ULTRAVIOLET-VISIBLE ABSORPTION SPECTROPHOTOMETRY
Properties and Errors in Spectrophotometric Systems

The fundamentals of ultraviolet-visible spectrophotometry have been demonstrated to you in past chemistry classes and laboratory experiments. You have explored the fundamental absorbance processes, dealt with absorbance measurements quantitatively using the Beer-Lambert law, prepared samples for analysis, and perhaps even made two-component quantitative measurements. These analyses and explorations were possible only because the analyte(s) conformed to several desired properties. These properties included

1) stability in solution,
2) adherence to Beer's law,
3) large molar absorptivities,
4) solubility of the analyte in the sample, and
5) sufficient separation of the desired analyte absorbance wavelength from interfering substances.

What do you do if you are required to measure a substance which does not conform to the ideal conditions? Many instances arise when a substance does not posses properties which are conducive to accurate optical measurement. In these cases, the substance is usually converted, by means of a chemical reaction using a chromogenic reagent, into a new species which has the desirable properties to quantitative spectrophotometry. The reaction can be illustrated with the following equation.

SAMPLE + CHROMOGENIC REAGENT $\rightarrow$ UV-VIS ABSORBING PRODUCT

The choice of a chromogenic reagent also has a great deal of importance in your analytical scheme. The reagent used should have as many of the following specific properties as possible. These properties include:

1) stability of the chromogenic reagent in solution,
2) rapid color development,
3) stoichiometric reactivity with the desired reagent,
4) transparency in the wavelength region,
5) selectivity or specificity to the sample reagent,
6) freedom from interference by other solution constituents which may or may not be present in all samples, and
7) capacity to function in a common solvent.

In this experiment we will explore some of the properties of chromogenic reagents and their usefulness in molecular spectrophotometry. In addition, using the iron(III)-thiocyanate complex, we will investigate instrumental performance characteristics and potential instrument errors which are commonly encountered in the ultraviolet-visible region.

EXPERIMENTAL PROCEDURE

Be sure to read the entire procedure before starting the experiment! If you follow the procedure step-by-step, you will not make use of your time effectively and it will be impossible to complete the experiment in the expected three hours.

Reagents:

- Iron (III) stock solution (~0.001 M Fe^{3+})
- Saturated NH_4SCN
- 0.5 M NH_4SCN
- Concentrated HCl
- 4 M NaOH
- Sodium Fluoride
- Sodium Oxalate
- Sodium Tartrate
- Potassium Dihydrogen Phosphate

I. Determination of the Wavelength of Maximum Absorbance:

Pipet 5 mL of the stock Fe^{3+} solution (~0.001 M) and 3 mL of saturated NH_4SCN into a clean 100 mL volumetric flask and dilute to volume. Scan the solution with the UV-VIS spectrophotometer to determine the wavelength of maximum absorbance and potential interferences. Did the wavelength appear where you expected it to be?

II. Effect of Time on Absolute Absorbance in the Iron(III)-Thiocyanate System:

Measure the absorbance versus time for the solution in Part I at approximately 30 minute intervals for a period of two hours. Plot absorbance versus time for all measurements made. Discuss the effects of time upon this system and the general need for monitoring time when performing these measurements.

III. Effect of Excess Reagent on the Iron(III)-Thiocyanate System:

Prepare solutions of Fe^{3+} containing varying amounts of SCN-. The solutions are made by pipetting the following quantities of stock Fe^{3+} and 0.5 M NH_4SCN into 100 mL volumetric flasks and diluting to the mark with distilled, deionized water.

mL of stock Fe^{3+}	mL NH_4SCN	Ratio SCN/ Fe^{3+}
5	0.3	30
5	0.8	80
5	3.0	300
5	10.0	1000
5	20.0	2000
5	30.0	3000

You may measure the larger quantities (10, 20, and 30 mL) in graduated cylinders. Make up one solution at a time and measure the absorbance as soon after the preparation as possible. It is recommended that this measurement be done consistently at the same time intervals after the addition and dilution of each solution. Use water as the blank for each measurement. Plot the absorbance vs. SCN-/ Fe^{3+} ratio for all the measurements made in this part.

IV. Effect of pH on Absolute Absorbance in the Iron(III)-Thiocyanate System:

a. pH=0: Note that the stock Fe^{3+} solution contains 0.5 M HCl. To 4 mL of Fe^{3+}, accurately pipetted into a 100 mL volumetric flask, add 2 mL of saturated NH_4SCN and 8 mL concentrated HCl, so that the final H+ concentration will be ~ 1 M after dilution to 100 mL. Mix thoroughly and measure the absorbance of the solution.

Note: Because of the fading of the color of the complex with time, the solutions of different pH should be prepared one-at-a-time and measured before making the next solution.

b. pH=1: To 4 mL of the stock Fe^{3+}solution in a 100 mL volumetric flask, add 2 mL saturated NH_4SCN and 13 drops of concentrated HCl. Dilute to the mark with distilled, deionized water. Obtain the absorbance and go directly to part V, below.

c. pH>1: Prepare solutions of various pH values in the following manner. To 4 mL of stock Fe^{3+} solution and 2 mL of saturated NH_4SCN in 100 mL volumetric flasks, add respectively 0 drops, 7 drops, and 9 drops of 4 M NaOH solution. Dilute to the mark and measure the absorbance of each. Test the pH of each solution.

Plot the absorbances versus pH for all of the measurements. Discuss the results.

V. Effect of Anions on Absolute Absorbance in the Iron(III)-Thiocyanate System:

After you measure the absorbance of the pH=1 solution in part IV, add a very small portion (about the size of a single grain of sugar) of NaF to the solution in the cuvette. Mix vigorously, covering the cuvette with parafilm, and measure the absorbance of the solution again. IMMEDIATELY RINSE THE CUVETTE THOROUGHLY WITH DISTILLED, DEIONIZED WATER AND YOUR pH=1 SOLUTION. Then add a small quantity of sodium oxalate. Shake and repeat the measurement. Repeat the procedure using sodium tartrate and potassium dihydrogen phosphate.

Report your observations as to the effect of each of the salts upon the absorbances of the iron(III)-thiocyanate solution. Discuss the merits of the thiocyanate ion as a colorimetric reagent for the analysis of iron. Be certain to include all pertinent factors that can influence the analysis.

VI. Linearity of the Beer-Lambert Law:

Make a series of standard concentration solutions (~5-6) of Fe^{3+} ranging from 1.0×10^{-3} M to 1.0×10^{-5} M. Develop the complex using the optimum quantities of reagents previously noted. Measure the absorbance of each of these solutions and plot absorbance versus concentration. Does your data adhere to the Beer-Lambert Law? What errors (chemical and instrumental) might limit the validity of the Beer-Lambert Law?

VII. Sensitivity of the Iron(III)-Thiocyanate System:

Set up a measurement to allow calculation of the sensitivity (in milligrams per liter and part-per-million per 0.01 absorbance units) for the iron(III)-thiocyanate complex. Use 2 mL of saturated NH_4SCN solution. Determine the detection limit of this method in ppm using experimental data. (A minimum of five blank readings) The reagent blank solution for the detection limit study should be prepared exactly the same as your iron(III)-thiocyanate solutions with the exception of including NO iron(III).

QUESTIONS

1. Describe the type of chromophore involved in the iron(III)-thiocyanate complex as it is related to the absorption of visible light.

2. What effect will the slit width of the spectrophotometer optics have on: a) stray light, b) molar absorptivity of the complex, c) calibration linearity?

3. What could cause a non-zero calibration intercept in a Beer-Lambert plot (using linear regression)?

4. Why use a reagent blank in a quantitative measurement?

5. Based on your results from this experiment, outline the considerations which must be made to allow for the accurate and precise determination of Fe^{3+} in tap water using UV-Vis spectrophotometry and thiocyanate as a chromogenic reagent. Briefly comment on the importance of each consideration.