LC TROUBLESHOOTING

Electrochemical-Detector and System Pressure Problems

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This month, an expert sheds some light on problems with reference electrodes used with electrochemical detectors, and a reader's report of a system pressure problem and its solution provide a good example of effective step-by-step problem isolation.

Problems with electrochemical detectors can seem especially puzzling because these detectors are not as well understood as other liquid chromatography (LC) detectors. This month, we look at a reader's problem with the reference electrode in an electrochemical detector. Only a few sources can cause problems of this sort. A second topic concerns the isolation of the cause of high system pressure.

ELECTROCHEMICAL-DETECTOR PROBLEMS

Q: We have been using an electrochemical detector for several years and have had few problems running it. During the past few months, however, the Ag/AgCl reference electrode goes dead after a few days in position.

For several years we have used a mobile phase of 2.7 mM sodium octylsulfonate, 20 mM dibasic potassium phosphate, 80 mM monobasic potassium phosphate, 2 mM EDTA, 25.8 mM hydrochloric acid, and 5% ethanol in distilled water (pH adjusted to 3.0). We allow the mobile phase to recycle for one week using flow rates of 0.3 mL/min at night and 1.0–1.5 mL/min during the day. The 3-µm d_p C8 column that we use is thermostated to 26 °C, and the detector is maintained at ambient temperature (24–26 °C).

The electrode problem began before we started using a column heater but lately seems to be worse. We work around the problem by soaking several reference electrodes in 3 M sodium chloride between uses; after several days of soaking, the electrodes are as good as new. We often observe a bubble in the reference cell housing, and removing the bubble restores electrode function. At other times, there is no bubble and the reference is dead. How can we correct this problem?

JWD: When I was getting started in LC, the most popular electrochemical detector was a $150 kit from Bioanalytical Systems (West Lafayette, Indiana). Electrochemical detectors have come a long way since then, but my skills in troubleshooting them have not. For this reason, I've asked an expert in the field, Dr. Peter Kissinger of Bioanalytical Systems, to respond to this question and to explain how to fix several commonly encountered problems with reference electrodes.

PK: It is unclear what the reader means by saying that the reference electrode is "dead." Knowing what indicates that electrode failure has occurred or is imminent would be helpful. Does the signal go off scale in a positive or negative direction? Is the failure gradual or sudden? Some things can — but rarely do — go wrong with reference electrodes in electrochemical detectors. Because the failure mode for the present problem is not specified, I list some possibilities.

- The electrode may have developed an open circuit; that is, it is no longer connected to the electronics because of a problem with a connector or cable. This situation usually results in an open-feedback loop that drives the auxiliary electrode potential to a high value and imposes very high currents on the working electrode. Such a condition is very deleterious to analyses using LC with electrochemical detection. In most cases, the working electrode must be cleaned, polished, or both before restarting the detector.
- The impedance may have become extremely high because the conductivity of the salt bridge frit, which typically is made of porous ceramic or Vycor glass (Corning Glass Works, Corning, New York), has become very low. Possible causes include an insufficient concentration of ions in the frit or a gas bubble sitting on the frit or otherwise blocking the pathway from the reference electrode compartment to the mobile phase. By moving around or responding to pressure changes, such bubbles can cause substantial baseline noise. Degassing the mobile phase beforehand often eliminates bubbles; however, helium sparging at a slightly elevated temperature is the most effective procedure. Vacuum degassers also work but are less effective. Using a very high percentage of organic solvent in the mobile phase can cause gel or salt to precipitate and clog the frit. In such cases (for example, in normal-phase LC), use a reference electrode with a nonaqueous filling solution. In rare instances, bacteria can grow on the frit, but I have never heard of bacterial growth completely inhibiting electrode function.
- The salt filling solution or gel may have been depleted of the proper concentration of electrolyte (in this case, chloride). Electrolyte depletion causes the reference electrode's potential to drift, but very slowly; it should not cause a sudden failure.
- An ionic conductance pathway may have developed between the reference-electrode contact pin and other parts of the detector cell because the reference-electrode cap is wet with mobile phase. In effect, this can short out the reference electrode. This problem can easily be avoided by keeping the electrode cap dry.
- The input stage of the follower amplifier that monitors the reference-electrode potential may have been damaged and is drawing current from the reference electrode, which in turn causes the interfacial potential to move far from its zero-current equilibrium position. This has been known to occur but is very unlikely to be the cause of the present problem.

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One solution to the reader's problem is to use a "pseudoreference electrode" rather than a true thermodynamic reference with a controlled filling solution. One can use a bare wire of platinum, palladium, silver, or a redox polymer coated on a carbon or metallic conductor. Such electrodes may be used in direct contact with the mobile phase without using a frit. The electronics continue to function, and the problems associated with the frit junction will disappear. On the other hand, the applied potential will not be comparable to data obtained using cyclic voltammetry and polarography, and the reference potential will vary somewhat (dramatically in some

cases) with mobile-phase composition. The theoretical and technical details of reference electrodes are outside the focus of this column. References 1 and 2 explain how the three-electrode (reference, auxiliary, and working) system functions and what role the reference electrode plays.

CASE STUDY: HIGH SYSTEM PRESSURE

JWD: A reader submitted the following case study, which illustrates the procedure for isolating a pressure problem (3).

> ***Besides being time-consuming and potentially dangerous, sonicating the frit provides no guarantee of removing trapped contaminants.***

Reader: One Monday a few weeks ago, I started my LC system after it had been shut down over the weekend. The pressure initially was twice what the final value had been on Friday, and it remained elevated. The LC system uses a 25 cm × 4.6 mm C18 column packed with 5-μm particles. Between the column and the sample-injection valve is a 3-in. guard column that contains pellicular C18 packing. A 10-in. length of $^1/_{16}$-in. o.d., 0.010-in. i.d. stainless steel tubing connects the injection valve to the guard column, and a 2-in. length of tubing connects the guard column to the analytical column. I was using a mobile phase of 10:0.5:89.5 (v/v/v) methanol–tetrahydrofuran–50 mM phosphate buffer (pH 3.8). A length of PTFE tubing connects the mobile-phase reservoir to the pump, and the end of the tubing in the mobile-phase reservoir is fitted with a 10-μm porosity inlet filter. I filter the mobile phase through a 0.45-μm porosity membrane filter before placing it in the reservoir.

To isolate the cause of the sudden pressure increase, I first prepared and filtered fresh mobile phase but obtained no improvement. Next, I disconnected the guard column from the system, and the pressure decreased to its usual level. Based on this result, I concluded that one or both of the frits in the guard column were plugged, so I removed them and sonicated them in 6 N nitric acid. Sonication, however, failed to resolve the problem, so I replaced both the upper (5-μm) and lower (2-μm) frits, but to no avail. I then repacked the guard column, but the problem persisted. The only option left was to change the short length of tubing between the guard column and the analytical column. Replacing this unit resolved the problem. Before the pressure problems occurred, I had left the short length of stainless steel tubing connected to the lower end of the guard column each time I disconnected it to expedite disconnecting and connecting it to the analytical column.

The obstructing particle must have come from the guard column because, had it originated in the mobile phase, it would have had to have passed through four filters.

JWD: You have clearly demonstrated the step-by-step problem-isolation procedure. By starting with the most probable source and working to the least probable, you increased your chances of isolating the problem quickly. Another procedure that many workers find fruitful is to loosen each of the system's connecting-tube fittings in a stepwise manner, working upstream from the detector. When the fitting just upstream from the blockage is loosened, the pressure will return to the expected level. Because this procedure usually is performed with the pump running, take extra care with eye protection — a small squirt of mobile phase could cause eye irritation or damage.

You mention sonicating the frits to clean them. Some workers find this effective, but I recommend replacing a questionable frit with a new one. Besides being time-consuming and potentially dangerous (I like to stay away from nitric acid — it tends to eat my lab coat), sonicating the frit provides no guarantee of removing trapped contaminants. If the contaminant is not removed during sonication and the frit is installed in the reverse direction (you can't tell the top from the bottom in most cases), the contaminant can be washed downstream when the LC system is restarted. If you do sonicate the frits, clean each frit separately in a marked beaker so you can keep their identities clear. The guard column under discussion here uses two frit sizes; installing the larger one on the outlet could result in guard-column leakage. Frits cost only about $4 each, so replacing a frit when blockage is suspected generally is more economical than sonication.

A final comment on this method has nothing to do with the problem at hand, but relates instead to the buffer. Buffering capacity decreases as the pH diverges from a buffer's pK_a. As a general rule in LC, buffers should be used within ± 1 pH unit of the pK_a. For phosphate, which has pK_as of 2.1, 7.2, and 12.3, the pH of the present mobile phase (3.8) falls outside the desired range. Better choices are acetate (pK_a = 4.8) and citrate (pK_a = 3.1).

REFERENCES

(1) A.J. Bard and L.R. Faulkner, *Electrochemical Methods* (John Wiley, New York, 1980).
(2) P.T. Kissinger and W.R. Heinman, *Laboratory Techniques in Electroanalytical Chemistry* (Marcel Dekker, New York, 1984).
(3) A.H. Anton, personal communication, 1991.

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