I. BACKGROUND

In this experiment, an unknown solid sample containing iron oxide (Fe₂O₃, FW=159.69) will be analyzed by two spectroscopic techniques. Both are based on transmitting light through the sample and measuring the fraction of light absorbed. Mathematically, the measurement can be expressed as:

\[ T(\lambda) = \frac{P(\lambda)}{P_0(\lambda)} \]

In Eq. 1, \( T \) is termed the transmittance, \( P \) denotes the light intensity transmitted through the sample, and \( P_0 \) is the light intensity incident on the sample. The \( P_0 \) value is measured by use of a blank solution that contains the sample matrix but contains no analyte. The values \( T \), \( P \), and \( P_0 \) are all a function of \( \lambda \), the wavelength of the light. The measured transmittance can be related to the analyte concentration through the Beer-Lambert law:

\[ A(\lambda) = -\log_{10}(T(\lambda)) = \varepsilon bc \]

In Eq. 2, \( A \) is termed the absorbance, \( c \) is the analyte concentration, and \( \varepsilon \) (absorptivity) and \( b \) (optical path length) are constants that define the linear relationship between \( A \) and \( c \). Absorptivity is an intrinsic property of the analyte, and the path length is a characteristic of the instrumental measurement. The value of \( \varepsilon b \) is determined by a calibration procedure that employs a set of known standards that bracket the desired concentration range.

Spectroscopic measurements are most easily performed with liquid samples. To convert the insoluble Fe₂O₃ in the unknown to a soluble species, an acid digestion will be performed. This will yield a solution of Fe³⁺. The two spectroscopic methods used here are: (1) an atomic spectroscopy measurement that is able to determine the iron directly in the liquid sample and (2) a molecular spectroscopy procedure that is based on complexing the iron with an organic ligand and measuring the absorbance of the resulting complex.

Atomic Absorption Spectroscopy

Atomic absorption spectroscopy (AAS) is a widely used technique for determining a large number of metals. In the most common implementation of AAS, a liquid sample containing the metal analyte is aspirated into an air-acetylene flame, causing evaporation of the solvent and vaporization of the free metal atoms. This process is called atomization. A line source (hollow cathode lamp) operating in the UV-visible spectral region is used to cause electronic excitation of the metal atoms, and the absorbance is measured with a conventional UV-visible dispersive spectrometer with photomultiplier detector. Figure 1 is a typical instrument block diagram. The light beam passes through the cloud of atomic vapor formed in the flame. The narrow spectral lines of gas-phase atomic samples necessitate the use of a line source as well as a high-resolution monochromator (i.e., a spectrometer capable of isolating a very narrow (e.g., < 1 nm) wavelength range). This helps to prevent interference from adjacent spectral lines of other atomic species present in the sample matrix. This allows a metal species to be analyzed directly in a complex sample without the need for performing separations. In this experiment, AAS in conjunction with flame atomization will be used to determine iron in the solution obtained through digestion of the unknown.
Molecular UV-Visible Spectroscopy

Molecular UV-visible spectroscopy is also typically performed by use of a transmittance measurement with a liquid sample. In this case, a molecular species in the solution is measured, so there is no need to perform the atomization step described above. Two key issues must be resolved, however. First, the analyte must either absorb strongly (i.e., have a large value of $\varepsilon$) in the UV-visible spectral region or must be made to absorb there through the use of a derivatization reaction. Second, the absorption must be located at a wavelength that does not overlap with absorptions from other species in the sample matrix. This is a larger concern in molecular spectroscopy than in atomic spectroscopy because the molecular absorption bands are much wider than the narrow spectral lines observed with gas-phase atoms. This also may motivate the use of a derivatization reaction in order to create a species whose absorption falls in a region remote from any interfering absorptions.

In this experiment, a complexation reaction is performed between ferrous iron and 1,10-phenanthroline to form an orange-colored complex that can serve as the basis for a sensitive and selective measurement. The absorptivity of the complex, $[(C_{12}H_8N_2)_2Fe]^{2+}$, is 11,100 L·mole$^{-1}$·cm$^{-1}$ at a wavelength of 508 nm. The intensity of the color is independent of pH in the range 2 to 9. The complex is very stable and the color intensity does not change appreciably over time. The Beer-Lambert Law is obeyed over a wide concentration range.

One complication is that the iron must be in the ferrous state, while most iron occurs naturally in the ferric state. Thus, a reducing agent must be added to the sample to convert $Fe^{3+}$
to Fe\textsuperscript{2+} before the complex is formed. Hydroxylamine, as its hydrochloride, can be used for this purpose:

\[
2\text{Fe}^{3+} + 2\text{NH}_2\text{OH} + 2\text{OH}^- \rightarrow 2\text{Fe}^{2+} + \text{N}_2 + 4\text{H}_2\text{O}
\]

The pH is adjusted to a value between 6 and 9 by addition of acetate ion.

**Calibration Design**

In this experiment, measurements with both instruments will be calibrated by use of prepared (known) standards and the assumption of a linear calibration model between absorbance (y-axis) and concentration (x-axis, units of ppm = mg/L). To ensure that the Beer-Lambert law holds, it is desirable to have absorbances $\leq 1.0$. Deviations from linearity can occur when the absorbance falls outside this range. Techniques of error propagation can be used to define an absorbance of ~0.4 as optimal in terms of the corresponding minimum relative error of concentration. Ideally, one wishes to prepare the unknown sample such that it produces an absorbance close to 0.4. With liquid samples, if enough analyte is present and the method has sufficient sensitivity, this can often be accomplished simply by adjusting the dilution scheme used.

Calibration models have highest precision in the middle of the concentration range spanned by the calibration standards. Thus, it is most desirable to design the calibration such that the unknown concentration falls somewhere in the middle of this range. Good calibration design thus requires some knowledge about the range of concentrations expected for the unknown. In this experiment, a preliminary evaluation of the unknown will be made in the first laboratory period to provide information for use in selecting the concentrations of the calibration standards. The final calibration will then be performed during the second laboratory session.

**II. REAGENTS**

The following reagents will be provided:

- 100 ppm Fe standard
- Reagent-grade water (Note: use only the water provided by the instructor)
- 2% (v/v) nitric acid
- Unknown solid sample
- 0.1% (w/w) 1,10-phenanthroline monohydrate
- 10% (w/w) hydroxylamine hydrochloride
- 0.01 M, pH 4 acetate buffer
- 50% (w/w) sodium hydroxide (NaOH)

**III. PROCEDURE**

**First Laboratory Period**

*Note: it is important that pipettes and volumetric flasks be very clean to prevent contamination by trace amounts of iron.*

A. Preparation of Unknown. The instructor will provide a solid unknown. Digest your sample by dissolving approximately 0.1 g (weigh accurately) in 10 mL of nitric acid (conc.), to which you add 30 mL of hydrochloric acid (conc.) in a beaker. Use a graduated cylinder to
deliver the correct acid volumes. **Do this in the fume hood.** Warm the beaker on a hotplate with a watch glass placed on top. **Watch carefully as the reaction may become violent; turn the heat down if necessary.** Once the digestion is completed, there should be no solid at the bottom of the beaker. Let the mixture cool and then add it slowly to a 100 mL volumetric flask containing approximately 30 mL of reagent-grade water. Rinse the beaker several times with reagent-grade water to ensure that all material is transferred. Dilute to the mark with reagent-grade water and mix well. Transfer the unknown solution to a plastic bottle. **Save this solution.**

B. **Investigation of Instrumental Parameters and Calibration Design.** Separate procedures are described below for molecular spectroscopy and atomic spectroscopy. Groups 1 and 2 should begin with molecular spectroscopy and Groups 3 and 4 should begin with atomic spectroscopy.

**Molecular Spectroscopy.**

1. Prepare the following solutions:
   
   1. **5 ppm iron standard.** Pipette 5 mL of the provided 100 ppm standard into a 100 mL volumetric flask. Add 1 mL of the hydroxylamine solution, 10 mL of the 1,10-phenanthroline solution, and 8 mL of the acetate buffer. These volumes can be delivered with a graduated cylinder. Dilute the solution to the mark with reagent-grade water and allow it to stand for 10 minutes.
   
   2. **Test solution of unknown.** In a 100 mL volumetric flask, pipette 5 mL of your unknown solution prepared in Part A, 2 mL of 50% (w/w) NaOH, 1 mL of the hydroxylamine solution, 10 mL of the 1,10-phenanthroline solution, and 8 mL of the acetate buffer solution. Dilute the solution to the mark with reagent-grade water and allow it to stand for 10 minutes.
   
   3. **Matrix blank.** Add 1 mL of the hydroxylamine solution, 10 mL of the 1,10-phenanthroline solution, and 8 mL of the acetate buffer to a 100 mL volumetric flask and dilute to the mark with reagent-grade water.

2. The instructor will review the operating procedures of the UV-visible spectrometer with you.

3. Using the matrix blank as the spectral background (P_0 in Eq. 1), collect spectra of the 5 ppm iron standard and the test solution of the unknown. Identify the wavelength of maximum absorbance (λ_max). Record the absorbance at this wavelength for both solutions.

**Atomic Spectroscopy**

1. Prepare the following solutions:
   
   a. **5 ppm iron standard.** Pipette 5 mL of the provided 100 ppm iron standard into a 100 mL volumetric flask and dilute to the mark with 2% nitric acid.
   
   b. **Test solution of unknown.** Pipette 10 mL of the unknown solution prepared in Part A into a 100 mL volumetric flask and dilute to the mark with 2% nitric acid.

2. Appendix 1 provides instructions for operating the atomic absorption spectrometer. The
instructor will review these procedures with you.

3. Using 2% nitric acid as the blank, record the absorbance of the two prepared solutions.

C. Final Calibration Design. (This step can be performed outside of class.) Separate procedures are required for the atomic and molecular spectroscopy measurements because of differences in sensitivity between the two methods.

**Molecular Spectroscopy**

The absorbance value obtained for the prepared unknown solution can be used to estimate an aliquot volume that will produce an absorbance of ~0.4. For this calculation, assume a linear relationship between absorbance and concentration. For example, if the 5 mL aliquot produced an absorbance of 0.2, assume that a 10 mL aliquot will increase the absorbance to 0.4. Round this volume such that it can be delivered with the available volumetric pipettes (0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 mL) in one transfer.

Use the absorbance obtained with the 5 ppm iron solution to design a set of four calibration standards that bracket the unknown and produce absorbance values that approximately span the range of 0.1 to 1.0. Try to achieve an even spacing between the concentrations. Again, round aliquot volumes of the 100 ppm iron standard to fit the available pipettes.

An additional complication arises from the fact that a chemical reaction is being performed and thus appropriate amounts of reagents must be present. Iron must be the limiting reagent in the reaction. For the amount of 1,10-phenanthroline added (10 mL of 0.1% (w/w)), the iron concentration must not exceed 9 ppm. If your design requires that one of the standards exceeds 9 ppm, the amount of 1,10-phenanthroline must be increased. If this is done, be sure to use the same amount of 1,10-phenanthroline in all solutions and in the matrix blank.

**Atomic Spectroscopy**

The recommended linear range of response for atomic spectroscopic measurements of iron is 2.5 to 10 ppm. Design a set of four standards that span this range with even concentration spacing. Round aliquot volumes of the 100 ppm iron standard to fit the available pipettes. Use the results from the 5 ppm iron standard to predict the absorbance range corresponding to 2.5 to 10 ppm iron. Use this information together with the measured absorbance of your unknown solution based on a 10 mL aliquot to select an aliquot volume that will produce an absorbance that you would predict to fall in the middle of the 2.5 to 10 ppm calibration range.

--- End of First Laboratory Period ---

**Second Laboratory Period**

*Note: it is important that pipettes and volumetric flasks be very clean to prevent contamination by trace amounts of iron.*

D. Calibration Design Check. Have the instructor verify your calibration design developed in Part C above.
E. Preparation of Solutions. Separate procedures are described below for atomic and molecular spectroscopy. Groups 1 and 2 should begin with molecular spectroscopy and Groups 3 and 4 should begin with atomic spectroscopy.

*Molecular Spectroscopy*

On the basis of your calibration design, use the 100 ppm iron standard to prepare four calibration standards. Use 100 mL volumetric flasks and add 1 mL of the hydroxylamine solution, 10 mL of the 1,10-phenanthroline solution, and 8 mL of the acetate buffer to each flask. Dilute to the mark with reagent-grade water. If your calibration design requires the iron concentration to exceed 9 ppm, adjust the amount of 1,10-phenanthroline accordingly.

Prepare the dilution of your unknown solution according to your calibration design. Pipette the calculated aliquot of the unknown into a 100 mL volumetric flask. To neutralize the large amount of acid present in the unknown, add a volume of 50% (w/w) NaOH according to the following formula:

\[
\text{[mL 50\% (w/w) NaOH]} = 0.4176 \times \text{[mL aliquot of unknown]}
\]

Round the volume up to the nearest mL. This calculation is based on the molarity of the NaOH solution and the moles of acid introduced through the use of 10 mL HNO₃ and 30 mL HCl in the digestion of the unknown. Add 1 mL of the hydroxylamine solution, 10 mL of the 1,10-phenanthroline solution, 8 mL of the acetate buffer, and dilute to the mark with reagent-grade water.

*Atomic Spectroscopy*

Use the 100 ppm iron standard to prepare four calibration standards according to your design. Use 100 mL volumetric flasks and dilute to the mark with 2% (v/v) nitric acid.

Prepare your unknown according to your design. Deliver the computed aliquot of your unknown solution to a 100 mL volumetric flask and dilute to the mark with 2% (v/v) nitric acid.

F. Measurements. Using the same procedures developed during the first laboratory period, obtain three replicate absorbance values for each calibration standard and for the unknown. Use the appropriate spectroscopic blanks. For the molecular spectroscopy procedure, measure each standard and the unknown once, and then cycle through the solutions two more times to obtain a total of three measurements for each solution. For the atomic spectroscopy procedure, aspirate each standard and the unknown once and then cycle through the solutions two more times to produce a total of three readings.

– End of Second Laboratory Period –

IV. CALCULATIONS

1. Summarize the calculations that produced your calibration design.

2. Calculate the exact concentrations of your calibration standards. Be sure to use the appropriate number of significant figures.

3. Tabulate the mean, standard deviation, and 95% confidence interval for your absorbance
data. Report values to the correct number of significant figures.

4. Make calibration models for each set of data. Use least-squares procedures to determine the slope and intercept of each calibration model. Report the value of $r^2$ and the standard error of estimate.

4. Include separate plots of absorbance (with error bars) vs. concentration (ppm) for each calibration model.

5. Use your calibration model together with your dilution scheme to compute the weight percent of iron in your original solid sample. Report as weight percent Fe$_2$O$_3$. Predict each replicate of the unknown separately, then compute a mean, standard deviation, and 95% confidence interval for the predicted amount. Repeat this calculation for both calibration models.

6. The limit of detection associated with each calibration can be estimated by use of the following procedure. The intercept of the calibration model is an estimate of the absorbance when no metal is present in the sample (i.e., the blank signal). The standard error of estimate is an estimate of the measurement noise (i.e., in the absence of systematic error, the variation about the least-squares line should be due solely to instrumental noise). A widely used definition of limit of detection is the concentration corresponding to an absorbance value equal to the blank signal plus three times the noise level. Thus,

$$A_{LOD} = b + 3s$$  \hspace{1cm} (3)

where $A_{LOD}$ is the absorbance corresponding to the limit of detection, $b$ is the intercept of the calibration model, and $s$ is the computed standard error of estimate. The limit of detection, $C_{LOD}$, can then be estimated as

$$C_{LOD} = (A_{LOD} - b)/m$$  \hspace{1cm} (4)

where $A_{LOD}$ are as defined above and $m$ is the computed least-squares slope of the calibration model. Equations 3 and 4 can be combined to yield

$$C_{LOD} = 3s/m$$  \hspace{1cm} (5)

Use your calibration data to estimate the limit of detection for each method.

7. In your discussion section, compare the atomic and molecular spectroscopic methods.
V. APPENDIX 1

Operating Instructions for Perkin-Elmer AAnalyst 300 Atomic Absorption Spectrometer

Using the Instrument for Flame experiment:

- Turn on the computer
- Turn on the instrument; switch **is on the right hand side**. Do not turn on the furnace (left hand side).
- Turn on the fan switch (on the wall behind the computer).
- Click on AA Winlab.
  - Software will check for connection.
- Make sure the technique says Flame in the bottom left corner.
- Click on “Use custom design workspace”. Choose Chem325.flm
  - 3 windows will appear: Results, Peaks, and Manual Analysis
- In the toolbar click on Lamps
  - Select the lamp you want to use by clicking in the setup column. A green light will appear.
    - Note: Cu and Zn are on the same lamp, so you must simply type the element you want to analyze. The software will change the wavelength and slit width accordingly.
- Close the lamp window.
- Make sure the method you are using corresponds to the element you want to analyze. (Top right corner) If not, click on method and select the appropriate method. All names are in the format: Ch325_ element.
- Click on the flame icon in the toolbar. **Make sure the aspiration tube is in reagent-grade water.**
- Open the AIR tank
- Open the ACETYLENE tank
  - Order for tank is not so important with this instrument because it has interlocks, but it is always a good habit to open oxidant first and then fuel. This reduces the possibility of explosions in instruments without interlocks.
- When interlocks turns green, turn flame ON.
  - The instrument has an igniter incorporated.

Analysis

- Place the aspiration tube in your blank.
- Press **Analyze Blank**
  - This will measure the absorbance of your blank and then automatically autozero the reading. This has to be done before each different element, but not in between samples analyzed for the same element.
- In the Manual Analysis window fill in:
  - Sample ID
  - Results data Set Name : Click browse and Type the name of your data file in Result Name.
    - Use format name325 then write a description so we know what can be erased later.
  - Check the Save Data box. Do not check the Print Log box.
  - Leave Sample Information file as “Untitled”
- Place the aspiration tube in your sample and press **Analyze Sample**.
The method is set so that a triplicate measurement of your data is acquired, leave the tube in your sample until the instrument is done and the result in the result window shows a mean value.

- Repeat as often as necessary, make sure to use an informative sample ID, so that you know which sample it was when you go back to write your report.

- If you wish to make measurements with more than one element, repeat the same procedure.
  - Change the Lamp.
  - Change the method.
  - Start data acquisition by running a blank.

- When you are finished with all your measurements:
  - Place the aspiration tube in distilled water, leave flame on for a minimum of 5 minutes.

- Collecting data in an Excel file:
  - Go to File, Utilities, Reformat.
  - Click Open Design, choose Chem325.
  - Browse to find your Data Set Name.
  - Click **Save Results**.
  - Your file is now in the chem325 folder on the desktop.

- Shut Down Procedure
  - Click Flame Icon.
  - Turn Flame off.
  - Wait for shutdown procedure to be completed.
  - Turn ACETYLENE off at the cylinder.
  - Turn AIR off at the cylinder.
  - Press Bleed Gases.
  - Turn off spectrometer.
  - Shut down Software.
  - Wait 5 minutes before turning fan off.