

LC Troubleshooting

Problems with Water

John W. Dolan

Water in the mobile phase can be a curse and a blessing.

Water and other polar additives are important components of the mobile phases used for liquid chromatography (LC); however, these additives are not problem-free. In the first of two reader-submitted problems, we see that too much water can cause poor chromatographic performance for reversed-phase separations. In contrast, the second case concerns problems encountered with an insufficient amount of water in a normal-phase separation. As a recent spoof about the hazards of dihydrogen monoxide illustrated (1), we find that the universal solvent can be the chromatographer's bane or boon, depending on the situation.

TOO MUCH WATER

Question: I just heard that reversed-phase columns collapse with less than 10% organic in the mobile phase. Is that true? If so, are there any ways around it?

John W. Dolan: Although I touched on this topic last month (2), this rumor should not be propagated, so let's take another look at the

topic. You should refer to Mary Wirth's excellent "Column Watch" discussion of this topic for further information (3). The most important thing to realize is that we are talking about the collapse of the stationary phase, *not* the column packing itself. The silica particles that make up the column packing are rigid and very stable under normal LC operating conditions. With modern column-manufacturing techniques, it is unlikely that the packing bed will collapse unless it is under extreme pressure (for example, more than 10,000 psi) or extreme pH conditions (lower than 2.5). On the other hand, the stationary phase is very flexible and can change configurations easily, depending on the local environment.

To offer a simplistic model, I like to think of the stationary phase as the pile of a deep-pile carpet. The bonded phase consists of a C₁₈ hydrocarbon chain (or other functional group) bonded to the silica surface at one end, just as the carpet pile is fastened to the backing at one end. The carpet pile has two extreme configurations: one is when the carpet is matted down; the other is when the pile stands up in an orderly manner with all fibers more or

less parallel to each other and perpendicular to the floor (after vacuuming).

The stationary phase has similar extended and collapsed configurations that vary with the mobile phase. With a high water content, the hydrophobic stationary phase collapses (to escape from the water); however, with a high organic content, the stationary phase extends, allowing free access of the mobile phase between the C₁₈ chains. At some point between these extremes, a transition occurs and results in intermediate configurations. Wirth cites evidence that for stationary phases with 80% or more acetonitrile in water, the silica surface is accessible to water in the mobile phase, suggesting that the stationary phase becomes extended with this mobile phase.

I've heard various reports of specific mobile-phase organic concentrations that resulted in significant phase collapse, but I won't quote any numbers for fear of a flood of mail contradicting my statements. From a practical point of view, a fairly linear relation exists between log retention factor (or retention time) and the organic content of the mobile phase within the 20–80% organic region.

Beyond this central region, retention changes may deviate from linearity. Of particular concern are mobile phases with less than 10% organic, in which the stationary phase is known to collapse. In this state, the stationary phase can trap mobile phase. The mobile-phase environment within the collapsed stationary phase can be quite different from the bulk mobile phase in the column, causing retention anomalies. Again, practical experience tells us that columns equilibrate slowly in high-water-content mobile phases, and, consequently, retention reproducibility suffers. For these reasons, I recommend that reversed-phase columns never be used with a mobile phase with an organic content of less than 5% unless you have specific evidence that weaker mobile phases are necessary. This guideline includes washing the column with straight water or buffer, as I mentioned in last month's column (2).

So let me summarize — just to make sure that your rumor does not become folklore. First, the physical stability of silica-based columns is very good unless you use conditions of extreme pressure (that crush the particles) or aggressive mobile phases (that dissolve the particles). Column collapse is rare with today's well-manufactured columns. Second, the stationary-phase configuration is very dynamic. The stationary phase can exist in an extended or collapsed condition and myriad intermediate orientations. The stationary phase is known to collapse in 100% water and become extended in 100% organic. With mobile phases of less than 5–10% organic, equilibration will slow, retention reproducibility will suffer, and your overall results will be less than satisfactory.

INSUFFICIENT WATER

Q: I have a normal-phase method that uses a silica column and a methylene chloride–hexane mobile phase. During a series of runs,

TABLE I: Solubility of Water in Selected Solvents^a

Solvent	Water Solubility (%) ^b
Isooctane	0.011
<i>n</i> -Hexane	0.010
<i>n</i> -Heptane	0.010
Ethyl ether	1.3
Isopropyl ether	0.62
Benzene	0.058
Methylene chloride	0.17
Ethyl acetate	9.8
Chloroform	0.072

^a From Table 6.1 of reference 4.^b Percentage weight of water dissolving in given solvent at 20 °C.

the peaks gradually drift to shorter and shorter retention times. If I make a fresh batch of mobile phase, the peaks come out later and gradually move to shorter retention times. What is happening here, and how can I fix the problem?

JWD: The source of this problem — somewhat related to the previous question — is water. With bare silica columns, retention is strongly dependent upon the amount of water in the mobile phase. Users commonly add water to deactivate the silica and stabilize retention. Although we normally don't think of water as being compatible with normal-phase solvents, water does have a low level of solubility in these solvents. Table I lists the solubility of water in some common solvents.

The sensitivity of silica-based retention to water in the mobile phase is such that retention can change as water is absorbed from the atmosphere. I once witnessed an experiment that showed changes in retention when the humidity of the laboratory changed. Your observations are consistent with absorption of atmospheric water. When you make up fresh mobile phase with dry solvents, the system resets itself, and water gradually is reabsorbed.

For detailed information about the preparation of mobile phases for bare silica columns, consult reference 4, from which I have drawn some of the following discussion. When the mobile phase contains approximately one-half (or more) of its saturation capacity of water, retention times are much less sensitive to the water content of the mobile phase.

For this reason, many workers prepare their normal-phase solvents by using mobile phase that is half-saturated with water. First, they blend the mobile phase (for example, methylene chloride–hexane) to the desired level. Next, they split the mobile phase into two aliquots, adding a little water to one aliquot, mixing it well, and allowing the phases to separate. This water-saturated portion then is mixed with an equal volume of dry mobile phase to obtain a half-saturated mobile phase. Typically, the water content of the mobile phase will be in the 0.005–0.01% region (see

TABLE II: Suggested Acetonitrile Concentrations for Half-Saturated Mobile Phases^a

Solvent	Acetonitrile (% Weight)
Hexane, heptane, isoctane	0.02
Benzene	0.12
Methylene chloride	0.34
Chloroform	0.14
Isopropyl ether	1.2

^a From reference 4, p. 382.

Table I). It is important to mix the mobile-phase components before adding water because the saturation capacity of the solvent mixtures is not a linear function of the percent-volume composition. Fully saturated mobile phases should not be used because small changes in temperature and other variables could cause the water to separate from the mobile phase and cause all sorts of problems.

Controlling the water content of nonaqueous mobile phases is tedious, and silica is overly sensitive to water, so analysts have developed alternative strategies. Today, adding a polar modifier such as methanol or acetonitrile is more popular than adding water to the mobile phase. When using these polar additives, you should add approximately four times as much as you would if you were using water, as shown in Table II. For mobile phases in which acetonitrile and methanol are readily soluble, a 0.05–0.2% addition of these additives is an appropriate level. I suspect that an addition of 0.02–0.03% acetonitrile to your present mobile phase will improve the performance of your method.

Using polar modifiers in normal-phase solvents requires some additional considerations. Equilibrating these solvent systems can be very slow. Figure 1 shows that several hundred column volumes of mobile phase may be necessary to equilibrate water-saturated mobile phases (5). The reason is that the stationary phase can adsorb 10% or more water by weight, whereas the mobile phase only can contain less than 1% water. When using acetonitrile as the additive, equilibration volumes can be reduced by an order of magnitude and further enhanced by injecting pure acetonitrile (for example, 30 μ L) several times during equilibration.

If you obtain the desired separation, you will find that normal-bonded-phase columns are much easier to use. These columns are much less sensitive to the water content of the mobile phase. (Consult reference 6 for tips about developing methods with normal-bonded-phase columns).

CONCLUSIONS

Many times we think of water as merely a component to make up the balance of an LC mobile phase. We have seen, however, that water is a very important part of many LC sep-

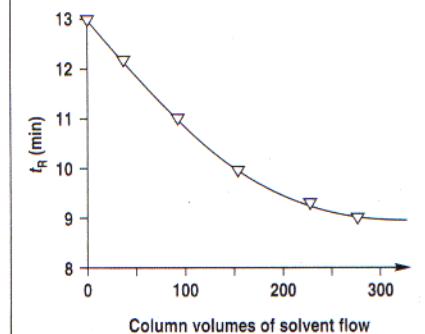


FIGURE 1: Equilibration of adsorbent column with water. Column packing: alumina; mobile phase: 35% methylene chloride–pentane; sample: quinoline; initial water saturation of solvent is 50%, then increased to 75%. (Reprinted with permission from *J. Chromatogr. Sci.*, Preston Publications, a division of Preston Industries, Inc.)

arations. With reversed-phase methods, water is vital to control the overall mobile-phase strength. It also has a strong influence on the apparent structure of the stationary phase. In normal-phase separations performed on bare-silica columns, analysts can use trace levels of water to control the activity of the silica. You must take care in controlling the water content if you want to obtain reproducible normal-phase methods.

Remember, although we need water to live, we can drown in it — and water in LC mobile phases presents the same dilemma.

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