EXPERIMENT #4
KINETICS OF THE OXIDATION OF ASCORBIC ACID BY HEXACYANOFERRATE(III) ION

The Effect of Sodium Nitrate on the Reaction Rate[1]

This experiment was designed by M. D. Gheorghiu.

I. PURPOSE OF THE EXPERIMENT

This is an integrated experiment that includes topics from inorganic, organic, analytical, physical, and computational chemistry. It is designed to introduce you to the basics of:

- how to acquire experimental kinetic data for a chemical reaction;
- how to perform data manipulation in order to extract information such as reaction order and rate constants from experimental kinetic data;
- how to assess the effect of the reaction environment upon rate constant. In the present case, how adding a salt modifies the environment.

Pieced together, correct information will elucidate the reaction mechanism. Also, the numerical results allow a convincing check of the validity of the mechanistic assumptions.

This experiment will contribute to improving your **lab technique** in the following areas:

- precise volumetric and gravimetric measurements
- correct handling of the UV-VIS instrument
- use **Microsoft Excel Solver (**the instruction in Appendix 3) that provides the graphical and numerical output resulting from your experimental data.

II. SAFETY

1. **Ascorbic acid (Vitamin C):** Pleasant, sharp acidic taste. Stable in air when dry. Aqueous solutions are rapidly oxidized by air. Alkalies, iron, and copper accelerate the reaction. Used as antimicrobial and antioxidant in foodstuffs. Not considered toxic except in immense quantities.

2. **Potassium Hexacyanoferrate (III**): The aqueous solution decomposes on standing. Avoid contact with acid. Harmful solid. *NOTE*: cyanide (CN^-) ions are highly toxic but are tightly

[1]Adapted after: Watkins, K. W.; Olson, J. A. *J. Chem. Educ.* **1980**, *57*, 158-159 and Martins, L. J.; daCosta, J. B. *J. Chem. Educ.* **1988**, *65*, 176-178. The original work is described in the following reference: Mehrotra, U. S.; Agrawal, M. C.; Mushran, S. P. *J. Phys. Chem*. **1969**, *73*, 1996-1999. See also: Sime, R. J. *Physical Chemistry: Methods, Techniques, and Experiments*, Saunders College Publishing: Philadelphia, PE, 1990; pp 628-640 (Experiment #29).

bound to the iron nucleus in this compound and so are not available in solution. (This is what happens when cyanide gets into your blood: it binds to the iron centers in hemoglobin making it impossible for red blood cells to carry oxygen to the organism, thus killing by asphyxiation.) But when hexacyanoferrate decomposes or hydrolyzes to release free CN^-, it forms a lethal poison.

3. **Nitric Acid**: Irritant, toxic, avoid eye contact, harmful if inhaled, causes severe burn. May be fatal if swallowed or ingested. Strong oxidizer. Contact with other materials may cause fire.

4. **EDTA**: Harmful solid, irritant.

5. **Sodium Nitrate**: Avoid eye contact, ingestion. Strong oxidizer, toxic, irritant. Target organs: Blood and nerves.

III. INTRODUCTION

A. Chemical Kinetics

One of the main goals in chemical kinetics is to understand the steps by which a reaction takes place. This series of steps is the reaction mechanism. Understanding the mechanism allows us to find ways to facilitate the reaction.

The reaction rate is defined as the change in concentration of a reactant or product, $\Delta[A]$, per unit of time Δt.

$$Rate = \frac{A_{t_2} - A_{t_1}}{t_2 - t_1} = \frac{\Delta[A]}{\Delta t} \tag{3.1}$$

1. Rate Laws.

Rate laws may be expressed in differential form or integrated form.

a). The differential rate law, often simply called the *rate law*, shows how the rate of a reaction depends on the concentration of reactant and product species:

Rate = *f*(concentrations)

b). The integrated rate law shows how the concentration of the species in the reaction depends on time.

Concentration = *f*(time)

The integrated rate law may be derived from the differential rate law, but closed-form analytic solutions cannot always be found.

2. Form of the Rate Law.

The first step in understanding how a chemical reaction occurs is to determine the form of the rate law.

Consider the reaction $aA \rightarrow bB$. The rate of this reaction may be given by:

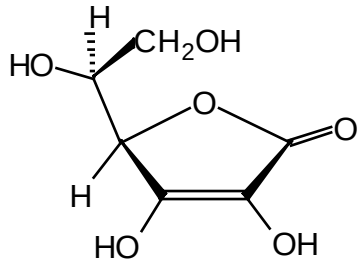
$$rate = -\frac{1}{a}\frac{d[A]}{dt} = \frac{1}{b}\frac{d[B]}{dt} = k[A]^n \qquad (3.2)$$

where k is the kinetic constant or rate coefficient and n is the order of the reaction with respect to A. For $n = 1$ the reaction is first order in A. For $n = 2$ the reaction is second order in A, and so forth.

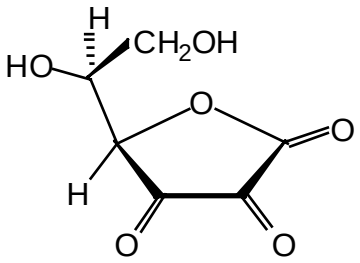
B. Ascorbic acid oxidation.

L-Ascorbic acid (vitamin C) is widely used in chemical and biological systems as a reducing agent, mostly in aqueous solution. Knowledge of the kinetic parameters that characterize the rates of ascorbate reductions, including the elementary steps, is actively expanding. Depending on the nature of the oxidant and the acidity of the reaction medium, either the ascorbic acid (H_2Asc), the ascorbate anion ($HAsc^-$) or the ascorbate dianion (Asc^{2-}) is the kinetically important species. Among the oxidants, which have been investigated, are metal ion-complexes, excited states of metal complexes and phenothiazene radicals.

The experiment described here involves the oxidation of the ascorbic acid ($C_6H_8O_6$) by hexacyanoferrate(III) ion[2] to dehydroascorbic acid ($C_6H_6O_6$).



Ascorbic Acid (H_2Asc)



Dehydroascorbic Acid (Asc)

The overall stoichiometry has been found to be (H_2Asc stands for ascorbic acid; Asc stands for dehydroascorbic acid):

$$H_2Asc + 2[Fe(CN)_6]^{3-} \rightarrow Asc + 2[Fe(CN)_6]^{4-} + 2H^+ \qquad (3.3)$$

[2] The hexacyanoferrate(III) ion is properly written as $[Fe(CN)_6]^{3-}$ as shown in equation 3.3. The brackets indicate the 6 CN^- ligands are bound directly to the metal center (coordination sphere). To simplify equations such as 3.4 where brackets also indicate concentration, the brackets indicating the coordination sphere will be omitted. Note that when brackets indicate concentration the entire species including charge in contained within the brackets.

The empirical rate law, **at constant pH**, is:

$$-\frac{1}{2}\frac{d[Fe(CN)_6^{3-}]}{dt} = k_{obs}[Fe(CN)_6^{3-}][H_2Asc] \qquad (3.4)$$

The mechanism that is consistent with the kinetic data comprises three steps:

$$\mathbf{H_2Asc} \underset{k_{-1}}{\overset{k_1(fast)}{\rightleftharpoons}} \mathbf{HAsc^- + H^+} \qquad \mathbf{3.5a}$$

$$\mathbf{HAsc^- + [Fe(CN)_6]^{3-}} \xrightarrow{k_2\,(slow)} \mathbf{HAsc^{\bullet} + [Fe(CN)_6]^{4-}} \qquad \mathbf{3.5b}$$

$$\mathbf{HAsc^{\bullet} + [Fe(CN)_6]^{3-}} \xrightarrow{k_3(fast)} \mathbf{Asc + [Fe(CN)_6]^{4-} + H^+} \qquad \mathbf{3.5c}$$

The ascorbate ion ($HAsc^-$) is formed by ionization of the ascorbic acid in a very fast process. The rate-determining step consists of an electron transfer from the ascorbate ($HAsc^-$) to the hexacyanoferrate(III) anion. The final step is a fast process involving the second electron transfer, this time from the ascorbate free radical (HAsc) to hexacyanoferrate(III) anion.

Note that the rate-determining step is an **<u>ionic reaction</u>** between $HAsc^-$ and $[Fe(CN)_6]^{3-}$ in which each of the reacting species carries a negative electrical charge. Changing the ionic strength of the solution will influence the measured rate constant, a phenomenon known as the "Salt Effect". This will be explained in more detail below.

C. <u>Determining the concentration of unreacted $[Fe(CN)_6]^{3-}$ with time</u>

The kinetics of the oxidation of ascorbic acid can be followed by determining the concentration of unreacted $[Fe(CN)_6]^{3-}$ with time. The concentration is assessed by its absorbance at 420 nm. According to the Lambert-Beer law, the amount of light transmitted by an absorbing sample is given by

$$\%\,T = I/I_0 = e^{-A} = e^{-\varepsilon cl} \qquad (3.6)$$

where the absorbance A is proportional to the concentration (c, in mol/L) of the solute, the length of the path the light travels through the sample (l, in cm), and the constant of proportionality, ε, called molar absorptivity coefficient (L mol^{-1} cm^{-1}) or molar extinction coefficient:

Aqueous solutions of hexacyanoferrate(III) are yellow, showing absorption of light in the blue-violet range, while ascorbic acid, dehydroascorbic acid and hexacyanoferrate(II) are colorless, and therefore they **do not interfere** in the spectroscopic determination of $[Fe(CN)_6]^{3-}$.

The unreacted fraction of $[Fe(CN)_6]^{3-}$ remaining at time *t* is A_t/A_o, where A_t is the absorbance due to $[Fe(CN)_6]^{3-}$ at time *t* and A_o is the absorbance at zero time. The concentration of $[Fe(CN)_6]^{3-}$ at time *t* is given by:

$$[Fe(CN)_6^{3-}] = \frac{A_t}{A_0}[Fe(CN)_6^{3-}]_0 \qquad (3.7)$$

where $[Fe(CN)_6^{3-}]_o$ is the initial concentration of hexacyanoferrate(III).

The concentration of ascorbic acid at time *t* is given by:

$$[H_2Asc] = [Asc]_0 - \frac{1}{2}\{[Fe(CN)_6^{3-}]_0 - [Fe(CN)_6^{3-}]_t\} \qquad (3.8)$$

In this experiment you will collect kinetic data on the oxidation of ascorbic acid by $K_3[Fe(CN)_6]$ occurring in four different environments differing in the ionic strength. Varying the concentration of added $NaNO_3$ produces the differences in the environments. Two important variables, temperature (chosen as room temperature), and pH (acidity) are kept constant throughout the experiment.

IV. PROCEDURE

CAUTION! Waste must be disposed only in appropriately labeled waste bottles; in this case "KINETICS WASTE". Accidental disposal in bottles dedicated to other wastes may cause severe explosion and fire, as well as costing MIT (i.e., YOU) a big fine from the E.P.A.!

Teamwork is an important part of research. In order to minimize the amount of chemicals required and reduce the amount of waste generated, solutions will be prepared by pairs of students and shared by the whole group. This way the importance of teamwork and shared responsibility is emphasized while keeping waste to a minimum.

A. The solutions: The TA will assign the preparation of the following solutions to different pairs of students (note that calculations of quantities required is part of the pre-lab):

For day one:

Solution A: One pair will prepare a 1.0×10^{-3} M $K_3[Fe(CN)_6]$ solution in a 250-mL volumetric flask. This solution will be used as a stock solution both for day one dilutions to verify Beer's Law and to determine molar absorptivity and for day two/three kinetics experiments.

For day two:

Four pairs will each prepare <u>one</u> of the following four solutions made by adding the appropriate quantity of solid $NaNO_3$ to the specified volumetric flask and diluting to the mark with stock **solution A**.

Solution B: $NaNO_3$ is added to a 50-mL volumetric flask in the amount necessary to obtain a concentration of 0.02 M.

Solution C: $NaNO_3$ is added to a 25-mL volumetric flask in the amount necessary to obtain a concentration of 0.05 M.

Solution D: $NaNO_3$ is added to a 25-mL volumetric flask in the amount necessary to obtain a concentration of 0.10 M.

Solution E: $NaNO_3$ is added to a 25-mL volumetric flask in the amount necessary to obtain a concentration of 0.20 M.

Solution F: At the beginning of <u>each</u> "kinetic day", the fifth pair of students will prepare a **2.5×10^{-4} M ascorbic acid solution** by adding the appropriate amount of solid ascorbic acid to a 250-mL volumetric flask. The volumetric flask is then filled with a stock solution consisting of **0.010 M HNO_3** and **0.001% disodium EDTA dihydrate** provided by the TA**. Since ascorbic acid reacts slowly with dissolved oxygen, the solution must be prepared and used the same day.**

All solutions should be within ±1 °C of room temperature.

B. Day 1: The Lambert-Beer equation and determination of ε

<u>Goals</u>

- Become familiar with the HP8532A UV spectrometer by recording the UV-VIS spectrum (λ = 360-550 nm) of each of the five solutions. Determine the λ_{max} for $K_3[Fe(CN)_6]$ in aqueous solution.
- Determine the validity of Lambert-Beer law by taking five UV spectra for known $K_3[Fe(CN)_6]$ solutions. Plot **Absorbance** at 420 nm *versus* **concentration**. The slope obtained by least square curve fit is your ε value. Report this value to your TA.
- Become familiar with **Microsoft Excel Solver.**

<u>Details</u>

On the UV-VIS spectrophotometer's computer select "General Scanning". Check that the parameter file is set correctly, and then take a background reading using a cell filled with distilled water. Step-by-step instructions for running the HP8532A UV spectrometer to obtain a UV-VIS Spectrum are provided in Appendix 1. Be sure the outer walls of the cuvette are dry and clean by wiping them with a Kim-Wipe.

Check the validity of the Lambert-Beer equation over a range of concentrations for $K_3[Fe(CN)_6]$ by recording the absorption maximum[3] for $K_3[Fe(CN)_6]$ solutions at differing concentrations. **Each** pair of students will determine the absorbance of **solution A**, followed by the absorbance resulting from 1/2, 1/3, 1/4 and 1/5 dilutions of **solution A**. There are different ways of carrying out the dilutions, remember more careful measurements leads to higher quality data.

Plot absorbance (A) versus concentration (c). Don't forget to include the (0, 0) data point in your calculations. If the Lambert-Beer equation is valid over this range of $K_3[Fe(CN)_6]$ concentrations, a straight line with slope ($\varepsilon \times l$) should result (the length, l, of the UV cell is 1 cm). Use this ε value throughout all your kinetic curve fitting calculations (see the instruction from Appendix 3).

C. Day 2: Kinetics

Goals

- Make the ascorbic acid solution and $K_3[Fe(CN)_6]$-$NaNO_3$ solutions.
- Run kinetics trials. Each pair of students runs all four trials.
- Get a printout with the graph $A = f(t)$ and the corresponding table for each kinetics trial.
- Edit your kinetics data (4 files) into your Athena account. Do some or all of the data fitting.

Details

On the UV-VIS spectrophotometer's computer select "Kinetics". Check that the parameter file is set correctly, and then take a background reading using a cell filled with distilled water. Details of running the HP8532A UV spectrometer for kinetics runs are provided in Appendix 2.

When ready to start the kinetics trials, pipet **3 mL** of one of the **$K_3[Fe(CN)_6]$-$NaNO_3$** solutions (solutions B,C,D,E) into an Erlenmeyer flask, and add **3 mL** of the **ascorbic acid-HNO_3-EDTA** solution (solution F)**.**

*****Swirling the solution of reactants for a few seconds improves mixing*****

Pour the solution into a UV-VIS cell. Dry and clean the outside walls of the cuvette by wiping it with a Kim-Wipe and place the cuvette in the spectrophotometer. Record the absorbance **at 1 minute intervals for 20 minutes**.

Transfer the data to your **Microsoft Excel Solver** and follow the instructions provided in the Appendix 3.

D. Day 3: Kinetics continued

[3] λ_{max} should occur at 420 nm. However, it may vary by ±3 nm due to variations in the instrument. If the maximum occurs within this range, use the value at that wavelength for your calculations.

- Bring your results. Consult your TA about the general appearance of your results. Decide whether any experiments should be repeated.
- Make **FRESH** ascorbic acid solution if kinetic trials need to be run.
- Run or re-run any remaining kinetics trials.

V. DATA TREATMENT

The rate constants are calculated for different ionic strengths by fitting the experimental $A = f(t)$ curves to the calculated $A = f(t)$ function, assuming that the ascorbic acid oxidation with hexacyanoferrate(III) follows the second order reaction kinetics as described by equation (3.4).

Consider the general chemical equation of the type:

$$b\mathrm{B} + c\mathrm{C} \rightarrow products$$

The integrated rate equation[4] is:

$$k_{obs}t = \frac{1}{\{c[B]_o - b[C]_o\}} \ln \frac{[B][C]_o}{[B]_o[C]} \tag{3.9}$$

In our case B = ascorbic acid, C = $K_3[Fe(CN)_6]$, $b = 1$ and $c = 2$. By using equations (3.7) and (3.8), equation (3.9) becomes:

$$A = \frac{A_f}{1 - \frac{A_o - A_f}{A_o} e^{-c_f k_{obs} t}} \tag{3.10}$$

where A is the current absorbance at time t, A_o is the value of absorbance at $t = 0$, A_f is the final absorbance ($t \rightarrow \infty$), c_f is the final concentration, and k_{obs} is the observed rate constant. The curve fitting with **Microsoft Excel Solver** will provide the corresponding k_{obs}, and A_f for each kinetic run.

Applying the steady-state approximation[5] to the elementary steps of the reaction mechanism (equations 3.5), and assuming $k_{-1}\left[H^+\right] >> k_2\left[Fe(CN)_6^{3-}\right]$, the theoretical rate law becomes

[4] Steinfeld, J.I., J.S. Francisco, and W.L. Hase, Chemical Kinetics and Dynamics, 2nd ed. (Prentice-Hall, Upper Saddle River, N.J., 1999), Chapter 1.

[5] Steinfeld, J.I., J.S. Francisco, and W.L. Hase, Chemical Kinetics and Dynamics, 2nd ed. (Prentice-Hall, Upper Saddle River, N.J., 1999), Chapter 2, Section 2.2.1.

$$-\frac{d}{dt}\left[Fe(CN)_6^{3-}\right]=\frac{2k_1k_2\left[H_2Asc\right]\left[Fe(CN)_6^{3-}\right]}{k_{-1}\left[H^+\right]} \qquad (3.11)$$

The real rate constant ($k_{real} = k_2$) can be determined from the observed rate constant (k_{obs}) if the concentration of H^+ due to nitric acid and the dissociation constant for ascorbic acid ($K_a = 6.76\text{x}10^{-5} = k_1/k_{-1}$) are known:

$$k_{obs} = k_{real}\frac{1}{[H^+]}K_{a_{AscorbicAcid}} \qquad (3.12)$$

The measured effect of ionic strength on the real rate constant, known as the *primary salt effect*, is then compared to the theoretical equations predicting the ionic strength effects[6]. Let us begin with a few words on the concept of ionic strength. Ionic strength was defined by G. N. Lewis as:

$$I = \frac{1}{2}\sum_i z_i^2 c_i \qquad (3.13)$$

Here z_i is the charge of the ion i and c_i its concentration, while the summation encompasses all the ions in the solution. For a simple solution of a univalent electrolyte, the ionic strength is equal to the molar concentration, but is greater than the concentration when the salt contains ions of higher valencies.

According to the Debye-Hückel theory for aqueous solutions of electrolytes[5], the rate constant at ionic strength I (k_{real}) is given by:

$$\log k_{real} = \log k_o + 1.02 Z_1 Z_2 \frac{I^{1/2}}{I^{1/2}+1} \qquad (3.14)$$

where k_o is the rate constant when the extraneous ionic strength is absent. The charges of the two reacting species (see the reaction mechanism scheme) are Z_1 and Z_2, respectively. The equation applies to dilute solutions ($c < 0.2$ M).

<u>Analysis and Discussion</u> (Be sure to include/discuss these in your report)

1. Draw the plot log k_{real} versus $I^{1/2}/\left(I^{1/2}+1\right)$. Calculate k_o and Z_1Z_2. Compare the calculated Z_1Z_2 with the expected value predicted by the reaction mechanism. If there are discrepancies, discuss the reason.

2. What is the effect of increased ionic strength upon the experimental reaction rate of ascorbic acid oxidation by hexacyanoferrate(III)?

3. What would be the added salt effect upon reaction rate between ions with opposite charges?

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[6] *ibid*., Chapter 4, Section 4.5.

4. If one species is neutral, what would be the effect of the added salt upon the reaction rate?

5. What would be the effect of changing the temperature of the solution on the reaction rate?

6. What would be the effect of changing the pH at constant ionic strength?

See Appendix 3 for Kinetics Analysis Using Non-Linear Curve Fitting with Microsoft Excel Solver.