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Review

Consolidation of particle beds and packing of chromatographic columns

Georges Guiochon*, Tivadar Farkas, Hong Guan-Sajonz, Joon-Ho Koh, Matilal Sarker[1], Brett J. Stanley[2], Tong Yun[3]

Department of Chemistry, The University of Tennessee, Knoxville, TN 37996-1600, USA
Chemical and Analytical Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831-6120, USA

Abstract

A variety of complementary evidence reviewed here demonstrates that the packing of chromatographic columns is heterogeneous. It is denser and less well organized close to the column wall than in the center of the column. The mechanism of particle consolidation under stress explains the origin of this phenomenon and provides a fundamental justification to what is known in column chromatography as "the wall effect".

Keywords: Column packing; Stationary phases, LC; Packing methods; Reviews

Contents

1. Introduction

In profound disagreement with conventional wisdom, there is overwhelming evidence that the beds of the conventional packed columns used in liquid chromatography are not homogeneous. Knox [1], Eon [2], Baur et al. [3,4] and Farkas et al. [5,6] have shown conclusively that the local velocity of the mobile phase and the local HETP, i.e., the local contribution to the apparent column efficiency, vary across the column. The importance of these variations is related to the column quality but, for modern columns of average performance, the mobile phase velocity may be 2 to 8% higher in the center than close to the wall and the column HETP may be 80 to 150% higher near the wall than in the central region [3–6]. While these results agree with those of Knox

*Corresponding author.
[1]Present address: Supelco, Bellefonte, PA, USA.
[2]Present address: Chemistry Department, California State University, San Bernardino, CA, USA.
[3]Department of Chemical Engineering, The University of Tennessee, Knoxville, TN, USA.

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[1] and Eon [2] for the radial distribution of the HETP, they are different for the distribution of the mobile phase velocity that the earlier authors [1,2] had found to be higher close to the wall than in the core region. This is explained by their use of a dry packing technique, while slurry packing methods were employed by the later authors [4–6]. During dry packing, the coarser particles tend to segregate close to the wall of the container. In slurry packing, the packing density is higher close to the tube wall. We see later why.

Nuclear magnetic resonance (NMR) imaging techniques have been used recently to record the shape of chromatographic bands during their migration along chromatographic columns [7–10]. The images of bands of gadolinium complexes in columns of moderate efficiency show a thin band profile with wrinkles of different shapes and amplitude, demonstrating the existence of significant radial perturbations of the local velocity, locally and over the whole cross-section. The minimum of the local value of the reduced HETP of several columns was slightly larger than one, while the overall reduced HETP measured with a conventional detector using the bulk eluent exiting the same columns exceeded three [8].

Anecdotal evidence suggests that column beds are sometimes unstable. Cavities of various sizes develop at the column inlet, resulting in a dramatic loss of efficiency due to intense, local convection of the liquid phase in these cavities. This is attributed to the packing being too loose at the beginning of column operation. In most cases, these troubles seem to be associated with the application of insufficiently intense vibrations to the bed when a dry packing technique is used or with an insufficiently high packing pressure (hence flow-rate) in slurry packing methods [11]. In both cases, the bed consolidates progressively, its packing density increases, its external porosity and its permeability decrease. This proves that the packing density of a column bed depends on the packing conditions and may vary significantly when those are adjusted. There are no reasons, however, for these conditions to be uniform throughout the bed in the absence of an appropriate averaging mechanism [12].

Three procedures are used for the packing of columns for high-performance liquid chromatography. Conventional slurry packing is used for most analytical and some preparative columns [11]. Dynamic mechanical compression, either in the axial [13–18] or the radial [19–24] direction, is used for the packing of wide bore columns for preparative applications (radial compression has also been used for analytical columns [19–21]). All packing techniques result in the application, during the operation, of a certain level of mechanical stress to the particles, either individually (in the slurry) or as a group (in the growing bed). This level is widely different depending on the axial and radial position. The local values of the packing density, the external porosity and the permeability are a function of the history of the local stress applied during packing and during the life of the column [12]. This could provide for a general mechanism explaining all the facts reported above. We discuss the applications of this concept to the different packing methods.

2. Axial compression columns

These columns are huge syringes, the barrels of which contain the packing material and the pistons are moved by a hydraulic jack which can apply a mechanical stress adjustable up to ca. 100 kg/cm^2 [14–18]. The column barrel is filled with a dilute slurry (ca. 30%) of the packing and closed with a frit and a bolted flange. Then the piston is moved to compress the slurry. The excess liquid exits through the end opposed to the piston and a consolidated bed builds up progressively. The force of the jack moving the piston causes a stress applied against one end of the packed bed. However, stress is not conveyed homogeneously in solids, whether divided or not, as it is in liquids and no mechanism guarantees that the local stress is constant. It has been shown by Train [25,26] and by Train and Hearsey [27] that the distribution of stress in a tablet during its compression was highly heterogeneous. Under a compression stress equivalent to 45 kg/cm^2, applied to the piston in a 5.3×14.5 cm die, the local stress at the end of the bed opposite the piston was approximately 15 kg/cm^2. It was approximately 27 kg/cm^2 in the geometrical center of the bed as well as against the piston, at its center. In a cylindrical region with a wedge-shaped cross-section, the stress increased rapidly when nearing the cylinder wall and

the piston. It exceeded 45 kg/cm^2 in the volume of a circular ring, close to the wall and the piston, which was approximately 2 cm high and occupied 60% of the cross-sectional surface area of the columns [26].

We have recently studied the consolidation of several conventional packing materials generously provided by their manufacturers (Hyperprep, Impaq, Kromasil and Zorbax) [12,28–31]. The mass of dry packing material used was measured before slurrying it. The volume of solvent expelled and the position of the piston were recorded while the axial stress applied to the bed was increased progressively from a few to nearly 100 kg/cm^2, by steps of a few to about 10 kg/cm^2. This allowed the determination of the packing density as a function of the axial stress applied. The results obtained depended much on the nature of the specific packing material studied and on whether it was a first compression or a recompression of a bed previously consolidated under the same or a higher stress and undisturbed in-between. Recompression of silica-based materials was elastic, reversible, and gave values of the Young modulus which were of the order of 200 (Kromasil) to 460 (Zorbax) MN/m^2 [30]. These values correspond to those of fine sands.

For a first consolidation, the behavior of the bed depended much on whether the particles of the packing material were irregular or spherical. A column of initial length 25 cm, packed with irregular-shaped particles, shrunk by nearly 25% when the axial compression stress was raised from 1 to 80 kg/cm^2 [10,28]; the corresponding Young modulus was an order of magnitude smaller than during further recompression [30]. Under the same conditions, a column packed with spherical-shaped particles shrunk by less than 10% [28]. The Young modulus was only 20 to 50% lower than during recompression of the bed [30]. Furthermore, the kinetics of shrinkage, following step increases of the stress, was different for irregular and round particles. It took several hours for a column packed with irregular-shaped particles to reach a constant length upon an increase of the compression stress. This consolidation took place by chaotic steps of random height occurring at random time intervals [10]. It took about 1 h for a column packed with either roughly spherical particles or rugous spherical particles to settle; during that time, the column length decreased both continuously and through a few random steps. Consolidation took less than 1 min for smooth, truly spherical particles and the process was continuous [28]. Finally, little breakage of the spherical particles was observed, while abundant fragmentation and chipping of irregular-shaped particles took place under axial compression stresses of 80 kg/cm^2 or less [10,28]. Aggregation of irregular-shaped particles into lumps that were stable under conventional ultrasonic irradiation has also been reported [28].

The packing density increases with increasing compression stress, while the external porosity and the permeability of the column decreases, in agreement with the correlation of Blake and Kozeny [32]. Particle breakages do not seem to contribute much to this effect [12,28]. On the other hand, the nature of the slurry solvent is important for the packing density [29]. The beds obtained with C_{18} silica particles are more compact when prepared with a slurry in *n*-heptane than with slurries in acetone, acetonitrile, 2-propanol or methanol. However, using methanol, isopropanol or acetone as the packing solvent produced better columns (i.e., presumably more homogeneous ones) than using acetonitrile or heptane, although these solvents wet the packing material well. No obvious correlation was found between the packing density and the column efficiency. The column efficiency, which was measured on the bulk eluent, not locally, is only moderately affected by the compression stress. It increases with increasing stress at low values of this stress and decreases at high values of the stress [17]. Values of the reduced HETP close to two, have been obtained with Kromasil [18]. For other packing materials, these values are usually closer to or larger than three [12,17,28,29].

3. Radial compression columns

These columns use a plastic cartridge closed at both ends by a frit and a stream distributor. The cartridge is filled with the packing material and is placed inside a steel cylinder. The design of the plastic cartridge and of the steel cylinder allows formation of a leakproof seal at both ends. A hydraulic fluid is introduced under pressure between

the plastic cartridge and the steel cylinder, allowing radial compression of the bed [19–24]. Implementations of this method of bed compression are available for analytical and preparative applications. The columns prepared with this procedure exhibit fine performance [2,23,24,33,34]. Measurement of the porosity and the permeability of these columns has demonstrated the strong influence of the intensity of the radial compression stress on these properties [23,24]. The external porosity of the bed decreases with increasing compression stress. The head pressure required to achieve a certain flow-rate increases. This result shows that the permeability follows the Blake-Kozeny correlation [32]. Because the instrument design allows a compression stress lower than in axial compression columns, the effects observed are smaller.

Although the geometry of the stress distribution in a radially compressed bed is more complex than in an axially compressed bed, a model has been proposed to relate the mobile phase flow-rate, the head pressure and the compression stress [33]. This model is in agreement with experimental results [23,24,34]. It shows that the radial compression stress applied should correspond to a hydraulic pressure in the compression chamber in excess of half the head pressure. Insufficient compression levels result in dramatic failure of the column's performance, with cracks forming in the bed [23]. This model could be used, together with independent data on the Young nodulus acquired with axial compression columns, to relate the intensity of the radial compression stress and the distribution of the packing density throughout the column bed. This issue is now under active investigation.

4. Slurry packed columns

These columns are packed by forcing a dilute slurry (ca. 8% packing material suspended in a solvent that is good at wetting and dispersing the material [11]) through the column, the end of which has been closed with a frit. A pushing solvent is pumped at a high flow-rate, under a head pressure up to 800 atm. Consolidation is achieved by percolating the pushing solvent for up to 15 or 30 min. The interaction of the particles and the high velocity stream has two origins. First, the particles in the slurry are carried rapidly by the stream and impinge vigorously on the rising surface of the bed. They get imbedded in it and find a stable position. Second, the viscous shear of the fast-moving stream of solvent pushes the particles forward and consolidates the bed. The extent of this consolidation is illustrated by the finding that the external porosity of the packed bed decreases linearly with increasing pressure of the packing solvent, from 0.434 to 0.399 (a 10% decrease) for columns packed with Zorbax 10 μm particles, under head pressures ranging from 72 to 770 atm [34]. At the same time, the column permeability is decreased by 45%, a value in agreement with the Blake and Kozeny correlation [32].

An estimate of the consolidation stress can be obtained by comparing the flow-rates obtained under a given head pressure and that achieved under inertial flow [36]. The former is given by the Hagen-Poiseuille law. The latter is the flow-rate that would be achieved when the mobile phase percolates through the column under its own weight in a huge gravity field (or in a centrifuge). If these two flow-rates are equal, the pressure gradient is equal to the inertia or gravity potential [36]. Hence, $\Delta P = \rho GL$, where ΔP is the head pressure, ρ the packing solvent density, G the intensity of the inertial field and L is the column length. The result is independent of either the nature of the solvent, i.e., its viscosity, or the particle size of the packing. For isopropanol ($\rho = 0.785$ g/cm^3) and a 10-cm long column packed under $\Delta P = 770$ atm (i.e., 10 000 p.s.i.), G is an enormous 100 000 g. It is no surprise that beds consolidate to a considerable extent under such conditions. One fact neglected in this calculation is that the bed is compressible and that the column length cannot remain constant under such a mechanical stress. When the flow is interrupted, at the end of the packing operation, the column length may rebound slightly, to contract again at resumption of the stress, i.e., when the flow is resumed. This issue will be discussed elsewhere.

Finally, the reproducibility of the external porosity of the bed or its packing density, hence of the other properties of columns packed by slurry methods, is only fair [30,31,35]. It is typically 1 and 2% for 4.6 and 10 mm I.D. columns, respectively [31]. Accordingly, the reproducibility of the retention factors

measured on a series of eleven columns is 2.3% (R.S.D.). Surprisingly, however, this is not much different from the day-to-day reproducibility of the retention factor on any one of these columns [31]. There seem to be several parameters that are not well enough controlled during the packing and the operation of HPLC columns.

5. Conclusion

The different packing methods result in beds that have quite different mechanical properties. For example, the lowest value of the external porosity for the series of ten 0.46×25 cm columns packed with Zorbax and discussed earlier was 0.390 [35]. For 9.8×1.0 cm columns packed with the same material, it was 0.386 [31]. In an axial compression column, under a stress of 900 N/cm^2 (approximately 90 atm.), the external porosity was 0.364 [30]. These variations affect significantly the phase ratio and hence modify the apparent thermodynamic data. The difference is sufficient to cause marked deviations between the experimental band profiles obtained on one of these columns and those calculated from the equilibrium isotherms measured on another one, although the same packing material is being used. This problem is serious in the optimization and the scale-up of preparative chromatography [28–31,35]. Still more important is the issue of the homogeneity of the column bed.

The results of recent theoretical calculations have shown that a significant cross-column variation of the local velocity of the mobile phase may affect the column efficiency, hence the separation performance [37,38]. A parabolic flow-rate distribution with a 5% higher velocity at the column center than along its wall was assumed. Depending on the composition of the feed, losses in recovery yield of between 1 and 12% can be experienced for the production of a 99% pure fraction of first component [38]. The loss in recovery yield is lower in the case of the second component.

The lack of homogeneity of the bed of stationary phase across the column is abundantly documented, as explained above, and this has been firmly established for twenty years. For a long time, chromatographers have alluded to a "wall effect", which has long remained mythical, for the lack of a suitable mechanism to explain it. We think that this effect is due to the friction of the packed bed against the column wall [25,26]. This friction prevents the slipping of an homogeneous bed along the wall during column packing and it causes the compression stress to be higher along the wall than it is in the column center. Because the packing is compressible, the bed becomes denser and less organized along the wall. This interplay would explain all the phenomena observed and reported above. It still remains to be fully demonstrated.

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