10 Real World Spectroscopy Labs for Real World Students

Applications in Spectroscopy

Monde Qhobosheane, Ph.D.

Instruction for ***Ocean Optics' SpectraSuite*** software included

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Ocean Optics, Inc.
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Preface

Students can now study the basic scientific principles on the same world-class equipment used by leading researchers in the university and government labs and more excitingly, NASA. With advances in electro-optics and their continuous impact on the sensing community, high-speed array detectors, inexpensive optical fibers and powerful computers have made optical spectroscopy the sensing technique of choice for many real-world applications.

The development and marketing of scientific instruments and methods have changed in an equally dramatic way; in the past, cutting-edge instrumentation started with expensive research devices that were accessible only to well-funded research and development enterprises, gradually, the technologies filtered into general laboratory use, application-specific instruments and now into the educational settings.

Our knowledge of spectroscopy has been based upon years of experimentation in a wide array of disciplines ranging from art to applied physics. All of the fields have strong roots in education, more specifically teaching and learning the basics of the field. It has been the experience of thousands of science educators that have utilized the Ocean Optics spectrometers with there own specific real world exciting experiments that the students in there classes lives have been enriched and have a greater appreciation for science.

It is important that today's science and engineering students appreciate the capabilities of optical sensing, the fundamental physics of the measurement process, the design trade-offs inherent in selecting and integrating components, and the discipline required to produce quality results. The goal of this lab manual is to provide a vehicle to allow future scientist to study the fundamentals of spectroscopy using modern research/industry instrumentation.

I would like to offer special thanks to the educators who contributed to this lab manual as part of the ongoing Ocean Optics grant program.

Note to Educators: If you would like to contribute to future compilations, please send an email to education@oceanoptics.com.

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1 Beer's Law of Potassium Permanganate

The Spectrometer and Beer's Law

The direct relationship between absorbance and concentration for a solution is known as the *Beer-Lambert Law*, or more commonly *Beer's law*. You can use Beer's law to test several samples of a solution, of known molar concentrations, and calculate a best-fit line equation to relate the absorbances of the solutions to their concentrations.

The primary objective of this experiment is to determine the concentration of an unknown potassium permanganate, $KMnO_4$, solution. You will first use a spectrometer to measure the absorbance of one $KMnO_4$ solution over the visible light spectrum and select the wavelength of maximum absorbance. You will prepare five potassium permanganate solutions of known concentration, which are your standard solutions. You will to measure the concentration of each standard solution to establish the Beer's law best-fit line equation for the $KMnO_4$ standards.

You will use the best-fit line function for the standard solutions to determine the molar concentration of a $KMnO_4$ solution of unknown concentration.

Materials

- Ocean Optics Spectrometer
- computer
- several cuvettes
- 1 dark cuvette
- 6 test tubes and test tube rack
- 10 mL graduated cylinders or pipettes
- 2.00×10^{-3} M potassium permanganate, $KMnO_4$, solution
- $KMnO_4$ solution, unknown concentration
- distilled water
- small beakers
- plastic Beral pipettes

Safety

Wear safety goggles and other appropriate safety gear (gloves or apron) during the experiment. Take all proper safety precautions when handling the solutions.

Procedure

1. Label five test tubes to use for your standard solutions. Prepare the $KMnO_4$ standards according to the chart below.

Test Tube	[$KMnO_4$] (mol/L)	mL of 2.00×10^{-3} M $KMnO_4$ (aq)	mL of distilled water
1	4.00×10^{-4}	2.0	8.0
2	8.00×10^{-4}	4.0	6.0
3	1.20×10^{-3}	6.0	4.0
4	1.60×10^{-3}	8.0	2.0
5	2.00×10^{-3}	10.0	0.0

2. Obtain a small amount of KMnO4 solution of unknown concentration. Set it aside to test after you have measured the absorbances of the standard solutions.

3. Fill a cuvette ~⅔ full with distilled water to serve as your blank.

4. Use a USB cable to connect a spectrometer to your computer. Start the SpectraSuite software.

5. Calibrate the spectrometer.

    a. On the toolbar, check the box next to "Strobe/Lamp Enable". Allow the spectrometer to warm up for at least five minutes before proceeding.

    b. Place the blank cuvette in the spectrometer. Open the File menu and choose New → Absorbance Measurement. The "Set Acquisition Parameters wizard" will appear. This process has four steps. First, confirm that your spectrometer has been identified, and then click Next >.

    c. In step two of this process, make sure that the box next to "Strobe/Lamp Enable" is checked. Manually select a suitable integration time by using the arrow keys to increase or decrease the integration time, in milliseconds. Click Next > to proceed.

    d. To record the absorbance spectrum of the blank (100% transmittance), click the Store Reference Spectrum icon, . Click Next > to proceed.

    e. Remove the blank cuvette and place the dark cuvette in the spectrometer. To save the dark reference (0% transmittance), click the Store Dark Spectrum icon, .

    f. Pour out the distilled water from the blank cuvette, rinse, and fill it with the 4.00×10^{-4} M $KMnO_4$ solution in Test Tube #1. Place the cuvette in the spectrometer. Click Finish to complete the acquisition parameters wizard.

g. A plot of absorbance *vs*. wavelength for your $KMnO_4$ solution is displayed.

6. Examine the graph and select the peak wavelength that you wish to use for your Beer's law experiment.

7. Set up the Beer's law experiment.

   a. Open the File menu and choose New → New Concentration Measurement. Select "Active Processing:", and then click Accept.

   b. The Concentration Measurement Setup wizard appears, and its default is the Beer-Lambert Law. Select "Calibrate from solutions of known concentration". Click Next >.

   c. The Range Selection defaults to a single wavelength. Enter the peak wavelength that you identified in Step 6, and then click Next >.

   d. The next dialog box sets up the Beer's law graph of your standard solutions. Type in the molar concentration of your first sample, 0.0004. Click Use Last Scan, and then click Add Sample. The absorbance of your first standard is saved and plotted on the small graph in the dialog box.

   e. Remove the cuvette and dispose of the solution as directed. Rinse and fill the cuvette with the KMnO4 standard solution in Test Tube #2. Place the cuvette in the spectrometer. Type in the molar concentration, click Use Last Scan, and then click Add Sample.

   f. Repeat step e for the remaining standards. When you complete the final standard solution ($2.00 \times 10–3$ M), type in the compound name and unit of concentration (mol/L). A best-fit line is calculated for your standards. Make sure the regression order is set to 1. Note: To delete a data point, choose a row of data and click Remove Sample. Click Finish to continue.

8. To measure the absorbance of the KMnO4 solution of unknown concentration, rinse and fill the cuvette ~⅔ full with the unknown sample. Click Single Update and write down the concentration of your unknown.

9. To close the SpectraSuite program, select File → Exit, and then click Yes.

Data Table

Test Tube	Concentration (mol/L)	Absorbance
1		
2		
3		
4		
5		
Unknown # ___		

Data Analysis

1. The graph of absorbance vs. wavelength for the KMnO4 solution shows two peaks that are fairly close together. Which wavelength did you measure the absorbance of the KMnO4 standard solutions? Explain.

2. Does the temperature of the solutions matter when conducting a Beer's Law experiment? Explain why or why not.

3. A student prepares the KMnO4 standard solutions and reads the volume incorrectly and low. For example, the student reads the volume as 10 mL when it is actually 11 mL. Explain how this error will affect the determination of a KMnO4 solution of unknown concentration?

2 Introduction to Reaction Rates

Introduction

An important piece of information one can learn about a chemical reaction is the reaction rate. While it is a huge oversimplification to think of reaction rate as the amount of time it takes for the products to be formed in a chemical reaction, it sometimes boils down to that very fact.

In this experiment, you will conduct a simple reaction between to household products: food coloring and bleach. The sodium hypochlorite in the bleach will react with the food coloring to form a nearly colorless product. A generic reaction equation is shown below:

$$\text{bleach} + \text{food dye} \rightarrow \text{products}$$

In this experiment, it will not be important to identify the exact molecular species that participate in the reaction. It is, however, important to record the change of the color of the reaction as it proceeds. This will be the job of the spectrometer.

The study of reaction rates is called **chemical kinetics**. When chemical kinetics is studied, it is common to limit the investigation of a reaction to one or two easily controlled variables. In this experiment, you will determine the effect of concentration on the reaction rate. To test the effect of the concentration of the food coloring, you merely have to measure the fading color over time. By selecting the proper wavelength, or wavelengths, the spectrometer will measure this for you. To test the effect of the concentration of bleach, you will have to conduct the reaction at least twice and change the initial bleach concentration. By measuring how the change in bleach concentration affects the time needed for the food color to fade, you can determine bleach's impact on the rate of the reaction.

Establishing the effect of concentration on reaction rate is called **rate order**. The goal of your data analysis will be to determine the order of the reaction with respect to the two reactants, bleach and food coloring.

Objectives

In this experiment, you will

- Measure the visible light absorbance spectrum of a food coloring solution to identify the wavelength of maximum absorbance (λ max).
- Measure the absorbance of the reaction between a food coloring solution and bleach at the λ max over a given length of time.
- Use your test results to determine the order of the reaction with respect to bleach and food coloring.

Materials

- ❑ Ocean Optics Spectrometer
- ❑ Food coloring

- Computer
- 1 cuvette
- 1 dark cuvette
- 50 mL and 150 mL beakers
- 10 mL and 25 mL graduated cylinders
- Household chlorine bleach
- 0.70 M sodium hypochlorite, NaOCl
- 0.70 M sodium chloride, NaCl, solution
- Distilled water
- Plastic Beral pipettes

Safety

Wear safety goggles and other appropriate safety gear (gloves or apron) during the experiment. Take all proper safety precautions when handling the solutions.

Procedure

1. Prepare your food coloring solution by adding 2 drops of food coloring to 50 mL of distilled water. Obtain about 10 mL each of 0.70 M NaCl solution and bleach (0.70 M NaOCl solution).

Part I: Set up the Spectrometer

2. Use a USB cable to connect a spectrometer to your computer. Start the SpectraSuite software.

3. On the toolbar, check the box next to "Strobe/Lamp Enable". Allow the spectrometer to warm up for at least five minutes before proceeding. If desired, skip ahead to Part II to prep your solutions for the first trial.

4. Fill a cuvette ~⅔ full with distilled water to serve as a blank.

5. Place the blank cuvette in the spectrometer. On the left hand side of the toolbar, manually increase or decrease the integration time so that the peak absorbance is slightly below 4,000 counts.

6. Record the absorbance spectrum of the blank (100% transmittance), by clicking the Store Reference Spectrum icon, (the yellow light bulb just above the graph).

7. Remove the blank cuvette and place the dark cuvette in the spectrometer. Save the dark reference (0% transmittance), by clicking the Store Dark Spectrum icon, (the gray light bulb above the graph).

8. Remove the dark cuvette from the spectrometer. Empty the distilled water from the blank cuvette, rinse and fill the cuvette ~⅔ full of the food coloring solution, and place the cuvette in the spectrometer. Identify the wavelength of maximum absorbance (λ max) for your food coloring solution and write it down. This is the wavelength you will use as you measure the absorbance of the reaction.

9. Click the Absorbance mode icon, A.

10. Open the File menu and select New → Strip Chart.

11. In the Chart Trend Settings dialog box, check the box next to Stop after this amount of time and change the time to 3 minutes.

12. On the right side of the dialog box under **Range Selection** select **One Wavelength** and select the λ max for your food coloring solution.

Part II: Conduct the First Reaction (Trial One)

13. Measure and transfer 18 mL of the food coloring solution and 1 mL of the NaCl solution to a clean and dry 50 mL beaker.

14. Pour out the food coloring from the cuvette and rinse the cuvette with small amounts of distilled water.

15. Measure and transfer 1 mL of bleach to the 50 mL beaker of food coloring solution. Swirl the beaker to mix the solutions. Use a plastic Beral pipette to rinse and fill the cuvette ~⅔ full of the reaction solution from the 50 mL beaker. Place the cuvette in the spectrometer.

16. Click Accept. A graph of absorbance vs. time will be displayed. If the Strip Chart Options dialog box appears, use your mouse to move it aside during the data collection.

17. After the run ends, export the emission measurements to Microsoft Excel.

    a. Click the Copy Spectral Data to Clipboard icon, ,on the toolbar.

    b. Open Microsoft Excel.

    c. In Excel, open the Edit menu and choose Office Clipboard…

    d. Click the spectral data file on the clipboard.

18. To store your data, click Save, . In the Save Trend dialog box, highlight the trend and click the Browse button, Browse... . Choose a location for the file, name it, and then click the Save button, Save. Back on the Save Trend dialog box, click Save. Click Close to continue.

Part III: Conduct the Second Reaction (Trial Two)

In Trial Two, you will increase the amount of bleach to measure its effect on the reaction rate.

19. Measure and transfer 18 mL of the food coloring solution to a new clean and dry 50 mL beaker.

20. Pour out the reaction mixture from the cuvette and rinse the cuvette with small amounts of distilled water.

21. As before, open the File menu and select New → Strip Chart. In the Chart Trend Settings dialog box, check the box next to Stop after this amount of time and change the time to 3 minutes. Under Range Selection select One Wavelength and select the λ max for your food coloring solution.

22. Measure and transfer 2 mL of bleach to the 50 mL beaker of food coloring solution. Swirl the beaker to mix the solutions. Use a plastic Beral pippette to rinse and fill the cuvette ~⅔ full of the reaction solution from the 50 mL beaker. Place the cuvette in the spectrometer.

23. Click Accept. If the Strip Chart Options dialog box appears, use your mouse to move it aside during the data collection.

24. Repeat Steps 17 and 18 to manage the data from Trial Two.

25. To close the SpectraSuite program, select File → Exit, and then click Yes.

26. Dispose of the solutions as directed by your instructor.

Data Analysis, Trial One

1. Prepare three plots: absorbance vs. time, ln abs vs. time, and 1/abs vs. time. Calculate the best-fit line equation for the plot that is the most linear, and write down the equation.

2. The order with respect to the food coloring can be determined by graphically applying the integrated rate laws. According to Beer's law, the absorbance of a food coloring solution is directly proportional to its concentration. Thus, the actual [food coloring] can be replaced by Amax, the maximum absorbance of the food coloring solution. A graph of Amax as a function of time will give a straight line if the reaction is zero order in food coloring. If the reaction is first order, then a graph of ln(Amax) as a function of time is linear. Finally, for the purposes of this experiment, if the reaction is second order, then a graph of 1/Amax as a function of time is linear. Based on the equation you selected to answer #1, what is the order of the reaction with respect to the food coloring – zero, first, or second order?

Data Analysis, Trial Two

3. Examine the graph of absorbance vs. time for both Trials One and Two. Select a brief period of time, 20-30 seconds, within the first minute of each trial and calculate the best fit line equation for Trials One and Two. Write down these two equations.

4. Because bleach does not change color in this reaction, you must analyze the initial rates of the reaction to determine the order with respect to bleach. In this instance, as with many others, during the first few moments the reaction proceeds as if it was zero order overall. Thus, the slopes of the best-fit line equations you calculated in #3 are also the reaction rates of Trials One and Two. Compare the slopes of Trials One and Two. If they are essentially the same, then the order of the reaction with respect to bleach is zero. If the slope of Trial Two is about twice the slope of Trial One, then the reaction is first order in bleach because doubling the bleach concentration caused the reaction to double. If the slope of Trial Two is closer to four times that of Trial One, then the reaction is second order in bleach.

5. Consult your textbook, if needed, to learn about rate laws. Write the rate law for this reaction.

6. Household bleach is 5.25% NaOCl (mass/volume). The qualitative (not perfectly accurate) concentration of food coloring is 0.0020 M. Use this information to calculate the value of the rate law constant, k.

One Final Question

7. Why was the NaCl solution included in this experiment?

3 Investigating the Atomic Spectrum of Hydrogen

Introduction

Hydrogen, with it lone electron, possess the simplest of all atomic spectra. When energy is applied to a hydrogen atom, the electron can be moved (an excited state) to a higher energy state but it doesn't remain in the higher energy state. When the electron returns to its original "position", a photon of light is released. The energy of this emitted photon is equal to the difference in energies of the two states. The Rydberg equation describes this situation mathematically:

$$\Delta E = -hcR_{H'}\left(\frac{1}{n_2^2}-\frac{1}{n_1^2}\right) = hc\upsilon', \quad n_1 = 1, 2, 3\ldots., \quad n_2 = n_1+1, n_1+2, n_1+3\ldots.$$

The variable υ' represents the wave number of the emitted photon. The speed of light, c, is measured in cm/s. When n1 = 1, the transitions occur in the ultraviolet light region, known as the Lyman series. When n1 = 2, the transitions occur in the visible light region, known as the Balmer series. Other values of n1 result in series that occur in the infrared light region.

In this experiment, we will focus our attention on the Balmer series of hydrogen – the visible light region. You will energize hydrogen gas that is contained in a sealed discharge tube (a specially designed light bulb, in essence). When you "turn on" the hydrogen discharge tube, you will see a colored light. The naked eye can see the Balmer series but what you see is a conglomeration of all the emitted photons. You will use an Ocean Optics spectrometer to not only measure the emitted photons but distinguish between them as individual lines at specific wavelengths in the visible region of light, ~400 nm - ~700 nm.

Mathematically, determining the wavelengths of light produced by the photons emitted in the Balmer follows the form shown below:

$$\lambda = \left(\frac{hm^2}{\left(m^2-n^2\right)}\right)$$

The variable m and n are integers, and h = 3654.6 × 10-8 cm. When you solve the equation with n =2 and m = 3, 4, 5, or 6, the calculated wavelengths represent the four emission lines in the Balmer series for hydrogen. These four wavelengths produce the color that your eyes can see in the energized hydrogen discharge tube.

In this experiment you will measure the emission spectrum of hydrogen gas and analyze the emission data to conduct important calculations describing the element hydrogen.

Objectives

In this experiment, you will

- Measure the visible light emission spectrum of a hydrogen in a discharge tube.
- Analyze the hydrogen emission spectrum to identify the Balmer series.

- Analyze the Balmer series data to determine the Rydberg constant and the first ionization energy of hydrogen.

Materials

- ❑ Ocean Optics Spectrometer
- ❑ computer
- ❑ fiber optic accessory
- ❑ hydrogen gas discharge tube

Procedure

1. Use a USB cable to connect an Ocean Optics or a Vernier Spectrometer to your computer. Make sure that the light source/cuvette holder has been detached from the spectrometer. Connect a fiber optic cable to the threaded detector housing of the spectrometer.

2. Start the SpectraSuite software.

3. Turn on the hydrogen gas discharge tube. Aim the tip of the fiber optic cable at the tube.

4. There are two methods of optimizing the graph of intensity vs. wavelength. Use the method that produces a better graph of the emission lines.

    a. Set the distance between the light source and the tip of the fiber optic cable so that the peak intensity on the graph is ~3500 counts.

    b. Adjust the Integration Time, which is located in the upper left hand portion of the SpectraSuite window. If the peak absorbance is off scale, reduce the integration time. If the peaks are too small, increase the integration time.

5. When you are satisfied with your emissions graph, click Save,  . In the **Save Spectrum** dialog box, click the Browse button,  . Choose a location for the file, name it, and then click the Save button,  . Back on the **Save Spectrum** dialog box, click  . Click  to continue.

6. To view the emission spectrum graph:

    a. Click the Overlay Spectral Data icon,  , on the right of the toolbar.

    b. Choose the file you saved in Step 5, and then click the Open icon,  .

    c. In the Load Spectrum dialog box, choose your file (which will be coded as "Processed"), and then click the Load icon,  .

7. To analyze your emission spectrum graph:

    a. Click anywhere on the graph to activate the cursor. Note the green vertical line marking a given wavelength on the graph; a box below the graph identifies the wavelength.

    b. Identify the four emission lines for hydrogen and write then down in the data table.

8. To close SpectraSuite, select **File → Exit**, and then click  .

Data Table

Complete the table below. You will have already recorded the wavelengths from examining the graph of the hydrogen discharge tube emissions.

To calculate the wavenumber, frequency, and photon energy:

- Wavenumber = 10^9 / (wavelength in nm)
- Frequency = (3×10^8 m/s) / (wavelength in m) **Note**: 1 nm = 1×10^{-9} m
- Photon Energy = (frequency) × h **Note**: $h = 6.626 \times 10^{-34}$ $m^2 \cdot kg \cdot s^{-1}$

Wavelength (nm)	Wavenumber (m^{-1})	Frequency (Hz)	Photon Energy (J)	n_i (Balmer Series)
				3
				4
				5
				6

Data Analysis

1. Use the equation described in the introductory remarks to calculate the Rydberg constant for the four lines in Balmer Series. For the Balmer series, n = 2. What is the average value for the Rydberg constant, based on your data.

2. Another equation is often used to analysis photon emitted from energized hydrogen.

$$\frac{1}{\lambda} = Rm\left(\frac{1}{n_f^2} - \frac{1}{n_i^2}\right)$$

   R_m is a constant called the Rydberg constant. It is an important part of the study of hydrogen. Calculate R_m for each of the four Balmer lines. In each case, $n_f = 2$, and n_i will be 3, 4, 5, or 6. What is the average value of R_m?

3. An accepted value of the Rydberg constant, Rm, is 1.097 × 107 m–1. Compare your value of Rm to the accepted value.

4. Use the Rm that you calculated in #2 to predict the wavelength of the fifth line in the Balmer Series (n = 7). Examine your graph of the hydrogen discharge tube emissions. Does the fifth Balmer line appear in your graph? Explain.

5. Use the data in your table to calculate the first ionization energy of hydrogen, in kJ/mol. Compare your calculated value with the accepted value of 1312.0 kJ/mol.

4 The Determination of the pKa of an Indicator Solution

Introduction

Generally speaking an indicator can be considered a weak acid. Its value as a visual indicator stems from the equilibrium it establishes and the different colors the indicator presents as its equilibrium is shifted. You will make use of this property of indicators to collect sufficient spectrophotometric measurements on your way to confirming the pKa of bromcresol green.

Historically, the pKa was found by preparing buffers of various values within the color transition range and calculating the pKa from the Henderson-Hasselbach equation. The chemical behavior of a buffer, HIn, can be expressed as shown below.

$$HIn \leftrightarrow In^- + H^+$$

Because the absorbance of a solution at a given wavelength varies directly with concentration (Beer's Law), one can determine the pKa of an indicator by carefully measuring the absorbances of five solutions: 1.0 M HCl, 1.0 M NaOH, and three buffers of specific pH values. The data analysis will allow you to prepare a plot of pH vs. $\log(k \cdot \frac{A_{In}}{A_{HIn}})$. The value of k can be calculated by taking and absorbance readings at λIn max after the equilibrium has been shifted, for all practical purposes, to the right by the addition of NaOH, and λHIn max after the equilibrium has been nearly 100% to the left by the addition of HCl.

Objectives

The objectives of this experiment are:

- Measure the absorbance spectrum of a series of five solutions of varying pH that contain the indicator bromcresol green.
- Determine the absorbance readings at λ_{max} for the five solutions.
- Calculate the pK_a of bromcresol green.

Materials

- Ocean Optics Spectrometer
- computer
- one cuvette
- one dark cuvette
- five test tubes and test tube rack
- 10 mL graduated cylinders
- 0.05 % bromcresol green solution
- 1.0 M sodium hydroxide, NaOH, solution
- 1.0 M hydrochloric acid, HCl, solution
- solutions of pH 4, pH 7, and pH 10 buffer
- distilled water
- plastic Beral pipettes

Safety

Wear safety goggles and other appropriate safety gear (gloves or apron) during the experiment. Take all proper safety precautions when handling the solutions.

Procedure

PART I: Prepare the sample solutions

1. Label five test tubes #1-5. Transfer 10 mL each of the following solutions to different test tubes: 1.0 M HCl to Test Tube #1, 1.0 M NaOH to Test Tube #2, pH 4 buffer to Test Tube #3, pH 7 buffer to Test Tube #4, and pH 10 buffer to Test Tube #5.

2. Add one drop of 0.05% bromcresol green indicator solution to each test tube. Add a second drop of indicator only if the colors in the test tubes are very faint and difficult to distinguish with the naked eye.

PART II: Determine the λ_{max} for HIn and In^-

3. The initial absorbance measurements are made with the acid and base solutions, in order to determine the λmax when the indicator's equilibrium has been shifted far to the right and far to the left. Follow the steps below to make these measurements:

    a. Use a USB cable to connect a spectrometer to your computer. Start the SpectraSuite software.

    b. On the toolbar, check the box next to "Strobe/Lamp Enable". Allow the spectrometer to warm up for at least five minutes before proceeding.

    c. Fill a cuvette ~⅔ full with distilled water to serve as a blank.

    d. Place the blank cuvette in the spectrometer. On the left hand side of the toolbar, manually increase or decrease the integration time so that the peak absorbance is slightly below 4,000 counts.

    e. Record the absorbance spectrum of the blank (100% transmittance), by clicking the Store Reference Spectrum icon,  .

    f. Remove the blank cuvette and place the dark cuvette in the spectrometer. Save the dark reference (0% transmittance), by clicking the Store Dark Spectrum icon,  .

    g. Remove the dark cuvette from the spectrometer. Empty the distilled water from the blank cuvette, rinse and fill the cuvette ~⅔ full of 1.0 M HCl solution from Test Tube #1, and place the cuvette in the spectrometer. Identify the wavelength of maximum absorbance (λmax). Write down the λmax, and the absorbance value, in your data table.

h. To save this graph, click  . In the Filename box, type in a name for your set of data. Click Browse... . Type in the name again and click Save . In the Save Spectrum dialog box, click Save , and then click Close . Your file will be saved in the default folder named "bin".

i. Empty, rinse and fill the cuvette ~⅔ full of 1.0 M NaOH solution from Test Tube #2, and place the cuvette in the spectrometer. Identify the wavelength of maximum absorbance (λmax). Write down the λmax, and the absorbance value, in your data table. Repeat step h to save the graph of the NaOH absorbance spectrum.

j. Examine the graph of the Test Tube #1 absorbance spectrum and write down the absorbance value at the λmax of the Test Tube #2 plot. Do the same examination of the graph of the Test Tube #2 solution. You will use these values to correct for background absorbance.

PART III: Determine the Absorbance Values at the λmax for HIn and In–

4. In Part III, you will measure the absorbance spectrum of each of the three buffer solutions (Test Tubes #3-5) and write down the absorbance of each buffer at the λ_{max} you determined for Test Tube #1 (Hin) and Test Tube #2 (In^-).

   a. Empty, rinse and fill the cuvette ~⅔ full of the pH 4 buffer solution from Test Tube #3, and place the cuvette in the spectrometer. Click anywhere on the graph to activate the cursor. Note the green vertical line marking a given wavelength on the graph; a box below the graph identifies the wavelength. Determine the absorbance value of the pH 4 buffer at the two λmax values you identified in Part II. Write these values down in your data table.

   b. Repeat step 3h to save the graph of the pH 4 buffer absorbance spectrum.

   c. Repeat steps a and b for the buffer solutions in Test Tubes #4 and #5.

5. To prepare individual graphs of your data for printing or inclusion in your lab report:

   a. Open the File menu and choose Open → Load Processed Spectrum.

   b. The default "bin" folder will appear. Select a file and click Open .

   c. Click  (Overlay Spectral Data). In the Load Spectrum dialog box, click on the first file listed (it will be designated as "Processed").

   d. Click Load . Your selected file will be plotted on the graph.

   e. (optional) If you wish to plot more than one set of data on the same graph, repeat steps a-d until you have the desired plots in place.

6. To close the SpectraSuite program, select File → Exit, and then click Yes .

7. Dispose of the solutions as directed by your instructor.

Data

PART II: Test Results for HIn and In–

Test Tube	λ_{max}	Absorbance
1 (HIn)		
2 (In^-)		

PART III: Test Results for Buffer Solutions

Test Tube	Buffer	Abs at λ_{max} for HIn	Abs at λ_{max} for In^-
3	4		
4	7		
5	10		

Data Analysis

1. Prepare two graphs. On the first graph plot the absorbance (Y-axis) as a function of pH (X-axis) for the five solutions at the HIn λmax value. One the second graph plot the absorbance (Y-axis) as a function of pH (X-axis) for the five solutions at the In– λmax value.

2. Calculate the best-fit line equation for each plot and identify the inflection point, which is the pKa.

3. Consult your text or other reference for the actual pKa of bromcresol green (it will be somewhere in the neighborhood of 4.5 – 4.8. Compare your value of the pKa with the actual value. Suggest reasons why your value is different from the actual value.

5 The Ksp of Copper Tartrate

Introduction

The solubility product constant, K_{sp}, of a slightly soluble salt is an example of aqueous equilibrium. The K_{sp} is defined as the product of the molar concentrations of the ions formed by the dissociation of the salt, each raised to the power of the coefficient of the balanced reaction equation. If a species in the equilibrium is colored or forms a colored complex in solution, the K_{sp} may be determined by visible spectroscopy. Copper (II) tartrate dissociates according to the equation:

$$CuC_2H_20_6(s) \leftrightarrow Cu^{2+}(aq) + C_2H_20_6^{2-}(aq)$$

Thus the solubility product constant is described by the expression: Ksp = [Cu2+] [C2H2O62-]

You will use an Ocean Optics Spectrometer to first measure the absorbance of a standard copper (II) tartrate solution at 676 nm. The copper 2+ ion has a characteristic blue color.

You will prepare five copper (II) sulfate solutions of known concentration (standard solutions) and measure their absorbance at a selected wavelength. When you graph absorbance vs. concentration for the standard solutions, a direct relationship will result, which is known as Beer's law.

Finally, you will measure the absorbance of a saturated copper (II) tartrate solution and use the Beer's law information from the standard samples to calculate the Ksp of copper (II) tartrate.

Objectives

In this experiment, you will

- Measure and analyze the visible light absorbance spectrum of a standard Cu^{2+} solution to determine the maximum wavelength of absorbance.
- Prepare and test the absorbance of five standard Cu^{2+} solutions.
- Calculate a standard curve from the test results of the standard solutions.
- Use the standard curve to determine the K_{sp} of copper (II) tartrate.

Materials

- Ocean Optics Spectrometer
- computer
- pipette pump or pipette bulb
- 1 cuvette
- Two 50 mL Erlenmeyer flasks
- Two 10 mL pipettes or graduated cylinders
- Two 100 mL volumetric flasks
- 5 test tubes and test tube rack
- 0.100 M copper (II) sulfate, $CuSO_4$, solution
- 0.100 M sodium tartrate, $Na_2C_2H_20_6$, solution
- distilled water
- filter and filter funnel
- quantitative filter paper
- tissues (preferably lint-free)
- centrifuge and centrifuge tubes
- Three 10 mL graduated pipettes

Safety

Wear safety goggles and other appropriate safety gear (gloves or apron) during the experiment. Take all proper safety precautions when handling the solutions.

Procedure

PART I Preparing a Saturated Solution of Copper (II) Tartrate

1. Prepare a saturated solution of copper (II) tartrate by mixing: 4 mL of 0.100 M copper (II) sulfate solution, 5 mL of 0.100 M sodium tartrate solution, and 1 mL of distilled water in a 50 mL Erlenmeyer flask. Mix thoroughly. Note that a precipitate forms. Allow the mixture to remain undisturbed for 15 minutes.

2. Centrifuge the liquid and keep the clear liquid layer. If the liquid layer appears slightly cloudy, centrifuge it a second time or filter the liquid through quantitative filter paper.

PART II Preparing Standard Solutions of Copper (II) Tartrate

3. Label five clean, dry, test tubes 1–5. Use pipettes to prepare five standard solutions according to the chart below. Thoroughly mix each solution with a stirring rod. Clean and dry the stirring rod between uses.

Test Tube number	0.100 M $CuSO_4$ (mL)	0.100 M $Na_2C_2H_20_6$ (mL)	Distilled H_2O (mL)	$CuC_2H_20_6$ Concentration (M)
1	2.00	5.00	3.00	0.020
2	1.80	5.00	3.20	0.018
3	1.50	5.00	3.50	0.015
4	1.20	5.00	3.80	0.012
5	1.00	5.00	4.00	0.010

4. Dilute 5 mL of 0.100 M NaC2H206 with 10 mL of distilled water. Fill an empty cuvette ¾ full with this dilute Na2C2H206 solution.

5. Use a USB cable to connect a spectrometer to your computer. Start the SpectraSuite software.

6. Calibrate the spectrometer.

    a. On the toolbar, check the box next to "Strobe/Lamp Enable". Allow the spectrometer to warm up for at least five minutes before proceeding.

    b. Place the blank cuvette in the spectrometer. Open the File menu and choose New → Absorbance Measurement. The "Set Acquisition Parameters wizard" will appear. This process has four steps. First, confirm that your spectrometer has been identified, and then click Next >.

    c. In step two of this process, make sure that the box next to "Strobe/Lamp Enable" is checked. Manually select a suitable integration time by using the arrow keys to increase or decrease the integration time, in milliseconds. Click Next > to proceed.

    e. To record the absorbance spectrum of the blank (100% transmittance), click the Store Reference Spectrum icon, . Click Next > to proceed.

    f. Remove the blank cuvette and place the dark cuvette in the spectrometer. To save the dark reference (0% transmittance), click the Store Dark Spectrum icon, .

    g. Pour out the distilled water from the blank cuvette, rinse, and fill it with the CuC2H206 solution in Test Tube #1. Place the cuvette in the spectrometer. Click Finish to complete the acquisition parameters wizard.

    h. A plot of absorbance vs. wavelength for your CuC2H206 solution is displayed. Examine the graph and select the peak wavelength that you wish to use for your Beer's law experiment.

7. Collect absorbance-concentration data for the five standard $CuC_2H_20_6$ solutions.

    a. Open the File menu and choose New → New Concentration Measurement. Select "Active Processing:", and then click Accept.

    b. The Concentration Measurement Setup wizard appears, and its default is the Beer-Lambert Law. Select "Calibrate from solutions of known concentration". Click Next >.

    c. The Range Selection defaults to a single wavelength. Enter the peak wavelength that you identified in Step 6g, and then click Next >.

    d. The next dialog box sets up the Beer's law graph of your standard solutions. Type in the molar concentration of your first sample, 0.020. Click Use Last Scan, and then click Add Sample. The absorbance of your first standard is saved and plotted on the small graph in the dialog box.

    e. Remove the cuvette and dispose of the solution as directed. Rinse and fill the cuvette with the CuC2H206 standard solution in Test Tube #2. Place the cuvette in the

spectrometer. Type in the molar concentration, click Use Last Scan, and then click Add Sample.

f. Repeat step e for the remaining standards. When you complete the final standard solution, type in the compound name and unit of concentration (mol/L). A best-fit line is calculated for your standards. Make sure the regression order is set to 1. Note: To delete a data point, choose a row of data and click Remove Sample. Click Finish to continue.

8. Write down the absorbance values for the standard solutions, as well as the best-fit line equation for the standards.

9. To measure the absorbance of the saturated CuC2H206 solution, rinse and fill the cuvette ~⅔ full with the saturated CuC2H206 solution you prepared in Part I. Click Single Update and write down the concentration.

10. To close the SpectraSuite program, select File → Exit, and then click Yes.

Data

Test Tube	Concentration (mol/L)	Absorbance
1	0.020	
2	0.018	
3	0.015	
4	0.012	
5	0.010	

Best-fit line equation: _____

Data Analysis

1. Determine the concentration of the saturated CuC2H206 solution.

2. Use your test results to calculate the Ksp for CuC2H206. Compare your empirical Ksp with the published (Lange's Handbook of Chemistry) value of 4×10^{-4}.

6 Spectrophotometric Analysis of a Buffer Solution

Introduction

Determination of pH is one of the most frequently performed measurements in chemistry. The potentiometric method with a glass electrode has been widely used for pH measurements but has drawbacks such as the need for a reference electrode, susceptibility to electrical interference and instrument drift. It is desirable to have alternative methods for pH determination. One such method is spectrophotometric measurement with the use of a pH indicator. In a spectrophotometric method, the pH of an unknown solution is determined by addition of a pH indicator and subsequent measurement of absorbance of the resulting solution. Because overlap exists between the spectra for the acid form (Hln) and base form (In-) of the indicator, it is necessary to determine individual molar absorptivities for each form at two wavelengths ($\lambda 1$ and $\lambda 2$), usually these are the wavelength peaks or absorption maxima of HIn and In-.

In this experiment, you will use an Ocean Optics spectrometer to determine the pH of an acetate buffer solution. You will measure the absorbance spectrum, over the 380 – 950 nm range, of the indicator bromcresol green when it is added to the acetate buffer that is subsequently treated with a basic solution and an acidic solution.

Objectives

In this experiment, you will

- Measure and analyze the visible light absorbance spectrum of an acetate buffer solution, containing bromcresol green indicator.
- Compare the spectra of a sample of acetate buffer that has been treated with acid to a sample of the buffer treated with base.
- Use your test results to calculate the pH of the buffer solution.

Materials

- Ocean Optics spectrometer
- computer
- 1 cuvette
- 5 mL and 10 mL pipette and pump
- 100 mL volumetric flask
- four 50 mL volumetric flasks
- 25 mL graduated cylinder
- solid sodium acetate, $NaCH_3COO$
- 1×10^{-4} M bromcresol green solution
- 0.10 M hydrochloric acid, HCl, solution
- 0.10 M sodium hydroxide, NaOH, solution
- 2.40 M acetic acid, CH_3COOH, solution
- distilled water

Safety

Wear safety goggles and other appropriate safety gear (gloves or apron) during the experiment. Take all proper safety precautions when handling the solutions.

Procedure

PART I: Prepare the acetate buffer solution

1. Pipette 5.00 mL of 2.40 M acetic acid, CH3COOH, solution into a 100 mL volumetric flask. Dilute with about 50 mL of distilled water.

2. Measure out 0.825 g of sodium acetate, NaCH3COO, and quantitatively transfer it to the volumetric flask. Swirl the flask until the solid completely dissolves.

3. Add distilled water to the volumetric flask to make 100 mL of buffer solution.

PART II: Prepare the sample solutions

4. Pipette 10.00 mL of 1×10–4 M bromcresol green solution to each of three 50 mL volumetric flasks. Label the flasks A-C. In flask A, use a graduated cylinder to add 25 mL of 0.10 M HCl solution. In flask B, add 25 mL of 0.10 M NaOH solution. In flask C, add 25 mL of the acetate buffer solution you prepared in Part I.

5. Add distilled water to each volumetric flask to make 50 mL of solution.

PART III: Test the sample solutions

6. Use a USB cable to connect a spectrometer to your computer. Start the SpectraSuite software.

7. On the toolbar, check the box next to "Strobe/Lamp Enable". Allow the spectrometer to warm up for at least five minutes before proceeding.

8. Fill a cuvette ~⅔ full with distilled water to serve as a blank.

9. Calibrate the spectrometer and measure the absorbance of the three flasks of solutions.

    a. Place the blank cuvette in the spectrometer. Open the File menu and choose New → Absorbance Measurement. The "Set Acquisition Parameters wizard" will appear. This process has four steps. First, confirm that your spectrometer has been identified, and then click Next >.

    b. In step two of this process, make sure that the box next to "Strobe/Lamp Enable" is checked. Manually select a suitable integration time by using the arrow keys to increase or decrease the integration time, in milliseconds. Click Next > to proceed.

    c. To record the absorbance spectrum of the blank (100% transmittance), click the Store Reference Spectrum icon,  . Click Next > to proceed.

d. Remove the blank cuvette and place the dark cuvette in the spectrometer. To save the dark reference (0% transmittance), click the Store Dark Spectrum icon,  .

e. Pour out the distilled water from the blank cuvette, rinse, and fill it with the mixture in Flask A. Place the cuvette in the spectrometer. Click Finish to complete the acquisition parameters wizard.

f. A plot of absorbance vs. wavelength for the sample in Flask A is displayed. Identify the wavelength of greatest absorbance (λ max) and write it down in your data table. If there is a secondary absorbance peak, record it in your data table.

10. To save the plot of the mixture in Flask A, follow these steps.

    a. In the toolbar across the top of the graph, click  to save the absorbance spectrum.

    b. In the Filename box, type in a name for your set of data.

    c. Click Browse... . Type in the name again and click Save .

    d. In the Save Spectrum dialog box, click Save , and then click Close . Your file will be saved in the default folder named "bin".

11. Pour out the Flask A from the cuvette, rinse, and fill it ~⅔ full with the mixture in Flask B. Place the cuvette in the spectrometer. Note that the absorbance plot for the sample is displayed. Identify and record the λ max and a secondary absorbance peak. Repeat Step 10 to save the plot.

12. Measure the absorbance of the mixture in Flask C, as you did with the other two samples, with one important change. The absorbance peaks of the buffer solution may be slightly different from the peaks of the acidic and basic solutions. Even so, you must record the absorbance values at the same two wavelengths as the acidic and basic solutions.

13. Dispose of the solutions as directed by your instructor.

14. To prepare a graph of any of the three plots, follow these steps:

    a. Open the File menu and choose Open → Load Processed Spectrum.

    b. The default "bin" folder will appear. Select a file and click Open . This will open the file, but not display the data on the graph yet.

    c. Click  (Overlay Spectral Data). In the Load Spectrum dialog box, click on the first file listed (it will be designated as "Processed").

    d. Click Load . Your selected file will be plotted on the graph.

    Repeat Steps a-d until you have the desired plots on your graph.

Data Table

Flask	Solution	1st Peak/absorbance	2nd Peak/absorbance
A	Indicator w/ Acid		
B	Indicator w/ Base		
C	Indicator w/ Buffer		

Data Analysis

1. Calculate the molar absorptivity of HIn and In^- at each wavelength peak, identified in your data table, for each sample.

2. Calculate the [HIn] and [In–] of the acetate buffer solution.

3. Calculate the [H+] and the pH of the buffer solution, using the Ka for bromcresol green: 1.60 × 10–5.

4. Another way to calculate the pH of the acetate buffer is to use the Henderson-Hasselbalch equation. Find this equation in your text and use it to calculate the pH of the buffer.

7 The Beer's Law Analysis of Erythrosin B

Introduction

There are a number of solutions and compounds that are excellent candidates for spectrophotometric analysis, but are toxic or expensive or difficult to handle. Two shining

examples are chromate and dichromate ions. The sodium or potassium salts of these ions are readily soluble in water, are relatively high molecular mass, and both ions are vividly colored in solution, making them the perfect candidates for conducting colorimetric analyses. If it were not for their severe toxicity, the title of this experiment most assuredly would include either "chromate" or "dichromate".

One of the important skills one practiced when conducting a colorimetric experiment with a chromate or dichromate compound was preparing solutions of very low molar concentration. A sodium dichromate solution of 5.00×10^{-4} M absorbs sufficiently strongly to conduct an accurate and precise Beer's Law experiment. Thus, the purpose of this experiment is twofold: (1) to prepare standard solutions of low molar concentrations; (2) to conduct a Beer's Law analysis of the standards and measure their effectiveness in determining the molar concentration of a solution of unknown concentration.

To replace a chromate or dichromate compound, one must identify a substance possessing low toxicity, high molecular mass, high water solubility, and bright color in aqueous solution. The choice in this experiment will be Erythrosin B, which is an FDA-approved (low toxicity) food dye (bright color, water soluble) with a molecular mass of 879.87 g/mol and a wavelength of maximum absorbance in the visible light range. Among other uses, Erythrosin B is used to color maraschino cherries. In fact, one of the objectives of the experiment will be to determine the amount of Erythrosin B found in a bottle of commercially available maraschino cherries.

Objectives

The objectives of this experiment are:

- Measure the absorbance spectrum of an Erythrosin B solution to determine the wavelength of maximum absorbance, λmax.
- Measure the absorbance of a set of Erythrosin B standard solutions at λmax.
- Determine the concentration of Erythrosin B in a container of maraschino cherries.

Materials

- ❑ Ocean Optics Spectrometer
- ❑ computer
- ❑ several cuvettes
- ❑ 1 dark cuvette
- ❑ 6 test tubes and test tube rack
- ❑ volumetric pipettes
- ❑ 100 mL volumetric flask
- ❑ Erythrosin B
- ❑ distilled water
- ❑ container of maraschino cherries
- ❑ small beakers
- ❑ plastic Beral pipettes
- ❑ balance, 0.001 g accuracy
- ❑ glassware as needed

Safety

Wear safety goggles and other appropriate safety gear (gloves or apron) during the experiment. Take all proper safety precautions when handling the solutions.

Pre-Lab Calculation

Calculate the mass of Erythrosin B needed to prepare 100 mL of 1.00×10^{-3} M solution.

Procedure

1. Prepare 100 mL of 1.00 × 10–3 M Erythrosin B solution. Use this solution as your stock solution. You will need to dilute this solution significantly to prepare the standard solutions, thus plan the most efficient method of preparing your standards and obtain the necessary concentrations.

2. Label five test tubes to use for your standard solutions. Use volumetric pipettes to measure your liquids. Complete the mixing chart based on you plans to use your stock Erythrosin B solution.

Test Tube	[Erythorsin B] (mol/L)	mL of dilute Erythrosin B	mL of distilled water
1	5.00×10^{-6}		
2	4.00×10^{-6}		
3	3.00×10^{-6}		
4	2.00×10^{-6}		
5	1.00×10^{-6}		

3. Obtain a small amount of the liquid from a container of maraschino cherries to serve as your unknown. Prepare a set of serial dilutions of this liquid so that you have one sample with a suitable absorbance for analysis.

4. Fill a cuvette ~⅔ full with distilled water to serve as your blank.

5. Use a USB cable to connect a spectrometer to your computer. Start the SpectraSuite software.

6. Calibrate the spectrometer.

    a. On the toolbar, check the box next to "Strobe/Lamp Enable". Allow the spectrometer to warm up for at least five minutes before proceeding.

    b. Place the blank cuvette in the spectrometer. Open the File menu and choose New → Absorbance Measurement. The "Set Acquisition Parameters wizard" will appear. This process has four steps. First, confirm that your spectrometer has been identified, and then click Next >.

    c. In step two of this process, make sure that the box next to "Strobe/Lamp Enable" is checked. Manually select a suitable integration time by using the arrow keys to increase or decrease the integration time, in milliseconds. Click Next > to proceed.

    d. To record the absorbance spectrum of the blank (100% transmittance), click the Store Reference Spectrum icon, . Click Next > to proceed.

    e. Remove the blank cuvette and place the dark cuvette in the spectrometer. To save the dark reference (0% transmittance), click the Store Dark Spectrum icon, .

    f. Pour out the distilled water from the blank cuvette, rinse, and fill it with the 5.00 × 10–6 M Erythrosin B solution in Test Tube #1. Place the cuvette in the spectrometer. Click Finish to complete the acquisition parameters wizard.

    g. A plot of absorbance vs. wavelength for your Erythrosin B solution is displayed. Examine the graph and select the peak wavelength that you wish to use for your Beer's law experiment.

7. Set up the Beer's Law experiment.

    a. Open the File menu and choose New → New Concentration Measurement. Select "Active Processing:", and then click Accept.

    b. The Concentration Measurement Setup wizard appears, and its default is the Beer-Lambert Law. Select "Calibrate from solutions of known concentration". Click Next >.

    c. The Range Selection defaults to a single wavelength. Enter the peak wavelength that you identified in Step 6, and then click Next >.

    d. The next dialog box sets up the Beer's law graph of your standard solutions. Type in the molar concentration of your first sample, 0.000005. Click Use Last Scan, and then click Add Sample. The absorbance of your first standard is saved and plotted on the small graph in the dialog box.

    e. Remove the cuvette and dispose of the solution as directed. Rinse and fill the cuvette with the Erythrosin B standard solution in Test Tube #2. Place the cuvette in the spectrometer. Type in the molar concentration, click Use Last Scan, and then click Add Sample.

f. Repeat Step e for the remaining standards. When you complete the final standard solution, type in the compound name and unit of concentration (mol/L). A best-fit line is calculated for your standards. Make sure the regression order is set to 1. Note: To delete a data point, choose a row of data and click Remove Sample. Click Finish to continue.

8. Write down the absorbance values and the best-fit line equation for your standard solutions in your data table.

9. Compare the serial dilutions of your unknown to your standard solution. Choose one of the dilute solutions to test. Rinse and fill the cuvette ~⅔ full with the unknown sample. Click Single Update. If the dilute solution has an absorbance that falls within the range of absorbance values you recorded for your standard solutions, write down the concentration in your data table.

10. Choose one or two other dilute solutions of your unknown to test in this way. The more measurements of absorbance and concentration for your unknown, the more accurate and precise your final determinations will be.

11. To close the SpectraSuite program, select File → Exit, and then click Yes.

Data Table

Test Tube	Concentration (mol/L)	Absorbance
1		
2		
3		
4		
5		
Unknown #1		
Unknown #2		
Unknown #3		

Best-Fit Line Equation: _____

Data Analysis

1. At which wavelength did you measure the absorbance of the Erythrosin B standard solutions? Was there another wavelength that you also could have used?

2. What was the concentration of the Erythrosin B in the container of maraschino cherries?

3. If Erythrosin B had been mixed with another food dye in the container of cherries, explain how you would analyze the liquid to determine the concentration of Erythrosin B.

8 Spectrophotometric Characterization of Spice Extracts

Introduction

Spices have a long a rich history around the world. In some senses (pun intended, although sorrowfully) the cuisine of a particular region identifies itself with specific local spices. Used to modify, in a positive fashion, the flavor of prepared foods, help preserve foods for later consumption, and act as herbal remedies for a wide variety of real and imagined ills, it is difficult to imagine a world without spices. Varieties of spices must indeed be the spice of life.

Along with the important role of contributing to the flavors of foods, spices are also employed to modify the color of foods. Turmeric adds a strong yellow tinge to curries and saffron offers a softer yellow to paellas. Paprika contributes a deep, vibrant red to chicken paprikash as well as presenting a vivid, flavorful spice garnish to simple cottage cheese. It is this property of spices, color, which you will investigate in this experiment.

You will use isopropanol, also known as rubbing alcohol, to prepare extracts of a selection of spices. You will then use an Ocean Optics spectrometer to measure the visible light absorbance spectrum of the spice extracts. And finally, you will test a few food products to determine whether or not a spice is present.

Objectives

In this experiment, you will

- Measure and analyze the visible light absorbance spectra of several spice extracts.
- Measure the visible light absorbance spectra of selected prepared foods.
- Analyze your data to identify the presence of specific spices added to the foods.

Materials

- ❑ Ocean Optics Spectrometer
- ❑ computer
- ❑ several cuvettes
- ❑ one dark cuvette
- ❑ 25 mL graduated cylinders
- ❑ several 50 mL beakers
- ❑ Plastic Beral pipettes
- ❑ spices, such as: paprika, turmeric, saffron, parsley, jalapeno, cumin, curry
- ❑ 70% rubbing alcohol
- ❑ unknown spice extract, prepared in
- ❑ 70% rubbing alcohol

Safety

Wear safety goggles and other appropriate safety gear (gloves or apron) during the experiment. Take all proper safety precautions when handling the solutions.

Procedure

1. Place a very small amount of each spice in a 50 mL beaker. Add 20 mL of rubbing alcohol (70% isopropanol). Swirl each beaker to mix the spices. Allow the mixtures to sit for 30 minutes so the colorants are well dissolved and any particulate matter settles to the bottom of the beakers.

2. Use a USB cable to connect a spectrometer to your computer. Start the SpectraSuite software.

3. On the toolbar, check the box next to "Strobe/Lamp Enable". Allow the spectrometer to warm up for at least five minutes before proceeding.

4. Fill a cuvette ~⅔ full with 70% rubbing alcohol to serve as a blank.

5. Calibrate the spectrometer and measure the absorbance of the spice extracts.

    a. Open the **File** menu and choose New → Absorbance Measurement. The "Set Acquisition Parameters wizard" will appear. This process has four steps. First, confirm that your spectrometer has been identified, and then click Next >.

    b. Place the blank cuvette in the spectrometer. In step two of this process, make sure that the box next to "**Strobe/Lamp Enable**" is checked. Manually select a suitable integration time by using the arrow keys to increase or decrease the integration time, in milliseconds. Click Next > to proceed.

    c. To record the absorbance spectrum of the blank (100% transmittance), click the Store Reference Spectrum icon, . Click Next > to proceed.

    d. Remove the blank cuvette and place the dark cuvette in the spectrometer. To save the dark reference (0% transmittance), click the Store Dark Spectrum icon, .

    e. Pour out the rubbing alcohol from the blank cuvette, rinse, and fill it ~⅔ full with the one of the spice extracts. Place the cuvette in the spectrometer. Click Finish to complete the acquisition parameters wizard. A plot of absorbance *vs*. wavelength for the extract is displayed

6. To save the plot of your first spice extract, follow these steps:

    a. Click to save the absorbance spectrum.

    b. In the Filename box, type in a name for your set of data.

    c. Click Browse... . Type in the name again and click Save.

    d. In the Save Spectrum dialog box, click Save, and then click Close. Your file will be saved in the default folder named "bin".

7. Pour out the extract from the cuvette, rinse, and fill it ~⅔ full with another spice extract. Place the cuvette in the spectrometer. Note that the absorbance plot for the yellow solution is displayed. Repeat Step 6 to save the plot.

8. Repeat Step 7 with your remaining spice extracts.

9. Obtain an unknown spice extract mixture. Repeat Step 7 to measure its absorbance spectrum.

10. Before you exit the SpectraSuite program, print copies of the absorbance spectrum graphs for each of your spice extracts and your unknown spice mixture. Alternately, keep SpectraSuite running and use it to analyze your graphs.

    To prepare a graph of any of the extracts, follow these steps:

    a. Open the File menu and choose Open → Load Processed Spectrum.

    b. The default "bin" folder will appear. Select a file and click Open. This will open the file, but not display the data on the graph yet.

    c. Click  (Overlay Spectral Data). In the Load Spectrum dialog box, click on the first file listed (it will be designated as "Processed").

    d. Click Load. Your selected file will be plotted on the graph.

    e. Repeat Steps a-d until you have the desired plots on your graph.

Data and Analysis

1. Print a copy of the absorbance spectrum for each of the spice extracts that you tested, as well as the unknown spice mixture. Note the distinguishing characteristics of the spectra that will help you identify the unknown.

2. Which spice, or spices, is contained in your unknown? Explain.

3. If distilled water had been used as the calibration blank for the chlorophyll test, would it have affected the absorbance measurements?

9 Determining the Manganese Concentration in a Steel Sample

Introduction

Steel is mostly iron, of course, but it can contain a variety of metal additives to modify its ductility, tensile strength, and other properties depending on the function the steel is to perform. One of the metals found in steel is manganese, which contributes to its tensile strength. However, the amount of manganese in steel is normally very small, less that 1% of the total mass of the steel. Thus, it is not easy to measure the amount in manganese in steel. One method does provide a suitable determination of the manganese concentration in steel, and that is a spectrophotometric analysis.

The method follows several steps in order to convert the manganese from its elemental form to an ion possessing a color that can be measured by a spectrometer. The first step is to dissolve a sample of steel in nitric acid, which oxidizes the Mn to Mn^{2+} ions, as shown in the reaction equation below:

$$3\ Mn + 2\ NO_3^- + 8\ H^+ \leftrightarrow 3\ Mn^{2+} + 2\ NO + 4\ H_2O$$

The product mixture is boiled and ammonium peroxydisulfate (also known as ammonium persulfate), $(NH_4)_2\ S_2O_8$ is added to remove the NO, a species that will interfere with the rest of the process. The reaction to cleanse the mixture of NO is:

$$2\ NO + 3\ S_2O_8^{2-} + 4\ H_2O \leftrightarrow 2NO_3^- + 6\ SO_4^{2-} + 8\ H^+$$

Unfortunately, when we knock out the NO we also disturb the Mn^{2+} ions. Thus, the next step involves adding sodium bisulfite, $NaHSO_3$, to reduce any manganese that was converted to MnO_2 by the ammonium peroxydisulfate. During the experiment, you will note the formation of MnO_2 as a brownish precipitate. It is hoped that a bare minimum of this precipitate forms. This step is done simply to prep the manganese for its final conversion to permanganate ion, MnO_4^-, and will not be shown here.

The final step, before you analyze the sample with an Ocean Optics spectrometer, is to oxidize the Mn^{2+} to MnO_4^- by adding potassium metaperiodate, KIO_4, as shown below:

$$2\ Mn^{2+} + 5\ IO_4^- + 3\ H_2O \leftrightarrow 2\ MnO_4^- + 5\ IO_3^- + 6\ H^+$$

Now the manganese is locked into an ion, MnO_4^-, that is a purplish color. By measuring the absorbance of several standard solutions of MnO_4^- with known concentrations you will be able to determine the amount of manganese in your sample of steel.

Objectives

The objectives of this experiment are:

- Chemically isolate the manganese in a sample of steel as MnO_4^-.

- Measure the absorbance spectrum of a standard MnO_4^- solution to determine the wavelength of maximum absorbance, λ_{max}.
- Measure the absorbance of several standard samples of MnO_4^- solution to establish a Beer's Law plot.
- Use the Beer's Law plot of the MnO_4^- standards to determine the concentration of manganese in a steel sample.

Materials

- ❑ Ocean Optics Spectrometer
- ❑ computer
- ❑ pipette pump or pippette bulb
- ❑ one cuvette
- ❑ four 125 mL Erlenmeyer flasks
- ❑ 50 mL graduated cylinder
- ❑ two 100 mL volumetric flasks
- ❑ two 50 mL volumetric flasks
- ❑ funnel and quantitative filter paper
- ❑ ice bath
- ❑ test tubes and test tube rack
- ❑ two steel samples, ~0.8 g each
- ❑ 6 M HNO_3 solution
- ❑ ammonium peroxydisulfate, $(NH_4)_2\ S_2O_8$
- ❑ sodium bisulfite, $NaHSO_3$
- ❑ potassium metaperiodate, KIO_4
- ❑ 2.00×10^{-4} M $KMnO_4$ solution
- ❑ concentrated phosphoric acid, H_3PO_4
- ❑ distilled water
- ❑ fume hood
- ❑ balance, 0.001 g accuracy
- ❑ hot plate

Safety

Wear safety goggles and other appropriate safety gear (gloves or apron) during the experiment. Take all proper safety precautions when handling the solutions.

Procedure

PART I Prepare and Test Standard Solutions of Potassium Permanganate

1. Prepare five standard **solutions** of potassium permanganate, $KMnO_4$, solution following the mixing chart below. Measure the volumes with pipettes and label five test tubes in which to store your standards.

Test Tube	[$KMnO_4$] (mol/L)	mL of 2.00×10^{-4} M $KMnO_4$ (aq)	mL of distilled water
1	2.00×10^{-4}	10.00	0.00
2	1.60×10^{-4}	8.00	2.00
3	1.20×10^{-4}	6.00	4.00
4	8.00×10^{-5}	4.00	6.00
5	4.00×10^{-5}	2.00	8.00

PART II Chemically Convert the Mn in a Steel Sample to MnO_4^-

2. Measure the mass of two steel samples, to the nearest 0.001 g. Write down the mass of each sample in your data table. Transfer each sample to its own 125 mL Erlenmeyer flask and place the flasks on a hot plate in a fume hood.

3. Transfer 40 mL of 6 M HNO_3 solution to each flask containing the steel samples. Heat the flasks to a gentle boil for about 5 minutes, and then cool the flasks to room temperature. Use an ice bath, if desired, to cool the samples more quickly.

4. Slowly and carefully add 0.1 g of ammonium peroxydisulfate to each flask and gently boil the reaction mixtures for 5 more minutes, followed by cooling to room temperature.

5. Add 0.1 g of sodium bisulfite to each flask, boil the mixtures for 5 minutes, and cool them to room temperature. If the solutions are clear, then quantitatively transfer each solution to its own 100 mL volumetric flask and fill the flask to the line with distilled water. If the solutions are not clear, filter each solution through quantitative filter paper to collect the residue and then quantitatively transfer the solutions to 100 mL volumetric flasks. Stopper the flasks and invert them a few times to mix the solution.

6. Use a pipette to transfer a 25.00 mL aliquot of solution from each of the volumetric flasks to two 125 mL Erlenmeyer flasks. Add 4 mL of concentrated H_3PO_4 to each 25 mL aliquot. Add 0.4 g of potassium metaperiodate, KIO_4 and gently boil the flasks for 2 minutes. Cool the flasks of mixtures to room temperature.

7. Quantitatively transfer the aliquots to separate 50 mL volumetric flasks. These samples are now ready to be tested. Stopper the flasks and set them aside until Part IV of the experiment.

PART III Measure the Absorbance of the Standard MnO_4^- Samples

8. Fill a cuvette ~⅔ full with distilled water to serve as your blank.

9. Use a USB cable to connect a spectrometer to your computer. Start the SpectraSuite software.

10. Calibrate the spectrometer.

    a. On the toolbar, check the box next to "**Strobe/Lamp Enable**". Allow the spectrometer to warm up for at least five minutes before proceeding.

    b. Place the blank cuvette in the spectrometer. On the left hand side of the toolbar, manually increase or decrease the integration time so that the peak absorbance is slightly below 4,000 counts.

    c. Record the absorbance spectrum of the blank (100% transmittance), by clicking the Store Reference Spectrum icon,  .

    d. Remove the blank cuvette and place the dark cuvette in the spectrometer. Save the dark reference (0% transmittance), by clicking the Store Dark Spectrum icon,  .

e. Remove the dark cuvette from the spectrometer. Empty the distilled water from the blank cuvette, rinse and fill the cuvette ~⅔ full of the $KMnO_4$ solution from Test Tube #5, and place the cuvette in the spectrometer. Identify the wavelength of maximum absorbance (λ_{max}). Write down the λ_{max}, and the absorbance value, in your data table.

11. Measure the absorbance of the standard $KMnO_4$ solutions.

    a. Open the File menu and choose New → New Concentration Measurement. Select "Active Processing:", and then click Accept.

    b. The Concentration Measurement Setup wizard appears, and its default is the Beer-Lambert Law. Select "Calibrate from solutions of known concentration". Click Next >.

    c. The Range Selection defaults to a single wavelength. Enter the peak wavelength that you identified in Step 6, and then click Next >.

    d. Type in the molar concentration of Test Tube #1 solution, 0.0002. Click Use Last Scan, and then click Add Sample. The absorbance of your first standard is saved and plotted on the small graph in the dialog box.

    e. Remove the cuvette and dispose of the solution as directed. Rinse and fill the cuvette with the KMnO4 standard solution in Test Tube #2. Place the cuvette in the spectrometer. Type in the molar concentration, click Use Last Scan, and then click Add Sample.

    f. Repeat Step e for the remaining standards. When you complete the final standard solution (2.00 × 10–3 M), type in the compound name and unit of concentration (mol/L). A best-fit line is calculated for your standards. Make sure the regression order is set to 1. Note: To delete a data point, choose a row of data and click Remove Sample. Click Finish to continue.

PART IV Measure the Absorbance of the Treated Steel Samples

12. Choose one of the two samples, stored in a 50 mL volumetric flask, to test. Using the same cuvette with which you measured the standard solutions, rinse and fill the cuvette ~⅔ full with the liquid from the volumetric flask. Click **Single Update** and write down the concentration of the first aliquot of treated steel.

13. Repeat Step 12 with the second sample in the 50 mL volumetric flask.

14. (optional) Upon the direction of your instructor, repeat Step 6 and 7 in Part II to prepare additional samples of the treated steel and test them as you did the first two samples.

15. To close the SpectraSuite program, select File → Exit, and then click Yes.

Data

PART III Standard MnO4– Samples

Wavelength of maximum absorbance (λ_{max}) = ___ nm

Test Tube	Concentration(mol/L)	Absorbance
1		
2		
3		
4		
5		

PART IV Treated Steel Samples

Steel Sample	Mass	Abs at λ_{max}	[MnO_4^-]
1			
2			

Data Analysis

1. Calculate the mass of manganese in the treated steel samples.

2. Calculate the concentration of manganese in the steel samples. Express your answer in mass of manganese per gram of steel.

3. Describe the sources of error inherent in this process of determining Mn in steel.

4. In Step 6 (Part II), concentrated phosphoric acid was added to the final treated samples so that any iron that found its way through the process would be rendered clear to the spectrometer. However, in some types of steel chromium and/or cerium are present. These two elements interfere in a very problematic manner because their absorbance maxima are very close to that of the MnO4– ion. Suggest a method for dealing with steel samples that contain Cr and Ce, when your goal is to determine the Mn concentration.

10 Investigating the Absorbance Spectra of Iodine

Introduction

A spectrometer is normally employed to measure the absorbance or % transmittance of a liquid sample bearing a color or absorbing light in the ultraviolet range outside the range of human eyes. In addition, an Ocean Optics spectrometer can measure light that is emitted from a source such as a discharge tube, light that is reflected off a solid surface, or light that resulted from fluorescing or luminescing materials. It can also measure gases, although other devices are more commonly used in these instances.

Say, for instance, that a very small amount of a solid was placed in a standard cuvette and the cuvette was sealed. If the solid sublimed at room temperature, and if the gas produced by the sublimation was also colored, then perhaps a spectrometer could measure the absorbance of the gas molecules. There is such a solid substance, iodine (I2). And, as it so happens, quite a lot can be learned by the simple measurement of the graph of data that appears when the visible light absorbance spectrum of iodine is analyzed.

The absorbance spectrum of gaseous iodine presents a set of data unlike that of a liquid sample, which is not a huge surprise, but is quite similar to that of emissions from a gas discharge tube. This makes perfect sense when you consider that the light source in the spectrometer is an excitation source for the gas molecules. An absorbance spectrum of iodine in solution can be easily achieved, as iodine is soluble in a variety of common solvents, including: ethanol, diethyl ether, chloroform, and glycerol.

Objectives

The objectives of this experiment are:

- Measure the visible light absorbance spectrum of gaseous iodine, I_2.
- Measure the visible light absorbance spectrum of iodine dissolved in ethanol.
- Compare and contrast the iodine spectra.

Materials

- ❑ Ocean Optics Spectrometer
- ❑ computer
- ❑ empty glass cuvette
- ❑ dark cuvette
- ❑ sealed glass cuvette of iodine vapor
- ❑ sealed glass cuvette of solution of iodine in ethanol

Safety

Do not open the sealed cuvettes. The vapors are harmful, and many people are allergic to iodine.

Procedure

1. Obtain an empty glass cuvette and two sealed glass cuvettes: one containing iodine vapor and the other containing iodine dissolved in ethanol. There may be a tiny nugget of solid iodine in the bottom of one cuvette; this will not affect your absorbance spectrum readings.

2. Use a USB cable to connect a spectrometer to your computer. Start the SpectraSuite software.

3. On the toolbar, check the box next to "Strobe/Lamp Enable". Allow the spectrometer to warm up for at least five minutes before proceeding.

4. After the spectrometer has warmed up, place the empty glass cuvette in the spectrometer. On the left hand side of the toolbar, manually increase or decrease the integration time so that the peak absorbance is slightly below 4,000 counts.

5. Record the absorbance spectrum of the blank (100% transmittance), by clicking the Store Reference Spectrum icon,  .

6. Remove the empty glass cuvette and place the dark cuvette in the spectrometer. Save the dark reference (0% transmittance), by clicking the Store Dark Spectrum icon,  .

7. Remove the dark cuvette from the spectrometer. Place the cuvette of iodine vapor in the spectrometer.

8. Follow the steps below to save the graph of the iodine vapor absorbance:

    a. Click  to save the absorbance spectrum.

    b. In the Filename box, type in a name for your graph.

    c. Click  . Type in the name again and click  .

    d. In the Save Spectrum dialog box, click  , and then click  . Your file will be saved in the default folder named "bin".

9. Remove the iodine vapor cuvette from the spectrometer. Place the cuvette of iodine-in-ethanol solution in the spectrometer.

10. Repeat Step 8 to save the graph of the iodine solution absorbance.

11. To close the SpectraSuite program, select File → Exit, and then click  .

Data Analysis

1. Print a copy of each of your two absorbance vs. wavelength graphs.

2. Examine the graph of absorbance vs. wavelength for iodine vapor. Speculate about the unusual plot of absorbance for the vapor.

3. Noting that the light source of the spectrometer can present excitation energy for the vapor, make a case for the plot of gaseous iodine representing quantum movement.

4. Describe the absorbance spectrum of the iodine solution. Do any of the absorbance peaks relate to the graph of iodine vapor? Explain.

Extension

The measure the absorbance of gaseous iodine is described in an excellent article published in the Journal of Chemical Education in 1987 – volume 64, number 11 (November), pages 919 – 921. Secure a copy of this article, and use your data from the absorbance spectrum of gaseous iodine to complete the following calculations:

(a) the fundamental oscillating frequency (ω)

(b) the force constant of the I – I bond (k)

(c) the anharmonicity constant (x)

(d) the dissociation energy (Do)