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On the reproducibility of column performance in liquid chromatography and the role of the packing density

Brett J. Stanley[a], Charles R. Foster[a], Georges Guiochon[b,c,*]

[a]*Department of Chemistry, California State University, San Bernardino, CA 92507-2397, USA*

[b]*Department of Chemistry, University of Tennessee, Knoxville, TN 37996-1600, USA*

[c]*Chemical and Analytical Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831-6120, USA*

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Abstract

The packing behavior of a typical 10 μm C_{18} stationary phase was studied in terms of the resultant column efficiency and capacity factor. The column-to-column reproducibility of these parameters under identical packing procedures is assessed. Correlation of these parameters and the column void volume to the column packing density is reported. Two regimes were studied; that of poor-and well-packed columns. For poorly packed columns, the column-to-column variability is high, but a concomitantly poor same-column reproducibility of measurement suggests that little statistically significant difference exists between different columns packed with the same procedure. There is also no statistically significant correlation between the column parameters and the packing densities, however, the poorest columns showed a degradation of performance after the drying procedures used to obtain the column masses. Well-packed columns showed much less degradation upon drying. For the well-packed columns, statistically significant column-to-column differences were observable, mainly due to a high same-column precision of measurement. Analysis of the results suggests that even well-packed columns are not optimally packed and that regions of high and low density coexist along the column. The results are compared to those achieved with semi-preparative columns packed with the same slurry procedure and preparative columns packed under dynamic axial compression. Poor day-to-day, same-column reproducibility (degradation) under ambient conditions was observed in conjunction with a high column-to-column variability for the semi-preparative columns.

Keywords: Stationary phases, LC; Packing density; Column efficiency; Retention factors; Preparative chromatography

1. Introduction

The slurry method of packing liquid chromatography columns was developed with the advent of high-performance liquid chromatography (HPLC) when it was realized that a highly dense and homogeneous column packing was required in order to achieve high efficiency [1–3]. It was soon after realized that the *A* term in the Van Deemter or Knox equation was an indicator of the homogeneity and density of the packing, where values approaching unity characterized a "good" packing or column, and high values indicated a "poor" column [2,4]. Several specific methods were developed to optimize the packing process, i.e. the homogeneity of the resultant packing, such as the balanced-density method [1,5] and the upward-slurry method [6].

The balanced-density method utilizes a slurry solvent with a density approaching that of silica, so

*Corresponding author. Address for correspondence at the University of Tennessee.

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that a more stable suspension of the packing material is produced prior to the packing process. The stability of the suspension is considered important in the packing process, so that the slurry is maximally homogeneous at the outset. The packing, or pushing, solvent would then accelerate the particles into the bed in the most homogeneous and reproducible manner. The upward-slurry method pushes the packing upward against gravity. This was argued to further increase the homogeneity of the resulting bed, as dense agglomerates would settle to the bottom of the packing chamber and presumably not enter the column but stay in the packing chamber [7].

It was further argued that the nature of the solvent in terms of its flocculating or deflocculating properties was of importance [2,8]. Flocculating solvents cause agglomeration of silica particles whereas deflocculating solvents cause a dispersion of particles. Agglomeration may indeed cause more dense regions in the column packing, and presumably result in high A values. Studies have shown that solvents such as acetone and n-heptane are deflocculating solvents for reversed-phase C_{18} particles, whereas methanol is a flocculating solvent [2,8]. This characterization can be explained by the colloidal surface chemistry of the particles, i.e. methanol does not wet the surface of C_{18} particles as well as acetone.

A recurring problem with column packing seems to be a relatively poor reproducibility of the column bed as measured by the column efficiency [9]. More drastic performance characteristics, such as the capacity factor and the selectivity coefficient, may show poor reproducibility with more specialized stationary phases and separation systems [10]. These problems may be related to the geometry of the packed bed, the reproducibility of the particles themselves, or of the surface modification thereof.

It is of interest to further the understanding and science of the packing process, as it appears to be still more of an art than a science at this point. In the field of preparative chromatography, this goal is especially important. Thermodynamic and kinetic parameters, needed to evaluate the potential performance of new packing materials and to model the performance of large-diameter columns for optimization of their performance, are most conveniently and economically obtained on analytical-sized columns. However, if the precision and accuracy of these variables is dependent on the packing geometry and density, poor scaleabililty to preparative systems will result. This problem will be exasperated if the precision and accuracy characteristics of the measurements on a preparative bed are significantly different than for an analytical bed.

These considerations lead to the conclusion that the chromatographic bed needs to be more scientifically characterized. At optimum flow-rates, with most columns currently available, the limiting term in the column efficiency can be shown to be the A term in the Van Deemter equation [11]. Characterization can proceed initially and relatively easily by obtaining the exact mass, and thus the density, of the stationary phase within the column after packing. More precise indications of internal and external porosity and column permeability may be obtained by independent analysis of the stationary phase density and pore volume. Future work on this subject should be dedicated to in situ observations of packing density and homogeneity by independent means. Analysis of local plate heights has already been initiated [12]. Correlation of these results with the packing method, solvent and equipment may then shed some light on the avenue that needs to be taken in order to further optimize column efficiency and resolution in HPLC as well as in preparative HPLC.

This work is an assessment of the reproducibility of column packing under identical packing conditions in terms of column efficiency and retention factor, with additional assessment of porosity and column density. The effect of packing solvent and column dimensions are also studied. This information is needed to better define the problem in practical terms. The exact same type and lot of stationary phase, typical of reversed-phase C_{18} used in analytical or preparative HPLC, is used for all characterizations; and the same packing equipment and procedure is used in the packing process. Thus the effect of particle and surface chemistry variability should be minimized as well as any contribution of system-to-system variability in the packing equipment and protocol used. This work does not attempt to rigorously define the statistics of optimally packed columns according to the current state-of-the-art, this project would be too poorly defined to attempt. It discusses, however, possible causes for

the column-to-column variability typically exhibited in HPLC for a well-defined surface chemistry.

2. Experimental

2.1. Materials

The reversed-phase C_{18} silica packing used in this study was Zorbax (BTR, Wilmington, DE, USA) with an average article diameter of 10 μm. The mobile phase used was methanol–water (40:60, v/v). Several solutes were tested: acetone benzyl alcohol, *p*-cresol and phenol. Uracil was used to measure the hold-up volume. All chemicals were of reagent grade and used as received.

2.2. Equipment

The columns were packed in-house using an air compressor in-line with a Haskel (Burbank, CA, USA) amplifier pump. Half-inch stainless steel tubing (1 in.=2.54 cm) was used as the packing chamber with standard fittings connecting the column of interest. Chromatograms were obtained on two systems: a Hewlett–Packard 1090 HPLC (Palo Alto, CA, USA) and a Gilson (Middletown, WI, USA) system utilizing Model 302 pumps with Model 5SC pump heads, Model 811B dynamic mixer and Model 621 Data Master interfaced to the Model 715 HPLC software. UV absorbance detection at 254 nm was used on both systems, a Spectroflow Model 757 (Kratos, Ramsey, NJ, USA) being interfaced with the Gilson hardware. Injection was performed remotely with a Valco actuator (VICI, Houston, TX, USA) on the Gilson system and with the system autosampler on the Hewlett–Packard system.

2.3. Packing procedure

Three methods differing in solvent composition were used. One method utilized methanol as both the suspension solvent and the pushing solvent. The other two methods used isopropanol–methylene chloride (70:30, v/v) for the suspension solvent. One of these latter methods used this suspension solvent as the pushing solvent as well, and the other used pure isopropanol as the pushing solvent (to minimize the amount of chlorinated waste). The rest of the procedure remained constant amongst the three methods. The first method is expected to give "poor" columns [1–4]. It was anticipated that a detailed comparison between the characteristics and performance of "poor" and "good" columns would be instructive.

The columns were first cleaned and rinsed with distilled water and the pushing solvent prior to packing. The stationary phase was dried, suspended in the suspension solvent and sonicated for approximately 5 min. This suspension was then poured into the packing chamber, which was subsequently topped off with the suspension solvent and closed. The packing pressure was increased to 6500 p.s.i. manually over a time interval of about 1 min (1 p.s.i.= 6894.76 Pa). This packing pressure corresponded to a nominal flow-rate of approximately 20 ml/min for the analytical columns and 80 ml/min for the semi-preparative columns through the duration of the packing process (the cross-section areas of the two columns types are in the ratio of 4.7 to 1). Note that, due to the reciprocating action of the (one cylinder) pump, the flow-rate during the active segment of the duty-cycle was substantially higher. The columns were allowed to pack for 10 min, at which time the pressure was reduced at the amplifier to zero and the valve above the packing chamber was closed. A few minutes were used to allow the pressure inside the column to reduce to atmospheric pressure. Then, the column connection was broken from the packing chamber, the excess silica trimmed from the top of the column with a razor blade and finally the frit and end-fitting were installed and the ends plugged.

2.4. Analysis

The columns were conditioned on the HPLC with methanol as the mobile phase at a flow-rate of 1.0 ml/min for the analytical columns, 3.0 ml/min for the semi-preparative columns, for 30 min. The mobile phase was then ramped to methanol–water (40:60, v/v) over a 10-min interval and sustained for a least 10 min before analysis. A mixture of either uracil, acetone, benzyl alcohol and *p*-cresol, or uracil and phenol was then injected to determine the column efficiency and capacity factors. Column

efficiency was calculated using the classical definition

$$N = 5.54 \cdot \left(\frac{t_R}{W_{1/2}} \right)^2 \quad (1)$$

where $W_{1/2}$ is the peak width at half-height, using an in-house program. The column temperature was maintained at either 37°C, 25°C or ambient temperature. The flow-rate was 1.0 ml/min for the analytical columns and 3.0 ml/min for the semi-preparative columns.

Injection volumes of 20 and 50 μl were performed without significant difference in results. Five replicate injections were performed to obtain average values and standard deviations for all measurements.

2.5. Column masses

The dimensions for the analytical-sized columns were 25×0.46 cm, and for the semi-preparative columns were 9.8×1.0 cm. The mass of stationary phase packed into the columns, i.e. the packing density, was determined by two methods. In the first method, an accurate dry mass of the silica placed into the packing chamber was obtained. The mass of silica leftover after packing the column was then determined by carefully collecting the remaining amount in the packing chamber and the amount trimmed from the top of the column. This collection was then dried and weighed. The difference in these two mass measurements was assumed to be the amount of silica packed into the column. This method neglects any loss of silica in the collection process, adhesion of silica in the packing chamber, or accumulation in the pre-column fittings.

In the second method, an accurate dry-mass of the empty column and fittings was obtained prior to the packing process. After packing and analysis, the column was dried in a GC oven under a nitrogen flow-rate of 5 ml/min for the analytical columns and 20 ml/min for the semi-preparative columns for 24 h at a temperature of 80°C. Note that the column was conditioned in methanol after the analysis in methanol–water (40:60, v/v). This procedure was checked by drying to constant mass and was determined to be adequate in entirely removing the mobile phase from the column. It was also checked by calculation using the ideal gas law, the void volume and the vapor pressure of methanol at 80°C. The total amount of nitrogen passed through the columns in the drying procedure was always at least 10 times greater than the volume of methanol vapor expected to be produced in the column. The dry masses of the packed columns were then obtained and the mass of stationary phase obtained by difference. Proper desiccation for 1 h after oven treatment was performed for all mass measurements in either method.

The second method neglects any accumulation of silica within the fittings during the capping procedure after packing. Comparison of the analytical stationary phase obtained by the two methods reveals a systematically higher mass measured with the latter method by about 30 mg for the analytical columns studied. It was subsequently assumed that this latter method of determining the mass of packing was more accurate and the difference in masses observed was attributed to losses incurred in the former method.

3. Results and discussion

3.1. Analytical columns

The results for four columns packed in methanol solvent are given in Table 1. The efficiency is described by the average number of theoretical plates for the solutes uracil, acetone, benzyl alcohol and *p*-cresol; the capacity factor is reported only for the moderately retained benzyl alcohol. The column temperature was 37°C. The standard deviation of the measurements made (5 in each case) are given in parentheses. Statistically significant differences in packing density were observed, based on the precision by which the measurements could be made. The results suggest that despite these differences, a correlation between column efficiency and stationary phase mass does not exist. Indeed, the column with the highest packing density (column 3) surprisingly yields the lowest efficiency. Such results are counter-intuitive. One would expect that a more homogeneously packed bed would correspond to a denser bed, considering that high pressures and flows are intentionally used to consolidate the bed as much as possible. Therefore, results such as these suggest that regions of high and low density may coexist within

Table 1
Column characteristics for methanol-packed columns

Column	M_s (g)	N_{avg} (plates) before heat	N_{avg} (plates) after heat	k' (BzOH)[a] before heat	k' (BzOH)[a] after heat	A[b]
1	2.9526	3240	876	1.484	1.470	1.7
	(0.0020)	(260)	(40)	(0.008)	(0.003)	(0.2)
2	2.9662	3501	3418	1.442	1.450	1.5
	(0.0020)	(46)	(58)	(0.001)	(0.006)	(0.1)
3	3.0056	2915	1045	1.457	1.494	2.0
	(0.0020)	(125)	(185)	(0.006)	(0.007)	(0.2)
4	2.9653	3306	3178	1.470	1.522	1.4
	(0.0020)	(69)	(104)	(0.001)	(0.064)	(0.4)

[a] BzOH = benzyl alcohol.
[b] A = asymmetry value calculated for small impurity peak.
Standard deviations of five succesive measurements are given in parentheses.

the bed. Such regions would affect the flow profile of mobile phase through the column, and in turn affect the A term in the Van Deemter equation by increasing the eddy diffusion. Van Deemter curves were not modelled to obtain A parameters in this work, due to the poor reproducibility and statistical significance of the fitted parameters. In terms of the column-to-column reproducibility of the packing density, as measured by the relative standard deviation (R.S.D.), the mass of stationary phase packed into these columns with this method is 2.972 g with a standard deviation of 23 mg, or a R.S.D. of 0.77%. Although these numbers sound quite good, the column-to-column R.S.D. of the efficiency is an order of magnitude worse at 7.52%.

Also shown in Table 1 are the efficiencies and capacity factors after the heat treatment under nitrogen flow. This treatment is required to measure the dry mass of packing. Although the capacity factors are generally reproducible (R.S.D. is 0.8% for all columns before and after heat treatment), two of the columns suffered drastic losses in efficiency (columns 1 and 3). Upon opening these columns, the existence of a void at the head of the column was observed. These two columns also showed the two lowest nominal efficiencies before heat treatment. After heat treatment, the column void volumes of the two degraded columns, as measured by the unretained uracil, were lower by 11% for column 1 and 4% for column 3 (see Table 2). This can be explained by the existence of marked channelling of mobile phase flow after the heat treatment. The precision of the efficiency calculations for each column is seen to vary widely. The two columns of lower efficiency which degraded under heat treatment also displayed the poorest same-column reproducibility of efficiency measurement.

The observation of a void space at the top of the two degraded columns suggests that these columns were not completely consolidated after the packing process. However, the two columns which retained their performance characteristics (and did not visibly consolidate further), did not necessarily possess a higher packing density than the poor columns. This suggests that all four columns were consolidated to

Table 2
Void volume and porosity of analytical columns studied

Column	Packing density (g/ml)	V_m (ml)	ε (V_m/V_c)[a]	ε $(1-V_s/V_c)$[b]
1	0.7107	2.713	0.653	0.606
1 after dry	0.7107	2.446	0.589	0.606
2	0.7139	2.704	0.651	0.604
2 after dry	0.7139	2.701	0.650	0.604
3	0.7234	2.733	0.658	0.599
3 after dry	0.7234	2.648	0.637	0.599
4	0.7137	2.700	0.650	0.604
4 after dry	0.7137	2.703	0.651	0.604
5	0.7204	2.738	0.659	0.600
6	0.7365	2.701	0.650	0.592

[a] Determined from void volume measurements of uracil.
[b] Determined from independent measurement of stationary phase density and internal pore volume [13].

various degrees, but half were stable in their particular packing geometry and half were not. This series of experiments also suggests a method for verifying the stability and quality of a packing. If the column can survive a heat treatment similar to that described here, the column is stable. It will most likely exhibit a higher efficiency also.

The average reduced plate height for the above 4 columns is 7.7, which signifies a poorly packed column. The efficiency can be improved dramatically by using a denser and more dispersing solvent such as isopropanol–methylene chloride (70:30, v/v). The results for two columns packed under these conditions are given in Table 3. The plate numbers increased by 77% on average. This increase in efficiency may be attributed to a more homogeneous slurry to begin with (because of the better wettability of the particle surface by the solvent), and to the use of a more viscous solvent to prepare the slurry (slower sedimentation rate of the large particles) and to consolidate the packing (albeit, at constant packing pressure, the drag force remains constant). Although plate height and column density were not correlated in the above experiments with methanol, the average density of the better columns packed with the isopropanol–methylene chloride (70:30, v/v) solution was also 1.8% higher. Column 6 possessed the highest density and also exhibited the highest efficiency and the highest capacity factor. The former correlation is suspected from theory, the latter results directly from theory.

As the packing density increases, the column void volume decreases, and the phase ratio increases. Coupled to this relationship, the column saturation capacity also increases with the packing density, i.e. if we assume a Langmuir equilibrium isotherm between the two phases (analytical applications being carried out in the concentration range in which the product bC is negligible), we have

$$k' = Fa = Fbq_s \tag{2}$$

where a is the initial slope of the isotherm, b the second numerical coefficient, q_s the column saturation capacity and F the phase ratio, with $F=(1-\varepsilon)/\varepsilon$ and ε is the total porosity of the column, V_m/V_c. V_c is the geometrical volume of the column and V_m the volume accessible to the mobile phase, or more precisely, to uracil. The volume occupied by the stationary phase is $V_c - V_m$. Assuming that the packing density does not affect the particle shape or the surface area accessible to the solutes, this volume as well as the column saturation capacity are proportional to the packing density. Accordingly, if we set $q_s = q'D$ and $V_c - V_m = vD$, with D the packing density and q' and v numerical coefficients independent of D, we obtain

$$k' = bvq' \cdot \frac{D^2}{V_c - vD}. \tag{3}$$

This equation explains that k' should increase somewhat faster than the square of the packing density. The results from columns 5 and 6 vary more proportionately with the packing density than with the square of the packing density. If the k' values are normalized to the packing density, a 3.0% increase in k' is reduced to a 0.77% increase. A 1.4% decrease in normalized-k' occurs if the k' values are normalized by D^2. The average same-column R.S.D. of the normalized capacity factor is 0.18%. This approximate proportionality (k' vs. D) was also observed for the semi-preparative columns which were studied (see later, below). This dependence is also in agreement with those of a recent systematic study of the influence of the packing pressure on the column performance [15].

Thus, although the stationary phase mass correlates with the capacity factor, the significant differences in efficiency under identical packing conditions (even though they were better packed overall) is intriguing. There is no published mathematical relationship between the column efficiency and the packing density, and no correlation between these

Table 3
Column characteristics for isopropanol–ethylene chloride packed columns

Column	M_s (g)	N_{avg} (plates)	k' (BzOH)[a]	A[b]
5	2.9931	5275	1.789	1.2
	(0.0020)	(74)	(0.002)	(0.2)
6	3.0600	6213	1.843	0.9
	(0.0020)	(91)	(0.004)	(0.2)

[a] BzOH = benzyl alcohol.
[b] A = asymmetry value calculated for small impurity peak.
Standard deviations of measurements are given in parentheses.

two parameters was observed in the following semi-preparative studies. Therefore, we cannot report any correlation in general between column efficiency and packing density in this work. The 29% difference in efficiency between the columns 5 and 6 is high enough to measurably affect resolution calculations [7].

Note that the same-column reproducibility of the efficiency measurements are better on average than for the lower efficiency, methanol-packed columns. It was noted in general that the poorly packed columns produced statistically insignificant column-to-column differences in results. The better packed columns produced statistically significant column-to-column differences because of this increased precision in elution profiles which accompanies a better packed column.

Comparison of Tables 1 and 3 indicates that better packed columns may have a higher column density. This still does not prove them to be fully consolidated. Regions of high and low packing density may still coexist, the homogeneity or random dispersion of which may affect the efficiency. Columns 1 and 3, which collapsed from the methanol packing, were "topped off" in the packing instrument to see if a further increase in packing density would increase the column performance. The efficiency increased somewhat for column 1 (1542 vs. 876 plates) and remained the same for column 3 (1045 plates). These values suggest that when the columns further consolidate after heat treatment and the void volumes at the top of the columns were corrected for, the packing geometry was still poor, perhaps less homogeneous than for the higher efficiency columns which apparently were not completely consolidated either (see below). Further studies would be needed to assess the statistical significance of the differences between the packing densities of the poor- and well-packed columns.

The isopropanol–methylene chloride (70:30, v/v) packed columns yielded significantly higher capacity factors (ca. 1.8 vs. 1.5) than the methanol-packed columns. According to the data in Tables 1 and 3, this result cannot entirely be attributed to a higher amount of stationary phase in the isopranol–methylene chloride (70:30, v/v) packed columns. It is improbable that either of the packing solvents could have modified sufficiently the chemistry of the adsorbent surface to account for this change. Only pure water (not used in this work) has been reported to be associated with changes in the retention factors [3]. On the other hand, the amount of packing material in columns 5 and 6 (Table 3) is barely 1 to 2% higher than in columns 2 and 4. Table 2 lists the void volumes for all the columns studied, which range from 2.70 to 2.74. The total column porosites, ε, are also given. The small differences between them cannot explain the large differences between the retention factors. The same is true for the phase ratio, $F=(1-\varepsilon)/\varepsilon$, which is also very close for all these columns. The difference between the porosity given by chromatography and that obtained from independent data on the density of the silica [13] is only marginally compensated for by the dead volume in the chromatographic system, and is currently under investigation.

The chromatographic porosities of columns 5 and 6 follow from the relative packing densities observed, i.e. packing densities and void volumes are inversely related (see later, e.g., Fig. 2c). However, the poorly packed methanol columns do not obey this relationship well. This could suggest that the flow through poorly packed columns might be heterogeneous and that through well-packed columns is more homogeneous. In the heterogeneous case, the flow would not sample the entire column equally at the same flow-rate, and the spatial distribution of this flow would not be reproducible from column to column. If the packing density is higher with poorly packed columns, this could be due to more tightly packed regions of the column. Flow through less dense regions would be favored, thus sustaining a somewhat higher void volume than might be expected. On the other hand, if the packing density is higher in well-packed columns, a lower void volume should be expected because the flow profile through the packing is more homogeneous in this case. The retention factor could conceivably increase with increasing degree of column homogeneity if the solute samples regions of lower density on the average for poorly packed, inhomogeneous, columns with respect to that sampled for wellpacked, homogeneous, columns. To account for the relatively close column densities amongst poor and good columns (Table 1), regions of high density in the poor columns must be higher on average than the

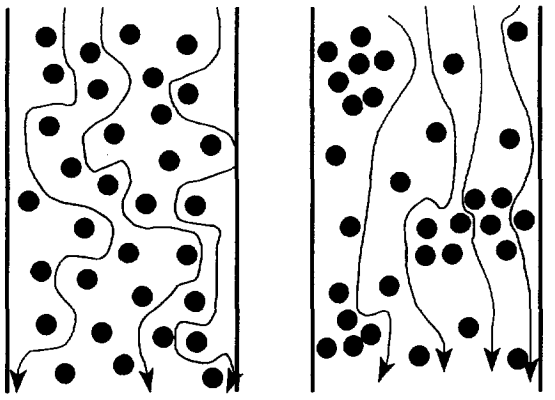

Fig. 1. Exaggerated, hypothetical representation of flow through homogeneously- and heterogeneously-dispersed packing resulting in similar densities and porosities. Efficiency would be higher for the more homogeneous packing.

density in the good columns. An exaggerated representation of this situation is depicted in Fig. 1.

Peak assymmetries are also reported in Tables 1 and 3 and support the notion of greater homogeneity of the columns packed in isopropanol–methylene chloride (70:30, v/v) with respect to the methanol-packed columns. The asymmetry in general decreases as the column efficiency increases, and is notably higher for those methanol columns which degraded. Further data are currently being collected to critique this hypothesis of packing homogeneity.

3.2. Semi-preparative columns

Short semi-preparative columns (9.8×1.0 cm) were packed with the same packing material in an effort to supply a batch of comparable columns for a simulated moving bed (SMB) preparative system used in another study. An isopropanol–methylene chloride (70:30, v/v) mixture was used as the slurry solvent and 100% isopropanol as the pushing solvent. The performance characteristics of the columns, using uracil and phenol as retention markers at ambient (ca. 25°C) temperature, are given in Table 4. The column efficiency appears to be very good on the average, with a reduced plate height between 2.3 (column D) and 3.6 (column E), and with three columns below 2.5 and eight columns below 3.0. However, the column-to-column reproducibility of efficiency for the 11 columns was only 13.6%. The

Table 4
Performance characteristics for semi-preparative columns slurried in isopropanol–methylene chloride (70:30, v/v) and packed in 100% isopropanol

Column	Packing density (g/ml)	N_{avg} (plates)	k'	V_m (ml)
A	0.7061	3713	1.945	4.926
B	0.7162	3185	1.944	4.992
C	0.7627	3366	2.037	4.800
D	0.7335	4358	1.984	4.912
E	0.7494	2763	2.010	4.799
F	0.7449	3185	1.993	4.836
G	0.7448	3863	2.004	4.828
H	0.7192	3407	1.878	4.968
I	0.7485	4073	2.036	4.852
J	0.7359	4224	1.989	4.923
K	0.7464	3611	2.006	4.900

retention factor reproducibility was 2.3%. This reproducibility is significantly worse than that observed for the analytical-sized columns. Again, the retention factor variability can be substantially decreased by normalizing to the mass of stationary phase within the column (Fig. 2a), but the column efficiency is in no way correlated to the packing density (Fig. 2b). The void volume is correctly seen to vary inversely with the packing density (Fig. 2c). The packing density observed for the semi-preparative columns was somewhat higher than that observed for the analytical columns (around 5%), thus further substantiating the claim that the above columns were not completely consolidated after the packing process. This is in agreement with previous results suggesting that the packing density increases with increasing column diameter [13].

The day-to-day reproducibility of the semi-preparative columns was studied and is reported in Table 5. The R.S.D. for k' was in general higher day-to-day for each column than the column-to-column R.S.D., but in any case it was less than 5%. It could be explained in part by fluctuations of the ambient temperature. The R.S.D. for N, on the other hand, was substantially higher for column-to-column, same-day profiles with respect to that obtained for day-to-day, same-column profiles. This again shows that the band profile is much more variable amongst columns than the precision by which the measurement can be made. These differences in efficiency can only be attributed to variations in the specific

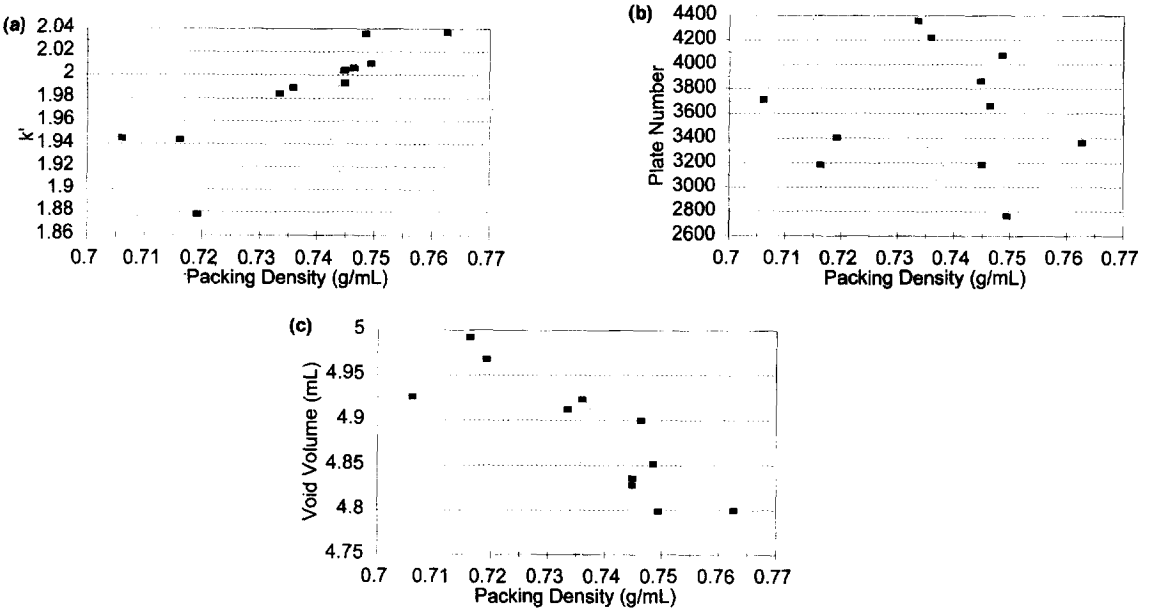

Fig. 2. Correlation of column characteristics with packing density. (a) Capacity factor, k', shows correlation; (b) Efficiency, N, shows no correlation; (c) V_m shows correlation.

packing structure of each column. For the case of semi-preparative columns, the R.S.D. of the efficiency is over 20%, when studied over a period of time.

The semi-preparative columns were seen to be less stable in general than the analytical-sized columns.

Table 5
Day-to-day reproducibility of 9.8×1.0 cm semi-preparative columns at ambient temperature

Column	k' (R.S.D.)	N (R.S.D.)
A[a]	3.4	39
B[d]	3.2	21
C	3.8	4.9
D	4.2	8.5
E[b]	0.6	12
F	4.3	4.8
G	4.0	4.5
H[c]	N/A	N/A
I	4.8	5.0
J	4.5	4.9
K	4.4	10

[a] Column degraded after 4th trial.
[b] Column degraded after 3rd trial.
[c] Column degraded during 1st trial.
[d] Column degraded after 5th trial.

Several columns degraded in performance during this study, eventually showing double peaks. Peak asymmetries were in general higher for the semi-preparative case as well, with values generally between 1.4 and 1.6. This degradation could be due to a shift in the packing structure which severely alters the mobile phase flow profile – becoming less homogeneous than what existed originally after the packing procedure ended. Such effects were not observed in analytical-sized columns, whose packing structure may interact more strongly with the column walls. As is shown below and argued above, there is apparently further room for consolidation in the packing structures for all the columns packed by the slurry-flow technique.

The packing densities of the analytical-and semi-preparative-size columns are compared to that achieved under dynamic axial compression in high-performance preparative liquid chromatography in Table 6 [13]. A higher packing density is observed for the preparative columns, showing that further consolidation is indeed possible beyond that achieved with the slurry-flow method. This supports the conclusions made earlier that the packing density is not maximized in conventional high-performance

Table 6
Comparison of packing densities as a function of column dimension and packing technique

Column dimensions (cm)	Packing density (g/ml)	Total porosity	External porosity
25.0×0.46[a]	0.7285	0.5962	0.3827
9.8×1.0[b]	0.7370	0.5914	0.3754
16×5.0[c]	0.7509	0.5837	0.3636

[a] Average data of two columns packed in isopropanol–methylene chloride (70:30, v/v).
[b] Average of data for 11 semi-preparative columns. R.S.D. for packing density is 2.3%.
[c] Packed by the dynamic axial compression technique.

liquid chromatography. The packing density reported for the preparative columns was obtained over a series of experiments where the axial compression pressure was increased in increments and the column was allowed to consolidate in between steps. The slurry-flow method does not have such a direct and mechanical method to force the consolidation of the stationary phase.

The consequences of the results of this study suggest that the band profile, as characterized by the column efficiency, exhibits poor column-to-column reproducibility in HPLC. Two columns packed successively following the same procedure can differ by as much as 30%. The observed differences in retention factor can also be significant, mainly but maybe not exclusively, due to differences in packing density. Moreover, these differences can only be accurately normalized if a measure of the mass of stationary phase is obtained. Thus, the practice of using thermodynamic data from analytical columns in scale-up calculations for preparative systems must be carried out carefully. Preparative columns may be packed at higher densities, and a small shift in D- or D^2-normalized k' may be possible with respect to analytical-sized columns, if significantly different packing structures are present which could alter the radial distribution of the mobile phase flow.

The result that columns may produce statistically significant differences in characteristics is disturbing from the standpoint of using such columns for scale-up information to preparative chromatography. The packing process may not be reproducible enough to allow analytical column information to be extrapolated to preparative system optimization. Further exacerbating the problem is that the packing of preparative columns may yield properties that have an entirely different set of statistics. The problem most likely lies in the packing procedures. Analytical columns are packed under a high inlet pressure and at elevated flow-rates, whereas preparative columns are packed under mechanical compression. They cannot be assumed to yield the same packing geometry in each case. The work presented here suggests that different packing geometries can indeed cause significant differences in column characteristics.

4. Conclusions

The reproducibility of the packing density in analytical-sized (0.46 cm I.D.) columns is better than 1% if packed with the same method. For columns packed with different solvents, which can produce "good" and "bad" columns, the reproducibility is still within 2%. The reproducibility of the packing density for semi-preparative-sized columns (1.0 cm I.D.) obtained by the same method as that used for the analytical case is about 2%. This demonstrates that at least one critical parameter of the packing procedure is not properly controlled. This variability has an effect on the capacity factor of the columns of similar magnitude, which can then be corrected for by normalizing the retention to the amount of silica within the column. This assumes, of course, that the stationary phase mass can be measured to an accuracy greater than these values.

The reproducibility of the column-to-column efficiency is significantly worse than the precision of the packing density and the same-column, day-to-day precision of the efficiency measurement. This variability is not correlated to the packing density for a given method, although better-packed columns of higher efficiency may exhibit a higher packing density than other columns packed with a different

method. This reproducibility can approach 10% for analytical columns and 20% for semi-preparative columns which are packed similarly. With such statistics, any two columns have a significant probability of being 30% different, which can significantly affect the reproducibility of performance critical to any application.

The poor reproducibility of the column performance is due to a variation of the specific packing structure or geometry obtained within each column. This variation can cause fluctuations in the radial distribution of the mobile phase flow velocity which percolates through these columns, which in turn affects the efficiency [12,14]. The columns packed with the slurry-flow technique are not fully consolidated, thus the packing density is not maximized. If the packing density was constant throughout the column, or even randomly variable, the efficiency or band profile would not vary as considerably as seen here. The packing density exhibits high and low regions throughout the column which are more or less inhomogeneous. The particular distribution of these regions in any given packing results in a particular imprint on the band profile.

Control of the packing density with respect to its spatial distribution within the column does not appear possible with the standard packing technology used in this study. Better homogeneity could conceivably be attained with other solvents and apparatus designs. However, the optimum solvent scheme and methodology may vary with the particular stationary phase being packed. This work examined a packing (i.e. C_{18}) which is considered as fairly homogeneous with respect to its surface chemistry. Results for some other types of stationary phases may be expected to be more variable. Packing technology needs to be further studied and optimized in order to further realize the potential of separation power of analytical HPLC and scale-up to preparative HPLC.

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