the Crucial Concentration

Investigating Unknown Quantities of Protein Using the Lowry Assay

Maryland Loaner Lab Teacher Packet

www.towson.edu/cse/beop

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Time Requirements:

Activity:	**Time needed:**
Preparing Reagents and Student Stations.	30 minutes
Pre-Lab Activity 1 - "Understanding Standards"	35 - 70 minutes
Pre-Lab Activity 2 - Micropipette Challenge	30 – 45 minutes
Main Laboratory Investigation.	45 minutes
Graphing and Analysis of Results	15-30 minutes
Analysis Questions.	Varies

List of Materials

Materials provided by MDLL:

The following amounts are for 1 class set; the amounts you receive may vary depending on the number of class sets you have requested. Please check the individualized paperwork that has been sent with your kit for the amounts included in your box.

Description	Quantity	Comments	Must be Returned
Teacher Binder	1 binder	Contains all info necessary for completing lab	Yes
Bag labeled "Materials for Pre-Lab Activity 1 – 'Understanding Standards'"	- 5 (15 ml) conicals of "Blue Dye" - 5 transfer pipettes - 10 (50 ml) conicals filled with unknown concentration of blue water labeled Unknowns A-E - Teacher Demo Set (8 (50 ml) conicals) - 1 flashlight	Each of 5 groups of students receives: - 1 (15ml) conical "Blue Dye" - 1 transfer pipette - 2 (50 ml) conicals of blue water of unknown concentration (NOT to be opened by students) For Teacher Demo Set of Standards: - Set of 6 (50 ml) conicals of blue water of known concentration and 2 conicals of blue water of unknown concentration	- return unused portion of stock colored water - Do NOT return transfer pipettes - DO return unknowns (should NOT be opened by students) Return Demo Set filled (do NOT empty). - Return flashlight
Bag labeled "25 empty 50-ml conicals for Pre-Lab Activity 1 – 'Understanding Standards'"	25 empty (50 ml) conicals	For Pre-Lab Activity 1. Allow each group to choose how many they want to use.	Yes, empty tubes rinsed and dried
Bag labeled "Materials for Pre-Lab Activity 2 - Micropipette Challenge"	- 10 (15 ml) conicals of red water - 10 (15 ml) conicals of yellow water - 10 (15 ml) conicals of blue water	For Pre-Lab Activity 2 - Micropipette Challenge. Each group gets a red, yellow and blue tube.	Yes. Return unused portions.
1000 ul micropipettes	10	Used to measure 100 ul – 1000 ul. (1 per group.)	Yes
1000 ul tips	5 boxes	1 box per 2 groups. (tips are blue)	Yes, unused tips
200 ul micropipettes	10	Used to measure 20ul – 200 ul. (1 per group.)	Yes

200 ul tips	5 boxes	1 box per 2 groups. (tips are yellow)	Yes, unused tips
Bag labeled "Solution 1and Solution 2 for Lab Activity"	- 1 bottle "Solution 1 (110 ml)" - 1 microcentrifuge tube "Solution 2 (220 ul)"	Sol 2 to be added to Sol 1 by teacher, see prep directions. Keep at room temperature.	- Empty Sol. 1 bottle rinsed and returned - Throw away Sol 2 microcentrifuge tube
Bag of tubes labeled "Cu Reagent (Solution 3) tubes"	10 empty (15ml) conical tubes labeled "Cu Reagent"	Teacher will prep Cu Reagent (Solution 3) and transfer 10 ml into each conical tube. 1 per group. MUST BE MIXED DAY OF LAB. Keep at room temperature.	Yes, empty tubes rinsed and dried
Bag of tubes of Folin-Phenol Reagent labeled "Folin-Phenol Tubes"	10 (15ml) conical tubes labeled "Folin-Phenol"	1 conical tube per group. Keep at room temperature.	Yes, empty tubes rinsed and dried
Bag labeled "Protein Samples for Lab Activity – Keep Refrigerated"	- 10 (15ml) conical tubes of unknown sample A - 10 (15ml) conical tubes of unknown sample B - 10 (15ml) conical tubes of unknown sample C - 10 (15 ml) conical tubes labeled "Stock Protein (0.5ug/ul)"	Each of 10 groups receives: - 1 (15ml) tube of Unknown A - 1 (15ml) tube of Unknown B - 1 (15ml) tube of Unknown C - 1 (15ml) tube "Stock Protein (0.5ug/ul)" MUST BE KEPT IN THE REFRIGERATOR	Yes, empty tubes rinsed and dried
Bag of tubes of distilled water labeled "dH_2O tubes"	10 (15ml) conical tubes labeled "dH_2O"	1 tube of distilled water for each of 10 groups	Yes, empty tubes rinsed and dried
Tupperware container labeled "Glass test tubes"	~150 test tubes	6 glass test tubes/group for Pre-Lab Activity 2 (Micropipette Challenge) and 8 tubes/group for main lab activity (extra to account for breakage)	- Only return UNUSED glass test tubes - DO return container they came in.
Bag of Sharpies labeled "Sharpies for students to label test tubes"	10 fine-tipped Sharpies	For students to label test tubes	Yes
Bag labeled "10 sets of cuvettes"	80 (10 sets of 8 cuvettes each, labeled "1-5, A, B, C")	8 per group to use in colorimeter	Yes, empty tubes rinsed and dried

Cuvette Rack	10	1 per group to hold cuvettes	Yes
Test Tube Rack	10	1 per group	Yes
Disinfectant Wipes	1 bottle	Please wipe down pipettes	Yes
Insulated Pack	1-2	Contains protein samples	Yes
Colorimeter and LabQuest Data Unit	3-6	Each kit will have at least 3 and up to 6 units, depending upon availability, to be shared among student groups	Yes
Kimwipes	3-6	Place one by each colorimeter to clean smudges from cuvettes	Yes

Materials provided by Teacher:

Description	**Quantity**	**Comments**
Tap Water	Varies	For use by students in Pre-Lab Activity 1 – "Understanding Standards"
Graduated Cylinder (10ml)	1	Used to measure Cu Reagent
Disposable Cups	10	1 per group – waste container
Containers of various sizes for Pre-Lab Activity 1 (optional)	Varies	See Pre-Lab Activity 1 directions for more explanation

SAFETY: The classroom teacher must instruct students with basic laboratory safety rules and provide gloves and goggles for student use with the laboratory activity.

Maryland Science Core Learning Goals

Goal 1.0 Skills and Processes

1.3	The student will carry out scientific investigations effectively and employ the instruments, systems of measurement, and materials of science appropriately.
1.4.4	*The student will determine the relationships between quantities and develop the mathematical model that describes these relationships. **
1.1.5	The student will explain factors that produce biased data (incomplete data, using data inappropriately, conflicts of interest, etc.).
1.2.1	The student will identify meaningful, answerable scientific questions.
1.2.6	The student will identify appropriate methods for conducting an investigation (independent and dependent variables, proper controls, repeat trials, appropriate sample size, etc.).
1.2.8	The student will defend the need for verifiable data.
1.3.1[NTB]	*The student will develop and demonstrate skill in using lab and field equipment to perform investigative techniques. **
1.3.2	*The student will recognize safe laboratory procedures. **
1.3.3[NTB]	*The student will demonstrate safe handling of the chemicals and materials of science. **
1.3.4[NTB]	*The student will learn the use of new instruments and equipment by following instructions in a manual or from oral direction. **
1.4.1	The student will organize data appropriately using techniques such as tables, graphs, and webs. (for graphs: axes labeled with appropriate quantities, appropriate units on axes, axes labeled with appropriate intervals, independent and dependent variables on correct axes, appropriate title)
1.4.2	The student will analyze data to make predictions, decisions, or draw conclusions.
1.5.4	The student will use tables, graphs, and displays to support arguments and claims in both written and oral communication.
1.6.5	The student will judge the reasonableness of an answer.
1.7.5	Students will investigate career possibilities in the various areas of science.

Goal 4.0 Concepts of Chemistry

4.1.2	*The student will gather and interpret data related to physical and chemical properties of matter, such as density and percent composition. (constructing data tables, graphing linear relationship, appropriate technology to analyze data) **

* Italicized CLG's are the primary focus of the laboratory activity. The other indicators are addressed, but can also be used as a guide to determine the focus of the lesson plans for the pre-lab and post-lab extension activities.

Crucial Concentration Module Overview

The Crucial Concentration Loaner Lab Module has three parts:

- A pre-laboratory classroom activity ("Understanding Standards") that challenges students to create a method for measuring the concentration of blue dye in a solution.

- A pre-laboratory classroom activity ("Roy, Gee, and Biv's Micropipette Challenge") that introduces students to the use of a micropipette.

- A main laboratory activity ("The Power Drink Challenge") that allows students to use the Lowry Assay to determine which of three sports drinks has the highest concentration of protein.

Scientists are often faced with the challenge of determining the concentration of a substance in a solution. For example, they may need to measure levels of proteins, cholesterol, glucose, or the rate of enzymatic activity. This investigation focuses on a colorimetric assay commonly used to measure protein concentrations called the Lowry Assay. The Lowry Assay requires a series of standard protein solutions to create a standard curve. The standard curve is used to measure the quantity of protein in an unknown solution. Because the protein used to make the standards is colorless, a chemical reaction is required to produce a color. The intensity of that color is in direct proportion to the amount of protein present.

The concept of developing standards for measurement is frequently applied to solve quantification problems. A standard is a tool made up of known increments or units, which is used to measure something. This idea is often quite familiar to students although they may not recognize it as such. When comparing lengths of objects, for example, a standard such as a ruler or tape measure is a common tool. Measuring the concentration of a substance in a solution also requires development of a standard.

This investigation is organized into two parts – two pre-laboratory activities ("Understanding Standards" and "Roy, Gee, and Biv's Micropipette Challenge") and a main laboratory activity ("The Power Drink Challenge").

The first pre-laboratory activity, "Understanding Standards", introduces students to the concept of using a standard to determine concentrations of unknown solutions by challenging them to determine the number of drops of blue dye in a bottle of water. Resources are provided for the students to design their investigation and they are encouraged to share information and ideas. In attempting to solve the problem, students discover that they need to make a set of standards of known concentrations to compare with the unknowns. In doing so, students also explore the concepts of variables and constants. The instructor's role is that of a coach, leading students by inquiry where necessary and making them aware of the concepts they apply, such as the use of constants, variables, and the creation of a set of standards.

The second pre-laboratory activity, "Roy, Gee, and Biv's Micropipette Challenge", will allow students to practice using micropipettes. Measuring volumes precisely is critical in this lab and this fun challenge offers students ample opportunity to practice their pipetting techniques.

Following the pre-laboratory activities, students apply the concepts that they learned to perform the main laboratory activity, “The Power Drink Challenge”. In this activity, students determine which of three sports drinks contains the highest concentration of protein. If students haven’t used colorimeters, then additional time will be needed to explain how they work and how to use them properly.

Pre-Laboratory Activity 1 - “Understanding Standards” - Explanation

The purpose of this pre-laboratory activity is to challenge students to create a method for measuring unknown concentrations of blue dye in a solution. It provides students with the opportunity to construct ideas about the creation of a set of standards, the need for constants and variables, and the use of chemical reactions to solve quantification problems.

The Objectives of Pre-Laboratory Activity 1 are:

- Develop a method to find the concentration of blue dye in a solution of blue dye and water
- Work cooperatively to solve a problem
- Record and execute procedures developed by each group
- Verbally communicate procedures and results
- Express data in graph form
- Create and use a standard curve

Pre-Laboratory Activity 1 Materials:

- To be shared by all students:
    - One large container of water (to supply students with water during the experiment)
    - We have provided 25 empty 50ml conicals for this activity, but you can give the students additional options as well, such as several empty containers of varying sizes. (Suggestions are beakers or Erlenmeyer flasks).
    - Measuring devices, such as graduated cylinders, tubes, or cups
    - Flashlight
- Each of the 5 groups needs:
    - 2 different (50ml) conicals with colored water of unknown concentrations
    - 1 (15ml) conical of “Blue Dye”
    - 1 plastic transfer pipette
    - Student lab notebooks, or a piece of paper to record protocol and results and graph paper to graph results (or students can draw their own graph paper, if necessary)

Pre-Laboratory 1 Engagement (5 minutes)

Organize students into 5 groups. At each group’s station, place the materials listed above. Challenge each group to develop a method to determine how many drops of blue dye are dissolved in the mystery solutions of unknown concentrations.

Pre-Laboratory 1 Exploration (5 - 10 minutes)

Set up a central table with the large container of water, empty 50ml conical tubes (and any other empty containers of varying sizes you choose to set out) and the various measuring devices. Tell the students they can use any or all of the resources available to solve the problem of determining the amount of blue dye in their unknown samples that were introduced in the first step.

Have the students record the procedure they develop for solving the problem and their experimental data in their lab notebooks. (Provide students with a sample of the laboratory notebook format to use). Each student is responsible for recording the procedure and data in their own notebook. Also, have each student group present their procedure and data to the class.

Pre-Laboratory 1 Explanation (10 - 15 minutes)

Require each group to tell the class what they did and what their results were. The most obvious method is to add drops of blue dye to identical containers with the same volume of water until the color intensity matches the colors of the unknowns. Going a step further, some groups may create a set of standards of known concentration to which they can compare their unknowns. Reinforce all logical attempts, but if no teams discover this "creation of standards" method, the teacher needs to suggest it. It is the same basic method that will be used to discover the concentration of protein in the unknown sample they will receive for the main laboratory activity.

Ask the students what variables (e.g., the number of drops of blue dye) there are in the investigation and list them on the board; list also those things held constant (e.g., volume of water, size of container, etc).

Explain that by creating a set of solutions with known number of drops of blue dye in the same volume of water as the unknown, they have created a set of standards. Ask for other examples of standards that are commonly used. For example, rulers are standards used to measure length. In this instance, we made a standard to measure concentrations of blue dye in water.

Demonstrate how a set of standards could be used to measure the concentration of an unknown. Place the Teacher Demo Set of Standards (6 (50ml) conicals with increasing concentration of color) on a table in front of the class in increasing order of the number of drops of blue dye. Inform the students how much blue dye is in conicals #1-6 (they are labeled). Hold up the 2 conicals of unknown concentration from the teacher demo set and ask the students to determine the number of drops of blue dye in each of them. There is bound to be some disagreement about where the bottle should be placed among the set of standards in conicals #1-6. Use this discrepancy to make the point that a more objective and sensitive way of comparing the bottles should be used. This can be accomplished by using a machine, such as a colorimeter, which provides reproducible scientific data.

Pre-Laboratory Extension (10 – 30 min)

To demonstrate how a colorimeter works, shine a flashlight through the conicals so that the light reflects on a white background. Ask the class to assign each color intensity a number from 1 to

10, 1 being the lightest and 10 being the darkest. Record the values assigned by the class for each standard. (This is what a colorimeter does). Next, have the students graph the results with the concentration on the x-axis and the number assigned for color intensity on the y-axis. When the best-fit line is drawn, they will have constructed a standard curve. Demonstrate how the graph can be used to estimate the unknown concentrations of the mystery solutions (based on Beer's Law- see explanation of Beer's Law on page 13).

We have purposefully not given the concentration of the unknown solutions in the pre-laboratory activity. This is because it is important for students to realize that when conducting authentic science, scientists cannot "check the back of the book" to see if their answers are "correct". If your students ask you what the 'right' answer is, use that question as an opportunity to discuss the nature of authentic science. You can ask the students how they think scientists handle this element of uncertainty when they conduct their own research. Ideas that may come up in the discussion include:

- Scientists may repeat an experiment a number of times to confirm their conclusions are reliable.
- Scientists must carefully record their protocols, procedures and results so they can carefully scrutinize them for consistency.
- Scientists often share their work and collaborate with others, allowing their methods, results and conclusions to be critiqued and validated.

Pre-Laboratory 1 Assessment (5 - 10 minutes)

Ask the students to write a summary of what they learned and to describe some applications for the technique of measuring concentrations.

Pre-Lab Activity 2: Micropipette Challenge Explanation (30-45 minutes)

"Roy, Gee, and Biv's Micropipette Challenge" is a second pre-laboratory activity that will allow students to practice using micropipettes. This activity may be performed the day of the main laboratory activity or any time in advance.

The goal of this lab is for students to use proper pipetting technique to move around different amounts of colored water. In the end, they should end up with six test tubes with the same amount of liquid in each. The colors will form a rainbow - hence the name, "Roy, Gee, and Biv's Micropipette Challenge". "Roy G. Biv" stands for "red, orange, yellow, green, blue, indigo, and violet", which are the colors in a rainbow spectrum.

By filling out the chart as they go, they can keep track of where they added liquid and where they removed liquid. This provides an important reference for checking for errors if they do not end up with the same amount of liquid in each test tube at the end. They then have an opportunity to practicing converting their units from µl to ml.

Since neither of the pipettes supplied can measure the necessary initial volumes (over 1000 µl) students will need to pipette in two steps. For example, in step 2 of the Micropipette Challenge, students are instructed to pipette 1900 µl. They therefore, must split the amount (e.g. 1000 µl and then 900 µl). TRYING TO TURN THE PIPETTES PAST THEIR MAXIMUM VOLUME WILL CAUSE THEM TO BREAK.

All students must be reminded about the proper usage of micropipettes to prevent damage to the equipment and also to provide students with accurate results during the main laboratory activity. Be sure that everyone understands how to operate the micropipettes. It is worthwhile to check each student for correct technique before beginning the main laboratory activity.

Student Technical Skills

Teachers should read through this section so they are able to explain proper use of equipment to their students.

Micropipettes

Micropipettes are precision instruments designed to measure and transfer small volumes. They are expensive and must be used with care. Their accuracy is dependent upon their proper use. Different brands of micropipettes vary in the range of volumes they will measure, the type of tips they fit, and the type of device used to set the volume.

Golden Rules of Pipetting

1. Don't rotate the volume adjuster beyond the upper or lower range of the pipette - this can damage it.

2. Don't use a pipette without a tip on it. If this happens, liquid gets into the opening of the pipette and can damage the mechanism inside.

3. Don't lay down a pipette that has a tip filled with liquid. If this happens, liquid can get inside the pipette and can damage it.

Setting the Volume

All micropipettes have a volume control dial. Determine whether the volume window on your pipette shows tenths of microliters (0.1 µl) or whole microliters in the smallest place, so that you can read the scale correctly (it varies with different brands of micropipettes). Each set of micropipettes comes with a laminated card with specific instructions for setting their volumes.

Drawing Up and Expelling Liquid

Micropipettes have 2 stops as you depress the plunger to expel liquid. The first stop corresponds to the volume set in the window. The second stop gives a little puff of air to blow out any remaining liquid upon delivery. To draw liquid into the pipette tip, depress the plunger control only to the first stop. If you go to the second stop, you will draw too much liquid into the tip. The most common pipetting error is to go past the first stop, to the second stop, for drawing liquid into the tip (which gives an inaccurate volume). You go to the second stop only when you are letting the liquid out of the tip.

Using the Micropipette

1. Select the pipette that includes the volume range you will need.
2. Adjust the pipette to the desired volume by turning the dial. DO NOT turn beyond the volume range for the pipette.

3. Press a new tip onto the pipette firmly (gently tap the pipette into a tip while the tip is in the box). Get a tip without touching it with your hands - this is to prevent contamination of the samples.

4. To draw liquid into the micropipette tip:
   - Depress the plunger to the first stop to measure the desired volume and hold it in that position.
   - Holding the pipette vertically, immerse the tip 1-3 mm into the liquid to be transferred.
   - Draw the fluid into the tip by slowly releasing the plunger. Wait 1-2 seconds to be sure that the full volume of sample is drawn into the tip. If you see air bubbles, there is a problem with your volume and you will need to repeat this step to get the correct volume (either your tip wasn't immersed far enough down into the liquid or you perhaps raised your arm while releasing the plunger).

5. To dispense the liquid:
   - Place the tip into the container where the liquid is to be released, near the bottom.
   - Slowly depress the plunger to the second stop to blow out all of the liquid in the tip. Be careful not to suck liquid back into the tip by releasing the plunger while the tip is in the liquid you just dispensed.
   - Eject the tip when done into a waste container by pressing the separate ejector plunger found on the top or side of the micropipette (depending on the brand of micropipette).

Main Laboratory Activity Explanation and Lesson Plan

The purpose of the main laboratory activity, "The Power Drink Challenge", is to use the Lowry Assay to determine which of three sports drinks has the highest concentration of protein.

The Objectives for the main laboratory activity are:

- Apply the general concept of quantification to a Lowry Assay
- Collect data using a colorimeter
- Graph data to produce a standard curve
- Using a standard curve, determine the concentration of a protein solution

Developing the Concept for the Assay

Give each student a copy of the student worksheets. Have them read the background information about "The Power Drink Challenge" and challenge them to find the concentration of protein in three unknown sports drink samples. Ask each group to develop an idea for solving the problem. Remind them of the exercise they did with the colored water. Lead a discussion about their ideas and record the highlights on the board.

Now show students the three protein solutions: "A, B, and C". Students should realize that, unlike the blue dye solutions, the protein solutions have no color and, therefore, cannot simply be visually inspected to indicate how much protein is in the solution. Inform students that a chemical reaction can be used to add color. The reaction they will use is a two-step assay called the Lowry Assay, diagrammed in their student worksheets. Once the Lowry Assay has been performed, a colorimeter can be used to measure the absorbency of each of the protein standards as well as the unknown samples. At this point, students will use Beer's Law to determine the concentration of their unknown samples. Beer's Law states that the concentration of a chemical substance is related to the amount of light absorbed by the sample. The equation for Beer's Law is

$$A = \varepsilon б c$$

where A = absorbance, ε is the molar extinction coefficient (L/mol●cm), б is the pathlength of the sample cell (cm), and c is the concentration of the substance that absorbs light. Notice that both ε and б must be known to directly calculate c from A. If ε and б are not known (as in this case), then a standard curve of absorbance vs. concentration, using standards of known concentration, can be constructed. The slope of the line is equal to the product, εб. The relationship between A and c must be linear to satisfy Beer's Law. In the Lowry assay, you are effectively constructing a standard curve assuming Beer's Law and determining the product, εб.

The Main Laboratory Activity - The Power Drink Challenge

Create the Protein Standards and Perform the Lowry Assay

Each group of students receives a stock protein solution (0.5 µg/µl BSA or Bovine Serum Albumin) and distilled water. They will create a set of standards by making a series of protein solutions of known concentrations. The students next perform the Lowry Assay on both the set of protein standards and three unknown protein solutions (labeled A, B, and C).

Use the colorimeter and record the data

Teachers must provide instructions to the students for using the colorimeters - follow the instructions on pages 16-17. Please also place a laminated set of colorimeter instructions next to each colorimeter/LabQuest unit. Students will next measure the absorbance of each solution (their standards and their unknowns) at 635 nm on a colorimeter. The data is recorded on the students' "Data Table".

Interpretation of Results

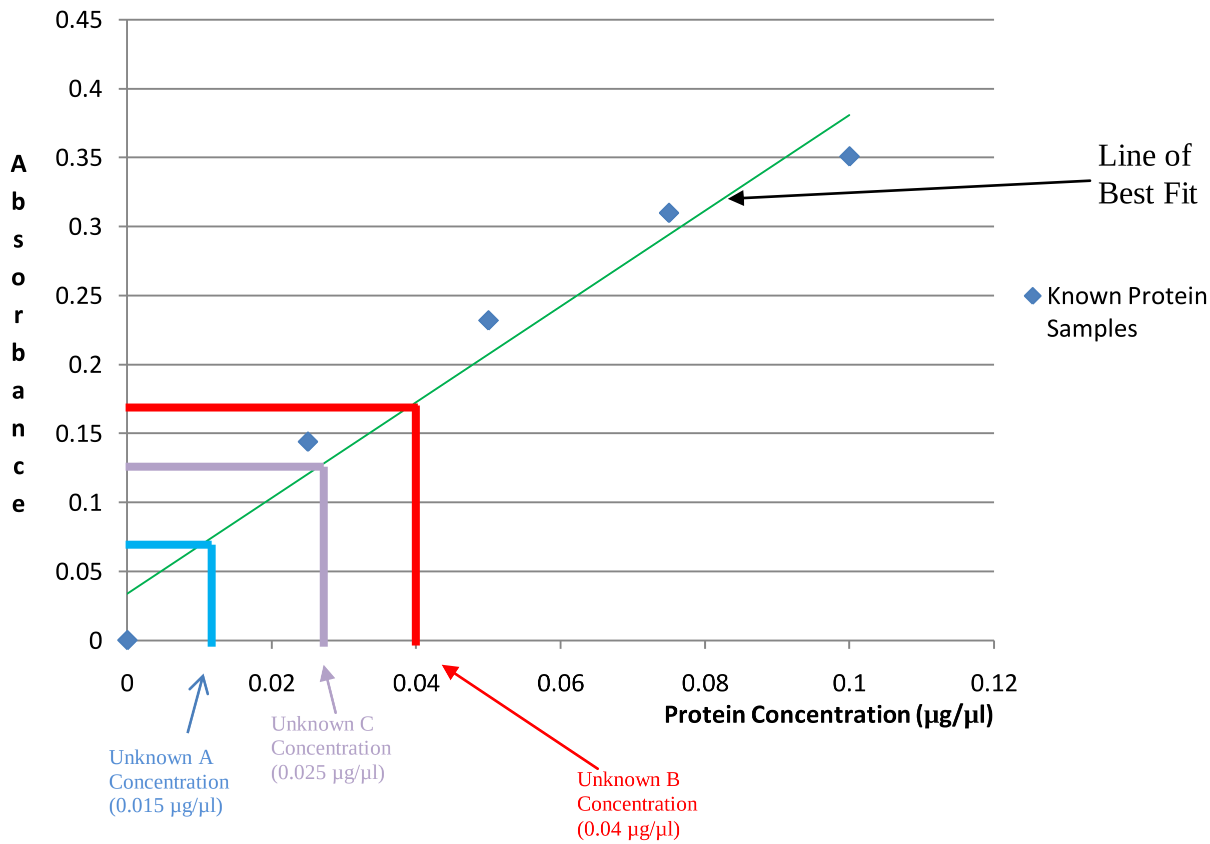
Students should graph their data to develop a standard curve and then use the standard curve to measure the concentration of protein in their unknowns (based on the relationship between absorbance and concentration as detailed in Beer's Law). Students should be able to conclude which of the three companies is accurate in its claim that its sports drink contains the most protein. Students should complete the analysis questions. The last analysis question asks students to write a report to each company explaining their results. They should provide evidence in their report to support their results.

Ask each team of students to post their results on the board. Discuss the reasons for discrepancies in the results (such as pipetting error, timing errors, limitations of graphing by hand, etc.). Calculate a class average for the concentration of each protein solution (A, B, and C) and determine the standard deviations.

Resist the temptation to give the students the 'correct answer'. In a research laboratory, a scientist would not be able to 'confirm' they had the correct answer, yet scientists are confident in their results. If your students ask you what the 'right' answer is, use that question as an opportunity to discuss the nature of authentic science. You can ask the students how they think scientists handle this element of uncertainty when they conduct their own research. Ideas that may come up in the discussion include:

- Scientists may repeat an experiment a number of times to confirm their conclusions are reliable.
- Scientists must carefully record their protocols, procedures and results so they can carefully scrutinize them for consistency.
- Scientists often share their work and collaborate with others, allowing their methods, results and conclusions to be critiqued and validated.

The following graph is what the final graph should resemble on the student worksheets. (This graph is provided to assist the educator in evaluating student progress; it is not meant to be shared with the students.)



The green line is the standard curve based on the points obtained from samples #1, 2, 3, 4, and 5, which are the set of protein standards. The blue line is unknown A (with a concentration 0.015 µg/µl of protein), the red line is unknown C (with 0.025 µg/µl of protein), and the purple line is unknown B (with 0.040 µg/µl of protein). Therefore, unknown sample B has the most protein.

Material and Reagent preparation BEFORE students arrive:

Pre-Laboratory Activity 1, "Understanding Standards", Set-up

- All of the solutions for this activity are provided. Please see page 8 for instructions on how to set up each student station. If you choose to provide extra containers of varying sizes for liquids (see page 8), you may want to collect them ahead of time.

Pre-Laboratory Activity 2 - Micropipette Challenge - Reagents:

- All of the solutions for this activity are provided. Please see page 17 for instructions on how to set up each student station.

Main Laboratory Activity Set-up

- Solutions 1 and 2 are pre-made and sent to the schools ready to be used by the teacher to make Cu Reagent (Solution 3).
- Folin-Phenol is pre-made and ready to be used.
- Stock protein (already aliquoted into 10 conical tubes) is ready for use and MUST BE KEPT REFRIGERATED until used.
- Unknown protein samples A, B, and C are pre-made and aliquoted into conical tubes and MUST BE KEPT REFRIGERATED until used.

Solution 1 (110 ml)- (Sodium Carbonate/Sodium Hydroxide)

Supplied by the Maryland Loaner Lab program; marked "Solution 1 (110ml)" in a small plastic bottle. It may be stored indefinitely at room temperature. This will be used to make Cu Reagent (Solution 3).

Solution 2 (220µl) (Cupric Sulfate/Sodium Citrate)

Supplied by the Maryland Loaner Lab program; marked "Solution 2 (220 µl)" in a microcentrifuge tube. It may be stored indefinitely at room temperature. This will be used to make Cu Reagent (Solution 3).

Solution 3 - Made by teacher (CU Reagent)

Using a 1000 µl micropipette and clean tip, add all of "Solution 2 (220µl)" to the bottle labeled "Solution 1 (110 ml)" - be careful to add the entire amount of Solution 2 as it is a very small amount. Cap the bottle and mix very well by inversion. This is now Solution 3, or "Cu Reagent". Using a graduated cylinder, aliquot 10 ml of Solution 3 into each of the 10 conical tubes marked "Cu Reagent" that are provided. There is one tube per group. IMPORTANT: This solution MUST BE PREPARED WITHIN 5 HOURS of being used. Keep at room temperature.

Folin-Phenol

1N Folin-Phenol Reagent ("Folin-Phenol") has already been aliquoted into 10 conical tubes. Keep at room temperature.

Stock Protein

Stock protein solution (Bovine Serum Albumin) labeled "Stock Protein (0.5 µg/µl)" has already been aliquoted into 10 conical tubes. IMPORTANT: Keep protein samples refrigerated until ready to use.

Unknown Protein Samples "A, B, and C"

30 conical tubes have already been filled with 1.5 ml of protein of unknown concentrations (A, B, and C). "Unknown A" has 0.015 µg/µl BSA, "Unknown B" has 0.040 µg/µl BSA, and "Unknown C" has 0.025 µg/µl BSA. IMPORTANT: Keep protein samples refrigerated until ready to use.

Colorimeter and LabQuest Data Collection Unit

Students will use a Vernier colorimeter, plugged into the handheld LabQuest data collection unit, to determine the absorbance values of their known protein concentration standards (test tubes #1-5) and their 3 unknown sports drink samples (test tubes A, B, and C). Laminated instructions are provided that can be placed next to each colorimeter for students to refer to. Instructions for using the colorimeter are as follows:

Fig. 1. LabQuest Data Collection Unit

1. Plug colorimeter into "CH 1" on the Vernier LabQuest (Fig. 1).
2. Turn the LabQuest on by pushing the power button in the upper left corner (Fig. 1).
3. Wait 5 minutes for the colorimeter to warm up.
4. Make sure the colorimeter is set to measure at wavelength, 635 nm (Fig. 2) - this will be indicated by a lit green light under the label "635 nm". If it is not at the correct setting, hit the < or > button to select "635 nm".
5. Students will have already transferred 2 ml of each of their protein solutions (test tubes 1-5 and A, B, and C) into their cuvettes (labeled #1-5, and A, B, and C). Please make sure students use the provided cuvette rack to transport their samples to wherever the colorimeter is located (the colorimeters are shared among groups). This will prevent spilling and loss of their samples. If a group does spill or otherwise lose their sample, we recommend that they take absorbance readings on their remaining samples so they understand how the colorimeter works, but it may be best to have them use another group's data when they complete their graph.

Fig. 2. Colorimeter

6. Each group will need to perform their own calibration. They will do this by putting the sample from their cuvette "1", which contains no protein, into the colorimeter. To do this they will:
    a. Open the colorimeter lid
    b. Clean the cuvette with a kimwipe and gently place cuvette "1" into the colorimeter with the clear side <u>pointing to the arrow on the top of the cuvette slot of the colorimeter</u> (Fig. 3). DO NOT force it down; gently insert it only as far as it easily goes. If you force the cuvette too far into the colorimeter, it becomes stuck in the plastic insert that holds the cuvettes and is very difficult to remove.
    c. Press the "CAL" (calibrate) button on the colorimeter (Fig. 2) and hold until the red LED begins to flash (this is usually very quick - less than one second). Check to see that the reading on the LabQuest unit is either 0.000 or 0.001 (see Fig. 1). You have now calibrated the colorimeter for the rest of that group's cuvettes.

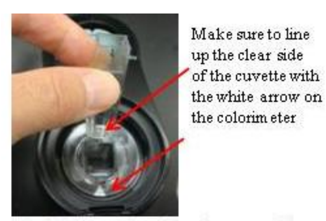


Fig 3. Line up clear side of cuvette with arrow on colorimeter

Remember, EACH group must recalibrate using their own sample # 1 (which contains no protein).

d. Remove cuvette “1”.
e. Insert cuvette “2”. Record the absorbance on the student data sheet. It may take a second or two for the reading to stabilize. Remember, you do NOT want to hit “CAL” again; you only do that with the first sample. Gently remove the cuvette.
f. Repeat above step (step e) for samples 3-5 AND for each unknown sample (A, B, and C). Make sure students record each absorbance reading on their data sheet.
g. Please make sure to gently rinse and dry the cuvettes as we do reuse them. Any scratches to the cuvettes will affect future absorbance readings.

Please note that while the LabQuest can be programmed to produce an Absorbance vs. Concentration graph (based on Beer’s Law), we are purposefully having the students plot their own graph. We have found this is an essential activity for students to fully understand the connection between the standards they made and how they are used to determine the concentration of unknown samples. It also provides an opportunity to reinforce the students’ graphing skills.

Student Workstation Set-up for Pre-Laboratory Activity 2 – Micropipette Challenge

Prepare Student Stations (10):

- Copy of Student Worksheet pages (S1-S10) for each student
- Test tube rack
- 3 conicals filled with colored water (red, blue and yellow)
- 6 empty test tubes for Micropipette Challenge
- 1000 µl micropipette
- One blue box of 1000 µl micropipette tips (1 box/2 student groups)

Main Laboratory Activity

Prepare Student Stations (10):

- Copy of Student Worksheet pages (S1-S10) for each student
- Test tube rack
- 8 empty test tubes
- Cuvette rack
- 8 empty cuvettes
- Sharpie
- 1000 µl micropipette
- One blue box of 1000 µl micropipette tips (1 box/2 student groups)
- 200 µl micropipette
- One yellow box of micropipette tips (1 box/2 student groups)
- One disposable cup (waste container for tips) (provided by teacher)
- Unknown Samples A, B, and C in conical tubes (2.0 ml each)*
- “Stock Protein (0.5 µg/µl)” sample in conical tube (1 ml) *
- “Cu Reagent” (Solution 3) in conical tube (10 ml aliquoted by teacher) *
- “Folin-Phenol” reagent in conical tube (2 ml) *
- Distilled water, “dH_20”, in conical tube (≥ 6 ml)

*Make sure all liquids are thoroughly mixed (by inversion) before placing at stations

Shared Equipment for Multiple Student Stations:

- LabQuest Data Unit and Colorimeter. A minimum of 3 and a maximum of 6 of both of these units (depending on how many we have available) are provided with each kit. Since the groups will have to share, we recommend having the colorimeters and attached LabQuest units set up in a central location and have the students bring their samples to the colorimeter. Place one of the laminated set of directions next to the colorimeter to assist students (but please also give verbal instructions as well). Place a box of kimwipes next to each unit to clean off the cuvettes before they are placed into the colorimeter.

Suggested Extension Activities:

1. Use the wavelength setting of 635 nm for the colorimeter to lead into a discussion about the electromagnetic and visible spectrums. Include a discussion about light absorption, reflection, and transmission, and why we see the colors that we do. Discuss why the colorimeter is set at 635 nm (which is at the red end of the visible spectrum) and why we see the Lowry Assay results in the tubes as a blue-gray color. (The proteins in the samples absorb light in the red end of the visible spectrum and what gets reflected to our eyes is light in the blue end of the visible spectrum, which is why the colorimeter is set at 635 nm.)

2. Have students use computers with graphing software or graphing calculators to graph the results. Compare best-fit lines from the computer/calculator to manually drawn best-fit lines. Discuss accuracy and importance of choosing various methods to analyze data.

3. Incorporate into the post-laboratory activity an in-depth discussion about the Beer-Lambert Law. A very helpful website is: http://teaching.shu.ac.uk/hwb/chemistry/tutorials/molspec/beers1.htm. Discuss why scientists generally prefer to express the Beer-Lambert Law using absorbance rather than % transmittance (%T). (It's based on the linear relationship that exists between concentration and absorbance, except at very high concentrations.) Have the students take the short Beer's Law quiz available at the above website.

4. Have students design an experiment to test the amount of protein in different foods. For example, the protein content of different cereals could be compared. This requires students to ask their own question and alter the procedures accordingly.

Crucial Concentration: Student Worksheet Answer Key

26. What is a standard?

A standard is a basis for comparison. It is a reference against which something can be evaluated. For example, a ruler is a standard used to measure length (inches or centimeters are the units). In this experiment, our set of protein solutions of known concentrations is the *standard (or set of standards) we can use to extrapolate the results of our unknown samples (the unit of measurement is µg/µl).*

27. How were standards used for this experiment?

In this experiment, our set of protein solutions of known concentrations is the standard (or set of standards). By plotting the absorbance results for this set of standards after performing the Lowry Assay on them, as with the unknown samples, we can use a graph to extrapolate the amount of protein in our unknown samples.

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

The copper reagent (Cu+2), when combined with protein, forms a copper/protein complex. The formation of this is the first step in a two-step reaction, called the Lowry Assay. The copper/protein complex is reduced when Folin-phenol is added, which ultimately produces a blue-gray colored solution in which the intensity of color is directly related to the amount of protein present in a sample. If protein is not present in a sample, then the copper reagent will not be reduced to Cu+1 and no color reaction will occur.

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

If protein was present in the sample, the solution turned a shade of blue-gray after adding the Folin-Phenol solution. This happened because the copper/protein complex is reduced by Folin-Phenol and this reduction results in a blue-gray color being produced. Note that Folin-Phenol does not react with Cu+2, so if protein is not present in a sample, then the blue-gray color isn't produced (as seen with test tube #1, which never received protein - only water).

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

The intensity of the blue-gray color produced is directly proportional to the amount of protein that was present in the sample (the darker the blue-gray color, the greater the amount of protein contained in the sample).

31. What does the colorimeter do?

The colorimeter is an instrument that allows us to quantify the amount of color in a solution. The colorimeter measures the amount of light that is able to pass through a solution (% transmittance) at a given wavelength. It then is able to calculate the amount of light that the sample was able to absorb based on Beer-Lambert's Law. The % transmittance and the calculated absorbance are inversely proportional to one another. Therefore, a dark-colored solution will not allow much light to pass through it and will have a lower % transmittance and a higher absorbance than a lighter-colored solution. In this experiment, the amount of blue-gray color produced by the Lowry Assay for a given sample is directly proportional to the amount or concentration of protein within that sample. The colorimeter, therefore, allows us to precisely quantify the amount of protein present in our unknown samples.

Name________________________________

Roy, Gee, and Biv's Micropipette Challenge

Gee, Roy, and Biv are having problems with their 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.

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	760 µl (Red)				
2	0 µl				
3	880 µl (Yellow)				
4	0 µl				
5	1000 µl (Blue)				
6	0 µl				

Hint: 1000 µl= 1 ml

Place a ✓ in each box as you complete the steps below.

Setting up your tubes:

- ☐ 1. Label the six test tubes at your station, 1-6.
- ☐ 2. Put 760 µl of red water into test tube number 1.
- ☐ 3. Put 880 µl of yellow water into test tube number 3.
- ☐ 4. Put 1000 µl of blue water into test tube number 5.

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

- ☐ 5. Take 160 µl from test tube number 1 and put it into test tube number 2.
- ☐ 6. Take 160 µl from test tube number 1 and put it into test tube number 6.
- ☐ 7. Take 160 µl from test tube number 3 and put it into test tube number 4.
- ☐ 8. Take 280 µl from test tube number 3 and put it into test tube number 2.
- ☐ 9. Take 280 µl from test tube number 5 and put it into test tube number 4.
- ☐ 10. Take 280 µl from test tube number 5 and put it into test tube number 6.

Crunching the numbers:

- ☐ 11. Calculate the total volume in each tube and record your answer in the table.
- ☐ 12. Convert your units from µl (microliters) to ml (milliliters). Hint: 1000 µl = 1 ml
- ☐ 13. What is the spectrum that you created?

The Power Drink Challenge: Lab Background

Background Information Scientists sometimes measure how much of something there is in liquids. For example, they may measure the amount of lead levels in drinking water or the amount of dissolved oxygen in the Chesapeake Bay.

The amount of a substance in a solution is often expressed as weight or volume. Concentration is an expression of how much of one substance is dissolved in another substance. It always has one unit divided by another. For example, if I use 3 cups of sugar in 1 gallon of lemonade, the *amount* of sugar is 3 cups but the *concentration* of sugar is 3 cups per gallon.

Nutrition Facts

Serving Size = 250 ml	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%

The Nutrition Facts Label on food and drinks contains information about the nutrients in the product. Look at the label to the right. (It is from a beverage).

1. How many grams of protein per serving are in this drink? (Your answer should include units).

2. What is the CONCENTRATION of protein in this drink? (Your answer should include units).

Laboratory Challenge Several companies are competing to produce a new product, *The Power Drink*. It is a high-protein drink for athletes to improve their 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.

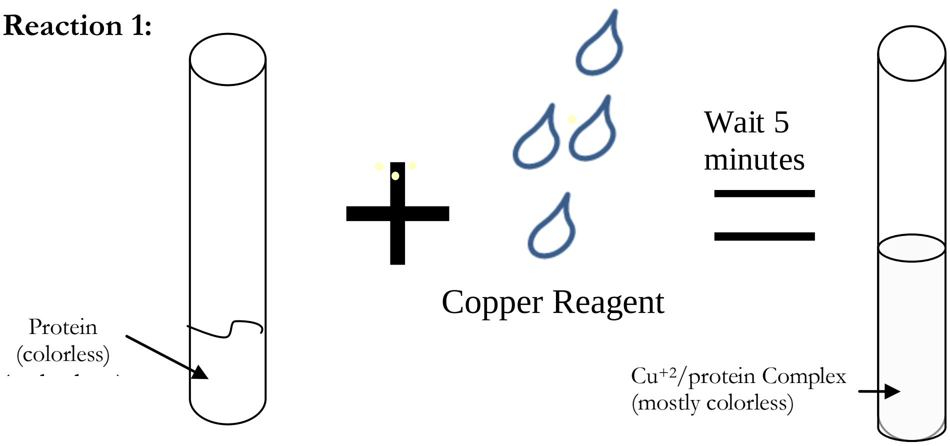
You and your partner can help Sharon find out which of the three companies can actually claim they make the drink with the highest amount of protein. Your job is to determine the concentration of protein in the three drinks. The results from each team will be compared and you will present your conclusions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

Crucial Concentration: Lab Background

How will you see the protein? Protein is colorless – you cannot look at each sample to see the amount of protein in it. But there is a chemical reaction that can make protein turn a blue color - the darker the color, the greater the amount of protein in the sample. It is called the Lowry Assay. Sharon has decided to use the Lowry Assay to help her determine the amount of protein in the different sports drinks.

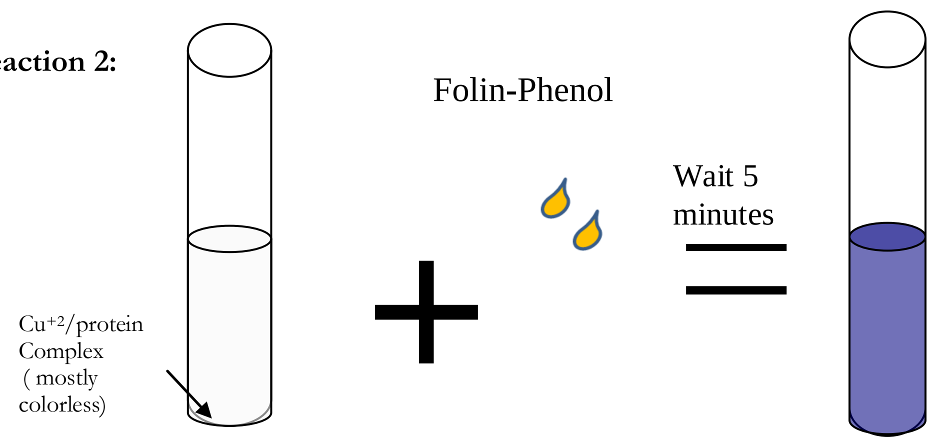
How does the Lowry Assay Work? The Lowry Assay uses chemical reactions to add color to protein in solutions. The Lowry Assay must be done in two steps since it involves two chemical reactions. (A chemical reaction is a chemical change that forms new substances).

Reaction 1:



The Copper (Cu) Reagent reacts with the colorless protein in the solution to form a copper/protein solution, which is still mostly colorless.

Reaction 2:



The Folin-Phenol reacts with the Copper/Protein Complex and a dark blue color is produced in direct proportion to amount of protein present. The more protein, the darker the solution.

3. What is the purpose of the Lowry Assay?

4. Why do you need to do the assay in two steps?

Crucial Concentration: Lab Protocol

Materials

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

- Three unknown sports drink samples, labeled A, B, and C, in conical tubes.
- One conical tube labeled "Stock Protein (0.5 µg/µl)" that contains protein of a known concentration. You will use this to make your set of protein standards.
- Eight empty test tubes: The ones that you will label #1-5 will be used to mix known concentrations of protein and distilled water to create a set of standards. The ones that you will label "A", "B", and "C" will be used for the unknown samples A, B, and C.
- One conical tube labeled "Cu Reagent" that contains the copper reagent.
- One conical tube labeled "Folin-Phenol" that contains the Folin-Phenol reagent.
- One conical tube labeled "dH_2O" that contains distilled water.
- 8 square plastic cuvettes, labeled #1-5, and A, B, and C, to be used with the colorimeter (in a cuvette rack)

5. What is the goal of this lab?

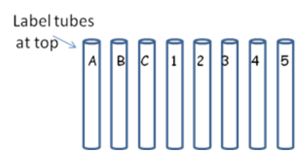
6. What assay are you going to use?

7. Describe the set of standards you will be making.

Protocol

Place a ✓ in the box as you complete each step.

Prepare Protein Standards:



☐ 8. Use a Sharpie to label your test tubes A, B and C, and 1-5.

☐ 9. In the next two steps, you will be preparing five different concentrations of protein to use as standards. How will a set of standards help you figure out how much protein is in the sports drinks? Write your answer below.

Crucial Concentration: Lab Protocol

☐ 10. First, add different amounts of dH_2O to test tubes #1-5 (see Column 2 in the data table on page S-7 for specific amounts).

☐ 11. Next, add different amounts of "Stock Protein (0.5 µg/µl)" to test tubes #1-5 (see Column 3 of the data table on page S-7 for specific amounts).

Calculate Concentration of Standards

☐ 12. Calculate the amount of protein (in µg) in each test tube. Write your answers in Column 4 in your data table on page S-7. Your answer should be in µg.

☐ 13. Calculate the final volume of each test tube in µl and fill in Column 5 in your data table on page S-7. Your answer should be in µl.

☐ 14. Calculate the final concentration of standards and fill in Column 6 in your data table on page S-7. Your answer should be in µg/µl.

How do I calculate concentration?
Concentration is a measure of amount of solute (in this case protein) in a solvent (in this case water).

1. You need to know concentration of the stock protein solution you added to each tube above (hint- look at column 3 heading in your data table and make sure to write your units) ______________
2. Next, you need to figure out the amount (in µg) of protein you added to each test tube. For example in Test Tube 2, you added 50 µl of 0.5 µg/µl. 50 ul X 0.5 ug/ul = 25ug of protein.
3. Next you need to figure out the final concentration in the test tube. This will be the amount of protein (in µg) over the final volume in the test tube (in µl). For example, in Test Tube 2 you had 25 ug protein/1000 ul or 0.025 ug/ul.

Prepare Unknown Sports Drink Samples

☐ 15. Add 1000 µl of each unknown sample (A, B, and C) from the conical tubes to their corresponding test tubes labeled "A", "B", and "C". These are the sports drink samples for which you want to determine the protein concentrations.

Perform the Lowry Reaction

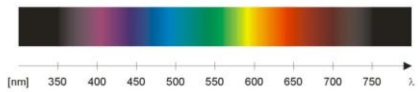
☐ 16. Add 1000 µl of "Cu Reagent" from the conical tube to each of your test tubes (# 1-5, and A, B, and C). Gently pipette up and down at least five times to mix each solution.

☐ 17. Incubate the test tubes at room temperature for 5 minutes.

☐ 18. Add 200 µl of "Folin-Phenol" reagent to each of your test tubes (# 1–5, and A, B, and C). Gently pipette up and down at least five times to mix each solution.

☐ 19. Incubate the test tubes at room temperature for 5 minutes.

Crucial Concentration: Lab Protocol



Analysis of the Protein Standards and Unknown Samples

using a colorimeter: The colorimeter measures absorbance, which is the amount of light absorbed by the color in the test tubes - the darker the color, the higher the absorbance value.

☐ 20. Transfer 2 ml of each of your samples from the glass test tubes to the small plastic cuvettes. Make sure to put your samples in the appropriately labeled cuvette. (Sample #1 should go into the cuvette labeled "1"). Use a NEW micropipette tip for each sample.

☐ 21. Make sure your 8 cuvettes (samples 1-5, and A, B, and C) are in the cuvette rack and take them to the colorimeter. Your teacher will demonstrate how to use the colorimeter. Groups must take turns on the colorimeter and should work as quickly as possible to allow the next group to use it.

22. Colorimeter instructions:

☐ a. Clean cuvette "1" with a kimwipe and gently place it into the colorimeter with <u>the arrow on the top of the cuvette slot on the colorimeter pointing to the clear side of the cuvette</u>. DO NOT force it down; gently insert only as far as it easily goes.

☐ b. Press the "CAL" (calibrate) button on the colorimeter and hold it until the red LED begins to flash (this usually takes less than one second). When the reading on the LabQuest unit is either 0.000 or 0.001, you have calibrated the colorimeter for the rest of your cuvettes.

☐ c. Remove cuvette "1".

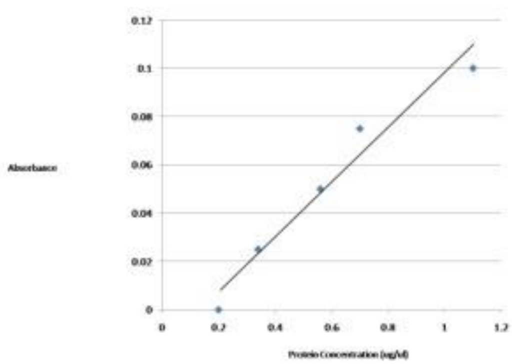
☐ d. Clean cuvette "2" and insert it into the colorimeter. <u>Do NOT hit "CAL"</u> anymore; that is only done for the first sample to calibrate the colorimeter for your samples. Record the absorbance on your data table. (It may take a second or two for the reading to stabilize). Gently remove the cuvette.

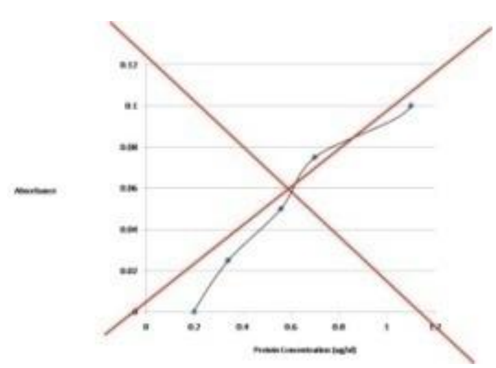
☐ e. Repeat above step (step d) for samples #3-5 AND for each of your unknown samples (A, B, and C). Don't forget to record each absorbance reading on your data table.

Graphing your Standards as Absorbance versus Protein Concentration

☐ 23. Plot absorbance versus protein concentration data for the five protein standards (samples #1-5) on the graph paper at the end of your packet. (Do NOT plot your unknowns yet).

☐ 24. Draw a "best-fit" line through the data points. (A best fit line is a STRAIGHT line that best describes the trend of the data. It will not 'connect the dots'.)

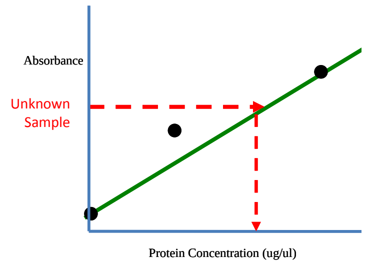




Crucial Concentration: Lab Protocol

Determine Concentration of Unknown Samples

25. To determine the concentration of protein in each of your unknown samples, find its absorbance on the y-axis and draw a horizontal line across the graph until you reach your best-fit line. Then, draw a vertical line from your line of best-fit down to the x-axis. The point where this vertical line intersects the x-axis will tell you the concentration of the unknown sample.



Beer-Lambert Law (or Beer's Law)
Beer's law describes the linear relationship between absorbance and concentration. The absorbance of a solution is directly proportional to its concentration.

$A = \varepsilon bc$

where A = absorbance
ε = the molar extinction coefficient (L/mol•cm)
b = the pathlength of the sample cell (cm)
c = the concentration of the substance (M) that absorbs light.

Notice that both ε and b must be known to directly calculate c from A. If ε and b are not known (as in our case) then a standard curve of absorbance vs. concentration, using standards with known concentrations, can be constructed. The slope of the line is equal to the product εb. The relationship between A and c must be linear to satisfy Beer's Law.

In the Lowry assay, you are effectively constructing a standard curve assuming Beer's Law and determining the product εb.

☐ a. Find the concentration of Unknown A; write the result in Column 6 of your data table.

☐ b. Find the concentration of Unknown B; write the result in Column 6 of your data table.

☐ c. Find the concentration of Unknown C; write the result in Column 6 of your data table.

DATA TABLE

Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7
Test Tube	Amount of Water to Add (µl)	Amount of Stock Protein to add (µl) **Stock protein concentration is 0.5 µg/µl**	Amount of protein in each test tube (µg)	Volume in Tube (µl) (add columns 2 and 3)	Concentration (µg/µl) Solute/Solvent	Absorbance
1	1000 µl	0 µl				
2	950 µl	50 µl				
3	900 µl	100 µl				
4	850 µl	150 µl				
5	800 µl	200 µl				
Unknown A				1000 µl		
Unknown B				1000 µl		
Unknown C				1000 µl		

Crucial Concentration: Lab Protocol

Analysis of results

26. What is a standard?

27. How were standards used for this experiment?

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

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

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

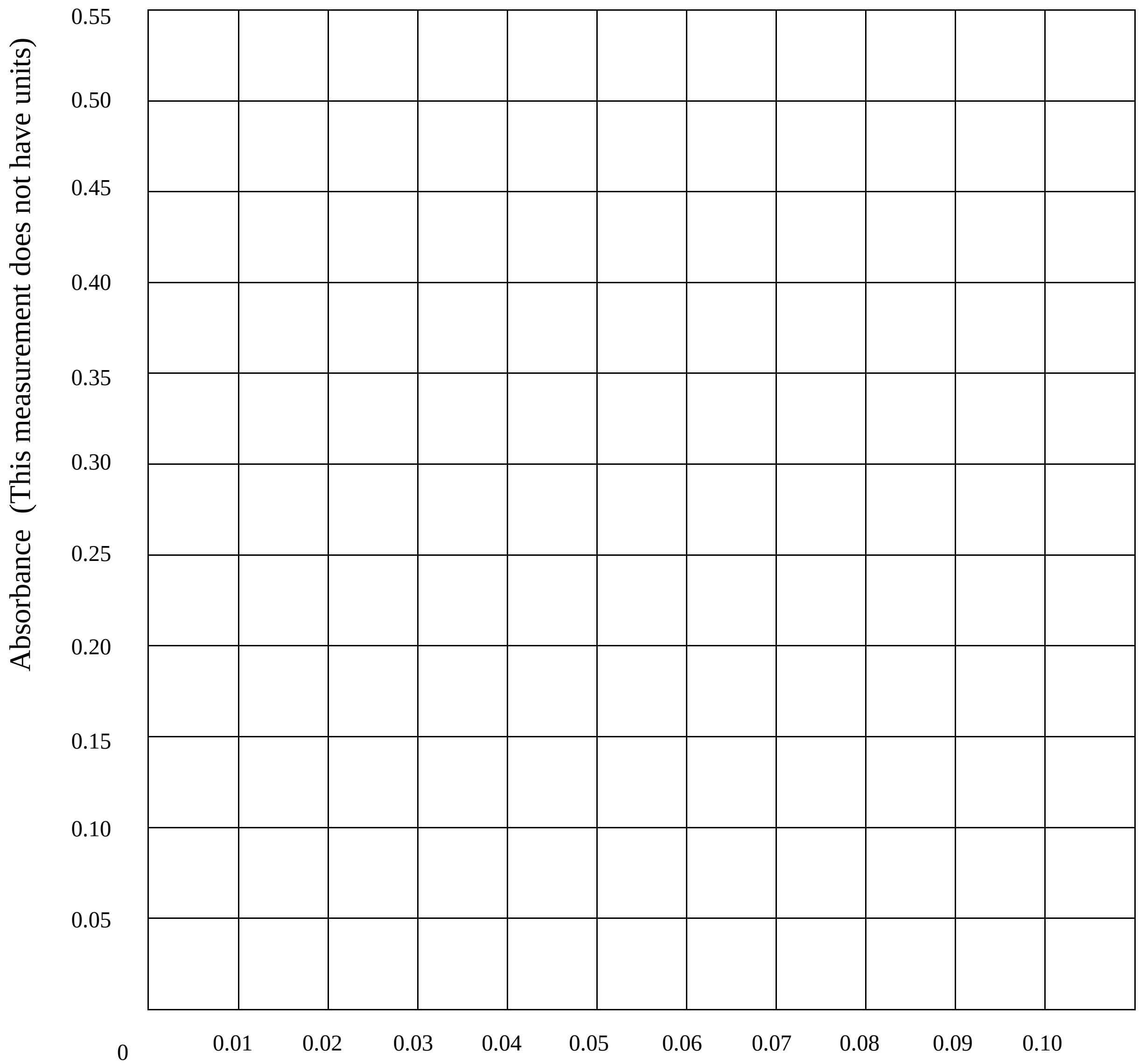
31. What does the colorimeter do?

32. What were the protein concentrations of the unknown sport drink samples? Which one had the highest protein concentration?

Crucial Concentration: Lab Protocol

33. Write a report below that summarizes how you determined which unknown sample had the most protein. Explain thoroughly, as this report will be given to the companies that make the sports drinks.

Crucial Concentration: Graph Paper



Protein Concentration
What are your units? Write them here ________________