Spectrophotometric Analysis

Abstract

Spectrophotometric analysis is employed to measure the amount of light that a sample substance absorbs. The instrument operates by passing a beam of light through a sample and measuring the intensity of light reaching a detector. In this experiment $KMnO_4$ (potassium permanganate) was mixed in aqueous solution at known concentrations in order to establish standard curve. This curve allowed us to determine the concentration of two unknown substances.

Introduction

One of the most common applications of spectrophotometry is to determine the concentration of an unknown substance in a solution. This application uses Beer's Law, which predicts a linear relationship between the absorbance of the solution and the concentration of the substance being analyzed. The benefit of this kind of measurement is that spectrophotometry is capable of detecting very low concentrations up to 10 e-7 molarity. It accurately measures the amount of light that is absorbed by a sample in the visible spectrum. This principle can be illustrated by examining a simple glass of ice tea mix.

When a glass of water is prepared with 1 tablespoon of tea mix, it changes color to an amber shade. When a second glass is prepared with 2 tablespoons of tea mix, the aqueous solution turns to a darker amber shade than the first one. The reason is that the second sample has twice as much tea mix and is therefore twice as concentrated. Part of the light that passes through the glass is being absorbed by every single light-absorbing particle in the solution. This minimizes the amount of light that passes through the fluid. Greater concentrations yield less light.

The main objective of this lab was to calculate the concentration of two unknown substances by establishing a baseline of potassium permanganate concentrations. This was done by preparing 6 samples of KMnO4 in differing concentrations, and constructing a line graph of the quantitative data as determined by the spectrophotometer. This experiment helped us to clearly see how the difference in color of a sample, is related to a numerical absorbance number given by the spectrophotometer. This lab also helps us to discover the concentration of an unknown substance by using the straight-line graph that we produced from the results of KMnO4.

Methods

Part A: Preparation of Materials

In order to establish a baseline, 6 samples of stock $KMnO_4$ at0.0050 M were diluted to 100 mL. The first sample was prepared by pipetting 1 mL of KMnO4 into beaker number one, and then pipette the remainder of the solution with deionized water, up to exactly 100 mL. The pipette was then rinsed with water to clear out any remaining KMnO4. The prepared solution was immediately capped with parafilm.

The next five samples were prepared the same way using 2 mL of KMnO4, then the next sample with 4 mL, then 6 mL, 8 mL, and finally 10 mL. After preparing the 6 samples, we prepared 2 additional samples of KMnO4 in unknown concentrations. This was done by adding 4 mL of each unknown into 2 beakers, and diluting them to exactly 100 ml as done with the previous samples. Each beaker was then labeled and capped.

Part B: Collecting the Data:

Each prepared sample was analyzed using a Hach DR 2400 spectrophotometer at a wavelength of 526 nm. The absorbance values were recorded in and added to an excel graph. In order to calculate the absorbance, the initial concentrations of the stock KMnO4 were converted from mL to molarity. 0.0050 x 1/100, for the 1 ml sample, 0.0050 x 2/100, for the 2 ml sample, and continuing with each concentration sample.

After plotting the chart with the values for the known concentrations, absorbance (y axis) versus concentration (x axis), a standard curve was constructed (Fig. 1). The data from the unknown samples was then compared to the standard curve. This allowed us to calculate the concentration of the unknowns.

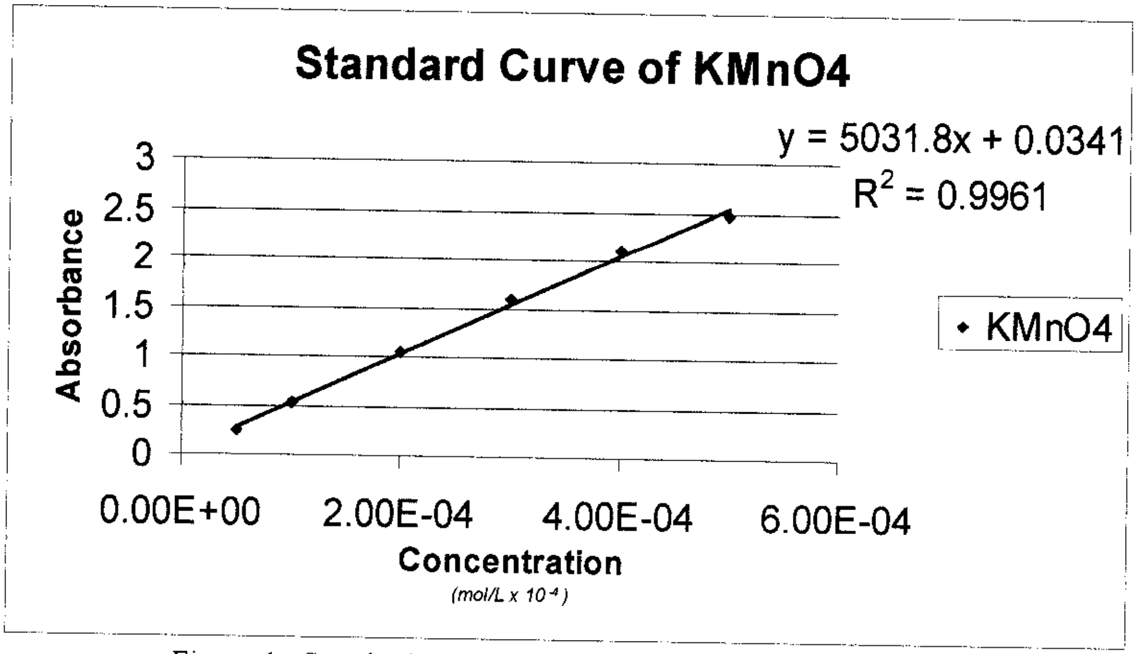

Figure 1. Standard Curve as related to collected data (table 1)

Solution #	mL Stock	Concentration	Absorbance
1	1	5.00E-05	0.253
2	2	1.00E-04	0.518
3	4	2.00E-04	1.057
4	6	3.00E-04	1.602
5	8	4.00E-04	2.105
6	10	5.00E-04	2.469
UNK	X		0.731
UNK	X		1.236

Table 1. Collected Data from Spectrophotometer

Results

As shown in the table above, the collected data from the six known concentrations, was plotted on a graph using the dilution factor:

0.00531 M x (XmL/100mL) = _____M

The subsequent figures were then plugged into the graph and resulted in a straight line, or standard curve. Again using the dilution factor the 4mL solutions of unknown concentrations were inserted in the graph and when compared were indeed on the curve. The concentrations of the unknowns were concluded to be 1.30 e-4 and 2.30 e-4.

Discussion

This lab illustrated the direct association between the concentration of particles in a solution and the amount of light that could pass through the solution. If development of color is linked to the concentration of a substance in solution, then that concentration can be measured by determining the extent of absorption of light at the appropriate wavelength. The analysis of the absorbency of these particles at certain wavelengths of light was demonstrated by this experiment using a spectrophotometer. While this seems to be a simple process it is easy to get bad data due to inaccurate measuring techniques and damaged equipment (scratched glassware). Over the course of this experiment two of the samples used had to be measured twice, their first measurement being grossly off what was becoming the standard curve of our data. This error was most likely due to the methods of measuring that were used to dilute the $KMnO_4$ to a 100mL solution.

Conclusion

This lab succeeded in illustrating the use of a spectrophotometer to analyze concentrations of substances in solution. In doing the calculations for the lab we learned that when monochromatic light (light of a specific wavelength) passes through a solution there is usually a relationship (Beer's law) between the solute concentration and the intensity of the transmitted light. This lab also showed the importance of accurate measurement, obviously if the dilution of the substance is different the concentration will be different. While using the spectrophotometer we learned that your equipment must be clean and serviceable, any scratched or dirty glassware could result in a false reading from the device.

References

Paradis, Jeffery (2006). *Hands on Chemistry.* Boston: McGraw-Hill.

Spectrophotometry Handout