TROUBLESHOOTING

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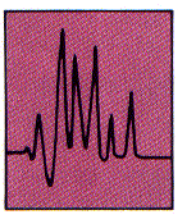

Beginning this month, "Troubleshooting" will change to a question and answer format to address specific problems that are encountered with LC operation. Readers are invited to submit questions or to contribute their troubleshooting tips. Write to: The Editor, *LC Magazine*, P.O. Box 50, Springfield, OR 97477.

FRIT BLOCKAGE AND VALVE MAINTENANCE

Q: I have a recurring problem of frit blockage at the top of the column. I routinely filter my samples, but this does not seem to help. When I remove the column end-fitting, the frit is covered with fine black material and the top of the column packing is gray. Replacing the frit causes the pressure to return to normal, but the pressure rises again in just a few days. Replacing the pump seals doesn't help either.

JWD: If you are filtering the samples properly, the problem must be arising from the system itself. The most likely cause is a deteriorating seal in the sample-injection valve. When the seal is damaged, it often wears abnormally fast, causing the flaking of black particulate material. Carefully dismantle the valve and inspect the sealing surface with a magnifying glass. If the surface is rough, or if you can rub off black particles with a soft tissue, the seal is worn out. The valve should be cleaned and the seal replaced (or return the valve to the manufacturer for rebuilding).

To clean the valve, wash it with a detergent solution in an ultrasonic cleaner and then rinse it with water followed by alcohol. Inspect the metal sealing surface for scratches or other damage. (If it is damaged, replace the valve or return it to the factory for reconditioning.) Reassemble the valve with a new seal according to the manufacturer's instructions.

To prevent subsequent valve failure, it is a good idea to determine the original cause so that it doesn't happen again. If the valve is quite old, wear may be normal. Automated valves may appear to wear more quickly than manually operated valves, but valve life as measured by the total number of samples injected should be the same (an automated valve usually injects more samples per month than does a manually operated valve). Valve damage can be caused by using the wrong type of syringe. Most liquid chromatography valves require square-ended syringes rather than the pointed type used for gas chromatography. A pointed syringe can scratch the valve seal. Drying of buffer salts in the valve also can cause seal scratches. Overtightening of the rotor can accelerate valve wear.

Preventive measures can be taken to minimize valve problems. First, flush all buffers out of the LC system when you are through for the day. Be sure to use a mobile phase containing water, because flushing the system with pure acetonitrile or methanol can sometimes precipitate buffers instead of washing them out. If the valve leaks when a buffered mobile phase is used, change to an aqueous mobile phase, but before tightening the valve, let the nonbuffered mobile phase leak out to wash any buffer residue from the valve. Use the proper type of injection syringe. Sample filtering is a good idea if the sample is cloudy or has particles in it. A guard column or submicron filter inserted between the injector and the column will often prevent damage to a very expensive analytical column when valve failure occurs.

VARYING PEAK SIZE

Q: When I fill a 20-μl loop on my valve with sample, I find that the variations in peak height or peak area are much too large (7% to 9% coefficient of variation). I understand that I should be getting coefficient of variation (C.V.) values of 1% or less. What am I doing wrong?

JWD: I suspect that you are not filling the loop with enough sample. To obtain the desired level of precision, you should overfill the loop by a factor of two to three. In other words, you should be using 40–60 μl of sample to fill a 20-μl loop. This is because of the washout characteristics of the valve. First of all, in some valves there is as much as a microliter of volume between the tip of the injection syringe and the sample loop. In this case, you could inject 20 μl and only get 19 μl into the loop. You will thus be operating with a partially filled loop and can expect lower precision. A second reason for overfilling the loop has to do with the physical characteristics of a flowing stream. When a liquid passes through the loop (or through any tube), there is a thin layer of liquid at the tubing walls that is not washed out as readily as is the liquid in the core of the tubing. It takes extra sample or extra wash solution to wash out this last bit of the loop's previous contents. This is the principal reason for overfilling the loop by two to three times its nominal volume.

If your sample is valuable, and you would like to use as little of it as possible yet retain good precision, there are two approaches you could take. First, determine exactly how much sample is required to give the desired level of precision. To do this, plot the C.V. against the volume of sample pushed into the loop. The C.V. will drop as sample volume is increased, and then will level off. You may find that 80 μl is required for maximum precision. On the other hand, if your assay requires only a 3% C.V., you may find that only 25 μl of sample is adequate. The precision attained will depend upon the particular combination of syringe, fittings, valve, and loop you use.

BUBBLES IN PUMP

Q: I can't seem to keep bubbles out of my pump. I degas the mobile phase with helium and leave a small stream of helium flowing when I am using the system. It doesn't seem to matter what mobile phase I use; I can use the low-pressure mixer on the chromatograph or manually mix the mobile phase. There are bubbles even when I use a pure solvent for the mobile phase. Can you help me?

JWD: Inadequate degassing of the mobile phase is the most common cause of bubble problems; however, your degassing technique should be adequate, so your problem is probably caused either by cavitation or by aspiration of air into the system. Cavitation is another word for a vacuum forming in the system, much like a vapor lock in an automobile. If the problem is cavitation, there is a constriction of mobile-phase flow on the low-pressure side of the pump. The pump tries to draw mobile phase, but it pulls a vacuum instead, causing a bubble to form in the pump or in the solvent inlet line. The most common cause of this problem is a blocked frit in the mobile-phase reservoir. This is easily checked. Just remove the frit from the end of the solvent inlet line and turn on the pump. If the problem goes away, the frit is blocked. Discard it and replace it with a new one. The frit can be cleaned in 50% nitric acid, but it is usually not worth the trouble unless you don't have a spare on hand.

Check for the cause of frit blockage before continuing. The inlet frits should last three to six months unless the chromatograph is in constant use. There are several possible causes of blockage. The mobile phase should be filtered before it is placed in the reservoir unless it contains only HPLC-grade solvents. Small bits of foreign matter in buffers or other reagents will gradually block the inlet frit. Some buffers, particularly acetate, are very good growth media for microorganisms. If you decide that frit blockage is associated with the buffer, replace the buffer daily, filter it before use, and store the stock solution in the refrigerator. Laboratory dust can also accumulate on the filter over time. Loosely cap the mobile-phase reservoirs to keep dust out. A piece of aluminum foil works well for this. If you use a screw cap on the bottle, be sure that it isn't fitted too tightly or a vacuum will form in the reservoir as mobile phase is removed . . . and you will have a cavitation problem again!

Some LC systems have frits in the check valves or in the low-pressure mixer that can become blocked and cause cavitation as well. These areas are seldom a problem if mobile phase is properly filtered and a frit is used on the inlet line.

Another possible, but unlikely, cause of cavitation is pumping at too high a flow rate. Some pumps are capable of pumping at flow rates above 10 ml/min, but the inlet tubing may be too small to accommodate the pump demand, so cavitation results. This is easily checked by lowering the flow rate to see if the problem disappears. To solve cavitation problems related to flow rate, either replumb the system with tubing of larger internal diameter or use a lower flow rate.

If you determine that cavitation is not the cause of the bubble problem, you can assume that air is being aspirated into the system at one of the tubing connections. An air leak can be difficult to locate. Check to be sure that all fittings on the low-pressure side of the pump are tight. Connectors for plastic tubing are usually the source of an air leak. These connecting fittings can gradually work loose under the influence of pump vibration. A lock nut will help minimize this problem. Overtightening of plastic fittings can also cause leakage. This is a common problem with fittings that use flared tubing. When the fitting is overtightened, the tubing is forced back through the hole and the seal is broken. If you consistently have a problem with one brand of plastic fitting, switch to another (most are interchangeable). A loose check valve or pump head can also be the source of an air leak. Some check valves seal against a hard plastic seat in the pump head. If this seat cracks, it can leak. Finally, check the connections of other components that contact the low-pressure stream. Low-pressure mixing systems often use diaphragm valves to meter the solvents. A pinhole, a tear, or a poor fit can cause the diaphragm to leak air into the mobile phase.

Finally, when you have corrected the problem, be sure to get all the air out of the system. Flushing the system with 50–100 ml of degassed methanol will usually remove any residual bubbles. Most LC pumps can accommodate an occasional bubble, but a small bubble that remains in the system will often grow and cause a problem later.

HIGH pH

Q: I need to run an assay with a mobile phase at pH 9. What can I do to maximize the life of my C18 analytical column?

JWD: Silica-based LC columns are not stable at conditions above about pH 7, so protective measures do need to be taken for your assay. First, use a precolumn or silica saturator column between the pump and the injector. Such a column is made by dry-packing the tubing from an old 15-cm or 25-cm analytical column with silica gel. Although LC-grade silica can be used, larger 60–200 μm irregularly shaped silica is less expensive and just as effective. This column packing preconditions the mobile phase and reduces the dissolution rate of the analytical column. Some workers find that placing a submicron filter after the precolumn prevents any fine particles from reaching the injection valve. Before the precolumn is put into service, flush it thoroughly with a strong solvent and then with mobile phase to remove any contaminants. In the beginning, check the precolumn daily to determine when it needs to be replaced, then follow a regular replacement schedule.

You should also use a guard column between the injector and the analytical column. In addition to the normal guard-column functions, this will provide a twofold benefit. First, it will trap any fine particles that might wash out of the precolumn. Second, it will act as backup to the saturator precolumn, thereby providing a little more protection for the analytical column.

ALTERNATIVE DETECTION

Q: I occasionally use my chromatograph for semipreparative separations. I do not have a refractive index detector, so I inject a small amount of compound into the chromatograph and determine its retention time with a UV detector. When I inject a large sample for collection, my detector becomes overloaded, so I rely on retention time for collecting my fractions. I would feel better if I could detect the sample in the run that I collect because I know that the retention time shifts under overload conditions. Is there a simple modification I can make to the UV detector so that the peaks can be detected?

JWD: You can effectively extend the upper range of a UV detector to solve your problem either by changing the detection wavelength or by installing a splitter. If you have a variable-wavelength UV detector, adjusting the wavelength is the easier approach. In the analytical mode, a wavelength near the absorbance maximum for a compound is used for maximum detector sensitivity. In your case, the optimum wavelength will be away from the absorbance maximum. If, for example, your compound has a fairly sharp absorbance peak at 278 nm, you may find that it has only 10% as strong an absorbance at 290 nm. In this case, setting the detection wavelength at 290 nm will give you an extra order of magnitude in detection sensitivity. If the absorbance peak is broad, you may need to move the wavelength 40 nm or 50 nm away from the maximum to achieve similar results. You can find the best wavelength by trial and error or by running a UV absorbance spectrum of the compound on a UV spectrometer. The linearity of the measurement is in question under these conditions; but linearity is usually not a concern in preparative separations. If you have a variable-wavelength detector, try this method first because the detector does not require any hardware changes and is capable of absorbing in different spectral regions by turning the wavelength-selection knob.

A splitter will extend the upper limit of detection for any detector, not just the variable-wavelength UV detector. This is especially useful if you use fluorescence or electrochemical detection, which generally are

aimed at the lower limit of detection. The splitter functions by sending only part of the sample to the detector; the rest is split off before the detector for collection. (You may also want to collect the portion that passes through the detector.) Hence, if 1% of the stream is directed to the detector and 99% is directed to the collection vessel, you should get approximately the same detector signal as you would for 100 times less sample without using the splitter.

The splitter can be constructed from a stainless-steel T and two pieces of 1/16-in. connecting tubing. The sample stream is directed to the center port of the T and a piece of tubing is attached to the two side ports. The split ratio is determined by the relative flow resistance created by the two pieces of tubing. If, for example, 1-cm and 9-cm lengths of tubing of equal internal diameter are used, 10% of the flow will go out the 9-cm piece and 90% will go out the 1-cm piece.

In practice, the flow resistance of the detector (with its associated heat exchanger and tubing) also needs to be taken into account. Rather than trying to measure detector resistance, adjust the splitter by trial and error until you get the desired ratio. In this case, replace the union between the column and the detector with a T (see Figure 1). You will have to experiment with the length of the tube on the open arm of the T. I suggest starting with about 20 cm of 0.010-in. i.d. tubing (you probably have a piece complete with fittings in your tubing drawer). Determine the split ratio by collecting the effluent from the detector and from this tubing. If too much of the stream is going to the detector, decrease the length of the collection tubing or, if you want more to go to the detector, increase it. I have tried using a needle valve to vary the resistance of the collection tubing, but it was very difficult to adjust and didn't seem to stay in adjustment very long.

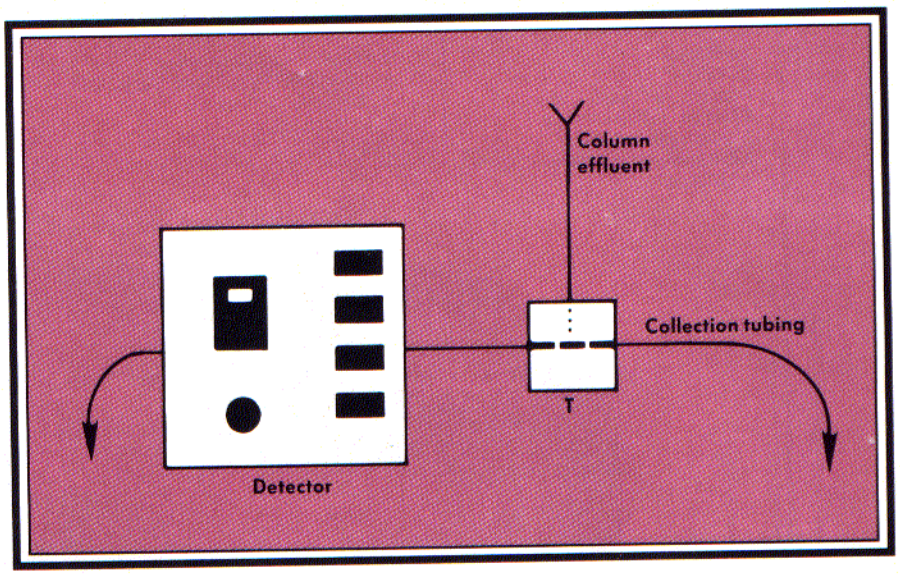

FIGURE 1: Diagram of stream-splitter setup.

One or the other of these two methods should allow you to use your UV detector in the semipreparative mode. You should also be aware that some manufacturers offer short-pathlength cells specifically for this purpose.

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