**Kinetics of the Reaction of Ascorbic Acid and Dichloroindophenol[1]: Stopped Flow Kinetics**

**Purpose:** Determine the order of the reaction, the rate constant at 25°-55°C, the activation energy, and the pre-exponential factor for the reaction of ascorbic acid with 2,6-dichloroindophenol.

## Introduction

Ascorbic acid, vitamin C, is an important nutrient that is present in many foods. About 10mg of the vitamin per day is necessary to prevent scurvy. Vitamin C is easily oxidized, especially in neutral or basic solution so that it may be used as a food additive for its antioxidant properties. Because of the ease of oxidation of ascorbic acid, it reacts with air making it difficult to preserve in foodstuffs. The analysis of ascorbic acid is an important analytical problem. The first method devised to determine vitamin C in foods is the redox titration with 2,6-dichloroindophenol (DCIP). The oxidation of ascorbic acid yields dehydroascorbic acid:

$$\text{ascorbic acid} \rightleftharpoons \text{dehydroascorbic acid} + 2\,H^+ + 2\,e^- \qquad (1)$$

DCIP is blue in neutral solution and pink in acidic solution. The reduced form is colorless, so the endpoint of the titration is signaled by the appearance of a faint color. The DCIP reaction is also suited to automated analysis techniques based on spectrophotometry, such as flow injection analysis. In flow injection analysis the rate of the reaction is an important parameter. The DCIP reaction is sluggish enough that careful attention must be paid to analysis conditions. This experiment is designed to study the kinetics of the DCIP-ascorbic acid reaction.

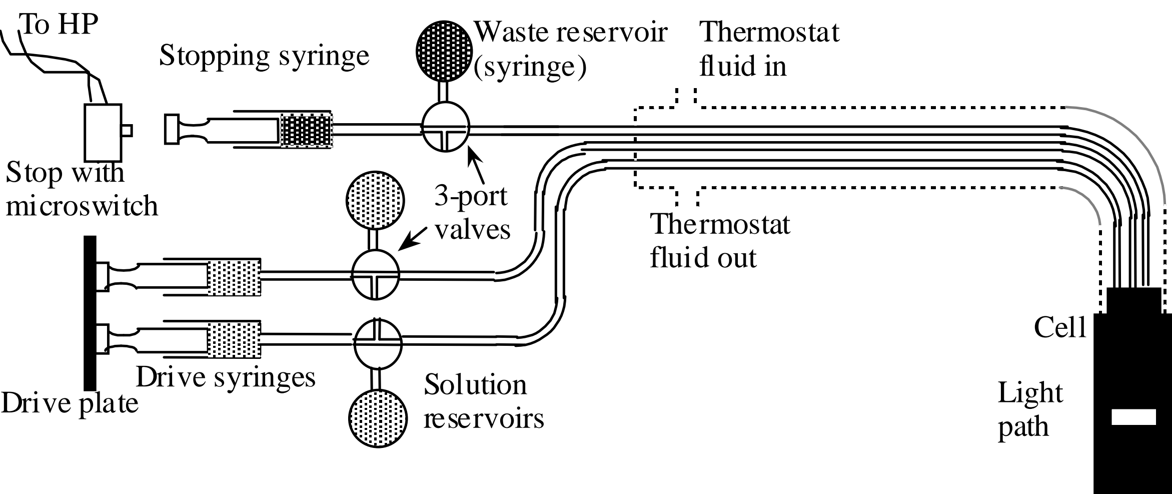



Figure 1. Stopped flow apparatus

While the reaction of ascorbic acid with DCIP is slow enough to be a problem in analytical determinations, it is too fast to study by classical kinetic measurements. In this experiment we will use the stopped flow technique. In the stopped flow technique, the reagents are mixed rapidly in a special spectrophotometer cuvette by forcing the solutions from syringes through jets in the cuvette mixing chamber[2]. The flow of reagents is stopped suddenly and the time course of the reaction is monitored spectrophotometrically (Figure 1).The limiting factor in the observation of fast reactions is the mixing time, the half-time of the reaction must be larger than the mixing time[3]. Hand operated stopped flow systems are available with mixing times near 20msec.

## Theory

Under the circumstances of this reaction the kinetics of the disappearance of DCIP may be written as:

$$\frac{d[DCIP]}{dt} = k\,[Ascorbate]^p\,[DCIP]^n \qquad 2$$

where p is the order of the reaction with respect to ascorbic acid and n is the order of the reaction with respect to DCIP. In this experiment the concentration of ascorbic acid will be greater than the concentration of DCIP and Eq. 2 can be rewritten to isolate the rate dependence on just the DCIP:

$$\frac{d[DCIP]}{dt} = k_{eff}\,[DCIP]^n \qquad 3$$

where $k_{eff} = k\,[Ascorbate]^p$. We will not be able to determine the order with respect to ascorbic acid in this experiment. However, we will be able to determine the reaction order with respect to DCIP based on on Eq. 3.

First order behavior gives rise to a time course for the reaction that obeys the relationship:

$$\ln\left(\frac{[DCIP]}{[DCIP]_o}\right) = -k_{eff}\,t \qquad 4$$

where $[DCIP]_o$ is the initial concentration and [DCIP] is the concentration at time t. Second order behavior for DCIP shows the time course:

$$\frac{1}{[DCIP]} - \frac{1}{[DCIP]_o} = k_{eff}\,t \qquad 5$$

In the temperature range near room temperature, the reaction has been found to follow Arrhenius behavior:

$$k_{eff} = A\,e^{-E_a/RT} \qquad 6$$

where $E_a$ is the activation energy and A is the pre-exponential factor. Taking logs to convert the equation to linear form:

$$\ln k_{eff} = \frac{-E_a}{RT} + \ln A \qquad 7$$

Appropriate plots of equations 4-7 will allow the determination of the reaction order, the rate constant as a function of temperature, and the activation energy for the reaction.

In this experiment, absorbances will be used for the plots to find k, instead of concentrations. Using the Beer-Lambert law at time t:

$$A_{DCIP} = a\ b\ [DCIP]_t \qquad 8$$

where $A_{DCIP}$ is the absorbance of DCIP, a is the molar absorbance coefficient at the chosen wavelength, and b is the path length of the cell. The total absorbance of the solution is the sum of the absorbance of the DCIP and any other absorbing species,

$$A = A_{DCIP} + A_{bkg} \qquad 9$$

where $A_{bkg}$ is the background absorbance of any other species. In addition, if the cuvette position is changed slightly, the background absorbance will be changed slightly. $A_{bkg}$ will also include these small changes from run to run. If the at the start of the reaction, t=0, with DCIP concentration $[DCIP]_o$ and at infinite time with concentration $[DCIP]_\infty$ we find:

$$A_o = a\ b\ [DCIP]_o + A_{bkg} \qquad A_\infty = a\ b\ [DCIP]_\infty + A_{bkg} \qquad 10$$

Assuming that $[DCIP]_\infty = 0$ gives:

$$\frac{[DCIP]}{[DCIP]_o} = \frac{A - A_\infty}{A_o - A_\infty} \qquad 11$$

substitution into equation 4 gives for a first-order process:

$$\ln \left(\frac{A - A_\infty}{A_o - A_\infty}\right) = -k\ t \qquad 12$$

or:

$$\ln (A - A_\infty) = -k\ t + \ln (A_o - A_\infty) \qquad 13$$

Multiplying equation 5 by $[DCIP]_o$ and substitution of equation 9 gives for a second-order process:

$$\frac{1}{\left(\frac{A - A_\infty}{A_o - A_\infty}\right)} - 1 = [DCIP]_o\ k\ t \qquad 14$$

or

$$\frac{1}{\left(\frac{A - A_\infty}{A_o - A_\infty}\right)} = [DCIP]_o\ k\ t + 1 \qquad 15$$

In summary, equations 12-15 show that absorbances may be used instead of concentrations for kinetics curve fitting.

## Procedure

**Equipment**
Hi-Tech stopped flow accessory
Ocean Optics Diode Array Spectrophotometer
Circulating water bath
500 ml volumetric flask
200 ml volumetric flask
4x 50-ml volumteric flasks
2x 10, 15, 20, 25-ml volumetric pipets
4x 30-ml beakers
long stemmed funnel

Stock solutions:
- 500 ml of pH 7.4 phosphate buffer (see Lange's Handbook or the CRC for concentrations[4])
- 100 ml of $1.0 \times 10^{-3}$ M sodium dichloroindophenol in pH 7.4 buffer
- 200 ml of $1.0 \times 10^{-3}$ M HCl

Prepare a dilution of the DCIP stock solution, using the volumetric glassware listed above, to produce a $5 \times 10^{-4}$ M solution of DCIP in pH 7.4 buffer. (Remember that the solutions will be mixed in equal volumes in the stopped-flow cuvette, thus halving their concentrations). Fill one of the stopped-flow reservoirs with $5 \times 10^{-4}$ M DCIP and the other with deionized water. Practice your injection technique with these solutions. Also determine the absorption spectrum of the dye using these solutions. Instructions for the use of the Ocean Optics Spectrophotometer are in the Appendix. Find the maximum wavelength of absorption. Use this wavelength for the following kinetic runs.

Prepare a stock solution of ascorbic acid that is $8.0 \times 10^{-3}$M in ascorbic acid in $1.0 \times 10^{-3}$ M HCl of 200ml volume. The stock solution is made up in dilute acid to retard air oxidation of the ascorbic acid. Keep this stock solution in the refrigerator for the same reason.

Prepare a dilution of the ascorbic acid stock solution using the volumetric glassware listed above to produce a concentration in the range of $1.6 \times 10^{-3}$M to $3.2 \times 10^{-3}$M of ascorbic acid in $1.0 \times 10^{-3}$ M HCl. In making up this solution dilute with $1.0 \times 10^{-3}$ M HCl in a 50-ml volumetric flask. Do a kinetics run at 25°C. The total time of the reaction should be about 6-10 sec. If total time of the reaction is not in this range, adjust the concentration of ascorbic acid accordingly. Use volumetric glassware and dilutions as above. Run each dilution immediately after preparation to avoid air oxidation of the ascorbic acid. Instructions for use of the stopped-flow accessory follow this write-up. The minimum cycle time for the spectrophotometer is 0.1 sec. Save the data file and make a printout of each successful run. Tabulate the time trace of the 25°C run.

Using the concentration determined in the last step, do at least three kinetic runs at roughly 10°C degree intervals above 25°C. Make up fresh ascorbic acid solution in $1 \times 10^{-3}$ M HCl immediately before each run.

## Calculations

Using equations 12 and 15, determine the reaction order and the rate constant for the 25°C run (only). Check that your fit value agrees with the value calculated by Logger Pro. Report both values and their uncertainties. Using the rate constants as a function of temperature determine the activation energy for the reaction and the pre-exponential factor. In your report include the two plots that you used to determine the order for the reaction and your Arrhenius plot. Include the uncertainties from the least squares curve fitting. Use propagation of errors rules to report the uncertainty in the activation energy and the pre-exponential factor.

Derive equation 11 from equations 9 and 10. Discuss the predominant experimental error in your measurements. Discuss the chemical significance of this experiment in general and your results in particular.

## References

1. B. Morelli, *J. of Chemical Education*, **1976**, *53*, 119-122.

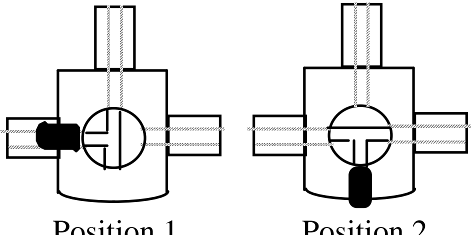
2. E.F. Caldrin, "Fast Reactions in Solution," Blackwell Scientific Pub., Oxford (England), 1964

3. G. G. Hammes (ed.)," Investigation of Rates and Mechanisms of Reactions" (Vol. VI of "Techniques of Chemistry", edited by A. Weissberger), 3rd ed., Part II, Chs. 2-6, Wiley-Interscience, New York, 1974.

4. J.A. Dean (ed.), "Lange's Handbook of Chemistry", McGraw-Hill, New York.

**Operation of the SFA-11**
**Stopped Flow Accessory**

There are two positions for the three-way taps. In position 1 the operating lever points horizontally towards the syringe, in position 2 the lever points vertically downwards.



Position 1 Position 2

There are two positions for the three-way taps. In position 1 the operating lever points horizontally towards the syringe: in position 2 the lever points vertically downwards.

1. Fit the open reagent reservoir syringes to the taps connected to the drive syringes, and fit the waste syringe complete with piston, to the tap connected to the stoppping syringe. Half-fill the open syringes with their reagent solutions.

2. Turn all taps to 1. Push and pull rapidly each drive syringe piston so as to fill the drive syringes and expel air bubbles. Pull the drive syringe pistons out as far as possible. Push the stopping syringe piston in as far as possible.

3. Turn all taps to 2. Push firmly on the drive plate. The best way to do all this is to squeeze the drive plate and the stopping block firmly together, using the thumbs and first fingers of both hands, one on each side.

4. Turn all taps to 1. Push the stopping syringe in as far as possible, and pull the drive syringe pistons out as far as possible.

5. Repeat 3 and 4 a few times with the taps set at 1, push and pull the waste syringe to and fro, so as to expel all the air bubbles.

6. To prepare the unit for a run, now that all air bubbles have been expelled, turn all taps to 1, fill the drive syringes and empty the waste syringe. Refill the reservoir syringes as required to prevent further air being drawn in.

7. To perform a run, turn the taps to 2, start the chart recorder and data-capture system, and squeeze the drive plate and stopping block firmly together. The spectrophotometer signal may vary slightly with how hard this squeeze is. For reproducibility adopt the same procedure for each run. If the run is complete in less than a minute or so, keep squeezing until the reaction is complete. If the reaction is slower, release the pressure as soon as the flow stops.

<u>Spectrophotometer Response Time</u>

A useful check on the spectrophotometer and data-capture system response time may be obtained by observing the initial part of the run. At the start of the push the old, reacted solution is swept out of the sample cell in about 20 milliseconds, and there is a rapid absorbance change as the

new, unreacted solution comes in. The observed time for this process will be the response time for the spectrophotometer and the data-capture system, which is normally much greater than 20 milliseconds.

## After Use

The flow circuit should be neutralized by washing through with distilled water and, ideally, flushed with air, nitrogen or acetone.
All the syringe pistons should be pushed right in, or removed if the accessory is not to be used for a long period of time.
Application of a hot cloth will usually free a stubborn piston seizure.

## Ocean Optics Spectrophotometers with Vernier Data Acquisition Software Instructions

### Introduction:

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

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* 3 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 in the 30 to 2000 range. Use more Samples for more precise absorbances. If you are using regular plastic cuvettes, set the wavelength range to 350 – 850 nm; UV plastic cuvettes, set the wavelength range to 260-850 nm; or UV quartz cuvettes, set the wavelength range to 200-850 nm. The Integration time will be set for you automatically when you do the calibration in the next step. Click on Close.

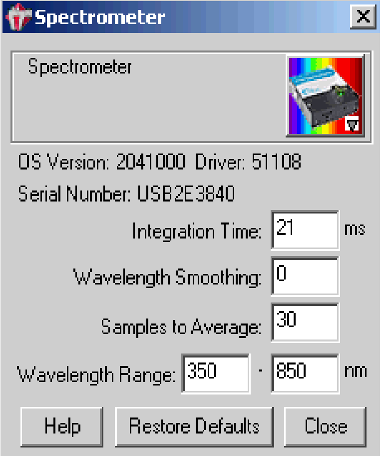



Figure 1. Settings for fast analysis with regular plastic cuvettes or work in the visible range.

4. To calibrate the Spectrometer, 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, use a cuvette filled about ¾ full with the solvent or buffer for

your reference, as instructed. Check to make sure the non-frosted, clear sides are in the light path. The cuvette should be inserted all the way through 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 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 Collect and then Stop.

2. You can read the absorbance using the Examine tool, by clicking on X=. 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 wavelength throughout your experiment.

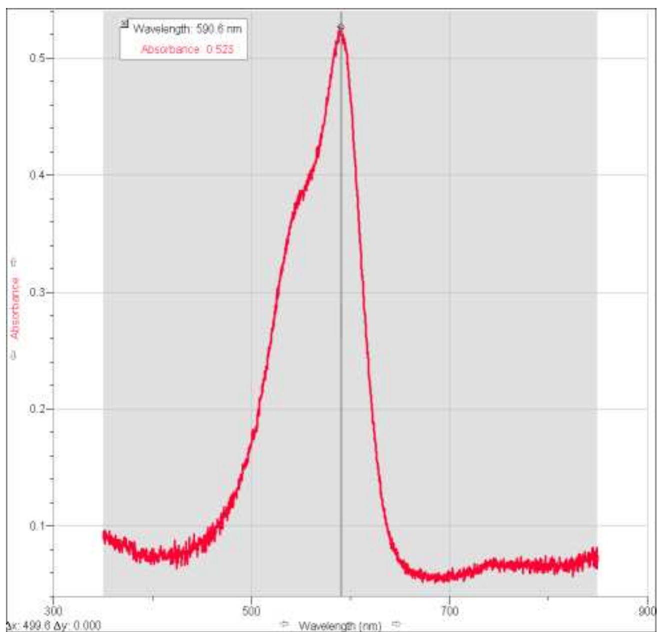



Figure 3. Spectrum of Crystal Violet with the Examine Tool.

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.

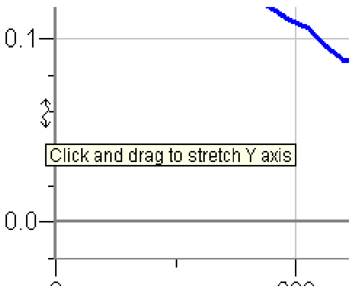



Figure 4. Move the cursor over the axis to change the axis scale.

*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.

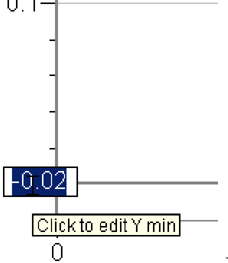



Figure 5. Click near the axis maximum or minimum to show the dialog box.

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.

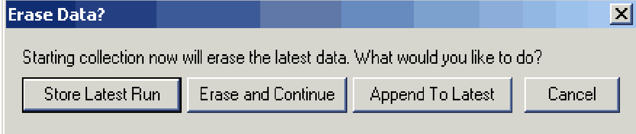
6. Replace the cuvette with another sample. You may need to calibrate again if there is a different reference solvent or buffer for the new sample. Return to step 1.

*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.

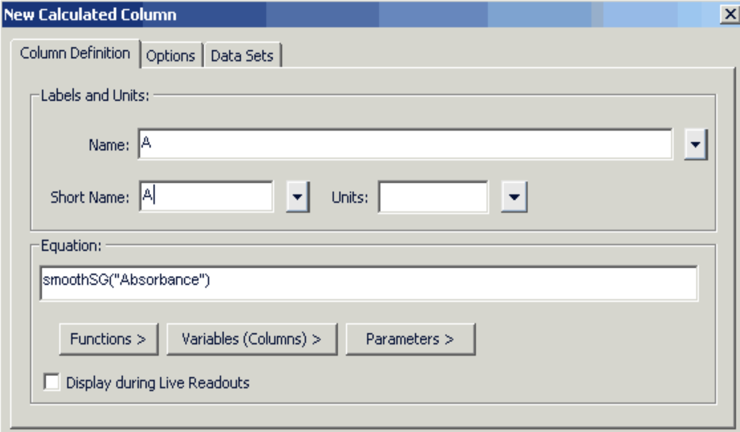
6. When you click on Collect this time, if you did not store the latest run to set-up for overlayed spectra in step 3, you will get a dialog box:




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.

8. If your spectrum is noisy, you can increase the setting for the number of Samples to Average in step I.3 and retake your spectrum. Alternatively, you can use spectral smoothing. Spectral smoothing averages together adjacent data points. To apply spectral smoothing, choose New Calculated Column from the Data menu. Enter "A" as the Name, "A" as the Short Name, and leave the units blank. Absorbance is unitless. Enter the formula for the column into the Equation edit box, by choosing smoothSg from the functions menu and "Absorbance" from the Variables menu, as shown below. Click Done.




Click on the y-axis label. Choose "A." A graph of smoothed absorbance will be displayed.

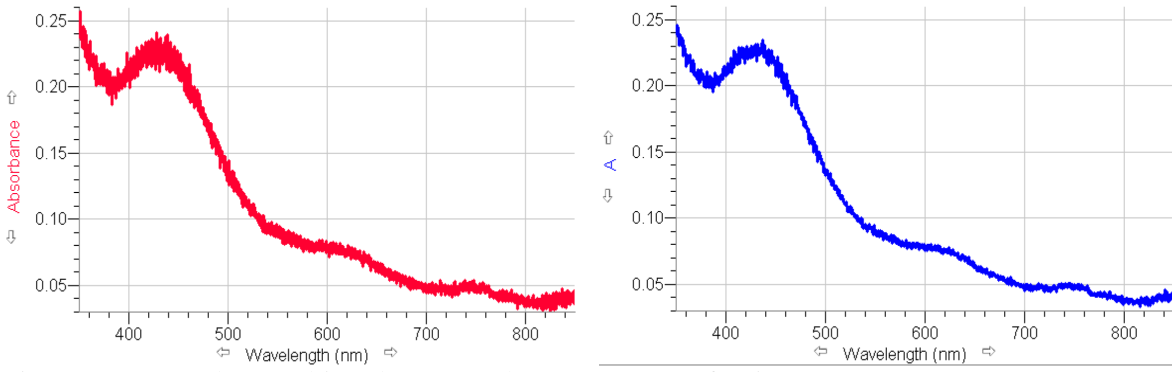



Figure 6. Spectral smoothing decreases the appearance of noise.

**<u>To finish up, see section IV below.</u>**

**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 7. 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.

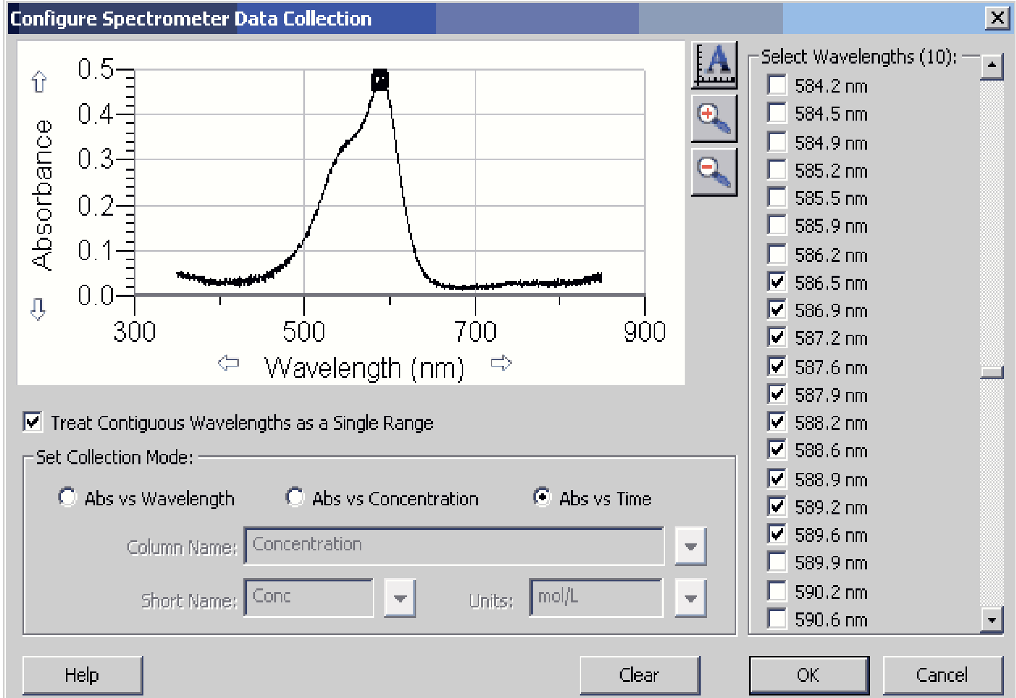


Figure 7. Averaging over several wavelengths to obtain better precision.

3. Click Data Collection . Choose a Length and seconds per sample as suggested in the lab writeup, Figure 8. 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.

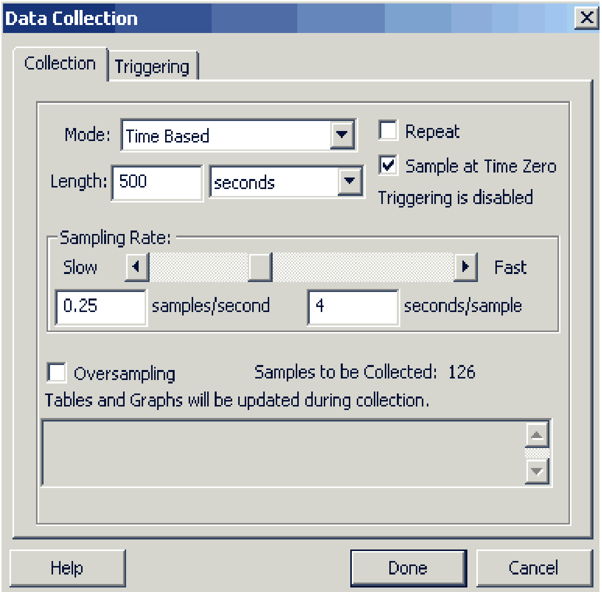


Figure 8. Setting the sampling time interval and total experiment length.

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 Collect 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 move the cuvette. Don't stick anything sharp into the cuvettes.

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

3. <u>Please unplug the spectrometer's transformer</u>. The deuterium lamp in the spectrometer has a limited lifetime and replacements are very expensive.

**Kinetic Data Analysis Using Vernier Software.**

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

- 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 = slope a b),

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. 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 when 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, 1/2 STAT. The mean of the selected data points will be listed. Record this value as $A_\infty$.
    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, $A_\infty$. 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.

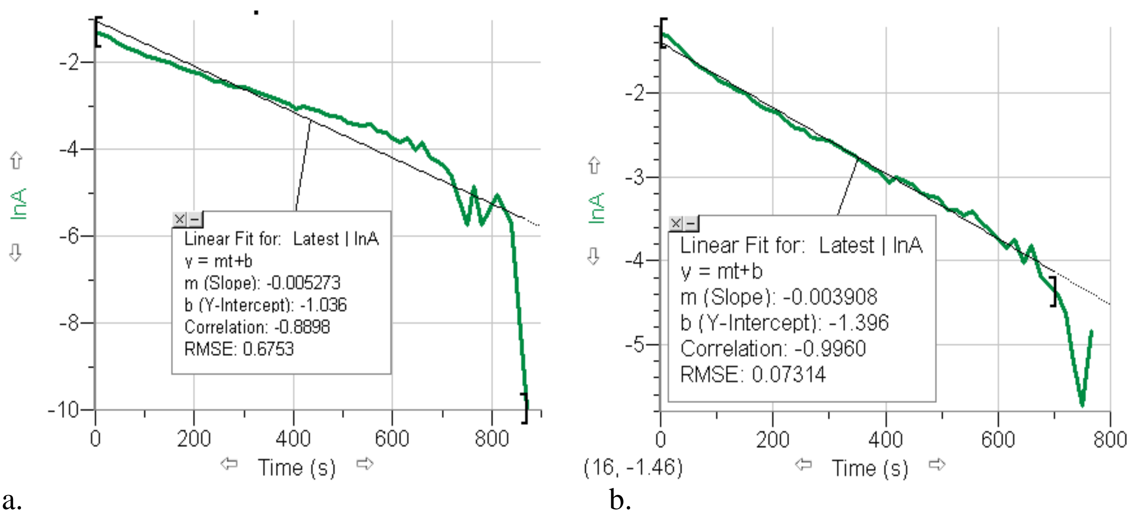



Figure 9. (a) Noisy points at the long-time end cause a large scale range. (b) The Strike Through Data Cells option is used to avoid plotting and fitting values at the end of the kinetics run where noise dominates. Notice the scale is expanded almost by a factor of two.

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 $A_\infty$ 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 $A_\infty$ 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 $A_\infty$ makes sense (i.e. estimate $A_\infty$ by eye and check your result). Choosing an incorrect $A_\infty$ value can distort the data plots so that you end up choosing the incorrect order.

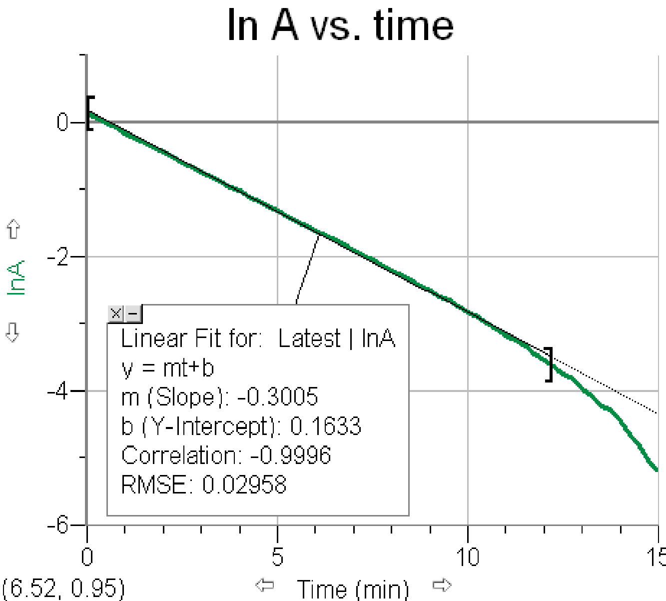



Figure 10. Kinetics plot with a shortened time range. The $A_\infty$ offset used was too large producing curvature at the very end of the plot.

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 [A]. To see if the relationship is linear, click the Linear Fit button, [R].

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.

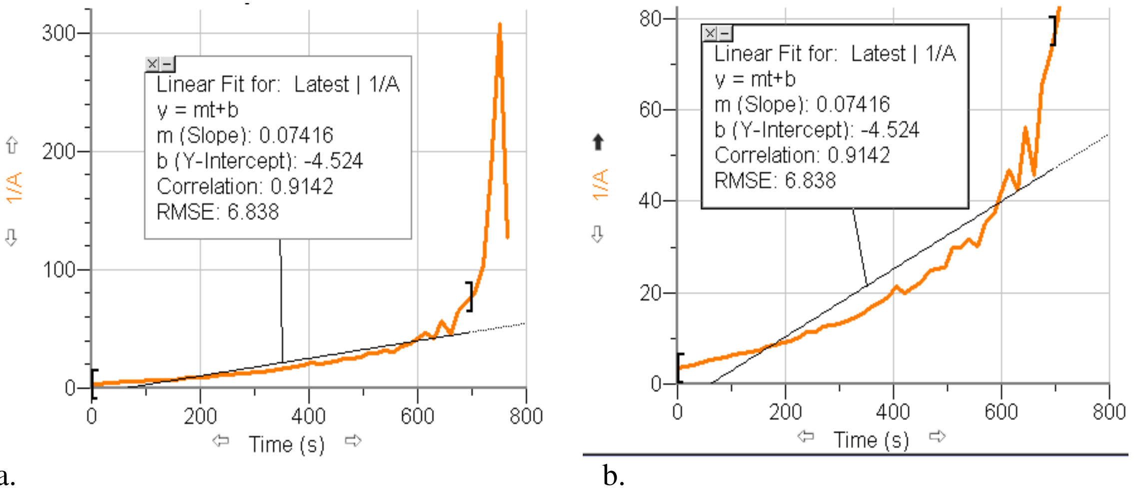



Figure 11. (a.) The y-axis range is too large because of noisy points at the long-time end. (b.) Expand the y-axis scale to get a comparable view to the ln A vs. t plot (compare with Figure 9 at right).

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 both ln A vs. t and 1/A vs. t plots in your report, since the comparison between the two plots determines the proper order.