Experiment 22: Spectrophotometric Determination of Iron in a Vitamin

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Purpose
You will use a spectrophotometer to determine the amount of iron in a multivitamin to see if the manufacturer's claim is correct.

Background
Iron itself is not a huge absorber of light, but when Fe^{2+} binds to phenanthroline, it forms a highly stable red/orange-colored species. By quantifying the color with a spectrophotometer, we can deduce the concentration of iron in the diluted sample solution and back-calculate the amount of iron in the original vitamin. This value can then be compared to the manufacturer's claim on the bottle.

The formation of the red/orange-colored iron-phenanthroline complex requires the iron to be in the Fe^{2+} form, and the procedure thus includes the reagent hydroxylamine hydrochloride that will reduce all iron in the sample to the Fe^{2+} form. Ammonium acetate is used to control the pH of the solution since this also affects the absorbance. The red/orange iron-phenanthroline complex absorbs light at 508 nm. To quantify the intensity of the color, you can use the spectrophotometer to measure the absorbance.

Iron standard solutions will be prepared and their absorbance of the 508 nm light measured. The known concentrations and absorbance values will be used to generate a calibration curve. The equation of the best-fit line on the calibration curve will be used to quantitate the iron in the known quality control solution and vitamin solution.

Chemicals

1.0 M ammonium acetate	1% hydroxylamine hydrochloride
0.30% o-phenanthroline	0.025 mg/mL $FeSO_4$ stock solution
1.0 M hydrochloric acid	Ferrous Ammonium Sulfate *(for QC check)*
Iron Vitamin tablet	

Equipment

Pipets, (4) 10 mL, (2) 1 mL	Beakers (5), 100 mL or so	Parafilm squares
50 mL vol. flask (4)	thermometer	Hotplate (use in the hood)
Test tubes, large	Test tubes, small	Spectrophotometer

Procedure

There are three parts to this experiment procedure:
A) The vitamin sample will be prepared for analysis. The solid sample must be dissolved, filtered, diluted, and chemically adjusted to produce a colored solution.
B) The known iron sample will be prepared for analysis as a way to check on the accuracy of the experiment (quality control). The solid sample must be dissolved, diluted, and chemically adjusted to produce a colored solution.
C) The standard iron solutions must be made from the stock solution and chemically adjusted to produce colored solutions.

Vitamin Sample Preparation

The vitamin sample preparation requires boiling the crushed iron vitamin in acid to release the iron, and filtering the solution to remove particulates that could interfere in the spectrophotometer measurements.

1) Obtain one vitamin tablet. Use the lab balance to find the mass of the tablet. Place the tablet on a tared piece of weighing paper.
2) Crush the tablet with a mortar and pestle until it is a fine powder.
3) Measure 0.050 g – 0.055 g of the vitamin powder onto a new piece of tared weighing paper. Record the exact mass and then transfer into a 100 mL beaker.
4) Add 15 mL of 1.0 M HCl solution to the beaker. Swirl to mix.
5) Heat the mixture on a hotplate in the fume hood. Swirl often. Do not leave your sample unattended. Use a thermometer to keep your sample at 70 – 80°C for approximately 10 minutes.
6) Bring your sample back to your lab bench and filter the sample directly into a 50 mL volumetric flask. Rinse the beaker with DI water several times to be sure to transfer all of the sample solution to the filter.
7) Dilute the sample to the graduation mark on the 50 mL flask, using DI water. Cover the flask with Parafilm and invert to mix (30 – 50 times).
8) Pipet **2.0 mL** of this solution into a new 50 mL volumetric flask. Dilute to the graduation mark with DI water. Cover with Parafilm and invert to mix, 30 – 50 times. ***This solution will be the vitamin solution you will use for the analysis.***

Known Iron Sample Preparation

The known iron sample requires the dissolving of the solid sample. Heating and filtering are not required.

1) Measure 0.080 g of the known iron sample onto a new piece of tared weighing paper. Transfer this mass into a 50 mL volumetric flask.
2) Add 15 mL of 1.0 M HCl solution to the volumetric flask. Swirl to mix and dissolve.
3) Dilute the sample to the 50 mL graduation mark on the flask, using DI water. Cover the flask with Parafilm and invert to mix (30 – 50 times).
4) Pipet **1.0 mL** of this solution into another 50 mL volumetric flask, and dilute to the graduation mark with DI water. Cover with Parafilm and invert to mix (30-50 times). ***This is the known iron sample solution that you will use for the analysis.***

Standard Solutions & Color Preparation for all Solutions

1. Label 8 large test tubes with the numbers 1 – 5, blank, Known Fe, and Vitamin.

2. Label 5 medium beakers with the names of the stock solutions: ammonium acetate, hydroxylamine HCl, o-phenanthroline, iron standard solution, and DI water. Fill these beakers with a slight excess of the needed volumes.

3. To each of the eight test tubes add 1 mL of each: ammonium acetate, hydroxylamine HCl, and o-phenanthroline.

4. To test tubes 1 – 5, add the volumes of iron stock solution and DI water indicated in Table 1. Record the volumes used in your notebook.

5. To the test tube labeled "Blank", do not add any iron standard, only the DI water volume indicated in Table 1.

6. To the test tube labeled Known Fe, use 5.0 mL of the diluted Known Fe solution prepared in step 4 of the **Known Iron Sample Preparation** directions. Add 2.0 mL of DI water as indicated in Table 1.

7. To the test tube labeled Vitamin, use 5.0 mL of the diluted vitamin solution prepared in step 8 of the **Vitamin Sample Preparation** directions. Add 2.0 mL of DI water as indicated in Table 1.

6. Cover the test tubes with Parafilm and mix well. Let the test tubes sit for 10 minutes.

Table 1. *(volumes are in mL)*

Standard	**Blank**	**1**	**2**	**3**	**4**	**5**	**Known Sample**	**Vitamin**
Vol. Na acetate	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Vol. hydroxyam HCl	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Vol. o-Phen	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Vol. Fe stock added (0.025 mg/mL)	0	0.30	0.50	1.0	1.5	2.0	Vol Known Fe soln 5.0	Vol Vitamin soln 5.0
Vol. DI H_2O	7.0	6.7	6.5	6.0	5.5	5.0	2.0	2.0
Total vol. in test tube	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Conc. Fe mg/L In the standard solutions	0	Calculate using the dilution equation: Mc * Vc = Md * Vd, solve for Md					????	????

Spectrophotometric Analysis

1. Get 8 small test tubes. Label each small test tube, and transfer some of each solution from its large test tube to its corresponding small test tube.

2. Set the wavelength on the spectrophotometer to 508 nm. Use the blank solution to zero the spectrophotometer.

3. Measure the absorbance of each solution in the remaining test tubes. Record the absorbances in your notebook.

Data Analysis

The absorbance measurements of the standard solutions are used to prepare a calibration curve. The equation of the best-fit line of the calibration curve is used to determine the concentration of iron in the diluted Vitamin solution and the diluted Known Fe solution. These results are then used to determine the amount of iron present in the original vitamin and the original Fe sample.

Follow these steps for your calculations of Fe in the Vitamin:

1. The concentration units for the calibration curve were mg/mL. So, the concentration of iron in the Vitamin solution is in units of mg/mL. You prepared 50 mL of the diluted solution, by using 2.0 mL of the digested solution. Reverse the sample preparation math to calculated the mg Fe in the original vitamin.

(mg/mL Fe from best-fit eqn.) (10 mL / 5 mL) (50 mL / 2.0 mL) (50 mL) = mg Fe

2. Before you can compare this value of mg Fe to the amount listed on the bottle, you have to consider that not all of the crushed pill was used to make the first solution. The mg of Fe determined are the mg in the crushed sample you put in the beaker.

(mg Fe) / (grams in beaker) = mg Fe / g used

3. Now you can calculate the mg of Fe in a whole tablet, which is how the Fe content is displayed on the bottle's label.

(mg Fe / g used) x (g of whole pill) = mg Fe in a whole pill

4. Calculate the % error for your result. This will give information as to how your results match up to the bottle label, rather than saying 'the results seem good' when you write your conclusion.

$$\% \text{ Error} = \frac{/ \text{ mass Fe label} - \text{mass Fe exp.} /}{\text{mass Fe label}} \times 100$$

Follow these steps for your calculations of Fe in the Known Iron Sample:

1. The concentration units for the calibration curve were mg/mL. So, the concentration of iron in the Known Iron solution is in units of mg/mL. Reverse the sample preparation math to calculated the mg Fe in the original known sample used to make the diluted solution that was put into the spectrophotometer.

(mg/mL Fe from best-fit eqn.) (10mL / 5mL) (50mL / 1mL) (50mL) = mg Fe in original sample used

2. Once you know the mg of Fe in the original sample used, calculate the %Fe as follows:

% Fe = (mg of Fe in original sample used) / (mg of original known sample used) x 100

3. Calculate the % error for your result. This will give information as to how your results match up to the known value, rather than saying 'the results seem good' when you write your conclusion.

$$\% \text{ Error} = \frac{/ \quad \% \text{ Fe True Value} - \% \text{ Fe exp.} \quad /}{\% \text{ Fe True Value}} \times 100$$