

Spectrophotometers with Vernier Data Acquisition Software

The absorbance of a sample is given as $A = \log \left(\frac{I_0}{I} \right)$, where I_0 is the intensity without sample present and I is the intensity with the sample in the light path of the spectrophotometer (spectrophotometer and spectrometer are used interchangeably). This calculation is done at each wavelength in the spectrum. The value of I_0 depends on wavelength and must be determined by placing a reference or blank sample in the instrument. The terms “**reference**” and “**blank**” are also used interchangeably. The **reference** is usually a cuvette filled with only the solvent or the buffer for the experiment. The process of acquiring the **reference** spectrum is called calibration in Logger Pro. Every time you switch solvents or buffers, a new **reference** spectrum must be taken, so the instrument must be recalibrated.

I. Getting Started and Calibration

Start Here for Experiment 1 & 2

1. Plug the wire coming from the computer into the spectrophotometer to start warming up the device. Log into your computer. Select the Logger Pro icon at the bottom of the screen.

2. The spectrophotometer should be automatically recognized by the software showing the colors of the visible spectrum in the data window with Wavelength as the horizontal axis.

If the visible spectrum is not displayed check your connections. If it is not a connection issue go to “Experiment” (top menu), select “Connect Interface” → “Spectrometer” → “Scan for Spectrometers”.

If you wish to disable the visible rainbow spectrum you click once on the graph then on “Options” (top menu), slide down to “Graph Options.” Unselect “Draw visible spectrum.”

3. Click on “Experiment” (top menu), slide the mouse down to “Set Up Sensors”, and go across to “Spectrometer: 1”, click. The Spectrometer dialog box will be displayed. Enter a zero for **Wavelength Smoothing** and enter a 3 for **Samples to Average**. For your cuvette the **Wavelength Range** should be set for 380 –750 nm. The **Sample Time** will be set for you automatically when you do the calibration in the next step. When done do not click on “Restore Defaults”, instead exit this window via the small upper left red button.

4. To calibrate the spectrophotometer, once again go to “Experiment”, slide the mouse down to “Calibrate”, slide across to “Spectrometer: 1”. The calibration dialog box will display the message: “Waiting...90 seconds for lamp to warm up.” (see Figure 1) The minimum warm up time once the device is plugged in, is two minutes. **For best results, allow the spectrometer to warm up for at least five minutes.** Following the instructions in the dialog box to complete the calibration, use a cuvette filled about $\frac{3}{4}$ full with the correct solution for your reference, as instructed by the Experiment hand-out. Check to make sure the non-frosted, clear sides are in the light path (length wise direction). The cuvette should be inserted as far down as it can be in the cell holder. You should feel that the cuvette is gently, but firmly, held in place so that you cannot twist the cuvette. Click Finish Calibration and then click OK.

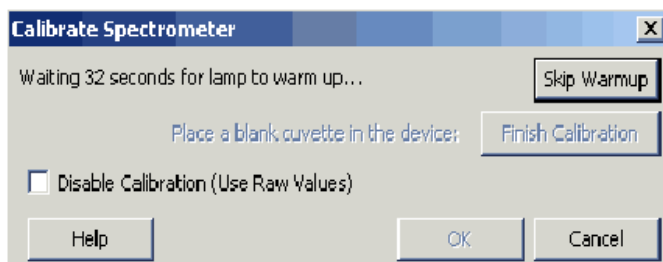




Figure 1. Calibration stores a reference spectrum.

The process of calibration sets the integration time for maximum sensitivity and also stores a reference spectrum.

II. Measuring Absorbance Spectra

1. Replace the reference cuvette with any sample. Click on the green button  to collect data. If asked, “What would you like to do?”, select “Erase and Continue”. Once a spectrum appears press the red button  to stop collecting data.

2. Right click the mouse when cursor is on any icon in the top menu bar. Slide over to select “Icon & Text”. The menu bar should now be clearly labeled. You can read the absorbance value by using the Examine icon. Now move the cursor along the spectrum until you are at the highest peak (with most area under the curve). The wavelength and absorbance will be displayed in the new dialog box in the data window, Figure 2. Determine the wavelength of maximum absorbance. Use this unique wavelength number throughout your experiment today.

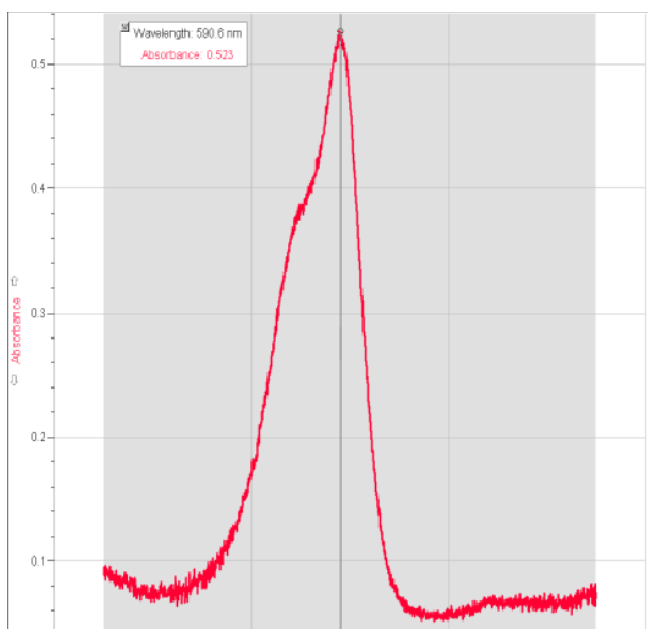



Figure 2. Spectrum of Crystal Violet with the Examine Tool.

3. **For Experiment 1:** Continue to read any cuvette sample as in step 1, but always record the absorbance only at this unique wavelength you found in step 2. All values need to be recorded in your lab notebook. Place used cuvettes (emptied and rinsed) in collection beaker at front lab table. Thanks!

III. Running a Kinetics Trial for Experiment 2

1. Click on the Configure Spectrometer icon labeled Wave, , located on the right hand side of the toolbar to open the Configure Spectrometer : 1Data Collection window.
2. The window will look similar to Figure 3 below. Click “Abs vs. Time” (found under Collection Mode). Click on “Single 10nm Band” and that will give an option to click on “Individual Wavelengths”. After that is done, move the mouse over to the right where a column of wavelengths is displayed. First click on “Clear Selection”. Then click on your optimum wavelength. If a checkmark appears you then proceed to average over a range of wavelengths for better precision by scrolling and clicking on 4 wavelengths above and 4 below this box. Now click on the box that will enable the program to **Combine Contiguous Wavelengths**. Click OK. If prompted with a question about “storing latest run before switching Collection Mode”, choose YES.

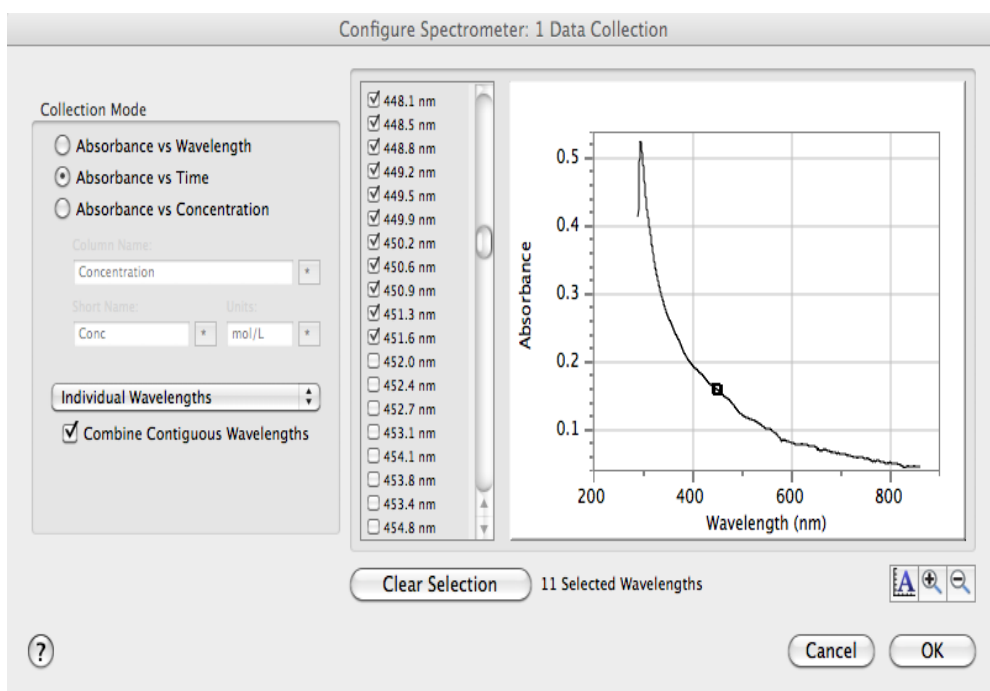



Figure 3. Averaging over several wavelengths to obtain better precision.

3. Click the Data Collection icon . A window similar to Figure 4 will appear. Choose a “Length” of **90** , keep the unit as **seconds**. Under “Sampling Rate” type in **0.5** for samples/ second, instead of what is shown in that window. Click Done.

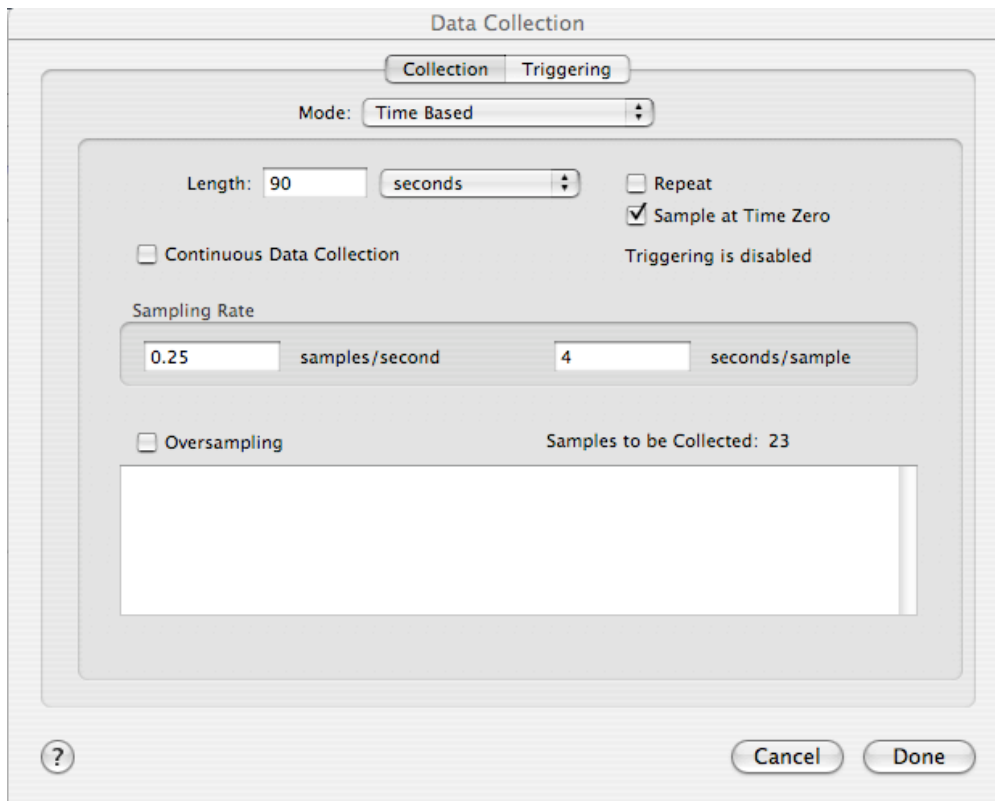







Figure 4. Setting the sampling time interval and total experiment length.

4. If you have already calibrated the spectrophotometer with water, it is now time to put exact CV and H₂O reagents and a round stir-bar into the cuvette. For more uniform mixing put the magnet’s crown pointing upwards inside the cuvette. Adjust the stirrer to allow for mixing without splashing. Check for bubbles in the cuvette. If present, tap the cuvette gently to remove the bubbles as they may impede the light path.
5. Add the correct volume of your final reagent (OH⁻) to the cuvette without allowing the pipette tip to touch the liquid in the cuvette. Count to five. Click on the  button. Data will start appearing on a graph. If not visible, you can auto scale the y-axis by clicking on .
6. After 90 seconds of data collection the automatic collection is finished.
7. Click on the Linear Fit icon, . This will display the linear equation and the correlation coefficient of the line. The line can be modified by clicking on the black bracket at the end of the line. Sliding the bracket will highlight the desired “new” portion of the line. Go to “File”, slide down to “Save As” and click. The file should be saved on the Desktop for now. Each time you run a new trial give it a new name.

Before you leave lab today you **MUST** save all your graphs to your fileserver account (Go, connect to server, connect, academics, chem, CH142, your email name). These files can only be opened and analyzed with the Logger Pro software in K405.

8. To run the next kinetics trial, close the linear fit window. Prepare the next sample (as you did in step 4 & 5) and when ready click on the collect button . Choose "Erase and Continue" if prompted with an "Erase Data?" question.
9. To exit the kinetics mode and return to taking full spectra, click on the Configure Spectrometer icon labeled Wave, . Click Abs vs. Wavelength (under the Set Collection Mode). Click OK.

IV. Finishing up

1. Make sure to rinse your cuvettes three times with water. Remember not to use paper towels to clean them. Don't stick anything sharp into the cuvettes, including a test tube brush. A collection beaker with soapy water is at the front lab table.
2. Make sure the area around the spectrometer is clean and dry.
3. Please unplug the spectrometer. The deuterium lamp in the spectrometer has a limited lifetime and replacements are very expensive. Thank you.

*When you have finally made five usable Concentration versus Time plots out of your data (page 6 of Exp.2 handout) then you will print those 5 graphs for week 1. Go to "File", slide down to "Print Graph" and click. You can set up print options by first clicking on "Print Footer", enter your choice of words to be printed at bottom of the graph, then click on OK to print your kinetics plot to the **Keyes 4th Floor Hallway** printer. You will print 5 new graphs for week 2 in the same manner.*

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