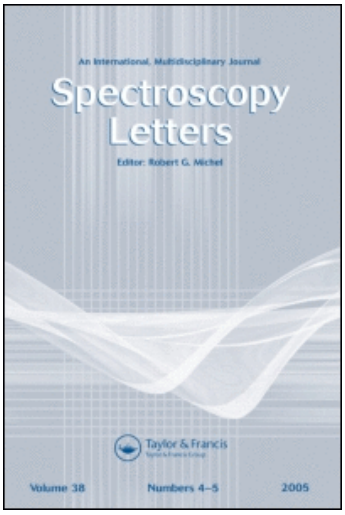

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Undergraduate Analytical Chemistry: To Use and Evaluate Organic Chelators for Spectrophotometric Determination of Iron

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Undergraduate Analytical Chemistry: To Use and Evaluate Organic Chelators for Spectrophotometric Determination of Iron

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Abstract: An analytical chemistry laboratory project to use and evaluate pyridyl- and triazine-containing chelators for spectrophotometric determination of iron is described. The "Iron Project" introduces students to UV-visible spectrophotometry and Beer's law via hands-on development and use of spectrophotometric methods for iron. Nine chelators are currently available for use. Students perform their proposed work, culminating in formal papers and posters. Student values of molar absorptivities (ε_{max}) and λ_{max} for ligands 1–9 agree with published values to within $\pm$15% (ε_{max}) and $\pm$3 nm (λ_{max}) for 75% of results obtained so far. Stoichiometries for Fe(II) chelates of ligands 1–5 are within $\pm$1 mole of ligand of the published ratios. Student work shows a basic understanding of Beer's law as well as a need to improve writing, problem-solving, and laboratory skills.

Keywords: Beer's law, calibration, chelate, iron, limit of detection, limit of quantitation, method of continuous variations, pyridyl- and triazine-type ligands, UV-visible spectrophotometry

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INTRODUCTION

Iron plays a prominent role in natural processes and human activity; thus, quantitative determinations of iron are of high interest for a variety of industrial, biological, medical, and environmental reasons.[1–4] Methods such as inductively coupled plasma (ICP)[5] and ICP coupled with mass spectrometry (ICP-MS)[5,6] are routinely used for trace and higher concentration determinations of iron, yet ultraviolet-visible spectrophotometric methods for iron are still widely employed[7–13] because of inexpensive instrumentation and the ability of many UV-Vis methods for iron to achieve subpart per million detection limits.

Ultraviolet-Visible (UV-Vis) spectrophotometry is a core topic in any undergraduate course on quantitative analysis. The basis of UV-Vis Beer's law,[14–16] which relates in a linear manner the relative amount of light transmitted by an analyte to its concentration. In addition to quantitative determinations of absorbing analytes, UV-Vis spectrophotometry can yield information about the stoichiometry of light-absorbing chelates.[16–19]

The author has found that use of a series of pyridyl- and triazine-containing organic ligands[12] (Figs. 1 and 2) for determination of iron (as Fe^{2+})

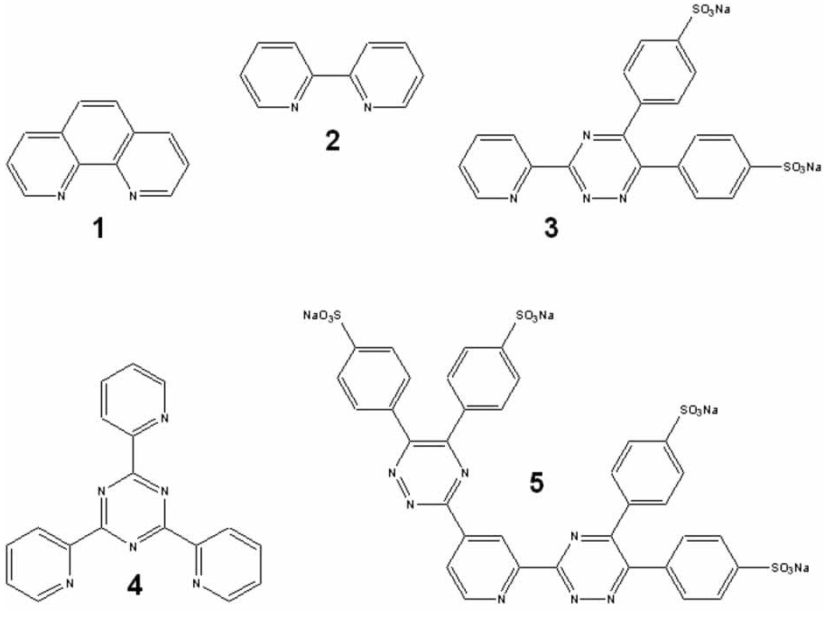

Figure 1. Ligands used in the Iron Project at UPG since fall term 2001: (**1**) 1,10-phenanthroline (1,10-phen); (**2**) 2,2′-bipyridine (2,2′-bipy); (**3**) 3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine disodium salt (PDTS, or Ferrozine); (**4**) 2,4,6-tripyridyl-1,3,5-triazine (TPTZ); (**5**) 2,4-bis(5,6-diphenyl-1,2,4-triazin-3-yl)-pyridine tetrasulfonic acid tetrasodium salt (2,4-BDTPS).

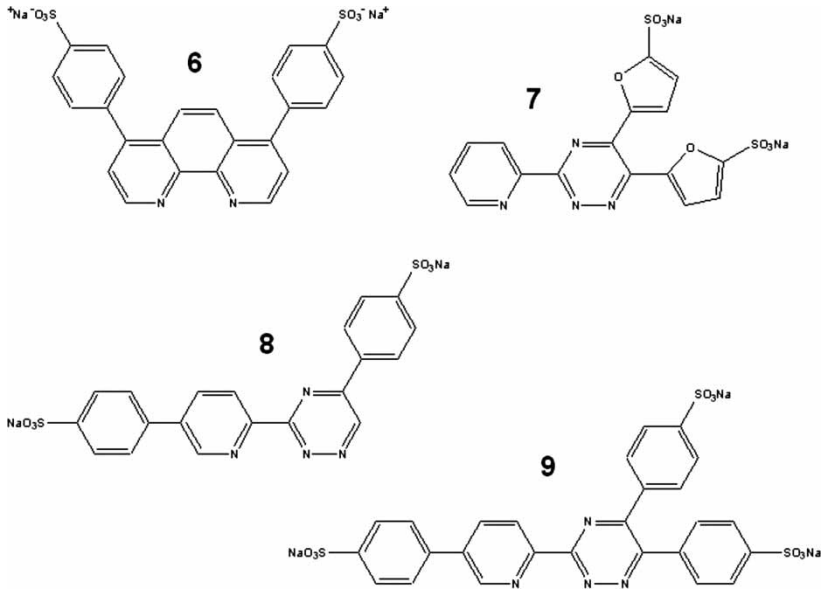

Figure 2. Ligands added to the Iron Project at UPG since fall term 2005: (**6**) 4,7-diphenyl-1,10-phenanthrolinedisulfonic acid disodium salt (bathophenanthrolinedisulfonic acid disodium salt, or BPDSA); (**7**) 3-(2-pyridyl)-5,6-difurylsulfonic acid-1,2,4-triazine sodium salt (PDFST, or Ferene S); (**8**) 3-(4-phenyl-2-pyridyl)-5-phenyl-1,2,4-triazine disulfonic acid disodium salt (PPTS); (**9**) 3-(4-phenyl-2-pyridyl)-5,6-diphenyl-1,2,4-triazine trisulfonic acid trisodium salt (PPDTS).

provides an excellent vehicle for introducing students to UV-Vis and Beer's law in a hands-on, guided-inquiry manner. The use of these iron chelators to illustrate the concepts of UV-Vis spectrophotometry has resulted in what is now known as the "Iron Project", which students enrolled in the CHEM 0260 (Laboratory for Introduction to Analytical Chemistry) course at the University of Pittsburgh at Greensburg (UPG) are required to perform as part of their course grade. This paper describes the Iron Project.

MATERIALS AND METHODS

Apparatus and Reagents

Spectral measurements were made using a Hitachi Model U-3010 scanning, double-beam UV-Vis spectrophotometer (Hitachi Instruments, Inc., San Diego, CA, USA) and matched 1-cm quartz cuvets (NSG Precision Cells, Inc., Farmington, NY, USA), and Sequoia-Turner single-beam spectrophotometers (Sequoia-Turner Instruments, Inc., San Jose, CA, USA) and cylindrical 1-cm

cuvets. Adjustments of pH of analysis solutions for optimum color formation were monitored with pH meters (Flinn Scientific, Inc., Batavia, IL, USA; Fisher Scientific, Inc., Pittsburgh, PA, USA). Ligands **1**–**9** (Figs. 1 and 2) were purchased from GFS Chemicals, Inc. (Powell, OH, USA). Other reagents include hydroxylamine hydrochloride, hydroquinone, ascorbic acid, sodium acetate, and NIST-traceable pH 7 buffer solution (Fisher Scientific, Inc.).

Solution Preparation

Aqueous solutions of hydroquinone were prepared at 1–10% (w/v) concentration. Aqueous solutions of sodium acetate were prepared at 0.1–10 M concentration. Dilute (1 M) aqueous solutions of HCl and NaOH were prepared from reagent grade 12 M HCl and NaOH pellets (Fisher Scientific, Inc.), respectively. Solutions of water-soluble sulfonated ligands were easily prepared. Ligands with poorer water solubility, such as 1,10-phenanthroline, 2,2′-bipyridine, and 2,4,6-tripyridyl-1,3,5-triazine (TPTZ) were made to dissolve in water by dropwise addition of 12 M HCl[12,23] to the solution before dilution to volume. Ligand solutions were prepared at concentrations of 5.0×10^{-3} to 1.0×10^{-2} M, to provide at least a fivefold excess of ligand with respect to Fe in the low-to-medium Fe concentration range. Standard aqueous Fe stock solutions (1000 mg Fe/L or 1.79×10^{-2} M) in 1 M H_2SO_4 or HCl (to keep Fe in solution for extended periods of time) were prepared from reagent grade iron wire or iron(II) salt [e.g., iron (II) ammonium sulfate hexahydrate, $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$] (Fisher Scientific, Inc.; GFS Chemicals, Inc.), according to established procedures.[12] Working standard Fe solutions (10–100 mg Fe/L or 1.79×10^{-4} to 1.79×10^{-3} M) were prepared according to established procedures.[12] Calibration standards ranging from 0.05 to 10 mg Fe/L (or approximately 10^{-6} to 10^{-4} M Fe) were prepared from the working standard. This range of Fe concentrations was selected on the basis of Fe concentrations that yielded calculated absorbances between 0.1 and 1.0 for the linear range [using Beer's law and literature values of the Fe(II) chelate molar absorptivities] and allowed for potential deviations from Beer's law at low and high Fe concentrations.

Experimental Procedures

Student Preparation and Optimization

There are common steps with all approaches for the determination of iron.[18] These are (1) pipetting an appropriate aliquot of the prepared sample solution into a small beaker containing a small volume of distilled or deionized water; (2) pipetting aliquots of the ligand solution, hydroquinone solution, and

sodium acetate; (3) adjusting the pH of the analysis solution to that required for optimum color formation; (4) quantitative transfer of the analysis solution to a small volumetric flask and dilution to volume, followed by spectrophotometric determination of iron. The procedures used by the students will vary in terms of the ligand used and sample preparation.

Wavelength of Maximum Absorption (λ_{max})

Obtain a spectrum for the metal ligand chelate from 300–800 nm (or 400–700 nm if the instrument has no UV capability) and determine the wavelength of maximum absorbance.

Absorbance–Concentration (Beer's Law) Relationships for Fe(II) Chelates

Prepare one or more reagent blanks plus a series of Fe(II) standard solutions, typically ranging in concentration from 1.0×10^{-6} to 1.0×10^{-4} mol Fe L^{-1}, for spectrophotometric determination of iron according to the common steps outlined under "Student Preparation and Optimization". Generate an absorbance–concentration curve, and perform linear regression of the Beer's law region to obtain ε_{max}.

Stoichiometries of the Fe(II) Chelates

Prepare up to 11 solutions of varying mole ratios of iron to ligand for the Job plot and analyze the solutions for Fe according to the common steps outlined under "Student Preparation and Optimization". Details concerning the method of continuous variations, or Job method, are described in the literature.[16–19]

Limits of Detection and Quantitation

Prepare a series of at least six reagent blanks and analyze for iron using the common steps outlined under "Student Preparation and Optimization". Calculate the average and standard deviation of the measured absorbances. Determine the limits of detection and quantitation according to established methods.[15,16,28]

Data Manipulation

All student calculations were performed using Microsoft Excel.

RESULTS AND DISCUSSION

Determination of the Maximum Absorption Wavelength (λ_{max}) of the Iron (II) Chelates

Table 1 gives the measured λ_{max} of each chelate of iron (II) with ligands **1** through **9** and the published λ_{max} for each chelate. Measured values of λ_{max} for ligands **1**–**3** and ligands **5**–**8** were found to be within ± 2 nm of the published values;[12,20–22,24–26] for ligands **4** and **9**, measured values were within ± 3 nm.[23,24] The differences between experimental and literature values of λ_{max} may be explained by the need for periodic wavelength calibration of the spectrophotometers, due to shifting of wavelength alignment over time. Wavelength calibration standards (e.g. holmium oxide glass) aid in the calibration. Instructors can use this module to ask students to consider potential errors in the measurement of λ_{max}, followed by introduction to wavelength calibration. Such an approach introduces students to one aspect of method validation in spectrophotometry and helps students to better understand the performance of the spectrophotometer.

Beer's Law Behavior of the Iron(II) Chelates

Table 2 summarizes the Beer's law behavior of the iron(II) chelates of ligands **1** through **9**. Linearity of absorbance with concentration for the chelates extended from one to two orders of magnitude over the Fe(II) concentration range of 1.0×10^{-6} to 1.0×10^{-4} mol Fe L^{-1} (0.056–5.6 mg Fe L^{-1}). Values of R^2 obtained by linear regression ranged from 0.970 to 0.998, indicating poor to excellent linearity. Depending on the concentration units

Table 1. λ_{max} Values for chelates of iron(II) with ligands **1**–**9**, measured by students

	Wavelength of maximum absorption (λ_{max}) of chelate (nm)				
Ligand	Fall 2001	Spring 2003	Fall 2004	Fall 2005	Published [12,20–26]
1,10-phen (**1**)	510	508	510	—	508
2,2′-bipy (**2**)	522	520	522	—	522
Ferrozine (**3**)	560	560	—	—	560
TPTZ (**4**)	590	596	595	—	593
2,4-BDTPS (**5**)	—	564	566	565	565
BPDSA (**6**)	—	—	—	535	535
PDFST (**7**)	—	—	—	591	593
PPTS (**8**)	—	—	—	564	565
PPDTS (**9**)	—	—	—	566	563

Table 2. Samples of student-calculated ε_{max} values for chelates of iron(II) with ligands **1–9**

Ligand	Term/year	Molar absorptivity[a] (ε_{max}) (M^{-1} cm^{-1})	R^2	Linear range (M)	Published ε_{max} (M^{-1} cm^{-1})[12,20–26]
1,10-phen (**1**)	Fall 2004	1.11×10^4	0.9944	1.00×10^{-6} to 1.00×10^{-4}	1.11×10^4
2,2′-bipy (**2**)	Fall 2004	7.72×10^3	0.9969	1.00×10^{-6} to 1.00×10^{-4}	8.7×10^3
Ferrozine (**3**)	Spring 2003	6.69×10^4	0.9972	1.00×10^{-6} to 2.00×10^{-5}	2.54×10^4
TPTZ (**4**)	Fall 2004	2.28×10^4	0.9762	1.00×10^{-6} to 2.00×10^{-5}	2.26×10^4
2,4-BDTPS (**5**)	Fall 2005	3.54×10^4	0.9975	1.00×10^{-6} to 2.00×10^{-5}	3.22×10^4
BPDSA (**6**)	Fall 2005	2.17×10^4	0.9976	1.00×10^{-6} to 2.00×10^{-5}	2.21×10^4
PDFST (**7**)	Fall 2005	4.10×10^4	0.9726	1.00×10^{-6} to 1.00×10^{-5}	3.55×10^4
PPTS (**8**)	Fall 2005	3.51×10^4	0.9891	1.00×10^{-6} to 2.00×10^{-5}	3.29×10^4
PPDTS (**9**)	Fall 2005	3.15×10^4	0.9701	1.00×10^{-6} to 8.60×10^{-6}	3.07×10^4

[a]Cuvet pathlength $b = 1.00$ cm.

(mol Fe L^{-1} or mg Fe L^{-1}) employed by the students in their absorbance–concentration curves, molar absorptivities (ε_{max}) at λ_{max} were obtained directly or calculated from the slopes of the calibration curves. Students were taught how to convert between mol Fe L^{-1} and mg Fe L^{-1} and then permitted to display the concentration axes of their plots in either unit. In all cases, the cuvet pathlength was taken to be 1.00 cm (based on the pathlength of the rectangular quartz cuvets used in our laboratory). Published values of molar absorptivity[12,20–26] are included in Table 2 for comparison.

The accuracies of the student-generated values of ε_{max}, expressed as percent errors, were calculated and found to range from $<0.9\%$ to 31% for the bulk of the determinations, with two outliers yielding percent errors of 81 and 164, respectively. Of the experimental ε_{max} values listed in Table 2, 10 of them agreed with the published ε_{max} to within 10%. These errors can be attributed to experimental conditions, such as selection of a wavelength that is slightly different from the λ_{max} for the published (i.e., known) ε_{max}; wavelength calibration of the spectrophotometer; preparation of stock, working, and calibration standards for Fe; and optimum color formation and premature or delayed measurement of absorbance of the calibration standards. The use of micropipets for dilution of standards can lead to errors in the experimental ε_{max} and poor (<0.99) values of R^2. Instructors may want to have their students compare experimental values of ε_{max} obtained by preparation of standards using micropipettes versus glass volumetric pipettes and, for example, serial dilution. The results can be used to review proper pipetting techniques and to emphasize careful attention to detail in preparation of calibration standards.

In two instances—formation of the Fe(II) chelates of ligands **1** (fall term 2004, $R^2 = 0.9944$) and **2** (fall term 2004, $R^2 = 0.9969$)—the students working with these ligands achieved linearity of absorbance with concentration over the range of Fe concentrations (1.0×10^{-6} to 1.0×10^{-4} mol Fe L^{-1}) used in the experiment. For ligands **1** and **2**, the published molar absorptivities of their Fe(II) chelates are 1.11×10^4 and 8.7×10^3 L mol^{-1} cm^{-1}, respectively.[20,21] Using Beer's law and the stoichiometries of the Fe(II)-ligand **1** and Fe(II)-ligand **2** chelates, students can determine that with a 10-fold excess of ligand, Beer's law is achieveable over the range 1.0×10^{-6} to 1.0×10^{-4} mol Fe L^{-1}. Compared with the values of ε_{max} for the Fe(II) chelates of ligands **3**–**9** (Table 2),[12,22–26] those of Fe(II)-ligand **1** and Fe(II)-ligand **2** are low enough to produce a linear calibration curve ranging up to 1.0×10^{-4} mol Fe L^{-1}. No such behavior was observed for the other seven Fe(II) chelates.

The concentration of chelating ligand used to form the colored chelate can influence the linearity of the absorbance-concentration curve and produce deviations from Beer's law,[27] usually at the high-iron end of the curve. The author uses the limiting reactant concept to remind students that complete chelation of iron is achieved by addition of ligand in excess of the amount dictated by the chelate stoichiometry. A 10-fold excess of ligand is more than sufficient to force chelation of all iron present. At the high-Fe

end of the concentration regime plus a fixed ligand concentration, it is possible for the ratio of Fe to ligand to become less than the stoichiometric ratio. Under those conditions, Fe may no longer be the limiting reactant and a linear absorbance–Fe concentration relationship no longer observed. One approach for students to explore the effect of ligand concentration on the linearity of the absorbance-concentration curve is to generate the curve at various ratios of Fe to ligand concentrations (e.g., 1:1, 1:3, and 1:10 Fe:ligand) and determine the linear range of each plot.

Determination of Limits of Detection and Quantitation

Students must learn that every analytical method has a limit of analyte concentration detectable by that method. Two important method validation parameters that define the lower limit of the linear range of absorbance with concentration in the spectrophotometric determination of iron are the *limit of detection* (LOD) and the *limit of quantitation* (LOQ). The LOD has been defined in several ways;[15,16,28] the generally accepted definitions of the LOD and LOQ used in the Iron Project are given in the footnotes in Table 3. The standard deviation of the blank or small analyte signal is given by s_{blank}; the slope of the calibration curve is defined by m.

Table 3 gives values of the LOD, LOQ, and standard deviation s for the determination of iron by UV-Vis using ligands **5**–**8**, as these were the only ligands for which LOD and LOQ have been determined to date. The LOD and LOQ values for Fe determinations using ligands **5**, **6**, and **8** are sub-milligram Fe L^{-1}. These sub-milligram Fe L^{-1} LOD and LOQ are expected for ligands **5**, **6**, and **8**, due to their large molar absorptivities (see Table 2). As for the LOD and LOQ associated with ligand **7**, contamination of the reagent blanks appears to be the source of the higher-than-expected values. The measured absorbances of the reagent blanks ranged from 0.066 to 0.174—considerably higher than one would want—and

Table 3. Limits of detection (LOD) and quantitation (LOQ) for determination of iron by selected ligands used in the Iron Project

Ligand used	Standard deviation of blank (s_{blank})[a]	LOD[c] (mg Fe L^{-1})	LOQ[d] (mg Fe L^{-1})
2,4-BDTPS (**5**)	0.0012	0.006	0.021
PPTS (**8**)	0.0019	0.010	0.031
BPDSA (**6**)	0.0043[b]	0.037	0.11
PDFST (**7**)	0.042	0.19	0.58

[a]Based on determination of Fe in six replicate reagent blanks.
[b]Five replicate reagents blanks were analyzed for Fe.
[c]Limit of detection = LOD = $(3.3)(s_{blank})/(m)$.
[d]Limit of quantitation = LOQ = $(10)(s_{blank})/(m)$.

with a high degree of scatter among the individual measurements (reflected by s_{blank}). Such results can be used by instructors to warn students about the need for careful attention to detail in a spectrophotometric determination.

Quantitative Determination of Iron in Samples and Recovery of Known Added Iron

Table 4 shows results from determination of iron in selected samples provided by the author and students. Typical samples analyzed for iron are runoff water samples from abandoned mine drainage sites, due simply to local interest in abandoned mine drainage and immediate availability of these samples. Percent relative standard deviations (RSD) range from 0.9% to 9.6%. Sources of high-percent RSD values may be attributed to experimental errors, such as incorrect pipetting techniques, errors in dilutions, solution preparation, and absorbance measurements.

Selected results of single-point standard addition for determination of iron recovery are presented in Table 5. The recovery of iron obtained for the student method utilizing ligand **6** indicates that nearly complete Fe recoveries are possible. The lower-than-expected Fe recoveries for the methods using ligands **5** and **8** may be attributed to typical experimental errors, such as measurement of the aliquot of standard Fe spike, incomplete reduction of Fe^{3+} to Fe^{2+}, pH variations during workup of the analysis solution and afterward, and errors in dilutions and absorbance measurements.

Students need to be made aware of the importance of validation of an analytical method in terms of its precision and accuracy. In particular, validation of the accuracy of the method is necessary to establish that essentially all of the analyte in the sample is detected and determined, ensuring reliable results. The results shown in Tables 4 and 5 indicate that, overall, students need to develop more careful laboratory technique to improve the accuracy and precision of their analytical methods.

Table 4. Results from determination of iron in samples from students

Sample type	Term and year	Ligand used	Mean Fe $\pm$ standard deviation
Mine drainage, Latrobe, PA	Fall 2004	1,10-phen (**1**)	10.8 ± 0.1^a
Mine drainage, Bolivar, PA	Fall 2004	2,4-BDTPS (**5**)	16.3 ± 1.1^a
Iron supplement tablet	Fall 2004	2,2′-bipy (**2**)	33.0 ± 2.3^b
Jacks run water, Greensburg, PA	Fall 2004	TPTZ (**4**)	$< 0.5^a$
Mine drainage, Mon Valley, PA	Fall 2005	BPDSA (**6**)	8.6 ± 0.2^a
Mine drainage, Cambria County, PA	Fall 2005	PPTS (**8**)	25.1 ± 2.4^a

[a]Concentration in mg Fe L^{-1}.
[b]Mass in mg Fe.

Table 5. Percent recoveries for Fe by UV-Vis/chelating agents by standard addition

Ligand used	Sample	mg Fe L^{-1} added	mg Fe L^{-1} recovered	Percent recovery
2,4-BDTPS (**5**)	Abandoned mine drainage, Coal Bluff, PA	11.2	9.2	82
PPTS (**8**)	Abandoned mine drainage, Conemaugh River Valley, PA	55.8	48.9	87.6
BPDSA (**6**)	Abandoned mine drainage, Forward Township, PA	8.4	8.3	99

Determination of Fe(II) Chelate Stoichiometries by the Method of Continuous Variations

Results for stoichiometries of the chelates of Fe(II) with ligands **1**–**5** obtained by the method of continuous variations[16–19] are presented in Fig. 3 and in the following discussion. Figure 3 illustrates the use of a Job plot to ascertain the ratio of Fe^{2+} ions to molecules of ligand **2**. Experimental Fe:ligand ratios for the chelates of Fe(II) with ligands **1**, **3**, and **5** were between 1:2–1:3, 1:2–1:3, and 1:3–1:4, respectively, versus a published ratio of 1:3 for each of these chelates.[12,20,22,24] Fe:ligand ratios with chelates of ligands **2** and **4** agreed with the published values of 1:3 (ligand **2**)[21] and 1:2 (ligand **4**).[12,23] The observed variations in the stoichiometries for the chelates of Fe(II) with

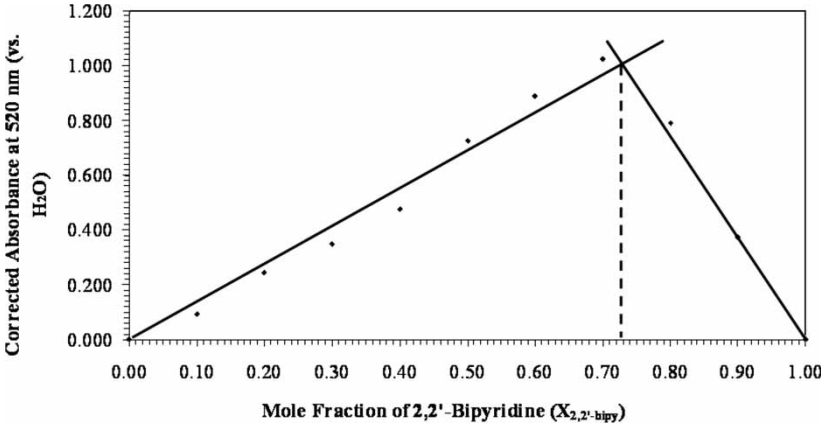

Figure 3. Determination of the stoichiometry of the chelate of Fe^{2+} with ligand **2**, using the method of continuous variations.

ligands **1**, **3**, and **5** may arise from inaccurate measurements of aliquots of stock iron(II) and ligand solutions taken for preparation of the Job plot solutions, changes in solution pH over time and lack of pH adjustment, improper or no correction of measured absorbances to the baseline,[16–19] and confusion in the order of the Job plot solutions upon measurement of absorbance.

The Job experiments were omitted from the Iron Project in the previous two courses due to greater emphasis placed on Beer's law, calibration, and method validation. With the introduction of the newer ligands **6** (Fall 2004) and **7**–**9** (Fall 2005), a revisit to the Job method may be in order.

Student Formal Papers and Posters

The culmination of the Iron Project is a formal paper and poster produced by each student or group. The format of the formal paper is that of a typical article published in a reputable analytical chemistry journal (*Analytical Chemistry* is one example),[6] in either a one- or two-column format. Students obtain sample copies of articles from the selected journal, either from hardcopy reserves or online library catalogs. The poster is prepared according to standard guidelines in the *ACS Style Guide*.[29] Students are required to use Microsoft PowerPoint to construct their poster presentations and submit their papers and posters to the instructor in electronic format.

In general, the formal papers and posters generated by individual students and groups tend to be well organized and neat. Most students, especially those exposed to written lab reports and research projects in introductory biology and chemistry courses, appear to have sufficient expertise with word processing, spreadsheet, and presentation software. Even more experienced students may still encounter at least some difficulty with the Iron Project in terms of basic grammar and writing skills, algebra and other math skills, and interpretation and discussion of data and results. Learning these skills is an ongoing process; feedback to students on the papers and posters is attempted as promptly as possible to expedite student learning in these areas.

FUTURE DIRECTIONS

The Iron Project has become an extensive laboratory research project for introduction of students to UV-visible spectrophotometry and its application to the determination of iron. Each CHEM 0260 course taught at UPG produces more ideas for project modules. Ideas for future consideration include analyzing other sample types in addition to the popular abandoned mine drainage, exploring various sample preparation techniques, emphasizing more in-depth statistical treatment of calibration curves (e.g., standard deviation of the slope and *y*-intercept, and the standard error of the estimate), exploring the possible deviations from Beer's law via Ringbom

plots, determination of optimum pH for the Fe(II) chelates, simultaneous determination of iron and another metal ion (e.g., copper) using ligands from the Iron Project, and the effect of cuvet pathlength on the absorbance–concentration curve of the Fe(II) chelates. Additionally, the effects of ionic strength[16,30] and variations in pH[12,20–26] upon the Beer's law behavior of the Fe(II) chelates are under consideration as well.

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