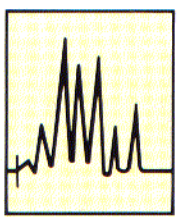
LC TROUBLESHOOTING

Readers' Questions

JOHN W. DOLAN



Readers' questions about frit replacement, biocompatible parts, detector lamps, and autosampler precision problems are discussed in this month's "LC Troubleshooting" installment. Also, two new product innovations are covered with an eye to their potential for improving the reliability of LC system operation. Readers who have experience with other such products are invited to share this information with other users. Write to The Editor, *LC•GC*, P.O. Box 10460, Eugene, OR 97440, USA.

A NEW ENDFITTING DESIGN

Frit blockage is one of the most frequently encountered problems with LC operation and is a primary cause of column failure. The symptoms of frit blockage are split or tailing peaks or high back pressure. In about one-third of such cases, these problems can be corrected by replacing the frit, but this is not without its own complications. As shown in Figure 1a, when the column endfitting is removed so that the frit can be replaced, the column packing is exposed. Unless the frit is replaced very carefully, the packing at the head of the column can be disturbed, possibly ruining the column. Also, with the increasingly popular cartridge columns, changing a frit is no longer even a possibility because in these columns the frit is pressed into the end of the column tubing. To avoid these difficulties, many chromatographers use an in-line filter between the sample injector and the column. The filter prolongs the lifetime of the column frit by trapping particulate matter before it reaches the column.

A recent development by Keystone Scientific (Bellefonte, Pennsylvania) called a Column-Plus frit addresses both of the above problems. The design is a logical extension of the use of cartridge guard columns that are coupled directly to an analytical column. In this case, however, the column endfitting is modified so that it contains a separate frit (Figure 1b). Now the frit can be changed freely without any chance of disturbing the column packing itself. This design should also work with cartridge columns that have pressed-in frits, in effect giving to a cartridge column the advantage of a removable frit. This product looks as if it should work well; I would like to hear feedback from anyone who has experience with it.

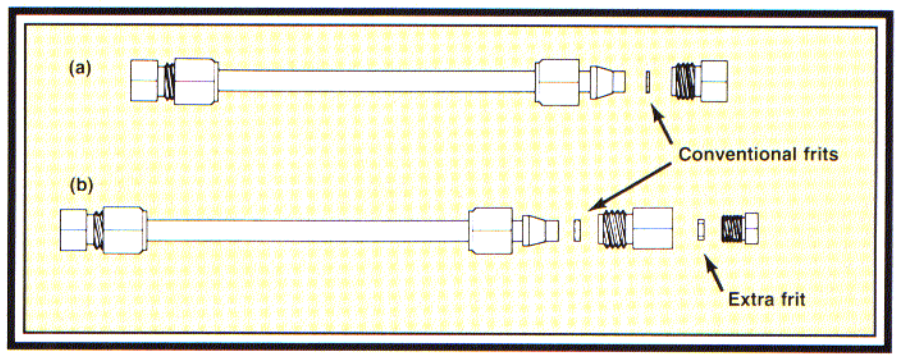


FIGURE 1: Column endfitting designs. (a) Conventional fitting in which the endfitting holds the frit on the end of the column. (b) Column-Plus fitting, which includes a frit that can be replaced without disturbing the column packing. (Courtesy of Keystone Scientific, Inc.)

A note of caution is in order for anyone using a product such as this or an in-line filter: These products are *not* a substitute for filtering samples before injection. Any sample that has visible particulates or is cloudy or opalescent should be filtered through a sample filter with a pore size of 0.5 µm to minimize problems created by injecting dirty samples.

PEEK TUBING

Q: I have been reading about biocompatibility of LC systems and have seen reference to a polymer called PEEK. Is this something new, and if so, why is it so great?

JWD: PEEK, a poly-ether-ether-ketone polymer, is said by some to be the future replacement for stainless steel in LC systems. This plastic is most commonly used today for ferrules in finger-tightenable fittings. The natural color is a chocolate brown, but it can be colored with inert additives. The plastic has better chemical inertness than Vespel and does not cold-flow like PTFE. Its pressure stability is also adequate for most LC applications. PEEK is claimed to be "biocompatible" — that is, to cause little or no protein denaturation. For this reason, some companies now offer columns packed in PEEK tubing for biochemical applications. Other LC components, such as connecting fittings and tubing, are becoming available in PEEK as well. Because PEEK can be molded rather than machined, production costs for PEEK fittings should be lower than those for stainless steel fittings. Once manufacturing is in full production, this cost reduction (we can hope) should be reflected in a lower cost of fittings to the user.

We recently replaced a length of $^{1}/_{16}$-in. o.d., 0.010-in. i.d. stainless steel tubing with PEEK to connect the column outlet to the detector. The tubing is cut easily with a razor blade, and, because of its flexibility, it can be routed between the column oven and detector without the problems created by the "springiness" of stainless steel tubing. Its high pressure resistance allows PEEK tubing to be used anywhere in the LC system, in contrast to PTFE, which must be limited to the low-pressure parts of the system. Although we chose to use PEEK tubing because of convenience, many users will choose it for biocompatibility.

DEUTERIUM LAMPS

Q: How can I tell when I need to replace the deuterium lamp in my detector? It seems to me that if I replace it on a calendar schedule, I lose a significant portion of its usable life. On the other hand, if I wait until it fails, I often lose a day of runs.

JWD: First of all, you should recognize the two signs of impending lamp failure: an increase in baseline noise, and a lamp that is difficult to start. Increased noise is most commonly noted by more "hash" or rapid pen deflections on the baseline, as well as more baseline "wander" when an isocratic mobile phase is used. Of course, noise is difficult to quantify unless you have records of what the baseline should look like when the system is

operating properly, so you should regularly save samples of a blank baseline. A sure sign of impending lamp failure is the presence of sharp pen deflections, or spikes, in the chromatogram. You may notice the second sign — a lamp that is hard to start — when you try to start the detector in the morning and the lamp will not light until after several tries. At other times, the lamp may shut off temporarily or permanently. The cause of the latter symptoms is obvious, but noise problems may be caused by other problems as well. One quick way to isolate the source of a noise problem is to shut off the mobile phase flow. If the noise or spikes persist, the lamp is suspect; if the problem disappears, look for air bubbles or mobile phase problems. Remember that some mobile phases, as well as lower detection wavelengths, will increase the baseline noise even with a new lamp.

Although it is not too difficult to determine when a lamp has failed, the trick is to be able to predict when its useful life is up. In the past, determining the lifetime of a deuterium lamp for a variable-wavelength UV or diode-array detector was largely a matter of guesswork. The general rule was that the lamp would last for 500–1000 h of use, which was estimated to be about six months in an average lab. Because of the inconvenience of recordkeeping, few users kept accurate logs of lamp on-time. The cost of a replacement lamp, especially those premounted in a lamp holder, often exceeds $500, so users put pressure on the manufacturers to provide a guaranteed lamp lifetime. The manufacturers responded with a 1000-h guarantee in many cases, and added a device to help document lamp usage. As a result, most deuterium lamps today come with a small meter attached to one of the leads, which looks like a miniature thermometer calibrated to 1000 h. As the lamp is used, the meter registers cumulative hours of use, thus relieving the user of the necessity of keeping these records.

As mentioned in earlier columns, deuterium lamps have a finite shelf life of approximately six months. In other words, it doesn't matter whether the lamp is in use in the detector or is sitting on the shelf in its original box — eventually the lamp will no longer work satisfactorily. This aging has to do with the leakage of gas, especially through the seals where the electrical leads pass through the quartz body of the lamp. For this reason, it is best not to stockpile lamps for future use, but to buy them just before you need them. This is where the lamp-life meter comes in handy. You should inspect the meter every few weeks, or whenever you take the cover off the detector. When the lamp has been used for 750–800 h, it is nearing the end of its life, and a replacement should be ordered. Now when the lamp fails to start, or causes excessive noise, you'll have a fresh lamp for replacement.

AUTOSAMPLER PRECISION

Q: I have observed what seems like inconsistent behavior in my autosampler. If I program it to inject from a partially filled sample loop, the precision is better than if I use

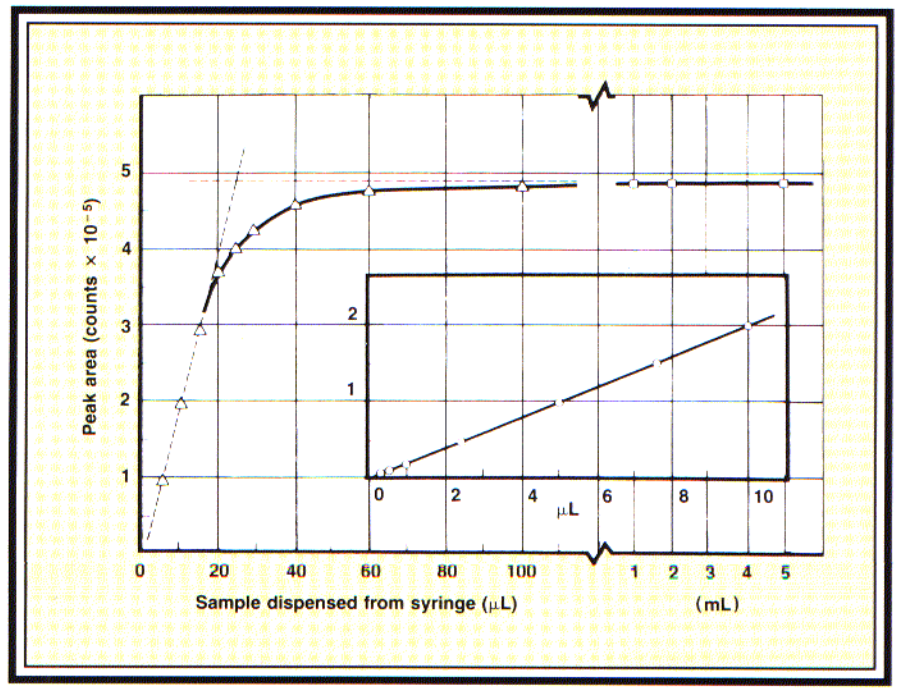


FIGURE 2: Plot of peak area vs. sample delivered to a 20-µL sample loop. Note nonlinear region between about 50% and 300% of the nominal loop volume. (Courtesy of Rheodyne Inc., Cotati, California.)

a filled loop. The autosampler uses a syringe to draw sample into the loop, then injects the sample. I have checked the LC system by making manual injections with the filled-loop technique, and the precision is much better than when the autosampler uses the same technique. What could be causing this problem?

JWD: As discussed previously (1,2), for maximum precision a sample loop should be filled to <50% or to >300% of its volume. Figure 2 shows that the volume between about 50% and 300% of the nominal loop volume has nonlinear filling characteristics. As long as you comply with these limits, you should be able to get acceptable precision from using a filled loop or a partially filled loop. (Of course the precision of the partially filled loop depends on the precision of sample delivery to the loop.)

Let's see how this information can help isolate the source of your problem. Because the precision is adequate for a partially filled loop, we can conclude that the loop is not being filled to more than ~50% of its volume. Poor precision for filled-loop injection can be explained if the loop is being filled in the low-precision region of 50–300% of its volume. This can be caused by one of three problems with the autosampler. First, if the loop were too large, the selected volume of sample might result in insufficient filling. For example, a 60-µL sample would adequately fill the 20-µL loop in Figure 1. If, however, a 50-µL loop were inadvertently installed, the loop would now be filled to only 120%, and lower precision would be expected. So one possibility is that you have the wrong size of loop installed in the autosampler. The second possible cause is that the syringe is drawing insufficient sample through the loop because of the use of the wrong syringe size. Most syringe-type autosamplers are calibrated internally for several different syringe sizes. For example, the syringe stepper motor might move 100 steps to draw 10 µL into a 100-µL syringe, whereas the same 100 steps might draw 50 µL into a 500-µL syringe. If the system were programmed to draw 60 µL into the 600-µL syringe to fill the 20-µL loop, good precision would be expected. This same program would draw only 12 µL of sample into the loop if the 100-µL syringe were accidentally installed, which would put the volume in the low-precision region of Figure 2. The third possible cause of the problem is that you have programmed the autosampler to withdraw a sample volume that results in imprecise injection (10–60 µL for the present example).

Any of the three problems discussed above could be causing the precision problem that you observed. You should also be aware that because of the inherent high precision of an autosampler (due to mechanical reproducibility of the sampling operation), the precision will probably be better than if you made similar mistakes with a manual injector. For more information on troubleshooting autosamplers, see references 2 and 3.

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"LC Troubleshooting" editor John W. Dolan is president of LC Resources Inc. of Lafayette, California, USA, and is a member of the Editorial Advisory Board of LC•GC.