TROUBLESHOOTING

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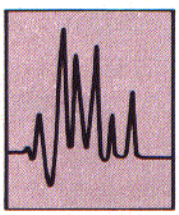

This month's column includes a discussion of a simple plumbing modification that will allow for rapid flushing of the reference cell of a refractive index (RI) detector. Pump seal replacement, method adjustment when the column size changes, and some possible sources of a ghost peak that one reader found in a gradient separation will also be discussed.

RI REFERENCE CELL FLUSHING

Q: Bubbles get into the reference cell of our refractive index detector when we flush it with fresh mobile phase. We flush the cell by disconnecting the column from the sample cell, connecting it to the reference cell, and pumping mobile phase through. When we are done, we disconnect the column and close off the reference cell with a loop of Teflon tubing. It is at this stage that we can't keep bubbles out. Is there a simple solution to this problem?

JWD: An inexpensive low-pressure valve can be added to the system to provide convenient and effective RI detector cell flushing. A sketch of the plumbing configuration is shown in Figure 1. I have used a model 5302 three-way slider valve (Rheodyne, Cotati, California) for this purpose, but any three-way valve should work. To link the valve with the detector, connect the center (or common) port to the outlet of the sample cell and one of the side ports to a waste line. The other side port is connected to the inlet of the reference cell. In normal operation, the valve would be positioned so that the sample cell effluent goes to waste. When you want to flush the reference cell, switch the valve to the other position. (Be sure no sample is in the column or you may contaminate the reference cell.) Now the reference cell is flushed with mobile phase. After the cell has been flushed thoroughly, switch the valve back to the waste position and proceed with your analysis. At this point, the inlet to the reference cell is sealed off by the valve.

It is helpful to maintain a slight back pressure on the reference cell. This can be accomplished by using about a meter of 0.010-in. to 0.020-in. i.d. Teflon tubing for the waste line that comes from the reference cell and by making sure that the outlet of this line is above the level of the reference cell.

Because no connections to the reference cell are made or broken when using this procedure, the likelihood of bubbles being introduced is minimized. Another advantage of this setup is that it makes flushing the reference cell very convenient, so you are more likely to flush it regularly.

GHOST PEAKS IN GRADIENT

Q: When I perform gradient separations using numerous C18 columns, a strongly absorbing peak elutes during gradient regeneration. The gradient used is 50% acetonitrile/water to 100% acetonitrile with detection at 254 nm or 280 nm. The peak appears when the mobile phase reaches a concentration of about 70% acetonitrile on the back side of the gradient. I use degassed LC-grade solvents. What is causing this ghost peak?

JWD: Occasionally, ghost peaks develop during gradient regeneration, and their cause can be difficult to track down. Fortunately, they seldom interfere with an analysis, and many workers ignore them. These peaks can be caused by very polar materials in the mobile phase. As long as there is water in the mobile phase, these materials elute rapidly; but, when a 100% organic mobile phase is approached, the polar silanol sites on the stationary phase may retain the materials. This is analogous to the salting-out phenomenon, in which solute solubility drops when high organic concentrations are used. When the gradient returns to a more polar condition on the back side of the gradient, the material elutes because it is attracted to the mobile phase. I can think of three possible sources of this problem, and perhaps readers can suggest a few more.

First, I would suspect the quality of your water. It may be "LC grade," but problems can still arise. Although it is unlikely, you could have a contaminated lot of solvent — this has happened to me on two occasions. Check for contamination by using water from another lot or from another vendor. Water can also become contaminated in the lab as a result of using improperly washed glassware or just by being left exposed for a period of time. If you generate your own LC-grade water using a Milli-Q (Millipore Corp., Bedford, Massachusetts) or a similar purification system, the system may not be purifying water to the degree you think it is. The activated carbon cartridges in these units can wear out, and yet the conductivity monitor will continue to give a normal reading and the final filter will continue to work properly. If this is the case, clean the system thoroughly and replace all of the cartridges. You should keep a log of maintenance and production volumes for a water system because most manufacturers recommend replacing cartridges either at certain time intervals or after a specific volume of water has been processed.

Second, check your degassing procedure because it can contaminate your solvents if not done properly. Helium sparging can contaminate solvents if the sparging frit is dirty (did you handle it with dirty hands or lay it on the bench top?) or if you are not using helium of sufficiently high purity. You can check for these problems by replacing the frit and all of the lines and by using a new tank of the highest grade of helium, or you can switch to another degassing method. Vacuum degassing can be a problem if the flasks are not cleaned properly or if backflow from the vacuum line contaminates the solvents when the vacuum is shut off. Also, be sure to use inert stoppers in the flasks: the black rubber stoppers used for other lab purposes can produce disastrous results for solvent degassing.

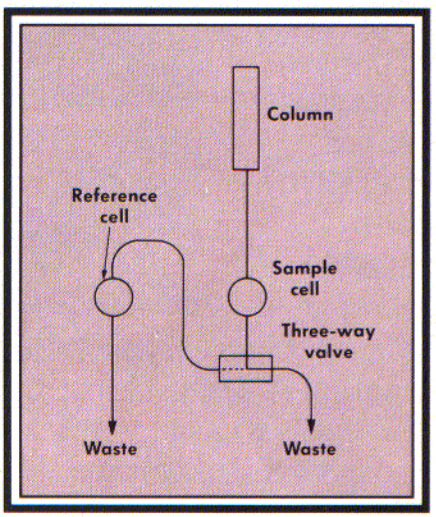

FIGURE 1: Plumbing diagram for using three-way valve to flush the RI detector reference cell.

Finally, it is possible that your chromatograph is contaminating the solvents. Although this problem is seen more often at lower wavelengths, you may see it at 254 nm. Inlet lines to the pump can become contaminated, especially when buffers are used because buffers often support microbial growth. You can check for inlet-line contamination by replacing the lines or by removing them from the pump and flushing them with a 30% nitric acid solution. Rinse the lines thoroughly with LC-grade water before reinstalling them.

PUMP SEALS

Q: I'm not sure when my LC pump seals need to be replaced. What signs should I look for, and what kinds of problems can develop with pump seals?

JWD: There are two signs of failure that are clearly related to pump seals. One is mobile phase leaking from behind the pump head, and the other is the inability of the pump to maintain steady high pressures. Unfortunately, by the time these signs are observed, it is well past the time when the seals should have been replaced. Many workers routinely replace the seals every three months because they are inexpensive and because early replacement can prevent other problems.

To replace a seal, first you must remove the pump head. When disconnecting the tubing from the inlet and outlet check valves, be sure to use a wrench to steady the check valve to avoid putting stress on the pump head itself. If the direction of flow through the head is not obvious, mark it on the head and on both check valves with a scribe. Next, loosen the bolts securing the pump head and carefully remove the head: do not twist the head at this point because you can break the piston.

After the head is removed, inspect the piston for buffer-crystal buildup. It is normal for the piston to be a little wet behind the seal because the seal does not completely prevent mobile phase from creeping along the piston. Wash off any crystal buildup with distilled water, rinse with methanol, and dry the piston with a lint-free tissue.

The procedure for removing the old seal from the pump head varies from pump to pump, so you should follow the directions for your particular pump. Removing the seal is sometimes difficult, although it can be facilitated by inserting a large screw into the center of the seal until it grips well. Twisting the screw will pull the seal out easily. Just be sure not to scrape the pump head.

Now remove the check valves. I don't recommend any maintenance for the check valves unless they have been leaking because it is very easy to contaminate them. Put them in a dust-free place until you are ready to reassemble the head, then wash the pump-head body thoroughly. Washing for a few minutes in an ultrasonic cleaner filled with methanol is usually sufficient.

Seals can be replaced without the aid of seal-insertion tools, but you will find seal replacement much easier if you use the guide tool sold by your pump manufacturer. Such tools allow you to slip the seal into the pump head without damaging the new seal. When you replace the seal, be sure it is inserted in the proper direction. The spring in the seal should face the high-pressure side of the head. The seal will be ineffective if it is reversed so that the spring side faces the main body of the pump.

When reassembling the head, follow an exploded diagram in the owner's manual to ensure that all washers and guides are inserted in the proper order. Check the inlet and outlet check valves for proper positioning. When pushing the head back onto the piston, it is helpful to wet the piston with a little methanol to lubricate it during reassembly. Again, be very careful not to twist the pump head at this point or you may break the piston.

Connect the inlet line to the inlet check valve and loosely insert the outlet line into the outlet check valve. Pump several milliliters of methanol through the pump to remove air from the head (wrap a laboratory wiper around the outlet check valve to absorb the methanol). When you can't see any more bubbles coming from the outlet check valve, tighten the fitting and proceed with normal operation.

There are a few points to keep in mind. First, not all pump seals are compatible with all solvents. I once used a seal that was supposed to be good for any reversed-phase eluent. It was — as long as there was water in the mobile phase; but the seal deteriorated when exposed to pure tetrahydrofuran and contaminated the system with UV-absorbing material. Therefore, be sure to use the seal recommended by your pump manufacturer for the mobile phases you normally use. Second, as was mentioned earlier, it is normal for the piston to be damp on the back side of the pump seal because seals are not 100% effective. When buffers are used, this leakage can result in the formation of buffer crystals on the piston. For this reason, at the end of each day you should pump water or nonbuffered mobile phase through the system at a pressure at least as high as the normal operating pressure for a few minutes to wash away any buffer from the sealing surface. Otherwise, buffer evaporation may cause the formation of abrasive crystals, which can drastically reduce seal life. Third, if downtime is critical in your lab, you might find it useful to keep a rebuilt pump head (complete with check valves) on hand. This spare will allow you to remove the head in use, replace it with a rebuilt one, and return to operation in just a few minutes. Now you can replace the seals in the old head at your leisure. Finally, order replacement seals and any other parts you've replaced so that you will have them on hand the next time they're needed.

ADAPTING SEPARATIONS

Q: I'm interested in trying some of the new, short 3-μm columns in methods that specify conventional LC columns, but I don't want to spend a lot of time redeveloping the methods. Are there some general guidelines that will help me make a rapid transition?

JWD: The conversion of a method designed for a column of one size for use with a column of another size is straightforward. The key to a successful conversion is using a new column of the same phase type from the same manufacturer and adjusting the flow rate so that the same linear velocity is used with the new column as was used with the old one.

It is important to use a column from the same manufacturer because this will ensure a column chemistry very close to or identical to that of the column you have been using. Manufacturers understand the importance of consistency in selectivity and try to control column chemistry so that you can change quickly from one column dimension to another. The same potential problems exist in changing from one manufacturer's 25-cm column to another manufacturer's 5-cm column as in changing from one manufacturer's column to a column of the same dimensions from another manufacturer. That is, as most workers know only too well, a C8 column purchased from one manufacturer is not necessarily equivalent to one purchased from another source.

To maintain the same linear velocity through the column, you will need to adjust the flow rate in proportion to the square of the column diameter. If, for example, you use a 4.6-mm i.d. column operated at a flow rate of 1 mL/min and want to change to a 6.8-mm i.d. column, you should adjust the flow rate by $(6.8/4.6)^2$, or 2.2 times, so the larger column is operated at a flow rate of 2.2 mL/min. Similarly, if you change from a 4.6-mm i.d. column to a 1-mm i.d. microbore column, you must drop the flow rate from 1 mL/min to 0.05 mL/min.

Once you have adjusted the linear velocity, you should be able to run your samples. Retention times will change in proportion to column length. For example, a 5-min retention time on a 25-cm column becomes 1 min on a 5-cm column.

After you confirm that the separation achieved on the new column is the same or very similar to that on the old column, you may want to adjust the conditions to improve the performance of the system. If, for example, pressure had been a limiting factor with a 25-cm column containing 5-μm particles, you may find that you can get a satisfactory separation at a much higher flow rate on a 5-cm column packed with 3-μm particles. The shorter column has a lower back pressure, and the efficiency of 3-μm columns is not affected by flow rate changes as much as are 5- or 10-μm columns. References 1 and 2 contain examples of techniques for obtaining similar separations when the column dimensions are changed.

REFERENCES

(1) M.W. Dong and J.R. Gant, *LC, Liq. Chromatogr. HPLC Mag.* **2**, 294–303 (1984).

(2) N.H.C. Cooke, K. Olsen, and B.G. Archer, *LC, Liq. Chromatogr. HPLC Mag.* **2**, 514–524 (1984).

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