Phase Collapse in Reversed-Phase LC

Matthew Przybyciel, ES Industries, West Berlin, New Jersey, USA,
and Ronald E. Majors, Agilent Technologies, Wilmington, Delaware, USA.

This month's "Column Watch" examines the topic of phase collapse in reversed-phase liquid chromatography. Phase collapse is best described as a dewetting phenomenon. It can cause a total loss of retention and chromatographic problems such as peak tailing, non-reproducible retention times and gradient regeneration delays. The authors offer suggestions for avoiding phase collapses and regenerating collapsed phases.

Reversed-phase liquid chromatography (LC) is the most popular mode of high performance liquid chromatography (HPLC).[1] Its continued popularity no doubt exists because of its versatility, its familiarity to most liquid chromatographers, the number and variety of commercial phases available, and the large number of published applications. Reversed-phase chromatography can be used to separate non-polar, polar and ionic compounds — sometimes in the same separation. The technique can also achieve a larger range of separations than all other modes combined because it enables users to manipulate the mobile phase by changing organic solvent type, solvent composition and pH; by adding modifiers such as surfactants, chiral reagents, competing bases and ion-pair reagents; or by adjusting experimental conditions such as flow-rate and temperature.

Reversed-phase chromatography is generally performed using octadecyldimethyl-silane (C18) or octyldimethylsilane (C8) stationary phases bonded to high-purity, spherical silica gel. Short-chain alkyl phases such as C2 and C4 and phenyl phases are used occasionally, but long-chain phases such as C30 are rarely used and only for specialized applications. Polymeric materials such as poly(styrene –divinylbenzene) (PS–DVB) find some use as reversed-phase media (e.g., with high-pH mobile phases) but generally provide lower column efficiencies than those of silica gel–based packings. Water, usually buffered, mixed with a water-miscible organic solvent (modifier), most commonly acetonitrile or methanol, is the preferred mobile phase. To retain most non-polar and semipolar organic analytes, chromatographers generally begin method development by varying the water–organic solvent ratio in the 20–80% range, often using gradient elution to find the optimum separation conditions more quickly.

Classic Explanation of Phase Collapse with Highly Aqueous Mobile Phases

With this type of approach, chromatographers can achieve a wide variety of separations. However, even the lower end of the 20–80% concentration range could be too strong for adequate retention for very polar analytes such as small organic acids or purine bases. For solubility reasons, many polar compounds separate better with an aqueous mobile phase and can be retained only with a minimal concentration, sometimes less than 5%, of organic modifier. Because the stationary phase in reversed-phase chromatography is usually quite hydrophobic — especially when a densely bonded, long-alkyl-chain phase is used in a highly aqueous and polar mobile phase — these oil-like phases tend to minimize their surface energy through an increased disposition to self-associate. The surfaces can dewet in this process, which occurs within the porous structure of the packing material. When this phenomenon occurs, the polar analytes can have trouble partitioning into or adsorbing onto the stationary-phase surface because of the poor wettability by water. From a chromatographic viewpoint, the stationary phase is believed to have collapsed. Others call this occurrence chain folding or stationary-phase matting. Figure 1 shows a rather simplistic view of this phenomenon. Of course, because most of the bonded phase is inside the pores of the silica gel, this collapse would occur within them rather than on the outer surface, as Figure 1 depicts.

The overall result of a phase collapse is that chromatography becomes more problematic with retention loss, retention irreproducibility, increased tailing, and long gradient regeneration times.[2] Many variables affect the rate and degree of retention loss, including the type of bonded alkyl ligand, the bonding density, and the pore diameter of the silica. Phase collapse is less of an issue when the stationary-phase coverage (bonding density) is low (less than 3 µmol/m^2), presumably because the silanols that remain on the surface allow the surface to be wetted with water molecules and allow the alkyl chains to continue to interact with the hydrophobic portion of the polar analyte. Phase collapse is also rare when very short, alkyl-bonded phases such as C3 are used. Because the available alkyl surface is determined by the high carbon density of compact bonded phase, little free volume exists between bonded chains, and little shielding of the silanols occurs. Although

chromatographers have speculated about what is happening mechanistically, the phenomenon has not been studied systematically.

Wetting Phenomenon for Alkyl Phases

Kazakevich, LoBrutto and co-workers[3,4] have studied the phenomenon from practical and theoretical viewpoints by investigating bonded alkyldimethylsilanes of different chain lengths from C1 to C18 on the same silica. They looked at the molecular volumes of bonded alkyl ligands, the decrease in pore volume with increasing chain length and bonded-phase coverage, and the measurements of the bonding density of different phases in the presence of typical HPLC mobile phases. Based on their observations, they concluded that alkyl phases are always in the most compact conformation (i.e., a collapsed state) regardless of the concentration of organic modifier. They found that the accumulated amount of eluent component in the stationary phase was practically independent of bonded alkyl-chain length. In addition, they observed that when flowing 100% aqueous mobile phase overnight through an alkyl phase column at a low flow-rate, the elution volume of the void marker was lower than a predetermined void volume. They attributed this behaviour to the inability of the 100% aqueous mobile phase to penetrate the silica pores because of water's high surface tension — a phase wetting problem. However, by resolvating the phase (flowing a high concentration of acetonitrile for at least 3 h), the elution volume of the void marker was restored.

This wetting phenomenon is very similar to the one described by Bouvier and colleagues[5] in their development of a novel reversed-phase sorbent for solid-phase extraction (SPE). A water-miscible organic solvent is normally used to wet a silica-based, reversed-phase SPE sorbent in a conditioning step before its use to extract an aqueous sample. If no conditioning occurred, then the hydrophobic sorbent inside the pores would not wet with water. After a sorbent is wetted, water or aqueous buffer can displace the filled pores. A dry sorbent requires very high pressure to cause aqueous solvent to enter the pores, according to the Laplace–Young Equation (Equation 1), which relates the intrusion pressure to the surface tension of the water and to the contact angle of the water and air in the sorbent surface:[6]

$$\Delta P = \frac{4\gamma \cos\theta}{d} \qquad [1]$$

where ΔP is the intrusion pressure required to drive liquid into the pores, γ is the surface tension, d is the effective pore diameter and θ is the contact angle made between water and air on the adsorbent surface. The actual measurement of contact angle is difficult with a non-uniform porous surface such as a bonded silica gel, but it has been approximated by Bouvier and co-workers[5] by considering a water–paraffin–air system.

To chromatographically illustrate the wetting–dewetting phenomenon and phase collapse, Przybyciel and Santangelo[7] used amoxicillin, a polar antibiotic, as a test probe. Figure 2 shows a series of chromatograms obtained using the same classic C8 endcapped column. The column was treated first with a water–acetonitrile mobile phase until they observed a stable baseline. Then, they switched the mobile

Figure 1: Illustration of the classic explanation of phase collapse in reversed-phase chromatography. Shown are the configurations of long-chain bonded alkyl phases (a) in water–methanol mixtures and (b) in 100% water.

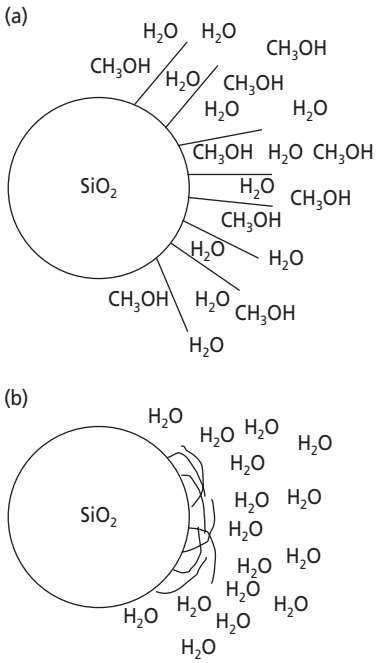
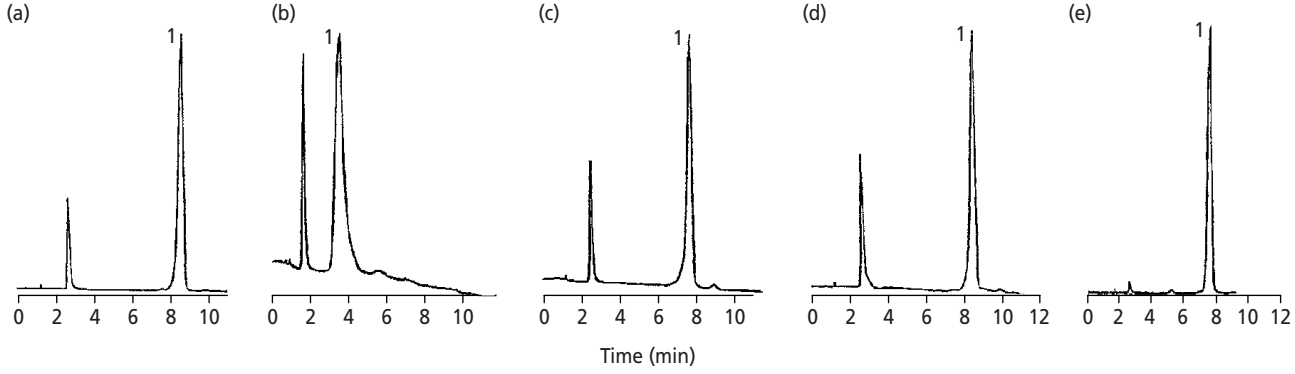


Figure 2: Chromatograms showing phase collapse in reversed-phase chromatography with highly aqueous mobile phase[7]. Mobile phase: 0.1% acetic acid in water; flow-rate: 1.0 mL/min; sample: amoxicillin dissolved in water. Shown are results from injection of an amoxicillin sample (a) onto a reversed-phase column that had been conditioned with a water–acetonitrile mobile phase (40:60 v/v), (b) after the pump was shut off for 10 min and flow was resumed, (c) after the column was pressurized to 270 bar with a restrictor and continuous flow, (d) after the column was conditioned with water–acetonitrile (40:60 volume %) and (e) onto a column with a polar-embedded bonded phase (alkyl phase containing ether linkage).

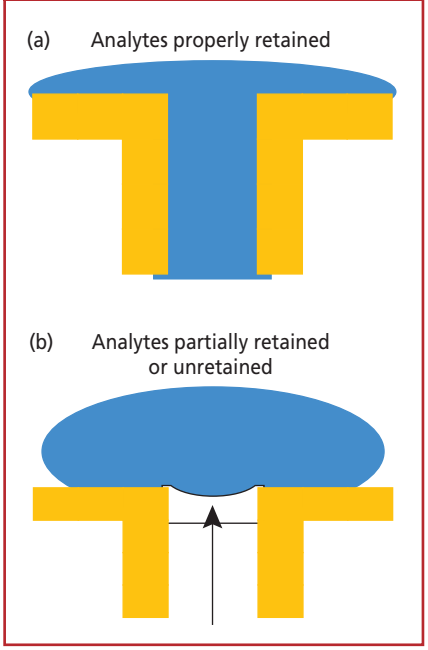


phase to 0.1% acetic acid in water (no organic solvent present). The initial chromatogram [Figure 2(a)] showed an amoxicillin retention time of 8.6 min. Next, they shut off the pump and allowed the system to rest for 10 min. They resumed the flow with the same mobile phase and injected another amoxicillin sample; Figure 2(b) is the resulting chromatogram. Note the retention time of 3.5 min for amoxicillin. The reduced retention time and poor peak shape can be attributed to the exclusion of water–acetonitrile from the pores with resultant dewetting (desolvation) of the bonded phase and subsequent collapse.

Restoring a Column to its Original State

A column can be restored to its original state by repressurization or resolvation. The Laplace–Young equation describes the pressure needed to drive water completely into the pore structure.[8] The contact angle with a 100% aqueous mobile phase and an alkyl-bonded phase in a silica-gel pore is greater than 90°; therefore, the surface is not wetted. Pressure must be applied to a column to cause water to be forced back into the pores.

Figure 3: Illustration of a possible mechanism of pore dewetting for reversed-phase chromatography in a highly aqueous mobile phase[8] in which (a) the alkyl chains are properly solvated with pressure using a 100% aqueous mobile phase and (b) the flow has been stopped to allow expulsion of water from the pores; with flow resumed the pores are still dewetted and analytes cannot enter pores and have little or no retention.



Several approaches in designing stationary phases can help retain polar analytes under highly aqueous conditions.

To restore the column retention for amoxicillin, Przybyciel and Santangelo[7] performed an experiment in which they pressurized a column to 270 bar and used a restrictor and the same 0.1% acetic acid in water as the mobile phase. The column was allowed to pressurize under flow conditions for 10 min, after which time the restrictor was removed but the original column flow was maintained. The amoxicillin sample was reinjected into the column. The chromatogram of amoxicillin [Figure 2(c)] now shows an increased retention time (7.8 min) for amoxicillin. They believed that pressurizing the column was sufficient to drive water into the silica pores and enable the interaction with the alkyl chain. The amoxicillin was not restored completely to its original retention time because the pressure needed to be greater.

They shut off the pump again and released the pressure. As expected, the retention time for amoxicillin was reduced again as the aqueous mobile phase was extruded from the pores. After restarting the flow, an aqueous mobile phase no longer entered the pores, and the accessible surface area was diminished greatly. Next, they exposed the column to a 60:40 (v/v) acetonitrile–water mobile phase for 30 min at 1 mL/min. They switched the column back to the 0.1% acetic acid in water mobile phase and injected amoxicillin. Figure 2(d) shows a chromatogram from this analysis. Note the restored retention time for amoxicillin. The water–organic solvent mobile phase easily wetted the C8 phase and occupied the pores of the silica. These pores contained mobile phase with a high content of acetonitrile, which sufficiently lowered the surface tension of the phase and allowed water to penetrate the silica pores at the column's normal operating pressures. However, if the 0.1% acetic acid mixture were allowed to flow through the restored column for a long period of time, the retention time for amoxicillin would be reduced because the acetonitrile in the pores would be removed slowly, and the water once again would be excluded from the pores. Other researchers have observed similar dewetting phenomena of alkyl-bonded phases.[2,8,9]

To overcome this wetting–dewetting phenomenon, Przybyciel and Santangelo[7] used a stationary phase engineered for use with high-water-concentration mobile phases. They obtained the chromatogram in Figure 2(e) with a 7.5 min retention time and a good peak shape for amoxicillin. Using this type of column, they achieved a stable retention time for amoxicillin under all operating conditions and independent of the column pressure. This type of column is designed to allow water to penetrate the pores at normal operating pressures using all mobile-phase compositions, including 100% water.

Figure 3 is a simple illustration of this proposed dewetting phenomenon.[8,10] If we start with a mobile phase of water and a water-miscible organic solvent such as acetonitrile so that the pore is wetted, bonded hydrocarbon chains are solvated, extended and ready to interact with the polar analyte. Normal retention occurs. With pressure and 100% water flowing through the pores because of the pressure, the bonded phase is in an extended state and can interact with analytes, and the analytes are properly retained inside the pore [Figure 3(a)]. However, if we stop the flow and the pressure that was forcing aqueous mobile phase into the pore ceases, the hydrophobic pore surface can expel the polar mobile phase and dewet the pore [Figure 3(b)]. Analytes can no longer enter the pore and interact with the bonded phase, and, even if the flow is restarted, the pores are still dewetted and the analytes are unretained. If an organic solvent such as methanol or acetonitrile or a water mixture with a substantial amount of organic solvent is pumped through the column, the pores can become rewetted and normal retention can recur.

Approaches for Successful Reversed-Phase Chromatography in Highly Aqueous Mobile Phases

Several approaches in designing stationary phases can help retain polar analytes under highly aqueous conditions, including using

- non-endcapped short-chain alkyl phases
- hydrophilic, polar-endcapped and polar-enhanced stationary phases
- polar-embedded alkyl phases
- long-chain alkyl phases
- wide-pore diameter phases.

In a future "Column Watch" column, we will discuss these approaches in detail and provide several examples of the successful use of reversed-phase columns in highly aqueous mobile phases — even 100% water — with excellent peak shape, good chromatographic retention and rapid gradient reequilibration.

Conclusions

In this month's "Column Watch," we described and demonstrated the phenomenon of phase collapse when using alkyl bonded phases (such as C8 or C18) in reversed-phase LC with water or mobile phases that have a low percentage of organic solvent. Because of surface tension, organic solvent is expelled from the pore unless the pressure is high enough to keep the phase in the pore solvated or the porous structure has a sufficient concentration of organic modifier to keep the phase solvated. Phase collapse is best described as a dewetting phenomenon. In its extreme, phase collapse (dewetting) can cause a total loss of retention. In normal practice, it can occur with organic solvent concentrations of less than 5% in the aqueous mobile phase and retention can be reduced gradually. Where and when it occurs depends upon the nature of the bonded phase, the density of the bonded phase and the pore diameter of the packing. Phase dewetting causes chromatographic problems such as retention loss, peak tailing, non-reproducible retention times and gradient regeneration delays. Instrument problems such as unreliable solvent compositions at low percentages of organic solvent in a binary gradient pumping system can also cause non-reproducible retention.

Phase collapse does not damage a column and washing a column with a 50% or higher concentration of the organic solvent can regenerate the column. However, after a period of time using a low percentage of organic solvent, phase collapse can recur. Phase collapse can be avoided by using columns especially designed for operation in highly aqueous environments. Many of these columns can also be used as regular reversed-phase columns throughout the entire range of aqueous-to-organic solvent mobile phase. These columns will be described in detail in the next instalment of "Column Watch."

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Matthew Przybyciel is vice-president and technical director for ES Industries, 701 South Route 73, West Berlin, NJ 08091, e-mail: info@esind.com. He directs research, development and manufacturing of HPLC column products.

"Column Watch" editor **Ronald E. Majors** is business development manager, consumables and accessories business unit, Agilent Technologies, Wilmington, Delaware, and is a member of *LC•GC Europe*'s Editorial Advisory Board. Direct correspondence about this column to "Column Watch," *LC•GC Europe*, Advanstar House, Sealand Road, Chester CH1 4RN, UK, e-mail: dhills@advanstar.com