T R O U B L E S H O O T I N G

Optical Detectors, Part II: Fixed-Wavelength UV Detectors

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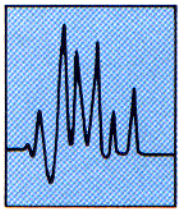

Perhaps the most common detectors used in liquid chromatography today are fixed-wavelength ultraviolet (UV) detectors, which measure either UV or visible light absorption. These detectors measure sample absorbance of one or more strong emission bands either from special lamps or phosphors that are irradiated by a 254-nm mercury vapor lamp source. The most common wavelengths used for detection are 214 nm with zinc lamps, 229 nm with cadmium lamps, 254 and 280 nm with mercury lamps, and 300, 313, 340, 365, 405, 434, 470, 510, 546, 578, 610, and 660 nm with mercury lamps that irradiate special phosphors. Lamps, filters, and phosphor combinations are designed to select a narrow, intense, and specific wavelength of light with minimal wavelength drift or miscalibration. The intense, narrow fixed wavelength of light provides maximum adherence to Beer's law. Thus, for a given cell path length, the photometer output (absorbance) is directly proportional to the sample size (concentration or volume) injected. Lamps have a relatively long lifetime, and detector costs are lower than those for spectrophotometers or UV/VIS detectors, which provide a continuum of light of which any wavelength can be selected. Variable-wavelength detectors will be considered next month.

DETERMINE THAT A PROBLEM REALLY EXISTS

The symptoms of a malfunctioning detector are limited. These may include the complete lack of a signal or a signal that cannot be brought on scale; peaks that are smaller than expected; a nonlinear relationship between peak area (or height) and sample size; a greater than expected baseline drift; and, finally, the appearance of noise. High-frequency short- or long-term noise may produce peaks and valleys that may be mistaken for peaks (1).

It is essential to know the optimum performance level of the detector to determine that a problem really does exist; then it is necessary to isolate the problem to the detector itself or to a system-detector interaction.

Establishing a set of typical chromatograms or calibration plots as described below, in which the optimum performance and noise level are estimated for the chromatographic conditions that you use, will make it much easier to determine whether your UV detector is operating properly. One method is to inject a minimum amount of sample at the most sensitive range and allow the baseline to run for 20–30 min with a well-equilibrated system. This scheme will allow calculation of the lower detector limit, baseline noise, and drift. Successive doubling of the sample mass injected to an amount at which peak height or area does not double will allow a practical estimate of the upper detection limit, linear range, and linear dynamic range.

Noise is measured as the width of the peak-to-peak baseline envelope and typically is 10^{-5} AU. Drift, the steady baseline movement up or down, is measured over 20–30 min and is typically 10^{-3}–10^{-4} AU/hr. The *lower detection limit* is defined as the mass of solute that provides a peak height equal to twice the baseline noise; the *upper detection limit* is the mass of solute that gives an absorbance reading that deviates from linearity by less than 5% (2). The *linear dynamic range* is defined as the ratio of the upper and the lower detection limits and usually is 10^3–10^4. Note that this number can be greatly increased with reduced noise. The *linear range* is the difference between the upper detection limit and the lower detection limit; at 254 nm, a typical value is 0.6–1.28 AU.

If a quick examination of the detector system using the following simple troubleshooting techniques does not reveal a problem, then it will be necessary to examine the detector more carefully to determine whether a problem exists. This examination is greatly facilitated if a standard chromatogram is available from the logbook for comparison. If solvent or column changes were made recently, detection problems may be traced to the system. If, for example, no signal is observed when a new solvent is tried, the solvent may not be transmitting light at the detection wavelength, or air bubbles may have been introduced during solvent changeover, thus blocking the detector window.

If a standard chromatogram is not available, it may be necessary to change the system to standard conditions of eluents and column to determine whether a problem exists. Shortcuts, such as substituting a functioning detector, are often faster. The standard conditions may be those that were originally provided with the instrument or those suggested by the column supplier. Standard samples are generally available from both sources.

At this point we will assume that you have determined that the detector or system-detector is not performing as expected based upon comparison with a known system.

SIMPLE TROUBLESHOOTING CHECKS

If a problem is apparent, first run through a checklist of these simple troubleshooting observations:

- Are the detector and recorder plugged in and turned on?
- Is the UV lamp on?
- Are the signal and ground cables properly connected?
- Is the detector head plugged in?
- Are the proper detector head, lamp, and phosphor in place?
- Is the control unit addressing the proper detector?
- Are the detector, printer-plotter, integrator, or recorder attenuations correct?
- Is the chromatograph running and connected to the proper detector without any leaks?
- Does the meter reading on the detector show the proper light-transmission and lamp energy?
- Does the meter on the detector indicate that a peak is coming through?
- Are the printer-plotter, chart recorder, and/or integrator operating properly?

ISOLATE THE PROBLEM

The photometer itself can be the cause of detection problems. An old lamp, dirty cell windows, or malfunctioning electronics can contribute to poor detector performance. Problems may also arise from the interaction between the system and the detector. These factors include high eluent absorbance, gas bubbles in the eluent or cell, eluent temperature or composition changes, and recorder/printer-plotter problems. It is critical in the troubleshooting procedure to determine whether the problem is originating from the detector itself or from the interaction between the system and the detector.

You might isolate the problem to the UV detector and not the system if a second detector, such as a variable-wavelength or RI detector, produces the expected signal. On the other hand, if a second UV detector (ideally, one that is identical to the instrument in question) is substituted in the system and produces the expected response, the problem is isolated to the first UV detector. In some detectors, component parts (such as detection heads or flow cells) may be readily substituted. Substitution may help to isolate a problem to a particular component.

We will now look in more detail at some typical symptoms, outline troubleshooting procedures for isolating the problem, and suggest remedies and preventive measures.

NO PEAKS, LOW PEAKS, OR REDUCED LINEAR RANGE

If simple troubleshooting checks reveal that the lamp is not lighting, then replacing the lamp is the first correction to be made. If, however, the lamp is on, but there are no peaks, peaks are lower than expected, or there is a greatly reduced linear range, then the problems are likely to arise from any of five causes: air in the cell, dirt on the cell windows, lamp aging, excessive eluent absorbance, or electronics failure. We will consider troubleshooting steps for each of these possible causes, from the easiest to the most difficult.

Air in the cell is detected either from the observation of bubbles in the effluent or in the cell itself, or from the observation of sharp spikes in the chromatogram. Procedures to eliminate the air were discussed in last month's column and elsewhere (1,3). This process may require solvent degassing, use of back pressure on the cell, or flushing the cell with another solvent.

If a weak or aging lamp is suspected, attempt to confirm the diagnosis by one of the following three methods. Many instruments provide a meter reading or plotting protocol to determine whether the lamp should be replaced. In addition, more modern instruments allow absolute absorbance through the cell (solvent, sample, or dirty windows) to be read directly. And, finally, some instruments have built-in calibration standards that can be rotated into the light beam to determine whether the detector is operating properly. These three capabilities and the possibility of viewing the cell can make troubleshooting very simple.

There are a few other means of checking for failure if your detector does not have these troubleshooting aids. Remove the solvent from the cell, blow the cell dry with nitrogen or dry air, and then check the noise of the system. If the noise is much more than two times the specified level, then the lamp should be changed. Or, if a highly absorbing solvent is suspected, measure solvent absorbance with a UV/VIS spectrophotometer at the wavelength you are monitoring. Finally, if the background absorbance seems high even with an empty cell, then the cell may be dirty. Visually inspect the cell for contaminants and use an acid cleaning procedure such as that recommended in the operator's manual. Note that acid or solvent cleaning will not remove silica or Teflon particles from a cell; cell disassembly is required to remove these particles.

If, after changing the lamp, checking the solvent absorbance, and cleaning the cell, you still observe a very low signal compared to a standard run in your logbook, contact the manufacturer for a possible service call.

DRIFT

Excessive drift of the baseline can be a problem, especially if low-wavelength or high-sensitivity detection is used. Many microprocessor-controlled instruments and some modern fixed-wavelength detectors will automatically reset the baseline to zero for each injection, making multiple unattended runs possible. Drift can be caused by a number of factors, such as insufficient instrument warm-up time.

Drift can come from chemical changes in the system, especially if low-wavelength or high-sensitivity UV detection is used. Slowly eluting UV absorbers used to pack a column or samples from previous runs can produce drift that lasts for several liters of eluent. Recycling gradients between weak and strong eluents and using eluents with scavenger agents such as triethylamine phosphate will speed equilibration and minimize drift. Follow the change in drift at a slow recorder speed to determine whether an absorber is the cause of the problem. UV contaminants eluting slowly from filter frits or gas bubblers can be especially insidious contributors to drift. It is safe practice to provide dedicated frits for each type of solvent; do not interchange them and discard them when they become blocked.

Changes in the bulk eluent can also cause drift. If solvents that consist of a mixture of weak and strong UV absorbers are rapidly purged with gas, preferential volatilization of one of the components will lead to drift. One safe method for preventing this problem is first to sparge the eluent rapidly in large, aluminum-foil-sealed containers with helium, thereby degassing them. Then maintain a slow flow of helium *above the solvent* to maintain helium saturation and to keep outside vapors from contaminating the solvent. Finally, mix the eluent with a magnetic stirrer to keep it homogeneous.

A problem often encountered in user-built systems is that dead-end components (such as Bourdon-tube pressure gauges or overpressure safety devices) can bleed material into the flow stream. If you encounter

this problem, carefully clean and flush these components, isolate them with a long narrow 0.010-in. i.d. tube, create a slight bleed through the Bourdon tube, or preferably, use a flow-through Bourdon tube or small-volume electronic pressure gauge to minimize this problem.

Environmental causes of drift were discussed last month in *LC* (3). Detectors or instruments located in direct sunlight or in an environment of changing temperature can lead to drift. To reduce or eliminate these effects, insulate the detector and shade the components.

NOISE: HIGH FREQUENCY

High-frequency noise causing a fuzzy baseline at high sensitivity can originate from the pulsations of the pump, from the detector electronics, from outside radio-frequency interferences, and from line voltage fluctuations. Often, this kind of noise is not a major problem, and simply by turning down the gain on recorders, using a resistance-capacitance filter, or using active filters, the problem can be minimized. You must ensure, however, that the decrease in response time does not affect apparent peak size. High-frequency noise can often be minimized by carefully grounding all components and matching the output impedance of the detector to the input of the recorder. If the cause of the noise is pump pulsations, these pulsations can be reduced with flow-through pulse dampeners (available from several vendors), Bourdon-type tubes (oval cross-section), or pressure gauges. If radio-frequency interference is suspected, a Faraday cage and thorough grounding of instruments may be necessary. Providing detectors with an isolated power line or a power-line stabilizer can minimize interference from other instruments that cycle on and off. The ultimate source of this noise is the detector and components themselves; the high-frequency noise level is being reduced continuously in modern instruments so that higher detection sensitivities can be obtained.

NOISE: SHORT- AND LONG-TERM

Short- and long-term noise that produces random or cyclic peaks and valleys is often of the same duration as sample peaks. This type of noise complicates detection and therefore quantification of peaks. The noise can originate from the cycling on and off of electrical devices such as oven heaters, refilling of slow-cycling pumps, cycling of the flow/feedback loop in some instruments, and poor mixing when two eluents are flow-mixed to create an intermediate composition (4), especially when high-sensitivity detection is used. It may also be necessary to apply some of the methods for eliminating drift and high-frequency noise to eliminate short- and long-term noise.

CONCLUSION

The common problems with fixed-wavelength photometric detectors originate from causes external to or inside the detector. Awareness of these problems and systematic, logical isolation of trouble spots enable rapid troubleshooting of problems that may arise in detector operation.

REFERENCES

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(3) J.W. Dolan and V.V. Berry, *LC* **2**, 290 (1984).
(4) V.V. Berry, *J. Chromatogr.* **290**, 143 (1984). ■

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.

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