Spectrophotometric Determination of Trace Iron in Solution

Measure the concentration of iron using a calibration curve

Introduction

Background

The ability to measure the concentration of iron in aqueous solutions in a quick and efficient way is important to many industries. Manufacturing industries where metal parts need to be cleaned may need to determine the level of iron in their waste streams for environmental compliance. Governments at all levels have an interest in testing wastewater, natural waters, and drinking waters to determine iron content to ensure compliance with the law and to ensure the safety of the water supply for wildlife and the human population.

Well-equipped, modern laboratories may perform iron content analysis by atomic emission spectroscopy in an inductively coupled plasma (ICP) spectrometer. Flame atomic absorption spectrometry also can be used, but iron solutions are notorious for clogging the burner with iron oxide when the concentration exceeds a certain level. Both of these techniques require a significant investment in instrumentation and a sustained laboratory infrastructure involving compressed gases and control of exhaust vapors. Fortunately, the solution chemistry underlying a colorimetric determination of iron content is simple enough to be reduced to kit form and performed in the field with hand-held equipment or in the lab with a low-cost visible spectrophotometer and simple glassware.

Preparing the colored iron complex

The colorimetric determination of iron content involves the measurement of the ferrous ion (Fe^{2+}) when it forms a complex with three molecules of 1,10-phenanthroline, also called *ortho*-phenanthroline or abbreviated as phen.

The chemical structure and numbering scheme for 1,10-phenanthroline is shown in Figure 1.

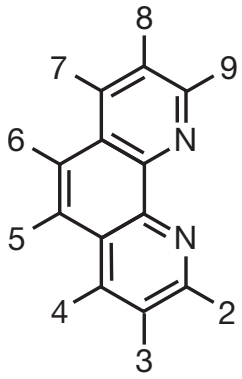

Figure 1. Numbering scheme for positions in 1,10-phenanthroline

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The complex formed by 1,10-phenanthroline and Fe^{2+}, ferrous tris(1,10-phenanthroline)iron(II) or $[Fe(phen)_3]^{2+}$, is a bright orange color. A 3D model of the structure of the complex is shown in Figure 2.

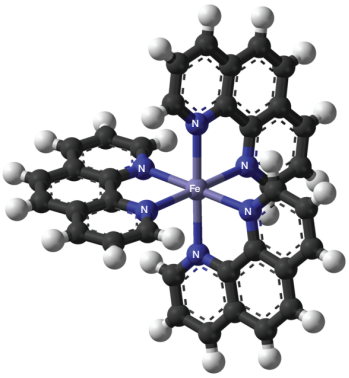

Figure 2. 3D model of the $[Fe(phen)_3]^{2+}$ complex

In addition to its Fe^{2+} (ferrous ion) form, iron also can exist in a Fe^{3+} (ferric ion) form. To perform a *total iron* measurement, it is essential to *reduce* any Fe^{3+} in solution to Fe^{2+} before adding the phenanthroline to form the complex. The chosen reducing agent in this experimental protocol is hydroxylamine hydrochloride, which reacts with Fe^{3+} by *Reaction 1*:

$$2Fe^{3+}_{(aq)} + 2NH_3OH^+_{(aq)} \rightarrow 2Fe^{2+}_{(aq)} + N_{2\,(g)} + 2H_2O_{(l)} + 4H^+_{(aq)} \quad \textit{Reaction 1}$$

Upon adding the phenanthroline, *Reaction 2* occurs:

$$Fe^{2+} + 3\ phen \rightarrow [Fe(phen)_3]^{2+} \quad \textit{Reaction 2}$$

Using Beer's Law to determine the concentration of an unknown

To determine the concentration of iron in an unknown solution, we must first *calibrate* the method with the spectrophotometer using Beer's Law:

$$A = \varepsilon bc \quad \text{Beer's Law}$$

where **A** = the absorbance reported by the spectrophotometer

ε = the extinction coefficient, a value that describes how strongly the particular compound absorbs photons at the particular wavelength, typically with units of ($L \cdot cm^{-1} \cdot mol^{-1}$)

b = the pathlength of the cuvette in cm, where typically a 1 cm pathlength cuvette is used

c = the concentration of the solution in mol/L ($mol \cdot L^{-1}$)

A *Beer's Law plot* can be constructed by preparing a series of solutions of known concentration and graphing the absorbance of each solution on the y-axis versus concentration on the x-axis:

Comparing the equation for Beer's Law to the plot, we see that the slope of the line is equal to ε. We can use the plot

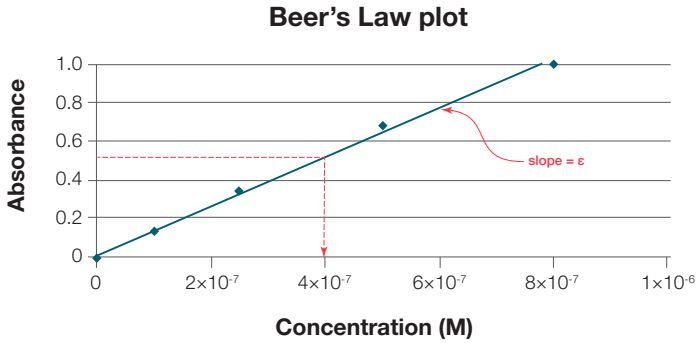

Figure 3. Example of a generic Beer's Law Plot

to calculate the concentration of an unknown solution in one of two ways:

1. Use the plot itself to select the point on the y-axis representing the measured absorbance. Trace a horizontal line from that point to the plot line, then draw a vertical line straight down to the concentration on the x-axis. The point where this intersects the x-axis represents the concentration of the unknown solution.

2. Use the equation of the line. If A = εbc and b=1, then c = A/ε. Use a spreadsheet program or a graphing calculator to plot your data and determine a best-fit line (trend line) to calculate the slope of your line. This slope equals ε. Divide the measured A value for the unknown by ε to calculate the concentration of the unknown solution.

Experimental

Purpose

In this experiment, you will perform an analysis of an iron-containing solution with an unknown concentration by reducing all the iron in solution to its ferrous form, determining λ_{max}, and creating a standard curve of absorbance versus concentration to calculate the concentration of the unknown solution.

Procedure

Making a measurement with the Thermo Scientific™ SPECTRONIC™ 200 Visible (Vis) Spectrophotometer*

1. Turn on the instrument and allow it to complete its startup sequence. Let the instrument warm up and stabilize for at least 30 minutes. Set up the experiment you want to perform in the spectrophotometer software. Obtain a square plastic cuvette or glass test tube to use in your experiments. If using a test tube cuvette, use a pen to place a mark near the top if the cuvette is not already marked with a white line. The mark allows you to ensure consistent placement into the instrument.

2. Add liquid to the cuvette until there is ~3 cm of liquid in the bottom (4 cm for test tubes). If plastic transfer pipettes are available, use one. The exact liquid level in the cuvette is not critical for good measurements as long as it is above 3 cm. Do not waste solution or risk spills by over-filling the cuvette.

3. Place the cuvette in the sample stage of the SPECTRONIC 200 Visible Spectrophotometer. If using a plastic cuvette, the clear sides should be on the right and left. If using a test tube cuvette, place it so that the mark faces to the right.

4. After the warm-up period, follow steps 2 and 3 using water or the appropriate "blank" solvent. Zero the instrument by pressing the autozero button.

5. For each subsequent measurement, empty and rinse your cuvette, shaking out as much of the rinse solvent as possible. When preparing samples, never return excess solution to the stock bottle. Pour all waste or excess into the appropriate waste receptacle. Follow steps 2 and 3 using your sample.

SPECTRONIC 200 Visible Spectrophotometer

*SPECTRONIC 200 Spectrophotometers are available on loan from Thermo Fisher Scientific™ at no cost. We will ship it to you, and you ship it back after one week. If you are interested in this program, please visit: www.thermofisher.com/spec200freetrial

Reagents

- Ammonium iron(II) sulfate, hexahydrate
- Hydroxylamine hydrochloride
- 1,10-phenanthroline
- Sodium acetate

Part 1. Prepare the reagents and standards

1. Prepare solutions prior to beginning this experiment, using deionized water for all dilutions.
   - **100 mg/L iron solution:** Dissolve 0.7022 g of ammonium iron(II) sulfate, hexahydrate in water in a 1 L volumetric flask.
   - **10 mg/L iron working solution:** Pipet 5 mL of the 100 mg/L iron solution into a 50 mL volumetric flask and fill to the mark with water.
   - **0.3 M hydroxylamine hydrochloride solution**
   - **0.25 % 1,10-phenanthroline solution:** Stir the solution to ensure that all solids have dissolved, using heat if necessary.
   - **1.0 M sodium acetate solution**

2. Set up six 50 mL volumetric flasks and pipette reagents into them as follows:

Flask no.	10 mg/L iron solution (mL)	0.3 M hydroxylamine hydrochloride solution (mL)
1	0.0	1.0
2	2.0	1.0
3	5.0	1.0
4	8.0	1.0
5	14.0	1.0
6	20.0	1.0

   Stopper each flask, then invert repeatedly for 2 minutes to allow the reaction to complete.

3. To each flask, add:
   - 5.0 mL of 1.0 M sodium acetate solution. Stopper and invert the flask to mix.
   - 5.0 mL of 0.25% 1,10-phenanthroline solution. Stopper and invert the flask to mix.
   - Add deionized water to the mark. Stopper and invert several times to mix.

Part 2. Determine the proper analytical wavelength

1. Pipet 3 mL of the solution from Flask 1 into a cuvette. This solution will be your blank.
2. Wipe the outside faces of the cuvette with a laboratory tissue and place the cuvette into the square cuvette stage of the SPECTRONIC 200 Spectrophotomer sample compartment with the clear faces pointing to the left and right.
3. Close the lid of the SPECTRONIC 200 Spectrophotometer. Set up a scan from 400 nm to 900 nm in ABS mode and press the 0.00 button to record a baseline. Wait until the hourglass icon disappears, which indicates the scan has completed.
4. Open the lid, remove the cuvette with the blank solution, and set it aside. If you only have one cuvette, discard the blank solution and rinse the cuvette with deionized water before reusing it.
5. Pipet 3 mL of the solution from Flask 6 into a cuvette. Wipe the outside faces of the cuvette with a laboratory tissue and place the cuvette into the square cuvette stage of the SPECTRONIC 200 Spectrophotometer sample compartment with the clear faces pointing to the left and right. Close the lid and press the round Enter button to record the scan.
6. When the scan appears on the screen, use the λ knob to move the green cursor line to the highest point on the peak. The left and right arrow keys can also be used. Record the wavelength of maximum absorption, known as λ_{max}.
7. Print the screen if your SPECTRONIC 200 Spectrophotometer is equipped with a printer.

Part 3. Prepare the Beer's Law plot

1. Calculate the concentration of iron in each of Flasks 1 through 6 and enter the values in the Lab Report.
2. Set the SPECTRONIC 200 Spectrophotometer to Live Display Mode with measurements in Absorbance at λ_{max} as determined in Part 2.
3. Follow the same directions for filling, wiping and orienting a cuvette given in Part 2, using a cuvette with blank solution from Flask 1 to record a blank value.
4. Prepare and measure the absorbance of cuvettes containing the solutions from Flasks 2 through 6. Record the values in Data Table 1 in your Lab Report.
5. Make a Beer's Law plot with your data. Determine the slope of the line and record this value as ε in your Lab Report.

Part 4. Determine the iron concentration in an unknown sample

1. Clean out one of the volumetric flasks.
2. Pipet 5.0 mL of an unknown iron solution into the flask. Follow the procedure used in Part 1, steps 2 and 3, to prepare the solution for measurement.
3. Record the absorbance of the solution at λ_{max} using the SPECTRONIC 200 Spectrophotometer.
4. Determine the concentration of iron in the solution mathematically using the value that you calculated for ε and the Beer's Law equation.
5. Remember to include the effect of diluting 5 mL of the unknown to 50 mL before you made your measurement when you calculate the concentration of the unknown.

Disposal of chemicals:

Check with your instructor before discarding any solutions. All solutions can be poured down the sink and rinsed with lots of water to dilute. Discard solids in the trash.

Lab Report

Spectrophotometric Determination of Trace Iron in Solution

Name: _____

Date: _____

Section No. or Lab Period: _____

Questions

1. **What is the wavelength of maximum absorption for your iron standard solutions?**

λ_{max} = _____ nm

Data Table 1

Flask no.	Fe^{2+} concentration	Measured absorbance
1		
2		
3		
4		
5		
6		

2. **What is the extinction coefficient for your iron standard solutions?**

ε = _____

(don't forget to include the units)

3. **What is the measured absorbance of the unknown $[Fe^{2+}]$solution?**

A = _____

4. **What is the concentration of the unknown $[Fe^{2+}]$ solution?**

c = _____

(don't forget to include the units)

5. **What is the absolute error between your value for the unknown $[Fe^{2+}]$ solution and the true value obtained by your instructor?**

6. **Calculate the percent error from question 4.**

7. **Why was it necessary to add hydroxylamine and sodium acetate to the solution used to record the blank?**

8. **Many concentrations in this experiment were given in units of mg/L. Express 10 mg/L iron using units of mol/L.**

Remember:

- Staple hand-drawn or printed graphs to your lab report

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