LC Troubleshooting

Air Problems

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Air is one of the biggest sources of headaches in LC system operation.

Some of the most irritating problems in liquid chromatography (LC) are caused by air in the system. Whereas many other problems are related to specific assays, mobile phases, or instruments, air problems are universal. Some LC systems are more tolerant to air than others, but I have yet to see a system immune to problems caused by air bubbles. This month's "LC Troubleshooting" looks at some of these problems and their corresponding solutions.

PUMP STARVATION

When you turn on the pump and no mobile phase flows from the pump outlet, you have a case of pump starvation. Pump starvation is caused by either an insufficient supply of mobile phase or an air lock in the pump. Instinctively, you first check the reservoir(s) to see if a sufficient supply of mobile phase is available. Because it is best not to allow the pump to completely empty the reservoirs, simply replenish the reservoirs before they reach the level of the sinker frit (solvent filter).

Once you have confirmed the presence of sufficient mobile phase, see if the pump will prime itself. Open the purge valve on the outlet side of the pump and increase the flow rate to 5–10 mL/min. Often this flow rate will be sufficient to purge the air from the pump and solvent inlet lines so that the pump will work properly. If self-priming is unsuccessful, disconnect the supply line at the inlet check valve (or at the low-pressure mixer if the system has one) and allow the tube to fill by siphon action. You may need to use a 5-mL syringe or a pipette bulb to start solvent flowing from the reservoir through the line. With a low-pressure mixing system, solvent generally will not flow from the outlet of the mixer unless the pump is running. Once solvent flows freely, reconnect the fitting and try priming the pump again. Some pumps require pressure from a solvent-filled syringe to aid priming. If solvent will not siphon freely from the reservoir, the line is blocked. The most common source of blockage is a blocked sinker frit in the reservoir. Remove this frit to confirm that it is the source of the problem, and, once confirmed, replace it with a new one (I recommend a 10-µm porosity solvent filter).

Another cause of pump starvation is a poorly vented reservoir. If the reservoir is not vented (or purge gas is not added) as solvent is removed, a partial vacuum can form, restricting the flow of the solvent.

A pump's ability to purge trapped air varies from model to model. Some pumps will self-prime from a dry inlet line, others will barely prime when you use every trick you can muster. If a purge prime doesn't work, you can remove the outlet check valve fitting (not the valve), turn up the flow, and sharply tap the pump head with a screwdriver handle to dislodge trapped bubbles. Often a slight head pressure on the solvent reservoir will help, too — just raise the reservoir above the level of the pump or use a helium degassing system that maintains a pressurized reservoir.

If none of the listed techniques primes the pump, try hand-priming it with isopropanol. Use a large (for example, 10-mL) syringe filled with isopropanol and connect it to the inlet check valve. You may need to make an adapter to connect the syringe to the check valve with a short piece of ⅛-in. o.d. plastic tubing. With pressure on the syringe, the pump should prime. If this does not work, remove the pump head to check for a broken piston and replace it if necessary. It is possible, but rare, that a total loss of pumping is due to a defective check valve. Usually bad check valves leak rather than fail completely (see below).

PRESSURE PULSES

More common than a pump that fails completely is one that exhibits large pressure pulses. With reciprocating-piston pumps, some level of pressure pulsing is common but should not exceed 10–20 psi at a 2000-psi operating pressure. When swings of 50–100 psi are present under these conditions, something is wrong. Pulses have one of two causes: either air is trapped in one or more of the pump heads, or one or more of the check valves is working improperly. Sometimes a high-flow purge (see previous section) will force the air out of the pump and restore reliable operation. More likely, however, the problem will return within a few minutes or hours. The best way to keep air from getting trapped in the pump head is to keep it out in the first place, which means degassing the mobile phase and preventing air leaks in the flow path between the mobile-phase reservoir(s) and the pump. Helium sparging is the most effective degassing technique, but vacuum degassing or other techniques may be adequate for your system. When degassed mobile phase is used, air problems in the pump are rare. Be sure that all of the low-pressure fittings are snug so that air cannot leak into the pump supply lines. Undertightened fittings can allow air to leak in; overtightened fittings can become distorted, also creating air leaks.

If purging the pump with degassed mobile phase fails to correct the pressure pulsing, check valves are the likely culprits. Often you can determine which pump head is failing by watching the pressure meter and correlating its response with the pump head that is delivering solvent. Replace the check valves one at a time until the problem is corrected (mark each check valve so you know where it was originally mounted). Purge the pump, then run it for ~15 min to see if the problem has been corrected. Once the problem check valve has been replaced, you can reinstall the other old check valves.

If changing the check valves fails to fix the problem, replace the pump seal(s). Leaky pump seals can also cause pressure pulsing, but not as often as check valves do.

CHROMATOGRAM SPIKES

Air in the LC system also shows up as spikes in the chromatogram. Usually these spikes are sharp, but they can also look like normal peaks or cause the baseline to shift to a new level (including off scale). Most commonly, the source of air is the mobile phase, and degassing the mobile phase will solve the problem. Mobile-phase degassing should be the first attempt at fixing chromatogram spikes and for most applications should be used routinely as a preventive measure. Other sources of air in the LC system are the injector, the fittings, the column, and mobile-phase outgassing.

Injectors: Air can be introduced when the sample is injected. When using manual injection, you can avoid injecting air by purging the air from the syringe with a solvent flush between injections. With autosamplers, be sure the sample vials are full enough and that the needle is in the correct position so that air is not drawn into the sample loop along with the sample. When filled-loop injection is used, it is best to flush the loop with three loop volumes of sample with each use, which not only purges air from the loop but ensures maximum reproducibility by flushing residual mobile phase from the loop. With partially filled loop injection, it is important that the waste line does not siphon the liquid from the loop in the fill position. If the liquid is siphoned from the loop, the next injection can consist of sample plus air. In general, the microliter or so of air that is inadvertently injected does not cause a problem, especially if a back-pressure regulator is used (see below). In fact, in one of my previous jobs we routinely injected an air-segmented sample consisting of perhaps 10 µL of air in a 100-µL sample with no ill effects.

Fittings: Although the usual failure is for liquid to leak out, high-pressure fittings can allow air to leak into the system. I can't explain how this happens, but I've seen it too many times to disbelieve it. The problem is usually corrected by slightly tightening (for example, one-quarter turn or less) each fitting in the system. I've seen several cases in which apparent air leaks were corrected by tightening the ¼-in. column endfittings. As with any leaky fitting, if you see any white deposits on or around the fittings (resulting from evaporated buffer), disassemble the fittings and rinse them before tightening. Tightening a fitting with buffer between the sealing surfaces can result in leakage or seizure.

Columns: Air can become trapped in the column if you inadvertently pump the column dry or leave the end caps off during storage. Often this will cause no column damage, but the trapped air can be difficult to remove. The mobile phase tends to pick up small bubbles, resulting in air spikes as they are swept through the detector cell. If you suspect or know that you have air in the column, try flushing it with helium-sparged mobile phase (50:50 methanol–water would be a good choice). Connect the column inlet to the LC system and leave the outlet free. Flush ~25 column volumes (~60 mL for a 25 cm × 4.6 mm column) of mobile phase through the column,

then connect the outlet to the system and see if the problem is corrected. Higher flow rates will result in higher pressures and may enhance the purging process by shrinking the bubbles in the column, allowing them to be swept out more easily.

Mobile-phase degassing should be the first attempt at fixing chromatogram spikes.

Mobile-phase outgassing: Mobile-phase outgassing can also be a source of air in the LC system, especially when using high-pressure mixing systems. When pumping pure solvents, degassing is often not required. When solvents are mixed, however, air is often released because the mixture has a lower capacity for dissolved air than do the pure solvents. If such outgassing occurs on the high-pressure side of the pump, however, the bubbles stay in solution because of the pressure. When the pressure drops back to near atmospheric pressure at the outlet end of the column, the bubbles may reform, causing spikes as they pass through the detector. Thorough mobile-phase degassing will often prevent this problem from occurring. Using a restrictor after the detector may also help.

BACK-PRESSURE REGULATORS

A back-pressure regulator, or restrictor, mounted on the detector waste line may help prevent many of the problems that result from occasional bubbles. Restrictors keep the pressure high enough to keep gas in solution until after the mobile phase exits the detector cell. Choose the back-pressure limit so that the detector-cell pressure limits are not exceeded. Commercial restrictors are available with defined back-pressure limits. You can make your own restrictor by placing a length of 0.005-in. i.d. tubing after the detector — you'll have to figure out how much tubing is required (for example, 1 m) to provide satisfactory back pressure. For such homemade restrictors, the back pressure is proportional to the flow rate, whereas the commercial devices are spring-loaded and give the same back pressure for any flow rate.

UNAVOIDABLE PROBLEMS

An occasional air spike is unavoidable. In my opinion, it is impossible to keep all air out of the LC system, so it is more reasonable to strive to minimize the problem when you can't eliminate it completely. If you see an extraneous air spike once or twice a day, it probably is not worth trying to correct. Unless the problem is reproducible, occurring at fairly regular intervals, you will have difficulty determining if you have fixed it. If you have tried the techniques listed above and still have an occasional air peak, I recommend just trying to live with it.

REQUEST FOR INPUT

I recently received a call from someone who had an extraneous peak in a gradient system with UV-absorbance detection at 215 nm. He was running a 40–95% B gradient between water (solvent A) and acetonitrile (solvent B) with 0.05% trifluoroacetic acid added to each solvent. Near the end of the gradient, he saw a large peak. This peak was independent of the sample because it appeared in the blank gradient as well. He tried the standard isolation schemes of using fresh solvents and another column to no avail.

A 5–80% gradient with the same solvents is commonly used for separating biomolecules. Generally, 80% B is all that is required to elute the samples. In our laboratory, when we extend this gradient, we often see an ugly peak near the end. We tend to ignore the problem because an endpoint of 80% B is sufficient.

As I hear of more workers encountering this problem, it makes me wonder how widespread it is and whether anyone has figured out a cure. If any of you have experience with this problem, please let me know by writing to me in care of *LC•GC*. Please include a description of your hardware, mobile phase, and column plus a fax or phone number so I can contact you if necessary.

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