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Visualization of the heterogeneity of column beds

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Abstract

Columns were packed with alternating layers of virgin Zorbax C_{18} and blue colored Zorbax, treated with the dye Cibacron Blue. After consolidation of the column packing, the beds were extruded off the column, examined and photographed as they were, then cut along an axial plane, and these sections were examined and photographed. Evidence of radial heterogeneity of the distribution of the layers of the two packing materials in the bed after consolidation was observed. Friction of the bed along the column wall during consolidation was demonstrated.

Keywords: Stationary phases, LC; Packed beds

1. Introduction

In a recent review [1], we have summarized the experimental evidence collected over the years which demonstrates that the beds of the packed columns used in liquid chromatography are heterogeneous. Most of this evidence, however, is circumstantial and indirect. Measurements of the radial distribution of the local mobile phase velocity and of the local column efficiency have been carried out at constant flow-rate, in different points of the cross-sectional area at the exit of slurry packed columns [2–5]. They have shown significant variations of the velocity, which is typically 2 to 3% higher in the column center than close to the wall, and important variations of the efficiency, which may be 80 to 150% lower close to the column wall than in its center. These results have been interpreted by assuming the existence of a "wall effect" which would affect the region of the packing close to the column wall. This layer, perturbed by the wall or supported by it would have a thickness of approximately 30 particle diameters [2,3]. All investigations made of the radial homogeneity of column packings report results which are in agreement with this effect [1]. However, no reasonable explanation of the formation of this layer has been suggested yet.

More direct evidence of the lack of homogeneity of column beds has been provided recently. NMR imaging of the bands of gadolinium complexes in a column of moderate efficiency have shown that the concentration profiles are very narrow in the axial direction, at any radial position [6]. These profiles exhibit many wrinkles, however, the bands progressing at a velocity which definitively depends on the radial position. In spite of some degree of randomization of the velocity along the axial direction, the migration distance during a time of the order of the hold-up time, t_0, varies significantly with the radial position. This explains why the column efficiency measured on the bulk effluent is markedly lower than the efficiency corresponding to the local width of the bands [6–8]. Furthermore, direct measurements of the local values of the axial apparent dispersion

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coefficients by pulsed field gradient (PFG) NMR show that the average of the local value of the height equivalent to a theoretical plate (HETP) is much lower than the average HETP value measured under the same experimental conditions on the column effluent [7,8]. NMR Imaging and PFG NMR give minima of the local values of the reduced HETP of several columns which are of the order of 1 while the minimum reduced HETP of these columns, measured in the conventional way, on the bulk eluent, is between 2 and 3 [6–8]. This evidence would be more compelling still if the spacial definition of these determinations were sufficient to afford the radial distribution of the axial dispersion coefficient. This is not yet possible in practice [8], however.

Thus, there are systematic heterogeneities in the radial distributions of the local migration velocity in the axial direction and of the efficiency of chromatography bands. These fluctuations are important on a spatial scale of the same order of magnitude as the column diameter. Their presence can be explained only by fluctuations of the local packing density of the column. The packing density controls the local values of the external porosity (hence of the permeability and of the local velocity), of the phase ratio (hence of the retention factor) and, as shown by Giddings [9], of the local value of the HETP. Still, the origin of such a heterogeneous distribution of the packing density has long remained elusive. Inklings as to the origin of this distribution can be found in the compression properties of particulate matter [1,10]. Although this is generally ignored by chromatographers, beds made of a large number of small particles are highly compressible, as is well known in soil mechanics [11]. An heterogeneous distribution of the packing density of the column of the order of the one observed [2–5] could easily be explained by heterogeneities in the distribution of the stress applied to the bed during its consolidation, i.e., during the column packing operation.

The results reported by Train [12] in the study of tablet consolidation demonstrate wide variations of the local stress in axial compression columns. Stress of comparable intensity but different geometric distribution arises inside radial compression columns. Simple calculations suggest that intense stress is applied to the column bed during slurry packing [1]. Any stress which tends to force a differential migration of part of the packing material relative to the rest of it generates differential stress, hence causes an heterogeneous packing density. This is especially important when packing material is forced to move along the wall as in slurry packing, consolidation under high flow-rate, or axial compression. In such a case, the friction of the bed against the wall could provide a suitable mechanism to explain the wall effect [1].

The purpose of this work is a detailed investigation of the phenomena which take place during the consolidation of a bed of packing material during the preparation of a chromatographic column. Experiments similar to those previously described by Train [12] were designed to illustrate the consequences of friction of the packing material along the column wall during bed consolidation. For easier visualization, a 5-cm I.D. preparative column was used and, for convenience, an axial compression column was selected. Obviously, because the wall effect does exist in columns obtained by slurry packing and radial compression, similar phenomena should be expected to take place.

2. Experimental

2.1. Equipment

All experiments were carried out using a Prochrom (Champigneulles, France) LC50.VE.500.100 dynamic axial compression column skid (5 cm I.D.), previously described [13–15]. When necessary, we also used a Kiloprep 100 PLC pumping system from Biotage (Charlottesville, VA, USA) and a Linear Scientific (Reno, NV, USA) UV detector equipped with a variable path length preparative cell.

2.2. Chemicals

All packing experiments were performed with isopropanol as the slurry solvent and methanol as the pushing solvent. Methanol was also used as the mobile phase when needed. These two solvents were purchased from Baxter (Atlanta, GA, USA).

Cibacron Blue 3GA (Sigma, St. Louis, MO, USA) was used as a dye to color part of the packing material. It is highly soluble in water, methanol, and

methanol–water (40:60) solutions but it is insoluble in isopropanol and *n*-hexane (solutions in these two solvents in equilibrium with the solid dye are colorless). 50 g/l solutions of the Cibacron Blue 3GA in methanol were prepared, filtered on 0.25 μm filters and used to dye the required amounts of C_{18} silica.

Zorbax C_{18} (BTR Separations, Wilmington, DE, USA), 10-μm average particle size, was used as the stationary phase.

2.3. Procedures

Columns were prepared following the axial compression procedure previously described [13,14]. Experiments made with an analytical column (25× 0.46 cm, 5000 plates for acetone, $k'=0$) showed that Cibacron Blue was hardly retained when the mobile phase was pure methanol and that a Gaussian peak was obtained, with an efficiency close to that for acetone. Accordingly, different procedures were used to dye the packing material, depending on whether the experiment was merely intended to visualize the consolidation process (solvent isopropanol in which the dye is insoluble) or whether percolation of the packed column with a stream of mobile phase (methanol) to collect chromatographic data was also planned.

2.3.1. Conventional band profiles

An axial compression of 89 atm (1 atm=101 325 Pa) was reached progressively and a 13.6-cm long column was obtained. Samples of 1% acetone in methanol were injected at different mobile phase velocities. Chromatograms were recorded in the conventional way and used for determination of the column efficiency. The same column was used for the visualization of a band of dye immobilized in the column (see Section 2.3.2).

2.3.2. Band profiles in the column

A 10-ml sample of a 50 g/l of Cibacron Blue solution in methanol was injected into the column at a flow-rate of 99 ml/min. Elution was interrupted after approximately 40 s by switching off the pump. This corresponds to a migration distance of nearly half the column length. The top flange of the column was taken off, the packing was pushed out and cut in half along the axial direction. Cakes of wet packings hold well and can be handled, although they are quite brittle. It is easy, however, to take away part of the packing by scratching repeatedly a cake with a large kitchen knife. The half cake obtained is photographed and stored in a 5.5 cm I.D. glass tube.

2.3.3. Visualization of packing migration. Wet–dry packing

Columns were packed under axial compression, using lots of silica impregnated with either pure methanol or methanol solutions of Cibacron Blue and superficially dried (methanol content, ca. 60%, v/v). Amounts of alternatingly virgin and dyed packing materials, corresponding to approximately 1.5-cm thick layers of the material, were introduced in the column. The top of each layer was gently tapped with a round-head Plexiglass taper which was slightly narrower than the column, to make it even. Axial compression was applied in four successive steps separated by several hours. Finally, the column was consolidated under a stream of isopropanol. During the entire process, the column length was monitored as previously described [13,14].

This process is not the conventional slurry packing process in which a 30% slurry is placed in the column and the slurry solvent eliminated by compression between the exit frit and the frit fixed on the compression piston [13]. It is not a dry packing process either [10]. The packing particles are wet and stick strongly to each other; they cannot roll. The material handled had the consistency of a thick mortar.

This experiment was repeated, with a minor change. A disk of Kimwipes paper (Kimbelly–Clark, GA, USA) slightly narrower than the column was placed between each successive layer of material. This prevents the mixing of dyed and undyed silica and allows the separation of the layers after extrusion of the packing bed out of the column.

2.3.4. Visualization of packing migration. Slurry packing

Two samples of packing material were slurried in isopropanol. One of them was prepared with the virgin packing material, the other one with a mixture of the virgin material and the finely ground dye. After each scoop of slurry is introduced in the

column, it is left to settle for an hour to let all the particle sediment and a clear layer of solvent forms on top. This layer is carefully pumped away with a syringe before introducing the scoop of new slurry, a layer of solvent approximately 1 cm thick remains when the new scoop is introduced. This liquid layer serves to prevent perturbation of the top of the bed when the new amount of slurry is poured over it.

3. Results and discussion

For further reference, Fig. 1 shows plots of the column length versus the axial compression pressure during consolidation of the columns obtained by wet–dry packing. These plots were recorded as described earlier [14], under the different sets of experimental conditions used here. The results obtained are similar to those we have already published [10,13–15].

3.1. Efficiency and dye band profile

A column prepared by axial compression with a methanol slurry is not a good column [15]. Fig. 2 shows the band profile obtained with a small sample of (unretained) acetone at a flow-rate of 99 ml/min.

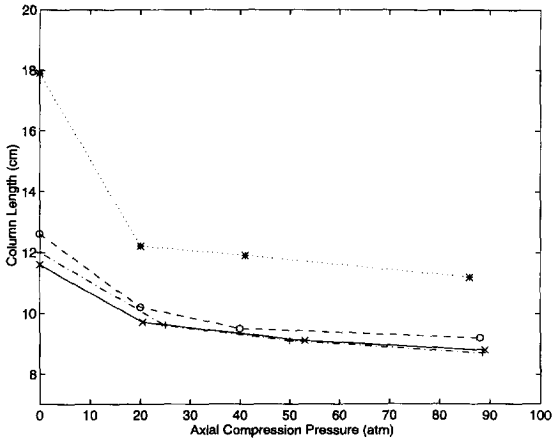

Fig. 1. Consolidation of the packing material in wet–dry and in slurry packing. Plot of the column length versus the axial compression stress applied. Solid line, wet–dry packing, without filter paper. Dash–dotted line, wet–dry packing, with paper disks between layers. Dashed line, slurry packing, first column. Dotted line, slurry packing, second column.

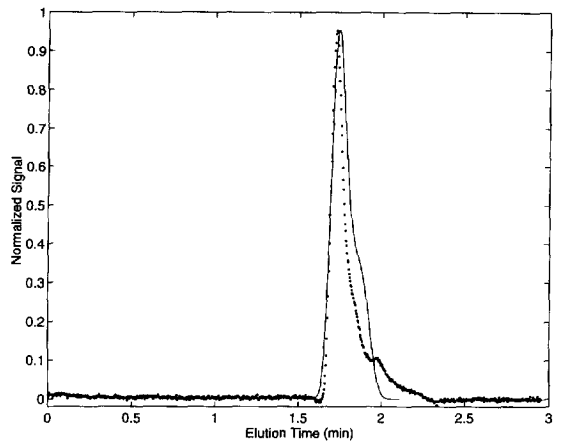

Fig. 2. Elution band profile of acetone on a Zorbax C_{18} (10 μm) column. Symbols, experimental data. Solid line, profile calculated for a nonhomogeneous column with the radial velocity distribution in Fig. 3.

The column length is 13.6 cm and its efficiency is 2300 (reduced HETP, $h=6$) theoretical plates if derived from the band width at half-height, 410 plates (reduced HETP, $h=33$) if derived from the peak second moment (average of three successive determinations). These results are in agreement with those obtained previously [15]. Obviously, an important tailing is observed (Fig. 2).

Quantitative determinations of the radial distribution of the mobile phase velocity were made, based on the scanning of the band profiles observed in the radial direction. For example, Fig. 3 illustrates such a velocity distribution as derived from the profile of the dye zone immobilized at approximately half elution, the radial cross-section of which is shown in Fig. 4 (see Section 2 for conditions). The mobile phase was moving upward in the column (the column inlet is on the piston side) and axial compression was applied in the upward direction (in all figures, the bottom of the figure corresponds to the bottom of the column and to its inlet). Although it is nearly flat in the center of the column, over almost three quarters of the column diameter, the radial profile is markedly slanted near the column wall. This confirms that, in a poorly packed column prepared by axial compression with an unsuitable solvent, there is a wall effect similar to the one reported previously [2–5] for slurry packed analytical columns. The mobile phase velocity varies

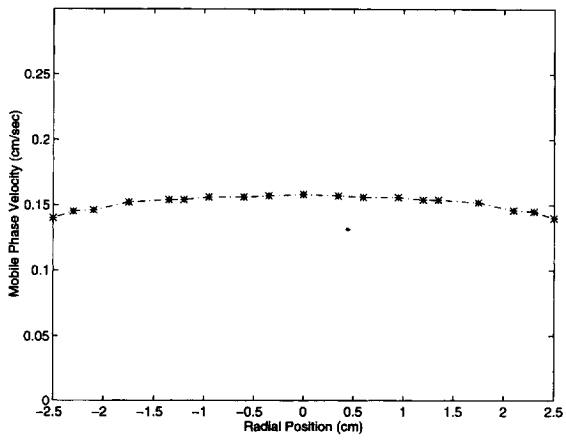

Fig. 3. Profile of a band of Cibacron Blue 3GA immobilized during its elution at a time equal to ca. 40% of the hold-up time. Radial distribution of the mobile phase velocity derived from the position of the band front in Fig. 4.

across the column. It is maximum in the central, core region and lower close to the column wall.

Fig. 3 shows that the mobile phase velocity close to the wall was 13% lower than in the center. Because the radial profile of the mobile phase velocity is not parabolic but rather flat, the volume of the column in which the velocity is significantly reduced is not very large and the global effect on the band profile is a marked tailing (Fig. 2). This observation is in agreement with the result obtained in a recent, independent work [16] in which measurements of the radial distribution of the mobile phase velocity were carried out using a method previously described [5]. The experimental band profile is compared in Fig. 2 with the profile calculated for a nonretained peak in a column exhibiting the radial distribution shown in Fig. 3 [17]. Constant velocity along the column axis for any radial position was assumed. The experimental and the calculated curves are in reasonable agreement, the difference between them suggesting that the thickness of the perturbed layer along the wall may be less than estimated here and the intensity of the perturbation somewhat higher.

3.2. Radial profiles of consolidated layers

3.2.1. Wet–dry packing

As seen in Fig. 1, the two columns had lost 31% of their initial length at the end of their consolidation. This result is in agreement with previous ones [10,14].

Fig. 5b and Fig. 6 show the radial distribution profiles of the packing material in the two columns. Fig. 5a shows the external surface of the packing cake extruded from the column, before cutting it in half to obtain the view shown in Fig. 5b. The layers of undyed and dyed materials are clearly seen. There is little mixing at the boundaries of successive layers, which was expected. The only possibility of an axial mixing arises when the new material is laid on top of the previous one and the thin layer of supernatant slurry solvent is perturbed. In both figures, we see that the profiles of the successive layers of packing materials are not flat; they are markedly curved. Note also that these layers are no longer homogeneous: the thickness of each layer varies with the radial position. This is especially clear with the layers which are closest to the compression piston. They are thinner close to the column wall than in the center region. The bed is not homogeneous in the axial direction either. The degree of consolidation [10,11] is higher close to the piston than at the other end of the column.

In all the figures, the part of the bed in contact with the upper flange of the column is at the top of the figure. This part of the bed cannot move much. On the other hand, the bottom part of the bed moves upward with the piston when the column is compressed and consolidated. It is clear on Fig. 5b and Fig. 6 that there is intense friction of the bed against the column wall. As a result, the part of the packing material which is close to the column wall is more intensely compressed and its average density is higher than that of the rest of the bed. This observation explains why the mobile phase velocity is lower in the cylindrical region close to the column wall, as has been observed with slurry packed analytical columns [2–5] and with axial compression columns [16]. It also suggests that the packing density in the perturbed layer along the column wall, which is responsible for the wall effect, is probably not homogeneous in the axial direction but is higher close to the piston than at the other end.

Because in the column shown in Fig. 6, the layers of different colors were placed on top of each other, without any physical separation, these layers made of

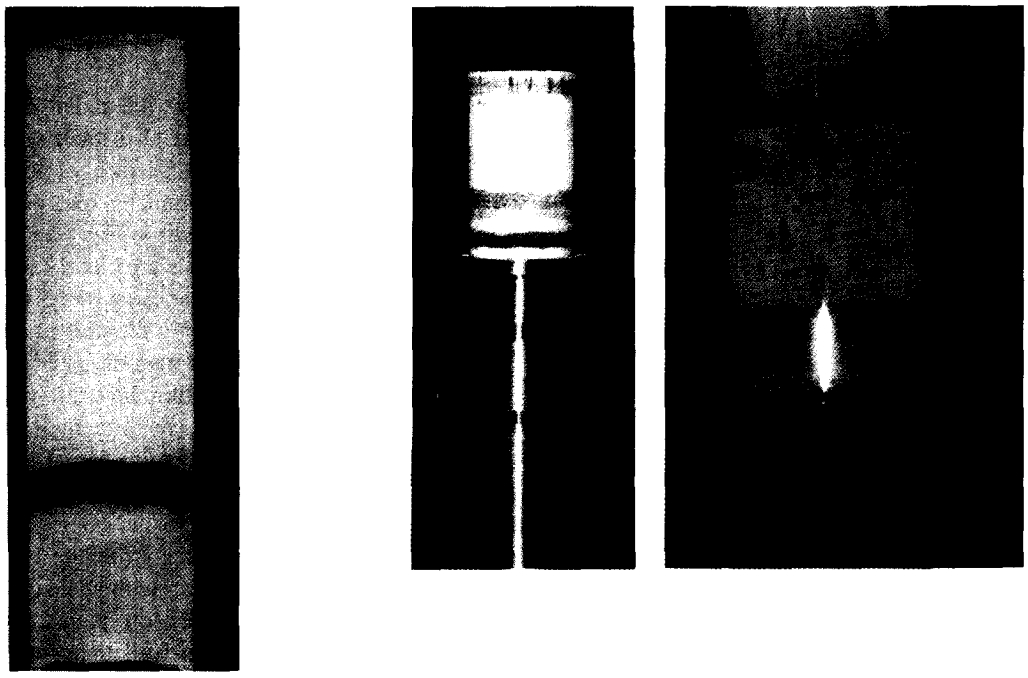

Fig. 4. Profile of a band of Cibacron Blue 3GA immobilized during its elution at a time equal to ca. 40% of the hold-up time. Photograph of the axial cross-section of the column.
Fig. 5. Column prepared by wet–dry packing. Adjacent layers are separated by a disk of filter paper. (a; middle) Photograph of the cake extruded from the column, before the cross-section is prepared. (b; right) Photograph of the axial cross-section of the column.

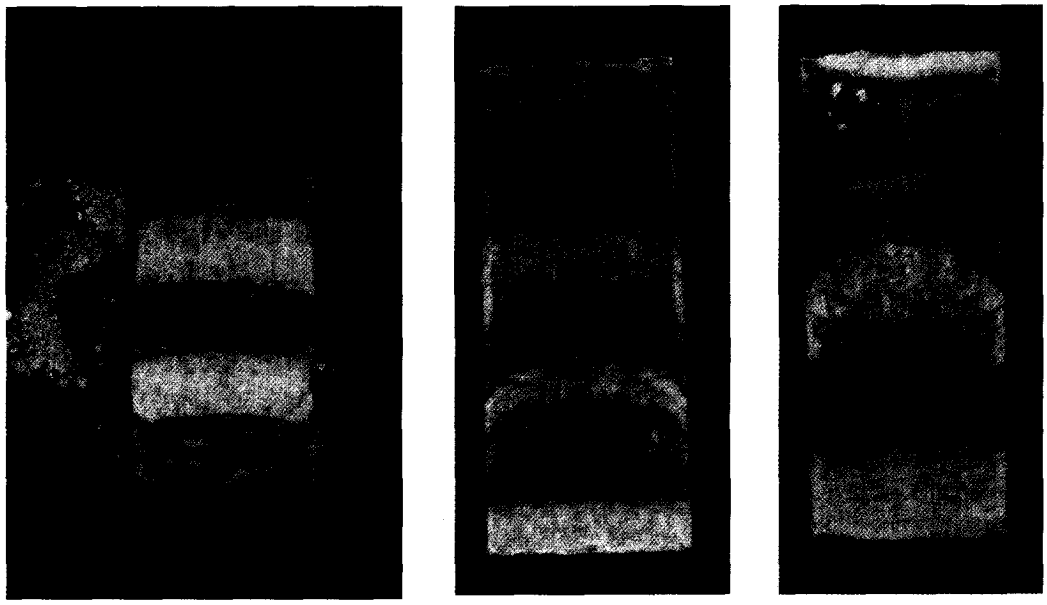

Fig. 6. Photograph of the axial cross-section of a column packed by wet–dry packing (no separation of adjacent layers).
Fig. 7. Photograph of the axial cross-section of a first column packed by slurry packing.
Fig. 8. Photograph of the axial cross-section of a second column packed by slurry packing.

the same packing material (Zorbax) stick to each other and the bed remains in one solid piece. By contrast, in the column shown in Fig. 5b, the successive layers were separated by a sheet of filter paper. In this case, the layers separate and warp slightly. A small void can be seen in Fig. 5a, between these layers and near the column edge. This indicates that there are some residual constraints inside these layers, after the consolidation has ended. A similar result was also observed by Train [12] in the compression of a multilayer bed at much higher pressures (2000 atm). Note also, in Fig. 5a, that a mere examination of the external surface of the packing bed does not give any clue regarding the actual degree of internal heterogeneity of the column.

3.2.2. Slurry packing

Plots of the column length versus the axial compression stress for the columns prepared by slurry packing are shown in Fig. 1. The degree of consolidation is comparable to the one achieved in the other series of experiments, previously described in this work. Figs. 7 and 8 show the distribution of the layers of packing material across two columns after their consolidation. As in the previous experiments, the radial distribution of the initially flat layers has been seriously perturbed by the compression. The material in the central region of the column has moved to a significant extent in the direction of the piston while the material which is close to the wall seems to have experienced less migration and a higher degree of consolidation. As in the case discussed previously, the degree of consolidation observed is higher close to the piston than at the opposite end of the column. This result is in agreement also with the observation that samples of packing materials recovered from axial compression columns exhibit more particle breakage when they are taken from the region close to the piston and the wall than when they come from any other part of the column [18].

The deformation of the successive layers appears much more intense in Figs. 7 and 8 than in Figs. 5 and 6. This is consistent with the assumption of intense friction between the bed of packing material and the column wall. The presence of isopropanol would reduce this friction, compared to what is experienced in pure methanol or in wet–dry packing. It seems, however, that the friction between particles has been reduced to a greater extent than the friction between wall and bed.

4. Conclusion

The experiments reported here are similar in nature to those described by Train [12] in his study of the axial compression of drug tablets. The results obtained are quite similar. They suggest that friction between the column bed and the column wall plays a major role in the formation of the wall effect and is critical in controlling the intensity of this effect.

Acknowledgments

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