Speciation and Determination of Low Concentration of Iron in Beer Samples by Cloud Point Extraction

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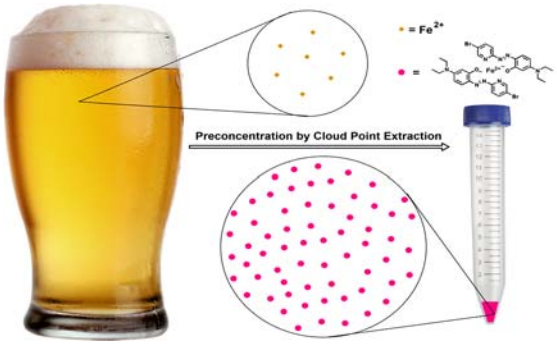
ABSTRACT

A laboratory experiment is described in which students determine the concentration and speciation of iron in beer samples using cloud point extraction and absorbance spectroscopy. The basis of determination is the complexation between iron and 2-(5-bromo-2- pyridylazo)-5-diethylaminophenol (5-Br-PADAP) as a colorimetric reagent in an aqueous micellar solution followed by cloud point extraction for preconcentration. Total iron and Fe(II) were determined with and without addition of ascorbic acid as a reducing agent, respectively. The determination of iron concentration in real beer samples using cloud point extraction provides an opportunity for students to become familiar with preconcentration, complexation chemistry, masking agents, and the speciation of an analyte. Students also gain hands-on experience with adapting methods from scientific literature, absorbance spectroscopy, and exploring quality control techniques, such as method detection limits and spike recovery.

GRAPHICAL ABSTRACT



KEYWORDS

Upper-Division Undergraduate, Laboratory Instruction, Analytical Chemistry, Hands-On Learning/Manipulatives, Micelles, Quantitative Analysis, and UV−Vis Spectroscopy

INTRODUCTION

Iron (Fe) plays a major role in the quality and physical properties of beer. Numerous references have demonstrated excellent correlation between the iron content and various quality factors of beer.[1] Fe is one of several metal ions essential for the respiration, activity, and growth of yeast and yeasts are responsible for fermentation and ageing of beer.[2] The level of Fe is related to physical properties of beer including the foaming quality, flavor stability, haze formation, and the color of beer by catalyzing some reactions in beer.[3] The average concentration of Fe is 200 μg/ml. Much higher values cause flavor instability, degradation of beer quality during beer storage, and lends a metallic taste to beer.[1,4] Sources contributing to the presence of iron in beer range from trace concentration of iron occurring naturally in water sources to Fe leached from metallic surfaces used throughout the brewing process.[2] Therefore, concentration of iron in beer is of special significance. One of the simple methods for determination of iron is complex formation and spectrophotometric measurement of complexes. However, under normal conditions, the iron content of fermented beer is below 3.5×10^{-6} M and a preconcentration method is necessary for spectrophotometric measurement. Cloud point extraction (CPE) is an effective technique for separation and preconcentration of various organic and organometallic compounds.[5] The key element of CPE is a surfactant; a molecule consisting of a hydrophobic tail and a hydrophilic head group. In aqueous solution, and at low concentrations, surfactant molecules are found as individual monomers. By increasing the concentration, the surfactant molecules find each other to form assemblies in which the hydrophilic heads organize at the surface while the hydrophobic tails align interior to the assembly.[6] These organized structures are called micelles and the certain concentration threshold for micellar formation for a given surfactant is called the “critical micellar concentration” (CMC). Aqueous micellar solutions are homogeneous in nature but consist of both hydrophobic and hydrophilic phases. From the analytical point of view, one of the most important properties of the micelles is their capacity to solubilize hydrophobic solutes in aqueous solutions by partitioning into its hydrophobic core.[7] (Figure1)

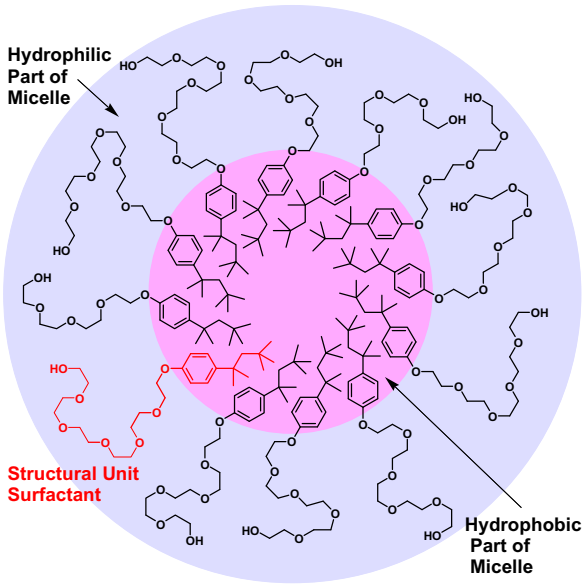


Figure 1. The structure of TritonX-114 as an example of non-ionic surfactant and its representative micellar structure [8]

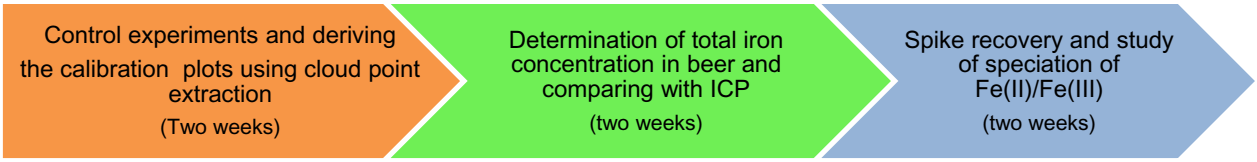
When the clear micellar solution is heated above the cloud-point temperature, it becomes cloudy. The cloudy solution includes two distinct phases: one is an aqueous phase and the other is a surfactant-rich phase that forms by aggregation of the micelles. The hydrophobic compounds initially present in the micelle interiors are extracted with the surfactant-rich phase and forms the basis for using CPE as a preconcentration technique. The volume of the surfactant rich solution is small compared to the initial aqueous phase and yields significant preconcentration of the solute of interest. CPE is known for its distinct merits of simplicity, lower toxicity to the environment, and higher enrichment factor compared to conventional extractions utilizing organic solvents.[6]

The measurement of low concentrations of iron in beer provides a simple and obvious experiment illustrating concepts of CPE and the utility of this technique as a preconcentration step in preparing samples for analysis.

EXPERIMENTAL OVERVIEW

This experiment was accomplished in an analytical chemistry laboratory course having 92 students. The laboratory was equipped with 25 spectrophotometers, and students worked in 25 groups of three and four in five different lab sections. Students met for lab activities twice a week; each

lab period meets for four hours. The project took a total of 48 hours of lab time to complete. Scheme 1 shows the project timeline for experimental activities.



Scheme 1. Project timeline within the semester. Each group of students provided the design proposal and a paper report before and after each part, respectively.

In the first part of the experiment, students developed a control experiment using 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol (5-Br-PADAP) as a colorimetric reagent to measure Fe(II).

Fe(II) reacts with 5-Br-PADAP yielding a pink/violet complex.[9] The color of 5-Br-PADAP is yellow/orange in the absence of Fe(II) and the complex formation yields a distinct purple color and absorbance changes (Figure 2)

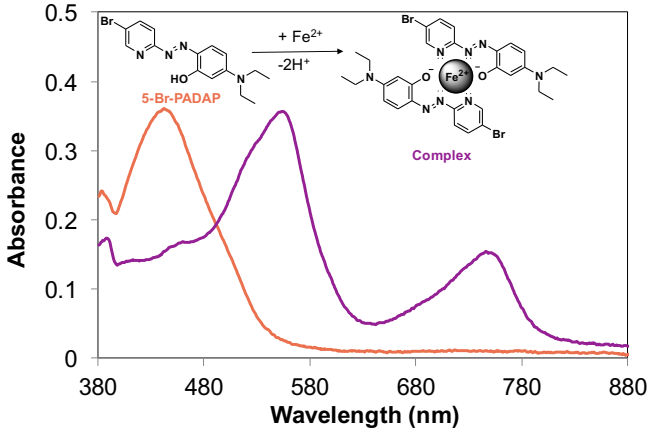


Figure 2. The absorption spectra for 1.0 × 10^{-5} M 5-Br-PADAP in the presence of ascorbic acid (0.05%w/v), acetate buffer (pH = 5), EDTA (0.01%w/v), Triton X-114 (0.25% v/v) and 1.8×10^{-4} M of Fe(II)

Both 5-Br-PADAP and its complex with Fe(II) are hydrophobic and insoluble in aqueous solutions so the use of the Triton X-114 surfactant to form a hydrophobic micellar media is necessary for their dissolution.[10] The result is suitable for CPE and measurement of Fe(II) after preconcentration in the surfactant rich phase. The experiment has good selectivity for determination of Fe(II) in the presence of Fe(III) utilizing EDTA in solution as a masking agent for Fe(III). The total iron concentration can be determined by reduction of Fe(III) to Fe(II) by using ascorbic acid as a reducing agent.[10] The designed protocol was considered as an experiment for determination of total iron and speciation of Fe(II) and

Fe(III) in beer samples. The students reported the results of their control experiments, their measurement of commercial beer samples, and the detection limits for their methodology. Inductively coupled plasma atomic emission spectroscopy (ICP-AES) analysis was used to cross-check student results reported in their control experiments. The students also designed a spike recovery experiment to test the matrix effects on analytical measurements and determined a conditional formation constant for Fe(II) and Fe(III) with 5-Br-PADAP under this micellar condition.

EXPERIMENTAL PROCEDURE

Students prepare stock solutions of Fe(II), 5-Br-PADAP, ascorbic acid, acetate buffer, EDTA and Triton X-114. Students then take the spectra of the 5-Br-PADAP in acetate buffer solution (pH = 5), in the presence ascorbic acid (0.05%w/v), EDTA (0.01%w/v) and Triton X-114 (0.25% v/v). They then measure the absorbance of a series of solutions with the same pH, same concentrations of 5-Br-PADAP and all other reagents and different concentrations of Fe(II) to construct a calibration curve for absorbance changes versus concentration of Fe(II). In order to achieve reproducible result, the best order for addition of the reagents is: Fe(II), 5-Br-PADAP, ascorbic acid, buffer solution, EDTA and TritonX-114. EDTA can be added 2 min after complex formation. The same order without Fe(II) is also necessary for absorbance measurement of 5-Br-PADAP. To perform CPE, aliquots of 10 ml of each sample solution are heated in a thermostatic bath at 50 °C for 5 min yielding the cloudy solutions. Separation of the two phases is accomplished by centrifugation for 10 min at 4000 rpm. By cooling in an ice/water bath for 5 minutes, the surfactant-rich phase, located at the bottom of centrifuge tube, becomes viscous. The aqueous phase is then separated completely by a plastic pipette dropper. The surfactant-rich phase is diluted by ethanol to 2 ml (To decrease the viscosity and facilitate sample handling) and the resultant solution is analyzed by spectrophotometry. Students used a SpectroVis plus with a wavelength range of 380−950 nm (visible range). Figure 3 shows the absorption spectra of 1.0×10^{-5} M 5-Br-PADAP in the absence and presence of different concentrations of Fe(II).

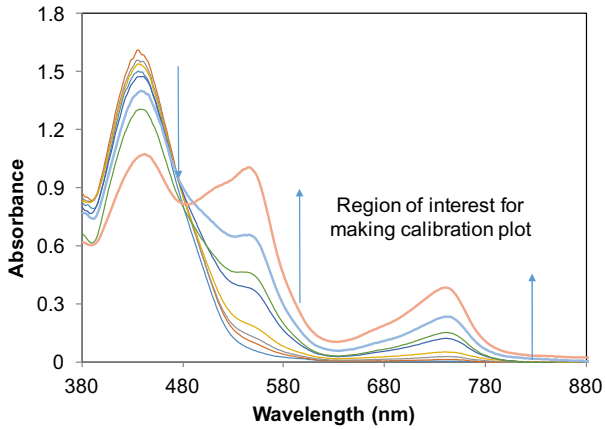


Figure 3: The absorption spectra for 1.0 × 10^{-5} M 5-Br-PADAP in the presence of ascorbic acid (0.05%w/v), acetate buffer (pH = 5), EDTA (0.01%w/v), Triton X-114 (0.25% v/v) and different concentrations of Fe(II) (a) 0, (b) 8.9×10^{-8} M, (c) 1.8×10^{-7} M, (d) 3.5×10^{-7} M, (e) 8.9×10^{-7} M, (f) 1.25×10^{-6} M, (g) 1.8×10^{-6} M and (h) 3.5×10^{-6} M

Figure 3 clearly demonstrates that by addition of Fe(II) to the 5-Br-PADAP solution, the absorption at 440 nm for 5-Br-PADAP solution gradually decreases and the absorption at 748 and 553 nm increase for the complex form of Fe(II) with 5-Br-PADAP. The calibration curves are derived by plotting the absorbance of complexes at 748 nm for Fe(II) versus the concentration of Fe(II) ion; one calibration for the micellar solution and one calibration plot for the ethanolic solution after CPE. (Figure 4)

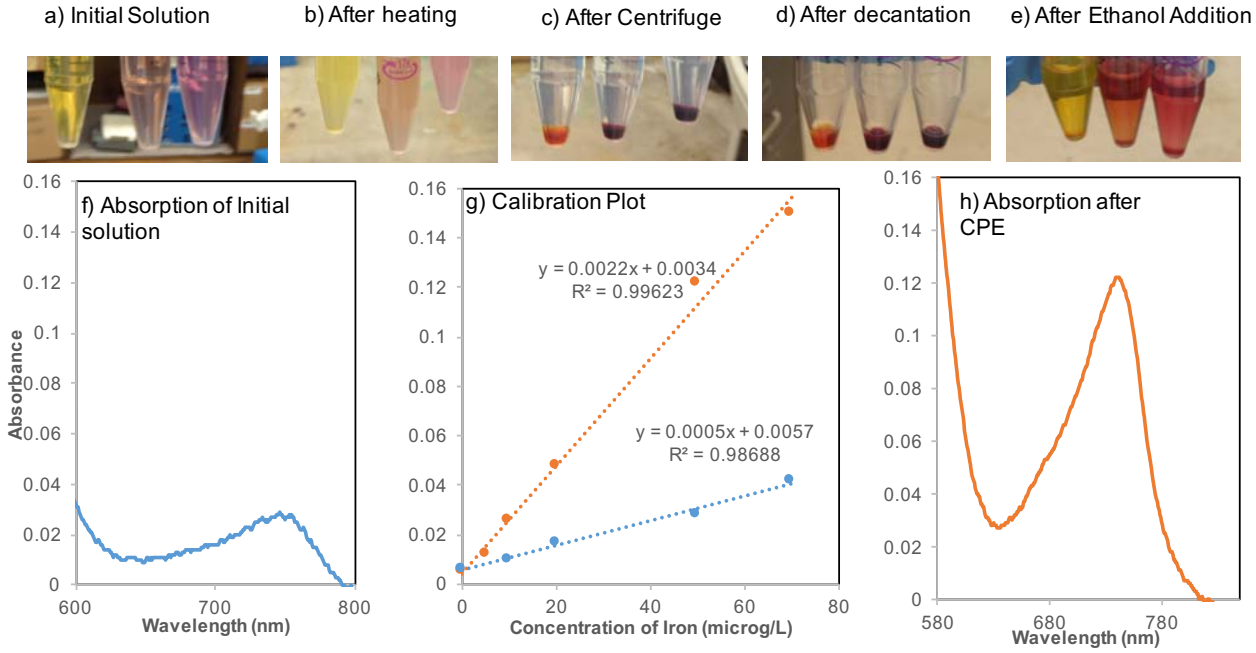


Figure 4. Representative example of the solutions for CPE and resulted calibration plots before and after CPE.

Thereafter, a sample of beer is subjected to the CPE analysis and students determine the total iron in samples using their calibration. For the speciation of Fe(II) and Fe(III), the students prepare solutions with and without ascorbic acid. Ascorbic acid reduces Fe(III) to Fe(II) so the measurement provides the total Fe concentration. Without ascorbic acid but in the presence of EDTA, the measurement provides the Fe(II) concentration. Subtracting the Fe(II) concentration from the total iron concentration provides the Fe(III) concentration. More experimental details are given in the supporting information.

HAZARDS

Proper laboratory clothing, gloves, and approved safety goggles must be used in a laboratory. Acetic acid can be a hazardous chemical if not used in a safe and appropriate manner. This liquid is highly corrosive to the skin and eyes and must be handled with extreme care. Laboratory chemicals including sodium acetate, ethanol, 5-Br-PADAP, $FeCl_3.6H_2O$, $(NH_4)_2Fe(SO_4)_2.6H_2O$, EDTA and ascorbic acid can cause eye and skin irritation. These chemicals must be handled using personal protection equipment. Flush eyes or hands with plenty of water in the case of eye or skin contact.

RESULTS AND DISCUSSION

As is shown in Figure 4(g), the absorbance of the complex shows a linear correlation with the concentration of Fe(II). Comparison of the calibration plots and the solution absorbance values before and after CPE clearly demonstrates preconcentration. The calibration plot after CPE enables the students to measure the low concentration of iron (total iron) in commercial beer samples. Students found concentrations of Fe in the beer samples in the range of 5.37×10^{-7} M (30 μg/L) to 2.68×10^{-6} M (150 μg/L) in various commercial beer brands, the spike test gave 65% to 140% (including some 98% to 102%) recovery. The measurements with CPE had good agreement with the measurements using the ICP analysis.

Figure 5 shows one of the examples of spike recovery test by a group of students and Table 1 demonstrates the results of the recovery test.

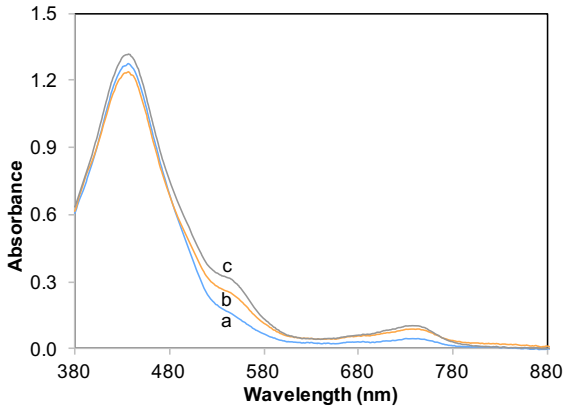


Figure 5: The absorption spectra of Miller lite beer (a) without added Fe(II), (b) spiking 8.9×10^{-7} M of Fe(II) and (c) 1.4×10^{-6} M of Fe(II). Condition 1.0×10^{-5} M 5-Br-PADAP, 0.05%w/v ascorbic acid, 0.01%w/v EDTA and 0.25% v/v Triton X-114 in acetate buffer (pH = 5).

The spike recovery calculated using equation (1) is:

$$\%R = \frac{F-I}{A} \times 100 \qquad (1)$$

To determine the %R of a spike, the sample is split into two portions and a known amount of a standard solution of analyte is added to one portion. The concentration of the analyte is determined for both the spiked, F, and unspiked portions, I, A is the concentration of analyte added to the spiked portion.

Table 1. Concentration of iron in unspiked and spike Miller Lite beer samples

Label	Fe (added)	Fe (found)	Recovery (%)
Unspiked	0	1.2×10^{-6} M(*)	--
Spike 1	8.9×10^{-7} M	2.1×10^{-6} M	101%
Spike 2	1.4×10^{-6} M	2.5×10^{-6} M	93%

*Realative Standard Deviation (RSD)=4%

ICP results revealed the concentration of iron in beer was 1.0×10^{-6} M, which correlates well with the student results reported in Table 1. The small difference in values may result from the color contribution of beer to the absorbance spectrum in the visible region.

The concentration and speciation of Fe for a variety of beers were obtained by the class. Table 2 shows the results of speciation for three beer brands by groups of students. The experimental procedure is provided in the supporting information. As Table 2 shows overall the concentration of Fe(III) is less than Fe(II) in all of the beer samples and varies from 1.67×10^{-7} - 1.00×10^{-6} M.

Table 2. Concentration of Fe(II) and Fe(III) after speciation for variety of beers

Brand of the beer	Hopalicious	Spotted Cow	Penguin Pale
packing	Canned (355 mL)	(bottle 355 mL)	(bottle 355 mL)
total Iron (M)	1.68×10^{-6} a	1.32×10^{-6} a	4.15×10^{-6} a
	2.04×10^{-6} b	2.23×10^{-6} b	1.59×10^{-6} b
	2.03×10^{-6} c	2.24×10^{-6} c	1.81×10^{-6} c
Fe(II) (M)	1.32×10^{-6} a	9.60×10^{-7} a	2.97×10^{-6} a
	1.62×10^{-6} b	1.82×10^{-6} b	1.39×10^{-6} b
	1.82×10^{-6} c	1.24×10^{-6} c	1.65×10^{-6} c
Fe(III) (M)	3.60×10^{-7} a	3.60×10^{-7} a	1.17×10^{-6} a
	4.13×10^{-7} b	4.10×10^{-7} b	2.10×10^{-7} b
	2.00×10^{-7} c	1.00×10^{-6} c	1.67×10^{-7} c

(a) to (c) represents the group 1 to 3
The RSD of the experiments for individual groups varies between 1.7 to 5.1%.

CONCLUSION

This lab experiment utilizes concepts of cloud point extraction and preconcentration in determining the low concentration of iron in beer samples. Accumulation of metal complexes in the hydrophobic interior of micelles highlights the importance of molecular interaction for extraction. Without CPE, spectrophotometric measurement of iron in beer is not possible. By employing absorbance spectroscopy and guiding students to develop and evaluate their own methods, students discover how color and absorbance changes can be used for quantitative analysis. Students must also understand the basic principles of masking agents and selective extraction, to report on the speciation of an analyte in a complex sample, such as beer. The students in this experiment also gain experience with analyses of data, calibration plots, measurements of concentration for both known and unknown samples, and spike recovery. This experiment could be extended for preconcentration and determination of variety of other metal ions in low concentrations and in different samples by spectrophotometric measurement, for example copper in beer also plays an important role in chemistry. [3,11] For small-scale brewers in

measuring the concentration of iron in their beer samples, this experiment provides an easy and cost effective test, and can be considered as a collaborative activity for iron measurement in their samples by the students.

Supporting Information

Experimental details, experimental student handouts, homework examples, and notes for the instructor are available via the internet at http://pubs.acs.org

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NOTES

The authors declare no competing financial interest.

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