# Consolidation of the packing material in chromatographic columns under dynamic axial compression
# III. Effect of the nature of the packing solvent on the consolidation and performance of axial compression columns

Matilal Sarker[a,b,1], Georges Guiochon[a,b,*]

[a]Department of Chemistry, University of Tennessee, Knoxville, TN 37996-1600, USA
[b]Division of Chemical and Analytical Sciences, Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA

## Abstract

Consolidation characteristics were studied for axial compression columns packed with five different solvents. All the columns were packed with nearly the same amount of the same packing material. Their lengths were close to each other. HETP curves were measured with acetone as the probe and methanol as the eluting solvent. The experiments reported here confirmed previous ones carried out with heptane and methanol. The consolidation process appears to depend on the nature of the solvent. The bed obtained is more compact with heptane than with methanol. The other three solvents give packing densities close to the density obtained with methanol. However, using methanol, isopropanol, or acetone as packing solvent produces better columns than using acetonitrile or heptane. No obvious correlation was found between the packing density and the efficiency of the columns.

Keywords: Stationary phases, LC; Dynamic axial compression; Packing consolidation; Axial compression columns; Consolidation; Packing solvent

## 1. Introduction

In previous studies [1–4] we have investigated the consolidation process of some common packing materials during the preparation of chromatographic columns, using the axial compression technology. These studies dealt with the development of procedures allowing the preparation of efficient columns [1,2] or with the study of the behavior of these packing materials during the consolidation of the column beds made from a slurry in the solvent most commonly used in practical applications of preparative chromatography, isopropanol [3,4]. Preliminary experiments showed, however, that the nature of the solvent in which the particles are slurried prior to

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* Corresponding author. Address for correspondence: Department of Chemistry, University of Tennessee, Knoxville, TN 37996100, USA.
[1] Present address: Supelco, P.O. Box B, Bellefonte, PA 16823, USA.

bed consolidation may have some importance [1]. Replacing the slurry solvent, isopropanol, by methanol caused a 2% reduction in the column length (Fig. 5 in Ref. [1]). Consolidation of dry packing materials followed by the wetting of the dry bed by a poor solvent then replaced with a good one [3] has demonstrated that a high degree of bed consolidation is achieved only when consolidation takes place from a slurry. Upon filling the void column volume of a dry-packed bed by a solvent which wets the packing material well, the apparent packing density increases by nearly 12% (Figs. 8 and 9 in Ref. [3]). Thus, the nature of the solvent which is used as the mobile phase may have a significant influence on the actual length of the column.

A change in the apparent density of the column packing could be related to a change in the surface tension of the solvent impregnating the bed [5]. Such a rapid change occurs when the packing solvent is replaced by the mobile phase. Absolom and Barford [5] have demonstrated that the length occupied by a certain amount of packing material depends on the surface tension of the solution in which it is let to sediment. Although the degree of packing consolidation achieved is drastically different in this experiment and in the consolidation of columns, a similar influence cannot be ruled out. However, the methanol–water solutions used by Absolom and Barford do not wet $C_{18}$ chemically bonded particles, so the experiment cannot be replicated with these materials. Furthermore, the surface tensions of the solvents used either as mobile phases or as slurry solvents are very close. The surface tensions of acetone, acetonitrile, heptane, isopropanol and methanol (at 20°C) are, respectively, 23.7, 29.3, 20.1, 21.7 and 22.6 dyne/cm (1 dyne/cm=0.001 N/m), respectively. This sets acetonitrile slightly apart. Finally, it is known that heptane is a much better dispersant of $C_{18}$ bonded silica particles than methanol, while isopropanol has an intermediate behavior.

The goals of this report are the presentation of the results of a series of experiments performed (i) to develop a procedure for the reproducible packing of efficient preparative columns using different solvents; (ii) to compare the performance of the columns so prepared; and (iii) to investigate the consolidation behavior of a given packing material in different solvents.

## 2. Experimental

### 2.1. Dynamic axial compression column

The LC.50.VE.500.100 axial compression skid unit was obtained from Prochrom (Champigneulles, France). The column is made of a stainless-steel cylinder (59.0×5.0 cm) with a maximum working pressure of 100 bar. The axial compression is applied by a hydraulic piston operated by a Haskel pump, assisted by compressed nitrogen.

### 2.2. Solvent delivery system

A Kiloprep 100 HPLC pump was obtained from Biotage (Charlottesville, VA, USA). This pump can 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. Phase

The column was packed with Shandon Hyperprep $C_{18}$ bonded spherical silica [Life Sciences International (Europe), Cheshire, UK]. The average particle size was 12.0 $\mu$m and the average pore size 100 Å.

### 2.4. The consolidation of columns

The columns were packed following the procedure previously described [3,4]. Packing material (238.0 g) was weighed in a beaker and a slurry was made with the desired solvent. The slurry was poured into the column and the stationary phase was left to settle and decant for 2 h. From time to time, the clear solvent was decanted from the top with a large syringe and the slurry was pushed up to the top of column. Then, the top flange of the column was closed. The initial column length was measured at this time. Then the desired axial compression was applied while monitoring the column length continuously.

### 2.5. The packing of axial compression columns

The columns used for the study of chromatographic performance were packed using the pro-

cedure described in a previous report [1]. A weighed amount of the stationary phase was taken in a beaker and slurried in the desired solvent. The slurry was transferred into the column. If there was any loss of material, it was noted so that the exact amount of stationary phase used to pack the column was known. The column volume was derived from its length, which is recorded during the entire experiment. From this volume and the known weight of packing, the packing density was derived. The packing pressure used for the packing of this stationary phase was 65.6 bar. This pressure was applied rapidly. All the experiments were carried out under this axial compression pressure, unless otherwise indicated, for some special types of experiments.

## 2.6. Chemicals

Acetone, acetonitrile, $n$-heptane, isopropanol and methanol were purchased from Baxter (Atlanta, GA, USA) and were 99.9% pure. Naphthalene (+99% pure) was purchased from Aldrich (St. Louis, MO, USA). Distilled water from the chemistry department plant was filtered on a 1.2-$\mu$m membrane before use.

## 2.7. Detector

A UV-Vis detector (Model 204, Linear Scientific, Reno, NV, USA) equipped with a variable-pathlength preparative cell was used to collect the chromatographic data. With a short cell pathlength the detector response remained linear up to much higher concentrations than the response of conventional HPLC detectors. The cell can be operated up to 138 bar at 500 ml/min flow-rate. For HETP measurements, the cell pathlength was kept to its maximum so that a reasonable response could be obtained with analytical size injections.

## 2.8. 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 that the inlet pressure ($P$, bar) and the voltage output ($V$, volt) are related by $P=(V-0.405)/(1.1745\cdot10^{-2})$. The reading of the pressure sensor under a zero pressure differential was checked periodically.

## 2.9. 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 2–9 V d.c. at 2.0–9.0 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 $d=(0.20\pm0.01)V+(0.18\pm0.06)$.

Unlike in our previous work the sensor was hanged from a stand and the steel target was clamped to the compression piston. Thus, an increase in target distance means an increase in column length. Depending on the movement of the piston, the distance between the sensor and the target increases or decreases. Because of the nature of the device, a change of 0.01 mm in the column length can be detected easily, while the actual column length is known within only 0.1 cm.

The actual length of a packed column was measured as follows. A long thin electric wire was attached to the piston rod just below the piston head. Then, the top flange was fixed with its chain. Then the piston was pushed up without any packing material in the column. When the piston stopped, a mark (a knot) was made on the wire against a fixed point on the frame while pulling the wire. This point is the indicator of zero column length. After a column is packed, the distance between the zero mark on the wire and the fixed point on the frame gives the actual length of the bed in the column. The length measured this way has an accuracy of 0.1 cm. Knowing the initial length of the column and the initial distance of the target from the sensor, it is easy to monitor any changes in the column length within 0.01 cm.

## 2.10. 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 able to simultaneously monitor 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 3.31 program, loaded in a NEC computer. All the data files were translated into ASCII format for further use and uploaded to the computer network of the University of Tennessee. For the treatment of these data, several DOS and VMS based software were developed in our laboratory.

## 2.11. Methods

Unless otherwise mentioned, the columns were tested using 100% methanol as the solvent or mobile phase, and acetone as the probe (this was not possible for HETP determinations at intermediate consolidation ratios when $n$-heptane was used as the slurry solvent, see later). All packing solvents were removed prior to column testing by washing the bed with methanol and the column was equilibrated for at least 5 h before testing. Sample volumes for HETP measurements were 1.5 ml, injected by filling an appropriate loop. For economic and waste management reasons, the solvents were pumped in closed circuit, with a 15–20 l reservoir on the solvent line. The solvent was replaced when the baseline absorbance became significantly higher than that of fresh solvent.

Three signal 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 [6,7], using a non-linear least-squares fit. The classical Wilke and Chang [8] correlation equation was used to estimate the diffusion coefficients of the compounds used and to calculate the reduced velocities.

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

# 3. Results and discussion

## 3.1. Consolidation experiments

Two identical 238-g samples of Hyperprep were consolidated successively, both up to an axial compression stress of 100 kg/cm$^2$, and recompressed from 0 up to 105 kg/cm$^2$. Our conventional procedure [3,4] was followed. Two different packing solvents, methanol and $n$-heptane, were used. The column length was monitored at each preset axial-compression pressure (AC) until equilibrium was reached. The compression and recompression curves are shown in Fig. 1. From the curves in this figure, it is clear that the consolidated column lengths, hence the packing densities, depend on the nature of the solvent used.

The curves corresponding to the first compression are parallel and 6 mm distant at AC pressures higher than 20 kg/cm$^2$. This corresponds to a 3.3% higher packing density in $n$-heptane. Both curves exhibit an
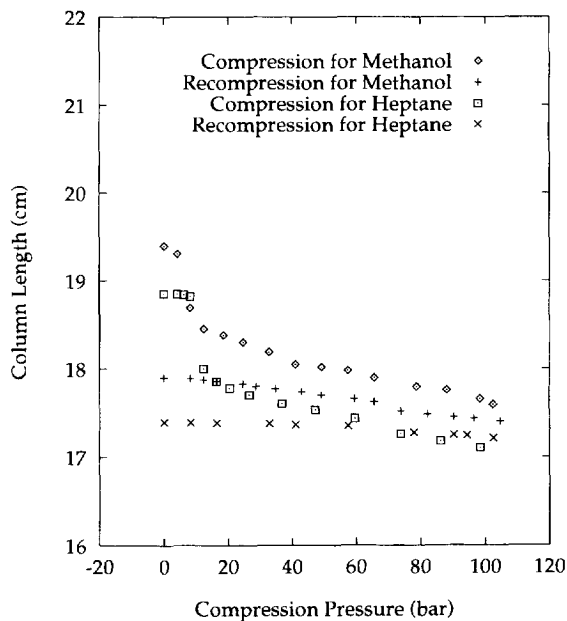


Fig. 1. Plot of the length (cm) of the consolidated columns versus the axial compression stress (kg/cm$^2$).

abrupt reduction in length by nearly 1 cm when the stress reaches ca 10 kg/cm$^2$. However, the jump is steeper and larger with $n$-heptane than with methanol. The recompression curves show that the rebound of the bed and the bed compressibility are lower in the case of $n$-heptane than with methanol. Note that the three data points obtained during the end of the first compression with $n$-heptane, at the highest values of the AC stress (70–100 kg/cm$^2$), are below the corresponding points obtained at the end of the recompression experiment, while they are expected to be the same or slightly higher.

The performance of each column was tested by measuring its efficiency and permeability at intermediate values of the AC stress. Fig. 2 shows the HETP curves obtained for acetone with the column consolidated in methanol under four successive values of the AC stresses. The four sets of data points obtained overlap and are scattered. No significant differences can be ascertained between these sets. The same behavior was observed in our previous work reporting similar results obtained with other stationary phases [4]. The values of the param-

eters of the best Van Deemter curves obtained by nonlinear regression of these data are widely scattered. We note that the minimum reduced HETP measured, 3.3, is only 50% larger than what can be obtained with a well-packed column, something which can be achieved only when the bed is settled quickly [2].

Fig. 3 shows plots of the inlet pressure needed to achieve a given flow-rate versus the flow-rate for the four different AC stresses. The slopes of the best-fit straight lines are inversely proportional to the column permeability. The latter decreases by 25% when the AC stress increases from 8 to 103 kg/cm$^2$. The corresponding decrease in column length is 6.7%. This is in agreement with the Kozeny–Carman prediction of a proportionality of the permeability to the external porosity raised to a power approximately equal to 4 [4]. The apparent particle sizes derived from Darcy's law would be 8.92±0.12, 8.41±0.12, 7.99±0.46 and 7.79±0.44 $\mu$m under AC stresses of 8.2, 41.0, 82.0 and 103.0 kg/cm$^2$, respectively.
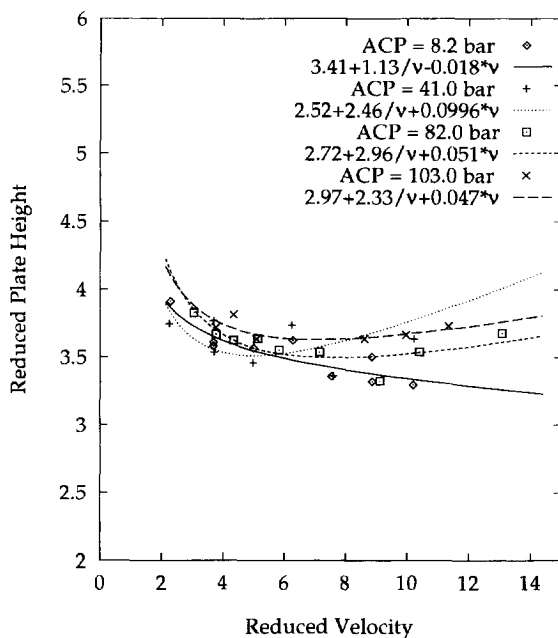
The column consolidated from a slurry in $n$-hep-

Fig. 2. HETP curves obtained with the axial compression column prepared with methanol at four different axial values of the axial compression stress. Mobile phase: methanol. Sample: acetone. The lines are the best fit of the data to the Van Deemter equation.
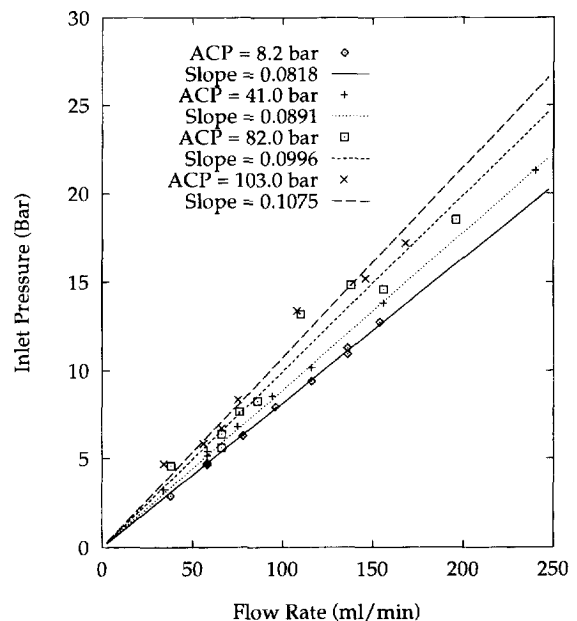
Fig. 3. Plot of the mobile phase (methanol) inlet pressure versus the flow rate at different AC stress for the column consolidated in methanol. The solid lines are the best fit of the data to a straight line passing through the origin. The slope of each line is inversely proportional to the column permeability.

tane could not be tested like the one just described. It was found impossible to change the solvent, from heptane to methanol and back, during the consolidation. The column was tested at different intermediate AC stresses by measuring the HETP curves for naphthalene in *n*-heptane. The data are reported in Fig. 4. Obviously, the column performance was very poor. The data are again scattered, but the spread of data obtained for different AC stresses is such that we may conclude that the column efficiency increased significantly with increasing AC stress (by 30% between 16 and 98 kg/cm$^2$). Nevertheless, a reduced plate-height of 14 is a very bad performance.

The correlation between the column inlet pressure and the mobile phase flow-rate are shown in Fig. 5. The values of the apparent hydrodynamic particle sizes derived from the slopes of these straight lines are 7.95±0.27, 7.68±0.27, 7.56±0.25 and 7.41±0.26 $\mu$m under AC stresses of 16.4, 36.9, 73.8 and 98.4 kg/cm$^2$, respectively. The trend is the same as the one observed for the column consolidated in

Fig. 5. Plot of the mobile phase inlet pressure versus the flow rate at different AC stress for the column consolidated in heptane. The solid lines are the best fit of the data to a straight line passing through the origin.

methanol, but the range of variation is narrower. The permeability decreases only by 7% when the AC stress increases from 16 to 98 kg/cm$^2$ (the packing density still increases by 4.7% in this stress range). If we compare Fig. 2 and Fig. 4 on the one hand, Fig. 3 and Fig. 5 on the other, we find that the data regarding the permeability of the columns under different AC stresses are much closer than those regarding their efficiency.

The column consolidated in *n*-heptane has a higher packing density than the one consolidated in methanol, as shown by the shorter length (Fig. 1) for a given mass of packing material and the lower permeability (Fig. 3 and Fig. 5; a viscosity correction is required but the viscosity of heptane, 0.40 cP, is lower than that of methanol, 0.55 cP). Nevertheless, the efficiency is considerably lower, the lowest reduced HETP in Fig. 4 being approximately four times larger than the average reduced HETP in Fig. 2. The reason for this poor efficiency are unknown at this stage, as is the possible mechanism leading to this surprising observation. The bed structure of a good column is most probably more homogeneous
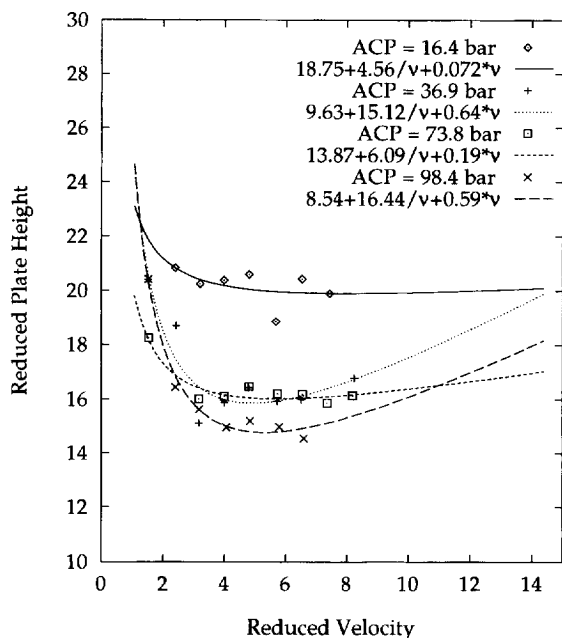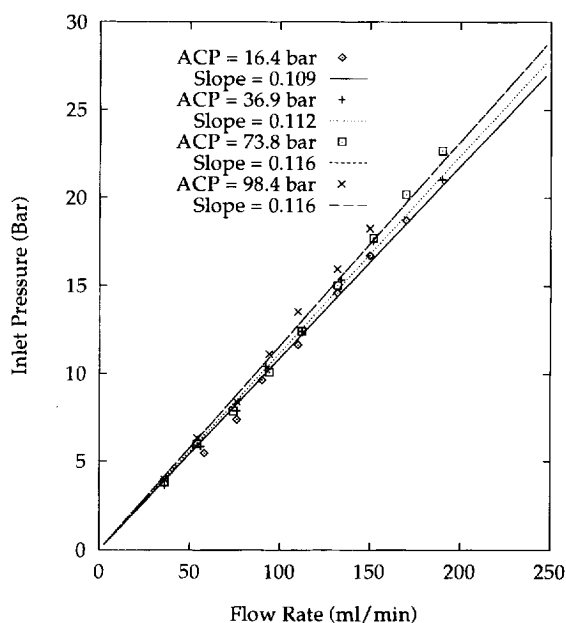
Fig. 4. HETP curves obtained with the axial compression column prepared with *n*-heptane at four different values of the axial compression stress. Mobile phase: *n*-heptane. Sample: naphthalene. The lines are the best fit of the data to the Van Deemter equation.

Table 1
Effect of the nature of the packing solvent on column consolidation

| Packing solvent | AC[a] (km/cm$^2$) | Bed volume (ml) | Total porosity ($\epsilon_T$) | Phase ratio (F) | Packing density (g/ml) |
|---|---|---|---|---|---|
| Methanol | 8.2 | 363.2 | 0.68 | 0.47 | 0.66 |
| Methanol | 41.0 | 354.4 | 0.67 | 0.49 | 0.67 |
| Methanol | 82.0 | 349.5 | 0.66 | 0.52 | 0.68 |
| Methanol | 103.0 | 347.5 | 0.62 | 0.60 | 0.68 |
| n-Heptane | 16.4 | 351.1 | 0.64 | 0.56 | 0.68 |
| n-Heptane | 36.9 | 345.7 | 0.65 | 0.54 | 0.69 |
| n-Heptane | 73.8 | 339.0 | 0.64 | 0.55 | 0.70 |
| n-Heptane | 98.4 | 339.7 | 0.64 | 0.57 | 0.70 |

[a] Axial compression pressure.

than that of a bad column. Our results confirm that a high column packing density does not guarantee an homogeneous bed nor an efficient column. These results do not seem to agree well with the general observation that n-heptane is a good dispersant of $C_{18}$ chemically bonded phases [9].

Table 1 summarizes the salient features of these two columns. Although the variation of the column length during compression is significant (Table 1), the change in packing density of the columns with increasing AC is not large. The final packing densities of the two columns are the same within 3%. It is interesting to note that the total porosity of the column consolidated in methanol decreases significantly with increasing AC, in the range from 10 to 100 kg/cm$^2$. In the same range, by contrast, the total porosity of the column consolidated in heptane does not show any significant changes. Since the packing density increases and the external porosity decreases (see data in Fig. 5), this means that the internal porosity must increase in the same time. A pressure dependence of the solubility of heptane in the $C_{18}$

bonded layer is the probable explanation. All these results should probably be correlated with the facts that heptane wets the silica surface better than methanol and better disperses the particles of chemically-bonded alkyl silicas [9].

## 3.2. Axial columns packed with different solvents

The results discussed above demonstrate clearly that the consolidation characteristics of the bed depend quite significantly on the nature of the slurry solvent used. Previous results have shown that the maximum efficiency achieved with a given stationary phase is obtained when using a rapid consolidation procedure [2]. In order to explore the possible influence of the nature of the slurry solvent used to pack the column bed on the column efficiency, it is necessary to use a different packing procedure. We packed a series of columns using the protocol previously published [1]. These columns were packed with a known amount of silica (Table 2), so that the packing density could be calculated exactly, and the amounts of material packed in each of the columns were very close. The packing pressure for all columns was 65.6 kg/cm$^2$. After packing the column bed, the slurry solvent was replaced with methanol, the column was equilibrated for 5 h by pumping methanol at 120 ml/min (under recycling mode), and the column performance was measured.

Fig. 6 shows the HETP curves obtained for the different columns. It is noted from this figure that n-heptane gives again, by far, the worst column of all, with an HETP which is 60% higher than the one achieved with isopropanol, arguably the solvent giving the best columns. Methanol gave a column which has nearly the same efficiency as the one obtained with isopropanol, the differences observed

Table 2
Characteristics of columns packed with different solvents

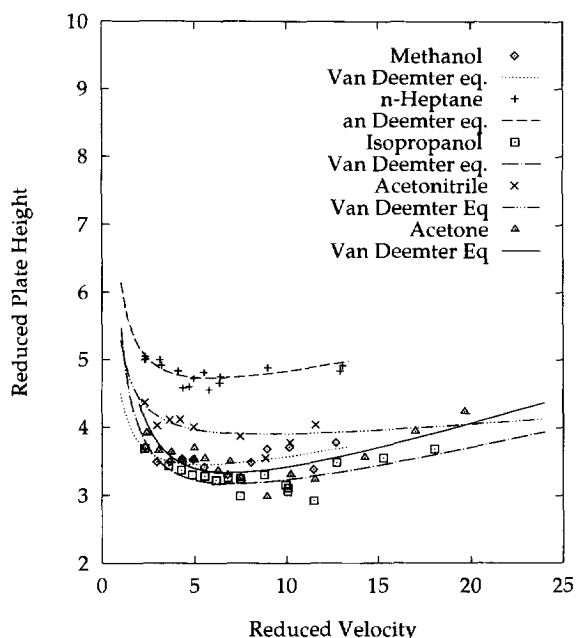| Solvent | Weight of silica (g) | Bed volume (ml) | Density (g/ml) | Volume of silica (ml) | Total porosity (silica) | Void volume (ml) | Total porosity (MeOH) |
|---|---|---|---|---|---|---|---|
| Heptane | 235.6 | 349.5 | 0.674 | 106.6 | 0.695 | 225.0 | 0.645 |
| Acetonitrile | 234.7 | 361.3 | 0.650 | 106.2 | 0.706 | 267.1 | 0.739 |
| Methanol | 233.6 | 359.3 | 0.650 | 105.7 | 0.706 | 231.5 | 0.644 |
| Isopropanol | 230.6 | 359.3 | 0.642 | 104.3 | 0.710 | 232.6 | 0.647 |
| Acetone | 230.6 | 359.3 | 0.642 | 104.3 | 0.710 | 230.9 | 0.642 |

Fig. 6. Plots of the HETP of acetone in methanol obtained with five axial compression columns packed with different slurry solvents. The lines are the best fit of the data to the Van Deemter equation.

being hardly significant. The observation that iso-propanol and methanol produce similar columns is consistent with our previous work [1]. The column prepared with acetone was slightly less efficient than the other two columns. Finally, acetonitrile produces a column whose efficiency is in between the two types just described. Note that all the columns have a minimum reduced plate-height of less than five, so they cannot be considered as really bad. Table 2 summarizes the other characteristics of these five columns.

To obtain the data in this table, we have estimated the volume of the silica in the column from its weight and from the density of silica (2.21 g/cm³). The calculated void volume is obtained by subtracting this volume from the column geometrical volume. This gives the total porosity of the columns (Table 2, column 6). The last column of Table 2 contains another estimate of the total porosity of the columns derived from the retention volume of acetone in methanol. We assumed the retention to be negligible, so this volume is equivalent to the hold-up volume. The values of the total porosity obtained

from the weight and density of silica are consistent, irrespective of the solvent used. With the exception of the acetonitrile column, the values of the total porosity obtained from acetone retention are also consistent with each other, but they are lower by approximately 8%. This is probably due to an underestimation of the volume occupied by the stationary phase, the bonded layer having a density three times lower than that of silica.

## 4. Conclusion

The consolidation of column packing in n-heptane gives a bed which is significantly more compact that the one obtained with methanol. However, the packing density is not correlated with the column efficiency and the columns packed with an n-heptane slurry are much less efficient than those packed with isopropanol or methanol. The consequences of these observations are under investigation.

## Acknowledgments

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