Experiment 1

Chemical Equilibria and Le Châtelier's Principle

A local theatre company is interested in preparing solutions that look like blood for their upcoming production of *Lizzie Borden*. They have hired Chemical Solutions Incorporated (CSI), to help them investigate the aqueous reaction of potassium thiocyanate with iron(III) nitrate that they have heard other companies are using as fake blood. You will investigate this equilibrium for CSI both *qualitatively* and *quantitatively*. The following useful information for these experiments is excerpted from reliable Web sites, and is reproduced with permission of the authors. You should also prepare for this experiment by reading about chemical equilibria and Le Châtelier's Principle (Chapter 15 in your textbook).

The Iron-Thiocyanate Equilibrium

When potassium thiocyanate [KNCS] is mixed with iron(III) nitrate [$Fe(NO_3)_3$] in solution, an equilibrium mixture of Fe^{+3}, NCS^-, and the complex ion $FeNCS^{+2}$ is formed (equation 1). The solution also contains the spectator ions K^+ and NO_3^-. The relative amounts of the ions participating in the reaction can be judged from the solution color, since in neutral to slightly acidic solutions, Fe^{+3} is light yellow, NCS^- is colorless, and $FeNCS^{+2}$ is red. If the solution is initially reddish, and the equilibrium shifts to the right (more $FeNCS^{+2}$), the solution becomes darker red, while if the equilibrium shifts to the left (less $FeNCS^{+2}$), the solution becomes lighter red or straw yellow. You will add various reagents to this reaction at equilibrium to see if/how those reagents shift the equilibrium position of the reaction using the color of the resulting solution.

$$\underset{\text{yellow}}{Fe^{+3}} + \underset{\text{colorless}}{NCS^-} \rightleftharpoons \underset{\text{blood red}}{FeNCS^{+2}} \qquad (1)$$

Quantitatively, the relative amounts of the two reactants and the product are related by the equilibrium constant for the reaction; in this case, the formation constant K_f, which is shown below. To precisely control the red color of the solution, it is necessary to know the value for K_f. K_f can be calculated through an experimental determination of the $[FeNCS^{+2}]_{eq}$ using a standard curve (week 2) and deduction of the $[Fe^{+3}]_{eq}$ and $[NCS^-]_{eq}$ by subtracting the amount of $FeNCS^{+2}$ produced from the known added initial amounts of Fe^{+3} and NCS^- (as that is how much was consumed during the reaction).

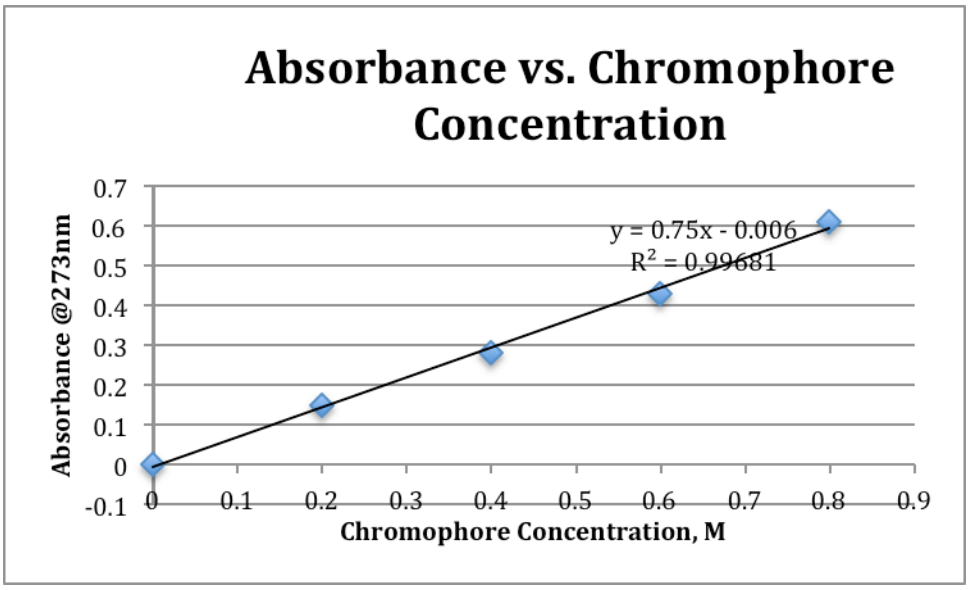
$$\frac{[FeNCS^{+2}]_{eq}}{[Fe^{+3}]_{eq}[NCS^-]_{eq}}$$

Use of a Standard Curve In this technique a series of solutions with known concentrations is prepared and then a parameter such as absorbance is measured. This parameter is then plotted versus concentration to yield a standard curve (in this case how absorbance varies with concentration), which is often a straight line with some degree of experimental error. Analysis of the data allows determination of the best-fit line (e.g., with Excel). A subsequent measurement of the absorbance for an unknown sample allows determination of its concentration using the equation for the standard curve. The unknown concentration should lie in the concentration range of the standard solutions used to construct the standard curve for an accurate concentration determination.

Beer's Law Beer's Law relates the experimental absorbance value for a chromophore (a substance that absorbs light) to the concentration of that chromophore in solution. Beer's law has many forms, the most common is: $A = \varepsilon\, l\, C$. In this equation A is the measured absorbance of the chromophore at a given wavelength (usually at a peak maximum, or l_{max}, determined from a spectrum spanning ultraviolet and/or visible wavelengths of light). The Greek letter epsilon, ε, stands for the molar extinction coefficient ($M^{-1}cm^{-1}$), an experimentally determined constant for the specific chromophore at a specified wavelength. The molar extinction coefficient is a quantitative measure of the light absorbance by the chromophore at that wavelength for a one molar solution and a one-centimeter path length. The value l is the path

length, or the distance the light travels through the solution in the cuvette (container) used for the absorbance measurement. Lastly, *C* is the molar concentration of the chromophore (mol/L) used for the measurement. Beer's law says that the relationship between the absorbance of the chromophore and its concentration is linear, allowing construction of a standard curve by plotting absorbance versus concentration, such as shown in Figure 1. This particular curve is most reliable for absorbance values between 0.0 -1.0. The concentration for an unknown solution can be determined by measuring its absorbance first. Then use the known absorbance value and the equation for the standard curve to solve for x, the concentration.

Figure 1. Absorbance vs. concentration (M) of a chromophore.



Pre-Laboratory Assignment

Week 1: Buy your lab notebook (see Lab Syllabus for specific requirements). This week (and every week), come to lab with a hand written outline of the experimental procedure in your lab notebook.

Week 2: In addition to the usual outline of the procedure in your lab notebook, please answer the following questions. **Due before 9 AM, Fri., Feb. 10th (in white bookcase by Keyes 310)**.

A. Calculate the concentrations of NCS^- and Fe^{+3} in each of the following solutions. Note that the final total volume is 10.0 mL in each case due to the addition of H_2O. As always, all work must be shown to receive full credit. Watch those units and sig figs.

volume 0.0020 *M* KNCS (mL)	volume 0.0020 *M* $Fe(NO_3)_3$ (mL)	total solution volume (mL)
1.0	5.0	10.0
2.5	5.0	10.0

B. 1. Construct a standard curve in Excel using the following data (and 0.0 micromolar concentration gives 0.000 absorbance) based on the absorbance at 275 nm of protein sample. Print your graph once it is titled (Y vs X), includes the equation of the line and the R^2 value, has a label for each axis and includes any appropriate units.

2. Use this graph to determine the unknown concentrations of two samples of the protein, showing your work.

3. Would you expect these determinations to be accurate or not? Explain your answer.

Concentration of Protein Sample	**Absorbance at 275 nm**
2.0 micromolar	0.460
1.0 micromolar	0.251
0.50 micromolar	0.117
0.20 micromolar	0.067
unknown concentration 1	0.178
unknown concentration 2	0.603

Experimental Procedure- Week 1, Feb. 6th-10th

For each of the external stresses described below, necessary information is provided regarding the manner in which one or more of the chemical species is affected. You will use a spot plate containing multiple wells and use a different well for each of the operations described, recording your observations of the color change of the solution.

In the Data section of your lab book, create a detailed table summarizing your observations for each of the reactions that you'll perform (see below) on the iron-thiocyanate equilibrium. As an example, if you added a drop of concentrated HCl to the standard solution, the blood-red color would lighten or perhaps even disappear altogether. This indicates that the $FeNCS^{+2}$ concentration has decreased. To explain this result, it is necessary to know that in the presence of a large excess of Cl^-, Fe^{+3} forms complex ions:

$$Fe^{+3} + 6\ Cl^- \boxed{} FeCl_6^{-3}$$

This change reduces the Fe^{+3} concentration, so in accord with Le Chatelier's Principle, some $FeNCS^{+2}$ dissociates to replace some of the Fe^{+3} removed by reaction with Cl^-. This would be summarized in your lab book as follows:

Stress	Observation	Reactions of Interest	Explanation
+1 drop HCl (6 M)	soln turned yellow (the original red color went away)	$Fe^{+3} + NCS^- \leftarrow FeNCS^{+2}$ $Fe^{+3} + 6\ Cl^- \rightarrow FeCl_6^{-3}$	Equilibrium shifted left in response to a decrease in $[Fe^{+3}]$, which was removed by its reaction with Cl^-.

A. Operations to Introduce an External Stress- Record all observations in your data table.

1. Add one drop each of 1 M $Fe(NO_3)_3$ and 1 M KNCS to 25 mL of distilled water. Mix well.

2. Add a few drops of this solution to each of seven wells of a spot plate. One well will serve as a color standard against which to judge color changes in the other wells. The other six wells will be for performing your operations to introduce an external stress. Record the appearance of the reaction before adding stress.

3. Add one drop of 1 M $Fe(NO_3)_3$ to one of the wells, mix, and observe. Record observation.

4. Add one drop of 1 M KNCS to a second well, mix, and record observation.

5. Add one drop of 0.1M $SnCl_2$ to a third well, mix, and record observation. Tin(II) ions reduce iron(III) ions to iron(II) ions:
$$Sn^{+2} + 2\ Fe^{+3} \boxed{} Sn^{+4} + 2\ Fe^{+2}$$

6. Add one drop of 0.1 M $AgNO_3$ to a fourth well, mix, and record observation. Silver ions react with thiocyanate ions to give a white precipitate of silver thiocyanate:
$$Ag^+ + NCS^- \boxed{} AgNCS(s)$$

7. Add one drop of 0.1 M Na_2HPO_4 to a fifth well, mix, and record observation. Hydrogen phosphate ions form a complex ion with iron(III) ions:
$$Fe^{+3} + HPO_4^{-2} \boxed{} FeHPO_4^+$$

8. Add one drop of 1 M NH_3 to a sixth well, mix, and record observation. Any base will form a precipitate or a colloidal suspension of iron(III) hydroxide when mixed with iron(III) ions:

$$NH_3 + H_2O \square NH_4^+ + OH^-$$
$$Fe^{+3} + 3\ OH^- \square Fe(OH)_3\ (s)$$

B. Effect of Heat on the Equilibrium- Also record your observations in the data table.

1. Pour about 4-5 mL of the iron-thiocyanate solution made earlier into three test tubes. Set one tube aside as a color standard against which to judge color changes in the other tubes.

2. Gently warm one tube in a hot water bath but don't boil the solution. Record observation.

3. Take the hot tube and stick it into an ice bath, along with an unheated tube of solution. Observe all three tubes and note how they compare. Is the reaction reversible? Do these results indicate that the forward reaction is exothermic or endothermic? Make notes to help you report back to the client on the significance of what you've seen today.

Experimental Procedure- Week 2, Feb. 13th-17th

Last week's phase of the experiment was qualitative, relying on the observation of color changes by eye, but this week you will be measuring the amount of color with a spectrophotometer. For this type of quantitative analysis, you need to be as exact as possible about the volumes that you measure. You will use 10.00 mL volumetric flasks to make up your solutions- with a volumetric flask you bring the total volume up to the white line to achieve a very accurate final volume. You will use automatic micropipettors to obtain known amounts of the reagents which are then dispensed into the volumetric flasks. Although many of you may have used these before in Biology laboratories, a few notes on their proper use follows. You will use a 200-1000 μL Finnpipette that we recommend setting to "500"- this is 500 μL or 0.5 mL. By dispensing 0.5 mL portions, you can conveniently add 0.5 mL, 1 mL, 1.5 mL, and so on by simply making multiple additions. Please make sure that you never dial the pipettor past the maximum volume, in this case 1000 μL. To draw up the sample into the pipette tip, first place a clean tip on your pipette. Pour some reagent into the provided beaker. Push the top pipette button downwards until you feel it catch on a notch, immerse the pipette tip in the sample, and slowly release the pressure you are exerting on the button. Check the tip to make sure you didn't capture any air bubbles. To dispense the sample, push the button all the way down. You can keep a pipette tip in the beaker and use it over and over. Each solution beaker can have one pipette tip and you'll be helping CSI to be green. **If you have any questions about the micropipettors, please ask your instructor or student assistant before use.** Micropipettors can be severely damaged if they are incorrectly used.

A. Determination of the Wavelength of Maximal Absorbance of the $FeNCS^{+2}$ Ion

You will establish a standard curve to calibrate the absorbance-concentration dependence of the $FeNCS^{+2}$ complex ion, but first you will need to determine the wavelength of maximal absorbance of the chromophore using the spectrophotometers. Refer to the "Spectrophotometer" handout (provided at each instrument) for detailed instructions on the use of these instruments. These very small spectrophotometers are interfaced to the computers in the laboratory for data acquisition during this experiment and Experiment II.

1. Using your 10-mL volumetric flask, prepare 10.0 mL of an iron-thiocyanate solution containing 1.00 mL of 0.200 M $Fe(NO_3)_3$ and 2.00 mL of 0.00200 M KNCS. Use the micropipettes to measure these volumes once you have poured the liquid into a beaker. ONLY pipette from beakers, never from bottles!
   Carefully add enough distilled H_2O to the flask to bring the final volume to the white etched line on the flask. Fill a cuvette three-quarters full with this solution. Be sure to wipe the cuvette with a Kimwipe to remove fingerprints.

2. Blank the spectrophotometer according to the handout. Use 0.200 M $Fe(NO_3)_3$ alone as your reference solution in its own cuvette.

3. Determine the wavelength of maximal absorbance (the λ_{max}) for the $FeNCS^{+2}$ solution and *use this wavelength in subsequent absorbance measurements*. If the peak has an absorbance that is greater than about 2, the peak may be somewhat flattened or "off-scale." In this case, it may be difficult to determine exactly what wavelength is the λ_{max}. You should therefore dilute the solution with water (by a known factor) until the absorbance reading is on scale. Record the absorbance reading- you can use this as one of the data points for your standard curve.

B. Generation of the Standard Curve

Ideally, for a good standard curve, the absorbance readings should be in the range of about 0.01->1.8 (an absorbance of 2 corresponds to 99% of the light being absorbed, which is at the upper limit of what can be measured accurately). You will have to select an appropriate range of $FeNCS^{+2}$ concentrations based on your observations from Part A. You will use a large excess of Fe^{+3} ion, which will drive the reaction to completion. Thus, you can assume that the final $FeNCS^{+2}$ concentration is equal to the initial concentration of KNCS, as the KNCS is the limiting reagent. Note that the $Fe(NO_3)_3$ solution is made up to include HNO_3 at a final concentration of 0.0100 M to prevent the formation of other iron complexes that may exist in the presence of base, but you do not have to account for this in any of your calculations.

1. Prepare five more solutions in 10-mL volumetric flasks containing varying concentrations of $FeNCS^{+2}$ in a total final volume of 10.00 mL. Each solution should contain 1.00 mL of 0.200 M $Fe(NO_3)_3$ and known, varying amounts of 0.00200 M KNCS. Add water to bring the final volume to 10.0 mL in each case. We recommend that in all cases your final concentration of KNCS is no higher than 0.8 mM, or the sample is likely to have an off-scale absorbance reading.

2. Take the absorbance at the wavelength determined in **Part A** for each of the solutions. If the absorbance readings are not within appropriate limits, make up more solutions until you have at least six concentrations that are within the 0.01 – 1.8 absorbance range.

3. Construct a standard curve using Excel as described in the **Data Analysis** section. Save & print the curve & data table for your client report.

C. Determination of the K_f

You will now make up a solution containing constant amounts of Fe^{+3} and varying amounts of NCS^- and use the standard curve to determine the final $FeNCS^{+2}$ concentration based on the measured absorbance. This will allow you to calculate K_f.

1. Mix five combinations of Fe^{+3} and NCS^- each in a total final volume of 10.00 mL. For each, use 5.00 mL of **0.00200 M** $Fe(NO_3)_3$ [this is a different solution than the one used above!], and known, varying amounts of the 0.00200 M KNCS, making up the total volume with water.

2. Determine the absorbance of the $FeNCS^{+2}$ complex ion for each solution after blanking appropriately (WHAT SHOULD YOUR REAGENT BLANK BE THIS TIME? THINK CAREFULLY.). Use the standard curve generated in **Part B** to convert the absorbance value into a concentration. This concentration reflects the $[FeNCS^{+2}]_{eq}$. Remember that your absorbance values should lie on the standard curve for best accuracy. If some of your trials are off the standard curve, make up new solutions until you have at least five absorbances that are on your standard curve, besides the blank.

Data Analysis

- In Excel, construct a standard curve of absorbance versus $[FeNCS^{+2}]$ (mol/L) for your standard solutions of **Part B**. Remember that in Excel the first data column corresponds to X and the second corresponds to Y; thus, your first column should be concentration, not absorbance. Note that [0, 0] should be included as a data point. Determine the equation of the best-fit line and the R^2 value. Make sure that you print your standard curve and have it to attach to your client report. Don't forget to have axes labeled, including units (and in this case include what specific wavelength was used). The convention for a graph title is "what's on Y axis" vs. "what's on X axis".

- From the standard curve, calculate the $[FeNCS^{+2}]_{eq}$ for each of your trials of **Part C.**

- Use the stoichiometry of the $FeNCS^{+2}$ complex ion formation to determine how much of each reactant was consumed and what the corresponding equilibrium concentrations of Fe^{+3} and NCS^- would be for your five trials of **Part C**.

- Use the equilibrium values of the $[Fe^{+3}]$, $[NCS^-]$, and $[FeNCS^{+2}]$ to calculate K_f as in equation (2) above for each of your solutions. Calculate an average K_f, , the standard deviation, the relative standard deviation, and the % precision for your trials of **Part C**. You may wish to refer to the Error Analysis (found on lab web page) for information on the relative standard deviation.

Client Report

Prepare a ONE-PAGE typed letter for the theatre client that hired you, summarizing your findings. You should attach to this letter all spreadsheets, graphs, and tables that you used to make your findings. You want to include both your qualitative and quantitative findings. Make sure that your report is at an appropriate level for your audience: in this case, the highly intelligent props manager at the theatre company who has not seen any chemistry since high school. Include a description of the underlying principle that explains the shift of the iron-thiocyanate reaction in terms of a response to the particular stress that was added. Using this theory, hypothesize whether the reaction is endothermic or exothermic and how the temperature of the theatre or of Lizzie Borden's victims could affect the equilibrium. Make sure that you include an error analysis in your report. Remember your Lab Syllabus gives you a guideline for the Client Report. Your success on reporting the findings to this client will likely bring CSI more business and recognition for your starring role!