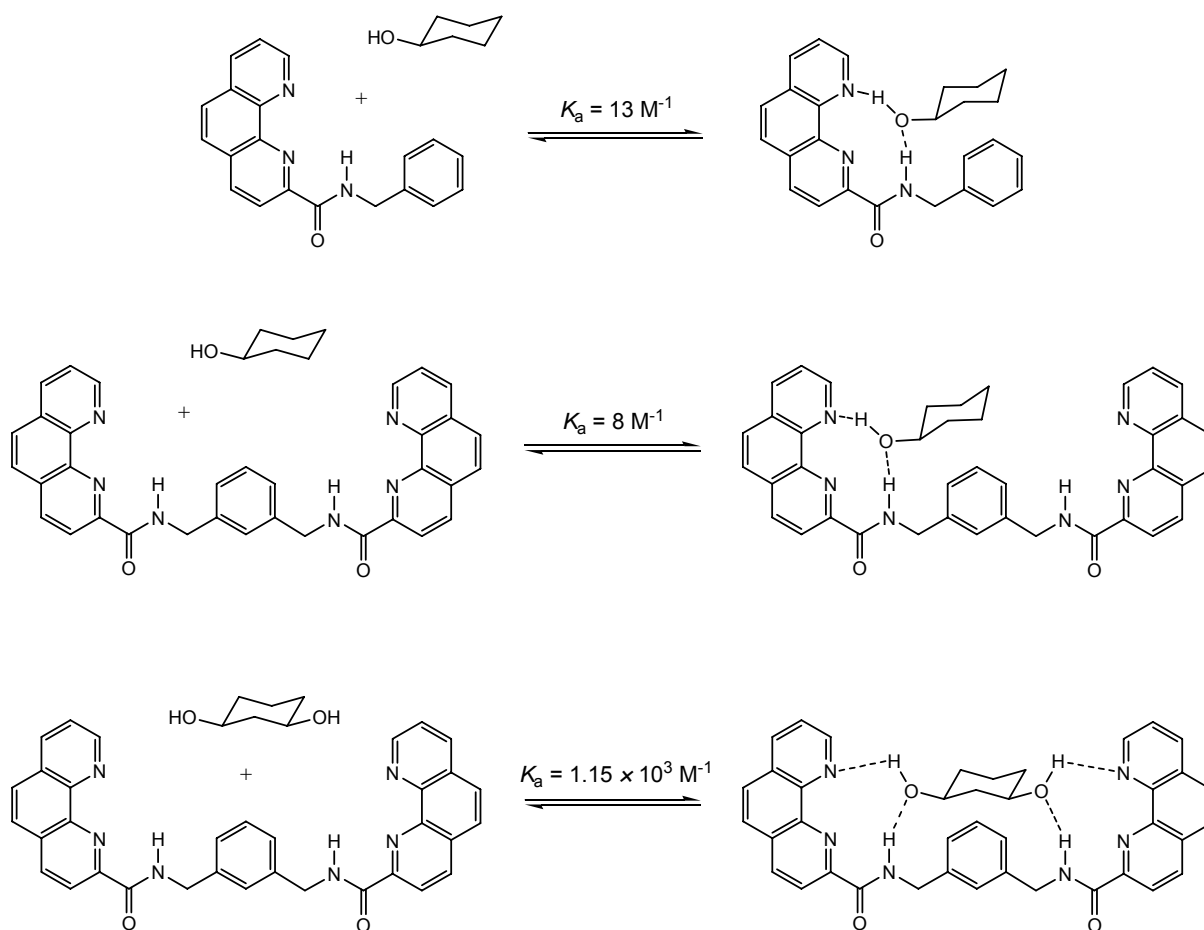


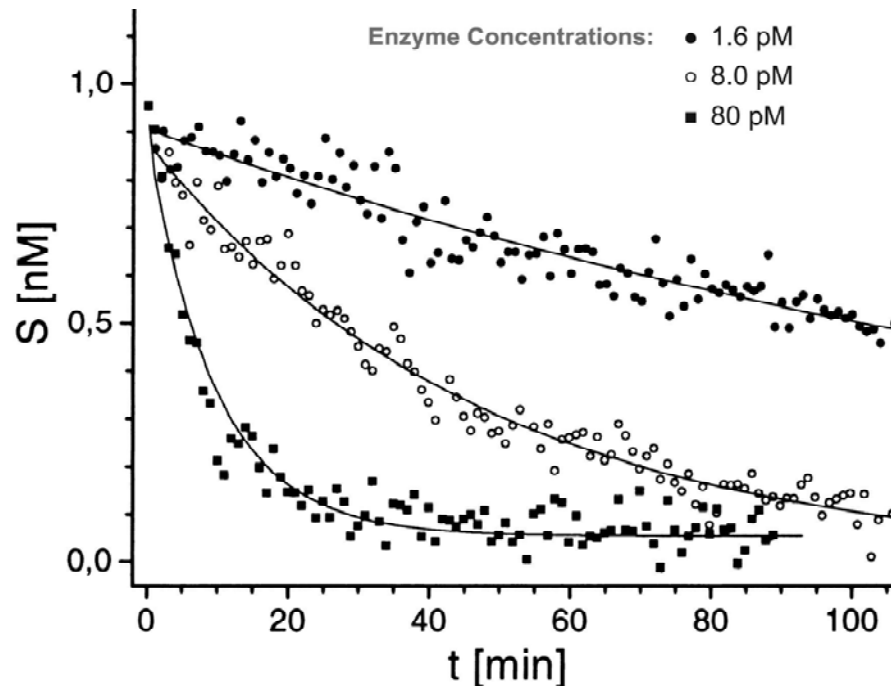
Problem Set 3

Due: In class, Friday, September 29

1. a) Based on the association constants shown below, and the definitions of cooperativity discussed in class and in *MPOC* Chapter 4, does the binding of *cis*-1,3-cyclohexanediol in the last equation show positive cooperativity? (i.e., is $\alpha > 1$ or < 1 ?)
- b) By how many kcal/mol is the binding cooperative in a positive or negative sense? Has engineering the entropy of this system helped or hurt?



2. The Method of Initial Rates isn't often used for reactions in which a reagent or product concentration can be monitored continuously; in these cases, the dataset is typically fit to an integrated rate expression (like in Problem 3 below). However, in cases where decomposition or complex successive reactions can follow an initial reaction of interest, the Method of Initial Rates can work better than data fitting. Kettling et al. used this method, for example, to show that fluorescence correlation spectroscopy could be used to characterize the activity of extremely low enzyme concentrations.¹ His data on the restriction enzyme *EcoRI*, which cuts a DNA substrate ("S") is shown below.



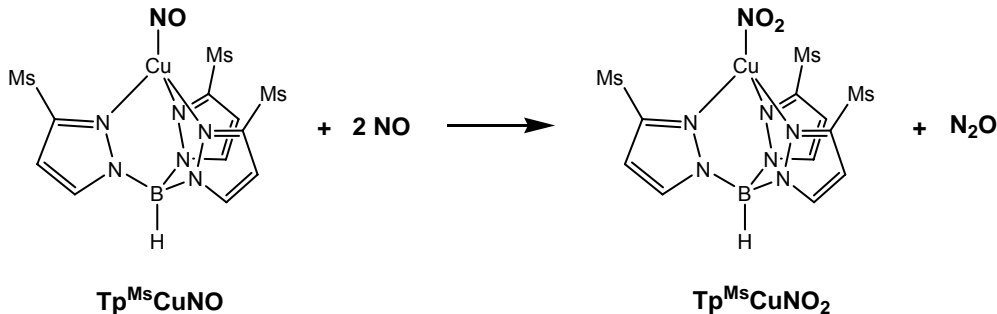
These curves didn't fit integrated rate laws well, but the Method of Initial Rates did work here. For

$$\frac{d[S]}{dt} = -k_{\text{obs}}[EcoRI]^x,$$

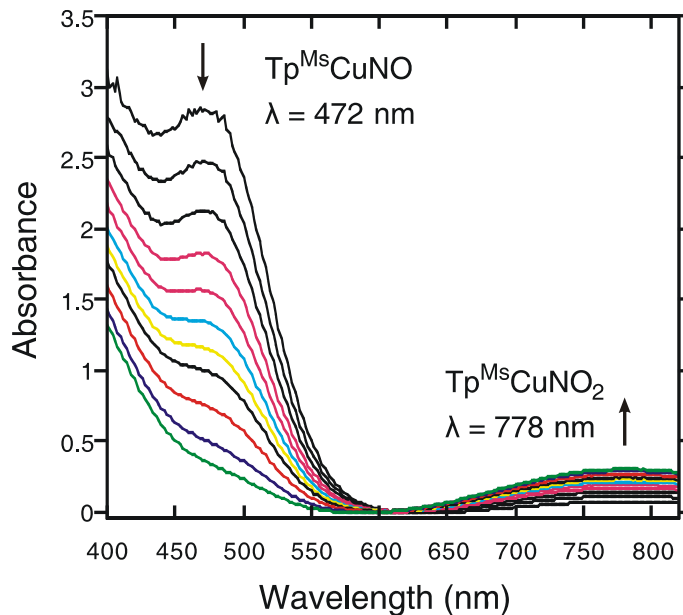
what are k_{obs} and x ?

¹ Kettling, U.; Koltermann, A.; Schwille, P.; Eigen, M. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 1416-1420.

3. The complex $\text{Tp}^{\text{Ms}}\text{CuNO}$ reacts with excess NO to yield N_2O and $\text{Tp}^{\text{Ms}}\text{CuNO}_2$.



The reaction can be monitored by UV-vis spectroscopy, as shown in the graph below. In this graph, the loss of $\text{Tp}^{\text{Ms}}\text{CuNO}$ corresponds to a decrease in absorbance at $\lambda_{\text{max}}(\text{Tp}^{\text{Ms}}\text{CuNO}) = 472 \text{ nm}$, and gain of product $\text{Tp}^{\text{Ms}}\text{CuNO}_2$ corresponds to gain in absorbance at $\lambda_{\text{max}}(\text{Tp}^{\text{Ms}}\text{CuNO}_2) = 778 \text{ nm}$. The reaction was carried out using a 4.8 mM solution of $\text{Tp}^{\text{Ms}}\text{CuNO}$ and a >10 fold excess of NO. A chart of absorbance vs. time data, collected at 472 nm and 778 nm, are posted on the class web site. In this problem, you will fit this data to integrated rate expressions; for each problem below, show your answer as a graph with the fit line and results on the graph (or at least somewhere that we can figure out which data goes with which fit).



a) If this process were first order in $\text{Tp}^{\text{Ms}}\text{CuNO}$, the integrated rate expression for this process would be

$$\frac{[\text{Tp}^{\text{Ms}}\text{CuNO}]_t}{[\text{Tp}^{\text{Ms}}\text{CuNO}]_0} = e^{-kt}$$

or

$$\ln\left(\frac{[\text{Tp}^{\text{Ms}}\text{CuNO}]_t}{[\text{Tp}^{\text{Ms}}\text{CuNO}]_0}\right) = -kt.$$

For this part of the problem, assume that the absorbance measured at 472 nm (A_{472}) is proportional to $[\text{Tp}^{\text{Ms}}\text{CuNO}]_t/[\text{Tp}^{\text{Ms}}\text{CuNO}]_0$, and that $A_{472} = 0$ for $[\text{Tp}^{\text{Ms}}\text{CuNO}] = 0$. Perform a *linear* least-squares fit of the A_{472} data to either of the expressions above, and use the output of the fit or the slope of the curve (for the second equation) to estimate k . You can do this in any number of programs, including Microsoft Excel, Kaleidagraph, and others, by using the pre-set fits that come with the program.

- b) Next, following either the instructions in Appendix 1 or 2 of this problem set, perform a *nonlinear* least-squares fit of the same data to the same equation to determine k . You may want to use Kaleidagraph to do this (instructions at the end of this Problem Set).
- c) As we discussed in class, it is possible to avoid the assumptions we made above by fitting to the phenomenological, first-order rate equation

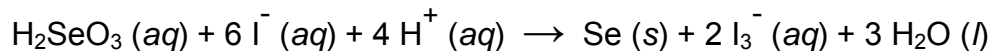
$$Y_t = Y_\infty + (Y_0 - Y_\infty)e^{-kt},$$

where Y is an observable (such as A_{472}). Fit the A_{472} rate data to this equation, using nonlinear least-squares and allowing the fit to optimize values for A_0 and A_∞ . What is your calculated k , and are the other variables reasonable? Does getting rid of outlier data points improve your fit?

- d) Repeat part (c) for the A_{778} data to evaluate the same rate constant k from $\partial[\text{Tp}^{\text{Ms}}\text{CuNO}_2]/\partial t$. Remember that the rate equation will not be exactly the same as the one in (c). How does your value for k compare?

Problems to try on your own:

- David Blauch (Davidson College) has created a Java applet (<http://www.chm.davidson.edu/ChemistryApplets/kinetics/MethodOfInitialRates.html>) that illustrates how a triiodide clock reaction, along with the Method of Initial Rates, can be used to determine the order of the following reaction:



Use the applet to determine the order of this reaction in $[\text{H}_2\text{SeO}_3]$ and $[\text{I}^-]$. Show your work; please don't just report values.

(For the website above to work, you must have Sun Microsystems' Java Runtime Environment installed on your computer; you can find this at <http://www.java.com>. The answer to this problem is posted in the Problem Set answer key.)

Chemistry 4011/8011

Problem Set 3, Appendix Nonlinear Least-Squares Curve Fitting with Kaleidagraph

There are not so many graphing programs that use nonlinear least-squares fitting; Synergy Kaleidagraph is one of the simplest. (You can also do nonlinear least-squares fitting on Microcal Origin, Mathworks MATLAB, or Wolfram Mathematica, if you have access to them and know how to use them.) By comparison, Kaleidagraph is much easier to use for curve fitting, but you will have to go to the Microcomputer Lab (176 Kolthoff), use the demo version, or purchase it to use it. (The demo version is available at <http://www.kaleidagraph.com/>. This version lasts for 45 days, and will only create graphs with “Demo Copy” in big letters written across them. Turning these in is just fine with me.) Excel, on the other hand, will require you to do more spreadsheet manipulation, but many of you probably own Excel already.

To use Kaleidagraph for nonlinear least-squares fitting:

1. Open Kaleidagraph and click **File, Import, Excel** to import your data spreadsheet.
2. Graph your data by clicking **Gallery, Linear, Scatter**. Pick the appropriate columns for the x- and y-axes, and create a New Plot.
3. To fit any of your data to a custom function, click **Curve Fit, General, fit1**. (Fit1 is already defined, but we will change it. If you want to create and save functions rather than just editing the one in the program, click **Curve Fit, General, Edit General** and create new functions.)
4. Check the data box you want to fit. Then, click **Define** to create your function. The function has to have a specific form in which the independent, x-axis variable (e.g., time) is named “m0” and all other variables to be optimized are named “m1”, “m2”, etc. The equation is then followed by initial guesses for the optimized variables. So, for example, if you were graphing UV-vis absorbance data and you wanted to fit it to the equation

$$A_{\lambda} = ce^{-kt} + b,$$

you might type

$$m1*e^{(-m2*m0)} + m3; m1 = 1; m2 = 0.01; m3 = 0.1$$

in the function box. Importantly, none of your initial guesses can be set to a value of 0, or you will get an error. In addition, as is probably obvious from the example above, the independent variable (A_{λ}) must be alone on the left-hand side of the rate equation to set up Kaleidagraph's fit.

5. Click **OK**, and a fit curve should appear in your data window, along with the optimized values and other information from your fit. If you don't like your fit, you can remove it from the graph by going back into **Curve Fit, General, fit1** and deselecting the check box for the fitted data. Click **OK**, and the fit curve and fit data disappear. If you get a "Singular Coefficient Matrix" error during your fit, it is probably because your initial guesses were really far off; try new guess values.