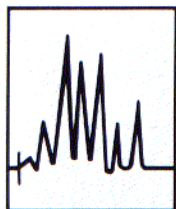


LC TROUBLESHOOTING

Readers' Questions

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A number of readers have written to comment on an error in a recent "LC Troubleshooting" column (1). It was stated that peak area is independent of flow rate. That statement is not true, unless a mass-sensitive detector is used. In fact, peak area decreases with increasing flow rate when a UV detector is used. Under the same conditions, peak height remains relatively constant. This is illustrated in Figure 1 (2).

This topic is worth a brief discussion because it helps us to understand more about the behavior of sample compounds as they pass through an LC system. With a concentration-sensitive detector, such as a UV detector, peak height is proportional to the concentration of sample in the detector cell. If we assume that peak volume is significantly greater than the detector cell volume, the height should remain constant as the flow rate is changed (as shown in Figure 1). Changes in flow rate change the peak *width* (in seconds). That is, higher flow rates result in narrower peaks. When these narrower peaks pass through the detector cell, the peak *area* will be lower (constant height \times smaller width = smaller area).

CARRYOVER IN THE SAMPLE INJECTOR

Q: I am finding unacceptable levels of carryover in my sample injector from one sample to the next. My sample concentrations are all about the same. I am using a manually operated injection valve with filled-loop injection. What could be causing this, and how can I correct it?

JWD: There are two main areas that you should look at to solve this problem. First, make sure that the loop is getting thoroughly flushed during each injection. It is a good practice to let 5–10 loop volumes of mobile phase pass through the loop when it is in the *inject* position. As has been discussed in previous "LC Troubleshooting" columns (3,4), the sample should be washed out of the loop in about three loop volumes. Thus, if you are using a 50- μ L loop and the flow rate is 1 mL/min, 30 s in the *inject* position should be sufficient. If you were injecting a partially filled loop (for example, 20 μ L in a 1-mL loop), increased flushing time would be required because the loop is larger, even though a small injection volume is used. Keep in mind that poor assembly of fittings could result in poorly flushed volumes in the valve. Make sure

that connecting tubing and loops are firmly bottomed in the fitting ports before the nuts are tightened.

Another source of your problem could be inadvertent contamination of the loop after the sample is injected. This could arise from any of several sources. If the waste line from the injector is positioned so that the contents can drain or siphon back into the loop, contamination of the loop with a previous sample can occur. This problem can be avoided by leaving the syringe in the injection port until after the valve is rotated to the *inject* position. (Remove the syringe after injection so that you don't accidentally bend it.) It is best, however, to configure the waste line so that no siphoning can occur and so that the waste line drains by gravity into the waste reservoir. Crimping a plastic waste line will sometimes help.

Residual sample in the injection port or syringe also can contaminate the next sample. It is good practice to rinse the syringe several times with mobile phase or injection solvent between each injection to completely remove the previous sample. If an external fill-port is used (that is, if you do not insert the syringe directly into the valve body), the connecting tubing between the syringe and the injector must be considered when the flushing volume is calculated. As has been discussed (3,4), you should flush the loop with three loop volumes of new sample to fill the loop completely. For flushing purposes, add the connecting tubing volume to the loop volume.

Many workers manually flush the injection valve with clean solvent between each sample. This is most convenient if a separate large syringe is reserved for flushing. For example, use a 500- μ L syringe to flush mobile phase or sample solvent through the valve between each injection. This flushes residual sample from the fill port and waste line, alleviating some of the problems discussed above.

AIR IN THE PUMP

Q: I have a problem with trapped air in the pump head, which causes the pump to lose prime and no longer work properly. I am using a single-pump system with low-pressure proportioning and mixing. Even if I place all three inlet lines in the same reservoir, use pure methanol, and continuously sparge with helium, it isn't long before a bubble gets trapped in the pump head and I have to re-prime the pump. What am I overlooking?

JWD: I suspect that you have either a restriction before the pump or an air leak. A restriction often will cause the solvent to cavitate so

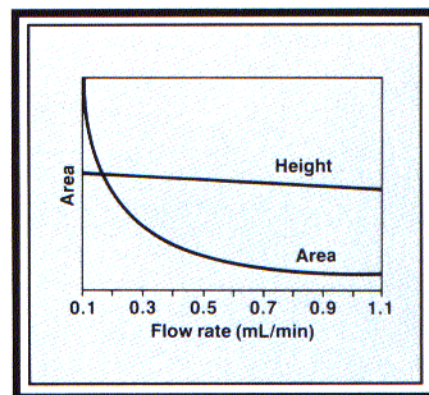


FIGURE 1: The effect of changes in mobile-phase flow rate on peak height and peak area with a UV detector. (Reprinted from reference 2 with permission.)

that air bubbles appear in the lines or the pump. Leave the system configured as you have it, with degassed methanol and all three lines in the same reservoir; make a cursory examination and tighten any obviously loose fittings. Consider the restriction problem first, because it is easier to find. Check to be sure that solvent is flowing freely to the pump. When the fitting on the inlet check valve is loosened, solvent should flow freely from the fitting. You may have to turn on the pump so that the proportioning valves open to allow solvent to flow through the mixing manifold. If solvent flows freely, there is no restriction, and you can skip to the discussion of air leaks. If solvent does not flow, or if it just dribbles out of the tubing, you need to locate the restriction. For troubleshooting purposes, divide the solvent inlet system into three parts: (1) before the proportioning valves; (2) the proportioning valves, manifold, and mixer (if any); and (3) between the manifold and the pump.

Begin looking for the problem at the upstream end. First, loosen the fitting for each solvent line at the inlet to the proportioning manifold. If solvent flows freely from each line, skip ahead; otherwise remove the inlet-line filters from the reservoir end of the lines. If the lines siphon freely, replace the filters with new ones (10- μ m porosity is recommended). If the problem persists, the lines are blocked or crimped. Isolate and clear the blockage or replace the lines.

Next, check the line between the manifold and the pump. Usually, you can loosen the manifold end of this line and place it in a

beaker filled with solvent. If the solvent can be pumped with no problems, the line is OK. Otherwise, locate the blockage and clear it or replace the line.

Once you have confirmed that all the feed lines are clear, you know that the restriction must be in the manifold or mixer. It is most convenient to check this by substituting a good unit for the suspect one. If you can't do this, carefully disassemble the unit and check for particulates in the passages. If there are filter or mixing frits, replace them. If that does not solve the problem, replace the manifold.

Air leaks on the low-pressure side of the pump can be difficult to find and eliminate. Start by *carefully* tightening all the fittings. Plastic fittings can be stripped quite easily, so be careful not to overtighten them. If that doesn't help, disassemble each fitting and inspect it for problems. The flared fittings used by many equipment manufacturers are prone to leakage if they are misformed or have become distorted during use. Remake any suspect fittings (you may want to use another style of low-pressure fittings at this point).

If you are sure that all the fittings are sealed properly, yet the air problem persists, check the gaskets and seals in the system. Once again, substituting a good mixing manifold is easier than checking out a questionable one. One insidious problem is the presence of pinholes in the proportioning valve gaskets. If you replace a single proportioning valve, check the system operator's manual to see if matched proportioning valves are required for adequate performance.

Occasionally, a severely worn pump seal can cause an air leak; if you haven't changed the pump seals recently, do so now. If the onset of the problem corresponded to a pump seal change, it is possible that you installed the seals backwards. Finally, some pumps use a plastic washer-seal where the inlet check valve seats against the pump head. If that seal is cracked, liquid can leak out or air can leak in. Visual inspection should reveal any cracks.

One of the steps discussed above should eliminate your air problem, whether it is air leaking into the system or bubbles created by cavitation.

VALVE-SEAL WEAR

Q: The seal on my sample injector is wearing out very quickly. I hear of people getting 10,000 or more injections before they have to replace the seal, but I rarely get more than 1000 injections. What can I do to extend the lifetime of the seal?

JWD: Valve-seal wear is caused by physical abrasion of the surface, which generally can be minimized by practicing a few preventive maintenance techniques. The seal lifetime is related to the pressure of the seal against the valve surface. As with any abrasive action, the harder the seal is pressed against the valve body, the faster it will wear. Most injection valves can be adjusted to operate at higher or lower pressures by adjusting one or more tensioning screws on the seal. Your valve should

be adjusted so that it will be leak-free to about 1000 psi above the maximum operating pressure of your LC system. For example, if you never run your LC system above 2500 psi, you can increase the seal lifetime by tensioning it to 3500 psi rather than 6000 psi. The instructions that come with the valve should tell you how to adjust the pressure limit (if not, contact the manufacturer for advice).

Dirt is the biggest enemy of injection valves. Any particulate matter that lodges between the seal and the body will grind the seal each time the valve is rotated. A 0.5- μ m porosity in-line filter just ahead of the valve should stop any particulate matter from getting to the valve. If a precolumn (saturator column) is used, however, this filter may not be sufficient to trap all the particulate matter. You should expect shorter valve-seal lifetimes when a precolumn is used. The other source of particulate matter that can contaminate the valve is the sample. Samples showing opalescence or particulates when held to the light should be filtered with a 0.5- μ m membrane filter before injection. As an experiment, you might want to filter all your samples to see if your valve lasts longer.

The final source of abrasive material is the mobile phase buffer. If buffers are allowed to stand in the LC system when it is not in use, abrasive crystals can form — and will quickly destroy a valve seal. Be sure to flush buffers from the system with unbuffered mobile phase when you shut down the system. If a valve leaks and buffers are being used, flush with unbuffered mobile phase before tightening the seal, or buffer can get trapped between the seal and the valve body and cause problems.

Finally, it is possible that your valve body has a rough sealing surface, causing seals to wear out prematurely. Replace the valve with a new one, or have it reconditioned by the factory.

A less-common source of seal problems is chemical damage to the seal. For example, one common seal material, Vespel, is not stable under strongly basic conditions. If you suspect the mobile phase is attacking the seal, contact the valve manufacturer for advice on alternative seal materials.

While it is true that some users get very long lifetimes from their injectors, many do not. It depends on operator technique, system configuration, mobile phase, and samples. If you consider the cost of valve-seal replacement in light of all other sample-related costs (labor, solvents, filters, and so forth), short valve-seal lifetimes may not seem so bad.

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