**Spectrophotometry**

**C. P. P. Uy1**

*1Chemical Engineering, College of Engineering*

*University of the Philippines, Diliman, Quezon City, Philippines*

Date Performed: 06/19/13

Instructor’s Name: Mariecris Banez

**ABSTRACT**

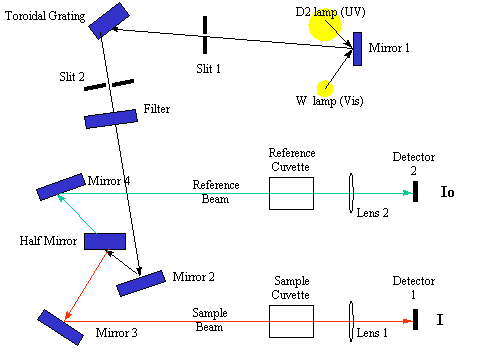
*Spectrophotometry can be used to determine the concentration of a chromophore by measuring the amount of light it absorbs at the wavelength of maximum absorbance and relating it with the Beer-Lambert's Law equation. In this experiment, the light absorbance of various concentrations of FeSCN2+ was measured using a UV-Vis spectrophotometer to get its concentration, and consequently the experimental Keq of the related reaction. This resulted in a Keq value with a 53.24% difference with its literature value. Due to this, the principle of spectrophotometry was proved to be a delicate but valid technique in determining chromophore concentration.*

**INTRODUCTION**

Spectrophotometry is concerned with how much light a certain chemical absorbs or transmits. The idea behind this is how each compound absorbs or transmits a distinct amount of light "over a certain range or wavelength"[1]. Through spectrophotometry, a chemical can be analyzed quantitatively for the concentration of light-absorbing substance it has.

This quality proves spectrophotometry to be useful over a wide range of fields, including but not limited to chemistry, biology, chemical engineering and medicine. For example, spectrophotometry can be used to determine the concentration of chlorophyll in the reactions in the processes of a certain plant. It can also be used to analyze blood or other highly pigmented fluids in people to diagnose for sickness. Because these substances are highly pigmented, they absorb light energy at a certain wavelength. The wavelength at which they absorb color from can be estimated by determining the color complementary to the observed color of the liquid: in the former example, chlorophyll can be said to absorb light at wavelengths nearing red (~700 nm) due to its green coloring.[1]

The absorbed light and the quantity absorbed are determined with the use of a spectrophotometer. There are two kinds of spectrophotometers, the UV-visible spectrophotometer which records light over the ultraviolet range (185 - 400 nm) and visible range (400 - 700 nm) of electromagnetic radiation spectrum, and the IR spectrophotometer, which uses light over the infrared range (700 - 15000 nm) of electromagnetic radiation spectrum.[1]



**Figure 1.** Optical System of a Spectrophotometer.

The spectrophotometer operates by passing a monochromatic ("light in which photons all have the same wavelength"[4]) beam of light through the sample of the analyte. The analyte is held in a container made out of a clear material such as plastic or quartz known as the cuvette. Afterwards, the light transmitted by the chemical is recorded. The spectrophotometer then "subtracts" the intensity of the transmitted light at the wavelength for which it has been calibrated to the intensity of the original beam of light to arrive at the amount of light absorbed by the chemical. Its basic mechanism can be seen through figure 1 above, which is enlarged in the Appendix.

A = εbc

**Equation** **1.** Beer-Lambert’s Law Equation

The amount of light absorbed and the concentration of the pigmented chemical can be relation through Beer-Lambert's Law (Equation 1). In this equation, A is the measured absorbance, ε stands for the molar absorptivity coefficient (determined experimentally), b is the path length in centimeters (length of the cuvette) and c is the molar concentration (also called the molar extinction coefficient[6]) of the analyte used.

A = εbc + Ao

**Equation** **2.** Equation of the Regression Curve formed by Beer-Lambert’s Law

Through performing a series of controlled tests using an analyte of known concentration, the molar absorptivity coefficient may be attained. The results can be graphed into a linear function (Equation 2). The addition of Ao is to account for errors in the data. While theoretically, Beer-Lambert's law should consist of only the variables in Equation 1, Ao is added in Equation 2 to account for possible errors (i.e., the cuvette material, fingerprints, or the water itself absorbing some of the light).

After getting the function of the line, solutions with the chromophore (the part of the solution that absorbs a significant amount of light) in it can be analyzed for concentration of the substance. This is the most often application for spectrophotometry. Through this information, various other data can be extrapolated.

In this experiment, spectrophotometry and Beer-Lambert's law were employed to indentify the analytical wavelength, equilibrium concentration and experimental Keq of FeSCN2+. The resulting data was compared with literature data and calculated for %error to measure for accuracy.

**METHODOLOGY**

**Part 1: Solution Preparation**

For the first part of the experiment, various stock solutions in different quantities were prepared from solids or solutions of higher concentration, specifically: (1) 1000 mL of 0.1 M HCl prepared from concentrated HCl; (2) 50 mL of 0.2 M KSCN solution prepared from solid KSCN; (3) 100 mL of 0.0002 KSCN prepared from solution (2); (4) 50 mL of 0.2 M FeCl3 prepared from solid FeCl3; and (5) 100 mL of 0.002 M FeCl3 prepared from solution (4).

Solution (2) was prepared by first weighing an appropriate amount of KSCN in a 50-mL beaker. About 20 mL of solution (1) was added. The mixture was then stirred to dissolve the crystals. Once all the crystals were dissolved, the mixture was transferred into a 50-mL volumetric flask and diluted to the mark with additional 0.1 M HCl. Solution (4) was prepared in the same manner, using an appropriate amount of FeCl3 solids in place of the KSCN solids. Solutions (3), (4), and (5) were diluted to their appropriate volume with solution (1).

**Table 1.** Preparation of Standard Solutions

|  |  |  |  |
| --- | --- | --- | --- |
| Solution | 0.002 M FeCl3 (mL) | 0.20 M KSCN (mL) | 0.10 M HCl (mL) |
| Blank | 0 | 1.00 | 9.00 |
| Standard 1 | 0.10 | 1.00 | 8.90 |
| Standard 2 | 0.25 | 1.00 | 8.75 |
| Standard 3 | 0.50 | 1.00 | 8.50 |
| Standard 4 | 1.00 | 1.00 | 8.00 |
| Standard 5 | 2.00 | 1.00 | 7.00 |

**Table 2.** Preparation of Unknown Solutions

|  |  |  |  |
| --- | --- | --- | --- |
| Solution | 0.002 M FeCl3 (mL) | 0.002 M KSCN (mL) | 0.10 M HCl (mL) |
| Blank | 0 | 5.0 | 5.0 |
| Unknown 1 | 3.0 | 5.0 | 2.0 |
| Unknown 2 | 4.0 | 5.0 | 1.0 |
| Unknown 3 | 5.0 | 5.0 | 0.0 |

The standard solutions were then prepared from the stock solutions. Table 1 shows all of the standard solutions to be made. The unknown solutions were also prepared, with each solution adequately described in Table 2.

**Part 2: Calibration of the UV-Vis Spectrophotometer**

*(Before calibration began, full understanding of how to properly use the Single-beam UV-Vis Spectrophotometer was necessary. Upon understanding, calibration was then started.)*

To begin, the cuvette was rinsed three times with distilled water. (Note: No shaking or tapping was done on the cuvette as it is fragile.) The cuvette was filled with the standard blank solution up to the mark, and AUTOZERO was then performed. To determine the wavelength of maximum absorption, Standard 5 was used as the value to which all subsequent readings were done.

The cuvette was removed from the spectrophotometer. Its contents were emptied into a waste beaker. After rinsing three times with distilled water, the cuvette was then rinsed with Standard 1. Its contents were disposed, and it was refilled with Standard 1 up to the mark. The sides of the cuvette were wiped with tissue paper to remove any moisture or smudges. The spectrophotometer was opened and the cuvette was placed inside the sample holder. After closing the spectrophotometer, the absorbance reading was recorded. These series of steps was then repeated for Standards 2 to 5.

**Part 3: Determination of Equilibrium Constant for the Formation of FeSCN2+**

After reading the absorbance for the standard solutions, the unknown solutions were then recorded. The cuvette was rinsed three times with distilled water, and then filled with the blank of the unknown solutions. The sides of the cuvette were wiped with tissue paper. AUTOZERO was performed.

The cuvette was emptied of the blank solution, rinsed thrice with distilled water, and rinsed with the Unknown 1 solution. It was filled with Unknown 1 up to the mark. After wiping the sides with tissue paper, its absorbance was also recorded. These steps were repeated using the Unknown solutions 2 and 3.

**RESULTS AND DISCUSSION**

The experiment aimed to determine the absorbance of FeSCN2+. However, as Fe3+ is yellow in solution. HCl was then introduced as the diluting chemical to enable the formation of FeCl3 through hydrolysis with HCl. This turns what would have been the yellow of Fe3+ alone into a clear color because of the hydrolysis reaction. In this way, only the FeSCN2+ was had a color in the solution, making it the sole chromophore.

In calculations, it was assumed that the Fe3+ reacted completely to form FeSCN2+ so as to determine the concentration of FeSCN2+ for the Standard solutions. This was done because the FeCl3 was the limiting reactant and therefore assumed to have been completely reacted. KSCN was also added in large excess, making the equilibrium of the reaction go forward. This left very little, if any, reactant in the standard solutions.

FeSCN2+'s maximum absorbance was machine recorded at the wavelength of 452 nm. As this figure is close to the literature value of 447 nm, accuracy of the recorded observations concerning the absorbed light can be assured. 452 nm is also seen as greenish-blue in visible light, which makes the absorbed spectrum of light logical as it is a color complementary to the reddish-orange hue of FeSCN2+. Absorbance should be always be measured at (or near to) the analytical wavelength to ensure that the maximum absorbance of the analyte is recorded.

|  |  |  |
| --- | --- | --- |
|  | [FeSCN2+] | Absorbance |
| Standard 1 | 0.00002 M | 0.007 |
| Standard 2 | 0.00005 M | 0.074 |
| Standard 3 | 0.0001 M | 0.132 |
| Standard 4 | 0.0002 M | 0.324 |
| Standard 5 | 0.0004 M | 0.668 |

**Table 3.** Results for Standard Solutions

The following values for the standard solutions obtained can be seen in the table above (Table 3). With the concentrations of the standard solutions as the values for x and the absorbed light as the value for y plugged into the Beer-Lambert's Law Equation, a regression line of y = 1733.x - 0.025 was attained. With its r2 having a value of .998, the r2 is sufficiently close to 1 to validate the regression line equation stated above as the best fit line.

Through the regression line equation, the molar extinction coefficient was attained at the value of 1733 and the y-intercept at 0.025. The literature values for FeSCN2+'s molar coefficient is 4.5 x 103 cm-1 mol-1. The experimental value has a 61.49% difference with the literature value. The y-intercept of 0.025 could be a possible error in the absorbance reading as discussed in the introduction.

**Graph 1.** The calibration regression line

The graph of the regression line equation is shown above, with a more detailed version in the Appendix. Through this graph, we can conclude that more light is absorbed as the concentration of the chromophore is increased. As the solution gets more colored, more light is absorbed. This follows with the theory that a solution that absorbs all light in the visible spectrum appears to be black, and a solution that does not absorb light in the visible spectrum appears to be white[4].

**Table 4.** Results for Unknown Solutions

|  |  |  |
| --- | --- | --- |
|  | [FeSCN2+] | Absorbance |
| Unknown 1 | 0.000170 M | 0.270 |
| Unknown 2 | 0.000203 M | 0.327 |
| Unknown 3 | 0.000259 M | 0.424 |

In the unknown solutions, a molar concentration of 0.002 M of KSCN was used. In comparison with the molar concentration of the KSCN in the standard solutions which is 0.2 M, this is a very small amount. This is to prevent the reaction from getting pushed forward by the relatively large amount of moles KSCN has. For the concentration of FeSCN2+ in the unknown solutions: through plugging in the values of absorbance in the regression line equation, the data in the table above (Table 4) was calculated.

By using the concentration values of FeSCN2+ at equilibrium from Table above, the concentration values for Fe3+ and SCN- could also be calculated by subtracting [FeSCN2+] from the initial concentrations of Fe3+ and SCN-. Through this Keq was calculated. The average Keq calculated was 458.22. In comparison with the literature value of the reaction Fe3+(aq) + SCN-(aq) ⇌ FeSCN2+(aq) which is 9.8 x 102 at 25 ºC [13], there was a 53.24% difference.

Various factors may have contributed to the relatively wide gap between the literature value with the experimental value. The literature value was recorded at 25 ºC, which may be different from the room temperature at which the solutions were measured for absorbency. Due to this, the actual value may differ.

Other factors may be from the improper handling of the solutions during the experiment. The solutions may have a slightly different concentration from the way they were prepared. It is also possible that the solutions were not mixed thoroughly, resulting in unequal distribution of the substances. These factors may increase or decrease the Keq value of a reaction.

The equipment may also have been improperly handled. The cuvette is very fragile, so the steps taken in rinsing the solution from the cuvette may not have been completely thorough. This can increase or decrease the concentration of the solution.

In filling the cuvette, errors may also have been made. The exteriors of the cuvette may have had fingerprints or moisture, which can affect the amount of light transmitted as they also absorb light. The walls of the cuvette may have also absorbed a small amount of light. An air bubble in the solution may have been present at the time of testing for light absorbance. Because of the difference in density of air and liquid, some of the light may have been refracted and thus not transmitted.

Machine errors may also be possible. During the experiment, it took several tries for the machine to correctly register the wavelength for maximum light absorbancy. This may be due to the age of the machinery used. However, the possibility of this is slight, as the UV-Vis Spectrophotometer used was recent and up-to-date.

**CONCLUSIONS AND RECOMMENDATIONS**

In this experiment, spectrophotometry was used to attain values for the [FeSCN2+]eq of the unknown solutions; consequently, the values for [Fe3+]eq and [SCN-]eq, and Keq were also attained. The experiment was successful; having experimental Keq values close enough to the literature values to prove the principle of spectrophotometry. However, the significant %difference of the two points out the delicate nature of spectrophotometry, which can only be performed accurately with careful precision and properly working machines.

During the preparation of stock solutions, rusty and aged weighing equipment was used. According to information, the equipment was accurate only up to two decimal points. This may have affected the concentrations of FeCl3 and KSCN, especially for FeCl3 in which such a small concentration was prepared. More accurate equipment is recommended for usage during the weighing of the reagents.

Spectrophotometers are expensive, which proves as a limitation to this experiment. Should the costs not be an obstacle, it is recommended to use a double-beam UV-Vis spectrophotometer for more accurate results.

The fragility of the cuvette may also be an issue. The washing of the cuvette calls for no shaking or stirring motions, which greatly impedes cuvette cleaning. Residual chemicals may be present in the cuvette which may affect the light absorbance readings. To assure more thorough cleaning, however, the method for washing the cuvettes may be modified, provided that the researchers have ample skill to clean the cuvette thoroughly without breaking it.

There was a significant Keq variation among the three unknown solution. To account for this, a greater number of unknown solutions are also recommended.

**REFERENCES**

[1] *Beer's law scatter plot and trendline (linear regression) - Biochemistry & ComputerScience.* biochemcs.com. Retrieved Last: July 31, 2013.

[2]  *UV-VIS Spectrophotometer.* bouman.chem.georgetown.edu. Retrieved Last: July 31, 2013.

[3] *Beer's Law Tutorial.* chem.ucla.edu. Retrieved Last: July 31, 2013.

[4] *Spectrophotometry*. chemwiki.ucdavis.edu. Retrieved Last: July 31, 2013.

[5] *Spectrophotometry: Absorbance Spectrum.* chm.davidson.edu. Retrieved Last: July 31, 2013.

[6] *Laboratory 6: Beer's Law.* lab\_handout\_Lab\_Lecture\_6.pdf. depts.washington.edu. Retrieved Last: July 31, 2013.

[7] *Determination of Equilibrium Constant.* files.chem.vt.edu. Retrieved Last: July 31, 2013.

[8] *Spectrophotometry for Quantitative Analysis.* Beers\_Law\_Spectrophotomotery.pdf. highered.mcgraw-hill.com. Retrieved Last: July 31, 2013.

[9] *Spectrophotometry.* ruf.rice.edu. Retrieved Last: July 31, 2013.

[10]  *UV-Vis Absorption Spectroscopy - Instrumentation.* teaching.shu.ac.uk. Retrieved Last: July 31, 2013.

[11] *UV-Visible Spectroscopy.* www2.chemistry.msu.edu. Retrieved Last: July 31, 2013.

[12] *Biology Laboratory Manual: The Importance of Spectrophotometry.* highered.mcgraw-hill.com. Retrieved Last: July 31, 2013.

[13] Petrucci, Ralph H.,William S. Harwood, and Geoffrey F. Herring (2002). *8th edition*. General Chemistry. Prentice-Hall.

[14] Zumdahl, Steven S. (2009). *6th edition.*Chemical Principles. Houghton Miffin.

[15] bilbo.chm.edu/CHM112/ tables/Kftable.htm. Retrieved Last: July 31, 2013.

[16] *Spectrophotometry: Analysis of an Unknown Solution.* chm. davidson.edu. Retrieved Last: July 31, 2013.

[17] *Spectrophotometry: Basic Principles.* chm.davidson.edu. Retrieved Last: July 31, 2013.

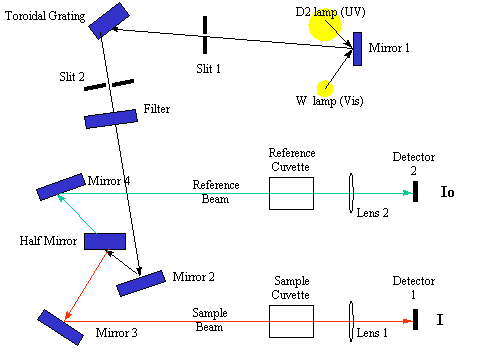
[18] *Spectrophotometry: Beer's Law.* chm.davidson.edu. Retrieved Last: July 31, 2013.

**APPENDIX**

|  |  |  |
| --- | --- | --- |
| Error | determinate/  indeterminate | gross/  systematic/  random |
| The machines used for weighing the FeCl3 and the KSCN are old; they may have been inaccurate. | determinate | gross |
| The solutions may have been prepared carelessly resulting in an actual concentration lower or higher than the intended value. | determinate | gross |
| The steps taken in rinsing the cuvette may not have been completely thorough, affecting the concentration of the solutions placed in the cuvette. | determinate | gross |
| The exteriors of the cuvette may have had fingerprints. The fingerprints may have absorbed light. | determinate | systematic |
| The cuvette may not have been wiped thoroughly with tissue; moisture may have been present. The moisture could have absorbed light. | determinate | systematic |
| Tiny pieces of tissue may have been dislodged into the exterior surface of the cuvette, blocking light. | determinate | systematic |
| There may have been an air bubble in the solution at the time of testing for light absorbance, refracting light. | determinate | systematic |
| The machine may have been flawed, registering a value different from the actual. | determinate | systematic |
| The room temperature was not exactly 25o C, the temperature in which literature values are often recorded. | determinate | random |

**Table 5.** Possible Sources of Error

**Graph 1.** The calibration regression line



**Figure 1.** Optical System of a Spectrophotometer.

**Calculations**

**Determination of [FeSCN2+]eq in standard solutions:**

Working Equation:

[FeSCN2+]eq= [Fe3+]i =

Standard 1:

[FeSCN2+]eq =

= .00002 M

Standard 2:

[FeSCN2+]eq =

= .00005 M

Standard 3:

[FeSCN2+]eq =

= .0001 M

Standard 4:

[FeSCN2+]eq =

= .0002 M

Standard 5:

[FeSCN2+]eq =

= .0004 M

|  |  |  |  |
| --- | --- | --- | --- |
| Fe3+(aq) + SCN-(aq)  FeSCN2+(aq) | | | |
| *i* | [Fe3+]i | [SCN-]i | 0 |
|  | -x | -x | x |
| *e* | [Fe3+]i –x | [SCN-]i - x | [FeSCN2+]eq |

**Beer-Lambert’s Law:**

(used for table 3.0)

A = εbc

Legend:

A = measured absorbance

ε = molar absorptivity coefficient

b = path length in centimeters

c = analyte molar concentration

**Calibration Regression Line Equation:**

y = 1733.x - 0.025

ε = 1733

Literature value of ε = 4.5 x 103

Percent difference = x 100%

= x 100% = 61.49%

**Determination of [FeSCN2+]eq in unknown solutions:**

Working Equation (From Calibration Regression Line equation):

[FeSCN2+]eq =

Unknown 1

[FeSCN2+]eq =

= .000170 M

Unknown 2

[FeSCN2+]eq =

= .000203 M

Unknown 3

[FeSCN2+]eq =

= .000259 M

**Determination of [Fe3+]eqand [SCN-]eq**

Unknown 1

[Fe3+]i = .0006

[Fe3+]eq = .0006 – .000170

= 4.3 x 10-4 M

[SCN-]i  = .001

[SCN-]eq = .001 – .000170

= 8.3 x 10-4 M

Unknown 2

[Fe3+]i = .0008

[Fe3+]eq = .0008 – .000203

= 5.97 x 10-4 M

[SCN-]i = .001

[SCN-]eq = .001 – .000203

=7.97 x 10-4 M

Unknown 3

[Fe3+]i = .001

[Fe3+]eq = .001 – .000259

= 7.41 x 10-4 M

[SCN-]i = .001

[SCN-]eq = .001 – .000259

= 7.41 x 10-4 M

**Calculation of Keq for the Unknown Solutions**

Working Equation:

Keq =

Unknown 1

Keq =

= 476.32

Unknown 2

Keq =

= 426.64

Unknown 3

Keq =

= 471.70

**Calculation of Average Keq of the Unknown Solutions**

Keq.ave. =

= 458.22

**Determination of Percent Difference from Literature Value**

Lit. Value of K = 9.8 x 102 [15]

Keq = 458.22

Percent difference = x 100%

= x 100% = 53.24%

**Answers to questions**

**Calibration**

**6. What is the importance of the blank solution?**

The blank solution contains everything that the other solutions have except FeCl3. In this way, the blank solution has all of the other substances except for the chromophore. The amount of light absorbed by these substances can then be subtracted from the absorbed light of the other solutions, making sure that the light absorption of only the chromophore (FeSCN2+) is recorded.

**Determination of Unknown FeSCN2+ concentration**

**13. Why was another blank solution prepared and used for this part of the experiment?**

Because the number of moles of KSCN in the solution had significantly decreased, the blank solution for the unknown solutions absorbs less light than the blank solution for the standard solutions. It is then needed for a more accurate measure of the light absorbance for the unknown solutions.