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

Optical Detectors, Part III: Variable-Wavelength UV Detectors

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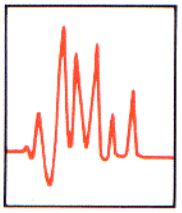

Recent surveys indicate that about 75% of the LC systems sold today contain variable-wavelength ultraviolet (UV) detectors (1). This dramatic reversal of the previous domination by fixed-wavelength UV detectors is attributed to the versatility of the variable-wavelength UV detector as well as to the improved sensitivity and reduced cost. Most chromatographers will sacrifice the order-of-magnitude loss in minimum detectability as compared to fixed-wavelength detectors for the ability to select the desired wavelength of detection.

DETECTOR TYPES

Variable-wavelength UV detectors fall into two categories: those that detect only ultraviolet wavelengths and those that detect ultraviolet and visible (UV/VIS) wavelengths. UV detectors usually have a detection range from 185–195 nm to about 350 nm. This range is extended into the visible region by UV/VIS detectors, usually to about 700 nm. Early designs incorporated a deuterium lamp for the UV region and a tungsten lamp for the visible region. Most of the popular detectors today use a single deuterium lamp for the entire detection range and rely upon the visible emission, albeit weak, from the deuterium lamp for the longer wavelengths. Two-lamp detectors either use a flip-mirror to change from UV to visible wavelengths in a manner similar to conventional UV/VIS spectrophotometers or direct the visible light through the deuterium source so that physical switching is not required. We will refer to all of these variations as variable-wavelength UV detectors for the following discussion, because the same troubleshooting procedures apply in most cases.

DETECTOR COMPONENTS

The optical path for variable-wavelength UV detectors is fairly simple (Figure 1). Ultraviolet light from the source is directed onto a diffraction grating that disperses the light into its various wavelengths. By proper positioning of the grating, a narrow range of wavelengths, from 5 nm to 10 nm in bandwidth, is directed to the detector cell. The light leaving the detector cell is collected at a photodiode and converted to an analog signal for output to the data-recording device. We can therefore divide the detector into discrete sections for troubleshooting purposes: light source, monochromator, cell, and photodiode and associated electronics.

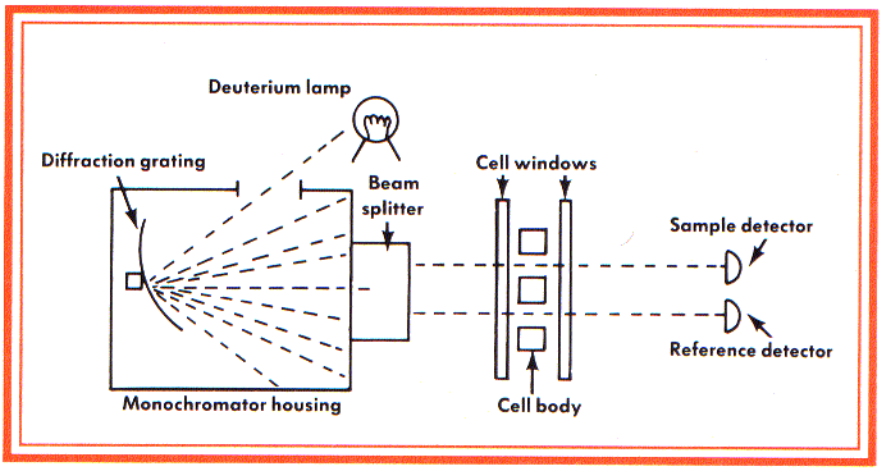

FIGURE 1: Optical path of variable-wavelength UV detector.

ISOLATE THE PROBLEM

When your detector is new, take reference data at standard conditions. These should be kept in your logbook with appropriate notations. The reference conditions should agree with the manufacturer's specifications. These tests are generally performed with a dry cell in a thermally stable environment and with a detector that has been on for a 30-min warmup period. Tests include measurement of short-term noise at 195 nm, 254 nm, and 350 nm (or as specified in the operator's manual), and drift for about 30 min. The noise, as was discussed in previous articles (2,3), is determined by drawing lines parallel to the top and bottom of the baseline peaks, measuring the perpendicular distance between the lines, and converting to absorbance units (AU). The best commercial detectors today have noise specifications in the range of $1–2 \times 10^{-5}$ AU. Drift, also discussed in previous columns, is the long-term wandering of the baseline and is usually measured in AU/hr, but is more properly measured in AU/°C. If the noise and drift values vary by more than about two times the specifications, then some corrective steps may need to be taken.

The first indication of detector problems will come not from a set of specifications tests, but from a deviation from normal operation. If you keep a regular record of operating conditions in your logbook, a problem will be readily apparent when noise spikes, excessive drift, or lowered sensitivity occur. At that point, you should isolate the problem to the detector by checking for obvious system problems such as leaks or column contamination, and then by checking detector operation under a standard set of conditions. If necessary, perform the noise and drift tests discussed above. One common problem that occurs when using detection wavelengths below 220 nm is changes in solvent absorbance, either with age or from lot to lot or vendor to vendor. If you perform analyses at low wavelengths

and high sensitivities, it is a good idea to keep a record of solvent lot numbers in your logbook for future reference. Finally, check recorder cables, time-constant settings, and range selection to ensure that the problem is not outside the detector.

OPTICAL SOURCE PROBLEMS

In contrast to fixed-wavelength UV detectors, in which the source seems to have an infinite lifetime, the deuterium lamp in variable-wavelength detectors may last only 400–1000 hr. The most common types of failure of a deuterium lamp are *failure to ignite*, *filament flutter*, and *low energy output*.

Failure to ignite is easy to diagnose because the purple glow of the lamp normally visible through the detector vents or cell compartment is absent. Use caution when viewing the lamp because the low-wavelength UV radiation can cause eye damage. Glass-lensed safety glasses are usually sufficient protection for short-term viewing of the source, but special goggles for UV use are recommended for longer viewing times. When replacing the lamp, leave the old lamp in place, connect the wires from the new lamp to the appropriate connections, and turn the new lamp on to test whether it lights. This method will prevent a sometimes tedious alignment procedure if the problem is in the lamp power supply or starting circuitry rather than in the lamp itself. If the new lamp works, disconnect the power and replace the old lamp. Care should be taken to avoid touching the lamp because fingerprints can etch the lamp window during operation. Also, be careful not to get burned by the hot lamp.

Filament flutter shows up as a significant increase in baseline noise and is sometimes accompanied by erratic spikes in the baseline. The solution to filament flutter is lamp replacement.

Low energy output is the most common type of deuterium source failure. This problem is observed as a gradual reduction of detector sensitivity. Many detectors have a meter, which allows you to monitor and record the output of the detector energy from time to time. The operator's manual should tell you the lower limit for acceptable lamp output. Again, lamp replacement is required when low energy output occurs.

One final note of caution: deuterium lamps do have a limited shelf life. The lamp will age dramatically in storage and may be unsuitable for detector use after six months on the shelf. Considering this factor and the several-hundred-dollar lamp cost, you can see that it would not be wise to keep a spare deuterium lamp on hand — order one when you need it.

The tungsten source of the visible portion of many UV/VIS detectors has a very long life and usually fails by burning out.

MONOCHROMATOR AND CALIBRATION PROBLEMS

The monochromator in most variable-wavelength UV detectors is factory aligned and sealed. It is unwise to open the monochromator compartment because this generally voids the detector warranty. The compartment contains the diffraction grating and one or more mirrors. Failure of the monochromator, aside from that caused by physical damage by dropping, is often a result of contamination on the optical surfaces. You are more likely to contaminate these delicate optical components than solve problems if you try to service the monochromator yourself. If you suspect a problem, contact the service personnel.

Some detectors use a beam splitter just in front of the detector cell to improve the uniformity of cell illumination. Beam splitters sometimes are mounted in such a way that rotation is prevented by a setscrew; however, the positioning of the beam splitter can be altered through vibration or during cell installation if it is not held firmly in place. The operator's manual usually has a clear procedure for beam-splitter alignment.

Much of the noise at very low wavelengths (in the 185-nm range) is caused by absorbance of the light by oxygen and ozone in the air. Because most of the optical path is within the monochromator, some detectors are designed to permit purging of the monochromator with nitrogen or another inert gas to enhance performance at low wavelengths. An example of this technique is discussed in reference 4.

Miscalibration is the most common problem in the monochromator section of the detector. This problem is especially apparent when comparing spectra around 200 nm from two detectors — a region in which most compounds have a steeply sloping absorbance curve. Differences in wavelength of 1–2 nm can make dramatic differences in detector response and reproducibility. Hence, two detectors that are accurate to within the wavelength selection specification may differ by two or more nanometers and, therefore, may measure responses that differ perhaps by a factor of two or more under nominally identical conditions. Your operator's manual will probably contain a discussion of methods for calibrating the detector with solutions of known absorbance maxima. A much simpler way to check the wavelength accuracy is to use a characteristic emission band of the deuterium lamp (656 nm). To check the calibration with this method, dial the wavelength to about 650 nm with the deuterium lamp on and adjust the detector range and zero so that the baseline is about 75% full scale on the recorder. Then, slowly adjust the wavelength to and through 656 nm. You should observe a minimum peak as 656 nm is passed. Repeat this procedure several times. Adjust the calibration dial if necessary, following instructions for your specific brand of detector. Check the calibration again and return to normal operation. You may confirm that the 656-nm calibration is valid by measuring the absorbance of one of the solvents used in liquid chromatography. Vendor data for LC-grade methanol, for example, indicate that absorbance is 0.5 AU at 210 nm (5). To check the calibration, simply zero the detector-output signal with a dry cell at 210 nm and then fill the cell with LC-grade methanol. At this point, the absorbance should read 0.5 AU.

CELL PROBLEMS

The construction of the detector cell in the variable-wavelength UV detector is similar to that of the fixed-wavelength UV detector discussed last month. The problems found with detector cells and eluent solutions are discussed in more detail in the last two articles (2,3) and are listed here for convenience. Tubing blockage in the heat exchanger or exit tubing is usually exhibited as increased system pressure or cell leakage. Air or immiscible liquid bubbles in the cell show up as spikes in the chromatogram and can be eliminated by careful cleaning, proper solvent changeover, and solvent degassing. Chemical contamination of the cell or cell windows may result from improper system cleaning, or it may be unavoidable with your particular type of sample. Contamination can usually be removed by cleaning the cell with solvents or acids, but cell rebuilding may be required in severe cases of contamination.

CELL CLEANING

The following discussion of cell cleaning is intended to be a supplement to the recom-

mended procedure for your specific detector. Because the general cleaning procedures for all optical-detector cells are similar, the following general discussion deviates from our focus on variable-wavelength UV detectors and includes all optical detectors.

Detector cell cleaning may take one of three forms: *backflushing*, *solvent cleaning*, and *acid cleaning*. You should consult the operator's manual for manufacturer's recommendations before using any of these procedures. Follow good laboratory safety practices, such as providing eye and skin protection, because all of these procedures create potentially hazardous conditions.

Backflushing the detector cell often effectively removes particulate matter that blocks the heat-exchanger tubing. Connect the LC pump (or column outlet) to the detector outlet. This reverses the flow through the detector cell. Direct the resulting waste stream from the detector inlet to a waste container. If high back pressure was an observed symptom, you will see the pressure drop when the blockage is removed. Use caution, especially with RI detectors, because excessive pressure across a flow cell can cause leakage, and repair may require a service call. An effective technique to avoid overpressuring the cell includes setting the maximum pressure cutoff for the pump below the pressure limits of the cell and gradually increasing the flow rate in increments of 0.1 ml/min. In some cases, cutting off a few millimeters of inlet tubing from the heat exchanger may remove a blockage.

Cleaning the cell with solvents is useful if you suspect the presence of an organic-soluble contaminant or droplets of an immiscible solvent in the cell. Proceed by drawing or pumping a series of solvents through the cell, each of which is miscible with the preceding and following solvents. Often, a series of solvents with different solvent properties is helpful in removing contaminants. For example, clean with methanol, then tetrahydrofuran, then methylene chloride, and then return to methanol; these solvents will remove contaminants that have a wide range of solubilities.

Acid cleaning is the most aggressive cleaning technique. In this technique, 10%–50% acid is drawn through the detector cell to remove contaminants, and then water is drawn through the cell to flush out the acid. Finally, water is pumped through the cell to rinse out the last traces of acid. Most chromatographers use nitric acid for cell cleaning, but you should be aware that UV-absorbing residues may be a problem at low wavelengths if the cell is not thoroughly flushed. Avoid halogenated acids, which attack the stainless steel in the cell. When the acid is drawn, rather than pushed, through the cell with the syringe, accidents are minimized because the acid is never under pressure, and contamination of the cell by materials in the syringe is avoided. You should use the specific procedure recommended in the detector manual for best results.

Finally, if the cleaning methods do not solve a contamination or blockage problem, the cell may need to be rebuilt or replaced. Some detector cells are readily rebuilt by the user, whereas others are sealed and must be factory serviced. Rebuilding kits are usually less expensive than a new cell and include new windows and gaskets. Consult the detector manual or the manufacturer's service department for specific recommendations.

PHOTODIODE AND ELECTRONICS PROBLEMS

To obtain maximum sensitivity from the detector, the preamplifier for the photodiodes is often mounted on the same circuit board as the photodiodes themselves. The photodiodes in turn are commonly mounted directly against the detector cell to minimize stray light. Use extreme caution when handling the photodiode-related components because the preamplifier is sensitive enough that fingerprints or capacitive discharge from your body may cause irreversible damage. If you cannot correct a problem that you suspect to be in the photodiode or electronics section of the detector by using the procedures in the operator's manual, you should call the manufacturer's service department for advice.

SUMMARY

The most common problems with variable-wavelength UV detectors are deuterium lamp failure and cell contamination. When isolating a problem to the variable-wavelength UV detector, be sure to consider solvent-absorbance effects, especially if the detector is operating at wavelengths below 220 nm. Reference chromatograms, noise measurements, and lamp-usage records in your logbook will greatly facilitate the solution of detector problems.

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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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