LC TROUBLESHOOTING

Baseline Noise: A Case Study

J. Paesen and J. Hoogmartens

Baseline problems can originate from nearly any component of an LC system, but methodical problem-isolation techniques will help you find the source.

When we set up our liquid chromatographic (LC) system for a specific analysis, we observed the baseline shown in Figure 1a. The magnitude of the baseline noise was much too great for the requirements of our assay. The LC system consisted of a pump, a manual injector, a polymeric reversed-phase column (25 cm × 4.6 mm, 8-μm particle diameter), a variable-wavelength UV detector (215 nm), and a laboratory integrator. Because the column was packed with a poly(styrene–divinylbenzene) copolymer, we used a water jacket to maintain column temperature at 60 °C to reduce the back pressure and improve the peak shape. Water was supplied from a water bath that was equipped with an immersion heater. The manually premixed mobile phase was 20:5:75 (v/v/v) 2-methyl-2-propanol/0.2 M potassium phosphate, pH 10.0/water. We observed a back pressure of ~1000 psi at a flow rate of 1.5 mL/min.

CHECK EACH MODULE

Our first suspicion was that, because of the cyclic nature of the baseline, the noise originated from a pump malfunction such as a faulty check valve or a trapped air bubble. We thoroughly checked the pump but were unable to find the source of the noise. We still suspected the pump, but when we tested it in another LC system, the pump worked properly. Finally, we exchanged the pump for one of another brand and the baseline problem persisted, so the pump was eliminated as a problem source.

Next, we replaced the injector and then the integrator with modules of the same type, but the baseline still did not improve. We exchanged the column for a new one and the detector for one of another brand. Neither of these changes helped. In each case, when a module was determined to be good, the original module was reinstalled in the instrument, so that the configuration was kept as close to the original as possible. To verify that the heating bath was not causing electronic interference with the detector, we isolated the circuit by connecting the water bath to a power source originating in another laboratory. As expected, this did not eliminate the problem.

MOBILE PHASE CONDITIONS

We next concentrated on the conditions within the column. First, we replaced the mobile phase with 50:50 (v/v) methanol/water to see if one of the mobile phase components could be causing the baseline noise, but no improvement was observed.

We saw the first sign of a cause–effect relationship when we reduced the flow rate from 1.5 mL/min to 0.5 mL/min. Under these conditions, the back pressure dropped, as expected, and the amplitude of the noise was reduced but did not disappear. This made us wonder whether the problem was related to flow and pressure, so we removed the column from the system and connected the injector directly to the detector. Without the column, the baseline returned to normal. At this point, we suspected that the stationary phase was not resistant to the temperature and pressure conditions we were using. This instability, however, did not make sense in light of the widespread use of polymeric reversed-phase columns. Furthermore, when the same column was tested with the same assay in another LC system, everything performed satisfactorily.

SYSTEM-TO-SYSTEM DIFFERENCES

Now that we had two LC systems — one that produced a good baseline and one that did not — we made a close comparison of the differences between the two. The only (apparently minor) difference was that in the "good" system, the column had been immersed in a water bath at 60 °C, whereas a water jacket had been used to heat the column in the "bad" system. When the water jacket was removed from the "bad" system and the column was immersed in the water bath that previously had supplied water to the jacket, the baseline of Figure 1b was observed. If the noise was an electronic phe-

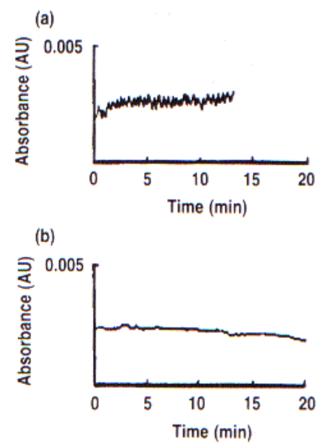

FIGURE 1: Baseline background noise recordings, including (a) unacceptable and (b) acceptable baseline tracings. System conditions are given in the text.

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nomenon, it should not have disappeared when the column was immersed in the water bath that made use of the same heating equipment, because nothing had changed in the electronic configuration. Further inspection revealed that in the immersion configuration, the PTFE detector-inlet tubing was immersed in the bath, but in the jacket configuration, part of the column endfitting and the PTFE tubing were not heated. The fact that the tubing was PTFE did not seem important because the same problem had been observed earlier when the detector was exchanged for one with a stainless steel inlet tube. However, when we used a third detector with the water jacket, no excessive baseline noise occurred. All of these observations seemed to point toward thermal effects as the problem source.

EXPLORING THERMAL EFFECTS

Several experiments were performed to further isolate thermal effects as the source of the problem. We adjusted the heating scheme so that the PTFE inlet tubing was heated while the water jacket was used; this change did not improve the noise problem. The entire water jacket, containing the column, was immersed in the water bath, and the noise persisted as long as water was circulating through the jacket. But when the flow through the water jacket was stopped, the noise also stopped, leading us to believe that the temperature of the water flowing into the jacket was varying slightly. This variation seemed possible because the water was drawn from the bath close to the heating element. We moved the water inlet from the end of the jacket to the top of the jacket. In this configuration, water flowed through the jacket in the same direction as the mobile phase, rather than entering at the column outlet end of the jacket and flowing in the opposite direction. This configuration improved the noise but did not eliminate it. In another experiment, we added a thermal time constant to the system by directing the flow of the heated water through a 1-L flask equipped with a stirrer before the water was pumped into the jacket; the noise disappeared. This configuration was left in place, and the LC system was put back in service. Finally, we observed that the frequency of the baseline noise corresponded to the cycle time of the heater (30 s/cycle).

CONCLUSION

We concluded that the baseline of Figure 1a was a temperature-related phenomenon originating from the proximity of the water-jacket inlet to the water-bath heating element. The appearance of this thermal noise differed from one detector to another. The third detector, which did not show the problem, had a tapered detector cell. The tapered-cell configuration reportedly reduces refractive index effects, such as those caused by temperature fluctuations. The lack of improvement in the baseline, even when the column jacket was immersed in the water bath, may have been a result of poor heat-exchange efficiency through the PTFE tubing. Decreasing the flow rate probably increased the heat-exchange efficiency, thus explaining the reduced noise at the lower flow rate. Finally, this example illustrates the difficulty of isolating thermal noise problems, especially when similar symptoms might be expected from electronic noise, which can be generated by a heating bath.

Editor's note: *This article serves as a good example of the fax machine's power to shrink the world. From my office in Oregon, I was able to work with the authors in Belgium to help track down the source of their problem. The total elapsed time was about two weeks, thanks to several fax exchanges that eliminated perhaps a month or more of time that would have been wasted had we relied on the postal services.*

This scenario also emphasizes a theme of my LC troubleshooting short courses: when you have a problem, don't be afraid to ask for help. When I first heard from the authors, they had rigorously performed many of the tests discussed above, but they had run out of ideas. Too often, we all fail to take advantage of available outside resources, such as instrument manufacturers. Some manufacturers advertise an "LC hot line" dedicated to solving field problems, but if you call and ask for "technical support," you'll get the same service. For the cost of a phone call, you'll talk to someone who has extensive knowledge of the particular piece of hardware. These experts also have contacts with numerous other users, so they can often share special tricks that will help you get your instrument back up and running. Sometimes the problem is solved by a free software or hardware update (that you would have received automatically had you remembered to send in the registration card for the instrument). Also, feel free to contact me (c/o LC•GC*) with your LC problems. I'll answer your inquiry promptly, even though it may not appear in the magazine for several months.*

"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. *Correspondence concerning this column can be sent to "LC Troubleshooting,"* LC•GC, *P.O. Box 10460, Eugene, OR 97440, USA.*

Bulletin

Microchemistry award recipient. Professor Peter W. Carr (University of Minnesota, Minneapolis) is the recipient of the American Microchemical Society Benedetti-Pichler Memorial Award for Microchemistry. Professor Carr will receive the award at the 29th Eastern Analytical Symposium (EAS), scheduled to be held 12–16 November 1990 in Somerset, New Jersey. Professor Carr is a member of the Editorial Advisory Board of *LC•GC*.