Complex Reaction Monitoring with UV-Visible Absorption Spectroscopy: $KMnO_4$ + Sugar

Introduction

Kinetic Analysis

The study of chemical kinetics involves the monitoring of reactant and/or product concentrations over time in order to better understand how quickly or slowly a reaction proceeds. By learning how quickly a product is formed, information about the optimal reaction conditions (*e.g.*, temperature, starting material concentrations, etc.) can be better understood. Furthermore, kinetic analyses can aid in understanding the overall mechanism of the reaction, as well as alternative reaction pathways.

One of the simplest examples is a first order reaction, as shown in Eq. 1, where one species (A) reacts to form a product (B).

$$A \xrightarrow{k_1} B$$

Equation 1.

k_1 refers to the rate constant for the reaction; it is a value which correlates the rate of a reaction to the concentration of the starting materials. The rate constant is the inverse of the reaction time constant (τ) and is used to describe how fast or slow a reaction proceeds. This value is also useful when comparing rates for reactions performed under varying conditions.

Using the chemical equation, a differential rate law can be written which describes the change in a species concentration as a function of time (Eq. 2),

$$\frac{d[A(t)]}{dt} = -k_1[A(t)]$$

Equation 2.

where *[A(t)]* is the concentration of the species of interest at a given reaction time; in this case (*A*) is the reactant from Eq. 1. When reactions are monitored experimentally, the integrated form of the rate law is needed. By integrating the differential rate law, a function can be generated relating the measured concentration of the species of interest as a function of time. This function will describe how the species is lost or produced overtime. For the generic first order reaction described in Eq. 1, *[A(t)]* will be lost according to an exponential function shown in the integrated rate law (Eq. 3).

$$A(t) = A_0 e^{-kt}$$

Equation 3.

As described previously, the rate constant (*k*) is often used as a descriptor for the speed of the reaction. Experimentally, by fitting the species concentration measured as a function of time, the integrated rate law is fit to the data and the rate constant can be determined from the fitting parameters. It is important to note that the reaction order (*i.e.*, first, second, etc.) is not determined by the chemical equation, but instead is determined experimentally. The chemical equation can aid in developing a model that can be checked by analyzing the measured data.

While a first order reaction is fairly simple, many reaction mechanisms can often be more complicated and involve multiple steps before the overall reaction is complete. For example, a starting material may initially react to form an intermediate species which can then react further to form a final product as shown in first order consecutive reaction outlined in Eq. 4.

$$A \xrightarrow{k_1} B \xrightarrow{k_2} D$$

Equation 4.

This consecutive reaction involves a more complicated analysis than a first order reaction as the intermediate (B) is involved in both steps of the reaction. Consequently, the rate constants k_1 and k_2 will both contribute to the rate law. The magnitude of these rate constants will have direct implications not only for the speed of the reactions, but for the maximum intermediate concentration possible.[1] This value is highly dependent on the rate of the first and second reaction steps. As shown in Figure 1, changing the rate constants alters the reaction profile drastically, including changing the maximum intermediate concentration produced over the course of the reaction.

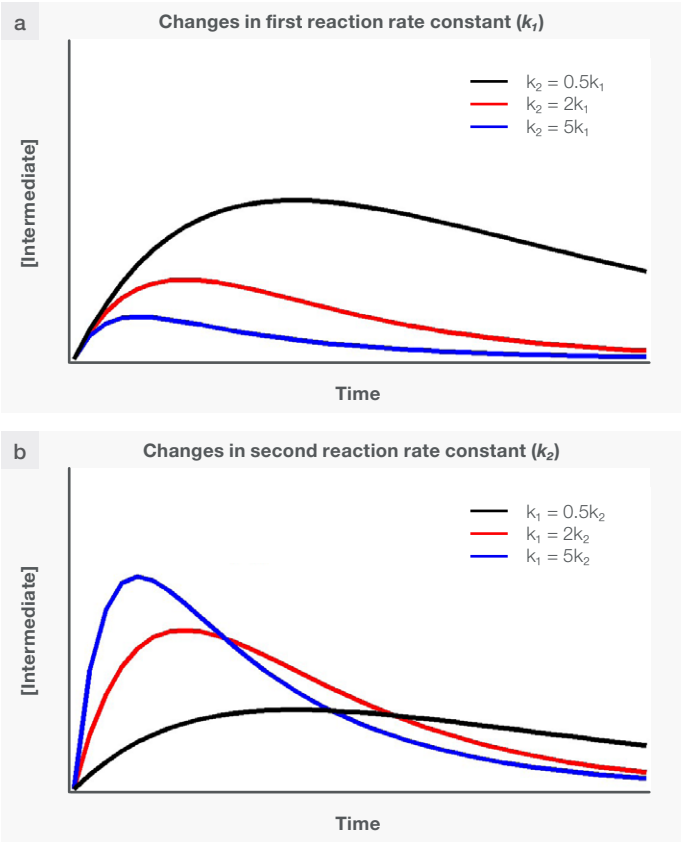

Figure 1. (a) Changes in the intermediate concentration over time for reactions with different k_1 and the same k_2. (b) Changes in the intermediate concentration over time for reactions with different k_2 and the same k_1.

In some circumstances, the intermediate species is the compound that needs to be isolated. Under these circumstances the reaction should be quenched before significant product is formed. Ideally, the reaction would be quenched when the maximum intermediate concentration is generated, requiring knowledge of when in the reaction process this occurs. As shown in Figure 1, the reaction profile is highly dependent on the rates of both first and second reaction, including the time at which the maximum intermediate concentration is formed. This value can be calculated if the rate constants are known for both steps of the reaction.

This concentration maximum correlates to the point at which the rate of change in the intermediate species is zero. Along with knowledge of the differential rate law for the intermediate species, this fact can be used to generate an equation (Eq. 5) relating the rate constants of the first and second reactions (k_1 and k_2, respectively) to the time at which the intermediate concentration reaches a maximum (t_{max}).[1]

$$t_{max} = \frac{1}{(k_1 - k_2)} \ln\left(\frac{k_1}{k_2}\right)$$

Equation 5.

UV-Visible Spectroscopy

As kinetic analysis requires the ability to monitor the concentration of compounds while a reaction progresses, one of the most common methods to monitor these reactions is UV-Visible absorption spectroscopy. In this technique, light spanning the UV-Visible range of the electromagnetic spectrum will interact with the sample. If the energy of incident photons are sufficient, electrons from the ground state can be promoted to the excited state; a process referred to as absorption (Figure 2).

As the energy levels for a small molecule are discrete, only photons with the appropriate energy can be absorbed by the molecule. The energy of a photon (E) is related to the wavelength of light (λ) though Eq. 6,

$$E = \frac{hc}{\lambda}$$

Equation 6.

where c is the speed of light and h is Plank's constant. Therefore, only specific regions of the electromagnetic spectrum can be absorbed by a molecule, leading to a unique absorption spectrum for the species studied. The energy spacing can be larger or smaller depending on the molecule studied, and will therefore result in different characteristic absorption spectra between different molecules. This can be helpful when measuring samples containing multiple species in solution, including reaction mixtures.

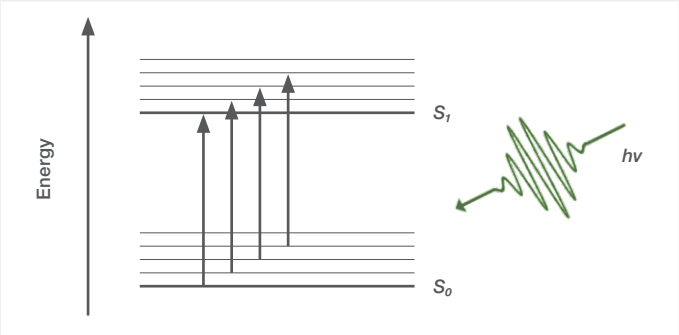

Figure 2. Generic energy level diagram depicting absorption of light for a small molecule.

Because of the absorption of photons by the sample, the intensity of the light observed exiting the sample (I) will be smaller than the initial intensity of the light before interacting with the sample (I_o) as shown in Figure 2. The loss of light due to absorption can be described through Beer's law (Eq. 7),

$$A = log\left(\frac{I_0}{I}\right)$$

Equation 7.

where the log of the ratio between the light intensity before and after the sample, I_0 and I, respectively, is equivalent to the absorbance of the sample (A). This is generally how a spectrophotometer calculates the absorbances of a measured sample. In these measurements, I_0 is typically established by measuring the intensity of a "blank" or a cuvette containing the solvent and all components other than the analyte of interest.

Beer's law can also be represented as Eq. 8,

$$A = cl\varepsilon$$

Equation 8.

where c is the concentration of the analyte, l is the path length the light travels through and ε is the extinction coefficient, a unique parameter for the substance studied. Through this form of Beer's law, absorbance is shown to be directly proportional to the concentration of the absorptive substance. By measuring the absorbance over time, the concentration of the species participating in the reaction can be directly monitored. However, it is important to know that not all molecules absorb in the UV-Visible range. Consequently, this analysis method is only pertinent when either one or all species participating in the reaction can absorb in the UV-Visible region.

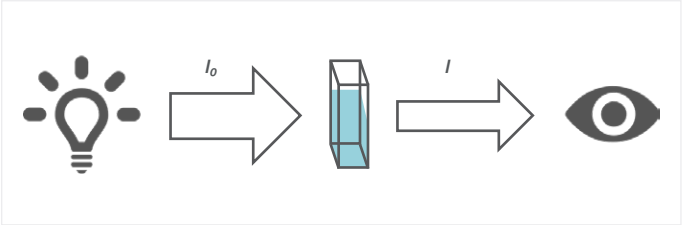

Figure 3. Generic depiction of light absorption within a liquid sample.

Reaction Scheme

Herein, the reduction of manganese-containing species will be studied using UV-Visible absorption spectroscopy in order to determine the rate constants for the reaction in the presence of different reducing sugars. While the mechanism is complex and dependent on a variety of parameters,[2,3] Eq.'s 9-11 describe one assumed reaction scheme.[4]

$$Mn(VII)\ (aq) + e^- \xrightarrow{k_1} Mn(VI)\ (aq)$$

Equation 9.

$$Mn(VI) + 2e^- \xrightarrow{k_2} Mn(IV)$$

Equation 10.

$$Mn(IV) \xrightarrow{k_3} MnO_2\ (s)$$

Equation 11.

In this scheme, the Mn^{7+} species (MnO_4^-, permanganate) is reduced to a Mn^{6+} species before the final reduction to a Mn^{4+} species by a reducing agent under alkaline conditions. Eq. 11 outlines the formation of insoluble MnO_2 from the soluble Mn^{4+} species.[4] Each manganese-containing compound absorbs strongly in the UV-Visible spectral region, allowing for all pertinent species to be monitored through this technique. Through the course of this experiment, this complex reaction will be monitored and the rate constants, k_1 and k_2, will be analyzed.

References

1. Engel, T.; Reid, P., *Physical Chemistry*; Pearson, 2014.
2. Simándi, L.I.; Jáky, M.; Savage, C.R.; Schelly, Z.A., Kinetics and Mechanism of the Permanganate Ion Oxidation of Sulfite in Alkaline Solutions. The Nature of Short-Lived Intermediates, *JACS*, **1985**, 107, 4220 – 4224.
3. Dash, S.; Patel, S.; Mishra, B.K., Oxidation by Permanganate: Synthetic and Mechanistic Aspects, *Tetrahedron*, **2009**, 65, 707 – 739.
4. Fernández-Terán, R.J.; Sucre-Rosales, E.; Echevarria, L.; Hernández, F.E., A Sweet Introduction to the Mathematical Analysis of Time-Resolved Spectra and Complex Kinetic Mechanisms: The Chameleon Reaction Revisited, *J. Chem. Ed.*, **2022**, 99, 2327 – 2337.

Experimental

Materials

- Thermo Scientific™ Evolution™ One Spectrophotometer
- Cuvette (1.0 cm, quartz. Need 3.0 mL volume)
- Pipetter and appropriate pipette tips
- Lint-free lab wipes
- Appropriate Glassware for preparing stock and sample solutions
- DI or Nanopure water
- Potassium permanganate
- Sodium Hydroxide
- D(+)-glucose
- D(+)-sucrose

Note: This analysis requires the ability for a student to fit the data with complex equations. Ensure students have access to data analysis software which allows for fitting to multiple exponential functions.

Safety

Eye protection and gloves should be worn at all times when handling reagents used in this experiment.

Potassium permanganate and sodium hydroxide are both corrosive substances. Potassium permanganate is also an environmental hazard and an oxidizer. Ensure students and any individual preparing or handling the chemicals described herein are using the correct personal protective equipment and are instructed in the proper waste disposal procedures. For more details, please refer to the Safety Data Sheet for each material prior to handling.

Instructions

Part A: Starting Material UV-Visible Spectra:

A1. Turn on the Evolution One spectrophotometer.

A2. Make the following stock solutions:

   a. 0.75 mM $KMnO_4$
   b. 400 M NaOH
   c. 700 mM D(+)-glucose
   d. 700 mM D(+)-sucrose

A3. Select the “Scan” application in the instrument software and set up the instrument using the following parameters in the settings window:

   a. “Measurement” tab:
      i. 100%T Baseline
   b. “Instrument” tab:
      i. Data Mode: Absorbance
      ii. Start Wavelength: 700 nm
      iii. End Wavelength: 300 nm
      iv. Data Interval: 1.0 nm
      v. Bandwidth: 1.0 nm
      vi. Integration Time: 0.1 s
      vii. Derivative: None
      viii. Smooth: None

A4. Prepare the following samples in DI water:

   a. 2.5 mM D(+)-glucose
   b. 2.5 mM D(+)-glucose
   c. 70 mM NaOH
   d. 0.25 mM $KMnO_4$

A5. Fill a clean 1.0 cm quartz cuvette with DI water to collect the blank measurement.

A6. Measure the blank solution, then empty and dry the cuvette.

   Note: Use gloves when handling the cuvette, and clean off the surface using a lint free lab tissue. Fingerprints and dust/lint can alter the blank and sample measurements. A light stream of air or N_2 can help dry the cuvette quickly.

A7. Measure each sample solution described in step A-4. Be sure to measure DI water as well.

A8. End the experiment and export the data as a .csv file (select “Spectrum, Comma Separated Values” for the raw data). This data will be used for your report.

Part B: Permanganate Reaction Kinetics:

B1. Prepare 3.0 mL of the following blank samples:

a. 70 mM NaOH, 2.5 mM D(+)-glucose

b. 70 mM NaOH, 2.5 mM D(+)-sucrose

B2. Select the "Kinetics" application in the instrument software and set up the instrument using the following parameters in the settings window:

a. "Type" tab:

i. Multiple Wavelengths

ii. Time Mode

b. "Measurement" Tab:

i. Time Units: Seconds

ii. Integration Time: 0.25 s

iii. Dwell Time: 0.75 s

iv. Stages: See Table 1

Stages	Start Time (s)	End Time (s)	Interval (s)
1	0	120	1.0 s
2	120	300	5.0 s

Table 1. Stages for $KMnO_4$ reaction in the presence of D(+)-glucose.

c. "Instrument" Tab:

i. Data Mode: Absorbance

ii. Bandwidth: 1.0 nm

iii. Wavelengths: 600 nm, 520 nm, 375 nm

B3. Fill a clean 1.0 cm quartz cuvette with the blank solution containing D(+)-glucose from step B-1a.

B4. Measure the blank solution, then empty and dry the cuvette.

B5. Measure a sample solution containing the following components:

a. Sample 1: 0.25 mM $KMnO_4$, 2.5 mM D(+)-glucose, 70 mM NaOH

Note: It will be easier to add the D(+)-glucose, NaOH and DI water to a cuvette and then place the cuvette in the instrument ***before*** *pipetting the $KMnO_4$ stock solution. As the reaction is rapid, the measurement should be started as soon as $KMnO_4$ is added to the solution.*

B6. End the experiment and export the data as a .csv file (select "Spectrum, Comma Separated Values" for the raw data). This data will be used for your report.

B7. Reserve the reaction product for analysis later.

B8. Rinse the cuvette three times with DI water. Make sure the cuvette is fully dried after the last rinse.

B9. Fill the cleaned 1.0 cm quartz cuvette with the blank solution containing D(+)-sucrose from step B-1b.

B10. Select the "Kinetics" application and set up the instrument using the following parameters in the settings window:

a. "Type" tab:

i. Multiple Wavelengths

ii. Time Mode

b. "Measurement" Tab:

i. Time Units: Seconds

ii. Integration Time: 0.25 s

iii. Dwell Time: 0.75 s

iv. Stages: See Table 2

Stages	Start Time (s)	End Time (s)	Interval (s)
1	0	120	1.0 s
2	120	600	5.0 s

Table 2. Stages for KMnO4 reaction in the presence of D(+)-sucrose.

c. "Instrument" Tab:

i. Data Mode: Absorbance

ii. Bandwidth: 1.0 nm

iii. Wavelengths: 600 nm, 520 nm, 375 nm

B11. Measure the blank solution, then empty and dry the cuvette.

B12. Measure a sample solution containing the following components:

a. Sample 1: 0.25 mM $KMnO_4$, 2.5 mM D(+)-sucrose, 70 mM NaOH

B13. End the experiment and export the data as a .csv file (select "Spectrum, Comma Separated Values" for the raw data). This data will be used for your report.

B14. Reserve the reaction product for analysis later.

B15. Rinse the cuvette three times with DI water. Make sure the cuvette is dried well after the last rinse.

Part C: Reaction Product UV-Visible Spectra:

C1. Select the "Scan" application in the instrument software and set up the instrument using the following parameters in the settings window:

a. "Measurement" tab:

i. 100%T Baseline

b. "Instrument" tab:

i. Data Mode: Absorbance

ii. Start Wavelength: 700 nm

iii. End Wavelength: 300 nm

iv. Data Interval: 1.0 nm

v. Bandwidth: 1.0 nm

vi. Integration Time: 0.1 s

vii. Derivative: None

viii. Smooth: None

C2. Fill a clean 1.0 cm quartz cuvette with DI water to collect the blank measurement.

C3. Measure the blank solution, then empty and dry the cuvette.

C4. Measure the two previously reserved reaction products from Part B (steps B7 and B14). Be sure to measure DI water as well.

C5. End the experiment and export the data as a .csv file (select "Spectrum, Comma Separated Values" for the raw data). This data will be used for your report.

**This procedure is based on the procedure outlined by Fernández-Terán et al.[4]*

Lab Report

Ensure that you have saved your experimental data and results where you can access them. Your results should be included in the final laboratory report, as well as the following:

1. Include plots of the absorption spectra of all starting materials, including DI water.
2. Include a plot of the absorption spectra of the final product for both reactions (D(+)-glucose and D(+)-sucrose).
3. Do the spectra of the final reaction products for both reactions match one another? Include additional observations for the absorption spectra included.
4. Using equations 9 – 11 from the introduction, solve and include the integrated rate laws for $Mn(VII)_{(aq)}$, $Mn(VI)_{(aq)}$ and $Mn(IV)_{(aq)}$. For the purposes of this experiment an integrated rate law for formation of $MnO_{2(s)}$ will not be required.

   Note: Electrons will not need to be included in the integrated rate laws.

5. Using the integrated rate laws and your knowledge of kinetics, fit the absorbance data as a function of time. Report k_1 and k_2 based on the fits for each data set. Do these results fit well to the data set? If not, why?

   Note: The data will need to be fit in a software program which allows fitting with single or multi-exponential functions. If the fit function does not appear to fit well, a constant offset may need to be added into the fit function to produce the appropriate fit (see Eq. 12 for an example).

$$[A] = A_0 e^{-k_1 t} + C$$

Equation 12.

   Hint: For some of the fitting functions, the fits may not need to span the full data set. Choose the appropriate region in which to fit the data.

6. Which reactant, D(+)-glucose or D(+)-sucrose, led to a faster overall reaction? Why?
7. Calculate and include t_{max} for each sample. Is there a trend in the t_{max}?
8. Based on the fit results, does the proposed mechanism in equations 9-11 make sense? Why/why not?
9. If the reaction needed to be slowed down, how could this be achieved?

Note: All axes for the reported graphs should be properly labeled, include appropriate units and significant figures.

Notes for Professors and Teaching Assistants:

Due to the complex nature of the permanganate reaction, as well as the overlap between the respective spectra of each manganese species analyzed in this experiment, the data does not perfectly fit to a standard two-step consecutive reaction if the entire data set is used. To avoid potential confusion, it is advised the data sets are fit using the time ranges outlined in Table 3. Fits can be performed in most commercially available data analysis software with curve fitting capabilities. Figure 4 depicts an example data set for the permanganate reaction performed in the presence of D(+)-glucose (Figure 4a) and D(+)-sucrose (Figure 4b).

Analysis Wavelength (nm)	Time Range (s)	
	Reaction with D(+)-glucose	Reaction with D(+)-sucrose
375	0 – 250	0 – 100
**520	0 – 20	0 – 250
600	0 - 300	0 – 600

Table 3. Recommended time ranges for curve fitting.

***Note, the loss and formation of the reactant and intermediate, respectively, are rapid for the permanganate reaction involving D(+)-glucose. Care should be taken to ensure the data fits only reflect the early dynamics, specifically for the reactant where late dynamics involve the growth of an additional species.*

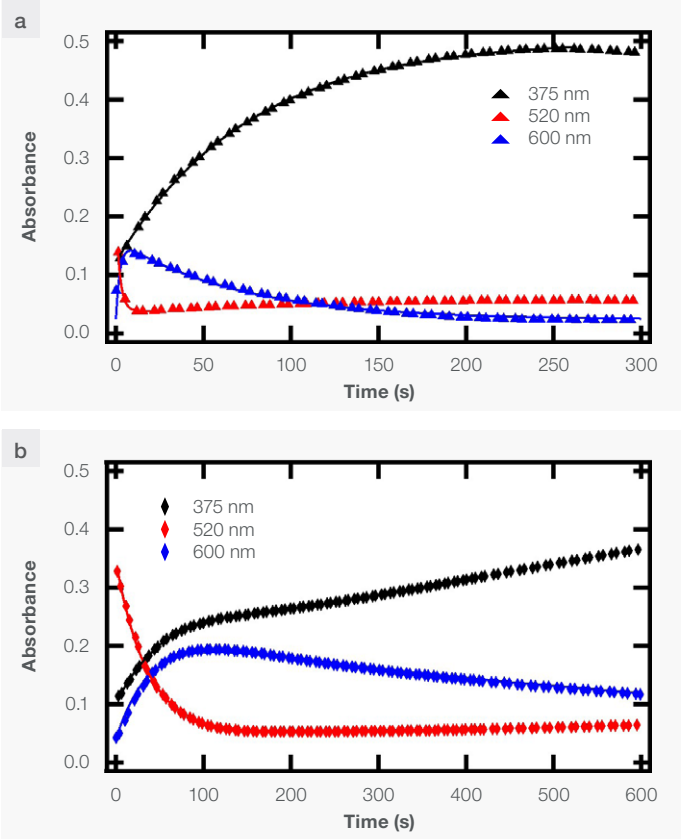

Figure 4 - Absorbance as a function of time for the permanganate reaction samples in the presence of (a) D(+)-glucose and (b) D(+)-sucrose. For a more clear visualization of the overlapping fit, some data points are excluded from these figures.

Learn more at **thermofisher.com/evolution**

thermo scientific