Spectrophotometric determination of aqueous iron concentrations with the ligand: 1,10-phenanthroline.

First Draft

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Abstract

There is a necessity amongst aquatic chemists and the public sector alike for a simple and reproducible method for determining heavy metal concentrations in aqueous solutions. This paper explores a method for determining iron concentrations in solution through a novel reduction with hydroxylamine hydrochloride followed by a complexation with the ligand: 1,10-phenanthroline. This complex was measured by spectrophotometry. An absorbance band was produced with an absorbance maximum at 510nm. The molar absorptivity of the complex was calculated as 12570 $L \cdot mol^{-1}cm^{-1}$ using Beer's law. The concentration of iron in an unknown was then determined to be $1.60x10^{-5} \pm 0.2x10^{-5}$ M, with a relative standard deviation of 12.53%.

Introduction

UV-Vis Spectrometry. Ultraviolet-visible molecular absorption spectroscopy, commonly referred to as UV-Vis spectrometry, is an important analytical method for identifying and quantifying a large variety of chemical species [1]. In UV-Vis spectrometry, a sample is exposed to a spectrum of light ranging from approximately

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200-800nm in wavelength and the instrument records the molecular absorption at each wavelength versus a blank. The instrument used in this experiment is a Perkin-Elmer Lambda 40 UV/Vis spectrometer. This instrument utilizes two lamps, one halogen and one deuterium, and a monochromator to scan through the working UV-vis spectrum of wavelengths [2]. The light beam is split by a beam splitter and reflects through a series of mirrors before passing through a sample and a solvent blank simultaneously. The absorbance is then recorded by two separate detectors and the sample absorption less the blank absorption is plotted versus wavelength. This type of electronic spectroscopy, with the application of radiation in the visible to near visible spectrum, is commonly referred to as spectrophotometry.

Absorption and Charge Transfer. Absorption of light occurs when an electron is briefly excited to a higher energy state by a photon. In aqueous transition metal complexes, this excitation takes the form of a charge-transfer absorption [1]. In charge-transfer complexes, an electron is transferred from an electron donor group to an orbital with mostly electron acceptor character when radiation is absorbed. In most inorganic complexes, the transition metal ion acts as the electron acceptor, receiving an electron from the complexed ligand. However, in the Fe(II) complex studied in this experiment, an electron is transferred in an opposite fashion from the metal to the 1,10-phenanthroline ligand when radiation is absorbed.

Beer's Law. In order to make determinations from molecular absorption spectroscopic data, Beer's law, an equation relating absorption to sample concentration, must be used. Beer's law states that

$$Abs = \varepsilon c l \qquad (1)$$

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Where c is the concentration of an analyte, l is the pathlength of the sample cuvette, and ε is a scaling constant for a specific compound called the molar absorptivity with units of $L \bullet mol^{-1}cm^{-1}$ [1]. Using this relationship is it possible to determine the unknown concentration of an analyte in solution by plotting the absorption of standards versus their know concentrations to calculate ε. The absorption of a sample is then measured and that value combined with the calculated value of ε is entered back into the Beer's law equation to determine concentration. The standard deviation of the molar concentration can be calculated using the equation:

$$s = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}(x_i - \overline{x})^2}, \qquad (2)$$

The relative standard deviation, also known as the coefficient of variation can be calculated using the equation:

$$\text{RSD} = s/(\text{x bar}) \text{ x}100\% \qquad (3)$$

Iron-Phenathroline Chemistry. The reaction of Fe(II) with 1,10-phenanthroline to form the $[Fe(phen)_3]^{2+}$ complex was first used in 1931 by Walden, et al. as a titration indicator because of the strong red coloration of the complex [3]. The strong coloration of this complex allows for the determination of the concentration of $[Fe(phen)_3]^{2+}$ and subsequently, Fe(II) by absorption spectrophotometry. This method was first developed and performed by Fortune and Walden in 1938 [4].

In this experiment, the standards and unknowns are prepared in a solution of MilliQ water containing sodium acetate, hydroxylamine hydrochloride, and 1,10-phenanthroline. In order to accurately determine the concentration of aqueous iron, all of the iron in the sample must be in the Fe(II) form in order for the $[Fe(phen)_3]^{2+}$ complex to

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form with the 1,10-phenanthroline ligand [5]. The hydroxylamine hydrochloride is added to the solution to lower the pH and act as a reducing agent to prevent the oxidation of Fe(II) to Fe(III) [4]. The sodium acetate is added to the solution to act as a pH buffer to maintain the pH in a range where all of the aqueous iron will reside in the Fe^{2+} oxidation state. The intensity of color has been shown not to change with proton concentration within the pH range of 2.0-9.0 indicating that this is the acceptable experimental pH range [4]. 1,10-phenanthroline ($C_{12}H_8N_2$) is a bidentate ligand, commonly abbreviated "phen", which is able to bind to transition metals through each of the ligand's two nitrogen atoms. Three equivalents of phen form a six coordinate complex with iron in the 2+ oxidation state [6]. The Iron(II) complex is stabilized by the 1,10-phenanthroline ligand because it is a π-acceptor [7].

Applications of Spectrophotometry for Iron Determinations. In the field of chemical oceanography, the presence of areas of high nutrient concentrations such as nitrate and phosphate and lower than expected chlorophyll levels are widely accepted to exist. A prominent high nutrient, low chlorophyll (HNLC) region exists in the equatorial Pacific Ocean [8]. The low chlorophyll levels indicate reduced biodiversity and low abundance of microorganisms compared to expected values in areas of similar nutrient concentrations. For years, the reason for this scarcity was unknown. In the early 1980's, following a series of reproducible low iron concentration measurements in the equatorial Pacific, it was hypothesized that limitation of iron as a micronutrient could be to blame.

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Iron is an important micronutrient for phytoplankton photosynthesis. Iron limitation has been shown to impair photochemical energy conversion by decreasing the quantum efficiency of the photochemistry in Photosystem II (PSII) which contains two

iron atoms [9]. The iron limitation hypothesis was tested though mesoscale iron-seeding experiments in which large amounts of free iron were added to an HNLC region of the Pacific Ocean [10]. Following seeding of the water with iron, samples were taken over a time series and determinations of nutrient, chlorophyll, and iron concentrations were made. A large phytoplankton bloom was observed following the seeding showing a spike in chlorophyll concentrations accompanied by a rapid decrease in macronutrients and a slow decrease of iron concentrations. This pattern proved the hypothesis that iron is in fact the limiting factor in HNLC regions. Without a practical method for measuring aqueous iron concentrations, this hypothesis could not have been formed and the explanation of HNLC regions could still remain a mystery.

Spectrophotometry is used by public utility companies to measure heavy metal concentrations in ground and drinking water. Although excess iron in drinking water does not pose a significant human health hazard, the Environmental Protection Agency (EPA) suggests that public water utilities monitor and maintain iron levels <0.3 mg/L for aesthetic and taste purposes [11].

Objectives. The objective of this experiment is to develop an understanding of the spectrophotometric technique for determining heavy metal concentrations in aqueous solutions through the reaction of the metal with a π-acceptor ligand to form a charge-transfer complex. This method can then be applied to subsequent concentration determination experiments.

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Experimental

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Reagents. Ammonium iron(II) sulfate [Mohr's Salt, $(NH_4)_2Fe^{2+}(SO_4)_2 \bullet 6H_2O$], Sodium Acetate ($CH_3CO_2Na$), Hydroxylamine hydrochloride ($NH_2OH \bullet HCl$), 1,10-Phenanthroline ($C_{12}H_8N_2 \bullet H_20$).

Stock Solutions. Solutions of Ammonium iron(II) sulfate (4.85×10^{-4} M, 0.380g brought to 2000 mL with 5.0 mL conc. H_2SO_4 and MilliQ water), Sodium Acetate (1.22 M, 200.g brought to 2000 mL with MilliQ water), Hydroxylamine hydrochloride (1.49 M, 20.7g brought to 200mL with MilliQ water), and 1,10-Phenanthroline (5.12×10^{-3} M, 1.015g dissolved in 50mL ethanol and brought to 1000 mL with MilliQ water) were prepared by the stockroom prior to the beginning of the experiment.

Instrument. Perkin-Elmer Lambda 40 UV/Vis spectrometer.

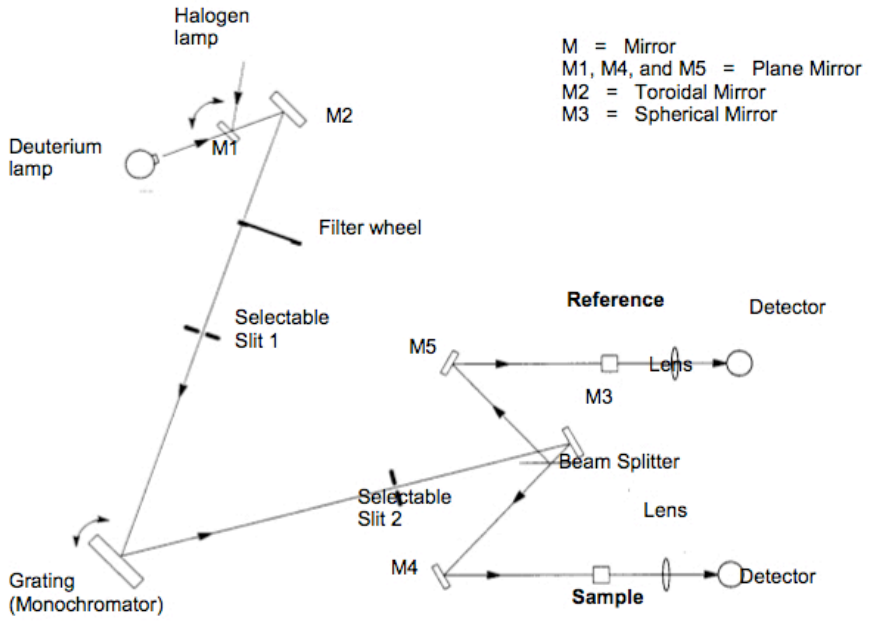

Figure 1. Schematic of Perkin-Elmer Lambda 40 UV/Vis spectrometer light path.

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Procedure. Four standards and one reference blank were prepared in 5—100 mL volumetric flasks. Into each flask was pipetted 10 mL sodium acetate solution, 1 mL hydroxylamine hydrochloride solution, 5 mL 1,10-phenanthroline solution and a varying amount of the Mohr's salt solution. To the four standards: 0.5, 3, 7, and 10 mL of Mohr's salt solution were added, with no Mohr's salt solution added to the blank. Each of the solutions were brought to 100 mL with MilliQ water. The blank was run on a UV-Vis spectrometer, followed by the four standard solutions. The wavelength of maximum absorbance and the absorbance at this value were recorded for each standard. Three solutions were then prepared containing 10 mL sodium acetate solution, 1 mL hydroxylamine hydrochloride solution, 5 mL 1,10-phenanthroline solution and 7 mL of an unknown iron solution. Each solution was run on a UV/Vis spectrometer and the

wavelength of maximum absorbance and the absorbance at this value were recorded for each solution.

Results and Discussion

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Table 1. Absorption data for standard solutions containing Fe(II).

Amount of Mohr's Salt solution added (mL)	Moles of Fe(II) in solution	Fe(II) molar Concentration	Absorbance	Wavelength of Maximum Absorbance (nm)
10	4.85E-06	4.85E-05 M	0.64	509.86
7	3.40E-06	3.40E-05 M	0.23	509.5
3	1.46E-06	1.46E-05 M	0.20	510.15
0.5	2.43E-07	2.43E-06 M	Below LOD	Below LOD

Color. The addition of Mohr's salt solution to each standard resulted in the appearance of a strong red color. The intensity of the color appeared proportional to the amount of Mohr's salt solution added. The linear relationship between color intensity and concentration permits the use of this method [4].

Absorption of standard solutions. Each standard solution was run on the UV-Vis spectrometer. Each standard produced an absorbance spectrum with a maximum absorption at a wavelength near 510nm. This closely compares to the literature value of 512nm for the wavelength of maximum absorbance for the $[Fe(phen)_3]^{2+}$ complex [12]. The standard with the lowest concentration of Mohr's salt had an absorption value below the limit of detection (LOD).

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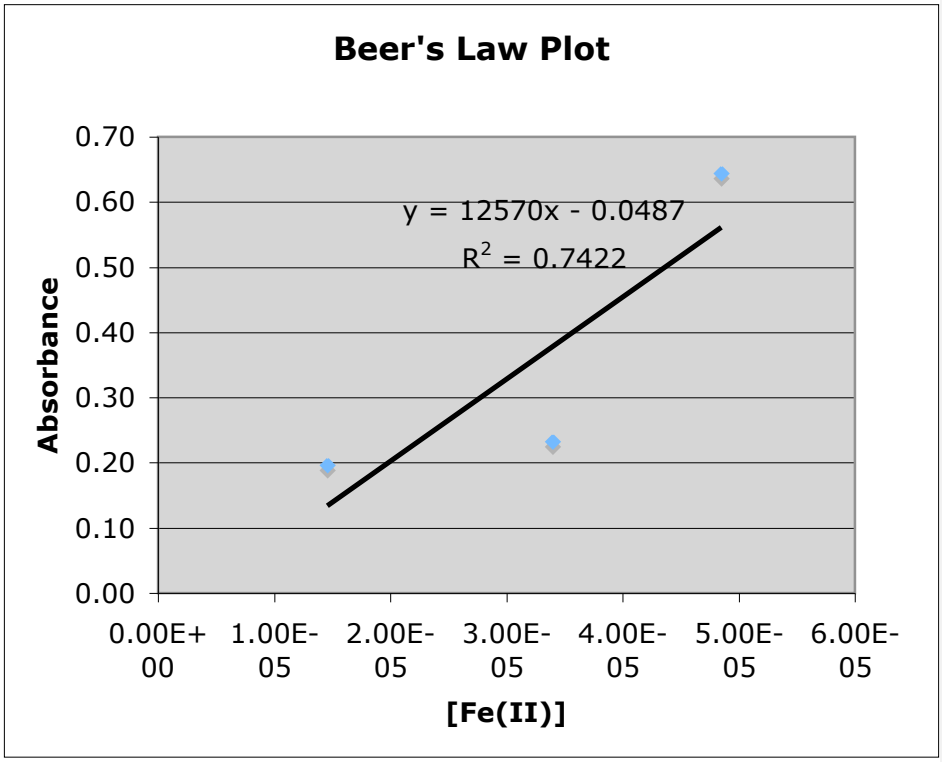

Figure 2. Beer's law plot of standard solutions of Fe(II).

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Beer's law plot. The measured values of absorption were plotted against the Fe(II) concentration of each standard. This plot was fitted with a trendline of the equation: y=12570x-0.0487 using linear regression. The R^2 value ($R^2 = 0.7422$) of this trendline is lower than desirable. This number, and subsequently the value of the plot, could be increased by measuring the absorbance of a greater number of standards of varying concentrations within the studied range. Using the slope, pathlength, and equation (1), the value for molar absorptivity, ε, was calculated to be 12570 $L \cdot mol^{-1}cm^{-1}$.

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Table 2. Parameters from Beer's law plot.

Slope	12570.
Path Length (cm)	1.0
ε	12570.

Sample preparation. Based on a comparison to the median intensity of the color of the standard solutions, three samples were prepared with 7mL of unknown iron solution. The intensity of the color of these samples most closely matched that of the $1.46x10^{-5}$ M standard.

Table 3. Absorption data for solutions containing unknown concentrations of Fe(II).

Unknown #	Abs	Wavelength of Maximum Absorbance (nm)
1	0.2138	509.88
2	0.2267	509.90
3	0.1770	509.19
	0.2058	509.66

Determination of iron concentration of unknown. The three samples were each run on the UV-vis spectrometer giving an average absorbance of 0.2058. The first two unknowns were close in value with less precision in the third measurement. This deviation is likely the result of a loss of unknown solution via spills during pipetting in the preparation of the samples. The concentration of each was then calculated using the previously calculated value of molar absorptivity, ε, and equation (1). The mean concentration was calculated to be $1.6x10^{-5}$ M. The standard deviation was calculated using equation (2). The relative standard deviation was calculated using equation (3). These values are reported in table 4.

Table 4. Calculated value for concentration of Fe(II) in unknown solution.

Concentration of Fe(II) in unknown solution	Relative Standard Deviation
$1.6 \times 10^{-5} \pm 0.2 \times 10^{-5}$ M	12.53%

The calculated value for concentration fell within the concentration range of the standards. The calculated concentration of the unknown was closest to the standard of which the color intensity matched most closely.

Evaluation of method. This method has the advantage of being able to accurately determine iron concentrations over a wide range of sample concentrations. However, the technique as a number of disadvantages as well. The method has a limit of detection of approximately 10 μM, below which the analyte cannot be detected. The other disadvantage of this technique is the necessity to form a complex for spectrophotometry. If any of the iron in solution is in a form other than Fe(II), it cannot complex with 1,10-phenanthroline and therefore will not be included in calculations of iron concentrations in the sample.

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Conclusions

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The method presented in this paper was demonstrated to be effective for accurately determining iron concentrations in aqueous solutions. This method is simple, reproducible, and effective over a wide range of concentrations. The maximum absorption of the complex studied in this experiment, at 510nm, falls in an ideal range for the type of spectroscopy used, providing further validity to the method. The results

presented in the paper indicate that this method could be applied to determine iron concentrations in water samples for use across a range of disciplines.

References

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Comment [25]: Good list. Follow format of ACS. 9/10