Liquid Chromatography Problem Solving and Troubleshooting

Question

Recently, I have seen several papers in the literature that discussed monolith columns. I am unfamiliar with this technology and would appreciate any information you could provide in helping me understand the advantages they offer compared to the conventional HPLC columns I now use.

Answer

Although the concept of monolith column construction started a little over a decade ago, most of the published accounts dealing with this topic have appeared within the last four or five years. To date, much of the research has been concerned with fundamental aspects of monolith construction, especially as it relates to producing different types of materials, including silica and several different polymer-based systems. Likewise, many of the fabricated materials have been in the form of 1–3-mm reticulated disks and have been used to separate larger molecular weight analytes, such as proteins in which slow diffusion results in significant mass transfer problems in conventional porous particle-based columns. The inherent advantage of reticulated monoliths is to minimize mass transfer effects that allow the separation to be run at high flow through velocities without the loss of separation efficiency.

In order to better understand the fundamental differences between monolithic and particle-based columns, it is important to briefly review the fluid dynamics of the mobile phase as it passes through these two different types of separation media. In conventional particle-based columns, irrespective of the physical nature of the particles in terms of their shape (i.e., irregular vs. spherical), size, or porosity, all are nonreticulated materials. Stated in slightly different terms, the pores within the particles are not interconnected to allow fluid to flow through them. As such, in order for the mobile phase to pass through conventional columns, the mobile phase must flow around the particles in the interstitial space between them. As the packing material is reduced in size, the flow channels formed between the particles also decrease in size. In contrast to the longitudinal flow of the mobile phase outside the packing, most of the interactive surface where the separation occurs is inside the pores. For standard small molecule separation media, the nominal pore size is between 60 and 100 Å, depending on the manufacturer.

As molecules migrate through the column, they access the internal surface by slow molecular diffusion, transferring first from the moving eluent to the nonmoving eluent trapped within the pores (i.e., typically referred to as the stagnant mobile phase), then to the surface, back to the stagnant mobile phase, and finally to the longitudinally moving eluent. This process occurs continuously as the solute migrates through the column

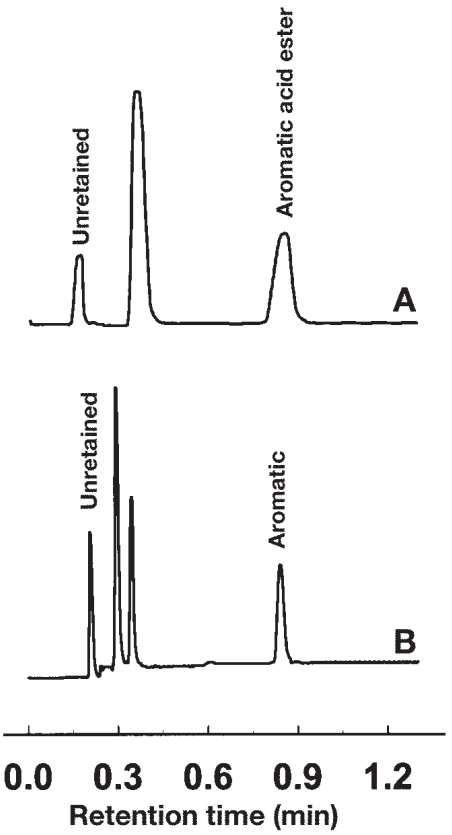

Figure 1. Reversed-phase separations of simple test solutes under fast analysis conditions. Chromatograms: (A) 50- × 4.6-mm i.d. column packed with 10-µm irregular octadecyl packing and (B) 100- × 4.6-mm i.d. RP-18 monolith column.

The purpose of *Chromatography Problem Solving and Troubleshooting* is to have selected experts answer chromatographic questions in any of the various separation fields (GC, GC–MS, HPLC, TLC, SFC, HPTLC, open column, etc.). If you have questions or problems that you would like answered, please forward these to the *Journal* editorial office with all pertinent details: instrument operating conditions, temperatures, pressures, columns, support materials, liquid phases, carrier gas, mobile phases, detectors, example chromatograms, etc. In addition, if you would like to share your expertise or experience in the form of a particular question accompanied by the answer, please forward it to: JCS Associate Editor, *Chromatography Problem Solving and Troubleshooting,* P.O. Box 48312, Niles, IL 60714. All questions/answers are reviewed to ensure completeness. The *Journal* reserves the right not to publish submitted questions/answers.

Roger K. Gilpin
Associate Editor

and is the basis for the theoretical plate concept. In order to minimize mass transfer effects within the stagnant mobile phase, the diffusion distances are decreased by the use of smaller and smaller diameter packings. In doing this, there is the tradeoff between gains in efficiency resulting from the use of smaller and smaller particles and decreasing the interstitial flow paths too much once these materials are tightly packed into beds. This latter effect results in the need for very high inlet pressures to force the eluent to flow through the column. For conventional 150–250-mm columns, 5-µm particles typically are used because they provide the smallest diameter and still allow acceptable fluid pressures to maintain sufficient flow rates. For high efficiency rapid analysis and columns that are typically 3–7 mm in length, 2–3-µm particles are used. The important underlying principle in producing high efficiency packed columns is to minimize diffusion paths within the particle while maintaining adequate flow channels that allow the mobile phase to pass through the column.

In contrast to the above, monolithic construction employs a different approach in that the interactive sites are located at the surface of flow through the pores. The result is to reduce the importance of mass transfer by minimizing the stagnant mobile phase component. There have been several strategies in producing chromatographically useful monoliths. In one of these, highly reticulated larger pores are created in relatively thin 1–3-mm disks. Several different types of these materials have been produced and they have been especially useful for carrying out rapid protein purification using a displacement/gradient elution approach. Additionally, monolithic silica phases have been prepared recently, in both capillary tubes and with dimensions similar to conventionally packed HPLC columns (i.e., 50-, 100-, and 250- × 4.6-mm i.d.). The latter types are commercially available products that can be operated at high flow rates because they are sparsely occupied by solid material that has adequate flow through channels to assure relatively low flow resistance (1 and the references therein). Currently, the columns are available as RP-18 materials; however the manufacturer is in the process of introducing other surface types.

Figure 1 shows two rapid analysis reversed-phase separations carried out using a short conventionally packed column operated at high flow rates (chromatogram A) and a monolith column (chromatogram B). The conditions used for the packed column only produce approximately 1200 theoretical plates, and for the monolith column, approximately 5400 theoretical plates. Clearly, in this example, the monolith column is superior in chromatographic performance. However, in making this comparison, it should be noted that the packed column is not optimized in terms of particle size to obtain maximum efficiency because it is filled with only a 10-µm irregular shaped packing. In more demanding separations, in which additional plate counts are needed, specially designed rapid analysis columns are available from several manufacturers that are packed with very high efficiency porous spherical materials with particle diameters between 2 and 3 µm. Likewise, nonporous particles have been developed recently as an alternative media for carrying out highly efficient fast separations. Under each of these latter conditions, higher plate counts can be obtained that are equivalent in performance to the monolith separation shown. Similarities in performance between monolith and conventional packed column construction for separating small molecules have been reported by others (2). However, advantages in speed have been noted for the monoliths, especially in terms of separating larger molecules such as proteins.

At this point, monolith disks and columns are an emerging separation technology. As such, like most new technologies their acceptance in terms of routine usage will be determined by tradeoffs between the need for increased performance and their long term ruggedness, reliability, and cost. To date, clearly there are some areas in which significant performance/speed advantages have been demonstrated, and there are other applications in which it is less apparent. Likewise, there are relatively few manufacturers currently producing them, and the number of surface types is limited. However, new materials are being developed and will be commercially available relatively soon.

References

1. D.V. McCalley. Comparison of conventional microparticulate and a monolithic reversed-phase column for high-efficiency fast liquid chromatography of basic compounds. *J. Chromatogr. A* **965:** 51–64 (2002).
2. B. Bidlingmeyer, K.K. Unger, and N. von Doehren. Comparative study on the column performance of microparticulate 5-µm C18-bonded reversed-phase columns in high performance liquid chromatography. *J. Chromatogr. A* **832:** 11–16 (1999).