Name:

The Crucial Concentration

Investigating Unknown Quantities of Protein Using the Lowry Assay

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Adapted from *The Crucial Concentration*, written by Donald A. DeRosa and B. Leslie Wolfe

Scientists sometimes measure the concentration of something in liquids. For example, they may measure the amount of lead levels in drinking water, a patient's cholesterol or dissolved oxygen in the Chesapeake Bay.

The concentration of a component in a solution is often expressed as weight or volume. The Nutrition Facts Label on food and drinks contains information about nutrients in the product. Look at the label on the right and determine how many grams of protein are in the drink per serving. This is also known as the concentration of protein.

Several companies are competing to produce a new product, *The Power Drink*. It is a high protein drink for athletes for improved physical performance. Three companies advertise that they produce a drink with the highest concentration of protein.

Sharon works for an independent testing agency and has been hired to settle the dispute among the companies. She will test the concentration of protein in each drink.

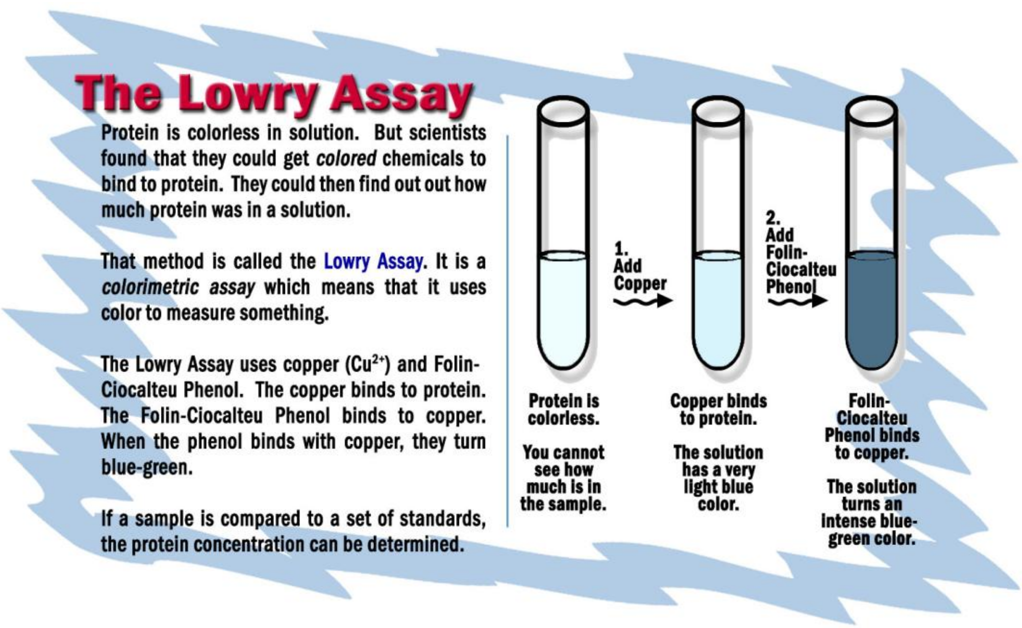
Nutrition Facts

	Amount per serving	
Calories (Energy)	190	
Calories from Fat	45	
		% Daily Value*
Total Fat	5g	8%
Saturated Fat	2g	10%
Trans Fat	0g	
Polyunsatured Fat	1g	
Monounsaturated Fat	2g	
Cholesterol	10mg	3%
Sodium	220mg	9%
Potassium	600mg	17%
Total Carbohydrate	24g	8%
Dietary Fiber	5g	20%
Sugars	13g	
Protein	15g	30%

You and your partner can help Sharon find out which of the three companies can actually say they make the drink with the highest amount of protein. Your job is to determine the concentration of protein in the three drinks. The results from each team will be compared and you will present your conclusions to the companies. You will have to defend your conclusions, especially to the companies that lost.

How do you see protein?

Protein is colorless – you cannot look at each sample to see the amount of protein. Sharon has decided to use the Lowry Assay. The Lowry Assay uses a chemical reaction to add color to protein in solutions - the darker the color, the greater the amount of protein in the sample.



Laboratory Protocol

Think of the activity you did with your class using food coloring and bottles. Your teacher asked you to develop a method to determine how many drops of food coloring are dissolved in a mystery solution. Sharon will use this same method to determine how much protein is dissolved in each of the three sports drinks. You and your partner will use the standards with known concentrations of proteins to compare the three sports drinks to.

To do The Power Drink Challenge, you will need several materials. The following is a list of the materials you will be using for the experiment:

Unknown A	Stock Protein	Spectrophotometer
Unknown B	Folin-phenol reagent	Copper reagent
Unknown C	Distilled Water	Tubes 1 - 5
	Timer	100 – 1000 µL Micropipette and tips

Prepare Unknown Samples

- ☐ 1. Label three of the empty test tubes A, B and C at the top of the tube, and label five of the empty test tubes 1, 2, 3, 4 and 5 at the top of the tube.
- ☐ 2. Add 1,000 µL of unknown A to the empty test tube labeled A.
- ☐ 3. Add 1,000 µL of unknown B to the empty test tube labeled B.
- ☐ 4. Add 1,000 µL of unknown C to the empty test tube labeled C.

Prepare Protein Standards

- ☐ 5. To create standards, add distilled water (dH_2O) to the tubes marked 1,2,3,4, and 5:

Tube Number	Amount dH_2O (µL)
1	1000
2	975
3	950
4	925
5	900

- ☐ 6. Using the table below, add the appropriate amount of stock protein to each test tube (The concentration of the stock protein is 1 µg/µl) to create your standards.

Tube Number	Amount Protein (µL)
1	0
2	25
3	50
4	75
5	100

QUICK CHECK: Why are the five tubes called “standards?” How will you use them for this experiment?

Lowry Assay Reaction – Step 1

- ☐ 7. Add 1,000 µL of the Cu^{+2} Reagent to the tubes marked 1 – 5, and to tubes marked A, B, and C. Vortex the tubes for three (3) seconds. Hold the tubes by the top of the tube between your thumb and forefinger to mix the samples.
- ☐ 8. Incubate the sample tubes at room temperature for 5 minutes.

  QUICK CHECK: Compare the eight tubes to each other. Did anything change visually after adding the copper reagent? What sort of reaction is happening in the tube?

Lowry Assay Reaction – Step 2

- ☐ 9. Add 200 µL of the Folin-Phenol reagent to the tubes marked 1 – 5, and to tubes marked A, B, and C. Vortex the tubes for three (3) seconds to mix the samples.
- ☐ 10. Incubate sample tubes at room temperature for 5 minutes.

  QUICK CHECK: Compare the eight tubes to each other. What happened to the samples when you added the Folin-Phenol reagent? Which of the unknowns A, B or C appears to have the largest amount of protein? How do you know?

Analysis of the Protein Standards and Unknown Samples

- ☐ 11. Take your samples to the spectrophotometer. An instructor will demonstrate how to use the spectrophotometer. The spectrophotometer measures absorbance, which is the amount of light absorbed by the color in the tubes.
- ☐ 12. Make sure the wavelength of the spectrophotometer is set with the dial to 750nm.
- ☐ 13. Insert sample tube 1 into the cuvette holder and press the 0 A/100 %T button to blank the spectrophotometer (Note ***A***=***A****bsorbance;* ***T***=***T****ransmittance*). Wait until the display reads 0.000. Each group must use their own tube 1 to blank the machine before reading their other tubes.

  QUICK CHECK: Why is sample tube 1 used to “blank” the spectrophotometer?

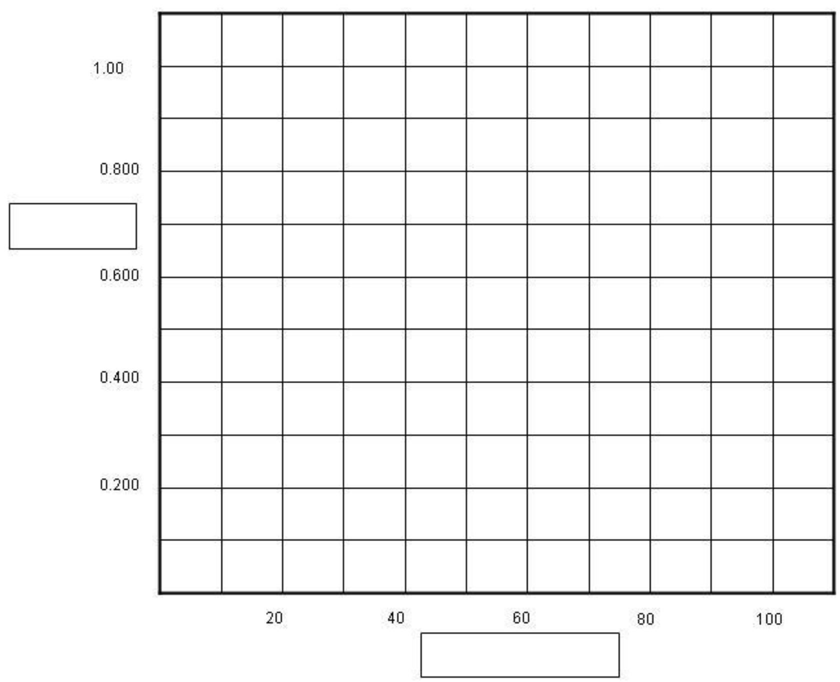
- ☐ 14. Take sample tube 1 out of the cuvette holder and insert sample tube 2 (**DO NOT PRESS ANY BUTTONS**). Record the absorbance displayed on your Data Sheet.
- ☐ 15. Remove sample tube 2 and read tubes 3, 4, 5, A, B and C. Record the absorbance for each sample on your Data Sheet (page 14).

Data Analysis

Use this chart to record the absorbencies indicated by the spectrophotometer.

Test Tube	Protein (µL)	Absorbance	Protein Concentration (µg/ul)
1	0		
2	25		
3	50		
4	75		
5	100		
A			
B			
C			

Using the absorbance values for the standards with known protein concentrations (0, 25, 50, 75, and 100 µL of protein in 1 mL of solution), create a graph with a trend line, or line of best fit. Make sure to fill in labels for your axes (what you are measuring *and* the units you are using). Use your graph to determine the amount of protein in your unknown samples by marking their absorbance on the graph, then estimate how many microliters of protein were in the samples. Finally, determine the concentration of protein in each of your samples (remember that the stock solution of protein was **1 µg/µl**). Record your answers in the table above.



Which of the samples, A, B or C had the highest concentration of protein? How do you know?

Review Questions

What is a standard?

How were standards used for this experiment?

Why is the Copper Reagent (Cu^{2+} Reagent) added to the test tubes?

What happened when the Folin-Phenol was added to the test tubes? Explain why.

How does the amount of color in the tube relate to the amount of protein?

What does the spectrophotometer do?