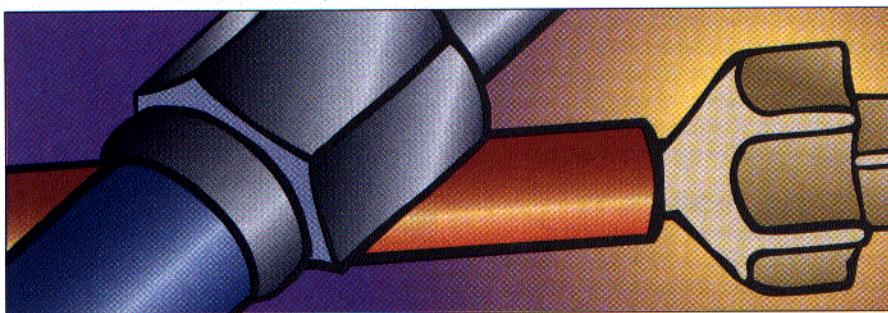


LC Troubleshooting



Mobile-Phase Degassing — Why, When, and How

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Inadequate mobile-phase degassing may be the single largest cause of LC problems.

Air bubbles in a liquid chromatography (LC) system's mobile phase can cause a variety of problems. Pumping reliability decreases when bubbles prevent proper check valve function or fill the piston chamber with air. Spurious peaks are generated when air bubbles pass through LC detectors. Fortunately, although bubbles may be the single largest cause of LC problems, eliminating bubble problems is a simple task when using degassed mobile phases. In this month's "LC Troubleshooting," I'll examine the sources of bubble problems and the techniques that can eliminate excess gas in the mobile phase.

THE SOURCE OF THE PROBLEM

The generation of gas bubbles when solvents are mixed is not unique to LC applications — it can happen whenever two solvents are mixed. Figure 1 demonstrates the source of the problem using dissolved oxygen as an example, but nitrogen is expected to behave in the same way (1). Analysts can generate a similar plot by taking flasks of water and methanol and letting them sit on the laboratory bench overnight so that they are fully equilibrated with the air in the room. If you blend these two solvents, you can expect to obtain a linear mixture curve. Just as the solvents are blended in a linear fashion, the gas contained in the solvents also will be blended in a linear manner (see the broken line in Figure 1).

The problem arises from the fact that the solubility of air in mixtures of water and alcohol is less than it is in the same proportion of the pure solvents. In other words, air-saturated pure solvents will generate supersaturated mixtures, as illustrated for the saturation curve of oxygen shown as the lower trace in Figure 1. As with any supersaturated solution, excess gas will bubble out of solution, a process that often is called *outgassing*. Outgassing can occur anywhere in an LC system, but it is most likely to occur either soon after the solvents are blended or where rough surfaces provide nucleation sites for bubble formation. Thus analysts often see bubbles clinging to the inlet-line frit in the solvent reservoir or in the transfer line between the mixer and the pump in a low-pressure mixing system.

Figure 1 illustrates the cause of the outgassing problem, but it also suggests the solution to it. If enough gas is removed so that the actual level of gas in the mobile phase is less than the saturation level, outgassing should be eliminated. If approximately one-half of the gas was removed, this condition should be satisfied. Therefore, practical mobile-phase degassing does not need to remove all of the gas from solution, only a fraction of it.

NOT ALL SYSTEMS ARE EQUAL

With an understanding of the cause of outgassing problems, chromatographers now can understand why some LC system designs are more prone to bubble problems than others.

For example, low-pressure mixing generally is more problematic in terms of bubble formation than high-pressure mixing.

Low-pressure mixing systems blend the solvents between the solvent reservoirs and the pump. At best, this procedure is done at atmospheric pressure, but if there is any resistance to flow between the reservoirs and the mixer, the mixing actually takes place at less than atmospheric pressure. This condition is similar to that of Figure 1, and outgassing can be expected from air-saturated mobile-phase components. The bubbles then move to the pump and can cause check valve and other pumping problems. The most common symptoms are excessive fluctuations in pressure and complete pump failure.

High-pressure mixing systems, on the other hand, blend the solvents after they go through the pumps. Each pump delivers a solvent from an individual reservoir. Outgassing problems in the pump are not expected because the solvents have not been mixed yet. Mixing takes place after the pumps in the high-pressure portion of the system, and although the mixture may be air-saturated at room pressure, the gas stays in solution at elevated pressures. No bubble problems can be observed until the mobile phase leaves the column and returns to near atmospheric pressure. At this point, air bubble formation will be exhibited as disturbances in the detector signal. A back-pressure

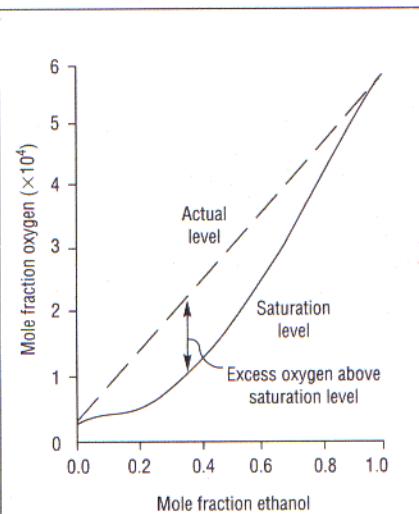


FIGURE 1: Plot of oxygen solubility versus oxygen concentration. The actual level represents the amount of oxygen in the admixtures, starting with air-saturated pure water and air-saturated pure ethanol. (Reprinted from reference 1 with permission.)

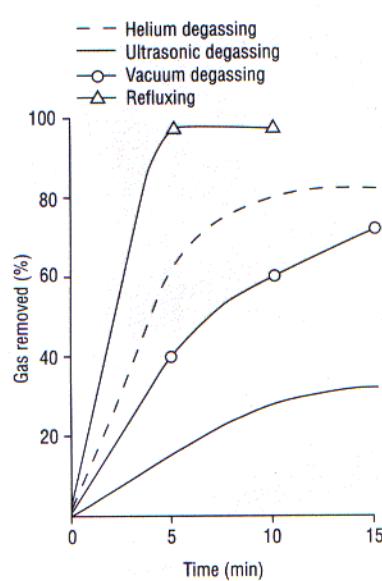


FIGURE 2: Plots showing the results from various techniques for removing oxygen from methanol. (Reprinted from reference 2 with permission.)

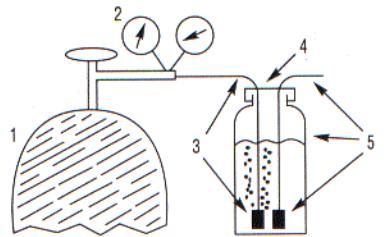


FIGURE 3: Schematic of a laboratory-built helium sparging apparatus. 1 = helium supply cylinder; 2 = two-stage regulator (second stage at 5 psig); 3 = helium line and sparging frit (for example, $\frac{1}{8}$ -in. o.d., $\frac{1}{16}$ -in. i.d. PTFE tubing with a 10- μ m porosity frit); 4 = vent; 5 = reservoir, inlet line to pump, and inlet-line frit. (Reprinted from reference 3 with permission.)

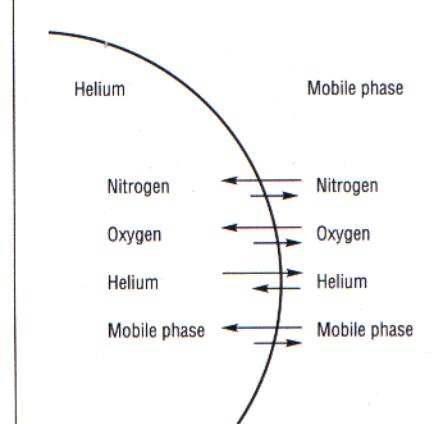


FIGURE 4: Diagram of helium sparging showing major gas equilibria.

regulator on the detector may reduce this problem, but it is unlikely to eliminate it.

Hybrid systems exist in which solvents are pumped into a low-pressure mixer or solvents are blended within the pump head. However, regardless of pump design, bubble problems will occur at one time or another if air-saturated solvents are used. In other words, every LC system will perform more reliably if the mobile phase is degassed.

DEGASSING TECHNIQUES

Figure 2 shows the effectiveness of several degassing techniques for reversed-phase solvent systems. The most effective degassing method — although it is inconvenient and impractical — is to boil the solvent and distill the gas from solution (upper trace, Figure 2) (2). Helium sparging removes approximately 80% of the air from solution, and vacuum degassing removes more than 60% of the gas, so either technique drops the gas content sufficiently for LC applications. The bottom trace of Figure 2 shows that sonication is an ineffective technique for removing gas, although when used in combination with vacuum, many workers find that more gas is removed than by vacuum alone. So for practical purposes, helium sparging and vacuum degassing are the best choices for degassing the mobile phase. Let's look at each technique in a little more detail.

Helium sparging: Workers can construct a serviceable helium sparging apparatus from readily available laboratory supplies, as illustrated in Figure 3 (3). Just connect a suitable

length of polytetrafluoroethylene (PTFE) tubing to the outlet of a regulator mounted on a high-purity helium cylinder. Attach a sparging frit to the end of the tubing. This frit can be an extra pump inlet-line frit of 10- μ m porosity, for example. Submerge the frit in the mobile phase and adjust the regulator to release a gentle stream of bubbles (for example, 100–200 mL/min flow rate). For water–organic solvent mixtures, more than 99% of the air will be removed by sparging with an equal volume of helium (4); that is, 1 L of mobile phase will be fully degassed with 1 L of helium.

When using high-pressure mixing, degassing in the manner described above usually is sufficient for a day's operation. In my laboratory, which uses high-pressure mixing LC systems, we degas mobile phases using a dedicated degassing station and then move the mobile phase to the instrument and proceed with analysis. With low-pressure mixing, bubble problems may occur as air redissolves in the mobile phase, so some form of continuous degassing may be necessary. The simplest way to keep the solvent degassed is to turn down the helium flow rate until only a small stream of bubbles is released and continue operation in this manner.

Excessive degassing with helium can cause a loss of the more volatile mobile-phase component. For example, an acetonitrile–buffer solution could lose a little of the acetonitrile over time. This occurrence is a rare, and most workers will never encounter problems related to selective evaporation if they take care to minimize the sparging rate.

One way to eliminate this problem is using a commercial helium degassing system that incorporates pressurized reservoirs. In these systems, the mobile phase is sparged in the normal manner and then the reservoir vent is closed and pressure builds up in the reservoir.

A pressure-relief valve prevents pressure from building to a dangerous level. As the pressure builds, helium gradually stops entering the reservoir. When the mobile phase is pumped out, a few bubbles of helium enter to replace the solvent. This type of apparatus has several advantages: it reduces helium usage, evaporation of solvent, and venting of solvent vapors into the laboratory. It also applies a small head pressure to the reservoir for more reliable check-valve operation.

You might wonder how bubbling a gas through the mobile phase can remove gas from solution — this statement seems to be a bit contradictory. The process is easy to understand with the aid of Figure 4. The figure shows one bubble of helium surrounded by mobile phase. The mobile phase starts out being saturated with air — mostly nitrogen and oxygen. Because the helium bubble contains no nitrogen or oxygen, the equilibrium across the gas–liquid interface favors transport of the air components into the helium bubble. The bubble rises to the top of the flask and takes its burden of air with it. A fresh helium bubble replaces it and repeats the process. This process is very efficient and requires only 1 L of helium to sparge 1 L of mobile phase, removing more than 99% of the air (4). Meanwhile, the mobile phase becomes saturated with helium, but the solubility characteristics of helium are such that outgassing is not problematic.

During helium sparging, some mobile phase will evaporate. Chromatographers may notice a slight change in retention caused by selective loss of the organic component of the mobile phase if they perform vigorous sparging for several hours. It is best to sparge for a few minutes and then turn the sparge rate down to a trickle or turn off the helium flow.

Vacuum degassing: Vacuum degassing can be performed in one of three ways: incidental

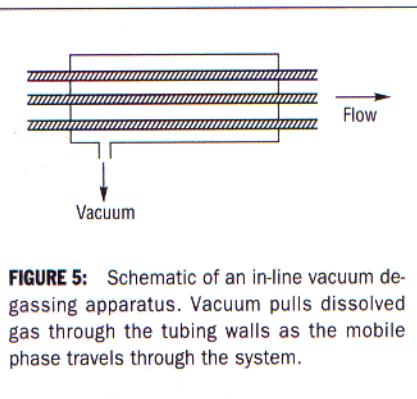


FIGURE 5: Schematic of an in-line vacuum degassing apparatus. Vacuum pulls dissolved gas through the tubing walls as the mobile phase travels through the system.

degassing, batch-wise, and on-line vacuum degassing. Let's look briefly at each technique.

Most workers vacuum filter the mobile phase through a 0.45- or 0.22- μm porosity membrane filter before use. Because the liquid is finely dispersed and exposed to a slight vacuum during this process, some degassing takes place. Although not suitable for all systems, degassing by filtration is sufficient for some systems. One high-pressure mixing LC system in my laboratory required only this form of degassing for reliable operation.

More commonly, vacuum degassing is performed in a batch-wise process by placing the mobile phase in a vacuum flask and then

drawing a vacuum with a water aspirator or simple vacuum pump. Some workers filter the solvent and then replace the filter funnel with a stopper to vacuum degas the mobile phase in the filter flask. A few minutes of this treatment, often aided with a stir bar, removes enough gas for the reliable operation of many LC systems.

On-line vacuum degassing is gaining popularity among chromatographers. Figure 5 is a simplified sketch of this type of apparatus. Thin-walled, porous polymer tubing, such as PTFE tubing, is passed through a vacuum chamber. The porosity of the tubing allows gas to pass through the walls, but the liquid remains in the tubing, much as a Gore-Tex raincoat sheds liquid water, yet passes water vapor. The combination of a sufficiently long piece of thin-walled tubing and the appropriate vacuum makes this technique quite efficient.

Several instrument manufacturers include on-line vacuum degassing as a standard part of their LC systems. Although these systems may remove sufficient gas for reliable LC operation, many workers prefer to briefly sparge the mobile phase with helium and then use the on-line degasser to keep the dissolved gas content low. As a note of caution, each solvent line in the on-line degassers can have 10 mL or more of holdup volume, so it is important to flush these lines thoroughly when changing from one solvent to another.

SUMMARY

Although air bubbles in the mobile phase can be one of the most troublesome LC problems, preventing bubble problems is accomplished easily by thorough degassing of the mobile phase. Vacuum degassing or helium sparging can be accomplished with inexpensive apparatus available in most laboratories. Using an on-line vacuum degasser or pressurized-reservoir helium degasser will require some investment, but these devices produce consistently reliable results, so the cost may be recovered through convenience and reduced downtime.

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