LC Troubleshooting

Aggressive Mobile Phases — A Case Study

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When two failures occur at nearly the same time, troubleshooting can be confusing.

When we talk about liquid chromatography (LC) troubleshooting, we often use five rules of thumb as reminders of proper troubleshooting techniques (1). The first of these, the Rule of One, tells us to change just one thing at a time when trying to fix a problem. This process helps to clearly identify a cause-and-effect relationship between an attempted fix and its result. Implicit in the Rule of One is the assumption that each problem is caused by one fault in the system. For the most part this assumption holds true. Sometimes, however, more than one part fails, either simultaneously or nearly simultaneously. When this happens, as in the case presented in this month's installment, the second problem is at first assumed to be another manifestation of the first problem. This confusion can delay a timely solution.

THE METHOD

We were using a method for separating proprietary oligonucleotides on a polymeric resin-based anion-exchange column. Solvent A was 10 mM sodium hydroxide and solvent B was 10 mM sodium hydroxide and 2 M sodium chloride. We ran a gradient of 75–100% B over 10 min and held at 100% B for a few minutes to ensure elution of the entire sample. Allowing time for column reequilibration, we could perform runs on a 30-min cycle using an autosampler. We operated the column at ambient temperature with a flow rate of 1 mL/min and detection at 260 nm. The sample size (40 μg in 20 μL) was reasonable for these conditions. We flushed the system with water at the end of each day's use.

PRESSURE PROBLEMS

After using the method for a couple of weeks, we began to see problems with the system pressure. We observed pressure increases of several hundred psi over the course of a day's operation. When we inspected the frit in the in-line filter, we found black particulate matter. We replaced the 0.5-μm frit in the in-line filter, and the pressure returned to normal but began to increase immediately.

At first we suspected the sample was the problem source because the pressure increased only when the autosampler made an injection. If we indefinitely extended the isocratic hold at the end of the run or the equilibration stage just before injection, the pressure remained constant. Injections of mobile phase alone, however, provided similar results, so we eliminated the sample as the cause. Our final step was to bypass the injector by connecting the tubing between the pump and the injector directly to the tubing between the injector and the column. When the system was run in this configuration, the pressure remained constant except for the expected within-run changes caused by the gradient.

Those experiments clearly identified the injection valve as the problem source. We removed the valve from the autosampler and disassembled it. Close inspection of the rotor seal showed a very dull surface along the valve channels. At this point we began to wonder if the mobile phase we were using was responsible for this damage.

When we discussed this with a representative of the valve manufacturer (2), we found that the rotor seal in our valve is made of Vespel, a polyimide blended with graphite and a fluorocarbon. Vespel has an excellent track record as a rotor seal material with more than 10 years of use in this application, but it has some limitations. The valve manufacturer's and the Vespel manufacturer's literature (3) clearly state that Vespel is not recommended for use at pH ≥10 because of loss of mechanical properties. (In our case, the high pH apparently attacked the surface, and the rotation of the valve displaced some of this material, which then flowed downstream with the mobile phase. When the valve was not rotated, mechanical displacement did not occur, and the pressure remained constant.) For high-pH applications, Tefzel, a fluorocarbon blend, is much more stable. Its operating range is pH 0–14, compared with pH 0–10 for Vespel. Tefzel, however, is softer and thus wears faster, requiring more frequent valve maintenance.

For these reasons, LC system manufacturers often use injection valves with Tefzel rotors for LC systems that use high-pH mobile phases. For other applications, valves with the more durable Vespel rotors are used. A newer polymer, PEEK (polyether ether ketone), is now being used in many LC parts, including injection-valve seals. PEEK has the same broad pH stability of Tefzel and appears to be more durable. PEEK valve parts are used primarily in valves designed for use in biochemical applications. PEEK someday may replace Vespel for LC valve applications, but it has yet to establish a comparable track record, so the change will probably be made with caution.

High-pH mobile-phase attacks on Vespel are the most common pH problems encountered with this material. High-pH samples can also cause problems, even if they are used with mobile phases buffered to lower pH values. Usually we ignore the sample pH when using buffered mobile phases because the mobile phase quickly dilutes and buffers the sample after injection. If the injector is used in the over-filled loop mode, some of the high-pH sample remains in the waste line (and perhaps the needle port) after the sample is injected. Sometimes this amount is sufficient to cause local damage to the rotor seal where the waste line contacts the seal. In severe cases, the high-pH sample solvent can drill a shallow hole in the seal surface. For this reason, when the sample pH exceeds 10, it is important to flush the valve with water or another suitable solvent after each injection.

We replaced the Vespel rotor seal with a

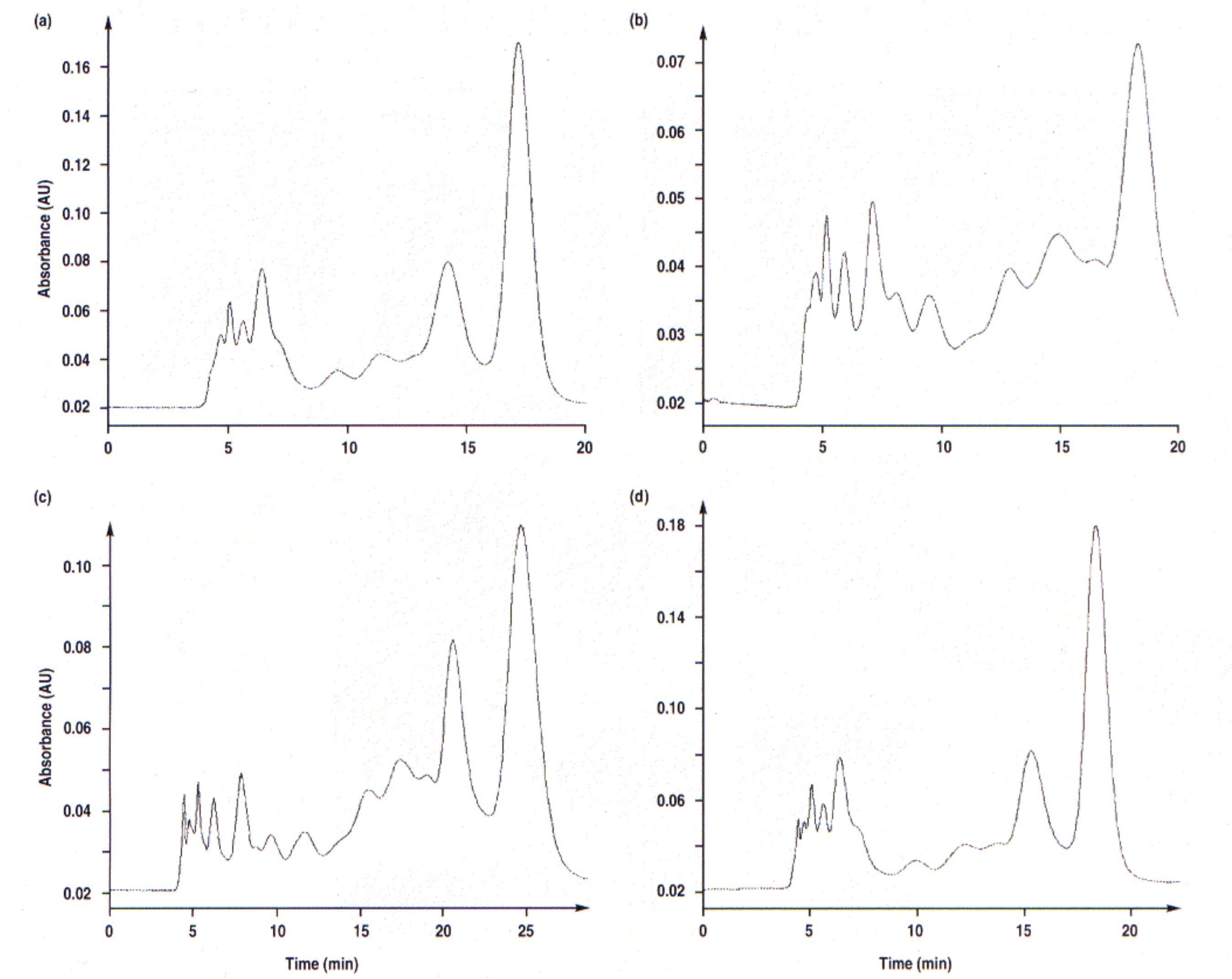

FIGURE 1: Chromatograms of an oligonucleotide sample obtained (a) under normal conditions, (b) with the same sample 7 h (14 runs) after (a), (c) after transferring the mobile phase, column, and sample of (b) to a second LC system, and (d) with the original system after replacing the mobile phase.

Tefzel one, and the pressure problem disappeared. Note that valve manufacturers use various materials for their rotors. The Rheodyne (Rheodyne, Inc, Cotati, California) valve in our system comes with a Vespel rotor seal as standard equipment. Valco's (Valco Instrument Co. Inc., Houston, Texas) LC valves use a carbon-filled PTFE rotor seal with a wide pH range; Valco uses Vespel in their high-temperature GC valves. If you have a question about the composition of your injection valve, look in the owner's manual or check with the manufacturer.

RETENTION DRIFT

Soon after we solved the pressure problem, we encountered a problem with drifting retention times. Normally the chromatograms looked like the one in Figure 1a, with run-to-run retention time reproducibility of ±2% or less. During one day's operation, however, we saw a dramatic shift in retention, as shown in Figure 1b. Note that the 18.2-min peak in Figure 1b is the same compound as the 14.2-min peak in Figure 1a. After 7 h (14 samples), retention had increased by almost 30%. First we checked the obvious problem sources. The pressure was fine, so this new problem appeared to be unrelated to the previous one. A leak or air in the pump would cause a symptom of low pressure, so these causes did not seem likely. Just to be sure, we measured the flow rate at the detector exit, and it was normal. Temperature changes can cause retention drift but generally not of the magnitude we observed. We operate our column at ambient temperature, and the temperature in the laboratory is steady.

Retention times can shift dramatically with changes in mobile-phase composition, so we pursued this avenue next. Selective evaporation of one mobile-phase component can cause retention drift, but we felt this was unlikely in our case — both the A and B solvents were aqueous, and the reservoirs were sealed. At this point we suspected a problem with the low-pressure solvent-proportioning equipment on our LC system. A malfunctioning proportioning valve or a partially blocked solvent inlet line frit can drastically modify the mobile phase. We first replaced the sinker frits in the solvent reservoirs. This change did not alter the system performance, so we eliminated blocked inlet frits as the problem source.

Our next task was to check the solvent-proportioning system. The easiest way to check for problems is to exchange the reservoirs and adjust the gradient program so that a B-to-A gradient is used rather than an A-to-B. If the proportioning system is at fault, the retention times should be dramatically different when the reservoirs are switched. But in our case, the chromatograms looked the same in each case. The next day we moved the column, solvents, and the sample to a nearly identical LC system. We were sure that this would isolate the problem, but we observed similar results, as can be seen by comparing Figure 1b with Figure 1c (note that the time axis is different in Figure 1c). Finally, we prepared new solvents and obtained the results shown in Figure 1d — the system returned to normal operation.

At this point we noticed that the original bottle of B solvent seemed to be less depleted than that of the A solvent, even though the B solvent was being used at a much higher rate. Was it possible that solvent was leaking back into the reservoir? We checked this by running the gradient using the A and C reservoir lines and leaving the B solvent line in an empty reservoir. Sure enough, we observed liquid leaking back into the B reservoir even though the B solvent was not included in the gradient program. We disassembled the proportioning manifold and found that the B poppet valve was visibly abraded and distorted compared with the other poppets. We replaced the defective poppet valve and the system performance returned to normal. We can't say for sure that the mobile phase was directly responsible for the proportioning valve failure, but high-pH and high-salt mobile phases do cause considerably more mechanical wear in LC systems than do more traditional mobile phases. We now take extra care in washing the salts out of the system at the end of each day's use.

CONCLUSION

What can we learn from this case study? First, we saw that the Rule of One really works. By isolating both of the problems in a stepwise manner, we efficiently identified the failed part. Second, when we change from traditional reversed-phase LC to a less used mode, we can expect additional problems. Third, flushing salts from the system remains important.

Should we have expected to avoid these problems by paying closer attention to the system limitations? Technically, yes, but from a practical standpoint, probably not. Unless you are endowed with an exceptional memory, it is unreasonable to expect to know all the limitations of an LC system under various conditions. And it is unreasonable to review all the product literature each time a new method is used. But before starting it is important to review the method carefully to see if specific conditions must be followed or avoided. And, yes, our method now includes a caution about using a Vespel valve rotor and recommends a thorough system flush each day.

REFERENCES

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