TROUBLESHOOTING

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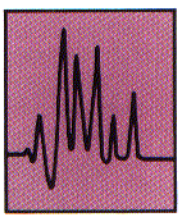

This month's Troubleshooting column addresses a problem with detector sensitivity and describes a technique that will help to reduce the cost of fittings. In addition, I have included some input from readers on methods they use to minimize LC problems. Some of these tips are presented this month; others will be presented in future columns. I encourage you to write to me (in care of *LC Magazine*), relating your own experiences in dealing with the problems described here or contributing other techniques you have found helpful for routine LC operation. Many of you have special tricks for extending LC component lifetimes. If you would like to share these ideas with others in the LC community, send them in and I will include them in future columns.

READER TIPS

When I was at the HPLC '85 meeting in Edinburgh this July, a reader mentioned that individuals in his lab routinely rinse *behind* the pump seal at the end of each day to ensure that no buffer salts remain that could damage the seal or scratch the piston. They flush 5–10 mL of water through the weep/vent holes at the back of the pump head, a procedure they claim greatly extends seal life. I checked the pumps at the exhibition and found that 12 of the 23 pump models on display had holes that would permit flushing in this manner, although only the new Kontron model 420 (Kontron Instruments, Everett, Massachusetts) was clearly designed for this purpose.

Readers are invited to contribute their troubleshooting tips to this column or to submit topics or questions for discussion in future articles. Write to: The Editor, *LC Magazine*, P.O. Box 50, Springfield, OR 97477.

Before trying this procedure, you should remove the head of your lab's pump and inspect the space behind the seal just to be sure that water would not be inadvertently directed into a bearing or other parts of the pump for which flushing is inappropriate.

Another reader indicated that he extends seal life by flushing the pump with hot water after using buffered mobile phases. I have recommended flushing the pump with water at a pressure higher than normal operating pressure to force the buffer out from under the contact area of the seal.

A third reader found that when ion-pairing techniques are used, the frit on the mobile phase inlet line occasionally can cause problems. You may encounter slow column equilibration as a result of buildup of reagent deposits in the frit. If irreproducible separations are a problem in ion-pair chromatography, consider removing the inlet frit altogether. With ion-pair chromatography, the mobile phase must always be filtered through a 0.5-μm or smaller filter just before use.

Finally, one reader solved a weekend breakdown problem ingeniously. He had broken a pump piston, had no spare, and had to have the pump running. He realized that nearly all LC pumps use pistons of the same diameter, but that the driving mechanism — and often the length — are different. Because his lab had more than one pump, he chose to replace the broken piston with a "spare." He unglued the broken piston from the drive assembly by heating the glue joint with a flame. Using the same technique, he removed the piston from a piston assembly that was intended for use in another brand of pump. Next, the good piston was cut to match the length of the broken one, then set in place with cyanomethacrylate glue and put back into service. Clever!

DETECTOR CALIBRATION

Q: My variable-wavelength detector gives absorbance readings that are far too high. If I

set the scale at 2.0 AUFS, the peaks go off scale. I checked the sample on another LC system with a spectrophotometer and got a maximum absorbance of about 0.05 AUFS. What could be the problem?

JWD: I believe the problem is caused either by improper settings or connections or by improper wavelength calibration. Let's look at the possibilities.

There are several points at which an error in the detector setup would cause an apparent calibration problem. Often, there are two sets of output terminals on the back of the detector: one intended for a strip-chart recorder and one for a data system. Typically, the strip-chart output is 0–10 mV full scale, whereas the data system output is 0–1 V full scale. If the cable is connected to the wrong set of terminals, the signal can change by a factor of 100. For instance, if you connect a strip-chart recorder to the data system terminals, the true 0.05-AU signal will instead appear to be 5.0 AU (off scale), just as you have observed. This may sound like a careless mistake, but it is an easy one to make because you often have to change the cable while leaning over the instrument — a position in which instrument labels are not clearly visible.

Some detectors have an output selector switch instead of two sets of terminals to determine the detector output range. Often, this is a toggle switch, which can be easily bumped into the wrong position when you reach around the detector to reposition a waste line or to plug in another module.

Most detectors are designed so that when the data system output is used, the detector signal bypasses the attenuation circuit. This means that you can set the attenuation switches on the front panel any way you wish, but the output remains the same — generally 1 V/AU. As a result, you must attenuate the detector signal using the data system. Here again it is easy to make an unintentional error: a "zero" attenuation on the data system may not really be zero. The attenuation circuitry of a data system is often designed to provide a small amount of amplification if desired. You may need to set the attenuation at 2^4 (data systems use binary settings), for example, to get an unattenuated signal (1 V/AU). You can see that in this case a setting of 2^0 actually represents a 16-fold amplification. Many strip-chart recorders also have range settings, so make sure the recorder is set at the same range as the detector output or it will make the detector appear to be out of calibration.

Once you have verified that all the connections and settings are correct, check the wavelength calibration. Most user's manuals for variable-wavelength UV detectors provide a calibration procedure that uses permanganate solutions or NBS standard solutions. To carry out the calibration, the detector cell must be disconnected from the chromatograph and filled with a calibration solution. Sometimes, it's difficult to flush the strongly absorbed calibration solution from the cell. (I have always had trouble perform-

ing these tests because bubbles become lodged in the cell when the solutions are drawn through.)

A much faster and more convenient way to check the wavelength calibration is to use the deuterium spectrum, which has a characteristic emission band at 656 nm. As you turn the wavelength dial to 656 nm, you should see the baseline drop to a minimum (valley) at 656 nm and then rise back to its original position. You will have to adjust the position of the baseline with the baseline zero knob while you change the wavelength in order to keep the baseline on scale; make coarse adjustments until you get to about 650 nm, then adjust the baseline to about 75% of full scale. Slowly turn the wavelength dial to 656 nm and note the wavelength indicated when the minimum pen position is reached. (You may have to try this a couple of times at different attenuation settings to keep the valley on scale.) If your dial is within ~2 nm of 656 nm, the detector wavelength is probably calibrated correctly. Nevertheless, minor miscalibrations can result in significant differences in the detector output, especially at low wavelengths (for example, 210 nm) or for compounds with sharp absorbance spectra. Check the detector manual for the adjustment procedure. Often, you just have to remove the wavelength selector knob, loosen a screw, turn the shaft to the true wavelength, and then retighten it. You may, however, want to verify the calibration by using a compound of known absorbance in the 200–300 nm region (for example, acetone, which absorbs at 294 nm). If you find that you cannot adjust the wavelength, check all the connections between the wavelength-selection knob and the grating to be sure there are no loose screws in the drive mechanism. Other variable-wavelength detector problems were discussed in an earlier Troubleshooting article (1).

ALTERNATIVE FERRULES

Q: I use a wide range of column and fitting brands in my laboratory and find that I have to keep on hand a large number of short adapter pieces to cover the possible combinations (for example, for connecting Rheodyne to Waters equipment). I have tried single-piece, finger-tightened fittings and found them to be very good, but they are rather expensive — especially given the number that I require. Are there any less expensive alternatives available?

JWD: As you must know, aside from the added housekeeping chores that come with keeping a large variety of adapters on hand, there is greater risk of adding unwanted dead volume to the LC system. I recommend using a polymeric ferrule in conjunction with a standard stainless-steel compression fitting. There are three polymeric ferrule materials commonly available: Vespel, Teflon, and nylon. All of these materials are less expensive than some brands of finger-tightened fittings, but may be more expensive than stainless steel.

To use one of these ferrules, just replace the stainless-steel ferrule with a polymeric one and make up the fitting in the standard way: slide the nut and then the ferrule onto the tube end and insert the tube end into the fitting body until the tube hits the bottom of the port. Tighten the fitting finger-tight, then tighten 1–1 1/4 turns farther with a wrench. This is about a half turn farther than is recommended for a stainless-steel ferrule, but the polymeric ferrule will deform more in the fitting. Overtightening doesn't seem to cause any damage. I have found that a fitting assembled this way will withstand 6000 psi with 1/16-in. o.d. tubing. For tubing with a larger outside diameter, the pressure limit is reduced.

The disadvantage of using polymeric ferrules instead of single-piece, finger-tightened fittings is that ferrules often stick in the compression fitting when it is disassembled. This is because there is more surface area on the fitting body for the ferrule to adhere to than there is on the tube. In some cases, the ferrule also distorts and is pushed into the threads of the fitting body. In either case, the ferrule can be easily removed with the universal lab tool — a bent paper clip! Straighten a paper clip, bend just the tip sharply, and insert it through the center of the ferrule; use the paper clip to grip the ferrule and pull it out. This usually destroys the ferrule and, if care is not taken, can scratch the sealing surface of the fitting body.

Which ferrule material is best? Vespel has the best properties: it is quite inert and is not easily distorted in the fitting, but it is the most expensive of the three. Teflon is quite popular, very inert, and intermediate in cost but tends to "cold flow." This means that Teflon ferrules can loosen with time (make sure you snug them up now and then), and they are easily distorted by overtightening; hence, they can only be reused a few times. I favor nylon ferrules (available from Crawford Fitting Company, Solon, Ohio) because they are quite inexpensive, do not distort easily, and, in most cases, perform at least as well as Vespel and Teflon. Nylon works well with most reversed-phase solvents, but it is not compatible with all LC solvents, so you should check solvent compatibility before use.

A final note of caution: if polymeric ferrules are used, be sure they are removed from the fitting when the fitting is disassembled. I once threaded a fitting with a preswaged stainless-steel ferrule into a fitting body that still contained the Teflon front ferrule from a previous use. The fitting sealed well, but there was a large dead volume.

If you would like more detailed information on this subject, see the January 1984 issue of *LC Magazine*, which contains a Troubleshooting article devoted to the proper use of tube fittings (2).

REFERENCES

(1) J.W. Dolan and V.V. Berry, *LC, Liq. Chromatogr. HPLC Mag.* **2,** 439–444 (1984).

(2) J.W. Dolan and V.V. Berry, *LC, Liq. Chromatogr. HPLC Mag.* **2,** 20–21 (1984).

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