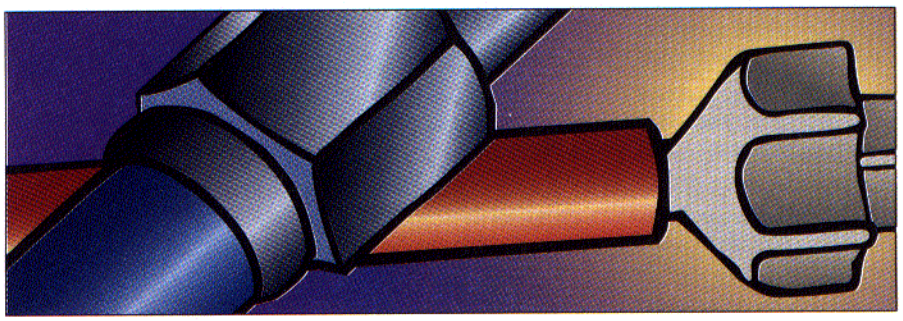
LC Troubleshooting



Column Packing — What's at the Bottom of It?

John W. Dolan

Silica packing particles are more than a place to fasten the stationary phase.

This month's "LC Troubleshooting" column reviews the development of reversed-phase packing materials. Understanding the reasons behind the development of the latest column packings helps analysts select the best stationary phase for a given application.

NORMAL PHASE

In the early days of modern liquid chromatography (LC), analysts performed most separations on columns packed with silica particles and a nonpolar mobile phase such as hexane modified with methylene chloride. Because this procedure was the normal way to operate the system, this type of separation earned the name *normal phase*. With normal-phase separations using silica as the stationary phase, the surface chemistry of the silica is extremely important for obtaining a desired separation, so control of the surface chemistry is essential.

Normal-phase separations on bare silica, however, are not without their problems. One particular problem relates to controlling the amount of water in the mobile phase. With hexane, for example, the solubility of water is nearly 0.2% under LC conditions. Changes in the water content of the mobile phase causes changes in peak shape and retention. In sensitive cases, a change in the humidity of the laboratory can change the chromatography. Another general problem with normal phase is the difficulty of applying this technique to aqueous samples. Most samples of environmental or biological origin are water soluble and contain considerable water in their natural state. Dissolving these samples in normal-phase solvents often involves cumbersome extraction and drying steps that could be eliminated by using an aqueous mobile phase.

LIQUID PHASES

One attempt to overcome the shortcomings of normal phase involved the use of liquid–liquid chromatography, in which the column was coated with an oily substance such as oxydipropionitrile. By changing from a nonpolar mobile phase such as hexane to a polar mobile phase such as methanol–water, analysts could add oxydipropionitrile to the mobile phase and obtain a fairly constant coating of oxydipropionitrile on the silica surface. Thus the first widespread use of reversed-phase chromatography was born. Why *reversed phase*? Well, LC normally was performed with a polar stationary phase and a nonpolar mobile phase, so what better name for the reverse process using a nonpolar stationary phase and a polar mobile phase? Readers familiar with ion pairing will see the shortcomings of liquid–liquid reversed-phase separations. Because the stationary phase loading relied on an equilibrium between the mobile phase and the stationary phase, the temperature, additive concentration, and mobile-phase composition all were important in controlling selectivity. Also, the presence of perhaps 30% of the additive in the collected fractions complicated the collection and recovery of sample.

BONDED PHASES

The next big advance in stationary-phase development was the advent of bonded phases. Silanizing reagents commonly were used to deactivate the surfaces of laboratory glassware. The surface of the silica particles also was covered with silanol (Si–OH) groups. The same chemistry could be used to bond an organic layer on the silica surface. Figure 1 shows the resulting reaction. The reacting group can be a halogen (chlorine is most popular, Figures 1a and 1b) or an ether (such as ethoxy, Figure 1c). Because the presence of two or three reactive groups allowed the silane reagent to react with more than one silanol group, di- or trifunctional silanes might appear to be advantageous. However, the second and third functional groups also can react with another silane reagent and create hard-to-control reactions. As a result, the most popular chemistry involves a single chlorine and two methyl groups (Figure 1a), which allows easy control of the reaction. This reaction is called *monofunctional silane chemistry*, and it is the easiest to use. Some manufacturers use di- or trifunctional silanes for special bonded-phase characteristics, but these reactions must be performed with extreme care if analysts want to obtain reproducible products.

On the nonreactive side of the silane reagent is the portion of the molecule important for retention (the R-group in Figure 1). A linear $C_{18}H_{37}$ (octadecyl) chain was one of the earliest functional groups used because it was readily available from other applications. Thus the C18 column was born. Since that time researchers have tried many variations, but the most popular phases are C18, C8, cyano, and phenyl.

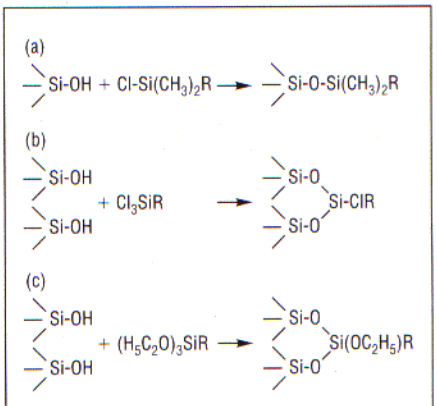


FIGURE 1: Formulas showing the reaction of surface silanols with (a) chlorodimethylsilane, (b) trifunctional silane, and (c) trifunctional alkoxysilane. (Reprinted with permission from reference 1.)

PARTIAL COVERAGE

It would be nice if the story ended here. Chromatographers would have a uniformly covered, stable, nonpolar surface. However, it isn't quite that simple. Because of steric crowding, only approximately half of the silanol groups on the silica surface react during the bonding process. Although there isn't room for another large C18 group to squeeze between the existing C18 chains, small sample molecules can fit in and interact during transit through the column. These interactions add complexity to the separation process and, in many cases, are responsible for peak tailing.

ENDCAPPING

The next chapter of the story is the development of endcapping. If no room existed to add another C18 group, perhaps a smaller silane could be used. Endcapping is the process of reacting a trimethylchlorosilane with a previously bonded surface to further bond the unreacted silanol groups. Endcapping does provide further reaction and helps to improve peak shape for some compounds. Even after endcapping, however, nearly half of the original silanol groups remain unreacted.

It is worth noting that the silica-based stationary phases are pH sensitive. At pH values lower than pH 2 the silylether bond attaching the bonded phase to the silica packing is unstable. At pH values higher than 8 the bonded phase is stable, but the silica dissolves from under it. So for the most part, manufacturers recommend that silica-based bonded-phase columns be used within a pH 2–8 range. Furthermore, evidence shows that shorter-chain bonded phases are less stable at low pH than longer ones.

You might think that endcapped columns would work better in all situations because of the reduced availability of free silanol groups, but the instability of the endcapping at low pH is a problem. It is common for users of endcapped columns with low-pH mobile phases to observe changes in column chemistry during the first 1000 column volumes of mobile-phase use. One common complaint is that a new column has significantly different selectivity than the one it replaced, but by running mobile phase through the column over the weekend, the desired selectivity is obtained. My first question is to find out if the column is endcapped and if an analyst is using a low-pH mobile phase. The answer inevitably is "yes." In these situations users are washing the endcapping off the column during the stabilization period. It would work better to start with a nonendcapped column. On the other hand, endcapping helps to stabilize the column, lowering the susceptibility of the silica to dissolve at high pH. For these reasons, I don't recommend using endcapped columns at low pH (lower than pH 4), whereas endcapping can be advantageous at higher pH (higher than pH 5).

POLYMER-BASED PACKINGS

Peak tailing results from more than one retention process occurring for a given solute. This situation commonly occurs for basic solutes that interact strongly with acidic silanol groups on the silica surface and also are retained by reversed-phase interactions with the bonded phase. If the column packing could be modified so that only one type of interaction was present, tailing from secondary retention processes should disappear.

One attempt to eliminate unwanted silanol interactions resulted in the development of nonsilica stationary phases. These column packings use polymeric particles instead of silica, and many of the same types of bonded phases are available. When these phases first were developed chromatographers were optimistic that they were the perfect bonded phase. Indeed, the silanol tailing and pH stability problems of silica columns are absent from the polymeric columns. Unfortunately, analysts discovered that, in general, these polymer columns could not provide separations as good as their silica cousins. Even when the desired selectivity is obtained, in my experience, plate numbers for polymer columns are much lower than the equivalent silica columns. Polymer-based columns are used widely today for applications outside the pH limits of silica columns, but they account for only a small portion of the total LC column business.

THE SILICA SURFACE

At this point of column development, a bit of a catch-22 resulted in terms of silanols. The residual silanols caused undesirable peak tailing for some compounds, but the elimination of silanols by the use of polymeric supports resulted in a loss of chromatographic performance. A closer examination of the chemistry of the surface silanols yielded some interesting observations. Whereas analysts commonly assumed that the surface was evenly covered with silanol groups, the silica surface was, in fact, heterogeneous. Figure 2 illustrates some of the silica characteristics.

Trace levels of metals present in the silica are a source of unwanted interactions, and aluminum is of particular concern. Some compounds can interact directly with the metals to produce undesirable tailing (Figure 2d). Metal ions also can withdraw electrons from adjacent silanol groups (Figure 2e), increasing their acidity and, thus, their unwanted interactions with basic solutes. In addition to metals, at least three types of silanol groups exist on the silica surface. Free silanols (Figure 2a) were assumed to be present, but geminal silanols (Figure 2b) were present as well. When silanol groups are properly positioned, they can hydrogen bond with each other to form an associated complex (Figure 2c). The geminal and associated silanols are less acidic than free or activated silanols and contribute much less to tailing. Older types of silica show a broad mixture of the groups illustrated in Figure 2, both in absolute numbers and distribution on the surface. These silicas are sometimes referred to as acidic or *type A* packings, as opposed to the newer *type B* packings discussed below.

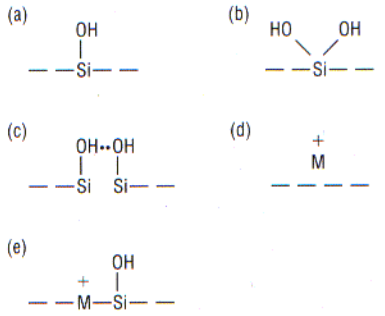


FIGURE 2: Schematics of various silica particle surfaces showing (a) a free silanol, (b) geminal silanols, (c) associated silanols, (d) metal exposed on the surface, and (e) activation of a silanol by an adjacent metal. (Reprinted with permission from reference 1.)

BETTER SILICA

The next big advance in packing development was the engineering of the silica particles so that the good silanols (geminal and associated) predominated and the more acidic bad silanols (free and metal-activated) were greatly reduced or eliminated. Many proprietary procedures for synthesis of silica particles and chemical treatments to generate desirable surfaces have resulted in a new generation of silica sometimes referred to as basic or type B silica. These less acidic packings are available from many column suppliers and often are named to reflect these properties (for example, Hypersil BDS column [Hypersil, Needham Heights, Massachusetts] for base-deactivated silica).

Column packings based on the type B silicas tend to cause less tailing, particularly for basic solutes. Figure 3 shows an example of the difference that might occur in the separation of four basic drugs. The column based on type A silica (Figure 3a) provides longer retention times and more tailing for the basic solutes.

BLOCKING THE SURFACE

Even with the type B silicas, nearly half the silica surface remains unbonded, and unwanted silanol interactions, although reduced in importance, still are present. The latest advance in stationary-phase chemistry has been the development of bonded phases designed to shield or interact with the residual silanols so they are less available for solute interactions. One process is steric protection, in which the two methyl groups of the chlorodimethylsilane reagent are replaced with a more bulky group such as a diisopropyl group. Figure 4 illustrates this effect by comparing a dimethyl-substituted bonded phase with a sterically protected one. The side groups shield the silica surface so that polar interactions with the unbonded silanols are minimized.

Another approach for suppressing unwanted silanol interactions is to make a bonded phase containing an amide or carba-

mate near the silica surface. It isn't clear exactly how these phases work, but Figure 5 illustrates a few of the possibilities. Whether the bonded phase interacts to block the polar silanol groups or provides a polar site for interaction with solutes, manufacturers have shown reduced tailing for basic solutes using these and similar bonded-phase chemistries.

THE BOTTOM LINE

So what does all this history mean in practical terms? The advent of reversed-phase LC with bonded packings opened up an enormous number of applications for biological, environmental, and water-soluble samples. Bonded phases overcame the shortcomings of liquid–liquid stationary phases and took bonded-phase preparation out of the laboratory and into a controlled production environment for a much more reproducible product. The elimination of silanol interactions through the use of polymeric supports lost more than it gained for many applications. However, the modification of the silica surface through new synthetic pathways and surface treatments resulted in silica particles with greatly diminished peak tailing. Newer bonded-phase chemistries have reduced unwanted silanol interactions further.

From a personal standpoint, I want all the cards stacked in my favor, so I'll have the highest chance of a successful method on the first try. For this reason, my first choice is a type B silica column, often one with additional surface protection. If I still see tailing of basic solutes, then I'll add 25 mM triethylamine as my next step in tailing reduction. Chromatographers should take advantage of the millions of dollars of research that have gone into developing a silica support that will provide the best chromatography possible — why start with less than the best?

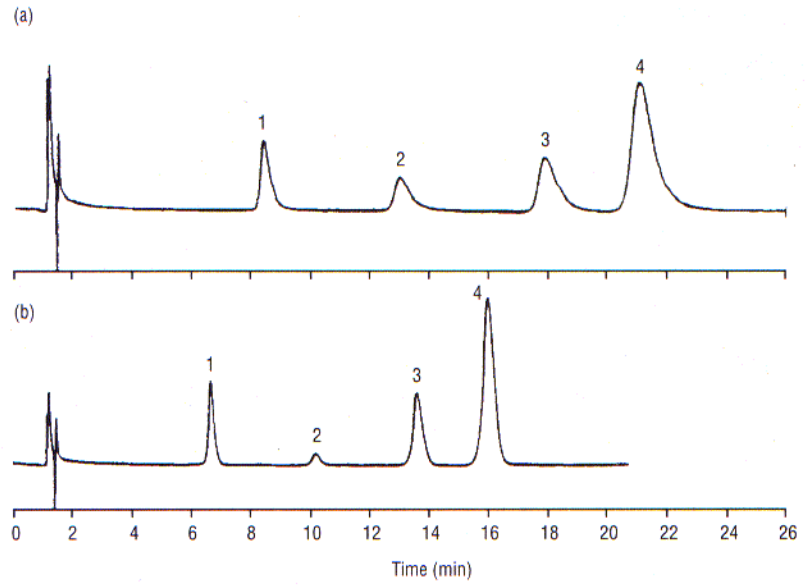


FIGURE 3: Separation of basic drugs using bonded phases attached to (a) type A and (b) type B silica supports. Mobile phase: 30:70:0.2:0.2 acetonitrile–sodium phosphate (pH 2.5)–triethylamine–trifluoroacetic acid. (Reprinted with permission from reference 1.)

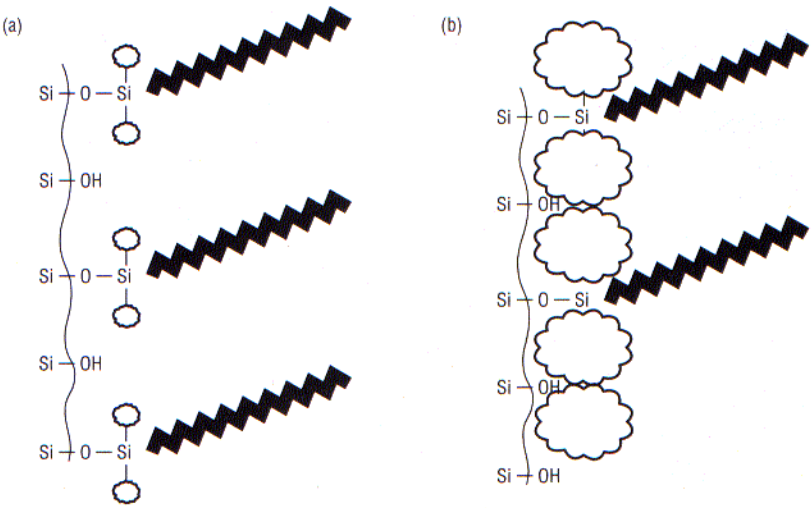


FIGURE 4: Shielding of the silica surface by bulky side groups for bonded phases prepared from (a) chlorodimethyloctadecyl silane and (b) chlorodiisopropyloctadecyl silane reagents. (Reprinted with permission from reference 1.)

ADDITIONAL READING

For more information about the chemistry of bonded phases, consult chapter 5 of reference 1 or reference 2. Column manufacturers also are an excellent source of more information about their columns.

REFERENCES

(1) L.R. Snyder, J.J. Kirkland, and J.L. Glajch, *Practical HPLC Method Development* (John Wiley & Sons, New York, 2nd ed., 1997), pp. 174–232.
(2) U.D. Neue, *HPLC Columns: Theory, Technology, and Practice* (John Wiley & Sons, New York, 1997).

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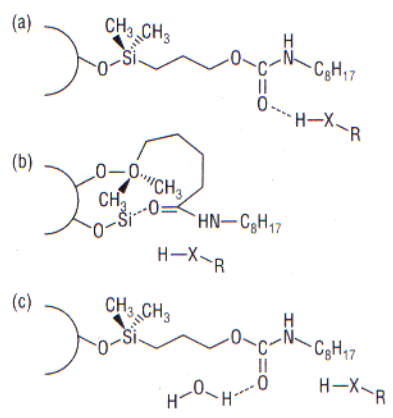

FIGURE 5: Structural diagrams showing possible mechanisms for an octylcarbamate bonded phase (SymmetryShield C8) in which (a) the analyte interacts with a polar group, (b) the analyte competes with a polar group for a silanol, and (c) a polar group increases the water concentration in the surface layer. (Courtesy of Waters Corp. [Milford, Massachusetts].)