

Determining the Concentration of an Unknown Protein Solution

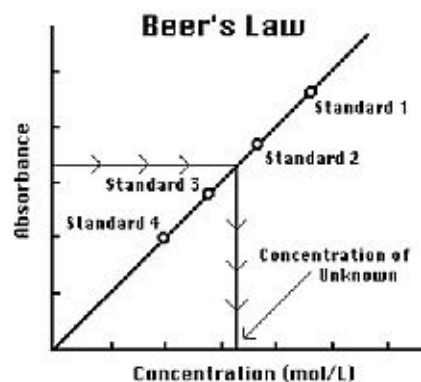
An Application of the Beer-Lambert Law

50 Points

Introduction

Scientists are often faced with the challenge of determining the concentration of a substance in solution. For example, they may need to measure levels of proteins, cholesterol, glucose, or the rate of enzymatic activity. This investigation will focus on a colorimetric assay commonly used to measure protein concentrations.

In this experiment, you will use a colorimeter to determine the absorbance values of five protein solutions of known concentrations (standard solutions). When a graph of absorbance vs. concentration is plotted for these solutions, a direct relationship should result, as shown in the figure. This relationship is known as the Beer-Lambert Law.



You can then determine the concentration of an unknown protein by measuring its absorbance, locating the absorbance of the unknown on the vertical axis of the graph, and finding the corresponding concentration on the horizontal axis (follow the arrows in the figure).

As you learned in the Identifying Organic Compounds Lab, Biuret reagent will turn a purplish color in the presence of protein. Biuret reagent contains copper ions that can interact with strands of protein molecules. These complexes form between the peptide bonds of different polypeptide chains. As a result, the intensity of the color change will be directly related to the amount of protein in solution.

Procedure

Part I: Establishing a Standard Curve

1. Add 15 mL of the 1% egg albumin (protein) solution to a small beaker.
2. Add 1 mL of Biuret to the beaker.
3. Add 15 mL of distilled H₂O to a second small beaker.
4. Label 5 clean test tubes and prepare the solutions according to the directions in the table below.

Test Tube	Albumin/Biuret Solution	Distilled H ₂ O
20%	1 mL	4 mL
40%	2 mL	3 mL
60%	3 mL	2 mL
80%	4 mL	1 mL
100%	5 mL	0 mL

5. Connect the Colorimeter to the LabQuest.
6. To use a Colorimeter correctly:
 - All cuvettes should be wiped clean and dry on the outside with a tissue.
 - Handle cuvettes only by the top edge of the ribbed sides.
 - All solutions should be free of bubbles.
 - Always position the cuvette with the smooth side facing toward the white reference mark at the right of the cuvette slot on the Colorimeter.
 - Be sure to close the lid.
7. Prepare a *blank* by filling an empty cuvette with 3 mL of distilled water.
8. Calibration:
 - Place the blank in the cuvette slot of the Colorimeter and close the lid.
 - Select Colorimeter from the Sensors → Sensor Setup Menu. Select OK.
 - Select Calibrate from the Sensors Menu.
 - Select Calibrate Now from the Calibration Menu.
 - Turn the wavelength knob of the Colorimeter to the 0% T position. When the voltage reading stabilizes, enter 0 as the percent transmittance. Select KEEP.
 - Turn the wavelength knob of the Colorimeter to the Red position (635 nm). When the voltage reading stabilizes, enter 100 as the percent transmittance. Select KEEP.
 - Select OK to return to the main screen.
9. Find the absorbance for the five standard solutions using the following method:
 - Fill a cuvette with 3 mL of the solution being analyzed.
 - Be sure to follow the instructions in step #6 above.
 - Record the absorbance in the data table provided after the data stops fluctuating.

Part II: The "Power Drink" Challenge

Several companies are competing to produce a new product nicknamed the "Power Drink." It is a high protein drink for athletes. Three companies in particular advertise that they produce a drink with the highest concentration of protein. As an independent testing agency, your company has been hired to settle this dispute by testing the concentration of protein in each drink.

Each team will be given samples from one of the drinks. The samples will be labeled A, B, or C. Your job is to find out the concentration of protein in your sample expressed as micrograms of protein per milliliter of sample ($\mu\text{g/ml}$). You will be given a procedure used to determine the concentration of protein. The results from each team will be compared and you will present your conclusions to the companies. Remember, you will have to defend your conclusions, especially to the companies that lost.

10. Find the absorbance of the unknown solution using the instructions in step #9.
11. Establish a standard curve and determine the concentration of your unknown:
 - Graph the five data points for your standards (x = concentration, y = absorbance).
 - Draw a best-fit line.
 - Follow the instructions in the introduction to determine the concentration of your unknown.

Results

Test Tube	Absorbance
20%	
40%	
60%	
80%	
100%	
Unknown Letter: _____	

Concentration of Protein in Unknown:

Conclusion (summary of experiment, analysis of data, discussion of error)

Questions

1. What is meant by a standard? How is a standard used?
2. How does an increase in protein concentration affect the color produced?
3. a. How does the concentration of protein in the unknown that you calculated compare to the actual concentration?

b. Calculate % error:
$$\% \text{ error} = \frac{\text{Experimental} - \text{Actual}}{\text{Actual}} \times 100\% =$$

c. Are the values close? If they are not, can you think of any reason why they would be different?
4. Could Biuret reagent be used to calculate the concentration of a mixture of amino acids in a solution? Why or why not?