LC TROUBLESHOOTING

Passivation of Liquid Chromatography Components

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This month's installment of "LC Troubleshooting" is a guest contribution that explains the requirements and procedures for stainless steel passivation. Ronald Shoup received his Ph.D. in analytical chemistry from Purdue University and is vice president for research at Bioanalytical Systems, Inc. (West Lafayette, Indiana). His research interests involve the miniaturization of liquid chromatographic systems for coupling to in vivo microdialysis, new detector technology, and selective extraction schemes for trace analysis. Michael Bogdan received his M.S. in analytical chemistry from Pennsylvania State University and is director of marketing at Interactive Microware, Inc. (State College, Pennsylvania). His interests involve automation of chromatographic techniques and training chromatographers in newly developed instrumentation and methodologies.

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Nearly all liquid chromatography (LC) systems rely on stainless steel construction for high-pressure connections and flow paths. Of the possible "inert" materials, stainless steel is one of the few that satisfy the criteria of cost, machinability, strength, and availability. Although plastics are now being used in moderate-pressure applications, they cannot be used in applications in which oxygen must be avoided. This month's "LC Troubleshooting" details the passivation procedures used to prepare stainless steel LC components that are used with electrochemical (EC) or UV detection systems.

WHAT IS PASSIVATION?

A metal may be designated "passive" if it corrodes at a very low rate during exposure to its chemical environment. Corrosion (the loss of metal to the environment by chemical attack) can be classified as "uniform" or "galvanic." We will focus on the latter type of corrosion (for a good review on corrosion technology, see reference 1).

Galvanic corrosion occurs when dissimilar materials in contact with one another create "batteries" of electromotive force that can ruin exposed surfaces. Even within an apparently homogeneous alloy of stainless steel (for example, Stainless 316) many such batteries may exist — principally at microcrystalline grain boundaries not visible to the unaided eye.

These boundaries are formed during creation of the bulk alloyed metal and are usually accentuated by the machining processes used to create the finished part. Where the tool cuts the metal, localized "hot spots" occur. The alloy can thus become heterogeneous at the atomic level. Surface crevices no longer resemble the parent alloy and can be attacked preferentially by corrosive chemicals. This intergranular corrosion can be attenuated by annealing the fabricated parts in a hot reducing atmosphere, a process that can redissolve the boundaries and recreate a uniform alloy.

Passivation of stainless steel — immersion of the component in an oxidizing medium such as nitric or chromic acid — results in high corrosion resistance, although corrosion is not eliminated. Technically, the purpose of the acid is to clean the steel by removing iron atoms, inclusion impurities, and other contaminants that may serve as initiation sites for corrosion. Once clean, an active metal surface spontaneously becomes passive in air or in oxygen-containing solutions.

WHY IS PASSIVATION NECESSARY?

Erosion, pitting, and crevice formation diminish the integrity of LC systems. The elimination of unalloyed iron is critical for successful LC–EC work. If iron is not masked by chelating agents such as ethylenediaminetetraacetic acid (EDTA), reactions such as

$$Fe^{2+}\ (aq) \rightarrow Fe^{3+}\ (aq) + e^{-}$$

can occur at the anode and cause prohibitively high detector background signals. Cleaning with oxidizing acids removes these pockets of unalloyed iron and allows the new oxide layer to form simultaneously.

Certain components are much more susceptible to corrosion than others. For example, we avoid tube fittings, including many types of compression-fitting ferrules, that have been "nitrided" for hardness. Corrosion-prone areas can easily be seen by immersing the ferrules in dilute HCl or HNO_3.

A typical symptom of corrosion is a slowly climbing background current with an induction period of about 24 h. In the case of ferrule corrosion, this is apparently the time needed for the mobile phase to diffuse into the ferrule zone and then diffuse back out into the flow stream. In most LC systems, these cause-and-effect relationships are hard to establish because ferrules are not directly in the flow path. Nevertheless, replacing the ferrules with nonnitrided components eliminates the problem. Because of their high surface areas, frits for columns and gas sparging also readily release trace amounts of iron. For this reason, the use of titanium frits is recommended.

In LC with UV detection, irregularities in an already increased background signal can be attributed to UV absorption of soluble greases and other contaminants. In this case, organic inclusions arise from the extrusion process used in manufacturing stainless steel tubing or during the machining of other parts. These inclusions must be eliminated before high-sensitivity LC–UV work.

A system that has been neglected or left inoperative should be returned to service only after careful attention to passivation.

HOW TO PASSIVATE LC SYSTEMS

Passivation can be performed in three basic steps: first, cleaning and degreasing; second, passivation; and third, reequilibration. The column and detector can be cleaned and degreased, but they should be removed before passivation. You may, however, wish to passivate the transfer line between the column exit and the detector. Also, leave the sample injection valve in the *inject* position so that the sample loop is passivated.

Cleaning and degreasing: The first step is to make sure that the LC components are capable of being wetted by the aqueous acid to be used. This is done by flushing the system of all remaining buffer salts with deionized water, followed by an increasing concentration of methanol. Then flush the system with a nonpolar solvent such as CH_2Cl_2 or hexane to remove grease in order to expose the unpassivated stainless steel; methanol alone will not do an effective job. (This especially applies to newly dedicated UV systems.) Next, reverse the procedure, ending with water. At this point, all components should be free of buffer salts, greases, CH_2Cl_2, and methanol. Recommended minimum volumes for cleaning and degreasing are shown to the right.

Passivation: The second step accomplishes the actual passivation, as detailed to the right. Because 6 N HNO_3 is pumped through the system, take care that no splashes or leaks occur, and be sure to wear eye protection. After the acid treatment, use at least three separate water rinses to remove all traces of HNO_3 from the system. Also give particular care to rinse headspaces where HNO_3 vapors can accumulate. You can easily confirm that the HNO_3 has been flushed through the system by checking the pH of the effluent. When it is about the same as the pH of the fresh rinse water, satisfactory flushing has been completed.

Reequilibration: The final step prepares the LC system for subsequent equilibration with the mobile phase. The column and the detector are connected and cleaned with 100% acetonitrile. This step is necessary for many hydrophobic stationary phases because variable wetting of the stationary phase by the mobile phase will cause variable elution times.

The final step is actual equilibration with the desired mobile phase. If the high background signals in the EC detector were caused by trace iron, a reduction in the background current will be observed. If the high background signal in the UV detector was the result of organics left by the machining or extrusion process, a similar drop in detection signal, as well as improved baseline stability, will result. If the high background signal in the EC or UV detector still persists, then the problem remains with the components of the mobile phase, the column, or the detector itself. The cause can be found by substituting each component separately.

Passivating an LC System

Cleaning and degreasing:

1) Flush the system with
   a. >30 mL 100% H_2O (remove buffer salts)
   b. >30 mL 50% MeOH (clean and degrease)
   c. >30 mL 100% MeOH (clean and degrease)
   d. >100 mL 100% CH_2Cl_2 or hexane (degrease)
2) Reverse the procedure, flushing with each solvent, ending with 100% H_2O
3) Remove the column and detector before proceeding to the next part

Passivation:

Flush the system with
   a. >30 mL 6 N HNO_3 (passivation)
   b. >100 mL 100% H_2O (remove acid); use three separate washes

Reequilibration:

1) Reconnect column and detector
2) Clean the system with >50 mL 100% ACN
3) Equilibrate with mobile phase

REFERENCE

(1) *Corrosion Causes and Control* (Carpenter Technology, Reading, Pennsylvania, 1979).

"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.