Liquid Chromatography Problem Solving and Troubleshooting

Question

Could you provide information about how reversed-phase columns are made and why I sometimes get very different results in performance from the test chromatograms that I receive with the columns? I am especially concerned with peak-tailing problems.

Answer

In answering your question it is helpful to point out a few details about the column manufacturing process and testing and how these can lead to actual and perceived differences in performance of the product by the user. In terms of column testing, there are two different opinions about the most useful solutes for evaluating a column's performance. On the one hand, it has been argued that well-behaved solutes (such as toluene) provide a truer measure of how the column is packed in terms of geometrical considerations (i.e., the maximum number of theoretical plates possible); however, others believe that solutes should be used that provide a better measure of the column's performance under less than ideal conditions. Irrespective of one's opinion on this matter, if well-behaved compounds are used to generate the test chromatogram, then significant differences in performance for more troublesome polar solutes are often observed. With this in mind, it is now useful to discuss briefly how columns are made.

Most of the commercially available reversed-phase materials are produced by chemically derivatizing some form of porous silica. Generally, the more commonly used alkyl phases (such as the octyl and octadecyl packings) are synthesized using either a mono- or trichlorosilane. From a chemical standpoint, monoreactive reagents are the easiest to control because trace levels of water in the reaction solvent and on the silica surface do not cause problems during bonding. Because of this, many manufacturers have switched to this route even though the resulting phases may be less stable when compared with those produced using trireactive reagents. However, they can be made with a greater degree of reproducibility as long as the base silica remains the same and the bonding reactions are driven to completion.

Column manufacturers fit into three broad categories: (*a*) those that buy the bonded phase materials from an outside source and only pack the columns; (*b*) those that buy the base silica from another source and carry out the bonding chemistry and packing in-house; and (*c*) those that produce the complete product from start to finish. Unfortunately, even if two manufacturers produce the same type of bonded phase using monoreactive chemistry, their columns can still differ dramatically. This is because of the complex nature of the underlying silica, which is an amorphous material that is structurally heterogeneous and contains several types of polar silanol groups on its surface (1,2). Structural heterogeneity refers to the nonuniform nature of the particle both in terms of its overall shape and size as well as its pore network. The particle shape and size and their distribution influence how well the particles can be packed into uniform beds. Small uniform materials (i.e., 3–5-µm spherical particles) are best because they lead to columns with good eluent flow and solute mass-transfer properties that can be operated within acceptable pressure ranges.

Over the years, the manufacturers of chromatographic-grade silica have become very skilled in making small uniform particles that are nearly equivalent based on an examination of their macroscopic properties, such as size, shape, and surface area. However, when alternate synthetic routes are employed, the microscopic heterogeneity of the resulting silicas can vary significantly (3). To the practicing chromatographer, differences in microscopic heterogeneity show up as manufacturer-to-manufacturer and batch-to-batch variability in column performance for a given type of stationary phase. This is coupled with the fact that even when monoreactive chemistry is used and the surface reactions are driven to completion, many silanol groups remain on the surface following the bonding process. These residual silanols are

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especially problematic for solutes that have polar functional groups that can interact strongly with them, such as amines and heterocyclic compounds.

In order to minimize the residual silanol problem, one of four approaches is generally used: postreaction end-capping, preparation of sterically blocking phases, electronic manipulation of the attached surface groups, and the use of mobile phase additives. The first three of these are processes employed during manufacturing. End-capping is the oldest approach and involves a resilanization of the column with a small reactive silane (such as trimethylchlorosilane) following the initial bonding process. The second approach utilizes surface ligands that can sterically block silanol accessability, such as the preparation of an octyl bonded phase using octyldiisopropylchlorosilane. The third approach involves imbedding a functional group within the bonded chain that can interact with neighboring residual silanols (e.g., alkylamide phases) and therefore serve as an internal silanol masking agent. Each of these three approaches has allowed manufacturers, as well as researchers, to prepare columns with enhanced chromatographic performance.

Clearly, the performance of commercially available reversed-phase columns has been improved using one of the mentioned approaches. In spite of this, not all peak-tailing problems have been eliminated completely, and as columns age the problem of exposed silanol groups increases even for high-performance bonded phases. Many of these unwanted effects can be minimized by secondary mobile phase additives. Alkylamines are the most commonly used compounds to mask silanol activity and thus to improve peak symmetry; however, in a few cases other compounds have been effective (4). Because of the importance of this topic, it will be covered in a future troubleshooting article in this series.

Reference

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