Quantitative Biochemical Analysis

By

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For

CHEM 4101-10x

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Introduction

Procedures within any laboratory require specific knowledge in the use of certain tools in order to obtain results specific to an experiment. In chemistry and biochemistry labs, the use of quantitative apparatus is a mainstay. This means that many experiments involve determining amounts or proportions of a constituent. Determination of mass, volume, and concentrations are a few examples of measurements commonly needed to complete an experiment. Equipment is available to make these measurements, i.e. a scale can measure mass. The purpose of this laboratory is to review some principles of quantitative analysis. Many of the tools used in this laboratory will be used in future experiments.

In this lab, we will concentrate on learning skills and using equipment that will be used in later exercises. Several new techniques that will be used are the creation of dilutions, use of the spectrophotometer, matching cuvettes and determining the mass of an unknown by use of the spectrophotometer. Dilutions are a method of changing a solutions concentration. The spectrophotometer is an instrument that is used to measure the intensity or absorbency of color at a specific wavelength of a substance. Matching is a method of calibration in order to make certain that absorption of light is equal in all cuvettes. We will then create a standard curve by creating known dilutions of a solution, taking absorbency readings of the dilutions, and then determine the concentration of an unknown. In many biochemistry and clinical laboratories, these skills are often used, i.e. for determining the relative amount of protein in plasma.

Materials

The following equipment was used in this lab: 0.1 mM $KMnO_4$, distilled water, kimwipes®, (5) identical cuvettes, (5) 20 ml culture tubes, Spec-20 Spectrophotometer, pipette with pump or bulb, and a small beaker or flask. A sample with an unknown concentration of $KMnO_4$ was also obtained by the TA.

Procedure

1) Spectrophotometer was turned on, warmed up for 15 minutes, and then set to zero absorbance.

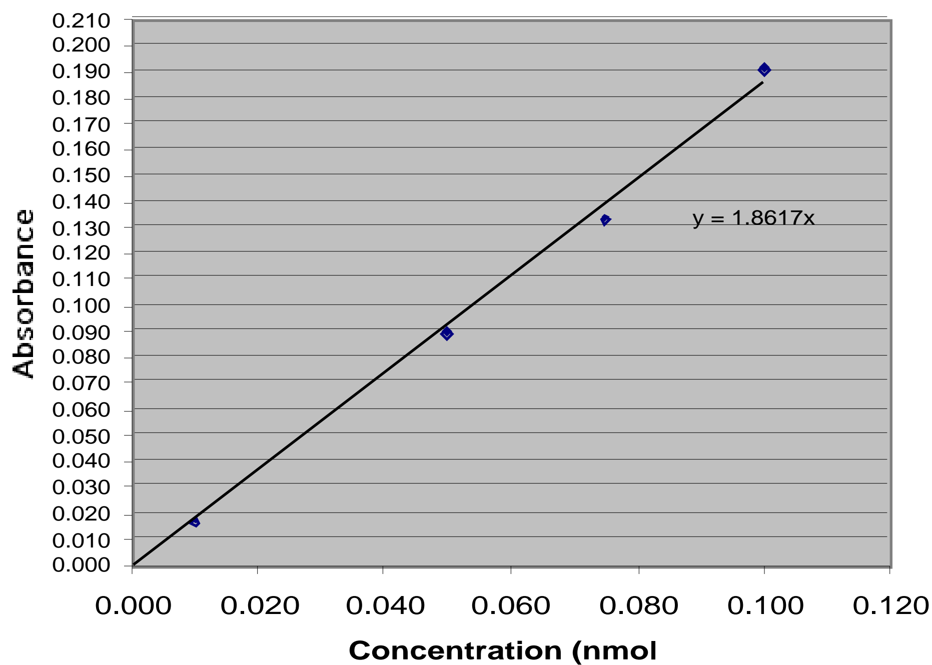
2) Each cuvette was filled with 3 ml of distilled water. Cuvettes were wiped clean and then placed into the Spec-20. All tubes were matched to zero.
3) 0.1 mM $KMnO_4$ was diluted in culture tubes at following concentrations, 0.1 mM, 0.075 mM, 0.050 mM, and 0.010 mM, each in total volume of 10 ml.
4) 3 ml of each sample was aliquot into a cuvette, and the absorbance was obtained for each concentration.
5) 3 ml of Unknown was aliquot into a cuvette, and absorbance was obtained.

6) A standard curve was created, and concentration in grams of the unknown was determined. Graph was Absorbance to mM.

Results

Table 1. Spec Absorbency Readings

Cuvette	Concentrations (mmol)	Absorbance
0	Water	0.00
1	0.100	0.192
2	0.075	0.134
3	0.050	0.090
4	0.010	0.018
A	?	0.058
B	?	0.043



Discussion

Table 1 illustrates absorbency readings obtained for various concentrations of $MnPO_4$. From this data, we can create a standard curve that can be used to determine concentrations of unknown solutions with the same chemical compound. This is done by plotting Concentration (x-axis) vs. Absorbance (y-axis) on a 2 dimensional X-Y Coordinate Graph. After plotting, it is necessary to determine the slope. To calculate the slope, the following formula is used:

$$y=mx+b$$

where, y is absorbance
x is concentration
m is the slope and
b is y intercept (zero)

Unfortunately, the plots do not "line up" perfectly as expected. For this reason we must determine a best-fit slope. My method was to draw a straight line that would best intercept most plots. I personally felt that sample 3 and sample 4 were to plots that closely resembled the slope. I then calculated the slope using the coordinates for those two samples and plugged them into the following equation:

$$\text{Best-fit Slope is } m = \frac{(y_2 - y_1)}{(x_2 - x_1)} = \frac{0.090 - 0.018 \text{ Unit}}{0.050 - 0.010 \text{ mmol}} = \text{slope of } 1.8$$

Upon determining the slope, a graph was constructed (Graph 1) and the concentrations of the unknowns could be determined. To determine the concentrations of the unknown, the following equations were used:

Solve for A:

$y = m(x) \rightarrow 0.058 = (1.8)(x) = 0.032$ mmol

Unknown A Concentration = 0.032 mmol (expected 0.030)*

Solve for B:

$y = m(x) \rightarrow 0.043 = (1.8)(x) = 0.024$ mmol

Unknown B Concentration = 0.024 mmol (expected 0.025)*

* TA Note: Expected value is what I originally thought I diluted the unknowns to. This Expected value would not normally be on your report, nor were you expected to solve for both unknowns. I am trying to illustrate the variability of the Spec-20 and let you know the expected concentration of each unknown.

With the concentrations of the unknowns determined in mmol, it was now possible to determine the concentration of chemicals in grams of the unknown solutions. This was determined by using the following equation.

$$\text{Mass of A} = \frac{0.032 \text{ mM} * 1 \text{ M} * 158.04\text{g/L} * 1000 \text{ mg}}{1000 \text{ mM} * 1 \text{ M} * 1 \text{ g}} = 5.05 \text{ mg/L or } 5.05\ \mu\text{g/ml}$$

$$\text{Mass of B} = \frac{0.024 \text{ mM} * 1 \text{ M} * 158.04\text{g/L} * 1000 \text{ mg}}{1000 \text{ mM} * 1 \text{ M} * 1 \text{ g}} = 3.79 \text{ mg/L or } 3.79\ \mu\text{g/ml}$$

In this lab, we have illustrated an ability to utilize quantitative biochemical analysis techniques. Often time biochemists are called upon to determine amounts of a specific substance. This can easily be accomplished by using the skills learned in this lab. By performing dilutions used in conjunction with a spectrophotometer, it is possible to create a standard curve for any substance based on the absorbent properties of that particular substance. From this curve, unknown concentration of that particular substance can be determined. Now, this method is not flawless. Inconsistency in the standard curve could exist. This is could be due to dirty cuvettes. However, not all spectrophotometer are 100% accurate. Thus, inconsistency could be the fault of the machine. This factor can be taken if the variance of the machine is noted beforehand. Also, the continuous monitoring of the readings on the spec using the blank will alleviate any problems.