The Lost Particles

Abstract:

Spectrophotometry has a wide variety of uses in the sciences including use in hospitals and laboratories to conduct vital blood tests. In this lab spectrophotometry was used to find the absorbance of known concentrations of $KMnO_4$ and this information was used to construct a standard curve. The standard curve was then used to find the molar concentrations of 2 unknown $KMnO_4$ solutions. According to the standard curve unknown #1 had a molar concentration of 1.45 E-4 and unknown #2 had a molar concentration of 2.47 E-4. Spectrophotometry and the standard curve are fairly accurate ways to analyze the content and concentration of specific solutions. Since spectrophotometry is probably the most widely used analytical technique, especially in the field of medicine it is important to understand its uses and application.

Introduction

This lab focused on using Spectrophotometry to determine the amount of light absorbed by different concentrations of $KMnO_4$. This method is based on Beer's law, which states that for a specific wavelength

$$A=ebC$$

A= absorbance, e= molar absorptivity constant, b= path length of light, C= concentration, mol/L. The more light absorbing particles in the solution, the greater the concentration, and the more the light is diminished as it comes through the other side of the test tube. This is defined as $T=I/I_0$. The "lost" light intensity has been absorbed and the spectrophotometer measures this absorbance. This effect is also dependent on how far the light must travel. The path length, b, is usually measured in centimeters and the standard curvette is exactly one centimeter wide.

The main objective of this lab is to prepare a standard curve for potassium permanganate ($KMnO_4$) and use it to determine the concentration of two different unknowns.

Methods

Part A: Preparation of Solutions

After first obtaining six 100-mL volumetric flasks with stoppers, a 1mL, 2mL and 10mL pipet, and a beaker of 60-75mL of stock $KMnO_4$ (with the molarity on the bottle recorded) the experiment was ready to begin.

The first empty 100-mL volumetric flask was filled with 1mL of KMnO4 by using the 1mL pipet. The $KMnO_4$ in the flask was then diluted to exactly 100mL with deionized water, capped and rotated 360 degrees to mix the solutions. The first flask was then set off to the side. The above procedure was then repeated for the other five flasks each containing one of the following concentrations of $KMnO_4$: (2mL, 4mL, 6mL, 8mL, & 10mL).

Part B: The Standard Curve

The six flasks previously prepared in part A were now available to be sent through the spectrophotometer to measure the absorbance of our standards (solutions of known concentration). During the experiment the HACH spectrophotometer DR2400 was used to read absorbance and set to a wavelength of 526nm. Before using the spectrophotometer to measure the absorbance of the 1mL flask, it needed to be zeroed. This was done by taking a small piece of glassware known as a cell and filling it with deionized water. Before pouring the solution into our cell, it required cleaning with a Kimwipe in order to ensure that the cell was devoid of all fingerprints and scratches. After cleaning the cell it was held using the Kimwipe as a barrier as to prevent any further fingerprints left upon the cell. Deionized water was added until the cell was at least ¾ full. The cell was then capped and placed into the spectrophotometer. After sliding the lid shut and making sure that it was measuring absorbance the read key was touched to zero the machine. When zeroing was completed the cell was removed (making sure to use a clean Kimwipe) and emptied of the deionized water. The cell was then rinsed with more deionized water and was now ready to receive the $KMnO_4$. The flask containing 1mL solution of $KMnO_4$ was then uncapped and poured into the cell until the cell was at least ¾ of the way full. The cell was then capped and placed into the spectrophotometer. After touching the read key and recording the shown absorbance the cell was then removed and emptied of the $KMnO_4$. Next deionized water was used to rinse out the cell and prepare it for the next concentration of $KMnO_4$. This process was then repeated for the other five values of the $KMnO_4$.

When the process was complete for all six of the known values, the two containers of the unknown solutions of $KMnO_4$ were put into the spectrophotometer using the same process as the known values.

Results:

As Table 1 shows, as the concentration of the solution increased, the absorbance also increased. The standard curve (Figure 1) that was created from the results of the lab was used to "read" the absorbance of the 2 unknown solutions. Using the standard curve and the absorbance of the unknowns it was found that the concentration of unknown #1 is 1.45 E-4 and the concentration of unknown #2 is 2.47 E-4. These concentrations were found using the equation $y = 4843x + .023$. The absorbance of each unknown was substituted for y, which gave the concentration (x). The slope of the standard curve was .9995 meaning that there was very little error in preparing the stock solutions and measuring absorbance.

Table 1. Absorbance and Concentration (M) of $KMnO_4$

Solution #	mL Stock	Absorbance	Concentration (M)
1	1	0.245	5.00E-05
2	2	0.514	1.00E-04
3	4	0.997	2.00E-04
4	6	1.5	3.00E-04
5	8	1.974	4.00E-04
6	10	2.421	5.00E-04
Unknown #1	X	0.726	1.45E-04
Unknown #2	X	1.219	2.47E-04

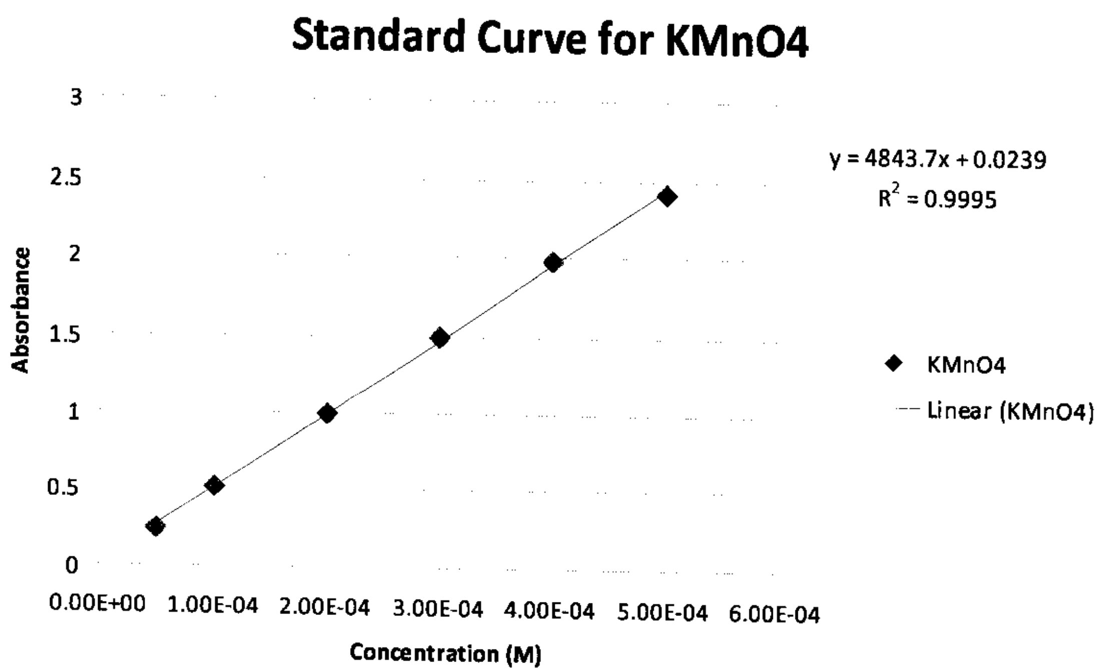

Figure 1.

Discussion

Results indicate that unknown # 1 is of a lesser concentration of $KMnO_4$ than unknown #2. This indicates that unknown # 2 has more light absorbing particles than does unknown # 1. When the light in the spectrophotometer is passed through the curvette the light is absorbed every time a light particle encounters a light-absorbing particle. Therefore, the more concentrated the solution, the more light absorbing particles will be in the solution and the more the light will be diminished when it appears on the other side of the curvette. Table 1 indicates that unknown #2 is more concentrated than the 1mL, 2mL, and 4mL stock solutions of $KMnO_4$. Table 1 also indicates that unknown #1 had a concentration of 1.45E-4 which means that it contained more light absorbing particles than the 1mL and 2mL stock solutions of $KMnO_4$.

The standard curve (Figure 1) created from the results obtained in the lab was used to determine the concentrations of the unknown solutions. The standard curve was created by plotting the absorbance on the y-axis and the known concentrations of the solutions on the x-axis. The unknowns were then analyzed by locating the absorbance of both on the standard curve line. The standard curve had an r-value of .9995, meaning that there was very little deviation from the slope.

It was very important to the accuracy of the identity of the unknowns that all of the solutions were prepared with care and precision. There is still human error that can skew the results, which is most likely why the r-value was .9995 instead of .10. There could have been error when the pipetting of the $KMnO_4$ was completed. There also could have been error when the 100mL of deionized water was added to the solution. There could have been small imperfections or dust on the curvette that was used in the spectrophotometer, which would distort the absorbance reading. Any or all of these things could have been sources of error during the experiment.

Conclusion:

The objective of the spectrophotometry lab was to collect data to use to make a standard curve and then use that standard curve to identify the concentration of two unknown solutions. The known concentrations of solutions were prepared and the spectrophotometer was used to find the absorbance. After the data was collected it was used to create a standard curve, which was used to identify the concentrations of the two unknown solutions.

From this lab we learned how to make a standard curve and gained a better understanding of Beer's law. From the observations we learned that unknown #1 had a molar concentration of 1.451E-4 and unknown #2 had a molar concentration of 2.469E-4 of $KMnO_4$. The standard curve and spectrophotometry have a wide variety of uses, including measuring a blood sugar level, the concentration of proteins, and concentration of DNA in various solutions (1). In fact, spectrophotometry is used in thousands of laboratory tests and can measure multiple substances in one sample. Finally, we learned that while spectrophotometry has a wide variety of uses, precision, accuracy, and conscientiousness are required to have exact results.

References:

1. Wikipedia Online Encyclopedia found on 10/23/2007, from http://wikipedia.org/