KINETICS OF THE REDUCTION OF METHYLENE BLUE BY ASCORBIC ACID

Theory

Understanding a chemical reaction requires one to specify the mechanism. The mechanism is given by a plausible set of elementary steps, each of which is specified by a rate constant and molecularity. Mechanisms can be quite involved, for example in the catalyzed decomposition of ozone or the formation of HCl from H2 and Cl2, even though the overall reaction may appear simple. Often the differential equations arising from a mechanism cannot be solved to obtain explicit time dependences of concentrations.

In the simplest single-step mechanism (often the representation of an elementary step), as shown below:

\[
A \rightarrow \text{Products}
\]  

(7.1)

the reaction is defined by the order, \(n\), and the rate constant, \(k_n\). The reaction velocity is given by:

\[
\nu = -\frac{dC_A}{dt} = k_n C_A^n,
\]

(7.2)

where \(C_A\) is the reactant concentration. One sort of reaction is one in which \(n = 1\), and the reaction is said to be of first order. For such a simple differential equation, the solution is very simple:

\[
\ln \left( \frac{C_A(t)}{C_A(0)} \right) = -k_1 t.
\]

(7.3)

A plot of the logarithm of the concentration versus \(t\) is a straight line, with a slope of \(-k_1\). From the slope of such a plot, one extracts the rate constant.

A more complex mechanism is a two-step reaction. In the following two-step mechanism, \(A\) is converted to \(B\), and \(B\) is converted to \(A\), both by first-order processes.

\[
A \xrightarrow{k_1} B \xrightarrow{k_{-1}} A
\]

(7.4)

The equation for the disappearance of \(A\) by this mechanism is:

\[
-\frac{dC_A}{dt} = k_1 C_A - k_{-1} C_B
\]

(7.5)

The equation for the rate of appearance of \(B\) is identical in form. The explicit solution of this equation is:
\[
\ln \left( \frac{C_A(t) - C_A(\infty)}{C_A(0) - C_A(\infty)} \right) = - \left( k_1 + k_{-1} \right) t, \quad (7.6)
\]

where the concentration at any time \( t \) is given by \( C_A(t) \). In particular, after a very long time (indicated by \( \infty \)), the reaction may produce a state in which both \( A \) and \( B \) are present. This state is the equilibrium state. For this mechanism, the linearized form of the equation is also logarithmic, however the argument of the logarithm is a difference of concentrations, so one has to form a function of concentrations to determine the rate constants, but these are available from the measured concentrations of \( A \). The situation is shown in Figure 7.1.

This mechanism illustrates the approach to dynamic equilibrium. It appears from equation (7.6) that the only information from such a graph is the sum of the rate constants. However, at dynamic equilibrium (i.e. at very long times), the rates of these two processes are equal, which allows one to determine the ratio of the rate constants.

\[
\frac{C_B(\infty)}{C_A(\infty)} = \frac{k_1}{k_{-1}}. \quad (7.7)
\]

From this equation and the slope of an appropriate logarithmic graph, one has two equations in two unknowns, which allows an independent evaluation of both \( k_1 \) and \( k_{-1} \).

Dynamic equilibrium is very important. Consider, for example, a reaction sequence in which the forward step is of first order in \( A \) and the reverse step is of second order in \( B \). While the mathematics of the solution of the equation at any time may be difficult, at equilibrium the two rates must be equal, which gives a relationship between the two rates constants and the equilibrium concentrations:

\[
\frac{C_A^2(\infty)}{C_A(\infty)} = \frac{k_1}{k_2}, \quad (7.8)
\]

in which the rate constant of the reverse step, since it is of second order, is labeled \( k_2 \).

Second-order reactions form an important class in kinetics. There are two kinds of second-order reaction steps, those that are of second order in a single species, and those that are of second order by virtue of being first-order in each of two distinguishable species.

Consider the simplest second-order mechanism of the first type. The reaction is:

\[
2 \ A \rightarrow \ \text{Products}, \quad (7.9)
\]

and the velocity equation is:

\[
v = -\frac{1}{2} \frac{dC_A}{dt} = k_2 C_A^2. \quad (7.10)
\]

The solution of this equation gives the time course of the reactant:

\[^1\text{In this equation, the velocity is defined according to the proper convention. In many texts, one sees equations that do not include the factor for the stoichiometric coefficient, }1/2. \text{ Those equations give a “rate constant” for the disappearance of } A, k_{\text{off}}, \text{ not the rate constant for the reaction.}\]
\[
\frac{1}{C_A(t)} = \frac{1}{C_A(0)} + 2k_2t = \frac{1}{C_A(0)} + k_{\text{eff}}t. \quad (7.11)
\]

This is the linearized form of the equation, so a plot of the inverse of the concentration of a reactant versus time should give a straight line, the slope of which is \(2k_2\), sometimes called \(k_{\text{eff}}\).

The second form occurs for reaction of two distinguishable species (each having a unique starting concentration) that react in a one-to-one step to form products:

\[
A + B \rightarrow \text{Products}. \quad (7.12)
\]

The rate equation derivable for this process is:

\[
\nu = -\frac{dC_A}{dt} = -\frac{dC_B}{dt} = k_2 C_A C_B. \quad (7.13)
\]

This differential equation is more complex but may be solved, subject to known initial concentrations, to give the following complex equation:

\[
\ln \left( \frac{C_B(t)}{C_A(t)} \right) = \ln \left( \frac{C_B(0)}{C_A(0)} \right) + (C_B(0) - C_A(0))k_2t. \quad (7.14)
\]

To solve for the parameters, one must plot a function of both concentrations as a function of time, which requires one to measure simultaneously and independently the concentrations of both reactants. If that can be done, a plot of the logarithm of the ratios of the concentrations gives a straight line with a slope of \((C_B(0) - C_A(0))k_2\), from which one may extract the rate constant.

The form of equation (7.14) becomes indeterminate for the condition that the initial concentrations of the two species are equal. One may show that, under these conditions, one need only follow the concentration of one species (since the other must always be of identical concentration). The equation for its change is similar to that of the identical-species second-order result:

\[
\frac{1}{C_A(t)} = \frac{1}{C_A(0)} + k_2t, \quad (7.15)
\]

except that the slope is the rate constant for the reaction. This is obviously mathematically simpler to analyze than equation (7.14), but the difficulty of ensuring that the initial concentrations of the two reactants are identical provides a practical barrier to using this technique.

**Limiting Reagents**

For more complex situations in which there are multiple reactants, one frequently approximates the rate law as a power series in the concentrations of the reactants. Consider the situation represented by equation (7.12) in which \(A\) reacts as first order and is present in small quantities; \(B\) reacts with an order \(n\). Under these conditions, with \(B\) present at concentrations many hundreds or thousands of times higher than \(A\), even though the concentration of \(B\) is changing, the changes are so slight that it may be considered, practically, constant over the course of the experiment. To a very good order of approximation, the rate equation for the change of \(A\) obeys equation (7.16):

\[
-\frac{dC_A}{dt} = k_{\text{eff}} C_A, \quad (7.16)
\]

where
\[ k_{\text{eff}} = k_n C_B^m(0). \]  

Equation (7.16) is of the same form as equation (7.2). The solution is of the same form:

\[ \ln \left( \frac{C_A(t)}{C_A(0)} \right) = -k_{\text{eff}}t. \]  

Under these conditions in which \( A \) is a limiting reagent, one may follow the concentration of the limiting reagent to determine the effective rate constant.

The idea of limiting reagents is much more general than just first-order reaction steps. For example, consider the reaction:

\[ m\ A + n\ B \rightarrow \text{Products}, \]  

which is not necessarily an elementary reaction, but denotes the relative stoichiometry. One may write an approximate rate equation for the loss of \( A \).

\[ -\frac{dC_A}{dt} = k C_A^m C_B^n, \]  

where \( m \) and \( n \) are the orders with respect to \( A \) and \( B \), respectively. Under the condition that \( B \) is present in large excess, this equation simplifies to:

\[ -\frac{dC_A}{dt} = k_{\text{eff}} C_A^m, \]  

where

\[ k_{\text{eff}} = k C_B^n(0). \]  

For example, \( m = 1 \) gives equation (7.18), but if \( m = 2 \), this technique gives a second-order dependence of \( C_A \) on time, and so forth. The use of limiting reagents gives a means to determine the effective rate constant from a plot, provided one can measure the concentration of the limiting reagent as a function of time.

Equation (7.22) also indicates another feature of the use of a limiting reagent. A series of studies of the effective rate constant for a reaction in which the excess reagent’s concentration is changed (but still remains in excess) gives a means of determining the order with respect to that reagent as well.

**Measuring Concentrations; Actinometry**

The biggest practical problem in chemical kinetics is relating what is measured to what needs to be measured. All equations derived above require the measurement of concentration of a single reactant or the concentrations of several reactants independently as functions of time. In some cases, one may measure the time dependence of the product’s concentration to obtain the same information as measuring the concentration of the reactant.

Reaction rates are monitored through measurement of a wide variety of variables. For gas-phase reactions, the measurement of total pressure as a function of time is relatively easy to do, however, one often has to determine the reactant concentration from this total pressure by a calculation of the partial pressure. For reactions in solution that change hydrogen-ion concentration, monitoring the pH of the solution as a function of time is a convenient means to determine the extent of reaction. In some cases, the reaction produces a change in volume of the solution, in which case the reaction may be followed by dilatometry. Ionic reactions may be followed electrochemically. Of particular interest in this experiment is actinometry, the determination of concentration by intensity of a colored solution. Any quality of a solution that is proportional to the concentration of a reactant may be used as a monitor of the concentration.
A consideration in choosing a measurement parameter is the measuring technique’s response time. It must be sufficiently short that the time dependence of the concentration can be adequately determined. For example, if a reaction reaches equilibrium in a time of the order of 0.5 second, a measuring device that requires 2 seconds to make a single measurement is not a useful probe of the course of the reaction. The response time must be fast enough that the concentration can be determined a large number of times during the course of reaction. With the desire to observe and characterize ever-faster reactions, this limitation comes into play in many of experiments.

Reduction of Methylene Blue

In this experiment, you investigate the spectroscopy of a dye and subsequently use the spectroscopy to monitor a reaction involving the dye. The reactant is methylene blue, the dye shown at the top of figure 7.2. It has a vivid blue color. Leucomethylene blue, produced from it by reduction, gives a colorless solution. In the experiment, you use actinometry to measure the concentration of methylene blue as the reaction proceeds.

The absorption of light by a solution is measured by the absorbance, \( A \):

\[
A = a b C,
\]

where \( C \) is the concentration of the absorbing species, \( b \) is the cell length, and \( a \) is a fundamental property of the molecule called the molar absorptivity. In a spectroscopic cell of constant path length, the absorbance is directly proportional to the concentration of the absorbing material. The absorbance is related to an intensity ratio:

\[
A = \log_{10} \left( \frac{I_0}{I} \right),
\]

where \( I_0 \) is the incident intensity of the radiation field and \( I \) is the intensity of the radiation field transmitted by the solution in the cell.

The absorbance depends on frequency, \( \nu \), or equivalently the wavelength, \( \lambda \), of the radiation. Some molecules absorb only in selected regions, which gives rise to the observed color. In a mixture such as one has in a reaction, it is possible that more than one component may absorb at a particular wavelength. In that case, the absorbance at a particular frequency may not represent the concentration of a single component. It is important to check the spectroscopy of all possible materials to ensure that the conditions one chooses to make the measurement allows a representative determination of the limiting reagent’s concentration. If there are interferences from other materials, it may be possible to measure concentration by extracting from the measured spectrum the absorbance due only to the limiting reagent. To be as sensitive

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Figure 7-2. Structures of components in the reaction to be studied.
as possible, one should choose to measure the absorbance in some region in which the reagent strongly absorbs.

Safety

The reagents for this experiment are methylene blue, a relatively safe material, and ascorbic acid (vitamin C). In addition, you are using hydrochloric-acid solutions. In addition to the usual safety precautions, you should ensure that you do not spill these materials on yourself or others in handling them, as you may get acid burns. To ensure your safety, take precautions to protect yourself against splashes.

General Remarks about Solutions for Kinetics Experiments and Experimental Technique

In this experiment, you carry out kinetic studies to determine the protonation of methylene blue by ascorbic acid. It is important to determine, as closely as possible, the start time of the experiment. Therefore, the way you make the reaction mixture is important for obtaining good results. **Always add the solution of methylene blue to the other material last** and mix several times before you immediately put it in the cuvette and then put the cuvette in the spectrometer. Detection should start as quickly as possible, so it is approximately at the time that mixing has occurred. Be certain that you add sufficient amounts to fill the cuvette.

You need several solutions. Make 50 mL of a stock solution of methylene blue in distilled water at an approximate concentration of $4 \times 10^{-4}$ mol/dm$^3$. Make another 50-mL stock solution of ascorbic acid in distilled water at approximately 0.100 mol/dm$^3$. There should be a stock solution of HCl available in the laboratory. From these, you must make a series of solutions, so it is important that you make the stock solutions carefully and know the concentrations precisely.

In the first set [A] of experiments, you hold all of the concentrations constant, the HCl concentration in the solution constant at some value near 0.06 mol/dm$^3$ (usually a concentration near that of the stock solution) and the ascorbic acid concentration constant at some value near 0.025 mol/dm$^3$. **(In advance, you must calculate how much of the stock solutions to add to ensure that the final concentration is an appropriate value.)** Make these solutions using 10-mL volumetric flasks, always adding the methylene blue last, mixing, and quickly add the material to the cuvette.

In the second set of kinetics experiments [B], you should fix the concentrations of HCl (at some concentration around 0.06 mol/dm$^3$) and the methylene blue (at some concentration near $1.5 \times 10^{-5}$ mol/dm$^3$), and vary the ascorbic-acid concentration over at least five different concentrations (from 0.01 up to about 0.04 mol/dm$^3$). Do each in triplicate. Again, add the methylene blue last immediately before running the kinetics run.

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3 Methylene blue can be very messy and stain clothing and counters. Handle it very carefully and clean all utensils thoroughly immediately after using them. No one makes a solution of exactly a certain concentration, so be sure to report your concentrations, including uncertainties.
In a separate set of runs, make the solutions with the same concentrations of methylene blue and hydrochloric acid, but without any ascorbic acid, so that you have a baseline measurement of rate of reaction without ascorbic acid present.

In the third set of kinetics experiments [C], fix the ascorbic acid concentration at some value near 0.04 mol/dm$^3$ and the methylene blue concentration at the concentration of $1.5 \times 10^{-5}$ mol/dm$^3$ of the previous runs. In this set, vary the HCl concentration from 0.01 to about 0.06 mol/dm$^3$. Do each run in triplicate.

The easiest way to create solutions is to determine how much of the stock solutions of HCl, ascorbic acid, and methylene blue must be added to the 10-mL volumetric flask for each solution. Add the HCl and ascorbic acid. When you are ready to start a run, quickly add the proper volume of methylene blue solution, mix and pipette to the cuvette, turn the cuvette once or twice to mix the materials, insert in the Agilent 8453 spectrophotometer and immediately begin taking data with the kinetics program. **To do this, you must have determined beforehand how much of each of the stock solutions you are to add to the cuvette to produce the appropriate concentrations and have the spectrophotometer ready to go.**

It is extremely important that you clean the cuvettes and glassware thoroughly between runs and before starting the experiments. Rinse all glassware including cuvettes with 0.1 mol/dm$^3$ HCl and re-rinse with copious amount of deionized water several times before use. If you do this, you should get very good, reproducible data.

**Procedure**

*The Visible Spectra of Methylene Blue and Leucomethylene Blue*

Before you determine kinetics, you must determine the spectra of methylene blue and the final product mixture. Click the Configure Spectrometer icon and select Absorbance vs Wavelength. For the spectrum of methylene blue, use a solution of methylene blue in water that is approximately $4 \times 10^{-5}$ mol/dm$^3$. Scan from 200 nm to 800 nm. Run a blank first, then the sample. To run the blank, go to Calibrate under the Experiment window. Select Spectrometer:1. You can use your discretion to decide whether or not the spectrometer needs a warm-up. When ready, place your blank in the instrument and hit Finish Calibration.

Save your data as a *.CSV file, which allows you to view the file with Excel. To do this, click on the spectrum you have obtained, then in the File menu, select Export Selected Spectrum . . . CSV Format. Save it on your flash drive with the name SMQP.CSV, where Q is the section number and P is your group. Thus, group 1 of section 10 would save the spectrum as SM1001.CSV.

You also must determine the spectrum of the product mixture over the same range. Create a solution that is approximately 0.06 mol/dm$^3$ in HCl, 0.025 mol/dm$^3$ in ascorbic acid, and $1.5 \times 10^{-5}$ mol/dm$^3$ in methylene blue. Let this sit on the desk for 5 minutes to allow reaction to go to completion. Place the cuvette in the spectrometer and determine the spectrum of the final product mixture over the same region as you did above. Save this file as SLQP.CSV, where Q and P are the section and group number respectively.

**Kinetics Experiments**

Be sure you know and record the temperature in the room. Rather than take the whole spectrum every time, decide where the maximum signal of the methylene blue solution is from the spectroscopy experiment, making sure it is a region where ONLY methylene blue absorbs,
and not also leucometylene blue. This wavelength is the point at which the spectrometer should be set to determine the methylene blue absorbance as a function of time in the kinetics runs. This setting is done in the *Wavelength vs Time* mode of the Vernier spectrophotometer program. Click on *Configure Spectrometer*, set the wavelength and choose *Individual Wavelength*.

Another parameter to set is the total time to determine kinetics. This is set in the *Data Collection, Time Based* field. The value depends on how fast the reaction proceeds. In set A, try a total time of 120 seconds; this should be sufficient to watch the reaction go to completion. In set B, in which the ascorbic acid concentration is varied, the reaction proceeds more slowly at concentrations below about 0.01 mol/dm$^3$, and you may need to use a longer time. The important thing is that you must be able to see that the absorbance has changed significantly. If not, repeat the experiment with a longer time. You must also set the *Seconds/Sample* at 1s. You may want to set this last parameter shorter for reactions that happen quickly, but a 1-s cycle time seems to work fairly well.

Introduce your blank first and blank the instrument as described above. When you are ready to do the kinetic measurement, click on *Data Collection* and assure the parameters are correct. Introduce the cuvette and click *Start* to record. The spectrophotometer should begin recording the absorbance as a function of time for the time you specified.

To export data to your diskette for later use, click on the graph. In the *File* menu, select *Export As* and *CSV Format* and follow the name structure given above.

**Calculations**

1. Plot the spectra of methylene blue solution and the solution of final products from 200 to 800 nm. The most convenient means for this procedure is with a program such as EXCEL. After bringing the data into EXCEL, you have a set of x and y data. These spectra should be in your report.

2. Analyze the kinetic data from [A] with a program like EXCEL. As in calculation 1, you have to generate x data. Before analysis, be sure to find the long-time absorption and subtract this from each point, so that you are looking at only the time-varying part of the absorption. For part [A], determine whether, under the conditions, the reaction is most appropriately considered a first-order or a second-order reaction in methylene blue. Calculate the average effective rate constant under these conditions from the experiments you have run in part [A]. (It is important that you know that the initial concentrations of HCl, ascorbic acid, and methylene blue are the same in these experiments. Report the concentrations in the cuvette of all three components for each run.) If the values of rate constants in the various runs are different, determine if they are significantly different from the average.

3. For part [B], you know the order with respect to methylene blue from the experiments of part [A]. All plots done in this experiment should follow the form which you found linear in part [A]. In this part, only take data for a sufficient time to be able to see the concentration of methylene blue change by approximately 10%; do not follow the reaction to completion as you did in part [A]. For each concentration of ascorbic acid (and being sure that the concentration of HCl is identical from sample to sample), extract the effective rate constant for each run. Do each concentration in triplicate, and do this for at least five (5) different concentrations. For each experiment, determine the effective rate constant from a plot. Make a table showing the

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4 If there is a residual signal after reaction is complete, remember that you will need to subtract this from the data at all times to give a signal that is proportional to the reactant concentration. If there is no (or a small) residual signal, then this is not necessary. You decide!
concentrations of HCl (which should be the same for each experiment!), ascorbic acid, and methylene blue (which should be the same for each experiment!) and the effective rate constant. For each concentration of ascorbic acid determine the average rate constant, averaged over the three runs. Be sure to get data at a sufficient number of concentrations of ascorbic acid (at least five, not including the one containing no ascorbic acid) so you can determine how the average effective rate constant depends on the ascorbic acid concentration. Make a plot of average effective rate constant versus ascorbic acid concentration.

4. In part [C], do a similar analysis as in part [B], except that you vary the HCl concentration while holding the ascorbic acid and methylene blue concentrations constant in the various solutions. Again, you only have to follow the initial part of the reaction. Again, do the experiment with the methylene blue and ascorbic acid concentrations constant, but with no HCl.

**Discussion Questions**

1. Describe the spectroscopy of methylene blue in terms of the molecule’s quantum structure. Ascribe any unique bands to transitions between quantum states. Be as specific as possible.

2. Based on the analyses of part [A], is the order with respect to methylene blue first or second? How did you decide this? What figures of merit did you use in making this decision?

3. Based on the analyses in parts [B] and [C], what are the orders with respect to ascorbic acid and hydrochloric acid? [NOTE: You may have to plot the results in various ways. Be clear in how you obtain the results. There may be other ways in which methylene blue is reduced without ascorbic acid, so in part [B], subtract the zero-concentration average rate constant from the rate constant at each other concentration to isolate the dependence on ascorbic acid concentration. Similarly, in part [C], subtract off the zero-concentration rate constant from each of the other rate constants to isolate the contribution from HCl.]

4. (a) From the results, write the rate law for this reaction as you know it from your experiments. (b) Using all of the data, determine the overall effective rate constant for the reaction, as generally given in equation (7.20). Be sure to include proper units.

5. Write a balanced equation for the reaction of methylene blue with ascorbic acid. Include full structures of all reactants and products, not abbreviations, in your equations. Think carefully about the chemistry involved in this reaction; are there other materials than those found in Figure 7.2 that are components of the reaction?

6. Propose a mechanism consistent with your observed experimental results. Explain the mechanism and how it gives the rate law you derived above. What assumptions about various steps in your mechanism are necessary to achieve agreement with your experimental rate law?

7. In solution, much is made about diffusion control. Estimate the effect of diffusion control on this reaction by estimating the diffusion-controlled rate constant from reasonable estimates of the diffusion coefficients and sizes of the reactants.