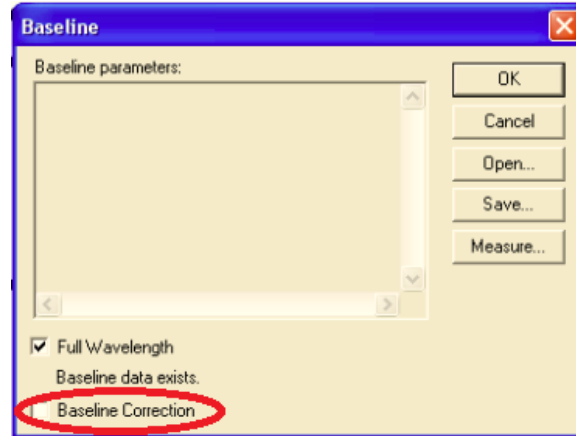


Jasco UV-Vis Operating Instructions

1. Carefully pipet your sample into the cuvette until the cuvette is $\frac{3}{4}$ full. A cuvette tips easily so never place it on the countertop by itself. Instead, always return it to a beaker or other stand.
2. Turn on all of the components of the instrument and allow it to sit before you begin. Ensure that the computer, monitor, spectrophotometer, cooling unit, and the temperature control unit are turned on. The monitor power button is underneath the right edge of the monitor and the spectrophotometer power button is on the right-hand side of the spectrophotometer, underneath the bar code. Turn these on first, along with the *Jasco Pbs* power supply, which is a small box to the left of the spectrophotometer. Then turn on the cooling unit underneath the counter. Flip both the “power” and the “cooling” switches. Make sure that the temperature is set below your analysis temperature. Lastly, turn on the temperature controller.
3. To insert your sample into the instrument, slide open the spectrophotometer cover and remove the black top on the sample chamber. Insert your cuvette into the sample chamber being careful not to touch the clear sides. The clouded sides should be facing the front of the machine so that the beam of light can pass through the clear sides of the cuvette.
4. Flush the sample chamber with nitrogen. Ensure that the black hose protruding from the front of the spectrophotometer is connected to the nitrogen tank found near the door of the instrument room. Turn on the nitrogen tank as instructed and adjust a small flow of nitrogen into the sample chamber. You can ensure that the nitrogen is flowing by directly feeling for the flow of nitrogen into the sample chamber. Be sure to place the cap back on the sample chamber and close the spectrophotometer lid.
5. Now, access the spectrophotometer software by clicking the Jasco V-570 icon on the desktop. Select the **Spectrum Manager** program and open **Spectrum Measurement**.
6. If you have dissolved your sample in a solvent you will want ‘Baseline Correction’ to be checked. If you are running pure sample that is not in solvent you do not need to take a baseline, which usually subtracts noise from the spectra caused by the solvent. To disable or enable this baseline, go to **measurement** → **Baseline**. Then **uncheck or check** ‘Baseline Correction’ and hit **OK** (Figure 1).

Figure 1. Disable the correction for solvent influence on the spectrum, known as 'Baseline Correction.'



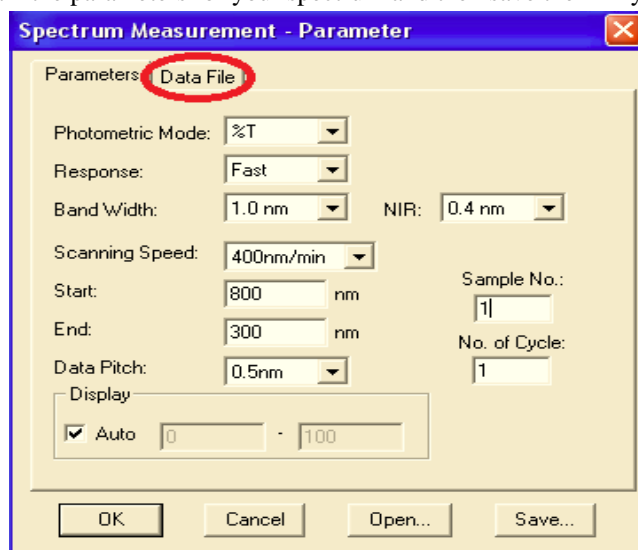
7. To program your spectrum, go to **Measurement** → **Parameter** and enter the following data (Figure 2):

Figure 2. Details for the spectrum parameters.

Photometric Mode: % T
Response: Fast
Band Width: 1.0 nm NIR 0.4 nm
Scanning Speed 400 nm/min
Start: 800 Sample number: 1
End: 300 No. of Cycle: 1
Display: check 'Auto'

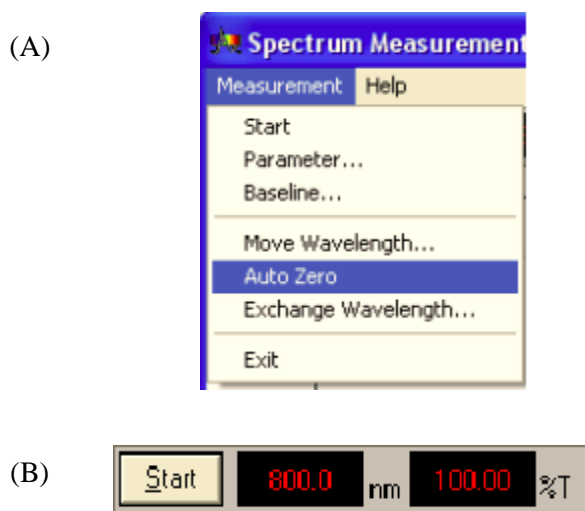
8. Then, go to the second tab in the **Parameter** window, called **Data File**. Click 'Browse' to save your file in a folder of your choice. Include your initials in your file name e.i. DKF_042312. Click 'Save' (Figure 3)

Figure 3. Program the parameters for your spectrum and then save them in your storage server



9. Now you must zero the transmittance. Go to **Measurement**→**Auto Zero** (Figure 3a). Find the percent transmittance reading at the top of the screen (Figure3b). This value may fluctuate by a few percent, but if it is more than 5% from 100% then re-zero.

Figure 3. Auto-zero the spectrum (A), and ensure that the transmittance is close to 100% (B).



10. To run your spectrum, click **Start**. The spectrum will take a few minutes to run. When the spectrum is complete, a **Spectra Analysis** screen will automatically open.
11. When the spectrum is complete, adjust the temperature control to the next appropriate temperature. Be sure to change the temperature as soon as the run is complete to allow your sample sufficient time to equilibrate.
12. Now save your transmittance spectrum by going to **File** → **Save As** → “//storage” → **Save** → *select your read-write folder*. Include important information of the spectrum in your file name i.e. DKF_biodiesel1_15degrees. Select **File Type: ASCII (*.txt)**. This will save your spectrum as coordinates in a text file that you can copy to Excel for analysis.
13. When you have finished collecting data, bring the sample chamber up to room temperature by adjusting the temperature control to 22 °C and raising the temperature on the cooling unit to above 20 °C. You need not wait more than five minutes for the sample chamber to warm up before you quickly remove your sample and insert the next sample, if it is ready, and close the chamber. If you are the last student to use the instrument, then close the chamber and turn of the nitrogen tank, cooling unit, temperature control, monitor, and UV/Vis Spectrophotometer.