L C T R O U B L E S H O O T I N G

Blocked Columns and Tubing

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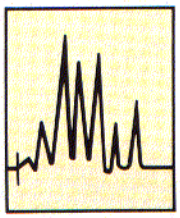

This month we look at various approaches to restoring the performance of blocked columns and at ways to avoid problems associated with small-bore connecting tubing. In addition, a method is presented to minimize sample carryover in injection loops.

STEPS TO COLUMN RESTORATION

Q: What is the best way to correct high column pressure? Some people say to replace the frit first, while others say to reverse the column before replacing the frit. Which is correct?

JWD: I don't think that there is a right or wrong way to proceed. A strong argument can be made for reversing the column before trying to replace the frit. To reverse the column, simply disconnect it and reconnect the original outlet end to the inlet tubing. Then pump mobile phase through the column to flush particulate matter from the old inlet frit. You should direct the column effluent into a waste container so that particulates are not pumped into the detector. Once the pressure drops, you can reconnect the column — still reversed — and continue with your work. If the pressure is still too high, you can replace the old inlet frit (now at the column outlet). Besides flushing the blockage from the frit, column reversal eliminates the risk of disturbing the column packing during frit replacement. Most columns today will tolerate reversal, although you might want to check with the column manufacturer before you do this the first time. Column reversal will correct the pressure problem about a third of the time.

Although column reversal is my first suggestion — particularly for newcomers to column repair — I have to admit that I first replace the frit. Replacing the frit has the advantage of allowing you to inspect the head of the column. If you observe a void, you can either discard the column or try to repair the void. The danger involved in replacing the frit is that you might disturb the packing and possibly ruin the column. Although a frit is simple to change, it is best to learn the procedure by watching someone else do it. There are several key points to observe. First, be sure that the column is securely supported. I recommend holding the outlet endfitting in a vise while working with the inlet end. Second, use a new frit of the same diameter and thickness as the old one. Don't try to clean or otherwise reuse old frits. This is false economy, as they cost more to clean than to buy. Third, clear packing particles from the sealing surfaces on the outside of the column, on the ferrule, and inside the endfitting before reassembly. A few bits of column packing on the ferrule can cause leakage; on the threads, they can cause binding. Fourth, don't overtighten the endfitting during reassembly. Finally, if you find that the frit is pressed into the endfitting, you will usually have to replace the entire fitting; if it is pressed into the column, you'll have to replace the column (you might try column reversal if you haven't done this already). As with column reversal, replacing the frit will solve the pressure problems about a third of the time.

I am not an advocate of filling voids at the head of the column, because this process is time-consuming and only marginally successful. However, if you do decide to fill a void, use packing from an old column with the same type of packing. Make a slurry of about 10% packing in solvent, and add this dropwise to the void, allowing the excess solvent to soak into the column. Finally, use a spatula to smooth the top of the packing so that it is even with the sides of the column. Replace the frit and endfitting, and flush the column. Usually, you will have to repeat the process several times until the void is filled with sufficiently compacted packing material. Photographs of this procedure can be found elsewhere (1).

If you decide to fill voids, you should also be aware of the work of Vendrell and Aviles (2), which showed that column performance could be restored successfully if the void was filled and the column was *reversed.* Reversing the column circumvented the need to repeatedly fill the void until the packing was compacted.

So the answer to your question is that you can choose either frit replacement or column reversal to correct high column pressure, depending on your personal preference. Either technique is equally likely to reduce a backpressure rise that is due to a buildup of particulate matter. If one method fails, try the other — the techniques are complementary. Of course, the best choice is to use an in-line filter plus a guard column to prevent blockages from occurring at the head of an analytical column. Abbott has provided a detailed discussion of column back-pressure problems (3).

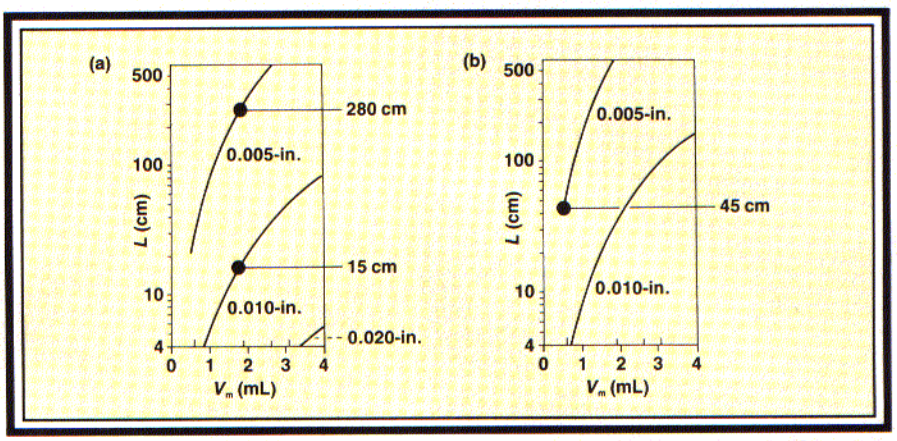

FIGURE 1: Tubing length allowable for 5% loss in column plate number, *N*. (a) Column with *N* = 10,000 (for example, 15 cm × 4.6 mm, 5 μm); (b) column with *N* = 5000 (5 cm × 4.6 mm, 3 μm). Assumes that appropriate detector cell volumes are used.

PLUMBING PROBLEMS

Q: I recently started using 3-μm columns, so I replumbed my entire LC system with 0.007-in. i.d. tubing to avoid band-broadening problems. I have found, however, that the tubing becomes blocked very easily, and as a result, the reliability of the system has worsened. Is this the price I have to pay for improved column performance?

JWD: It is true that 0.007-in. i.d. tubing is more prone to blockage than is 0.010-in. i.d. tubing, but using 3-μm particles does not mean that you have to use smaller i.d. tubing.

Figure 1 shows the amount of tubing of various diameters that can be added to the

system before a 5% decrease is observed in the plate number (you generally will not notice a smaller decrease). Figure 1a shows an example for a column generating 10,000 plates (such as a 15 cm × 4.6 mm, 5-μm column). To determine the allowable tubing length, find the column volume V_m on the *x*-axis corresponding to your column (1.5 mL is the example used here). Draw a vertical line to intersect one of the tubing plots and the corresponding tubing length on the *y*-axis. The example in Figure 1a indicates that you can use ~15 cm of 0.010-in. i.d. tubing without noticeably degrading the separation. Figure 1a also shows that a much longer piece (280 cm) of 0.005-in. i.d. tubing can be used before peak broadening will be noticed. Data for 0.007-in. i.d. tubing falls between the two plots.

The 3-μm column generating about the same plate number (N = 10,000) would be about 10 cm long, with $V_m \cong 1$ mL. Figure 1a shows that we would be limited to ~5–6 cm of 0.010-in. i.d. tubing, which should be sufficient for many system configurations.

If you use short 3-μm columns, you do need to pay close attention to the plumbing. Figure 1b shows data for an N = 5000 column. For example, a 5 cm × 4.6 mm, 3-μm column ($V_m \cong 0.5$ mL) could use up to 45 cm of 0.005-in. i.d. tubing before peak broadening becomes a problem. However, 0.010-in. i.d. tubing in any length would be unsuitable.

In practice, many routine separations do not generate 10,000-plate peaks; 5000 to 8000 plates is more usual. For these separations you can use results between those recommended for Figure 1a and Figure 1b. Consequently, with 3- or 5-μm columns that are 10 cm or more in length, practical lengths (for example, 10–15 cm) of 0.010-in. i.d. tubing can be used to connect the column to the injector and detector.

All this means is that using a 3-μm column doesn't preclude the use of 0.010-in. i.d. tubing. (In the above discussion of tubing length, it is assumed that the detector cell volume is selected so that extracolumn effects are minimized.)

You also can take other steps to minimize problems you might encounter when using 0.007- or 0.005-in. i.d. tubing. First, don't use such tubing where it is not required. Generally, this means that small-bore tubing should be used only at those locations where sample integrity is important — that is, between the injector and the column, and between the column and the detector. The plumbing before the injector should be 0.020-in. i.d. to minimize problems. Second, protect the system from particulate contamination. Particulates can enter the system from three major sources: the solvents, the pump, and the sample. Solvents should be of HPLC quality and should be filtered if they contain buffers. If your pump occasionally sheds bits of seal debris, install an in-line filter just ahead of the injector to prevent the debris from reaching the narrow-bore tubing. You might also need to filter the samples before injection to keep sample particulates out of the system. You can provide further protection from particulates by installing a zero-volume in-line filter directly after the injection valve. This will trap any stray particulates before they reach the narrow-bore tubing.

My general advice is to use tubing smaller than 0.010-in. i.d. only when it is absolutely necessary. As discussed above, there seems to be some sort of exponential relationship between the number of problems encountered and the reciprocal of the tubing diameter.

MORE ON SAMPLE CARRYOVER

In the March "LC Troubleshooting" column (4), we covered some ways to minimize carryover by proper loop-flushing techniques. A reader wrote to remind me that injecting a small air bubble will effectively purge the sample from the loop for all but the smallest loops. The "leading-bubble" technique is a good way to precisely inject small amounts of sample with a large loop. In this technique, the syringe is filled with sample to the desired volume, and then it is drawn back further to include a small air bubble (for example, 0.2 μL) at the syringe tip. When the sample is pushed into the loop, this bubble is injected first, forming a seal between the previous loop contents (the mobile phase) and the sample. Because the bubble prevents the sample from mixing with the mobile phase, laminar-flow conditions do not exist, and samples up to the loop volume can be injected precisely. When the valve is rotated to the *inject* position, the bubble will dissolve in the mobile phase, causing no problems. The technique requires that the injection valve be plumbed so that the loop is backflushed onto the column. Experimental data to support this technique can be found in reference 5.

REFERENCES

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(2) J. Vendrell and F.X. Aviles, *J. Chromatogr.* **356,** 420 (1986).
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(4) J.W. Dolan, *LC•GC* **6,** 222–224 (1988).
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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.

Readers are invited to contribute their troubleshooting tips to this column or to submit topics or questions for discussion in future columns. Write to The Editor, *LC•GC*, P.O. Box 10460, Eugene, OR 97440.