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Evaluation of the secondary consolidation of columns for liquid chromatography by ultrasonic irradiation

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Abstract

The consolidation of packed analytical chromatography columns was carried out under ultrasonic irradiation. Columns were first packed using a conventional high pressure downward slurry method. Then, they were subjected to further bed consolidation in the presence of ultrasonic vibration. This process of further bed consolidation is referred to as secondary consolidation. Secondary consolidation was observed to occur more readily in solvents of low viscosity and at low flow-rates (low pressures). Column efficiency was not observed to be a factor affecting the process of secondary consolidation of the packed bed.

Keywords: Secondary consolidation; Ultrasonic irradiation; Packing procedures; Bed consolidation

1. Introduction

In the 1980s, column packing was described as an ''art'' [1]. Column to column reproducibility was poor. Trial and error procedures yielded a seemingly endless array of ''optimal'' packing methods. Yet, advances in column packing procedures have been slow. The only significant progress made in the last twenty years is the high degree of reproducibility of the performance of commercial columns belonging to a given lot or batch [2–5]. Unfortunately, the progress achieved in this area by column manufacturers remain trade secrets. Critical factors, such as the friction of the packed bed along the wall [6] and the friction between the particles of the packing material [7], have only recently been identified. These factors, the dispersive and viscosity properties of the packing solvents and of the slurries used to pack the column, and the density match between the packing solvent and the particles all play a contributing part in the packing process, in the radial homogeneity of the columns obtained, hence in their efficiency [1]. Furthermore, channeling, associated with high solvent linear velocities, also contributes to the effects of poor packing [8]. How each of these factors relate to one another still remains very speculative. The ideal packing procedure is yet to be found, with reduced plate heights consistently falling below the apparent limiting value of 2. Column efficiencies, if highly reproducible for commercial columns, are no better now than they were twenty years ago. The progress accomplished arise more

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from the improved nature of the silica with respect to its particle size distribution, the particle shape and their surface roughness than to a better understanding of the packing procedure. It is disappointing that the important progress made in the manufacturing of homogeneous, reproducible, high quality packing materials has resulted in negligible advances on the column efficiency front [9].

Guiochon et al. demonstrated recently that, while a bed consolidates when mechanical stress is applied to it, this bed is elastic and returns to almost exactly its original length when the stress is removed [6]. These authors were also able to show that friction of the bed along the column wall contributes to the radial heterogeneity of the column bed which is easily visualized on actual beds after their extraction from the column [10,11], or, while in operation, by nuclear magnetic resonance imaging [12] or by simpler optical means [13,14]. Bed/wall friction has both considerable inconvenients and great advantages [6]. Because of this friction, the stress distribution throughout the bed is heterogeneous. This causes the radial heterogeneity of the column bed because, under the increased stress caused by friction, the packing density is higher near the wail. Without friction, however, the bed would not stick to the wall. The mobile phase might by-pass it, preventing chromatography from taking place at all. Furthermore, the bed would contract with increasing head pressure to expand again to its original length when this pressure is relieved. This would lead to void formation at the inlet of the column when it is in use. To some extent, this phenomenon takes place every time a column is put to use, the bed compacting under stress. However, friction slows, delays and dampens the bed movement when pressure is relieved. This is illustrated by the modulus of elasticity of the bed depending on the column length [6,15,16]. With prolonged use, voids eventually appear at the inlet of the column and its efficiency decreases. To overcome all void formation arising from bed compressibility, we must achieve an initial bed structure which is unable to undergo further compression because it is as compact as possible. This requires that axial friction forces be canceled at some stage during packing.

An analogy of the packing procedure was made by Jaeger et al. [17]. To illustrate the difficulties encountered to consolidate a packed bed, they called it the ''parking lot'' effect. Voids or regions of low packing density within the bed occur due to the limited possibility of any individual particle to move around, in much the same way as cars left in a parking lot. In order completely to fill a parking lot with equal sized cars in unassigned lots, one irregularly parked car may require the coordinate movements of several other cars in order to accommodate just one more vehicle. If a densification of the car parking is to take place, it requires the cooperation of many vehicles. This is the same in a bed of close packed spheres, where several or many spheres may have to be moved in order to accommodate one more sphere. Jaeger and coworkers [17] studied the movement of spheres, investigating the effect of vibration on assemblies of grains. They found that different conditions exist for the movement of grains, depending on the container shape and on the particles size-distribution. Sonic vibrations provided a means to rearrange particles and to force the bed structure to flow. Then, particles were segregated along the bed, based on their size. Friction between particles and the container wall was shown to have a contributing effect to particle movement. Under the influence of sonic vibrations, particles moved faster along the column wall when friction was increased [17]. This flow under sonic vibrations of particles that are otherwise more or less fixed in the bed structure may be of interest to chromatographers and we decided to investigate it.

In addition to friction and vibration, the dispersive nature of the solvent allows particles to flow smoothly without forming agglomerates. This minimizes the ''packing lot'' effect. If agglomeration during column packing and the formation of a bed of agglomerates can be avoided, a more homogeneous bed structure would be obtained. Stabilization of the slurry can be achieved in many different ways described by Verzele and Dewaele [1].

The application of vibrations during the packing process has attracted attention in the past. As early as 1972, Kirkland [18] recommended an undefined ''tap-fill'' method and Halász and Naefe [19] suggested to vibrate analytical columns at 60 Hz. An optimum tapping method was described by Klawiter et al. [20] but the best reduced plate height obtained by these authors exceeded 5. More recently,

mechanical devices were used that vibrate columns through tapping at controlled frequencies [21,22]. Ultrasonication was also employed for the consolidation of micro-columns after packing [23,24], with Tong et al. [24] reporting that bed consolidation takes place during ultrasonication, resulting in a 3–5% decrease in the bed length. No reports on the effects of bed consolidation in an ultrasonic field on analytical size columns have yet been published, to our knowledge.

The goal of this study is a systematic evaluation of the consolidation of a packed bed under ultrasonication, a process that is known to enhance the movement of particles, and which, as a consequence, alters the instantaneous frictional forces between particles and between particles and the wall. Columns were prepared using a conventional downward packing procedure at high pressures and high flowrates, but without vibration under any form. The columns where then tested, sonicated to produce secondary consolidation, and the development of inlet voids was examined by inspection of the column inlet.

2. Experimental

2.1. Equipment

The chromatographic system used consisted of two Waters model 510 dual piston pumps controlled by a Waters Automated Gradient Programmer (Waters, Milford, MA, USA), a Rheodyne model 7010 injection valve fitted with a 20 μL loop (Rheodyne, Cotati, CA, USA), and a Linear UVIS model 205 variable wavelength detector (Linear Instruments, Fremont, CA, USA), set at 254 nm with a rise time of 0.1 s. Data acquisition was achieved using a Lawson Labs model 203 Serially-Interfaced 20-bit data acquisition system with custom ±1 volt gain range (Lawson Labs, Malvern, PA, USA) collecting at 20 Hz and a 486 PC. Ultrasonication experiments were obtained using a Branson 2200 ultrasonic bath (40 kHz) (Branson Ultrasonics, Danbury, CT, USA). Columns were packed using a Haskel air-driven fluid pump (Haskel International, Burbank, CA, USA). Photographs were recorded on Kodak 200 speed Elite color slide film using a 35 mm Pentax ZXM SLR camera fitted with a Makinon 80–200 mm macro zoom.

2.2. Chemicals

HPLC grade methanol and dichloromethane were obtained from Fisher Scientific (Fair Lawn, NJ, USA). HPLC grade acetonitrile, and reagent grade acetone were supplied by J.T. Baker (Phillipsburg, NJ, USA). Ethyl alcohol (200 proof) was obtained from Aaper Alcohol and Chemical Company (Shelbyville, KY, USA). All mobile phases were sparged with helium. The stationary phase materials used for the preparation of columns in this study were either Zorbax Cl 8 10 pm particle size with a 15 nm pore size (Dupont, Remington, DE, USA) or Novapak C_{18}, 6 pm particle size with a 6 nm pore size (Waters, Milford, MA, USA). Both stationary phase materials were used as supplied by the manufacturer without further size segregation. Benzamide, used as the unretained sample was obtained from Aldrich (Milwaukee, WI, USA).

The viscosities at 25°C of the solvents used here are 0.35 cP for acetonitrile, 0.45 cP for dichloromethane, 0.55 cP for methanol, and 1.04 cP for ethanol. Using an equation due to Arrhenius and valid for mixtures of solvents with no hydrogen bonding [25], the viscosity of a 50/50 dichloromethane/acetonitrile mixture is calculated at 0.39 cP.

2.3. Preparation of the columns

A simple downward slurry packing procedure was employed for this study. The 4.6 mm I.D. columns were either 10 cm or 15 cm in length. A variable length pre-column section was inserted prior to the analytical column so that the efficiency of the packing procedure could be varied. For the Zorbax material, 6 g of stationary phase was suspended in 35 mL of acetone, while for the Novapak material 8.5 g was suspended in 35 mL of acetone. The slurries were stirred for 30 min and ultrasonicated for a further 15 min, followed by a further 30 min of stirring. A dichloromethane displacement solvent was employed in the column blank and the packing solvent was methanol. The columns were packed at 7000 p.s.i. allowing 400 mL of solvent to be flushed

Table 1
Reduced plate heights for each of the Zorbax columns

Reduced velocity (u)	Reduced plate height (h)	
	Column 1	Column 2
1.52	4.5	2.9
5.12	4.7	3.1
10.2	4.2	3.3
15.2	4.3	3.7
19.2	4.5	3.9

through the Zorbax columns and 200 mL through the Novapak columns.

3. Results and discussion

To observe the effect of ultrasonication on bed consolidation we packed a number of columns under different conditions. Two of these columns were packed in such a manner so as to yield one column with a poorer efficiency than the other (Table 1, columns 1 and 2). These columns were packed with the Zorbax packing material and, prior to use, they were flushed with methanol at high flow-rate (3.0 mL/min) for 30 min. This was to ensure that secondary consolidation would not occur under our normal operating conditions. Therefore, consolidation during ultrasonication, if it did occur, would be as a consequence of the ultrasonic vibration, not merely an effect of the flow of a high velocity stream of mobile phase under high inlet pressure.

In the first instance, we evaluated the poorly packed column. Reduced plate heights at several reduced velocities are given in Table 1. The elution profile of benzamide (unretained) was quite unsymmetrical. To evaluate the degree of secondary consolidation of the bed, we submersed the column into an ultrasonic bath. This necessitated increasing the length of the extra column tubing, thereby affecting the band dispersion. Band shape was measured before, during, and immediately after sonication, these measurements being made consecutively. To avoid excessive heating during the column sonication, flow-rates were restricted to greater than 0.7 mL/min. Elution occurred within a time period of insignificant temperature variation. Fig. 1 illustrates the effect of the sonication on the elution

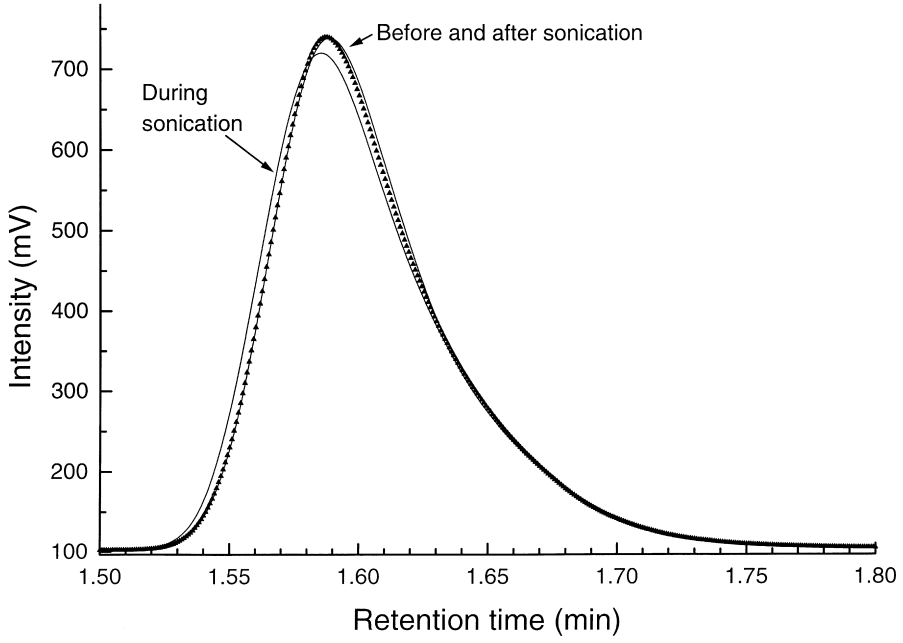

Fig. 1. Band profiles of benzamide eluting from the column with lower efficiency as recorded in Table 1. These profiles illustrate the changes arising from ultrasonication. Flow-rate=1.0 mL/min.

profile at a flow-rate of 1.0 mL/min. Similar profiles were observed for a range of flow-rates. The profiles before and after sonication were almost identical, but the profile of the band eluting during the sonication was different. The elution time and the peak height decreased slightly while the band width and peak tailing increased. This effect was not due to an increase in the dispersion as a result of increased mixing and diffusion in the presence of the ultrasonic field. Fig. 2 shows almost identical profiles for a sample that was allowed to pass through a piece of tubing (with no column) in the presence of the ultrasonic field (triangles) as opposed to sample in the absence of the ultrasonic field (solid line). These curves overlay almost perfectly.

The occurrence of some changes in the bed structure during sonication is therefore suspected. A forced circulation of the solid phase along the column is not impossible. This phenomenon was reported under different experimental conditions, with a bed consolidated to a much lesser extent [17]. However, given the observations reported later, it is unlikely under the conditions of our experiments. The peak profiles in Fig. 1 indicate that the bed would return to its original structure almost as soon as the ultrasonic field was removed. This return to the original form indicates that no extensive size segregation of the particles in the column took place and that particle breakage was not responsible either for the change in shape and position of the elution profile reported. Inspection of the column inlet revealed that no void formed at the column head. In fact, no inlet void was observed even when the ultrasonication period was increased to 8 min at each flow-rate, over a range in flow-rates giving a total period of 1 h of ultrasonication. Nevertheless, the changing form of the elution profiles verified that sufficient energy was being applied to the system in order to bring about some changes to the bed structure of the column under ultrasonic irradiation.

Our initial assumption was that, in order to bring about a complete secondary consolidation of the bed, the applied energy must overcome the friction between particles inside the bed. Now, the friction stress depends on the friction coefficient, which is difficult to alter for a given packing material, and of the normal stress applied by the bed against the wall. This mechanical stress is a function of the external

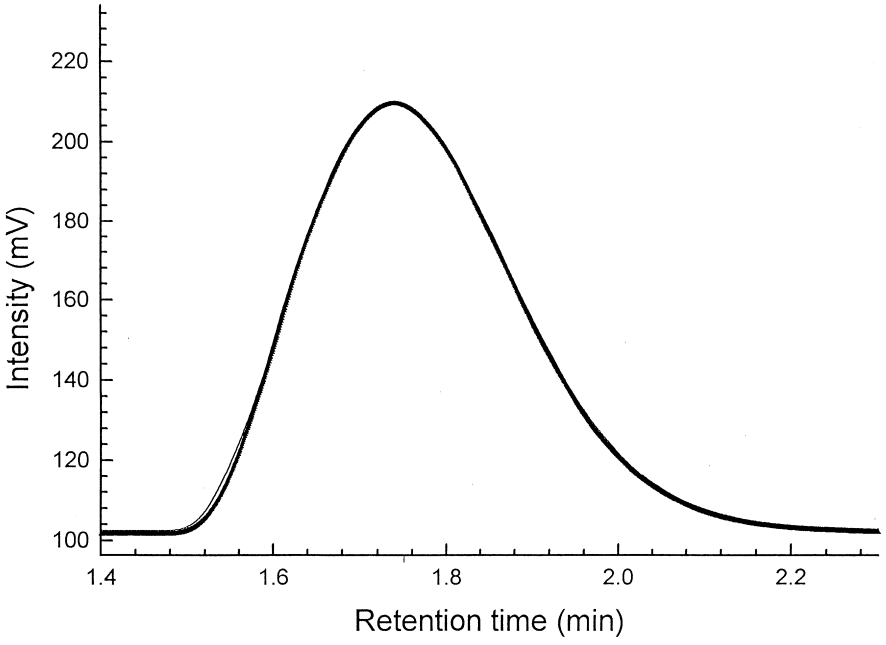

Fig. 2. Comparing the effect of ultrasonication versus no ultrasonication on the band profile of benzamide migrating through tubing without a chromatography column. The two curves overlay almost exactly.

stress applied to the bed. In an analytical column, this stress is caused by the head pressure forcing the mobile phase to percolate through the column [6]. In other words, the inlet pressure should be reduced to alleviate the force applied to the column wall. Secondary bed consolidation was then confirmed by changing the mobile phase to a less viscous mixture of 50/50 dichloromethane/acetonitrile and using a flow-rate of only 0.5 mL/min. After 10 min of ultrasonication, a large void formed at the column head, as illustrated by the photograph in Fig. 3. The unfortunate consequence of such an experiment is the destruction of the column when a positive (i.e., consolidation) result is obtained.

The reduced plate height of the second column (Table 1) illustrates that this column was better packed than the first. Secondary consolidation was tested in the same manner as for the first column, except we did not record the elution profiles since little differences were expected and we were interested only in the possible formation of an inlet void. Following the assumption that wall friction was reduced by decreasing the column inlet pressure, we used first methanol as the mobile phase, at flow-rates between 2.0 and 0.2 mL/min. Total sonication time was equivalent to 1 h. After sonication at each flow-rate, the column inlet was inspected. No significant void was observed after this treatment, except for the formation of a small, meniscus-like, indentation. Methanol was then replaced by dichloromethane and the flow-rate set to 2.0 mL/min. After 10 min of ultrasonication, there was no void other than the meniscus surface already observed. The operation was repeated at 0.5 mL/min with similar results. The column was sonicated, then equilibrated with 50/50 dichloromethane/acetonitrile at a flow-rate of 0.5 mL/min and again sonicated for 10 min, as before. Inspection of the inlet showed the presence of a large void, similar to that observed in Fig. 3. Both columns showed significant and similar secondary consolidation under proper ultrasonication conditions although their initial efficiencies were different, indicating that column efficiency is not only a function of the how much packing can be placed within the column container. The selection of the experimental conditions of the ultrasonication appears to be most important.

Fig. 3. Photograph of the inlet void for the poorly packed 15 cm Zorbax column (Table 1).

To test the contribution of the flow-rate (and/or the inlet pressure) to secondary bed consolidation with ultrasonication, a 10 cm column packed with Novapak was subjected to ultrasonication at differing flow-rates. The column was first flushed with methanol at 3.0 mL/min for 30 min. The end cap was removed and the inlet inspected for any void formation. A photograph of the column head showing a very small void is shown in Fig. 4a. The column was then reconnected to the HPLC system and equilibrated with acetonitrile at a flow-rate of 2.0 mL/min. The column was ultrasonicated for 10 min after which the flow was stopped and the inlet again inspected for any void. No change was observed, as shown in Fig. 4b. The column was reassembled, equilibrated with acetonitrile at a flow-rate of 0.2 mL/min and ultrasonicated for 10 min, after which the inlet was inspected again. At this point, a large void was present, as shown in Fig. 4c. From this result, we conclude that secondary consolidation takes place more readily at low flow-rates, most likely because the radial mechanical stress applied by the bed to the column wall is also reduced, thereby allowing the ultrasonic vibration to overcome the reduced wall friction. Under this reduced stress, the energy associated with ultrasonication also increases the ability of the particles to undergo the rearrangements required to overcome the effect described in the ''parking lot'' analogy.

At this stage, we have established that the mobile phase flow-rate is an important contributor to the secondary bed consolidation and that it should not be

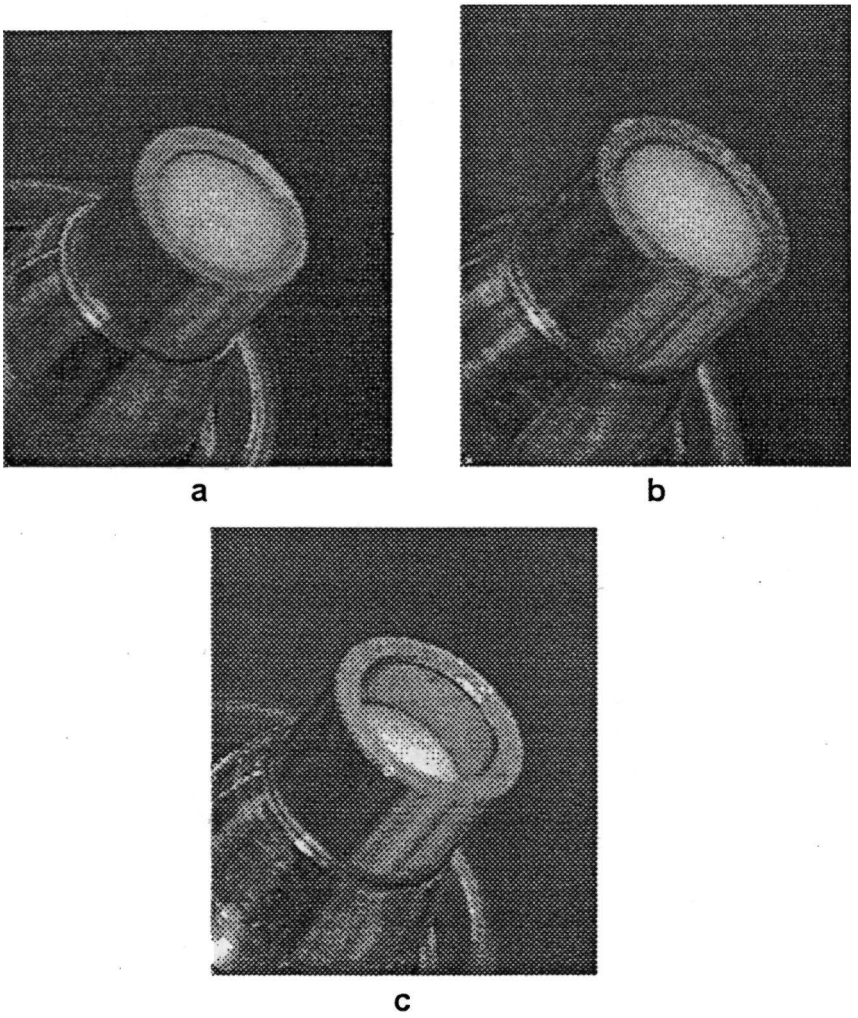

Fig. 4. Photographs showing the development of the inlet void as a function of the flow-rate for a 10 cm Novapak column. (a) original bed (b) mobile phase: 2.0 mL/min acetonitrile and (c) mobile phase: 0.2 mL/min acetonitrile.

too high, at least during part of the consolidation process. This result contradicts the popular belief that, in this field, more is better and that columns should be consolidated after packing under as high as possible an inlet pressure and a solvent flow-rate. We need now to investigate the influences of the solvent flow-rate and of the inlet pressure, hence the solvent viscosity, on this phenomenon of secondary or post-packing consolidation. A large number of columns were tested during this study and all results cannot be reported in detail. Methanol was initially employed. The first column tested underwent secondary bed consolidation almost immediately. Only one other column during the course of this study, however, showed any void formation following its consolidation in methanol. Hence, we decided to study systematically the nature of the solvent, starting with the most viscous usual solvent and working toward the least viscous ones.

A 10 cm Novapak column was equilibrated in pure ethanol, at a flow-rate of 2.0 mL/min for 10 min. The inlet cap was removed and the head of the column inspected for any voids. Fig. 5a shows that the head remained intact, no void being present.

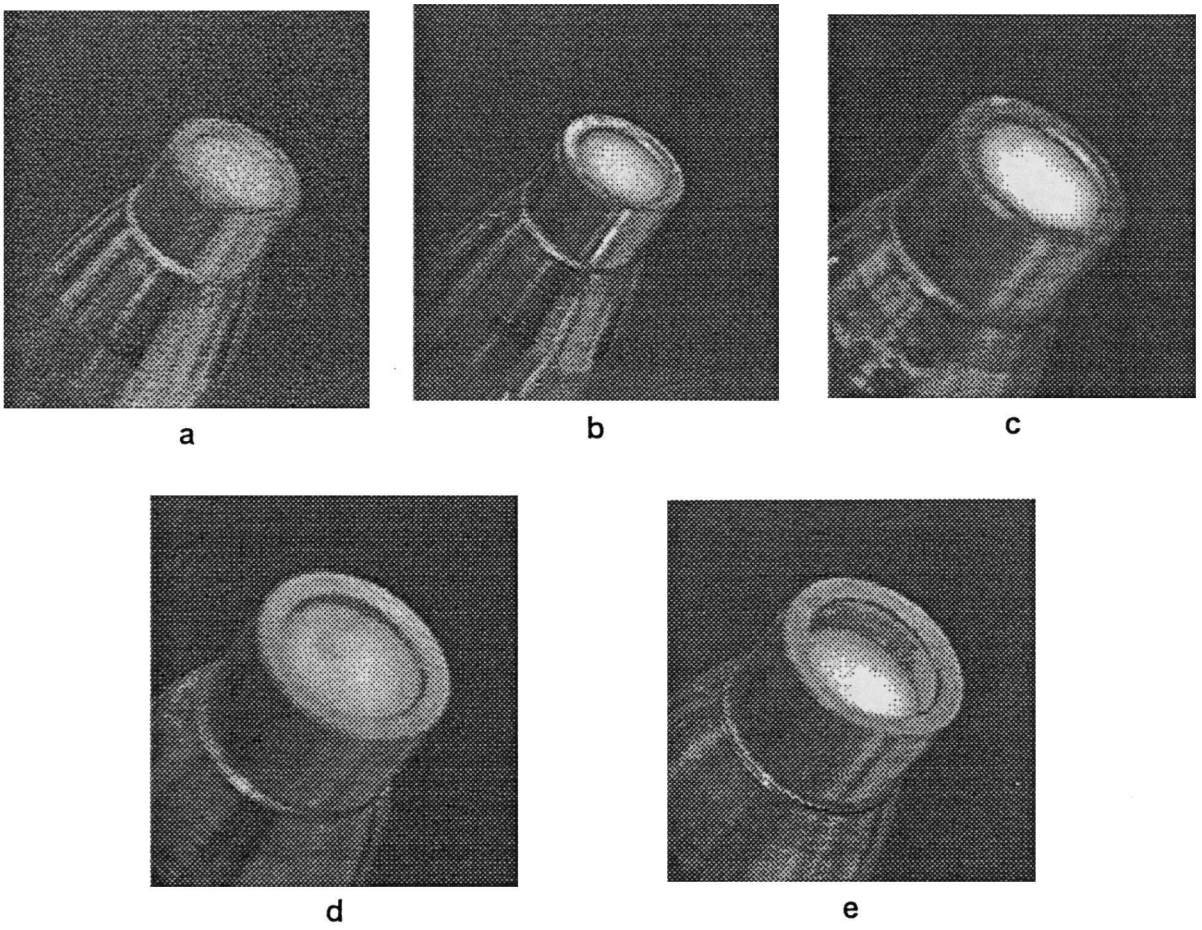

Fig. 5. Photographs showing the development of the inlet void as a function of the solvent for a 10 cm Novapak column. (a) Ethanol flow-rate=2.0 mL/min. (b) After 10 min sonication in ethanol at a flow-rate of 1.5 mL/min. (c) After 10 min sonication in methanol at a flow-rate of 0.1 mL/min. (d) After 10 min sonication in dichloromethane at a flow-rate of 0.1 mL/min. (e) After 10 min sonication in acetonitrile at 1.0 mL/min.

Following, the column was reassembled and placed in the ultrasonic bath. Under an ethanol stream at a flow-rate of 1.5 mL/min, the column was ultrasonicated for 10 min, after which the head of the column was again inspected for voids. As Fig. 5b shows, none was formed. The mobile phase was then changed to pure methanol and the column equilibrated, then ultrasonicated for 10 min under a methanol stream at a flow-rate of 1.5 mL/min. After which the inlet of the column was inspected for voids and none was found. The column was then re-equilibrated in ethanol and the flow-rate adjusted to 1.0 mL/min. The column ultrasonicated for 10 min and the inlet inspected for voids. This process was repeated, alternating ethanol and methanol as mobile phases and decreasing the flow-rate, then inspecting for voids, until finally flow-rates of 0.1 mL/min for both the ethanol and methanol were tested. Inspection of the inlet for each case showed that no voids were present in either mobile phase. This result is illustrated by the photograph in Fig. 5c, taken after flushing the column with methanol at 0.1 mL/min.

The same column was then equilibrated with a stream of dichloromethane at a flow-rate of 1.5 mL/min. After ultrasonication of the column for 10

min, the inlet was again inspected for void formation and no voids were found. However, when the test was repeated at a flow-rate of 0.5 mL/min, inspection of the column inlet showed a slight local void and a deformation of the surface of the packing. This deformation increased in size after the test had been repeated at a flow-rate of 0.1 mL/min of dichloromethane, as shown in Fig. 5d. Although a slight inlet void was apparent, secondary consolidation was not as significant as in previous experiments, so the mobile phase was changed to pure acetonitrile, the flow-rate set to 1.0 mL/min, and the column equilibrated, then ultrasonicated for 10 min. Inspection of the column inlet revealed the important void shown in Fig. 5e. Clearly, void formation was greatest for the least viscous solvent (see viscosities in Experimental section). On several other occasions, however, voids were observed to form in dichloromethane/acetonitrile mixtures, while solvents such as dichloromethane or methanol almost always failed to produce a significant extent of secondary consolidation.

In order to examine whether secondary consolidation of the bed in the presence of an ultrasonic field leads to a more efficient column, we filled the void which formed at the column inlet after secondary consolidation. Fig. 6 (curve a) shows the profile of the benzamide peak eluting from a Novapak column prior to its ultrasonication at a flow-rate of 1.0 mL/min of methanol. The column was then ultrasonicated in acetonitrile at a flow-rate of 0.1 mL/min for 10 min. The inlet cap was removed to confirm the formation of a void. This void was then filled by joining a 5 cm section of 4.6 mm stainless steel tubing to the column inlet. The 5 cm section was filled with a slurry of Novapak in acetonitrile and the assembly ultrasonicated for 4 h under percolation with a stream of acetonitrile at 0.1 mL/min. The pre-column tubing was disconnected and the column inlet cap reassembled in the usual manner. The column was flushed with methanol and the efficiency evaluated. Fig. 6 (curve b) illustrates the elution profile of benzamide following this whole procedure. Clearly there was a significant loss of efficiency, despite 4 h of sonication, a period that was thought sufficient to ensure bed homogeneity. Either this was not the case or the consolidation process resulted in a bed which was more heteroge-

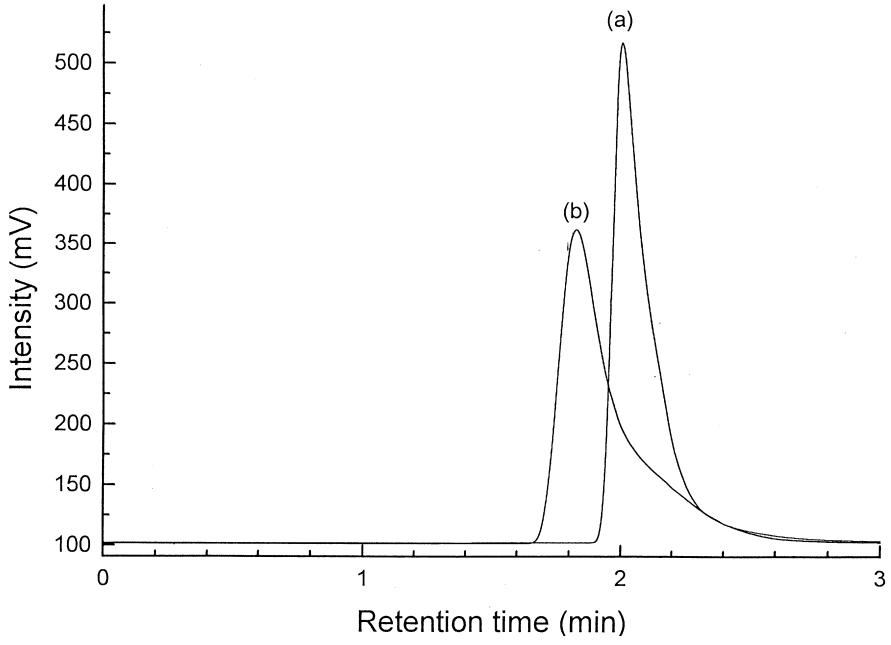

Fig. 6. Band profiles of benzamide eluting from a Novapak column (a) before ultrasonication and secondary bed consolidation. (b) after ultrasonication, secondary bed consolidation and having filled the void.

neous than the initial one. Note that the retention time of the nonretained compound (peak first moment) decreased during the process, indicating a decrease of the column void volume which was nearly proportional to the volume of the void formed.

This last result was disappointing. An improvement in column efficiency would have been favorable. However, we are not sure that (1) voids do not appear elsewhere than at column inlet; (2) that the entire bed was consolidated during the last step, when the inlet void was filled. We saw a void at the column inlet and filled it but other voids may have appeared at intervals along the column and the bed may actually be fully consolidated only in certain sections. Wall friction may be uneven along the column because of possible ''greasy spots'', contributing to only partial bed consolidation. The actual process of bed consolidation over the entire column length may be a very slow process. Finally, previous workers have shown that particle segregation along the bed length takes place during column sonication [17]. If this sorting out of particles by size causes radial heterogeneity, it may explain the poorly efficient and tailing peaks observed.

4. Conclusion

Rather surprisingly, a column packed following the current, conventional recipes applied in academic laboratories is not consolidated to its maximum possible packing density. The packed bed can be further consolidated under ultrasonication after the high pressure downward slurry packing procedure was completed. The percolation of the column under the combination of a high flow-rate stream and a high inlet pressure, through the use of a viscous solvent, is not sufficient to form the most densely packed bed possible. Furthermore, our results show that secondary bed consolidation is independent of the column efficiency and cannot be taken as a measure of the column performance nor as an objective function for its optimization. The development of better packing procedures may aim at achieving denser columns because they are more mechanically stable but the way columns are packed, the kinetics of the process, is extremely important.

Yet, we confirmed the importance of bed/wall friction in the packing of chromatographic columns by obtaining a series of results which are all explained and were in part predicted by this effect. Secondary consolidation takes place more rapidly and more readily at low than at high flow-rates, with less than more viscous solvents. Consolidating the columns and ''filling the void'' did not improve column efficiency, however. Further studies are in process, attempting to incorporate bed consolidation by ultrasonication in the packing process since we now know that ultrasonication does supply sufficient energy to assist in this process.

Finally, if conventional procedures produce columns which are not fully consolidated, these columns are metastable, not stable. The packing may collapse, causing the formation of an inlet void associated with a major loss in the column efficiency. This observation may explain many reports of columns losing their efficiency after gradient operation with acetonitrile or after transportation. Under such conditions, a metastable column might be extremely sensitive to vibrations or shocks.

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