Gee, Roy and Biv's Micropipette Challenge

Laboratory science often involves working with very small volumes of liquid; frequently millionths of liters are used. One millionth of a liter is equal to one microliter, abbreviated 1 µl.

1 liter = 1,000 ml = 1,000,000 µ l

It would be very difficult to measure such small volumes without a very accurate and precise instrument. The instrument most often used to measure microliters is called a micropipette. Different micropipettes are used to measure different volumes.

Gee, Roy, and Biv are having problems with there science lab. Their teacher is asking them to construct a model of a spectrum, but none of them have a clue as to what a spectrum is, let alone how to make one. Use the following table and the directions that follow to help them by constructing your own spectrum. It is important that you follow the directions and use the best pipette technique possible.

USE THE FOLLOWING TABLE TO RECORD YOUR ADDITIONS AND SUBTRACTIONS TO YOUR TEST TUBES:

Test Tube Number	Starting Volume (color to be added or subtracted)	Amounts Added or Subtracted to the Starting Volume		Total Volume at End in microliters (µl) (color of tube)	Total Volume at End in milliliters (ml)
1	1900 µl (Red)				
2	0 µl				
3	2200 µl (Yellow)				
4	0 µl				
5	2500 µl (Blue)				
6	0 µl				

Setting up your tubes:

1. Label the six test tubes at your station, 1-6.
2. Put 1900µl of red water into test tube number 1.
3. Put 2200µl of yellow water into test tube number 3.
4. Put 2500 µl of blue food coloring into test tube number 5.

Constructing ROY's Spectrum: Make sure your record your actions in the table above.

5. Take 400 µl from test tube number 1 and put it into test tube number 2.
6. Take 400 µl from test tube number 1 and put it into test tube number 6.
7. Take 400 µl from test tube number 3 and put it into test tube number 4.
8. Take 700 µl from test tube number 3 and put it into test tube number 2.
9. Take 700 µl from test tube number 5 and put it into test tube number 4.
10. Take 700 µl from test tube number 5 and put it into test tube number 6.

Crunching the numbers:

11. Use your data table to find the total volume in each tube and record your answer in the table.
12. Use the conversion factor to convert your units from microliters to milliliters.
13. What is the spectrum that you created?

The Power Drink Challenge

Several companies are competing to produce a new product called the *Power Drink*. It is a high-protein drink for athletes for improved physical performance. 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.

You will work in teams of two. Each team will be given samples from the drinks; the samples will be labeled A, B, or C. Your job is to determine the concentration of protein in your sample expressed as micrograms of protein per milliliter of sample (μg/ml). You will be given a laboratory protocol that you will follow to determine the concentration of protein in the three different sports 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.

Caution: Do NOT taste the samples

Crucial Concentration: Laboratory Protocol

Introduction

This is the protocol used to determine the amount of protein in the sports drink samples (A, B, and C).

DID YOU KNOW?
conical tube: The largest plastic tube with a screw-cap blue lid, the bottom is shaped like a cone
test tube: Standard glass tube without a lid
microcentrifuge tube: a very small plastic tube with an attached lid

You will find the following tubes and samples at your workstation:

- Three unknown sports drink samples (1.5 ml) labeled "A, B, and C" in microcentrifuge tubes.
- One microcentrifuge tube labeled "Protein" that contains 0.5 ml of protein of a known concentration. (You will use this to make your set of protein standards)
- Eight empty test tubes: Five will be used to mix known concentrations of protein and distilled water to create a set of standards. Three will be used to test the unknown samples A, B, and C.
- One conical tube labeled "Cu" that contains 10 ml the copper reagent.
- One microcentrifuge tube labeled "FP" that contains 1.8 ml Folin-Phenol reagent.
- One conical tube labeled "dH_2O" that contains 6 ml of distilled water.

Note: when working with micropipettes be sure to change tips when working with new liquids or after another liquid touches the tip. This will prevent contamination of samples. Discard tips once you are finished using them. When handling tubes hold them near the top to prevent putting fingerprints and oils onto the glass, which will be read through the spectrophotometer.

Prepare Unknown Samples:

1. Using a wax pencil or lab marker, label three test tubes "A, B and C", and label five of the test tubes "1, 2, 3, 4 and 5" all at the top of the tubes. (Tubes are labeled at the top because light needs to pass through the center of the tube while in the spectrophotometer)

2. Using the 1000 ul micropipette and a clean blue tip for each, add 1000 ul of each unknown sample ("A, B, and C") from their microcentrifuge tubes to their corresponding test tubes labeled "A, B, and C".

Prepare Protein Standards:

3. Using the 1000 ul micropipette and a clean blue tip for all tubes, add the following amounts of distilled water ("dH_2O") from the conical tube to test tubes labeled "1,2,3,4, and 5":

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

4. Using the 200 ul micropipette and a clean yellow tip for each tube, add the following amounts of "Protein" from the microcentrifuge tube to the bottom of the test tubes labeled "1,2,3,4, and 5": **Note**: **you must make sure the small volume of protein gets added to the water in the tube.**

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

Lowry Assay:

5. Using the 1000 ul micropipette and a clean blue tip for each tube, add 1000 ul of the "Cu Reagent" from the conical tube to each of the 8 test tubes labeled "1,2,3,4, and 5" and "A, B, and C". Hold each test tube at the top and mix the solutions by shaking gently.

6. Incubate the test tubes at room temperature for **5 minutes**.

7. Using the 1000 ul micropipette and a clean blue tip for each tube, add 200 ul of the "FP" (Folin-Phenol reagent) from the microcentrifuge tube to each of the 8 test tubes labeled "1,2,3,4, and 5" and "A, B, and C". Hold each test tube at the top and mix the solutions by shaking gently.

8. Incubate the test tubes at room temperature for **5 minutes**.

Analysis of the Protein Standards and Unknown Samples:

9. Take your 8 test tubes to the spectrophotometer. Your teacher will demonstrate how to use the spectrophotometer. Groups must take turns on the spectrophotometer and should work as quickly as possible to allow the next group to use it. The spectrophotometer measures absorbance, which is the amount of light absorbed by the color in the tubes.

10. Make sure the wavelength of the spectrophotometer is set to 750 nm.

11. Insert test tube labeled "1" into the tube holder and follow the manufacturer's instructions to blank the machine. When the instrument is blanked correctly, the absorbance for test tube labeled "1" will be a reading of "0.000". (**Note**: Each group must use their OWN test tube labeled "1" to blank the machine because of the possibility of pipetting error between groups).

12. Remove test tube "1" from the tube holder and insert test tube "2". **IMPORTANT: DO NOT PRESS ANY BUTTONS**. Record the absorbance displayed, on your Data Sheet.

13. Remove test tube "2" and read test tubes "3, 4, 5, A, B and C" in the same way. Record the absorbance for each sample on your Data Sheet. Follow the directions for graphing your data.

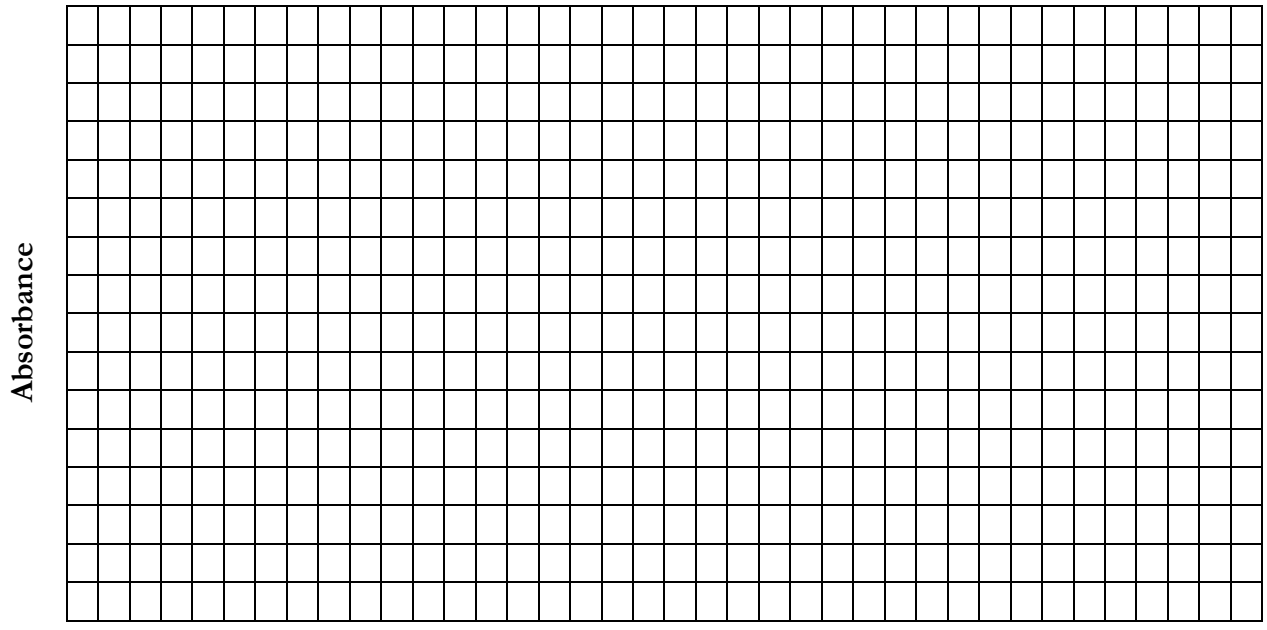
Name:_____

Crucial Concentration: Data Sheet

Use this chart to record the absorbencies indicated by the spectrophotometer for all 8 tubes. Estimate the amount of protein in each of the unknown samples based on the standard curve created when you graph the absorbencies for the set of protein standards (see below).

Test Tube	Protein (µl)	Absorbance
1	0	0.000
2	25	
3	50	
4	75	
5	100	
A		
B		
C		

Graph the absorbance data for the five protein standards (tubes 1,2,3,4, and 5) on the graph below. Draw a "best-fit" line (an average) through the data points. To determine the concentration of protein in each of the unknown samples, find its absorbance on the y-axis and draw a horizontal line across the graph. From the point where this line intersects the "best-fit" line, draw a vertical line down to the x-axis. The point where this line intersects the x-axis will tell you how many microliters of protein were in the one milliliter of sample. Record your answers in the table above. Your teacher will discuss converting ul/ml to ug/ml of protein.



Name:_____

Crucial Concentration: Student Worksheet

1. What is a standard?

2. How were standards used for this experiment?

3. Why is the Copper Reagent (Cu Reagent) added to the test tubes?

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

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

6. What does the spectrophotometer do?