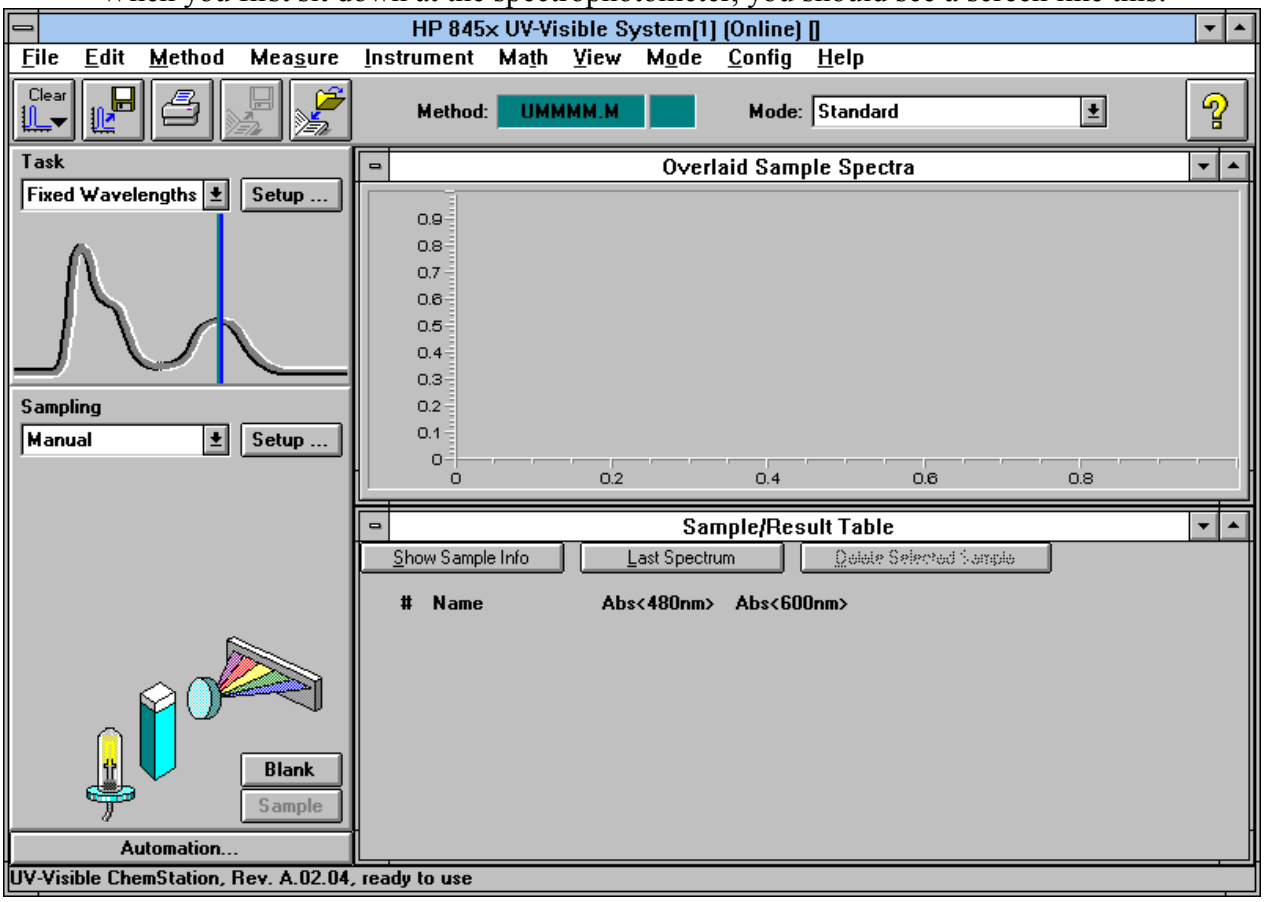
THE DIODE ARRAY SPECTROPHOTOMETER

The Hewlett Packard Diode Array Spectrophotometer is the latest (and some say greatest) instrument development in the field of UV-Visible Spectrophotometry. It features an easy to use graphical interface, quick full spectrum scans, and the ability to conduct kinetic studies.
Note: actions you take are in *italics*. Items on the screen are referred to in **bold** type.

TO START OFF:

When you first sit down at the spectrophotometer, you should see a screen like this:



If you don't, it means the software program has yet to be activated. Refer to the handout entitled " STARTING FROM SCRATCH-EVERYTHING **IS** DARK".

As you can see, the monitor screen is divided into a few areas:

TASK (upper left of screen) - lets you choose what type of scan you want to do- a look at the spectrum, or a look at just a few wavelengths (up to 6 different wavelengths can be monitored simultaneously)

SAMPLING (lower left of screen) - you can choose manual sampling in which you tell the instrument each time when to start collecting data, or set up an time based measuring procedure in which the instrument scans on a periodic basis that you choose.

OVERLAID SAMPLE SPECTRA (upper right) - Each time you scan your sample, the resulting spectrum is displayed. Scans are overlaid each other, each scan being displayed in a different color. Can get pretty muddled!

SAMPLE/RESULT TABLE (lower right of screen)- Sample #, name, absorption are displayed..

STEP- BY- STEP

1. The first task to choose the appropriate task. Duh.

a. *Click* on the window underneath **TASK** and *choose* **Spectrum/Peaks**. We must first verify where the peaks are we want to monitor, and it also is a good idea to have a look at the whole spectrum to see if anything looks out of whack.

b. Usually the **SPECTRUM/PEAKS SETUP** window will pop up, If it doesn't, *click* on **SETUP** window to the right of **TASK.** A setup window will open with some options for your to decide.

2. Enter the appropriate data in the **SETUP** window:

You can have the computer label the 3 most prominent peaks and/or valleys by *clicking* on the correct box or boxes.

In this experiment you want to measure Absorbance, so *choose* the correct entry in the **DATA TYPE** box.

You can choose what part of the spectrum you want displayed on the screen. *Enter* in 300 in "from" box and 800 in the "to" box. *Make a note* in your lab notebook explaining why these numbers are sensible

3. Close the **SETUP** window by *clicking* OK

4. Now *choose* **MANUAL** in the sampling menu

a. *Click* on the **SETUP** box to the right of the **SAMPLING** window. A **PATH LENGTH** window will appear. Check that the path length is set to 1 cm- *change it if necessary*, then close the window.

5. To turn on the source lamp, *position* the cursor arrow on the **picture of the lamp bulb** (lower left of screen). The arrow should change into a hand with a pointing finger. *Click* the mouse button and select **LAMP ON** in the window that pops open. When the lamp is on, the graphic of the lam bulb has a yellow glow in the middle. It takes about 90 seconds for the lamp to light- you'll hear the instrument hum and click a bit...

6. Now *insert* your blank cuvette in the cell holder of the spectrophotometer. The cuvette has 2 smooth sides and two ribbed sides. Which sides should the beam pass through? Why? Ask somebody if you aren't sure.

Click on the box labeled **Blank** in the lower left of the screen. The spectrophotometer will make some rumblings and clicking noises, and then display the blank spectrum. Note the y-axis of the displayed scale. It should be a fairly low absorbance value.

8. *Replace* the blank cuvette with your sample cuvette and *click* on the **Sample** box below the **Blank** box .

9. The spectrophotometer will burp once and then, in about then seconds, the absorption spectrum of your sample will be displayed in the upper right of the screen. IF you chose annotation in the **SETUP** window, the three most prominent peaks and/or valleys will be labeled. You can *position* the **cursor arrow** near the spectrum, *click* on the right mouse button, and see the white arrow change to a black arrow. *Move* the arrow by *sliding* the mouse around, noticing that as you do so, the wavelength, absorption, and standard deviation are displayed on the bottom of the screen. *Click* on the right mouse button again to return the arrow to its previous state.

10. OK, now that you have your spectrum, decide what wavelength(s) you want to monitor during the experiment. *Click* on the **TASK** box again, and *choose* **FIXED WAVELENGTH**. Then *click* on the **SETUP** button to the right, and enter the wavelength(s). The data type should continue to be Absorbance, and you can *choose* whether or not to display the spectrum in addition to the fixed wavelength(s) value(s), and whether or not to be prompted for sample info each time a scan is started.

11. Now all you have to do is *click* on the **Sample** box on the lower left of the screen when you want to scan a sample. Do it a few times to get the hang of it, and note how the display changes in the **Sample/Results Table.**

12. You can print your results by *clicking* on the **printer icon** at the top of the screen, or by *selecting* one of the print options available under the **File** command in the menu bar at the top of the screen.