SpectroVis Plus Spectrophotometers with Vernier Data Acquisition Software Instructions

Introduction:

The absorbance of a sample is given as $A = \log\left(\frac{I_0}{I}\right)$,

where I_o is the intensity without sample present and I is the intensity with the sample in the light path of the spectrophotometer. This calculation is done at each wavelength in the spectrum. The value of I_o depends on wavelength and must be determined by placing a reference or blank sample in the instrument. The terms reference and blank are 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

1. Start the Logger *Pro* software.
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, select Connect Interface →Spectrometer →Scan for Spectrometers from the Experiment menu.
3. Select Set Up Sensors →Spectrometer: from the Experiment menu. The Spectrometer dialog box will be displayed. Set Wavelength Smoothing to 0 and Samples to Average to 20. Using more Samples to Average gives more precise absorbances. Set the wavelength range to 480 – 700 nm. The Integration time will be set for you automatically when you do the calibration in the next step. Click on Close.

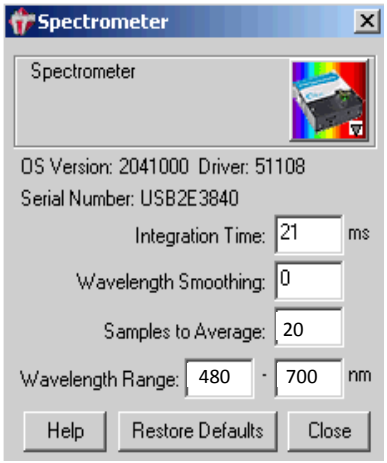


Figure 1. Settings for fast analysis with regular plastic cuvettes or work in the visible range.

4. To calibrate the Spectrometer, use a cuvette filled about ¾ full with water for your reference. Avoid touching the sides of the cuvette are in the light path. Choose Calibrate →Spectrometer from the Experiment menu. The calibration dialog box will display the message: "Waiting…60 seconds for lamp to warm up." (see Figure 2) The minimum warm up time is one minute.
NOTE: 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. Click Finish Calibration and then click OK.

Figure 2. Calibration stores a reference spectrum.

After the first calibration, the lamp should be warmed up enough that you can skip the warm-up period on subsequent calibrations. 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 your sample. Click on 

and then 

.

2. You can read the absorbance using the Examine tool, by clicking on  . Then move the cursor along the spectrum. The wavelength and absorbance will be displayed in the new dialog box in the data window, Figure 3. Determine the wavelength of maximum absorbance. Use this same wavelength throughout your experiment.

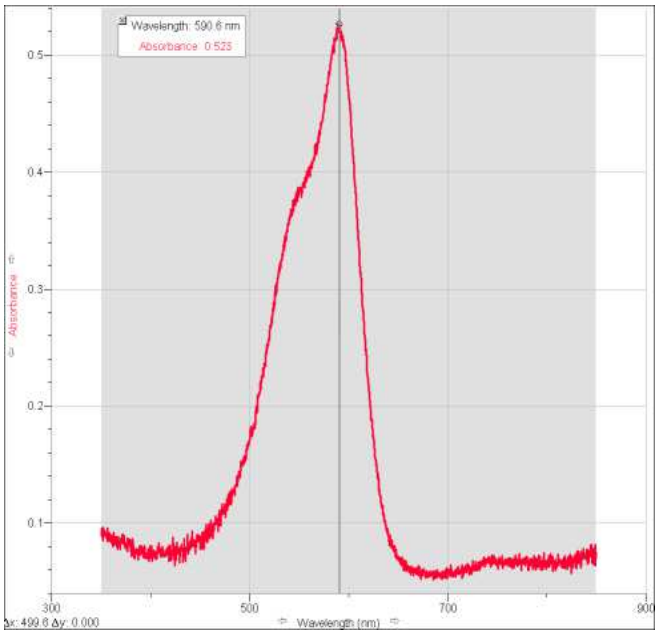


Figure 3. Spectrum of Crystal Violet with the Examine Tool.

Once you have determined the wavelength of maximum absorbance, you can use the Examine tool to read off the absorbance at that same wavelength and the absorbance at 700 nm for each sample. However, you might find it easier to read the absorbances from the spreadsheet output at

the left of the screen. You can scroll through the spreadsheet data to determine the absorbance at the maximum wavelength and for the baseline at 700 nm.

3. ***Expanding the Axes:*** If you need to expand the x or y axis to see your spectrum better, use one of three methods:

Automatic scaling: Click on the Autoscale icon  .
Using the cursor: Position the cursor over the axis you want to expand. The cursor will change shape, Figure 4. Drag the mouse to change the scale expansion.

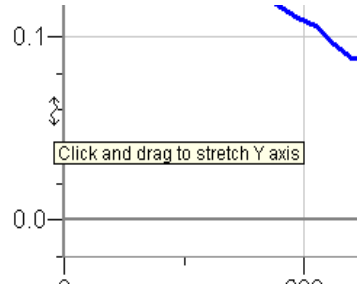


Figure 4. Move the cursor over the axis to change the axis scale.

Direct input: Click near the maximum or minimum of the axis you want to change. A dialog box will appear, Figure 5, and you can the type in the value that you want for the scale limit.

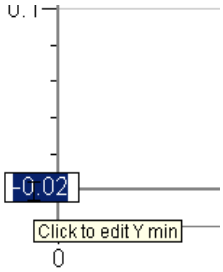


Figure 5. Click near the axis maximum or minimum to show the dialog box.

When working with multiple samples, you may choose to plot each spectrum separately or you can overlay successive spectra. Normally you plot each spectrum separately.

Plotting each spectrum separately:

4. Save the data file to the disk by pulling down the file menu and choosing Save As… Save your data files to the Documents directory.

5. Pull down the Data menu and choose Clear All Data.

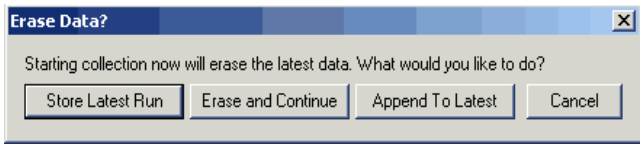
6. Replace the cuvette with another sample. You may need to calibrate again if there is a different reference solvent or buffer for the new sample. Return to step 1.

Overlayed Spectra

4. If you wish to overlay several spectra, choose Store Latest Run from the Experiment menu. This step allows you to overlay the next spectrum on top of the current spectra, with all showing. However, this step does not save the data file to the disk.

5. Replace the cuvette with another sample.

6. When you click on Collect this time, if you did not store the latest run to set-up for overlayed spectra in step 3, you will get a dialog box:



To overlay the next spectrum on top of the last spectra, click on Store Latest Run.

7. If you overlay your spectra, remember to save your combined spectra to disk. To save the data file to the disk pull down the file menu and choose Save As… Save your data files to the Documents directory.

III. Conduct a Kinetics Experiment (Absorbance *vs.* Time)

1. Click on the Configure Spectrometer Data Collection icon,  , located on the right hand side of the toolbar to open the Configure Spectrometer Data Collection display.

2. Click Abs *vs*. Time (under the Set Collection Mode). Click on Treat Contiguous Wavelengths as a Single Range. The wavelength of maximum absorbance will be automatically selected. First, if you don't want to use this automatic wavelength, press the Clear button. We want to average over a range of wavelengths for better precision, Figure 6. To average over a range of wavelengths, drag the mouse on the spectrum to select the analysis wavelengths. You should select at least 5 wavelengths on both sides of the maximum. In choosing a wavelength range, you should choose a narrow enough range that the absorbance is relatively constant (i.e. a relatively "flat" region near the maximum). Alternatively you can Scroll the Select Wavelengths list box to a wavelength near your absorbance maximum and then click on about 5 wavelengths on both sides of the maximum. Click OK.

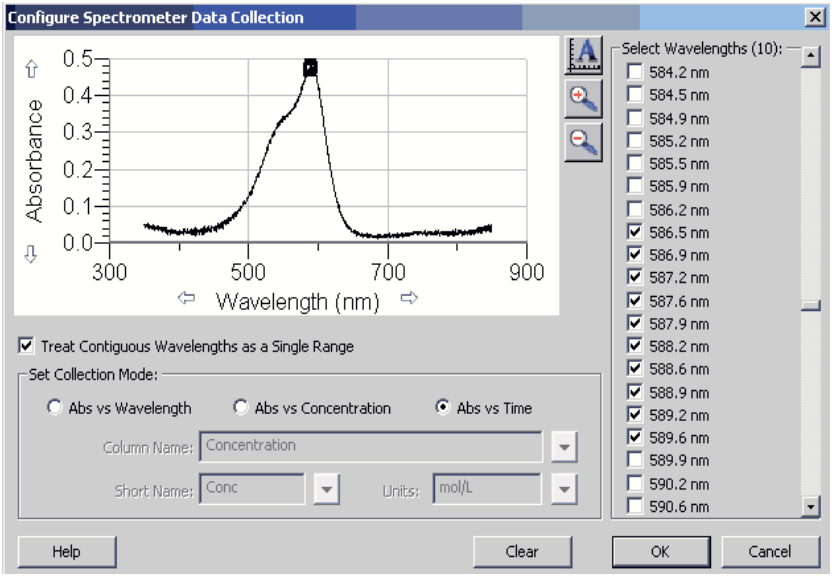


Figure 6. Averaging over several wavelengths to obtain better precision.

3. Click Data Collection  . Choose a Length and seconds per sample as suggested in the lab writeup, Figure 7. You will probably need to change these settings to fit your own particular runs after your first trial data set. If you don't have a good idea which values to set, try a Length of 500 sec and 4 seconds per sample as a starting point. Click Done. You can extend a run, during acquisition, by pulling down the Experiment menu and choosing Extend Collection.

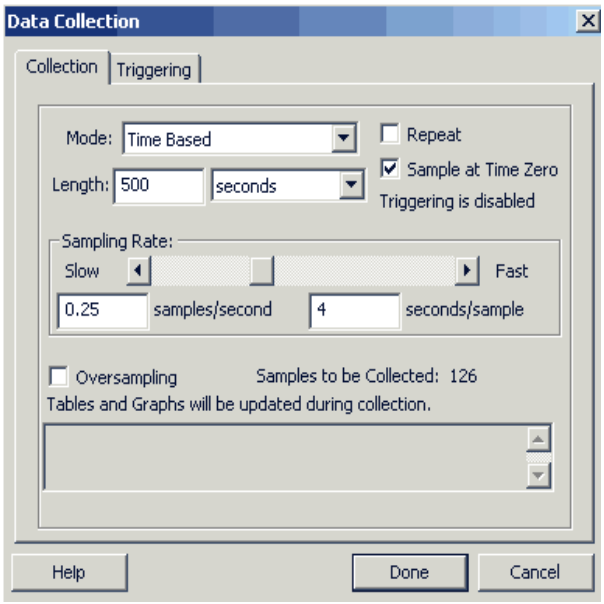


Figure 7. Setting the sampling time interval and total experiment length.

4. Add a special cuvette-stirbar to your sample and adjust the stirrer control (probably to "low") on the stirplate to allow very quick mixing of the sample when the other reactants are added. Look inside the cuvette to make sure the stir bar is spinning. BE SURE THAT THE HEATING ELEMENT OF THE HOTPLATE IS TURNED OFF! Look for bubbles in the cuvette. If bubbles are present, tap the cuvette gently to remove the bubbles as they might block the light path.

5. You must perform a calibration as described above in **Part I**.

6. Add the correct volume of your final reagent to the cuvette. Immediately click on the 

button. You will probably need to autoscale the y-axis by clicking on  .

6. When an appropriate time has elapsed (see lab handout), click on the **Stop** button.

7. Save your data file to the Documents directory.

8. Analyze your data using the instructions below.

9. To run another kinetics trial, select Clear All data from the Data menu.

10. To return to taking full spectra, click on the Configure Spectrometer Data Collection icon,  . Click Abs *vs*. Wavelength (under the Set Collection Mode). Click OK. You can then return to section II, above.

IV. Finishing up

1. Make sure to rinse your cuvettes three times with reagent grade water. Remember <u>not</u> to use paper towels to clean the plastic surfaces, only use ChemWipes. Don't stick anything sharp into the cuvettes, including a test tube brush.

2. Make sure the area around the spectrometer is clean and dry.

Kinetic Data Analysis Using Vernier Software.

Outline: Absorbance is proportional to the concentration of the colored reactant, $A = \varepsilon \ell c$, where ε is the molar absorptivity (or extinction coefficient) and ℓ is the path length (Beer's law). Absorbance will be used in place of concentration in plotting the following three graphs:

- Absorbance *vs*. time: A linear plot indicates a *zero order* reaction (k = –slope).
- ln Absorbance *vs*. time: A linear plot indicates a *first order* reaction (k = –slope).
- 1/Absorbance *vs*. time: A linear plot indicates a *second order* reaction (k = $\varepsilon \ell$ slope)

The integrated rate law equations assume that the concentration, and therefore the absorbance of the solution, approaches zero for long times. However, misalignment of the cuvettes and differences between the reference cuvette and the sample cuvette can cause an offset. To do the kinetic curve fitting this small offset must first be subtracted from the raw absorbance data.

1. If you didn't wait long enough for the kinetics trace to appear "flat" at the long-time end, skip this step. If your long-time absorbance does not approach zero to within 2% or you have negative absorbance values, use the following instructions to subtract the offset. Care must be taken to avoid zero or negative values, since the logarithm of zero or a negative number is undefined. LoggerPro skips these points in its plots, so your plot may be worse than it appears if some of the points are missing.

   a. Use the mouse to highlight the long-time, flat portion of your plot then click on the Statistics icon,  . The mean of the selected data points will be listed. Record this value as A_∞.
   b. Choose New Calculated Column from the Data menu.
   c. Enter "A" as the Name, "A" as the Short Name, and leave the unit blank. Absorbance is unitless.
   d. To enter the correct formula for the column into the Equation edit box, choose "Absorbance at…." then subtract off the long time absorbance value, A_∞. In the Equation edit box, you should now see displayed something like "Absorbance at 588.0-592.4 nm"-0.0532. Click Done.
   e. Click on the y-axis label. Choose "A." A graph of corrected absorbance *vs*. time should now be displayed.

2. Follow these directions to create a calculated column, ln A, and then plot a graph of ln A *vs.* time:

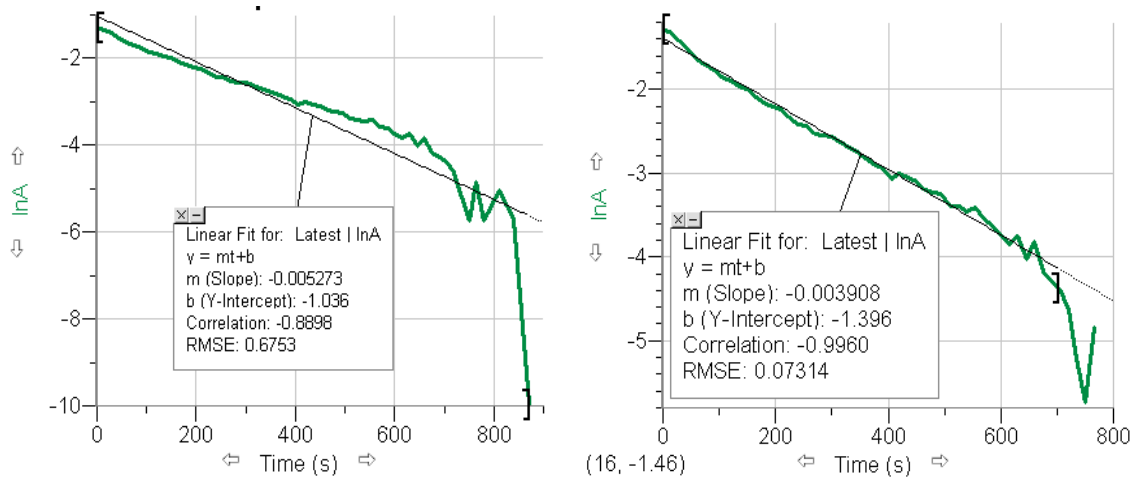
   a. Choose New Calculated Column from the Data menu.

   b. Enter "ln A" as the Name, "ln A" as the Short Name, and leave the unit blank. A logarithm is always unitless.

   c. To enter the correct formula for the column into the Equation edit box, choose "ln" from the Function list. Then select "A" from the Variables list, if you did the offset correction in step1. If you didn't do the offset correction, then select the entry that is similar to "Absorbance at 588.0-592.4 nm." In the Equation edit box, you should now see displayed: ln("A") or ln("Absorbance at 588.0-592.4 nm"). Click Done.

   d. Select Additional Graphs→Strip Chart from the Insert menu. Click on the y-axis label in this new Strip Chart. Choose ln A. A graph of ln absorbance *vs.* time should now be displayed. Autoscale the y-axis by clicking on  . To see if the relationship is linear, click the Linear Fit button,  .

   e. You will probably have some values in the long time portion that will make it difficult to get a useful vertical axis scale. To avoid plotting these points, in the data table scroll down to the bottom of the table and locate the first negative A value. Click on the row number one or two rows before the first negative A value. Then shift click on the last row in the data table. Pull down the edit menu and choose "Strike Through Data Cells." Those chosen cells will no longer be plotted and Autoscaling the plot should work better to help you set the vertical axis expansion, Figure 9. You can also select rows in the data table by using the mouse to drag over the corresponding range in the data plot.



a. b.

Figure 9. (a) Noisy points at the long-time end cause a large scale range. (b) The Strike Through Data Cells option is used to avoid plotting and fitting values at the end of the kinetics run where noise dominates. Notice the scale is expanded almost by a factor of two.

   f. The very long-time behavior of your plot may be noisy and may have some curvature, Figure 10. This curvature may be caused by not knowing the exact A_∞ offset from step 1,

above. You can narrow the range for the linear curve fit by dragging the] at the right-hand side of the plot. However, keep the fitting interval as wide as possible. (If the] handle isn't showing, remove the current curve and fit again.) Use the same time interval when comparing the curve fit for the 1/A vs. time plot, to make a fair comparison. Alternatively, you can adjust the A_∞ in the calculation for the A column to get a longer linear range. You can edit the formula for a column by double clicking the column label in the data table. Adjusting the offset also makes a fair comparison, since the same offset is used in both curve fits. However, make sure that this A_∞ makes sense (i.e. estimate A_∞ by eye and check your result). Choosing an incorrect A_∞ value can distort the data plots so that you end up choosing the incorrect order.

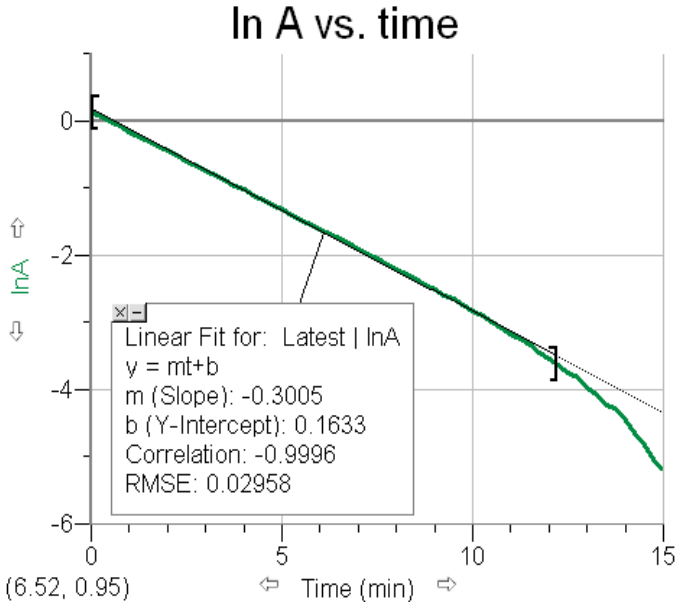


Figure 10. Kinetics plot with a shortened time range. The A_∞ offset used was too large producing curvature at the very end of the plot.

g. Print a copy of your graph. Be sure the linear regression curve is displayed on the graph, as well as the regression statistics box. Record the fit values and the correlation coefficient. Remember that the closer the correlation coefficient is to 1.0 or -1.0, the better the fit.

h. If you used the Strike Through option on any data cells, you can include any of those excluded points again by highlighting the data cells in the data table and pulling down the Edit menu and choosing "Restore Data Cells."

3. Follow these directions to create a calculated column, 1/A, and then plot a graph of 1/A *vs.* time:

   a. Choose New Calculated Column from the Data menu.

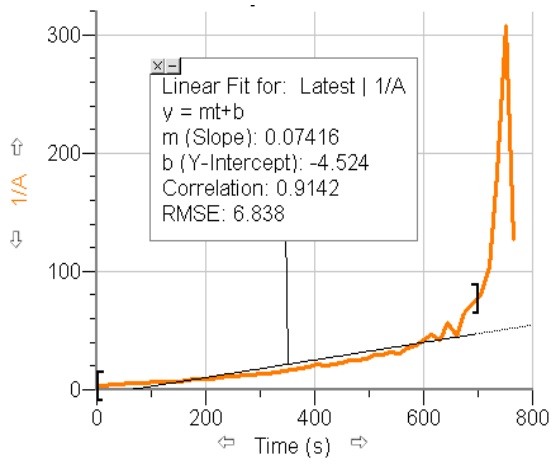
   b. Enter "1/A" as the Name, "1/A" as the Short Name, and leave the unit blank.

c. Enter the correct formula for the column into the Equation edit box: to do this, type in "1" and "/". Then select "A" from the Variables list. In the Equation edit box, you should now see displayed: 1/"A". Click Done.

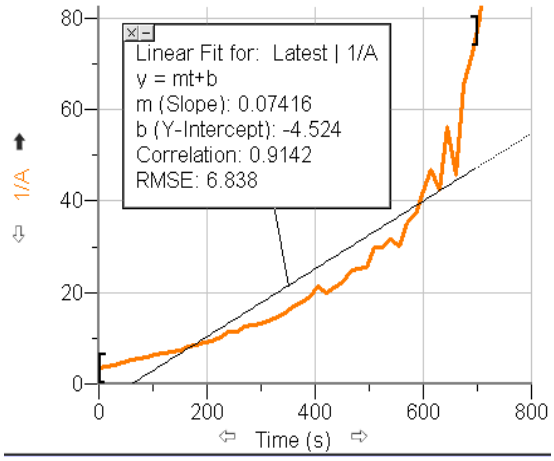
d. Select Additional Graphs→Strip Chart from the Insert menu. Click on the y-axis label in this new Strip Chart. Choose 1/A and uncheck any other boxes. A graph of 1/A *vs.* time should now be displayed. Autoscale the y-axis by clicking on  . To see if the relationship is linear, click the Linear Fit button,  .

e. If you have a few data points in the long time portion that are very different from the rest of the data points due to noise in the data, it may be difficult to get a useful y-axis scale expansion. You may use the instructions in step 2e to "Strike Through" a few more data points.

f. When you compare the ln A and 1/A plots, use the same time interval for your linear fit as you did for the ln A fit. Make sure to expand the y scale so the y-values during the chosen time interval cover the full y-axis. In other words, the long time y-values can be off scale. By greatly expanding the y-axis you will be better able to judge the linearity over the chosen time interval in a comparable scale expansion to your ln A vs. t plot, Figure 11.



a.



b.

Figure 11. (a.) The y-axis range is too large because of noisy points at the long-time end. (b.) Expand the y-axis scale to get a comparable view to the ln A vs. t plot (compare with Figure 9 at right).

g. Print a copy of your graph. Include this graph in your report. Be sure the linear regression curve is displayed on the graph, as well as the regression statistics box. Record the fit values and the correlation coefficient.

4. Copies of the plots should be in both partners' lab notebooks. Report the order and rate constant, k. Make sure to include <u>both</u> ln A vs. t and 1/A vs. t plots in your report, since the comparison between the two plots determines the proper order.