DETERMINATION OF AQUEOUS [Fe^{II}] & [Fe^{III}] with FERROZINE

Background

While the total concentrations of various metals in aqueous samples are typically measured with atomic absorption spectroscopy (AAS) or inductively coupled plasma mass spectrometry (ICP–MS), sometimes the detection of specific chemical oxidation states of a metal ion in water samples is required. For example, Cr(III) ingestion (poisoning) is much more serious than exposure to small amounts of Cr(VI). For the case of Fe(II) and Fe(III), Fe^{2+} and Fe^{3+} are absorbed by the body differently[1]. Additionally, remediation of iron waste in mine run-off water is handled differently depending on the oxidation state of the iron[2]. As such, determining not only the total iron concentration in water, but also the oxidation state of the iron in water is beneficial.

In order to determine the amounts of Fe^{II} or Fe^{III} in water, reagents must be found that react selectively with just Fe^{II} or just Fe^{III}. Ferrozine is one such reagent.
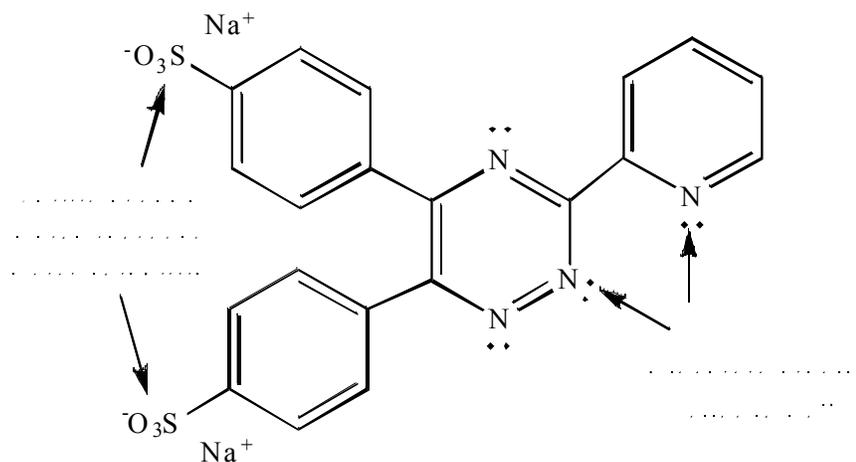

Figure 1. Ferrozine

Ferrozine is a bidentate ligand that binds to Fe^{II} (not Fe^{III}) in a 3:1 ratio (3 ferrozine molecules bind to one Fe^{II} center) to create the colored octahedral transition metal coordination compound, Na$_4$Fe(ferrozine)$_3$ (shown in Figure 2). As a bidentate ligand, ferrozine creates a more stable Fe^{2+} complex than will a monodentate ligand (e.g. SCN$^-$). This enhanced stability is known as the *chelate effect*. Also, the two sulfite (-SO$_3^-$) groups on each ferrozine keep the resulting complex soluble in water – an important asset when working on environmental or biological systems.

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[1] Hoffman R, Benz E, Shattil S, Furie B, Cohen H, Silberstein L, McGlave P. Hematology: Basic Principles and Practice, 3rd ed. Ch. 26: Disorders of Iron Metabolism: Iron deficiency and overload. Churchill Livingstone, Harcourt Brace & Co, New York, 2000

[2] Current Information on Mine Waste Treatment Technologies , Environmental Protection Agency, http://www.epa.gov/aml/tech/appenc.pdf, accessed, April 4, 2014
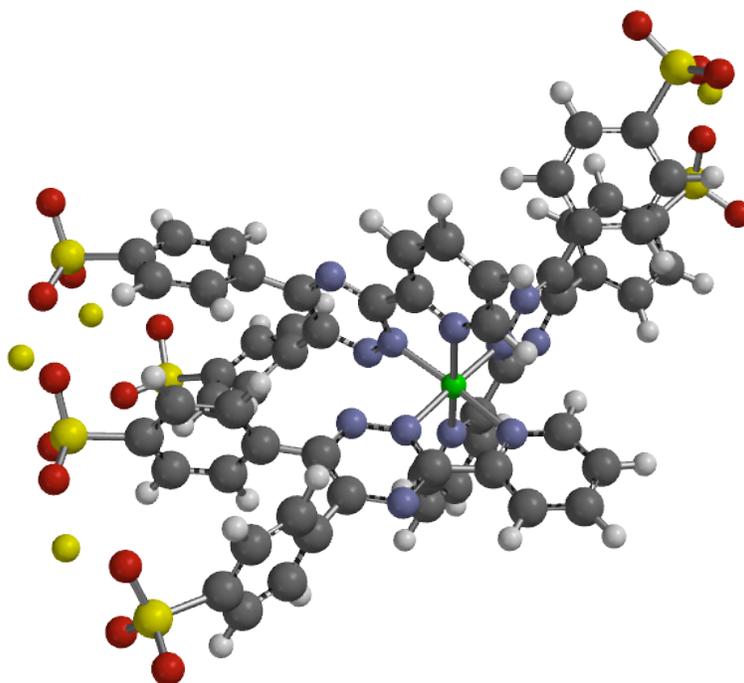
Figure 2. $Na_4Fe(ferrozine)_3$

Most importantly for this experiment, the formation of a colored complex allows for the determination of the iron concentration with **visible spectroscopy** (using Beer's Law). The procedure below is derived from a U.S. Geological Survey (USGS) protocol used to determine if dangerously high levels of Fe^{2+} and Fe^{3+} are being released from abandoned iron mines into nearby rivers and ground water.

Safety

Safety goggles and aprons must be worn at all times. All chemicals used are hazardous if ingested.

Procedure

A. Stock & Standard Solution Preparation.

1. *Fe^{II} stock solution 1* (Your TA will assign one group to make this for the entire class) – In a 50 mL volumetric flask, prepare a 5.0 mM ferrous ammonium sulfate hexahydrate $(Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O)$ solution. Before diluting to volume with deionized water, add 1.0 mL of 6 M HCl to the flask. (Acidification will prevent iron hydroxide from precipitating out of solution.)

2. *Fe^{II} stock solution 2* – In a 25 mL volumetric flask, dilute the solution above to 0.2 mM. Before diluting to volume with deionized water, add 40 mg ferrozine and 1.25 mL ammonium acetate buffer. Let the solution sit for 2 minutes and observe the color change. *This solution must be used within 30 minutes of its preparation to avoid precipitation of*

insoluble, red Fe^{III} oxide. If a considerable amount of solid forms, prepare another solution before moving forward.

3. *Fe^{II} standard solutions* – Using the 0.2 mM solution just created above, prepare four 25.0 mL solutions with the following concentrations of ferrous ammonium sulfate hexahydrate: 0.04 mM, 0.024 mM, 0.016 mM, and 0.008 mM.

B. Calibration Curve Creation.

1. Plug the spectrometer into your computer and calibrate with DI water. Pour the first standard solution mixture into a prerinsed cuvette and take the absorbance spectrum. Determine the λ_{max} you will use in this experiment and report its absorbance.

2. Take the absorbance spectrum of all remaining Fe^{II} standards. Create a calibration curve of absorbance (at λ_{max}) versus Fe^{II} concentration. Save the spectrum and calibration curve on your computer and print it out.

C. Molecular Weight Determination of Unknown Fe^{II} salt.

1. Obtain an unknown Fe^{II} salt from the stockroom, record the unknown number and mass it (0.050g or less).

2. Put the salt into a 25 mL volumetric flask and add 1.0 mL of 6 M HCl before diluting to volume with DI water.

3. Transfer 1.0 mL of the solution in previous step (C.2) to a 100 mL volumetric flask, add 1.25 mL buffer and 40 mg ferrozine and dilute to volume with DI water.

4. Take absorption spectrum of the solution in previous step (C.3) and record the absorbance at λ_{max}. If the absorbance is too high for your calibration curve, perform quantitative dilution(s) until the absorbance is in range. Once the absorbance is in range, take 2-3 measurements (with different aliquots of unknown solution).

5. Calculate the concentration of the unknown using the calibration curve created earlier (use the average of your 2-3 measurements). From the concentration and the known mass, calculate the molecular weight of your salt. Show all work and record the final number in your notebook.

   The five possible unknowns are: $FeSO_4 \cdot 7H_2O$, $FeCl_2 \cdot 4H_2O$, $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, $FeBr_2 \cdot 6H_2O$, and $FeI_2 \cdot 6H_2O$. Match the unknown you were given to one of these.

D. Determination of Fe^{III} Concentration Determination in an Aqueous Sample

1. Request a ferric ammonium sulfate dodecahydrate ($NH_4Fe(SO_4)_2 \cdot 12H_2O$) aqueous solution of unknown concentration from your TA. (Your TA will need to prepare this for you, as rust precipitates out of solution over time.)

2. Place 1.0 mL of the ferric ammonium sulfate dodecahydrate solution in a 25 mL volumetric flask. Add 40 mg ferrozine and 1.25 mL of ammonium acetate buffer. Then dilute volume with DI water. Take a visible spectrum of this solution.

3. Place another 1.0 mL of the original ferric ammonium sulfate dodecahydrate solution in a 25 mL volumetric flask. Add 1.5 mL hydroxylamine hydrochloride ($H_3NO \cdot HCl$, 10% w/v) (a reducing agent). Stir by swirling the flask for at least one minute. Add 40 mg of ferrozine and 1.25 mL of ammonium acetate buffer. Then dilute volume with DI water. Wait for 5 minutes, watching for a color change. Record changes in your notebook. Take a visible spectrum of this solution.

4. If the absorbance is outside of the range of your calibration curve, prepare a new solution with more or less ferric ammonium sulfate dodecahydrate solution, recording the exact amount used. Once the absorbance is in range, take 2-3 measurements (with different aliquots of unknown solution). Using this information, calculate the concentration of Fe^{III} according to your calibration curve (use the average of your 2-3 measurements) and calculate the concentration of the undiluted salt in the solution obtained in Step D.1.