Experiment 18
Determination of Iron by Visible Spectrophotometry

PRELAB DISCUSSION
Visible spectrophotometry (spectral analysis) is a method of measuring the concentration of colored solutions by the amount of light absorbed by or transmitted through a colored solution. The absorbance of white light by a solution containing a colored compound is directly proportional to the concentration of the colored compound. The constant of proportionality contains the path length of the sample through which the light passes and a constant that is determined by the color of the solution.

The operation of the Spec 20 will be demonstrated in the laboratory. BUT the fundamental operation of the Spec 20 is a lamp providing white light is separated into component colors by a diffraction grating and is focused and then is passed through a sample where some of the light is absorbed. The remaining light is detected by a phototube and the quantity of light absorbed (or transmitted) is read directly from the meter. On the Spec-20 meter, transmittance is a linear scale, while absorbance is a logarithmic scale. When a Spec-20 meter has a digital reading each can be read with a turn of a switch.

Experimentally, it has been found that the quantity of light absorbed is directly proportional to the molar concentration of the solution. This is represented by Beer’s Law, \( A = \varepsilon \ell c \) (the equation is \( A = abc \) on the AP Equation and Constant sheet), where \( A \) is the absorbance, \( \varepsilon \) is the molar absorbptivity, \( \ell \) is the path length the light travels through the solution, and \( c \) is the molar concentration of the solution. If the quantity of light that enters the solution is represented by \( I_0 \) and the quantity of light leaving the solution is represented by \( I \), then the internal transmittance of the solution can be represented by \( T \), and that the relationship between the quantity of light absorbed \( A \) and the quantity transmitted \( T \) can be expressed as: \( T = \frac{I}{I_0} \) and \( A = -\log_{10} \frac{1}{T} \).

It makes sense that if the light travels through more sample solution, it will encounter more absorbing species and the absorbance will increase. The constant of proportionality, or molar extinction coefficient (\( \varepsilon \)), depends mostly on the characteristics of the absorbing species and on the particular wavelength of light being absorbed. Thus, the Beer-Lambert Law can be written as \( A = \varepsilon \ell c \) (the equation is \( A = abc \) on the AP Equation and Constant sheet), where \( A \) is absorption, \( \varepsilon \) is the extinction coefficient, \( \ell \) is the path length of the light through the sample, and \( c \) is the concentration of the absorbing material. Since the path length is usually specified in centimeters and the concentration in molarity, the units of the extinction coefficient must be M\(^{-1}\)cm\(^{-1}\) (also maybe shown as L mole\(^{-1}\)cm\(^{-1}\)).

In this experiment we will not attempt to determine the values for \( \varepsilon \) and \( b \). Rather we will draw a graph of absorbance, \( A \), determined from the spectrophotometer vs. \( c \), the molar concentration of the solution and use it as a calibration curve or a conversion curve. From the calibration curve we will be able to determine the concentration of an unknown solution. The effect of “interfering” species (solvents, other molecules that are in the sample system, and the test tube) can be accounted for by running a blank. For a complete discussion of the spectroscopic theory of this experiment, see Appendix Three of the Zumdahl textbook.

You will be provided with a standard iron solution that contains acidified ferrous ammonium sulfate such that 1 mL = 50.0 µg Fe\(^{2+}\). Any iron (III) is reduced to iron (II) by the hydroxylamine hydrochloride. The element iron in water solutions forms salts of Fe\(^{2+}\) (ferrous iron) and Fe\(^{3+}\) (ferric iron). Ferric iron, however, forms a very insoluble hydroxide, Fe(OH)\(_3\), so that ferric salts remain in solution only in very acidic solutions, where the OH- concentration is very low. In a neutral solution (pH 7), the maximum possible concentration of free Fe\(^{3+}\) ions is about 10\(^{-17}\) M. While the stomach provides an acidic environment, the intestines (where iron is absorbed) provide a basic environment. Therefore, only ferrous iron can be absorbed and used by the body. We will also
be converting the ferric iron to the ferrous form in this lab. The redox reaction is: $4 \text{Fe}^{3+}_{(aq)} + 2 \text{NH}_2\text{OH}_{(aq)} \rightarrow 4 \text{Fe}^{2+}_{(aq)} + \text{N}_2\text{O}_{(aq)} + 4 \text{H}^+_{(aq)} + \text{H}_2\text{O}_{(l)}$

Like other transition metal ions, Fe$^{2+}$ forms many coordination compounds in which the iron ion is surrounded by various Lewis bases, called ligands. Many of these coordination compounds are colored, unlike aqueous ferrous ion itself. If the colored complex is stable and forms quantitatively, we can use the amount of color generated under a standard set of conditions as a measure of the amount of iron present. The ligand used to form a colored complex in this exercise is 1,10-phenanthroline, or o-phenanthroline, a bidentate chelating agent, which forms a stable red complex with ferrous iron: Complexing of Fe$^{2+}$ with C$_{12}$H$_2$N$_2$(o-phenanthroline): $\text{Fe}^{2+}_{(aq)} + 3 \text{C}_{12}\text{H}_2\text{N}_2_{(aq)} \rightarrow [(\text{C}_{12}\text{H}_2\text{N}_2)\text{Fe}]^{2+}_{(aq)}$ an orange-red complex

This procedure is selective for Fe$^{2+}$, even in the presence of other metals, for several reasons. Fe$^{2+}$ reacts rapidly with o-phenanthroline compared to many other metals, and the complex is exceptionally stable. Most important, it is the only phenanthroline complex that has a strong absorption in the visible spectrum. Therefore, the development of the intense red color due to absorption of light at a set wavelength is specifically due to Fe$^{2+}$, whatever other cations or complexes may be present. The complex we are forming has a notable orange-red color. This means that this complex ion maximally absorbs (minimally transmits) wavelengths that are complimentary to orange-red, which is in the blue-green region of the visible electromagnetic spectrum.

In our preparation of solutions to be analyzed colorimetrically, we will also include two additional reagents: ammonium acetate, (NH$_4$)$_2$(CH$_3$COO$^-$), is used as a buffer to maintain an appropriate pH (3.5) conducive to formation of the complex, and hydroxylamine HCl, (NH$_3$OH$^+$Cl$^-$) is added as a reducing agent to intercept oxygen and prevent oxidation of ferrous iron to ferric iron. The procedure to be followed consists of four major parts:

I. Making prepared diluted solutions from a given standard (concentrated) iron (II) solution
II. Determine the optimum wavelength for the phenanthroline iron complex
III. Making colorimetric measurements on carefully prepared solutions containing known amounts of Fe$^{2+}$ to construct a calibration curve;
IV. Determining the amount of iron in an "unknown" by preparing a Fe$^{2+}$ solution from a multivitamin tablet and analyzing the amount of iron contained.

**PRELAB QUESTION**
1. This lab is called a spectroscopic analysis, Labs 5 and 6 are collectively called a gravimetric analysis and Lab 17 is called a volumetric analysis. How are these labs similar and how are they different?
PROCEDURES

Part I: Solution Preparation

The calibration curve is determined from the following solutions that must be prepared as follows:

Place 1 mL, 2 mL, 5 mL, 10 mL, 12 mL, and 15 mL of standard iron solution (Stock Fe^{2+} solution: 1mL = 0.050mg Fe (II)) into seven properly labeled 100 mL volumetric flasks. Then to each flask add:

i. 2.00 mL of 1M ammonium acetate
ii. 2.00 mL of 10% hydroxylamine hydrochloride
iii. 25.00 mL of 0.30% o-phenanthroline solution (the complexing agent)
iv. dilute to a volume of 100.00 mL with distilled water.

Mix well and allow the color to develop for 20-25 minutes.

Part II: Determination of the optimum wavelength

Measure the transmittance at the associated wavelengths at 15 nm intervals for the range of the instrument using one of the intermediate solutions to determine an approximate optimum wavelength. For the report, prepare two separate graphs (smooth curve of “connect-a-dot”) of wavelength (of the entire spectrum) vs. absorbance and wavelength (of the entire spectrum) vs. transmittance to show the wavelength at which maximum absorbance occurs and minimum transmittance. Report out this number and this will then be the wavelength setting used for the next two parts.

Part III: Calibration Curve

Set the instrument at the optimum wavelength for the solution. Measure the absorbance for each prepared solution. Graph absorbance vs. concentration (µg/mL) to prepare the calibration curve. The concentration for each solution will need to be calculated using the dilution formula. This graph should be a xy-scatter plot on which the regression line is superimposed. Include the equation of the regression line and the correlation coefficient.

Part IV: Determine the amount of iron in a vitamin

Your old AP Chemistry teacher is concerned about the quality of the vitamins at a local discount pharmacy, but then, paranoia is common in old chemistry teachers. Unfortunately, you are now obligated to confirm the amount of iron in an iron supplement.

Record in your notebook the description of the tablet you are analyzing, including label information on the supposed quantity of iron present. Put the tablet in a 150 mL beaker, along with 30 mL of 6 M. HCl. CAUTION: Boil slowly for 5-7 minutes. Do not allow any solution to spatter out of the beaker. This procedure must be performed in the fume hood.

Dilute the mixture with 5 mL of water, and filter while still hot directly into a 100 mL volumetric flask. Wash the beaker, filter, and residue with distilled water, so as to transfer all soluble material into the volumetric flask. Allow the solution in the volumetric flask to cool to room temperature, and add distilled water to bring the volume to the calibration mark of the volumetric flask. This is Solution A. (This solution should contain all of the iron in the pill). Pipette 5 mL of Solution A into a 100 mL volumetric flask, and dilute to the mark with distilled water. This solution, Solution B, now contains one-twentieth of the iron present in the tablet.
Pipette 10.00 mL of Solution B into a 100 mL volumetric flask, and add 1.0 mL of NH₄C₂H₃O₂, along with 1.0 mL of hydroxylamine hydrochloride solution and 3.0 mL of phenanthroline solution. Dilute to 100 mL to make **Solution C**. Allow the solution sit for 20 minutes to permit formation of the iron complex. (Solution C contains one tenth of the iron in Solution B and therefore, one two hundredth of the amount in Solution A.)

Once the iron in each solution has developed for half an hour, measure the concentration of the iron in solution by visual inspection. Next, determine the absorbance solution in the Spec 20 at the optimum wavelength for each solution. Compare the Fe²⁺ concentration that is determined by the visual inspection method to that of the concentration determined by the calibration curve from Part B. **Calculate back to the amount of Fe²⁺ (in mg) in the original tablet.**

**Post Lab 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 H₂SO₄ 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?
2. If a student 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?
3. Beer’s Law states that A=ε l c. How does the visual inspection colorimetric method demonstrate this Law?
# Experiment 18: Determination of Iron by Visible Spectrophotometry

## Grading Protocol

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