm I.D.) has been studied using IMPAQ C_{18} silica (average particle size 17 μm) as the stationary phase and water–methanol solutions as the eluent. Provided the frits used at both column ends are properly cleaned, columns of the same efficiency as analytical columns are easily and reproducibly obtained. The efficiencies of several columns for acetone (poorly retained), phenol (moderately retained) and cresol (strongly retained) were found to remain unchanged over periods of 100 to 200 h.

The column length was monitored during operations. It was found to decrease slowly over time, and to change when the packing solvent is replaced by the experimental eluent, and during gradient elution. It is smaller when the eluent contains more water. The total porosity and the permeability of the axial compression columns were lower than those of analytical columns.

1. Introduction

Preparative high-performance liquid chromatography has become a method of choice for the extraction, the separation and the purification of fine chemicals and biochemicals from complex mixtures in the laboratory and at the production-scale level [1]. In the pharmaceutical industry, it is the only general-purpose method available for the production of pure enantiomers. It permits the purification of antibiotics, peptides and proteins to extremely high levels of purity. Its main inconvenience is the large cost of this process. Considerable effort has been devoted recently to its improvement, especially in the areas of application selection [1–3], and of the choice of the proper chromatographic system [1–4], as well as in the fundamental theory of the process [5–7], its modes of application [7,8] and in the optimization of the experimental conditions for maximum production rate or minimum cost [7,9–11]. Surprisingly, however, the properties of the column itself have been largely ignored. Few studies have been devoted to packing technologies, the structure and the homogeneity of the bed, and its main properties, i.e., its porosity and permeability.

The packing should be optimized for stability, efficiency and low hydraulic resistance. Operators have long complained that after a certain period of satisfactory operation, the performance of a conventional packed column

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and particularly its efficiency decrease rapidly. If the column is open, large voids appear at the top of the bed. Turbulences develop easily in these voids and are deleterious for the separation. This phenomenon does not appear in dynamic compression columns because voids cannot form when the bed settles. This explains their popularity among practitioners. It has even been shown that restoring dynamic compression after some interruption can mend the effects of voids or cracks formed inside the column bed during this interruption and restores the initial efficiency [12].

Three different compression technologies have been developed, annular [13], axial [14–16] and radial [17,18] compression. Although each of these approaches could be used either in the static or in the dynamic mode, instrumentation is available only for axial and radial dynamic compression. The former has been used with columns 2.5 to 80 cm in diameter, the latter for analytical columns and for preparative columns up to 20 cm in diameter. In a separate paper, we have reported on the behavior of radial compression columns [12]. The goal of the present work is a study of the packing performance of dynamic axial compression columns.

The original design of the axial compression column is due to Godbille and Devaux [14,15]. No systematic investigation of the properties and performance of these columns has been published to the best of our knowledge. However, Kroeff et al. [19] have demonstrated their usefulness in the purification of recombinant proteins, and more specifically, of human insulin. This type of column is widely used in industry. A number of reports mentioning their preparation [20] and systematic use can be found [21]. However, evidence regarding the superiority of dynamic compression columns over conventional ones is still mainly circumstantial. In this work we report on studies made with a dynamic axial compression column (5.0 cm I.D.). The work involves the packing of these columns, the determination of their efficiency and the study of their stability.

2. Experimental

2.1. Dynamic axial compression column

The unit used consists of an LC.50.VE.500.100 Column Skid, obtained from Prochrom (Champigneulles, France). The column is made of a stainless-steel cylinder (59.0 cm × 5.0 cm), with outer grooves at 15° angles and an inside cone at 10° angles at both ends. The grooves assist in fitting the top flange with a metallic clamp and the base with metallic braces. The inside cone at each end assists the entry of the piston chevron seals. The maximum working pressure of this column is 100 bar.

The compression unit consists of a Haskel (Burbank, CA, USA) pump, a pump oil reservoir, a three-way hydraulic valve and a hydraulic jack, all components being housed in a four legged stand. The Haskel pump drives the hydraulic jack and is assisted by compressed air from a cylinder. The upward or downward movement of the hydraulic jack is controlled by the three-way distribution valve which directs the oil flow. The piston is connected with the jack. The piston head contains a sample distributor and the inlet frit. It is connected to a tubing for the incoming mobile phase. The outer layer of the piston head contains chevron V-seals for proper sealing. The top flange contains a sample distributor, the outlet frit and an O-ring for proper sealing of the frit against the column wall.

2.2. Solvent-delivery system

A Kiloprep 100 HPLC pump was obtained from Biotage (Charlottesville, VA, USA). The pump is designed to deliver solvents up to 500 ml/min at a maximum pressure of 138 bar. The flow-rate is set manually. The system includes also two solvent ports and an injection valve with an injection loop.

2.3. Stationary phase

The column was packed with IMPAQ RG1020C18 a reversed-phase C_{18} bonded silica

(BTR Separations, Wilmington, DE, formerly The PQ Corporation). The product specifications provide for irregular particles having an average size of 16.7 μm and an average pore size of 100 Å.

A 10 × 0.46 cm analytical column was packed in this laboratory using the same stationary phase, and a conventional slurry packing method at 345 bar. The characteristics of these columns are summarized in Table 1.

2.4. Chemicals

Acetone, *m*-cresol, phenol, methyl benzoate, toluene, acetonitrile, nitric acid, ammonium hydrogenfluoride, 2-propanol and methanol were purchased from Baxter (Atlanta, GA, USA) and were 99.9% pure. L-Isoleucine, L-phenylalanine and L-tryptophan were purchased from Sigma (St. Louis, MO, USA). Distilled water from the chemistry department plant was filtered on a 1.2-μm membrane before use.

2.5. Detector

A UV–visible detector (Model 204; Linear Scientific, Reno, NV, USA) equipped with a variable-pathlength preparative cell was used to collect chromatograms. With a short cell pathlength the detector response remains linear up to much higher concentrations than with conventional HPLC detectors. The cell can be operated up to 138 bar at 500 ml/min flow-rate. For height equivalent to a theoretical plate (HETP) measurements the cell path length was kept to its maximum so that reasonable response could be obtained with analytical size injections. For overloading experiments the cell path length was set to its minimum.

Careful detector calibration was done with solutions of known concentrations of phenol in methanol–water solutions (40:60, v/v). The column was disconnected from the solvent line, the whole delivery line was then purged with each new solution of phenol, and the detector response recorded while flowing the solution at 50 ml/min. A calibration curve was constructed by plotting the known concentration (C) versus the detector response (R, volt). A fourth-degree polynomial ($C = 2\,054\,441R^4 - 405\,263R^3 + 28\,252R^2 - 737.2R + 5.295$) gives a good fit to these data.

2.6. Pressure sensor

The column inlet pressure was measured with an Omega pressure transducer Model PX603-2KG5V (Omega, Stamford, CT, USA). This transducer gives a 1–5 V d.c. linear output at 0 to 138 bar. Its response time is 1 ms. The output was adjusted to read 0.402 V d.c. for 0 bar and 2.022 V d.c. for 138 bar, for compatibility with the data acquisition system. Calibration shows

Table 1
Characteristics of axial compression columns

Column properties	Column 8	Column 9	Column 10	Column 0[a]
Dimensions (cm)	15.6 × 5.0	16.2 × 5.0	20.0 × 5.0	10 × 0.46
Hold-up volume (ml)	181.0	212.0	258.4	1.13
Total porosity (solvent A)	0.60	0.67	0.66	0.68
Phase ratio (solvent A)	0.69	0.50	0.52	0.47
Total porosity (solvent B)	0.57	0.63	0.62	0.71
Phase ratio (solvent B)	0.77	0.63	0.62	0.41
k'(acetone)	0.37	0.38	0.40	0.26
k'(phenol)	2.72	2.90	3.17	2.50
k'(cresol)	6.10	6.59	7.41	6.29

Solvent A = pure methanol; solvent B = methanol–water (40:60, v/v).
[a] Analytical column.

that the inlet pressure (P, bar) and the voltage output (V, volt) are related by $P = (V - 0.402)/(1.1745 \cdot 10^{-2})$.

2.7. Displacement sensor

Dynamic changes of the column length were measured with an Electro-Mike displacement sensor Model PAA1555 (Reagan Controls, Charlotte, NC, USA) which includes a displacement transducer and a transmitter with analog output of 2 to 9 V d.c. at 2.0 mm–9.0 mm range. The output voltage was attenuated to 2.2 V d.c. for our data system. Calibration of the sensor output with known targets shows the response to be linear in the 2–9 mm range, d (distance between target and sensor, mm) and V (output, V) being related by $d = (0.20 \pm 0.01)V + (0.18 \pm 0.06)$.

The sensor was fixed to the four-legged stand holding the column and the steel target to the compression piston. Thus, an increase in target distance meant a decrease in column length. Depending on the movement of the piston the distance between the sensor and the target increases or decreases. The resolution of the sensor is approximately 0.01 mm. Its drift (when recording the length of a metal bar) is less than 0.01 mm/day. However, the actual column length, which is measured directly, is known within only 2 mm.

2.8. Data acquisition system

The data system consists of a Waters system interface module (SIM) with two A/D convertors (Milford, MA, USA). This SIM is capable of simultaneous monitoring four sensors and/or detectors and can control three HPLC pumps. The digitized data from the SIM was collected by a Waters Maxima 820 version 3.3 loaded in a NEC computer. All the data files were translated to ASCII format for further use and uploaded to the computer network of the University of Tennessee. For treatment of these data, several DOS- and VMS-based software programs were developed in our laboratory.

2.9. Methods

In the experiments reported here, several solvents were used as eluent. The primary mobile phases were methanol (solvent A) and a mixture of methanol–water (40:60, v/v; solvent B). Other solvents used were water and acetonitrile–water (70:30). The primary test samples were low-concentration solutions of acetone, phenol and *m*-cresol in the eluent. Other test samples contained low concentration solutions of isoleucine, phenylalanine, uracil, methyl benzoate and toluene in appropriate solvents. Sample volumes for HETP measurements were 1.5 ml, injected by filling an appropriate loop. The sample concentrations for overloaded experiments were 200 g/l phenol or higher. Typical sample volumes for these experiments were 10 ml injected from a loop. For obvious economic and waste management reasons, the solvents were pumped in closed circuit, with a 15–20-l buffer reservoir on the solvent line. The solvent was replaced when the baseline absorbance became significantly higher than that of fresh solvent.

Three outputs were recorded in most of the experiments. These were: the UV detector, the displacement and the pressure sensors. The chromatographic data were used to calculate column efficiencies, from the width at peak half-height, and retention factors. The reduced velocities and reduced plate heights were fitted to the Van Deemter equation [22], using a non-linear least-squares fit. The classical Wilke–Chang [23] equation was used to estimate the diffusion coefficients of the compounds used. The adsorption isotherm of phenol was derived using the ECP method [7].

The data from the pressure transducer were converted to pressure units, and the output from the displacement sensor to changes in the column length.

2.10. Procedure for packing an axial compression column

The procedure described below is recommended by the manufacturer. It has been fol-

lowed carefully for all the columns whose performance are discussed in this report. It involves the following steps:

(1) The flow distributors and the frits are positioned inside the top flange and the piston head. If frits are already in place and were used for a previous column, *they must be cleaned carefully before being used again* (see next section).

(2) The column is fixed to its base. Then, the piston is pushed inside, and all the way up through, until it protrudes at the column exit. The seal bolts are checked. They should be snug and are adjusted if necessary. Too much tightening can break the seals and too little tightening cause leaks.

(3) The tubing between the pump and the piston head is disconnected and closed with a sealing nut. A small stainless tubing is fixed to the top flange to drain out the excess solvent while packing the column.

(4) The desired amount of silica (usually ca. 200 g) is measured in a 2-l beaker, with about 800 ml acetone. The slurry is stirred and left settle for about 15 min. The supernatant is decanted, or drained with a large syringe, to remove the fine particles still suspended. This procedure for removing fines is repeated two or three times (optional). Finally the slurry volume is adjusted to 800–900 ml with acetone.

(5) The piston is positioned at a certain distance from the top of the column. The distance is calculated so that the space above the piston can hold all the slurry.

(6) The air pressure in the Haskel pump is adjusted to give the desired oil pressure (100 bar) in the hydraulic manometer. The equivalent piston pressure inside the column is 41 bar.

(7) The slurry is poured into the column and the height of liquid adjusted to the top of the column by moving the piston. The top flange is placed and secured with the metallic clamp.

(8) The piston is moved upward by the pump, compressing the slurry and expelling the excess solvent which is collected in an empty beaker. When the air pump stops resetting, or the piston stops moving, the packing is done.

Unfortunately, columns packed with acetone are not as good as columns packed with 2-propanol, while use of the former solvent is recommended to eliminate the fine particles. Steps 5–8 are the most practical procedure to eliminate acetone. The column is then unpacked immediately, the extruded stationary phase suspended in 2-propanol, and the column repacked following steps 5–8.

Wu and Lohse [20] recommend a simpler procedure which applies when the packing material does not contain fines. They also recommend the use of a higher packing pressure, 60 to 80 atm (1 atm = 101 325 Pa), and the closing of the inlet valve to prevent the mobile phase from flowing from the column at both ends during compression of the slurry. There is a close compromise to adopt between an insufficient compression pressure which does not give a good, stable column, and too large a compression pressure which causes excessive particle breakage. The optimum pressure depends on the packing material.

A convenient variant of the packing procedure consists in removing the fine particles with 2-propanol, by suspending the stationary phase in 1 l of this solvent, waiting ca. 30 min, decanting the supernatant from the top of beaker, adding an equivalent amount of 2-propanol to the slurry, and repeating the procedure two or three times. Finally, the volume of slurry is adjusted to 800 ml and the column is packed following steps 5–8. Then, it is no longer necessary to repack the column.

2.11. Cleaning and installing frits

Frits are held in position in a machined groove. At room temperature the frit has a diameter slightly larger than the cavity where it is housed. To be installed, the frit must be shrunk by cooling it in liquid nitrogen; cooling in dry ice is insufficient. Once cold, the frit is pushed inside the flange or the piston head. Top flange frits are wider than piston frits and cannot be exchanged.

Before replacing a frit, it is necessary to remove the old one. Prochrom supplied us with an adapter that permits cooling the frit alone

while it is inside the piston head or the top flange. After cooling for about 15 min, the frit is removed with a pusher device. The process of removing old frits is more difficult than the process of putting new frits in.

There are two types of frits available, a sintered stainless-steel plate, or a fine mesh grid. In both cases, it is important to use only new frits, or to clean old ones very carefully. The grid frits are more difficult to foul and easier to clean than the sintered metal plates. Cleaning the frits can be done quite effectively by following a very simple procedure. The top flange and the piston head are removed from the column assembly. The top flange and then the piston head is connected to an erlenmeyer flask through a rubber cork and a tubing, while the side arm of the flask is connected to a water suction pump. The cooling adaptor is connected to the flange or the piston head. Liquids poured into the adaptor are sucked into the flask. The following solutions were used in this order: (1) filtered distilled water (200 ml), (2) methanol (200 ml), (3) filtered distilled water (200 ml), (4) 20% ammonium hydrogenfluoride in water (400 to 500 ml), (5) 0.5 *M* nitric acid (200 ml), (6) filtered distilled water (200 ml) and (7) methanol (200 ml).

This cleaning procedure is more complicated but less hazardous than the washing with hot concentrated sodium hydroxyde. Its efficiency seems comparable. As shown below, it gives excellent results.

2.12. Unpacking an axial compression column

Axial compression columns can be achieved, following a simple, easy procedure.

(1) The column must be flushed first with an organic solvent. Otherwise, the pressure necessary to unpack the column may be very high.

(2) The control valve is set on neutral, and the pressure set to zero by switching off the air pressure in the Haskel pump. This results in a drop of the hydraulic pressure only after the hydraulic valve is brought to the downward position. When the oil pressure reads zero, the valve is returned to neutral.

(3) The system is left for 10 to 15 min to achieve hydraulic equilibrium, and the tubing is disconnected from the top flange, which is unfastened and open up slowly to avoid that residual pressure pushes it out to some extent.

(4) The piston pressure is set to ca. 41 bar and it starts pushing the bed out of the column. If necessary, the piston pressure is increased gradually.

(5) Usually, the stationary phase comes out as one piece, but it may break very easily.

3. Results and discussion

3.1. The learning curve

Learning to install, pack, operate and use a dynamic axial compression column was most simple. The only noteworthy difficulty that we encountered is in understanding how much care should be devoted to the process of cleaning the frits. Different criteria could be used as a measure of the learning process. A standardized procedure was used to test all the columns made. The column efficiency turns out of be the most difficult characteristic to reproduce and does not vary much from one compound to another one. The retention factors tend to be related only to the nature of the stationary phase and to its amount, i.e., to the packing density. The separation factors, loadability and production rate are too specific of the compounds selected. Thus, the column efficiency, or rather its HETP, was selected for this study.

The reduced HETP obtained for acetone with the first ten columns packed are shown in Fig. 1. In our opinion, the learning process should be much faster, as our early failures had nothing to do with the learning process itself. The first two columns were tested using water as the mobile phase, and isoleucine, phenylalanine and tryptophan dissolved in water as samples. The efficiencies of these columns were poor, but the efficiencies of analytical columns are as poor under such experimental conditions. Water was abandoned and replaced by methanol, with acetone as the sample (column 3). The next three columns (4–

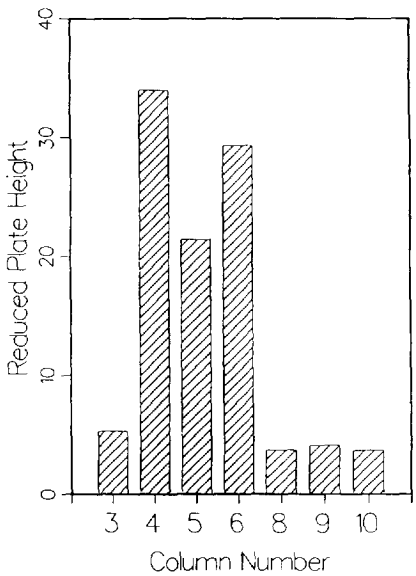

Fig. 1. Learning curve. Plot of the minimum reduced plate height achieved versus the rank of the column packed in the laboratory.

6) were packed with a laboratory-made top flange in which a thermocouple (0.16 mm O.D.) was inserted through a hole, to measure possible thermal effects in the column. Clearly, this affected the column efficiency much more than expected. Probably channeling develops along the thermocouple. Also during the packing of these three columns, cracking noises were heard coming from inside the column.

The next column (7) was never tested. While packing it crushing noises were heard again, the packing process was interrupted, and the column was opened. Similar crushing sounds were also heard while unpacking this column. However, the core obtained looked homogeneous, dense and no harm seemed to have occurred to the packing material. So, that same packing material was redispersed as a slurry and used to pack column 8, which was a good column (Fig. 1). The frit cleaning procedure described above was developed after this column 7 was aborted. The crushing noises were not heard again, but other investigators have reported similar experience.

3.2. Effect of the extra-column volumes

The apparent column efficiency is derived from the recorded chromatograms. The band widths of the peak obtained result from a combination of all the sources of band broadening, including axial dispersion and mass transfer resistances inside the column, which we are interested to know, and a number of possible extra-column contributions, which we must eliminate or correct for in order to determine the separate contributions of axial dispersion and mass transfer resistances. The theory of signals shows that, if the effect of several parts of the instrument combine following a shift-invariant convolution, which is the case in linear chromatography [24], the contributions of the extra-column volumes could be corrected by calculating separately the moments of the concentration distributions given by the detector with and without columns. The moments are given by the following equations

$$\mu_0 = \int_0^\infty y(t)\mathrm{d}t$$

$$\mu_n = \frac{1}{\mu_0} \cdot \int_0^\infty t^n y(t)\mathrm{d}t$$

$$\mu_1 = \frac{1}{\mu_0} \cdot \int_0^\infty t y(t)\mathrm{d}t$$

$$\sigma^2 = \frac{1}{\mu_0} \cdot \int_0^\infty (t-\mu_1)^2 y(t)\mathrm{d}t$$

where t is the time, $y(t)$ is the detector response, μ_n is the nth moment, and σ^2 is the variance of the peak. The zeroth moment is the peak area, the first moment the retention time of the mass center of the distribution, and the second moment is the variance of this distribution [25]. In linear chromatography, the first two moments are additive. The measured values of the retention time and the band variance are the sums of the contributions of the column and the instrument. This allows the calculation of the true retention time and the column efficiency, by subtracting the contributions of the extra-column volumes

$$\sigma^2_{\text{measured}} = \sigma^2_{\text{column}} + \sigma^2_{\text{extra-column}}$$

$$t_{\text{R,measured}} = t_{\text{R,column}} + t_{\text{R,extra-column}}$$

It is easy to estimate the contribution of the extra-column volumes to the measured efficiency by replacing the column with a short length of narrow empty tube having a comparable hydraulic resistance and injecting the same samples, provided the detector response time is sufficiently fast [26]. Chromatograms were recorded with acetone (non-retained) as sample, and pure methanol as the eluent, with and without the column. The variances of all the peaks obtained were derived from their second moment. The results are shown in Fig. 2. The instrument contributions to the apparent column efficiency are entirely negligible, which was expected since the total volume of the sampling valve, detector cell, and tubing is small compared to the column hold-up volume (ca. 210 ml). Accordingly, the results reported below are not corrected for the extra-column volumes.

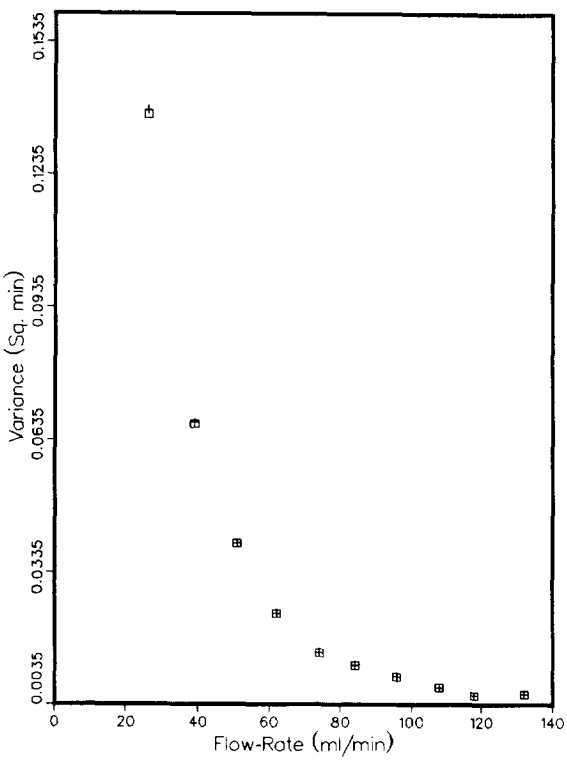

Fig. 2. Plot of the corrected column variance and the apparent column variance (sum of the variance contributions of the column and the extra-column parts of the equipment) versus the mobile phase (methanol) flow-rate for acetone. The variances were calculated from the second moment. □ = Column variance; + = measured variance.

3.3. Column test results

Columns 8–10 were tested immediately after packing, by measuring their efficiency for unretained acetone with methanol as the eluent. Then the column was subjected to aging under different conditions, for extended periods of 100 to 200 h, and the efficiency measured periodically. A selection of the results obtained is given in Table 2 which contains the best parameters a and c of the Van Deemter equation obtained by fitting the experimental data to this equation with $b = 1.80$. The last column of Table 2 gives the approximate time during which the individual column had been used prior to the measurement.

3.4. Column performance with pure methanol

In this series of experiments, pure methanol was used as the eluent and a 1.5% solution of acetone in methanol as the sample. The characteristics of the columns studied are reported in Table 1. The column lengths were determined with methanol as the eluent. Acetone is not retained in pure methanol, and its retention time was used to measure the column void volume. The total porosity of the columns, derived from the hold-up volumes of the negative water peaks with water–methanol solutions as the eluent, are also reported. There are some differences between the values of the total porosity and the phase ratio[1] measured with these two different solvents, but these differences are the same on the three preparative columns and on the analytical column. They are related to the excess isotherm and the dependence of the (small) amount of organic solvent adsorbed from the

[1] Phase ratio, $F = (1 - \epsilon)/\epsilon$, with ϵ = total column porosity.

Table 2
Van Deemter parameters for representative axial compression columns

Col	Solvent	Sample	a	c	h_{min}	ν_{opt}	Hours used
8	A	Acetone	2.94	0.134	3.92	3.67	18
8	A	Acetone	2.61	0.185	3.76	3.12	26
8	B	Acetone	3.85	0.093	4.67	4.40	37
		Phenol	4.31	0.099	5.15	4.27	
		Cresol	4.08	0.076	4.82	4.87	
8	B	Acetone	3.97	0.093	4.79	4.40	46
		Phenol	4.63	0.091	5.44	4.45	
		Cresol	4.61	0.064	5.29	5.30	
8	B	Acetone	3.46	0.116	4.37	3.94	109
		Phenol	3.87	0.096	4.70	4.33	
		Cresol	3.57	0.114	4.48	3.97	
9	A	Acetone	3.30	0.083	4.07	4.66	10
9	B	Acetone	5.02	0.080	5.78	4.74	68
		Phenol	6.12	0.044	6.68	6.40	
		Cresol	6.68	0.073	7.40	4.97	
9	B	Acetone	5.40	0.073	6.12	4.97	183
		Phenol	6.81	0.072	7.53	5.00	
		Cresol	6.42	0.063	7.09	5.35	
10	A	Acetone	3.13	0.133	4.11	3.68	10
10	A	Acetone	2.87	0.165	3.96	3.30	39
10	B	Acetone	4.48	0.104	5.35	4.16	111
		Phenol	7.69	0.087	8.48	4.55	
		Cresol	11.11	0.054	11.73	5.77	
10	B	Acetone	4.58	0.100	5.43	4.24	132
		Phenol	6.67	0.101	7.52	4.22	
		Cresol	7.03	0.068	7.73	5.14	
0	A	Acetone	2.71	0.118	3.63	3.91	NA
0	A	Acetone	2.76	0.120	3.69	3.87	NA
0	A	Acetone	2.38	0.174	3.50	3.22	NA

aqueous solution on the composition of this solution [27,28].

The optimum values of the reduced plate heights obtained with the three preparative columns and with the analytical column are very close, within the margin of error. A plot of the reduced plate height versus the reduced velocity for column 10 and for the analytical column is shown in Fig. 3. These plots are very similar.

The range of variations of the values obtained with acetone for the parameter a (2.38 to 3.30, average 2.84, relative standard deviation 10%) is

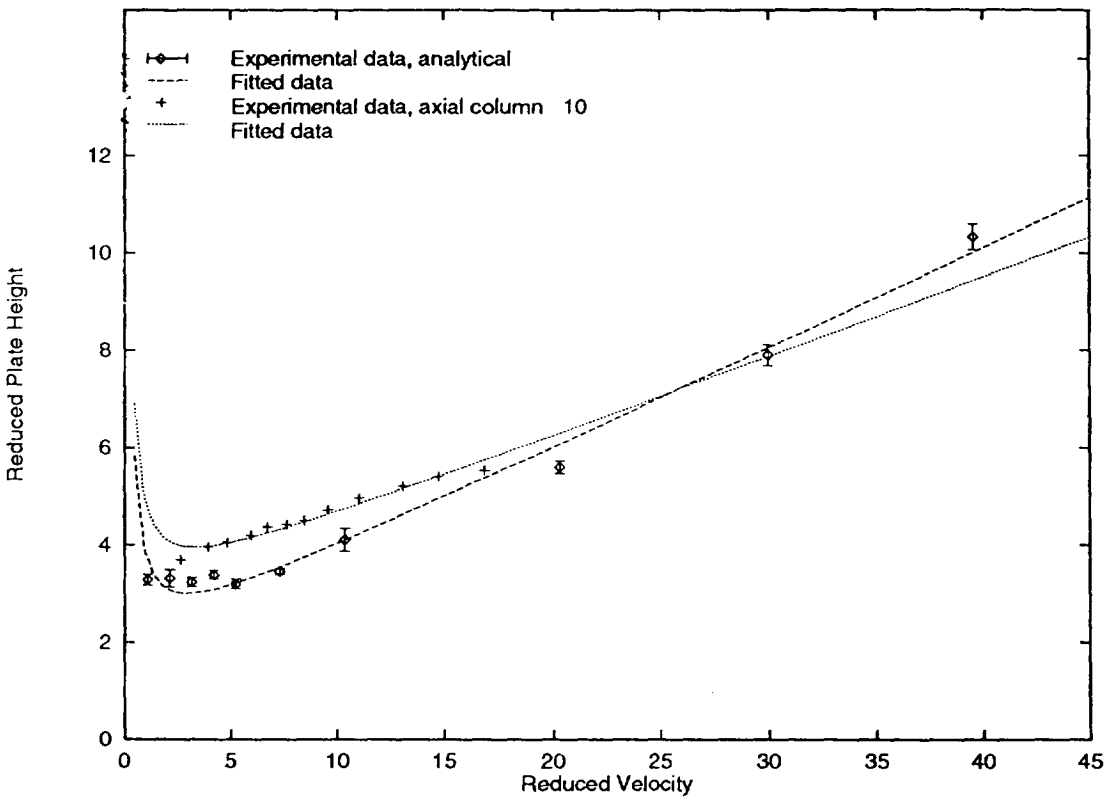

Fig. 3. Plot of the reduced plate height for acetone versus the reduced velocity of the mobile phase (methanol). Axial compression column number 10 (19.80 cm × 5.00 cm) after 39 h of column use. The analytical column (10.0 cm × 0.46 cm) was packed in the laboratory with the same stationary phase.

much narrower than that of the parameter c (0.083 to 0.185, average 0.139, relative standard deviation 24%), probably reflecting the fact that the range of mobile phase velocities within which the HETP data were acquired is too narrow to afford a good reproducibility of this parameter [29].

3.5. Column performance with methanol–water (40:60)

In all further series of experiments, a methanol–water (40:60) solution (solvent B) was used as the eluent instead of pure methanol. A 1.5-ml portion of a low-concentration solution of acetone (15 ml/l), phenol (4.3 g/l) and *m*-cresol (2.5 ml/l) dissolved in the eluent was injected as the sample. The sample also contained excess water, whose negative peak was used to determine the hold-up time. A representative chromatogram of this sample is shown in Fig. 4, with the experimental conditions described in the caption.

The column efficiency was measured and the data fitted to the van Deemter equation. The results are compiled in Table 2. Because almost no data points were acquired at flow velocities below the optimum for maximum efficiency, the precision on the parameter b would be very poor. Accordingly, a constant value of 1.80 was assumed for b in the regression of the experimental data to the Van Deemter equation. The values of the other two parameters of the reduced plate height equations are practically unaffected by the use of the columns for 100 to 200 h. The results obtained with column 9 are

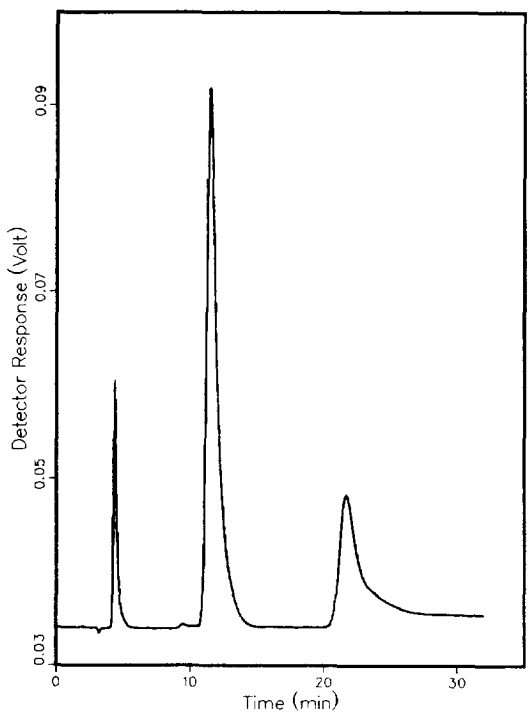

Fig. 4. A representative chromatogram obtained with axial compression column 10 (19.75 cm × 5.0 cm). Experimental conditions: 1.5 ml sample containing acetone (15 ml/l), phenol (4.3 g/l) and *m*-cresol (2.5 ml/l) dissolved in methanol–water (40:60, v/v) with a slight excess of water. The small negative peak is due to the excess water in the sample.

discussed separately in a further section. The values obtained for the parameter a for the three compounds on any given column are not constant but exhibit a significant dependence on the retention. On the other hand, the values obtained for the parameter c for a given compound on the different columns but with the same stationary phase are different. These apparent inconsistencies with the prediction of a simple model probably reflect, at least in a large part, the empirical character of the Van Deemter equation [7, p. 200].

3.6. Effect of replacing 2-propanol by methanol after completion of the column packing

As we are interested in the bed structure and the parameters which may affect it, it was interesting to follow the change in column length, at constant compression pressure and flow-rate, associated with the replacement of the packing solvent, 2-propanol (surface tension, 21.7 dyn/cm at 20°C; 1 dyn = 10^{-5} N), by the standard eluent, methanol (surface tension, 22.6 dyn/cm at 20°C). The experiment was done on column 8, just after completion of its packing. The signals of the three detectors, the UV detector set at 254 nm, the pressure and the position sensors, were monitored simultaneously during 90 min. The mobile phase flow-rate was set at 66 ml/min, and isopropanol was abruptly replaced by methanol 0.15 min after data acquisition began. The eluent was wasted for the first 15 min, then recycled. The initial column length was 16.0 cm and the initial target distance of the displacement sensor was 1.14 mm.

The variations of the column length, inlet pressure, and UV-detector signal are reported in Fig. 5. The UV-detector signal originates from impurities in the solvents which are not HPLC grade and do not have the low UV-cutoff wavelength of the highly pure solvents. The trace of the detector signal shows that all the isopropanol is washed out of the column within less than ca. 7 min. A steady baseline is then obtained, corresponding to the elution of pure methanol. The total volume of methanol passed during that time corresponds approximately to 2 column volumes (V_0 = 181 ml, Table 1; F_v = 66 ml/min), which is not surprising as it includes the amount of mobile phase needed to wash the pump and all the connecting tubings. The replacement of 2-propanol by methanol is accompanied by a rapid oscillation of the inlet pressure, which increases from 0.2 bar (pump restarts) to above 11 bar (column still almost completely filled with 2-propanol), then decreases to 4 bar in about 5 min (column fully washed with methanol), and finally stabilizes at 4.5 bar, after about 15 min. The viscosities of 2-propanol and methanol are 1.77 and 0.510 cP at 30°C, respectively.

The variation of the column length is more complex. It remains stable during the first few minutes, then increases slightly when the 2-propanol is nearly gone, to fall very rapidly, by 2 mm, while the 2-propanol finishes to be washed away. After a short pause, the column length

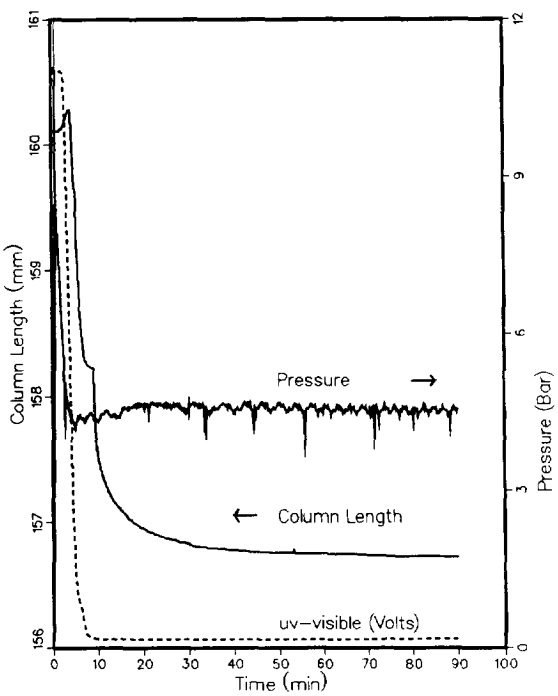

Fig. 5. Plot of the dynamic changes in column length, inlet pressure and UV–visible absorption (UV range 0–1.6 V) versus time when the packing solvent (isopropanol) is replaced by the mobile phase used (methanol). Flow-rate, 66 ml/min. The newly packed axial compression column 8 (16.0 cm × 5.0 cm) was used and the solvent change was a step gradient. The resolution of the position sensor is approximately 0.01 mm and its drift less than 0.01 mm/day.

begins to decrease again, and is not completely stabilized after 90 min. The total reduction in column length is approximately 3.5 mm, or 2%. The column length record is in agreement with the behavior of the dynamic compression unit. The Haskel pump resets at the 4th, the 5th and the 9th minutes, confirming that the column shrinks rapidly during a minute or so, about 4 min after the beginning of the operation, then reaches another equilibrium. It is difficult to explain the details of the column length record, but the trends is clear. Whether because of the increase in surface tension of the mobile phase, or for another reason, the packing density increases when 2-propanol is replaced by methanol, and it takes time for the particles to settle in the bed.

3.7. Variation of the column length during a concentration gradient

After 2-propanol was replaced by methanol, the efficiency of column 8 was measured with pure methanol as the eluent and acetone as the sample (Section 3.4). Three successive sets of experiments were done to acquire all the data needed. After approximately 26 h, the column length was stable at 15.54 cm. In order to carry out the next series of experiments, pure methanol (A) has to be replaced by a methanol–water (40:60) (B) solution. This was done using an exponential concentration gradient, generated by siphoning 4 l of solution B into a 0.5-l flask of A (connected to the pump), at a constant flow-rate equal to the set flow-rate of the pump (40 ml/min). The column length was recorded during that time.

This experiment took over 100 min. After 67 min the solvent in the bottle of A was replaced by fresh solution B. This set-up provided a slow gradient. The signals of the three detectors were acquired and the results are shown in Fig. 6. The pressure rises progressively, corresponding to the increase in viscosity (0.60 cP for methanol, 1.7 cP for B at 20°C, non-linear variation). The column length decreases by about 0.13 mm. The irregular aspect of the curve corresponds to the definition of the sensor, ca. 0.01 mm. This change is parallel to the concentration gradient. It is of a much lesser magnitude than the one observed in the previous case (replacement of 2-propanol by methanol). Note that, from the end of the experiment described in the previous section (Fig. 4) to the beginning of the one discussed here, the column length has been reduced by 1.3 mm (in ca. 26 h). There must be a limit to the packing density which can be reached without breaking particles but it is unclear whether this limit has been reached in any of our columns.

3.8. Relationship between flow-rate and inlet pressure

Measuring the column inlet pressure as a function of the flow-rate serves two main pur-

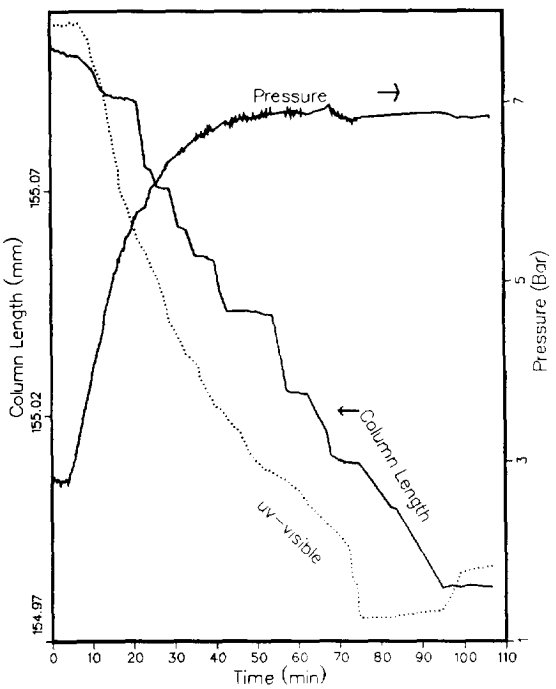

Fig. 6. Plot of the dynamic changes in column length, inlet pressure and UV–visible absorption at 290 nm (detector output in V; UV range 0.018–0.065 V) versus time during a methanol–water gradient (100% to 40% methanol). Axial compression column 8 after 26 h of use with and stabilized length (15.54 cm × 5.00 cm). See text for further details. The resolution of the position sensor is approximately 0.01 mm and its drift less than 0.01 mm/day.

poses in liquid chromatography. First, by reference to previous values obtained with the same column, it permits the detection of blockages occurring anywhere in the system. Secondly, it permits the calculation of the column permeability which, in turn, gives an estimate of the hydrodynamic particle size. The first determinations lead to an estimated particle size which was much lower than the expected value of 16.7 μm given by the supplier and confirmed by previous determinations [12].

Since there was no evidence of particle breakage to the significant extent which could explain this low value of the column permeability (see later), we measured the permeability of the instrument itself, including the tubings, the valves and the frits used in the axial compression column (nominal average pore size, 3 μm). An empty column was obtained by placing the two frits 14 cm apart, with no slurry in the column, and setting the hydraulic valve to neutral. The inlet pressure was measured at different flow-rates for solvent B, and a calibration curve constructed with these data. An excellent fit was obtained a third-degree polynomial with a forced y intercept at zero, giving $P = 0.0715F_v + 1.367 \cdot 10^{-4}F_v^2 - 1.19 \cdot 10^{-7}F_v^3$, with P = inlet pressure (bar) and F_v = flow-rate (ml/min). This calibration curve is used to correct the pressure readings for all columns. We have not observed any significant variation when an old frit is replaced by a new one. As an illustration, Fig. 7 shows the raw data obtained on column 10 after different periods of time, and the data obtained with the empty column.

Obviously, the contribution of the equipment is not negligible. This is not surprising as the diameter of the tubing connecting the pump and the column is relatively small (1 mm). With this diameter, it is possible to achieve a short residence time in the tubings and a rapid radial mass

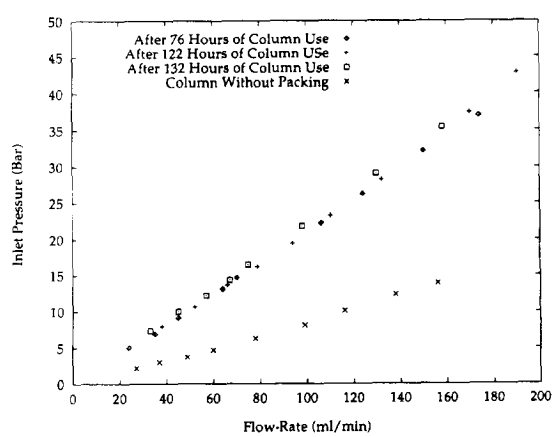

Fig. 7. Relationship between mobile phase flow-rate and column pressure drop. Plot of the column inlet pressure versus the mobile phase flow-rate. See symbols in figure. Top line, uncorrected data without correction for axial compression column 10 after 76, 122 and 132 h of column use. Lower line, same plot for a column without packing (i.e., the flow resistance is due only to the connecting tubings and frits). The relative standard deviations on the measurements of the inlet pressure and the flow-rate are 1.1 and 1%, respectively.

transfer because of secondary (i.e., radial) flow arising at the flow-rates typically used with the column (around 50 ml/min or larger). Turbulent flow takes place in the connecting tubings for a flow-rate[2] of the order of 120 ml/min. Turbulent flow minimizes the dispersion of the front and rear boundaries of the injection at high flow-rates. As a consequence of the narrow diameter, however, the pressure drop in the connecting tubes is not negligible and the data should be corrected to obtain the actual column permeability.

When the measured column permeability is corrected for the equipment contribution, and a value of 1000 is used for the specific permeability, estimates of 19.5 ± 0.4 μm, 19.1 ± 0.2 μm and 18.5 ± 0.4 μm were obtained for the average particle size, after the column has been used for 76, 122 and 132 h; respectively. The apparent decrease in particle size is a consequence of the progressive compaction of the packing reported above. The whole three sets of data are shown in Fig. 8. The data points in this figure appear slightly scattered, but the systematic variation corresponding to the progressive decrease in apparent average particle size indicated above is included in these fluctuations. Nevertheless, we see that the inlet pressure does not change much over the duration of the experiment. The solid line in this figure is the linear least-square fit of the data, with a slope of 0.124 ± 0.002 and an intercept of 0.326 ± 0.154. The particle sizes obtained from these experiments are in good agreement with previous results [12].

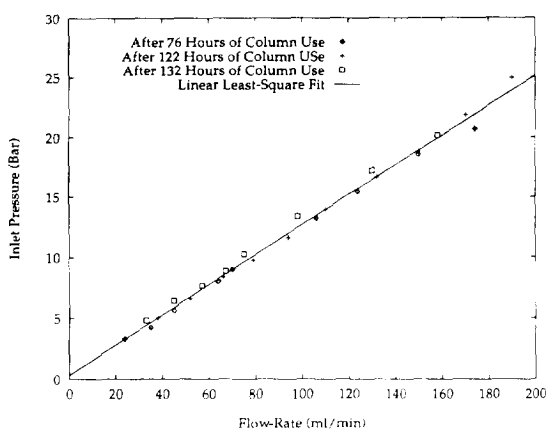

Fig. 8. Relationship between mobile phase flow-rate and column pressure drop. Plot of the column inlet pressure versus the mobile phase flow-rate. Same data as in Fig. 7, after correction for the pressure drop inside connecting tubes and column frits.

[2] $Re = (ud\rho)/\eta = (4F_v\rho)/\pi d\eta)$. For $F_v = 120$ ml/min, $\rho = 0.8$ g/ml, $\eta = 1$ cP, and $Re = 2000$, $d \approx 0.10$ cm.

3.9. Variation of the total column porosity with time

The total porosity of axial compression columns is smaller than that of analytical columns or radial compression columns [12]. With IMPAQ as a stationary phase and solvent B as the mobile phase, typical values are 0.69 to 0.71 for analytical columns, and 0.68 to 0.70 for radial compression columns, while the values obtained for axial compression columns range between 0.57 and 0.67. Careful measurements were performed to study the variation of the total porosity of one column during a change of the mobile phase. This column was packed with the IMPAQ material recovered from a radial compression cartridge (column 2 in our previous work, Ref. [12]) after it was open to take samples for particle size analysis. As the results of this analysis had showed that no particle fragmentation had taken place [12], and an analytical column packed with the material had exhibited the same efficiency as columns packed with fresh packing, the material was used to pack axial compression columns.

A plot of the total porosity of this column as a function of time is shown in Fig. 9. The inset of this figure shows a plot of the column length versus time. The first four data points were derived from the retention time of the non-retained acetone peaks in pure methanol as eluent. The last five data points were derived from the retention time of the negative peak of water in a methanol–water (40:60, v/v) solution. The total porosity decreased rapidly during two periods. First, during the first 20 h of column use, then when the eluent was changed from pure methanol to a more polar (aqueous) solution (same effect as illustrated for column 8 in Fig. 6). This effect is certainly in relation with

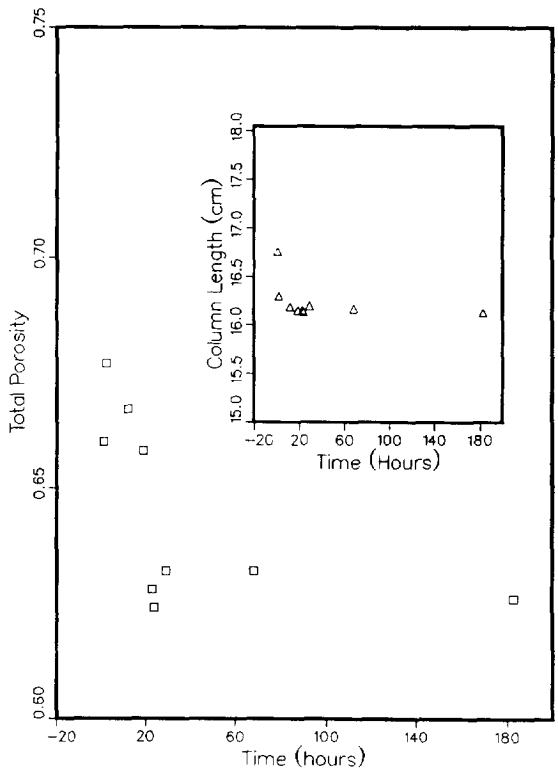

Fig. 9. Plot of the total porosity of the column versus the time of column use for axial compression column 9. Inset: plot of the column length versus the time of column operation. The resolution of the position sensor is approximately 0.01 mm and its drift less than 0.01 mm/day.

the surface tension of the eluent, but its exact origin is unknown, and further work is necessary to understand it. Because methanol is only very weakly adsorbed by the stationary phase (the retention factor of methanol in pure water is lower than 1), the change in measurement procedure should not affect the value of t_0 significantly. Furthermore, the porosity drop at 21 h is in agreement with the decrease of the column length observed during the replacement of pure methanol by a methanol–water (40:60) solution, previously documented for column 8 (Fig. 6).

3.10. Evolution of the particle size distribution

The particle size distribution were measured by Miller [30] using a Coulter Multisizer using 5% LiCl in methanol as the electrolyte, as in a previous report [12]. The experimental data obtained are reported in Table 3 and illustrated in Fig. 10. The relative standard deviation of the determinations of the average particle sizes is between 1 and 2%. The repeatability in a series of measurement is better by nearly an order of magnitude (Table 3). Most of the samples (2–7, included) were from column 8 (Table 2), open after 160 h of operation. They were taken from the entrance, exit and middle, along the wall and in the center. Sample 1 was scrapped from the piston frit of column 8, samples 8 and 9 were from the center of column 9 and sample 10 was a reference. Column 8 was packed with the material previously used to pack column 7. Column 8 was first used for various conventional determinations and operated for approximately 150 h; then a study of the effect of the axial compression pressure on the column performance was undertaken. These experiments were done in the pressure range of 20 to 65 bar. During the study, the column bed was compressed several times for several hours under pressures exceeding the value recommended by the manufacturer for irregular-shaped particles (41 bar). Column 9

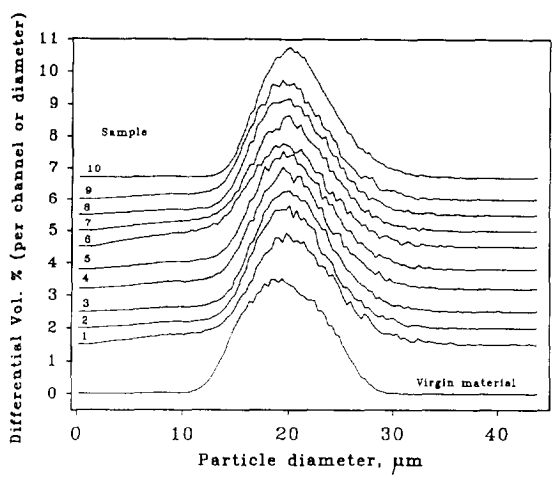

Fig. 10. Particle size distribution of samples of the stationary phase. Differential volume distributions obtained with a Coulter Multisizer counter. Samples: 1 = scraped from the piston frit of column 8; 2 = column 8, outlet, center; 3 = column 8, outlet, wall; 4 = column 8, middle, wall; 5 = column 8, middle, center; 6 = column 8, inlet, wall; 7 = column 8, inlet, center; 8 = column 9, middle, wall; 9 = column 9, middle, center; 10 = virgin material.

was packed with material recycled from the content of the cartridge 2 previously studied in the radial compression equipment [12]. In both cases, the stress applied to the packing was higher than what should be expected under standard operation.

The distributions based on the particle volume is shown in Fig. 10. They are all nearly identical and do not show a significant increase in the volume fraction of small particles. The only differences are a slight ramp from 0 to 12 μm for the samples 1 (scrapped from the piston frit, i.e., at the column entrance) and 6 (column entrance, along the wall), instead of the baseline. Except for these two samples 1 and 6, there is almost no difference between the values of D_v10/D_v90[3] for the virgin material and the samples. By contrast, however, the distributions based on the number of particles (not shown) exhibit an increase in the number of fine particles which is significantly larger than observed with the radial compression column [12], as expected since the packing material used with the axial compression column was recycled from a radial compression cartridge. Given this origin of the samples, explained above, this result indicates that the packing material is reasonably resistant to abrasion and crushing but suggests also that a given batch of stationary phase can be used only a limited number of times. We note also that the ratio D_v50/D_p50, which is the ratio of the medians of the distributions of the particle volumes and numbers, is about the same for all our samples, except for the virgin material and for the packing material on the piston side of the column or at the entrance (samples 1 and 6), regions which seem to have suffered more than the rest. Since the column entrance is the location where the mechanical stress applied by the piston to the packing is concentrated, this result was expected. Our results do not agree with those of Marme et al. [31] who had reported an enrichment in fine particles towards the exit of an axial compression column after 30 h of operation. In our experiments, the fine particles do not seem to migrate significantly but are slightly more abundant in the region where the mechanical stress is higher.

The extent of particle fragmentation during the various operations carried out is somewhat more important with axial compression than it has been observed with radial compression [12]. The difference is not very large, however. No decrease in column performance has been observed, either by us or by others, which could be related to the increase in the proportion of small particles. As a matter of fact, excellent column efficiencies are reproducibly observed. Nevertheless, attention should be paid to this phenomenon, especially when studying the suitability of new column packing materials.

4. Conclusions

As often reported by others at scientific meetings [1], we have found that it is simple and easy to obtain axial compression columns with a reproducibly high efficiency. The learning procedure is fast. If the packing material needed is available, an experienced operator can pack a new column, make a few simple tests and be ready to operate it for the separation required in about an hour. This, however, requires that the stationary phases used need no elutriation, or have been so treated in advance. The most critical issue is the cleanliness of the frits which must be extreme. Fortunately, a simple procedure which gives good results is available. As with all other packing methods, there is no other way to find out whether a newly packed column is good but to test it, although if sounds (e.g., crushing sounds) are heard coming from inside the column while packing it, it is usually poor.

Once packed, the columns remain stable and efficient for as long as we could operate them, 200 h at most, as safety regulations prevented us to operate the instrument unattended overnight or during week-ends. Columns could be packed with stationary phase reused from previous columns, either radial compression cartridges or other radial compression columns. The station-

[3] D_v10 and D_v90 are the particle sizes above which are found 10% and 90%, respectively, of the volume of packing material.

ary phase did not seem to suffer any serious degradation due to its packing, unpacking, and repacking operations. The efficiency of columns obtained with recycled stationary phase is the same as the one achieved with axial compression columns obtained from fresh packing. The slight increase observed in the number of fine particles (Fig. 10) is insufficient to affect the performance.

Because the length of all columns decreases slowly over an extended period of time, so do their total porosity and their permeability. Thus the apparent particle size decreases. The effect is not due to the fragmentation of the packing particles. It is caused by the slow increase in bed consolidation. Thus, it is not surprising that the total porosity and permeability of axial compression columns is lower than that of analytical and of radial compression columns packed with the same phase. The effect is masked in part by the low permeability of the frits used on the instrument. The consequences of this effect remain reasonably easy to manage, as the axial compression pressure required for optimum column performance is moderate. The dependence of the flow-rate on the compression pressure is uncertain at this stage. The phenomenon appears complex, as the column length is also related to the composition of the eluent. Further investigation are in progress.

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