TROUBLESHOOTING

Optical Detectors Part I: General Principles

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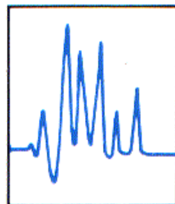

This article is the first in a series on troubleshooting for LC detectors. We will discuss some of the general troubleshooting techniques used for nearly all optical detectors. Future columns will cover specific problems that occur with UV, fluorescence, refractive-index, and electrochemical detectors. Readers are invited to contribute their troubleshooting tips to this column or to submit topics or questions for discussion in future articles. Write to: The Editor, *LC* Magazine, P.O. Box 50, Springfield, OR 97477.

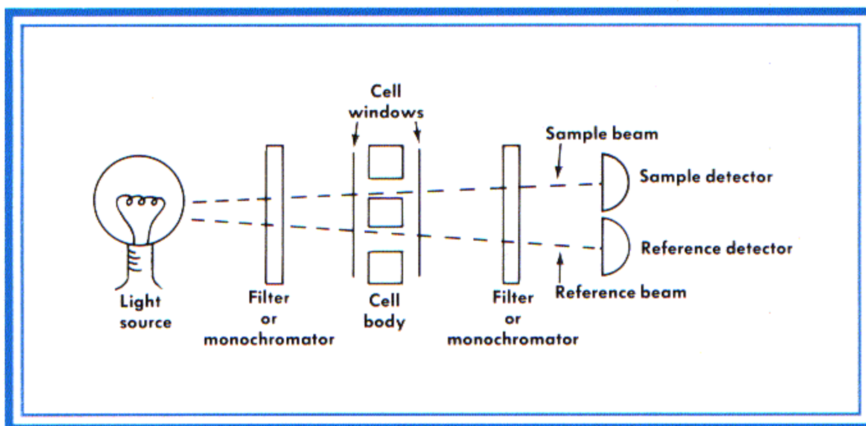

FIGURE 1: Generalized optical detector.

OPTICAL DETECTOR COMPONENTS

The most popular detectors used in liquid chromatography today measure the optical properties of the sample in the presence of bulk mobile phase. The block diagram in Figure 1 shows the major components of the optical detector. The optical source has a spectrum of wavelengths of light available for use in the detector. The sources may emit intense lines at specific wavelengths, such as the 254-nm Hg line commonly used in fixed-wavelength UV detectors, or a continuum of light over a broad range, such as that emitted by the deuterium lamp used in variable-wavelength UV detectors or by the tungsten lamp used in refractive-index (RI) detectors. There are also specialty lamps with wavelengths desirable for particular applications, such as the zinc lamp used for 214-nm detection or the variety of fluorescent tubes used in filter fluorometers.

The next component in the optical path is a device that selects a specific wavelength. A monochromator is used in spectrophotometric UV and fluorescence detectors, or a bandpass filter is used in fixed-wavelength UV and fluorescence detectors. When the filter is mounted directly next to the detector cell, stray light is minimized.

Following the optical path, the next part of the detector is the detector cell itself. For the UV detectors, this component is usually a block of stainless steel or fluoropolymer with holes drilled through it. Quartz windows are mounted on the two ends of the cell, and appropriate provisions are made for connecting the column to the cell. The windows may be either flat plates or lenses that focus the light into and out of the cell. Fluorescence detectors for the most part use right-angle detection techniques; the cell is therefore constructed of a piece of quartz tubing to allow the emitted light to be collected at right angles to the excitation light. Light emitted from the detector cell then passes to the detection element. A filter may be mounted in this part of the optical path to minimize interfering light or to select the desired wavelength, as in fluorescence detection.

The detector element is usually a photodiode, or pair of photodiodes if a sample and reference signal are measured simultaneously. In some detectors, photomultiplier tubes are used. These tubes are easily damaged by excess light and are less rugged than photodiodes. Diode-array detectors have a row of 256 or more diodes for detection of specific wavelengths of light. The electrical signals from the detectors are usually amplified to provide output to the recording device.

Most detectors have two different output signals — computer and recorder signals. The computer signal is generally about 1 V per absorbance unit (AU) for UV detectors and is unattenuated; that is, it is not affected by the range setting on the front of the detector. The recorder signal is typically 10 mV full scale, where the full-scale value is defined by the range setting selected by the user. Solving electronic problems with the detector output is usually beyond the capability of the user and requires a service call. Before requesting a service call, you should check to see that the proper output channel has been selected and that the signal cable is connected properly. Substituting a signal cable that is known to be good for a questionable one should allow you to detect a faulty cable.

HEAT EXCHANGERS

Although an optical detector may consist only of the components in the optical path and a means of getting the sample into and out of the cell, most detectors have ancillary components to enhance the detector's performance. The most important of these are heat exchangers. Nearly all optical detectors are sensitive to temperature fluctuations that result in changes in the refractive index of the mobile phase as it passes through the detector cell from the column.

Some UV detectors have tapered or stepped internal chambers that may compensate for part of the refractive-index effects. Because temperature affects refractive index, RI detectors are the most susceptible to this problem.

For UV detectors, the heat exchanger often consists of a mass of metal that forms the cell, so that effluent stays at a relatively constant temperature. Tubing, typically of 0.010-in. i.d. and 10–100 cm long, is wrapped around the metal cell housing and coated with a heat-transmitting material. Most RI detectors use a heat exchanger immersed in an externally controlled constant-temperature water bath kept 10–20 °C above room temperature.

For maximum temperature stabilization, the heat exchanger should provide for enough mixing so that all the liquid has adequate contact with the thermal mass for temperature stabilization. The heat exchanger can be detrimental to chromatographic performance because added volume in the system can reduce chromatographic efficiency. Consequently, a compromise is made between refractive-index effects and band-broadening tolerances.

Two major problems with heat exchangers are blockage and band broadening. Blockage problems become increasingly common as the diameter of the heat exchanger tubing is decreased. Tubing of a smaller diameter is used to minimize extra-column dead volume, but it also increases the susceptibility of the tubing to blockage. Burrs left from tube-cutting operations and crimps in the tubing can reduce the effective diameter significantly from the specified 0.010-in. i.d. A bit of packing leaking from the column, a burr from a poorly cleaned piece of tubing, or a piece of polymeric ferrule can easily lodge in the heat-exchanger tubing and increase the back pressure or block the tubing completely. If the system pressure is high, and loosening the connection at the detector inlet causes the pressure to drop to normal, a blocked heat exchanger is the probable cause. Unfortunately, most detectors do not allow for replacement of the heat-exchanger tubing, so the entire cell assembly must be sent to the factory for repair or replacement. If you are lucky, cleaning or backflushing the cell will solve the problem, but a totally blocked cell is rarely salvageable.

Band broadening in the heat exchanger is another common problem, especially today when columns of decreasing size are being used and when equipment is being used for longer periods of time before being replaced. If you have converted to columns that give much smaller elution volumes — for example, a 100 mm x 4.6 mm column — you may encounter band-broadening problems with the detector. Measure the column efficiency according to the manufacturer's recommendations. If the resulting plate count is significantly below the expected value, replace the column with a standard analytical column, such as a 250 mm x 4.6 mm column, and repeat the evaluation. If the performance is good with the longer column, the detector may not be suitable for use with the smaller column.

With some detectors, you can reverse the flow direction by connecting the column outlet to the detector outlet and the detector inlet to waste. The detector outlet tubing is generally a short, direct path from the detector to waste. If the diameter is small enough, you may be able to eliminate effectively 10 cm or more of heat exchanger by this method at the expense of a slight elevation in the noise level. Careful jacketing of the transfer tubing between the column and detector will minimize temperature-related problems. In some detectors the outlet tubing may be 0.020-in. i.d. or larger, and the technique of flow reversal may not provide any advantage.

TIME CONSTANTS

The *time constant* of an LC detector refers to the electronic noise-filtering circuitry in the detector. Most often this is a simple resistor-capacitor circuit, but some units have more sophisticated active filters. Generally, two or three time-constant values are available and can be selected by a switch on the rear panel of the detector. Common values are 0.5, 1.0, and 5.0 sec. You will find that newer detectors, which accommodate microbore columns, will have lower time constants, perhaps in the 0.05-sec range, to allow for detection of narrow peaks. Higher time-constant values mean that more filtering will take place and, thus, a smoother baseline will be observed. Too much filtering, however, will tend to decrease peak heights, or will even filter out narrow peaks entirely from the chromatogram. For analytical work, selecting a time constant of 0.5 sec or 1.0 sec is usually adequate, although smaller values are needed for microbore work. If you notice peaks that are broader than usual, be sure to check the time-constant setting on the detector for the proper value. We know of at least one 2500-mile service call that was made only to find that the time-constant switch had been bumped from 0.5 sec to 5.0 sec when the recorder was connected.

NOISE DETERMINATION

As we have seen with other parts of the LC system such as the column, standardization is essential in determining whether a problem really does exist. Measurement of baseline noise is one of the key parameters used to standardize LC detectors. The simplest way to check the detector noise level is to operate the detector under defined conditions at the most sensitive range for 10–20 min, recording the detector output on a strip-chart recorder. Short-term noise appears as a fuzzy baseline. Measure the width of the baseline by drawing a line parallel to the detector trace and tangent to the tops and bottoms of the noise spikes on the trace. The distance between these two lines is then converted to absorbance units or refractive-index units for comparison with the manufacturer's specifications. *Drift* is defined as the slow movement of the baseline up or down and is measured as change in absorbance units or refractive-index units per hour or per degree Celsius.

The conditions used most frequently to measure detector noise are either having the detector cell filled with dry nitrogen or with air; the operator's manual should specify the proper conditions. If the observed noise is within a factor of two of the manufacturer's specification, the detector is probably operating properly. As with any standardization method, you should test the detector noise when the instrument is new, record the value in your logbook, and keep the trace. It is good practice to check the noise level again whenever detector service, such as cell cleaning or lamp replacement, is performed.

AIR BUBBLES

Another problem commonly encountered with all detectors is bubble formation. Symptoms can be an off-scale signal if a bubble becomes lodged in the cell, or very sharp spikes in the chromatogram if a train of bubbles forms in the effluent. Bubble formation in detectors is encouraged by the presence of sharp corners in the flow path that act as nucleation sites. Bubbles caused by mixing two air-saturated solvents together pass through the column in solution, but upon reaching the low pressure in the detector cell, the bubbles come out of solution and stick in the cell. Another contributing factor to bubble formation is the heating of the detector cell by the source lamp.

A bubble can usually be removed from the detector cell by increasing the flow rate or momentarily blocking the outlet tubing of the detector. In more persistent cases, you may need to pump thoroughly degassed mobile phase through the cell or change to a mobile phase that has very low surface tension, such as methanol.

After ensuring that the problem is not caused by leaky fittings, there are two simple methods that can be used to eliminate bubble formation in the detector cell. The first is to degas the solvents. Chromatographers who routinely degas solvents prior to use have significantly fewer problems with bubble formation in all parts of the system. The second method is to delay bubble formation until after the solution passes the detector cell by applying back pressure to the cell. Be careful not to exceed the pressure limits of the detector cell as specified by the manufacturer, or the cell may be damaged. A few inches of 0.010-i.d. tubing connected to the detector outlet often provide enough back pressure to prevent bubbles from forming in the cell. Other workers use 0.010-in. i.d. Teflon tubing as a waste line or crimp a Teflon outlet line in several places with a pair of pliers. Several LC supply vendors sell back-pressure restrictors that create 50–100 psi back pressure to prevent bubble formation in detector cells. Another means of providing back pressure is to place one or more used 5–10 μm guard columns at the detector outlet.

Do not confuse a droplet of immiscible solvent with a bubble in the cell. The symptoms are often the same: spikes in the chromatogram or a visible liquid bubble in the cell. If the system is not completely rinsed between runs with two immiscible solvents by using a miscible intermediate solvent, droplets of the first solvent may remain in the system for some time and bleed tiny droplets into the cell. If this problem is encountered, flush the system with isopropanol or some other solvent miscible in both organic and aqueous solvents.

ENVIRONMENTAL CONDITIONS

The operating environment of the detector can play an important role in its optimum operation. Baseline drift can often be associated with changes in the detector environment. Temperature extremes or changes, such as those caused by direct sunlight on a lab bench, may cause significant detector drift. You can often test a liquid chromatograph for temperature-related drift problems by placing a protective cover, such as a large cardboard box, over the unit to eliminate drafts. Liquid chromatographs operated in fume hoods are often subject to temperature-stability problems. One method to minimize temperature-stability problems when the environment cannot be controlled is to operate the column at a slightly elevated temperature, for instance at 35 °C, and carefully insulate all connecting tubing. This procedure controls the direction of temperature fluctuation and often provides for a more stable chromatogram.

The detector should be protected from drafts. Creating this situation may require instrument relocation or construction of air deflectors to prevent heating or air conditioning ducts from blowing directly on the detector. High humidity may create problems with electronic components, especially in a cold-room environment.

SUMMARY

We have seen that several different types of optical detectors for LC have components of similar construction. Troubleshooting, especially for the cell and related plumbing, is performed in a similar manner regardless of the detector type or manufacturer. The operator's manual will give many helpful hints specific to your detector. ASTM Publication E685-79(1) also provides a standard method for testing LC detectors. Careful record keeping in your logbook will enable you to track recurring problems and establish a performance level for your detector.

REFERENCE

(1) *Standard Practice for Testing Fixed-Wavelength Photometric Detectors Used in Liquid Chromatography*, ASTM Publication E 685-79 (ASTM, Philadelphia, 1979). ■

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