Experiment 9

Spectrophotometry

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ABSTRACT

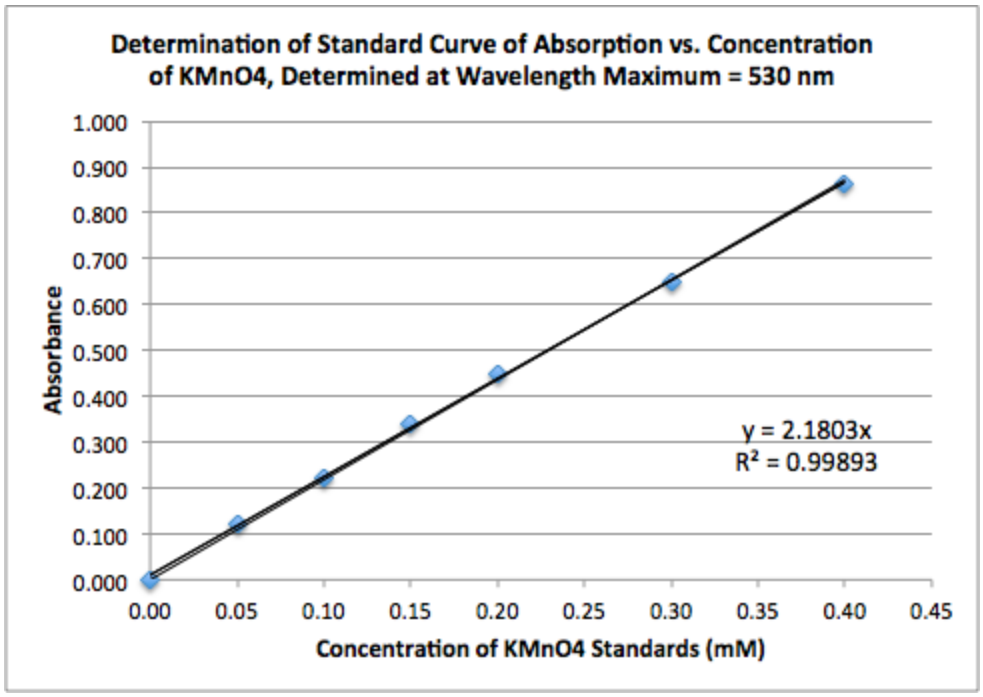
1. Demonstrate the techniques of using a spectrophotometer to determine the absorbances of solutions.
2. Understand the concept of standard curves and be able to apply this concept in solving other similar problems.
3. Understand the concepts and principles of Beer's law.
4. Learn how to manually draw graphs of experimental data and how to use Excel program to graph and perform linear regression analysis.

Part I: Answer the following questions. Include questions 1-10 in your report.

1) Copy your completed Table 3 into your report. Use Excel to generate a standard curve of absorption vs mM of KMnO4. Make sure you set the intercept at zero and include the equation and R2 value on your graph.

Table 3: Determination of Standard Curve of Absorption vs. Concentration of KMnO4, Determined at Wavelength Maximum = 530 nm

	Concentration of KMnO4 Standards (mM)	Absorbance
Tube A	0.00	0.000
Tube B	0.05	0.120
Tube C	0.10	0.224
Tube D	0.15	0.340
Tube E	0.20	0.448
Tube F	0.30	0.648
Tube G	0.40	0.863



2. Explain the purpose of the standard curve you made in question 1.

The standard curve shows the correlation between the absorbance of the solution to a given concentration, which can be used to calculate the concentration based on a measured absorbance. Specifically, the standard curve of absorbance of different concentrations of KMnO4 at wavelength 530 nm can be used to determine the concentration of an unknown by measuring its absorbance at the same wavelength of 530 nm.

3.Based on the equation generated by the standard curve and the absorption values recorded in Table 4, calculate the concentration of your unknown.
Absorbance = 2.1803 (Concentration of unknown Tube #8)
0.464 = 2.1803 (Concentration of unknown Tube #8)
(Concentration of unknown Tube #8) = 0.213 mM

4. **Write out Beer's law and define each of the terms it contains. Then calculate the molar absorptivity at the λmax of KMnO4 using Molar (M: mole/Liter) as the unit of concentration. (2pts) Note: The unit of concentration for KMnO4 in question 1 is mM (mmole/Liter). You need to convert the unit to M (mole/Liter) first.**

Beer's Law: A= ELC
Where A = absorbance (no unit)
E = absorptivity (L/mol cm)
L = pathlength (cm)
C = concentration (mol/L)

A = ELC
E = A/LC = 0.464/ (1cm * 0.000225mol/L) = $2.06X10^3$ L mol^{-1} cm^{-1}.
Where A = 0.464
E = absorptivity (L/mol cm) = ? = $2.06X10^3$ L mol^{-1} cm^{-1}.
L = 1 cm
C = concentration (mol/L) = 0.000225mol/L

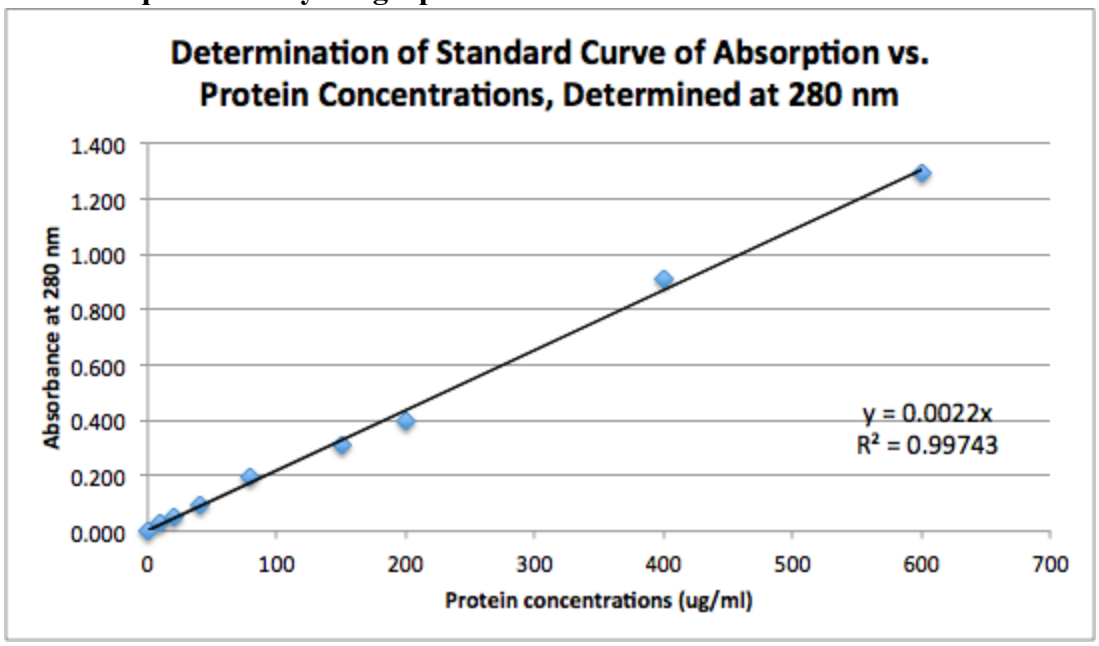
Thus E, the molar absorptivity at the λmax of KMnO4, is $2.06X10^3$ L mol^{-1} cm^{-1}.

5. The concept of a standard curve is very important. It is routinely used in biology and chemistry laboratories to determine unknown parameters. The following exercise demonstrates the use of a standard curve in determining the concentration of a protein solution. The standard curve is based on data showing how absorbance of UV light at 280 nm correlates with protein concentration:

a. Use Excel to make a scatter diagram using data in the table above; include the table in your report;

Protein concentrations (ug/ml)	Absorbance at 280 nm
0	0.000
10	0.033
20	0.052
40	0.095
80	0.195
150	0.312
200	0.400
400	0.912
600	1.290

b. Then calculate the best fitting straight line by using linear regression. Present the graph with the equations on your graph.



c.What makes you conclude that the protein concentration and UV absorbance are linearly related over this concentration range of protein?

There is a direct correlation between the protein concentration and the absorbance; As the protein concentration increases, the absorbance increases by a factor.When checked using excel (linear regression best-fit line), the high r^2 value (0.99743) indicates that the data is almost the perfect linear fit, in which the r^2 value would equal 1.

d. If the absorbance of a protein solution whose concentration is unknown is 0.45, what is the protein concentration of the solution? Show your calculation.

0.45=0.0022x

x= 204µg/ml