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Inorganic Chemistry
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Colorimetric Determination of Iron

Introduction

As an essential nutrient in the human diet, iron is soluble in certain situations. Many people take iron supplements in the form of vitamins. In this lab we will determine the amount of iron present in a sample supplement using colorimetric determination. Iron surrounds itself with Lewis bases (ligands) and forms a red colored complex. The red colored complex forms quantitatively and can be measured using a Spectrometer 20.

The ligand used to form a colored complex is 1,10-phenanthroline, or o-phenanthroline. Fe+2 reacts exclusively with o-phenanthroline and absorbs in the visible spectrum (around 510 nm). This reaction occurs under acidic conditions. Ammonium acetate will be used to buffer the solution that the complex will form in and hydroxylamine HCl will be used to prevent oxidation.

Formation of the soluble Iron:

$$4\ Fe^{3+}_{(aq)} + 2\ NH_2OH_{(aq)} \rightarrow 4\ Fe^{2+}_{(aq)} + N_2O_{(aq)} + 4\ H^{+}_{(aq)} + H_2O_{(l)}$$

Complexing of Fe^{2+} with $C_{12}H_2N_{2(l)}$ (o-phenanthroline):

$$Fe^{2+}_{(aq)} + 3\ C_{12}H_2N_{2(aq)} \rightarrow [(C_{12}H_2N_2)_3Fe]^{2+}_{(aq)}$$ an orange-red complex

After making colorimetric measurements on various concentrations of solutions, a calibration curve will be constructed. An unknown will be analyzed for iron as well. The Beer-Lambert Law can be used to determine that absorption ($\mathbf{A=\varepsilon lc}$) of the unknown and iron supplement. The concentration of the unknown will be determined by extrapolation to the calibration curve and by using visual inspection.

Materials

- 10mL graduated pipette
- (2) 50mL volumetric flask
- 100mL volumetric flask
- 50mL burette (1 per group)
- (4) equivalent test tubes (to be distributed)
- Hotplate
- Millimeter ruler
- (2) Cuvettes for Spec20
- Stock Fe solution: 1mL = 0.050mg Fe (III)
- 1M $NH_4C_2H_3O_2$
- 10% Hydroxylamine HCl
- 0.3% o-phenanthroline.
- 3M H_2SO_4
- 6M HCl

MSDS Important Information

Compound	MSDS Information
Ammonium Acetate	May cause eye and skin irritation. May cause respiratory and digestive tract irritation. May cause central nervous system effects. Hygroscopic (absorbs moisture from the air).
Hydroxylamine HCl	Very hazardous in case of skin contact (irritant), of eye contact (irritant), of ingestion, of inhalation. Slightly hazardous in case of skin contact (corrosive, sensitizer), of eye contact (corrosive).
o-phenanthroline	May be harmful by inhalation, ingestion,or skin absorption. Causes eye and skin irritation. Material is irritating to mucous membranes and upper respiratory tract.
3M H_2SO_4	Hazardous in case of skin contact (irritant), of eye contact (irritant). Slightly hazardous in case of ingestion.
6M HCl	POISON! DANGER! CORROSIVE. LIQUID AND MIST CAUSE SEVERE BURNS TO ALL BODY TISSUE. MAY BE FATAL IF SWALLOWED OR INHALED.

PreLab Questions: (see scanned document below)

1. (a) The standard recipe used in this exercise uses 3.0 mL of 0.25% ophenanthroline solution in 100mL of final solution to generate the red ironphenanthroline complex which is determined colorimetrically. What is the initial molarity of o-phenanthroline in the diluted solution? [0.25% means 2.50 g. of ophenanthroline (molar mass 180) per liter of solution.]

(b) What is the maximum concentration of iron-phenanthroline complex that can be formed from this amount of o-phenanthroline, assuming complete complexation?

(c) What does this concentration of iron correspond to in mg/L?

(d) Is this enough for the amounts of iron (up to 20mL of stock solution) we are using? (i.e.: What is the limiting reagent in these reactions?)

2. Determine the concentration of the iron complex for the known iron solutions used in this lab.

3. A Russian scientist poses the following question:
.Take a ruble and put it in the bottom of a tall coffee mug, such that the coin completely covers the bottom of the mug. Add just enough coffee so that the coin is completely obscured by the coffee and cannot be seen any more. How much vodka would I need to add to the coffee in order to see the coin again:

a) one drop,
b) an equal volume of vodka to the volume of coffee that had been added, or
c) all the vodka produced in Russia, and more.

Using your knowledge of Beer's Law, answer and be prepared to discuss in class the day after lab.

Pre-lab Questions

1a) Molarity = $\frac{\text{mol solute}}{\text{L solution}}$

$\frac{\frac{2.5\text{g o-phenanthroline}}{180\text{ g/mol o-phen}}}{1\text{ L soln}} = 0.0139\text{ M o-phen}$

1b) $Fe^{2+} + 3C_{12}H_8N_2 \rightarrow Fe^{+2}(C_{12}H_8N_2)_3$

$0.0139\text{ M o-phen} \times 0.003\text{ L} = 4.17\times10^{-5}\text{ mol total o-phen}$

$4.17\times10^{-5}\text{ mol total o-phen} \times \frac{1\text{ mol complex}}{3\text{ mol o-phen}} = \frac{1.39\times10^{-5}\text{ mol complex}}{0.1\text{ L}}$

$= 1.39\times10^{-4}\text{ M complex}$

1c) $\frac{1.389\times10^{-4}\text{ mol complex}}{\text{L}} \times \frac{1\text{ mol Fe}}{1\text{ mol complex}} \times \frac{55.8\text{g}}{1\text{ mol Fe}} \times \frac{1000\text{mg}}{1\text{g}} = 7.75\text{ mg/L Fe needed}$

1d) up to 20mL ...

$20\text{mL stock} \times \frac{0.05\text{ mg Fe}}{1\text{mL}} = 1\text{mg Fe}$ (I have...) — o-phen limiting

$1\text{mg Fe} \times \frac{1\text{g}}{1000\text{mg}} \times \frac{1\text{mol}}{55.8\text{g}} = 1.79\times10^{-5}\text{ mol Fe (I have)}$ — Fe in excess

$4.1607\times10^{-5}\text{ mol} \times \frac{1\text{ mol Fe}}{3\text{ mol o-phen}} = 1.389\times10^{-5}\text{ mol Fe (needed)}$

2) $M_1V_1 = M_2V_2$ $\quad M_2 = \frac{M_1V_1}{V_2}$ $\quad M_1 = 0.05\text{ mg/mL}$, $V_2 = 50\text{mL}$, $V_1 = \text{varies}$

$M_2 = \frac{(.05\text{ mg/mL})(5\text{ mL})}{(50\text{mL})} = 0.005\text{ mg/mL}$

$M_2 = \frac{(.05\text{ mg/mL})(10\text{mL})}{(50\text{mL})} = 0.01\text{ mg/mL}$

$M_2 = \frac{(.05\text{ mg/mL})(15\text{mL})}{(50\text{mL})} = 0.015\text{ mg/mL}$

$M_2 = \frac{(.05\text{ mg/mL})(20\text{mL})}{(50\text{mL})} = 0.02\text{ mg/mL}$

3) $A = \varepsilon l c$ (constant ↑↓)

c) all the vodka brc as the concentration of coffee decreases, the length of the liquid increases keeping the absorbance the same. (cont.)

Pre-Lab questions continued:

3. (cont.) Normally, on a Spec 20 the length of the sample stays the same. Therefore, absorbance and concentration would be linearly related on a graph. However, when the length of the tall coffee mug increases, the absorbance stays constant.

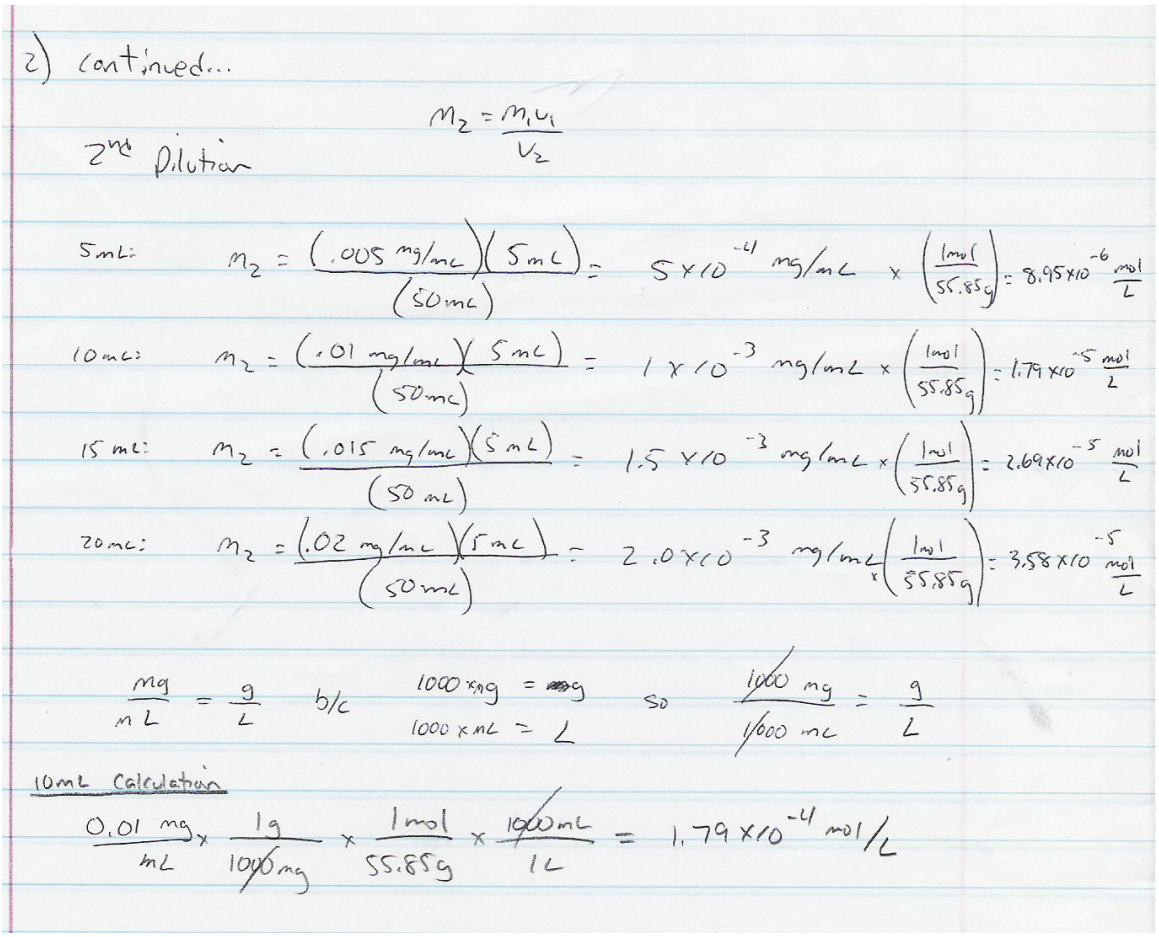
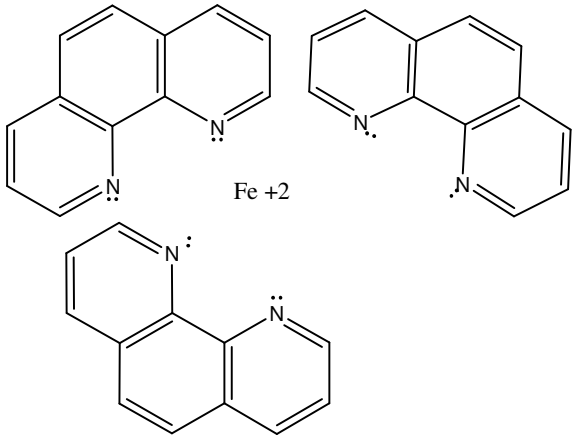
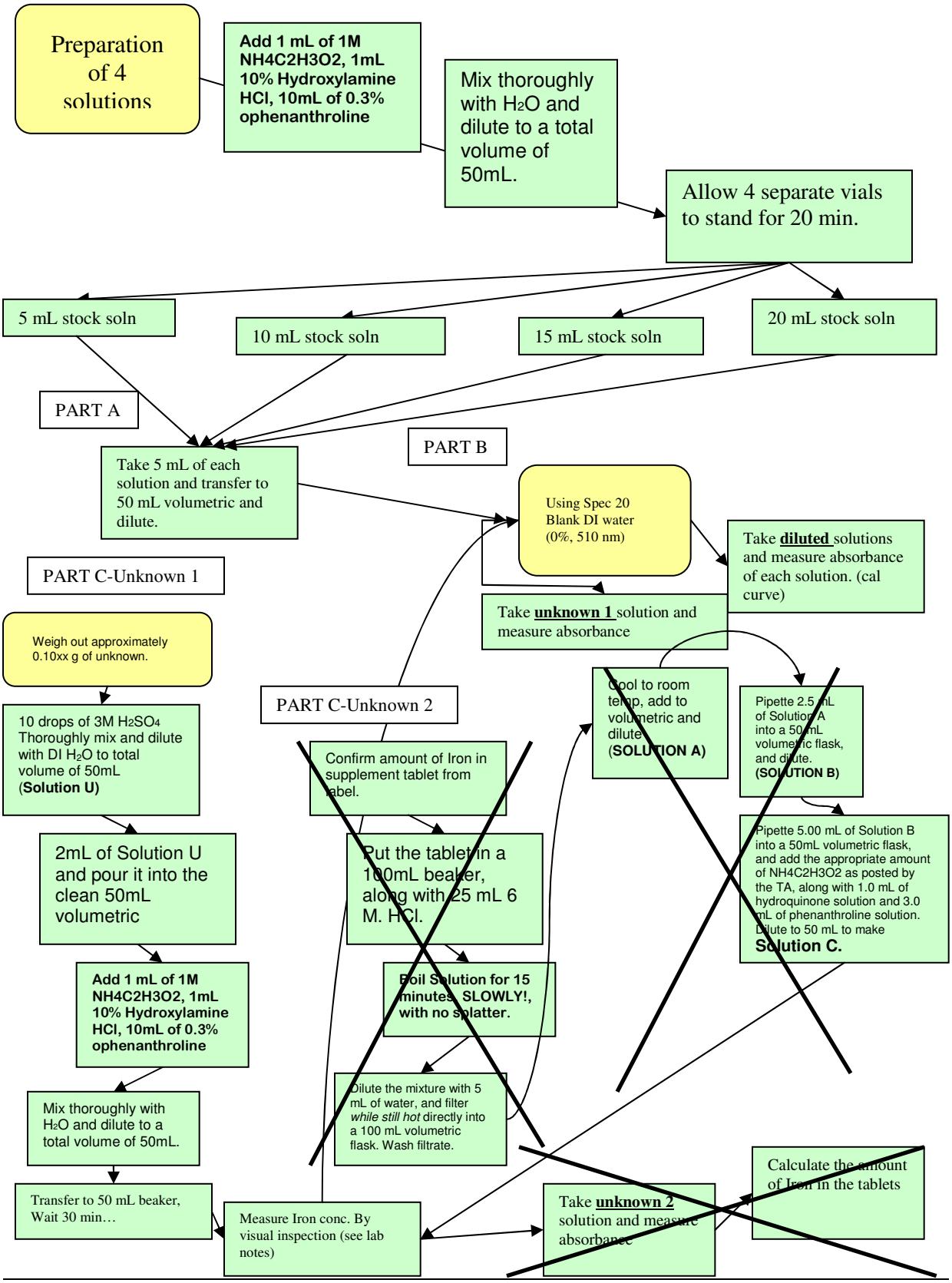


Figure 1: Structure of Iron Complex

Iron (II)-o-phenanthroline



Prodecure/Flow Chart



(Working in groups of 2 for Part A of the lab, and individually thereafter. Each group can share one burette, which can be rinsed with the stock Fe (III) solution before using)

(You will work in pairs instead of groups of four for Part A. This will help us get the same set of data points and move through the lab a bit faster. Each pair will randomly draw the two known concentrations for which they will be responsible.)

PART A: Preparation of Fe(II) solution for calibration curve data

1. Mix thoroughly with H_2O and dilute to a total volume of 50mL.

2. Each member of the group of ***2*** will make a solution using 5,10,15,20 mL of stock Fe solution.
3. Add 1mL of 1M $NH_4C_2H_3O_2$, 1mL 10% Hydroxylamine HCl, 10mL of 0.3% ophenanthroline to each sample. Mix thoroughly with H_2O and dilute to a total volume of 50mL.
4. Transfer to a clean, DRY beaker and label with the concentration of the iron complex. Allow each solution you prepare to stand for at least 20 minutes at room temperature, to provide time for formation of the iron-phenanthroline complex.

(start on Part C while you wait)

5. After allowing the iron complex to form, take 5mL of this solution into a 50mL volumetric flask, and dilute to the total volume of 50mL. This solution will be used for the remainder of Part B. Put the concentrated solution to one side until Part C.

   (Each member of the group should have made TWO of 4 solutions. Distribute these dilute solutions in the group for Part B.)

PART B: Determination of the Calibration Curve

1. Mark the top of a cuvette with a marker, and add DI water to the cuvette as a blank.
2. Align the mark at the top with the mark in the Spec 20 cuvette holder. This will be your blank solution. Use the blank solution to set the 0% absorbance on the spectrophotometer at 510nm.
3. Using another cuvette, also marked at top for alignment, measure the absorbance of each solution at 510nm, using some of the solution to be tested as a rinse for the cuvette between uses. Be sure to align the cuvette the same way each time. (Establish calibration curve in Data Section)

PART C: Unknowns (Unknown B was chosen for the lab analysis)
Unknown 1: Preparation and colorimetric determination of Unknown 1

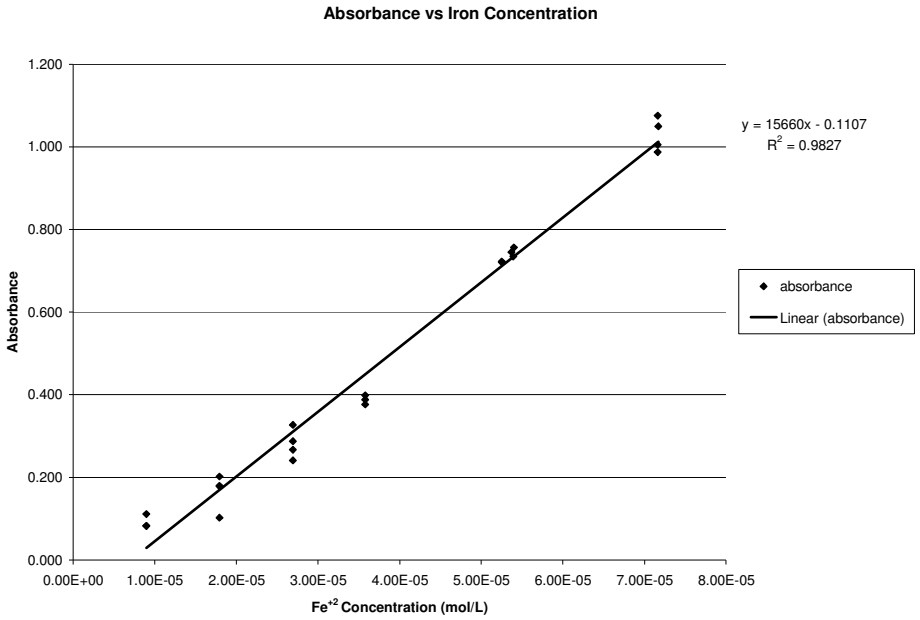
1. Weigh out approximately 0.10xx g of unknown and place into a clean 50mL volumetric flask.
2. Add 10 drops of 3M H_2SO_4 (from reagent bottles on shelf.)
3. Thoroughly mix and dilute with DI H_2O to total volume of 50mL. This will be called **Solution U** to prevent confusion later on. This may be transferred to a clean, DRY, and labeled beaker.
4. Using a graduated pipette, take 2mL of Solution U and pour it into the clean 50mL volumetric flask.
5. Follow the steps to complex the iron by adding 1mL of 1M $NH_4C_2H_3O_2$, 1mL 10% Hydroxylamine HCL, and 10mL of 0.3% o-phenanthroline.
6. Mix thoroughly with DI H_2O and dilute to a total volume of 50mL.
7. Once thoroughly mixed, the 50mL solution can be transferred to a clean, DRY, and labeled beaker, where the Colorimetric Determination of Iron reaction will develop over half an hour (30 mins).
8. Repeat this step once using the same Solution U
9. Once the iron in Solution U has developed for half an hour, measure the concentration of the iron in solution by visual inspection.
10. First, prepare two cylindrical paper sleeves, which can be slipped over the large test tubes to exclude side lighting, by wrapping strips of paper around a test tube and securing the edges with gummed labels. In a test tube, add enough of the designated concentration of iron complex as your referent. (*Your TA will indicate which solution from Part A should be used for this referent.)*
11. Add enough to cover 5cm length of the test tube. With the paper sleeves in place, arrange the referent test tube and another test tube so as to look lengthwise through the solution toward a good diffuse light source (preferably a light box.)
12. Measure one of the other solutions by adding small amounts to the comparison test tube until the color intensities appear exactly the same when viewed lengthwise through the tube.
13. Reverse the viewing positions of the tubes for better comparison, and make final adjustments with a pipette.
14. Measure the depth of each solution to the nearest millimeter, taking all measurements from the bottom of the test tube. Save your referent for the second unknown.
15. After you have finished with the colorimetric inspection, check the absorbance of your unknown with the Spec 20, using the same procedure as in Part B. (Note: It is recommended that you measure the absorbance of a second sample of the same diluted unknown solution (Solution U). If the absorbance is the same as the first (within the precision of the absorbance measurement), that is evidence both that you waited the appropriate length of time for the formation of the complex and that you mixed the solution to a uniform composition. If the two do not agree, one or the other of the above may not be true. In the latter case, you may wish to prepare a new dilution of your unknown solution.

Data

Concentration	Transmittance (%)	Absorbance = 2-log*T	Length of column (cm) with visual inspection
10 mL stock soln (assigned)	79.0	0.102	41.0
15 mL stock soln (partner)	57.4	0.241	n/a
Unknown 1	77.3	0.112	56.0

Unknown # B
0.1048 g

Figure 2: The calibration curve graph



Slope = 15660 = ε1 ℓ1
Equation = y = 15660x-0.1107

My unknown Absorbance = 0.112

Concentration of unknown sample from extrapolation: $1.42 * 10^{-5}$ mol/L

Data Analysis

Visual Inspection Method:

Known Absorbance of Stock 10 mL Solution = Unknown Absorbance
ε1 ℓ1 c1 = ε2 ℓ2 c2
ℓ1 c1 = ℓ2 c2

(41.0 cm)($1.79*10^{-4}$ mol/L) = (56.0 cm)*([Fe unknown])
[Fe unknown] = $1.31*10^{-4}$ mol/L

Questions

1. Iron (III) reacts with water by a hydrolysis reaction. In order to prevent this hydrolysis, acid has been added to the standard solution (and you add H_2SO_4 to the unknown sample). How would your results change if no acid had been added to the standard iron solution or to the unknown solution?

If no acid was added to the standard iron solution or the unknown solution, the Fe^{+3} would not be soluble. Adding the acid will allow the Fe^{+2} to form, thus allowing it to form the complex.

2. A MCE student, Matt, omitted the hydroxylamine HCl from the solution he prepared to determine the amount of iron in his unknown. What effect is that omission likely to have on his result?

Hydroxylamine HCl, ($NH_3OH^+Cl^-$) was added as a reducing agent to intercept oxygen and prevent oxidation of ferrous iron to ferric iron. If Matt omits hydroxylamine HCl, oxidation will occur and the Iron complex would not form.

3. Beer.s Law states that $A=\varepsilon l c$. How does the visual inspection colorimetric method demonstrate this Law?

By visual inspection colormetric method, two of the same solutions are compared under a light box and the amount of solution (ℓ) are adjusted so that the absorbance of solution #1 (standard) = absorbance #2 (unknown). Also, each solution has a defined concentration. As the length of solution varies in each test tube the amount of light traveling through each solution will be equal to each other.. The length of each test tube will be proportionate to the concentrations of each solution. We know ℓ_1, c_1, ℓ_2. Therefore, we can determine the concentration of the unknown solution. (Refer to data section)

$A=\varepsilon l c$ $\qquad \varepsilon_1 \ell_1 c_1 = \varepsilon_2 \ell_2 c_2$

4. During our colorimetric determination by visual inspection, we are using test tubes with rounded bottoms. How does this affect your results?

Round test tubes do not affect our results greatly because we are consistently using round test tubes for each solution. The visual inspection method is comparing two samples to each other not to a standard. Therefore the rounded bottom, which will affect the length of the solution column, does not affect our calculations of concentration. Also, if one measures the length of the test tubes from the same bottom point on each test tube, the roundness should not affect our results greatly. Both tubes were treated as “identical”. The light was allowed to enter the filled tubes the same way and the length was measured the same way using a millimeter ruler.

Conclusion

In this lab, Iron (III) was acidified in order to produce a soluble Iron (II) solution. The Iron (II) was then added to o-phenathroline to form a stable soluble complex, Iron (II)-o-phenanthroline (See Figure 1). This soluble complex was made in several solutions with known concentrations. The Beer-Lambert Law (**$A=\varepsilon lc$**) was used to determine the absorption of the unknown. A calibration curve was constructed using data from 13 other sources and used to compare to the concentration of an unknown sample (See Figure 2). The concentration via extrapolation of the unknown compound was $1.42 * 10^{-5}$ mol/L. Visual inspection was used as well to compare the known concentration to the unknown concentration. The length of the solutions in the test tubes were adjusted to give similar concentrations. The concentration for the unknown using visual inspection was $1.31*10^{-4}$ mol/L. The visual inspection method and extrapolation method gave values for the unknown that were off by a power of 10. Human error must have played a role in the visual inspection method. Reasons why the numbers were so far off cannot be fully explained at this time. The extrapolation method is much more reliable in determining the concentration of the unknown.

References

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