

Agilent 8453 Diode Array UV-Visible Spectrophotometer

Diode array spectrophotometers are capable of acquiring complete UV/Visible absorbance spectra in as little as 100 msec. The key is that the grating of these instruments is fixed, and rather than moving the grating to acquire spectra, hundreds to thousands of detectors are placed at the exit of the monochromator. The detectors are all integrated on a single silicon chip called a photodiode array. The diodes act as capacitors that discharge in proportion to the incident light flux. The capacitance of each diode is converted to a binary word that is input to a computer. The Agilent 8453 simplifies the operation of the spectrophotometer even further by using a deuterium discharge lamp for the full UV and visible range, rather than a deuterium lamp for the UV and a tungsten incandescent lamp for the visible, as in done in most other instruments.

The system is a single beam instrument, which means that you first run a scan on a cuvette containing just the solvent to determine the intensity of the lamp at each wavelength, $I_0(\lambda)$. Then you put in your sample in the same cuvette and scan the spectrum again. The absorbance is then calculated from the ratio of the two spectra:

$$A(\lambda) = \log \left(\frac{I_0(\lambda)}{I(\lambda)} \right)$$

The plot of $A(\lambda)$ verses λ is the spectrum of the solution.

Instrument Start-up

Turn on the monitor, printer, computer, and the diode array system. Use the Administrator Account (password 3000hanover). Double Click on the Instrument 1/online icon. Cancel the login dialog.

Taking a Spectrum

1. Place your background sample in the cell holder and rotate the cell holder lever down.
2. In the "Sampling" Window, on the right-hand side, click on blank. The background spectrum will then be displayed.
3. Place your sample in the cell holder and rotate the cell holder lever down.
4. Click on the Sample button in the "Sampling" window to take each spectrum.
5. Click in the spectrum window. Pull down the file menu and choose Print and choose Selected Window.
6. Continue with the next sample.
7. Save your spectra by pulling down the File menu and choosing Save....
8. To read out specific absorbances using the cursor, click on the spectrum curve that you wish to measure. Click right while the cursor is on the spectrum curve; the cursor should change shape. Move the mouse to read out the wavelengths of interest. The data is listed in the status bar at the bottom of the window. To change to a different spectrum use the \uparrow and \downarrow cursor buttons on the keyboard.
9. To delete a spectrum, click on the spectrum curve that you wish to delete. Then click the Delete Selected Sample button in the Sample/Result Table.