

Hewlett Packard 8452A Diode Array 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 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, $I_0(\lambda)$. 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 $A(\lambda)$ versus 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. When 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 "Main" icon in the Program Manager window. Then double click on the HP8452A icon to start the diode array operating software. Next click on "General Scanning" in the displayed list. 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. Click on the "Meas. Blank" menu button. The screen will change to the **Graphics** window and the background scan will be displayed.
3. Rinse the cuvette twice with your sample and fill the cuvette. Place the cuvette, now containing your sample, in the cell holder.
4. Click on the "Meas. Sample" menu button. The spectrum will be displayed in a few seconds.
5. You can read out the absorbance of your sample by clicking on the "Cursor" menu 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 middle of the screen.
6. You can have absorbance values listed when you print the spectrum by marking each wavelength of interest. You mark wavelengths by pressing the left mouse button at each desired point in the spectrum.
7. To quit the "Cursor" mode, click the *right mouse button*.

8. To print the spectrum, click on the "Hardcopy" menu button. The **Hardcopy** dialog box will be displayed. Click on "Print Spectra". The spectrum will then be plotted on the HP printer. Click *right* to close the **Hardcopy** dialog box. The absorbencies of any wavelengths that you marked in step 6 will be listed below the spectrum automatically.

Saving Spectra to the disk

9. Click on the "Return" menu button to return to the **Main** window. Click on "Files"; the **Files** dialog box will be displayed. Double click on "Store Data File". Notice the prompt line in the middle of the screen, just above the menu buttons. The prompt should read "Enter Data File Name." 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. Press [Enter]. Click *right* to close the **Files** dialog box.

10. To return to the display of your spectrum, click on the "Graphics" menu button.

Overlaying Spectra-The use of Registers

11. To overlay the next spectrum on the last, click on the "Registers" button.

12. In the **Registers** dialog box, the current active register is denoted with a -> in the left hand column. Press the ← cursor key and then click on the <F4 Make Current> button to make the next register current. Click *right* to close the dialog box.

13. Put in your next sample, and click "Meas. Sample". 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 moving the mouse up and down, while moving the mouse left and right selects the wavelength. If you mark any wavelengths for printout, you should mark the same wavelength in each register.

15. To print the overlaid spectra follow step 8.

16. If you are saving spectra to disk, remember to save each register before changing to a new register.

Finishing Up

17. If you used registers, please clear them as a courtesy for the next student. To clear all registers, click on the "Registers" menu button. The **Registers** dialog box will be displayed. Click on the <F3 Clear All> button. Click *right* to close the **Registers** dialog box.

18. Click the "Return" menu button to get back to the **Main** window.

19. 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. If you are in the General Scanning software, exit the **Main** window by clicking on the "Exit" menu button. Then click on "Exit" in the small window that is next displayed.
2. Double click on "HP 8452A Diagnostics" line to start the calibration program.
3. Press the [L] key on the keyboard to run the Lamp Check.
4. Press [Q] to Quit.
5. You can then return to the General Scanning software and your spectra should not have any spikes.