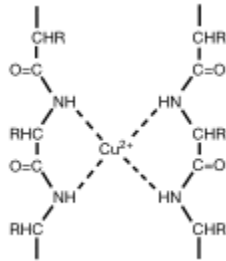


Name: _____ Block: ____ Date: _____

DETERMINING THE CONCENTRATION OF PROTEIN IN EGG WHITE

Background:

The quantity of protein in a sample can be determined by reacting it with Biuret Reagent and measuring the color change using a spectrophotometer. Biuret reagent contains Cu^{2+} ions in an alkaline solution. Molecules, such as proteins, that contain at least 2 peptide bonds form a tetravalent coordination complex with the copper ions. This complex creates a violet-purple color.



In lab a spectrophotometer will be used to calculate the saturation of purple color by determining the amount of light absorbed by the samples at 540nm. A spectrophotometer is an instrument that allows light to be filtered to very specific wavelengths and directs them through a sample at a light meter. The transmittance or absorbance of light can be used to determine concentrations of various solutions.

Beer's Law is used to calculate the appropriate wavelength and readings when using the spectrophotometer. Calibration curves are created by analyzing known standards (samples of known protein concentrations) and graphing the values based on the absorbance of light. Note: dilutions maybe required if the absorbance is too high for an accurate reading on the spectrophotometer (keeping the readings inside the range of linearity).

Absorbance varies linearly with both the cell path length and the analyte concentration. These two relationships can be combined to yield a general equation called **Beer's Law**.

$$A = \epsilon l c$$

If you plot absorbance versus concentration, the resulting graph yields a straight line. The equation for the straight line (termed regression line) can be used to determine the concentration of an unknown solution once the %T (percent transmittance) has been measured. Absorbance and transmittance are related by the following formula:

$$A = 2.00 - [\log (\%T)]$$

Purpose:

This lab will be used to expose the student to the use of a spectrophotometer, creation of a regression line and determine the concentration of protein in an egg white based on the line of regression.

Materials:

Biuret Reagent	Protein Standard Solution (1% w/v)	Egg White Solution	Micropipetor
Pipette tips	test tube rack	test tubes	cuvettes
Spectrophotometer			

Procedure:

1. Turn on spectrophotometer and set wavelength to 540 nm.
2. Using a clean 50 ml beaker, obtain 3.0 ml of a 1% (w/v) of an albumin solution. (1% (w/v) is the same as 1 g of solute in 100 ml of solution)
3. Obtain seven clean 13 x 100 mm test tubes, label 1 - 5 for your standards. Mix the appropriate amounts of protein solution, water and biuret solution as directed in the table below.
4. Label test tubes 6 – 7 for your egg white solution.
5. Obtain 1.0 ml of egg white and blend thoroughly with 9.0 ml of water. Add the egg white solution, water and biuret as directed in the table below.

Tube No.	ml of protein soln.	ml of egg white soln.	ml of water	ml of biuret soln.
1	0	0	1.0	2.0
2	0.1	0	0.9	2.0
3	0.5	0	0.5	2.0
4	0.9	0	0.1	2.0
5	1.0	0	0	2.0
6	0	0.5	0.5	2.0
7	0	0.5	0.5	2.0

6. Mix the contents of each tube by gently shaking or flicking.
7. Allow the test tubes to rest for 5 minutes.
8. Use the distilled water to set the 100% transmittance.
9. Determine the absorbance of each of the test tubes at 540 nm, record the results in the data table provided.
10. Graph the results of the standard solutions.
11. Determine the concentration of test tubes 6 and 7 using the graph.
12. Determine the concentration of protein in an egg white by averaging the concentrations of test tubes 6 and 7.

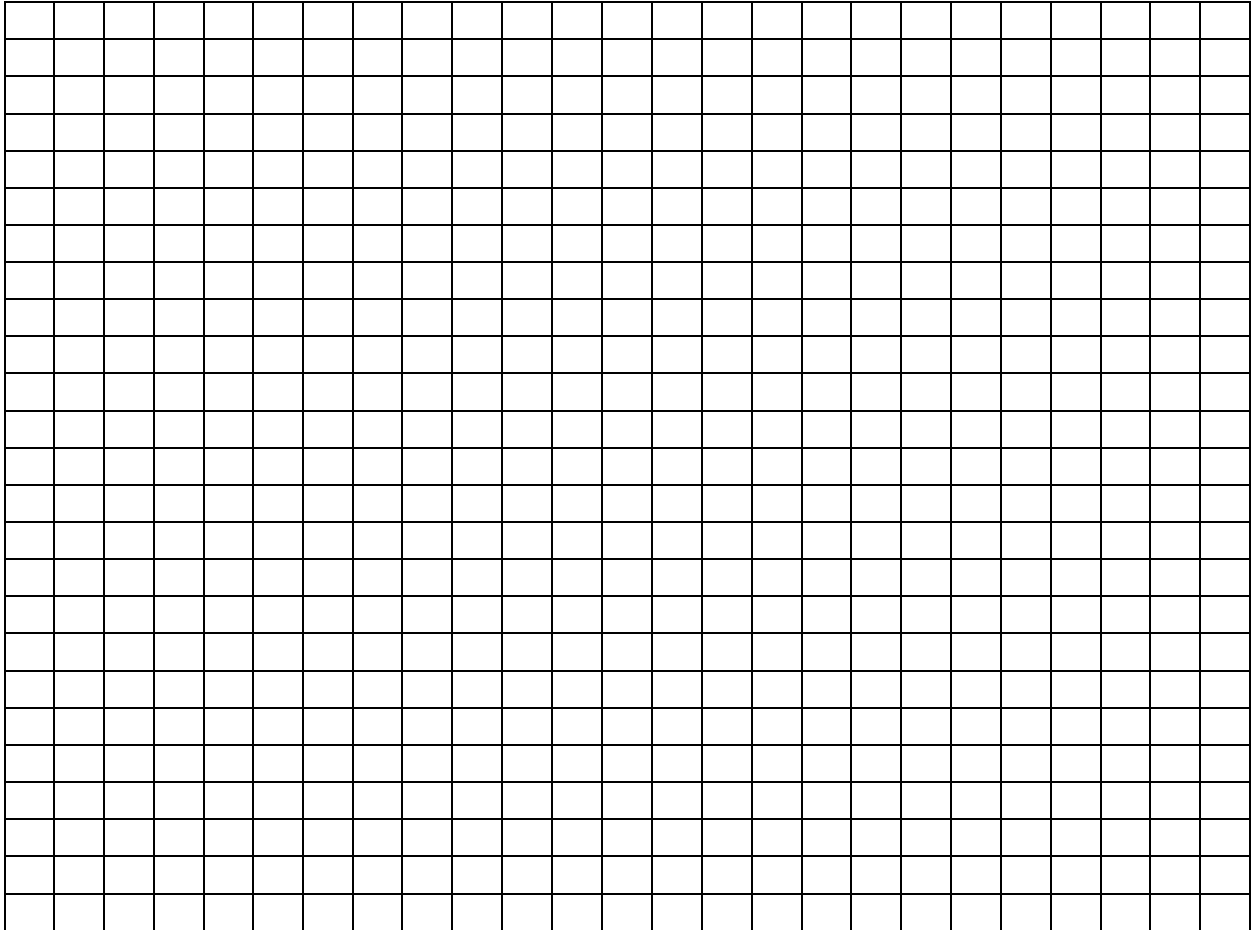
Calculations: Use the space below to determine the concentrations of your standards based on g/ml.

Data Table:

Tube No.	Protein Concentration (g/ml)	Absorbance Reading
1		
2		
3		
4		
5		
6		
7		

Average protein concentration for egg white: _____ g/ml

Graph (Remember to give your graph a title.)



Discussion Questions:

1. Why might it be necessary to dilute the standards with water?
2. If there was a protein composed of only two amino acids would it give a positive Biuret test? _____ Why?
3. If the predicted concentration of protein in an egg white is 0.124 g/ml what is your percent error?
4. When working in a lab it is important to evaluate the process as well as the results. What parts of the lab today may have led to your group needing additional assistance or obtaining less than perfect results?