Abstract

The main objective of this lab is to learn how to use spectrophotometry to explore and understand Beer's Law. After using the spectrophotometer to identify the absorbance of four known concentrations of $KMnO_4$, the absorbance of two unknown concentrations of $KMnO_4$ will be measured and recorded. The overall findings show that as the concentration of a solution increases, the absorbance of the solution increases at the same rate. Using this knowledge, a standard curve of concentration vs. absorbance can be created using our known solutions, and then the unknown concentrations can be identified by locating where the absorbance meets with the standard curve. During this lab students will learn how to use a spectrophotometer for quantitative analysis, how to properly make dilutions, and how to use effective pipetting techniques.

Introduction

This lab is designed to help students understand the concept of Beer's Law. Beer's Law states that, for a specified wavelength: A=EbC (A is absorbance, E is molar absorption constant, b is path length of light, and C is concentration in mol/L). It tells us that, as the concentration of a solution increases, the absorption increases. In simple terms, if you only add a few specks of Kool-Aid to a gallon of water, the water will only have a slight coloration and most light will come through (low absorption). If you add half of a pack of Kool-Aid, the water will be darker and less light will come through (high absorption). The object of this lab is to use quantitative analysis to record exactly how much the absorption changes as the concentration changes. The purpose of doing the lab is to gather our own results and compare how they relate to Beer's Law.

Methods

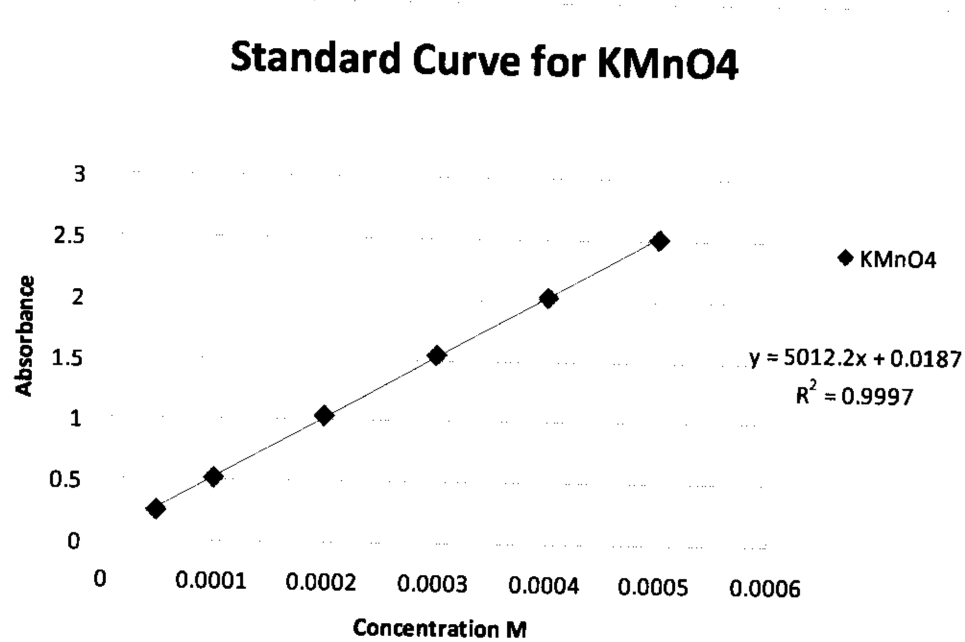
In order to establish a standard curve, the absorbances of 6 known concentrations of KMnO4 were measured. First approximately 40mL .005 $KMnO_4$ was acquired in a glass beaker. Six 100mL volumetric flasks were lined up and the first was labeled as 1mL .005M $KMnO_4$. Using a graduated pipette, 1mL of $KMnO_4$ was dispensed into the first volumetric flask. The volumetric flask was diluted to exactly 100mL with diH_2O flask, capped, and then mixed thoroughly. This was repeated for the next 5 volumetric flasks only using the following volumes of $KMnO_4$: 2mL, 4mL, 6mL, 8mL, and 10mL.

Once the spectrophotometer was on and the wavelength was set at 526 nm, the machine was blanked using diH_2O. Starting with the most dilute solution, the absorbance of each solution was measured and the values recorded on the report sheet (the cuvette was pre-flushed with a portion of the next sample each time so to not contaminate the next sample). Once all six of the known solutions were run, the unknown samples were obtained and the codes were written on the report sheet. The absorbance of each of the unknowns was measured and recorded just like the first six samples.

The concentrations of the 6 known solutions were calculated by applying the appropriate dilution factor and the results were recorded on the report sheet. By graphing absorbance of each of the known solutions vs. the concentration of each solution, the best possible line (the standard curve) was drawn between each point (ignoring any "bad" data). Finally, the concentrations of the unknown solutions were found by locating the absorbance of the unknown and following it horizontally over to the standard curve line. By finding the x value of the intersection, the concentration value of the unknowns were calculated and recorded on the report sheet.

Results

Chart 1.



On chart 1, the results from the lab show that the standard deviation derived for the six known concentrations of $KMnO_4$ is 99.97% accurate. The results can also be used to determine that the concentration of the first unknown is approximately $1.5x10^{-4}$ mol/L and the concentration of the second unknown is approximately $2.5x10^{-4}$ mol/L. Applying these numbers, the dilution factor can be used to calculate that the first unknown contains approximately 3mL $KMnO_4$ stock solution and the second unknown contains approximately 5mL $KMnO_4$ stock solution.

Discussion

The findings in this lab strongly support the ideas behind Beer's Law. According to the equation given by Beer's Law, the relationship between concentration and absorbance should be directly proportional to each other. The data supports this because when the concentration is doubled, the absorbance is doubled and when the concentration is halved, the absorbance is halved. By knowing a standard deviation of any substance, this concept can be used to identify unknown concentrations of the substance.

Conclusion

In this lab we learned that if the standard curve of the absorbance of a particular substance is available, then when unknowns are presented either the absorption or concentration can be found. This is due to Beer's Law which says that that absorbance and concentration are directly proportional. That is why spectrophotometry has proven to be an integral technique used in the medical world as well as everyday life.