Hewlett Packard 8452A Diode Array Spectrophotometer
HP Chemstation Version

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 of detectors are placed at the exit of the monochromator. The HP 8452A has 512 detectors. 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 HP 8452A 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, $I_o(\lambda)$. 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:

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

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

In the instructions below, clicking using the mouse is done with the **left mouse button**, unless instructed otherwise. The *right mouse button* is used for *closing windows*. When you are asked to press a key on the keyboard, the key is indicated in brackets: [F4], or [Enter], etc.

Start-up

Turn on the monitor, printer, computer, and the diode array system. Type “win” at the C:> prompt. If the system asks if you want to log on to the network, click "OK" if the system is attached to Ethernet and "Cancel" if the system is not. The Windows operating environment will be loaded. Double click on the “HP UV-Visible Chemstations” icon in the Program Manager window. Then double click on the “Instrument 1 online” icon to start the diode array operating software. Put in your operator name; no password is necessary. Click OK. The system will then start the spectrum acquisition program and display the **Main** window.

Acquiring a Spectrum

1. Lift the cell holder lever to open the holder. Place a cuvette with your solvent in the sample compartment. Press the cell holder lever down to secure the cuvette.
2. Pull down the Measure window and choose Blank. The screen will show the **Blank Spectrum** window and the background scan will be displayed. This spectrum will look very noisy, but notice the very narrow absorbance range chosen to display the plot.
3. Rinse the cuvette twice with your sample and fill the cuvette. Place the cuvette, now containing your sample, in the cell holder.
4. Pull down the Measure window and choose Sample. The spectrum will be displayed in a few seconds. Type the name of your spectrum in the “name” box in the lower left-hand window. (This name might be a concentration or amount of some substance.)

5. You can expand the spectrum by dragging a box around the region of interest using the left mouse button. To return to the full spectrum, double click the left mouse button.
6. You can read out the absorbance of your sample by positioning the cursor on or near the spectral trace and then clicking the ***right*** mouse button. The cursor changes shape to an arrow that marks the current wavelength. Move the mouse to the desired wavelength. The wavelength and absorbance are printed in the status bar at the bottom of the screen. You can step through the spectrum using the left (<) and right (>) cursor keys.
7. To quit the "Cursor" mode, click the ***right*** mouse button.
8. To print the spectrum, highlight the spectrum and then pull down the Edit menu and choose Print Highlighted Window.

Saving Spectra to the disk
9. Pull down the Edit menu sldie right on Save as and choose Samples. Type in the file name you wish, however this file name should follow DOS naming conventions: 8 characters or less, no spaces, no punctuation symbols (no - / \ * . & _ , etc.), and start with a letter. Click on Save.
10. If you want to overlay several spectra, go on to the next section. To clear the current spectrum (spectra) pull down the Edit menu, slide right on Clear and choose Samples.

Overlaying Spectra-The use of Registers
11. To overlay the next spectrum on the last, don't clear the previous spectrum and then just repeat the steps above under "acquiring a Spectrum."
12. The new spectrum will be placed in the active register.
14. To read out the absorbencies of the spectra, follow the instructions in steps 5-7 above. Note that you can move from register to register by using the up "^" and down "v" cursor keys.
15. To print the overlaid spectra follow step 8.
16. If you are saving spectra to disk, all the registers will be saved under the single file name..

Finishing Up
17. Clear the current spectra as a courtesy for the next student. To clear the current spectrum (spectra) pull down the Edit menu, slide right on Clear and choose Samples.
18. Rinse the cuvette with lab detergent and three rinses with deionized water. Never use a brush or other hard object in a cuvette. Dry the exterior with a ChemWipe.

If Your Spectra Has A Sharp Spike Near 656 nm:
Every analytical instrument needs to be calibrated for use. One of the calibration steps is to calibrate the wavelength scale by measuring the spectrum of the deuterium lamp. The deuterium lamp has several sharp peaks in its spectrum, including a major emission line at 656 nm. This line is so bright that the diodes at this wavelength are masked. When the wavelength scale is miscalibrated, this bright line misses the masked diodes and shows up as a spike in your spectrum. If sharp spikes show up, you need to recalibrate the lamp.

1. Pull down the Measure menu and choose Diagnostics, slide right and choose "Dark Current, Intensity, Stability…."
2. Click on the Intensity button.
3. Note if any wavelength are too low in intensity. If so the deuterium lamp will need to be replaced, if you need to use the spectrophotometer at those wavelengths.
4. Click on Close. Your spectra should now not have any spikes.