How Science Works

Student Notebook

High School Biology

Module 1

Class Question:

Do macromolecules in milk change as it spoils over time?

Scientist (Your Name): ________________________________

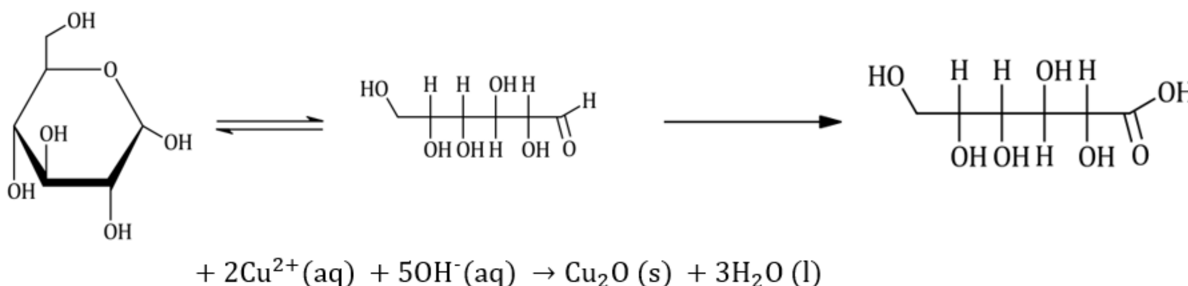
Teacher's Name: ____________________________________

SciTrek Volunteer's Name: ____________________________

OVERVIEW & BACKGROUND INFORMATION

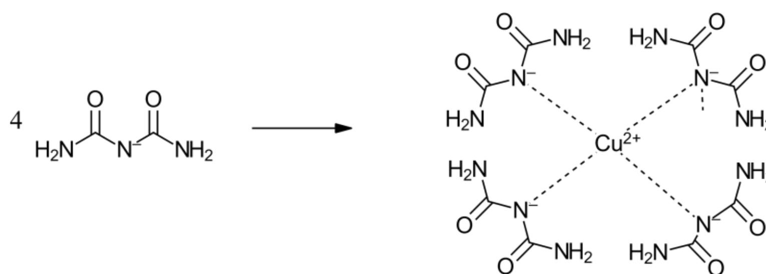
Complex Chemical Reagent Info for the Curious:

Benedict's test for Simple Sugars - Benedict's is a hydrated solution of copper (II) sulfate ($CuSO_4$). In the presence of reducing sugar, copper sulfate reacts to become copper (I) oxide (Cu_2O).



This reaction changes the Cu^{2+} ions which appear blue into Cu^{+} ions which appear red. The color transition creates a gradient from clear light blue to green to orange to red precipitate. Benedict's test is used to quantify glucose levels in blood and urine and test for diabetes.

Biuret test for Proteins - Biuret is a mixture of sodium hydroxide (NaOH), hydrated copper (II) sulfate ($CuSO_4 \times H_2O$), and potassium sodium tartrate to stabilize the copper ions. Cu^{2+} ions from the copper (II) sulfate has a blue color. In the presence of protein, the Cu^{2+} ions interact with **peptide bonds,** which link amino acid monomers together to make a protein polymer.



This interaction changes the Cu^{2+} ions which appear blue (left side) into a copper complex with a purple color (right side). The deeper the purple color, the more copper-peptide complexes have been formed.

Day 1 <u>Introduction, Macromolecules, and Organism Game</u>

Welcome to SciTrek everyone! Today we are going to get started by introducing you to macromolecules and how they change as food changes. What are some experiences can you think of that show a change in food over time (spoilage, ripening, or fermentation)?

The lead will introduce the big investigation. This will be the "big question!" It is important to keep the big question in mind as we go through the module! Our first activity will be an introduction to the macromolecules. This introduction will be done by your teacher! Make sure you are answering the questions as he goes through the PowerPoint!

Introduction to Macromolecules

Scientists categorize molecules as either "organic" or "inorganic" or both

Organic - compounds that contain both carbon and hydrogen atoms
Inorganic - compounds that DO NOT contain both carbon and hydrogen
There are four classes of organic compounds that are central to life on earth.
1. Carbohydrates 2. Lipids 3. Proteins 4. Nucleic Acids

Carbohydrates (Sugars and Starches) –

- Compounds made up of C, H, and O which give us_________________.
- Carbohydrates can be simple sugars; the monomer of this macromolecule is_________ ____________________.
- Carbohydrates can be used as structures; in plants, ________________is a carbohydrate that is used to for structural support in plant cells.

Lipids (Fats, Oils, Waxes) - compounds made of C, H, and O which store energy. Some lipids have structural functions: Plant wax is a lipid that keeps plants from dehydrating, and cholesterol is a lipid found in membranes of cells and organelles (your cells have this). Lipids are mostly **nonpolar**, meaning they do not mix well with polar molecules. Lipids are not polymers; A common lipids called a triglyceride consists of 3 fatty acids and one molecule of glycerol.
Not really a monomer.. repeating unit(s): _________________________________
Not really a polymer: ____________________________

Proteins (long chains of amino acids) - compounds made of C, H, O, and N which carry out many functions and are an energy source. The monomers of proteins are called **amino acids**, and the bonds that hold amino acids together are called **peptide bonds**. Proteins are used to build and repair hair, nails, and muscle tissues. A special class of proteins, called **enzymes,**

are used to speed up the rate of chemical reactions. Enzymes are used for digestion, respiration, reproduction, vision, movement, and other various tasks.
monomer: ____________________ polymer: ______________________

Nucleic Acids (made up of nucleotides) - compounds made of C, H, O, and N. Subcategories include DNA and RNA. The monomers of nucleic acids are called **nucleotides** which are composed of a nitrogenous base, a 5-carbon sugar, and a phosphate group. A really important nucleic acids called DNA stores genetic information. Another important nucleic acid called RNA makes proteins.
monomer: ____________________ polymer: ______________________

Organism: The Trading Card Game

Objective: You and your group are working together as a plant cell. You have limited resources (element cards) that you need to build a copy of yourselves (reproduce). Trade with the other cells in your class so that you have enough biomolecules to survive and reproduce.

Types of cards:
Stage 1 – elements - hydrogen, carbon, oxygen, nitrogen
Stage 2 – sugar
Stage 3 – lipids, carbohydrates, protein, DNA
Stage 4 – organism

Recipes:

To make:	You need:
Sugar	2 hydrogens + 1 carbon + 1 oxygen
Lipid	2 sugars
Carbohydrate	3 sugars
Protein	2 sugars + 2 nitrogens

DNA	2 sugars + 2 nitrogens
Organism	1 lipid + 1 carbohydrate + 1 protein + 1 DNA

Rules:

1. Shuffle the element cards.
2. Each group is provided 25 random element cards and an instruction card to begin (take out one nitrogen card to make 125 element cards total). During this time, the groups can sort out their cards.
3. With the cards given to each group, consult the formula on the instruction card to use your elements to construct your sugar molecules first.
4. After constructing sugar molecules, the groups can trade in their sugar molecules for larger molecules from a SciTrek volunteer (the volunteer will have the sugar and macromolecule cards)
5. Groups are allowed 45 seconds to discuss trading plans to trade any cards for other cards with another group (ex. Give up nitrogen for 2 hydrogens). Start a timer for 45 seconds.
6. Once the 45 seconds are up, have each group send one representative to the front of the room. The representatives will trade with each other for 1 minute, then return to their groups.
7. Repeat steps 5 and 6 until a group forms an organism. The first group to form an organism wins.

Questions after the Game

1. Circle the following macromolecules that are considered “building blocks” of organisms.

Carbohydrates **Proteins** **Lipids** **LEGO** **Nucleic Acids**

2. Why does your body need **carbohydrates** and where can you find them?

____
____
____
____

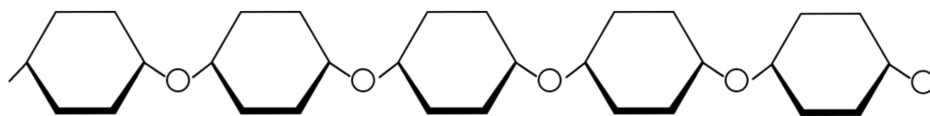
3. Why does your body need **proteins** and where can you find them?

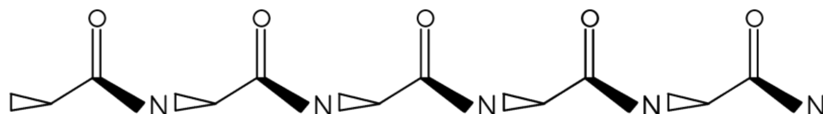
____
____
____
____

4. Why does your body need **fats** and where can you find them?

____
____
____

5. It takes a lot more than one molecule each of carbohydrates, proteins, fats, and DNA to make an organism. What actually happens is that carbohydrates and proteins will form long chains called **polymers** made of individual repeating units called **monomers.** Given the pictures of a carbohydrate chain and a protein chain, box and redraw the repeating monomer unit.





6. A SciTrek volunteer is having a hard time trying to get lipids to dissolve in water. Why would lipids and water typically not want to mix together? What might you add to help the lipids dissolve in water? Hint: why do you use shampoo to wash your hair? What are you trying to get rid of?

____

____

____

DAY 2 Testing for Biomolecules

(Caution: Concentrated acids should only be used by the volunteers. Acids should be placed in a safe area when not in use. Affected areas should be washed with soap and water for 10 minutes.) It's going to be Legend- wait for it- Dairy

Testing for the Other Macromolecules

Congratulations! What you just did was used for a long time to determine protein amounts in dairy and other foods. It works with large samples but takes a bit of time. Modern methods used for example in the food industry make use of **spectrophotometry**, where the concentration can be determined using chemical indicators.

Gravimetric analysis is great and effective when you have a relatively large amount of sample, but if you look at the weigh scales we have, they only go up to two decimal points. What if I want to measure out something in milligrams or track a small change in carbohydrates, protein, or sugar content? We use a spectrophotometer.

How does the spectrophotometer work?

When light is passed through a sample, some of the light is absorbed by the sample, and the intensity of light that passes through will change. Think about shining a flash light through clear water versus through muddy water. The more a sample contains of something which absorbs, the less light gets through. We usually refer to a particular wavelength of light, which is something a spectrophotometer can be used to measure. The relationship between how much of a particular molecule you have in a sample and the amount of light that is absorbed allow us to determine the concentration of the molecule..

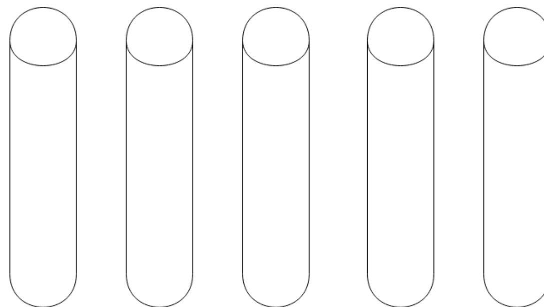
Demonstration: Bradford Reagent for Testing Protein Amount

Which has more protein, chicken or butter?

A few drops of Bradford reagent were added on top of the chicken. What do you notice about the color of the Bradford drops on the chicken compared to butter?

1. The test tube with 0% protein is called a **control**. What is in the control then? What does the "control" tell you? Why is it needed?

2. What is the trend of how the solution color changes with increasing protein concentration? Draw and color the series of solutions below



0.01% protein | 0.001% protein | 0.0001% protein | 0.00001% protein | 0% protein

Percents are given in mass per volume, meaning that 0.1% protein solution was made by adding 0.1g (one tenth of one gram) to 100mL water.

3. We typically call a series of varying concentration of solutions a **gradient**. How could we use a gradient to figure out how much protein is in an unknown solution? What do we need to know to make this gradient?

4. Why might this test not work if you were testing a sample (food) that was colored blue...like blue berries? What other problems can you identify that might interfere with this test?

____

____

____

The SciTrek lead will take samples of each solution into a cuvette and measure their absorbance at 610 nm. After getting a series of absorbances, the lead will make a table on the board that looks like the one below. Copy the values into your notebooks.

The solution concentrations are given in percent by volume, meaning that
1% protein = 1 gram of protein/100 mL of water.

Concentration of Protein	Control (0%)	0.0001	0.00001	0.000001	0.0000001
Absorbance of Sample @ 610 nm					

What is the shape of the graph formed when the absorbance is plotted against concentration? Paste or redraw the graph and write down the equation corresponding to it. What is the R-squared value for this line? What does that tell you about the tightness of your data points.

The Separation Problem

The SciTrek lead will add Bradford reagent directly to the milk to see if we can use it to make a calibration curve for protein concentration. There's however a little problem that might come up. What's the problem when Bradford is added to milk? Draw and record your observations below.

Milk without Bradford reagent	**Milk with Bradford reagent**

THE MILK HAS GONE BAD

"HOW DO I KNOW?" YOU ASK?
© 1995 PAWS, INC. All Rights Reserved.

THE FACT THAT IT'S SITTING THERE WITHOUT A GLASS IS A CLUE
JIM DAVIS 12-2

DAY 3 Calibration Curves

Colorimetric Calibration Curve Activity

How do we know that living things (i.e. food and ourselves) are made of varying amounts macromolecules? Is it possible for us to quantify the macromolecule content in a sample?

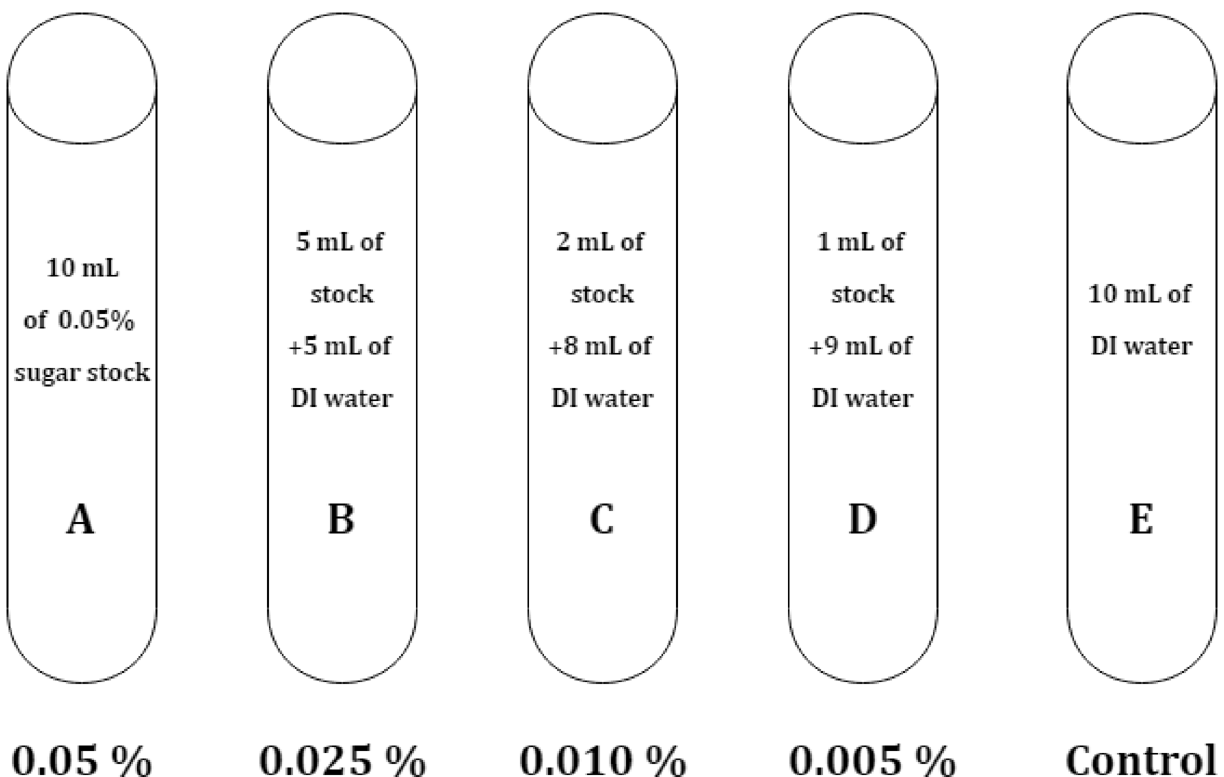
Test #1: Benedict's Reagent for Carbohydrates

(Caution: Hot objects ~ test tubes and hot plate ~ can lead to painful burns. Be careful!)
The first reagent that we will be using is the Benedict's test, which tests for reducing sugars.

Materials:

- Hot plate + beaker of hot water (~250mL H_2O @ boiling) + stir bar (prepared beforehand)
- Thermometer
- Benedict's solution
- 0.05% dextrose solution prepared beforehand
- DI water
- 10mL graduated cylinder + 50mL beaker
- Plastic pipette
- Labeled test tube rack + 5 labeled test tubes (A, B, C, D, and E)
- Spectrophotometer + cord
- Laptop
- Cuvettes

Prepare a test tube rack labeled like the diagram on the previous page.



Percent Concentration of Sugar Solution

(grams of sugar/mL of water) x 100%

Procedure for Benedict's Gradient

1. To prepare the calibration curve, start by adding 5 mL of H_2O into test tube **B**, 8 mL of H_2O into test tube **C**, and 9 mL of H_2O into test tube **D**. Add 10mL of H2O to test tube **E**. Do not add water to test tube **A.**
2. Obtain 18 mL of 0.05% dextrose stock solution from your Scitrek volunteer.
3. Take 10mL of the newly made dextrose stock and add this to tube A.
4. Take 5 mL stock and add it to test tube **B**. Mix by swirling.
5. Add 2 mL of stock to tube **C**. Swirl to mix.
6. Add 1 mL of stock to tube **D** and mix.
7. Test tube **E** will just have water.
8. Add 1 mL of Benedict's solution to each of the test tubes, then carefully lower them into the pre-prepared water bath for ~ 3 minutes.

9. Carefully remove the tubes (only grasp the top of each tube since the bottom may be hot) and replace them on their rack.
10. To measure the absorbance of each sample, use a plastic pipette to fill a clean cuvette with sample until the sample volume reaches at least ⅔ of the cuvette height. Give the cuvette to your table's designated lead who will help you measure the absorbance of your sample (750 nm) and record your data in a table on the same page. The concentrations corresponding to the dilutions can be found in the table at the end of this section. Repeat this for each of your samples.
11. Rename "Data Set _" with "Benedict's Calibration Curve" and your table number. Press the three dots in the upper right hand corner next to the table's title to change the table name. If you've labeled your table well, you'll be able to determine the corresponding absorbance values for each sample.
12. Clean out your cuvette by emptying it into a waste beaker. Add water to it, squeezing and releasing the pipette bulb to flush out any remaining sample, and empty out the cuvette into the waste beaker once more. Do not leave sample in your cuvette since precipitate will get stuck inside it.
13. Report your absorbances to the lead at the front of the class to plot your data.

	Test Tube A	**Test Tube B**	**Test Tube C**	**Test Tube D**	**Test Tube E**
Sugar Concentration	Solution A = 0.050% dextrose (most sugar)	Solution B = 0.025% dextrose	Solution C = 0.010% dextrose	Solution D = 0.005% dextrose	Solution E = 0% dextrose (least sugar)
Benedict's Amount	1 mL	1 mL	1 mL	1 mL	1 mL
Color Display					
Sample Absorbance @ 749.7 nm					

Record the data from the spectrophotometer and send one person from the group up to the lead to relay the information. As you collect data, graph your values below, making sure to properly label everything. Compare your graph to the class average. What differences can you see?

Title:____

D

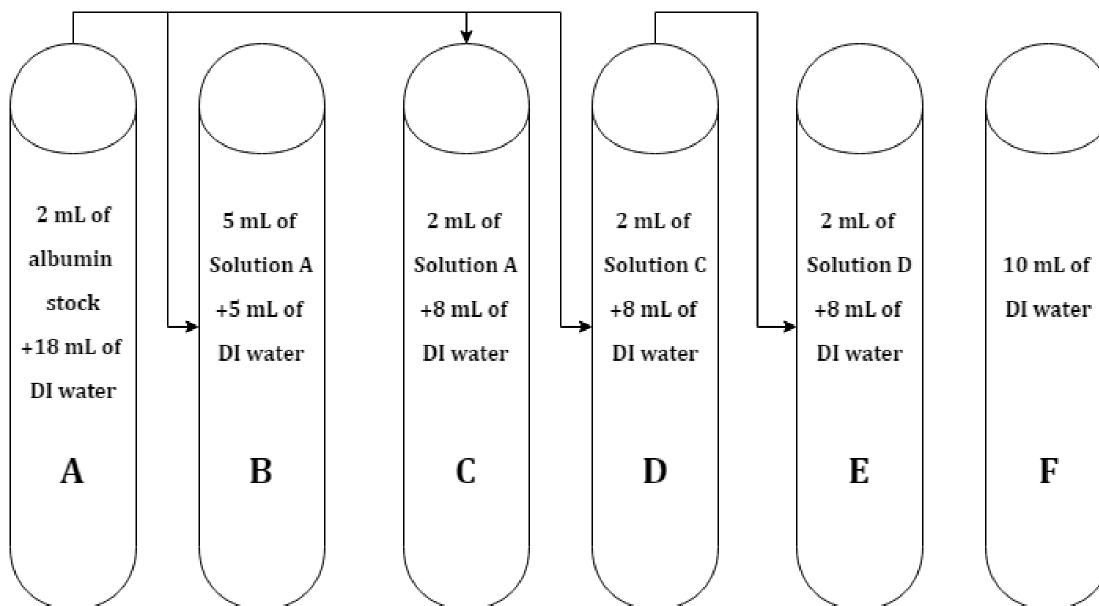
Independent Variable:__________________________

Test #2: Biuret Reagent for Proteins

The second test that we will be doing is testing for proteins using Biuret solution.

Materials:

- Labeled test tube rack, Biuret solution, Plastic pipettes, DI water
- Albumin Solution (200 mg/ml)
- 10mL graduated cylinder
- Plastic pipette
- Labeled test tube rack + 6 labeled test tubes (A, B, C, D, and E)
- Spectrophotometer + cord
- Computer
- Cuvettes



20 mg/mL 10 mg/mL 4 mg/mL 8 mg/mL 0.16 mg/mL Control

Concentration of Protein Solution

(grams of protein/mL of water)

Procedure for Biuret Assay:

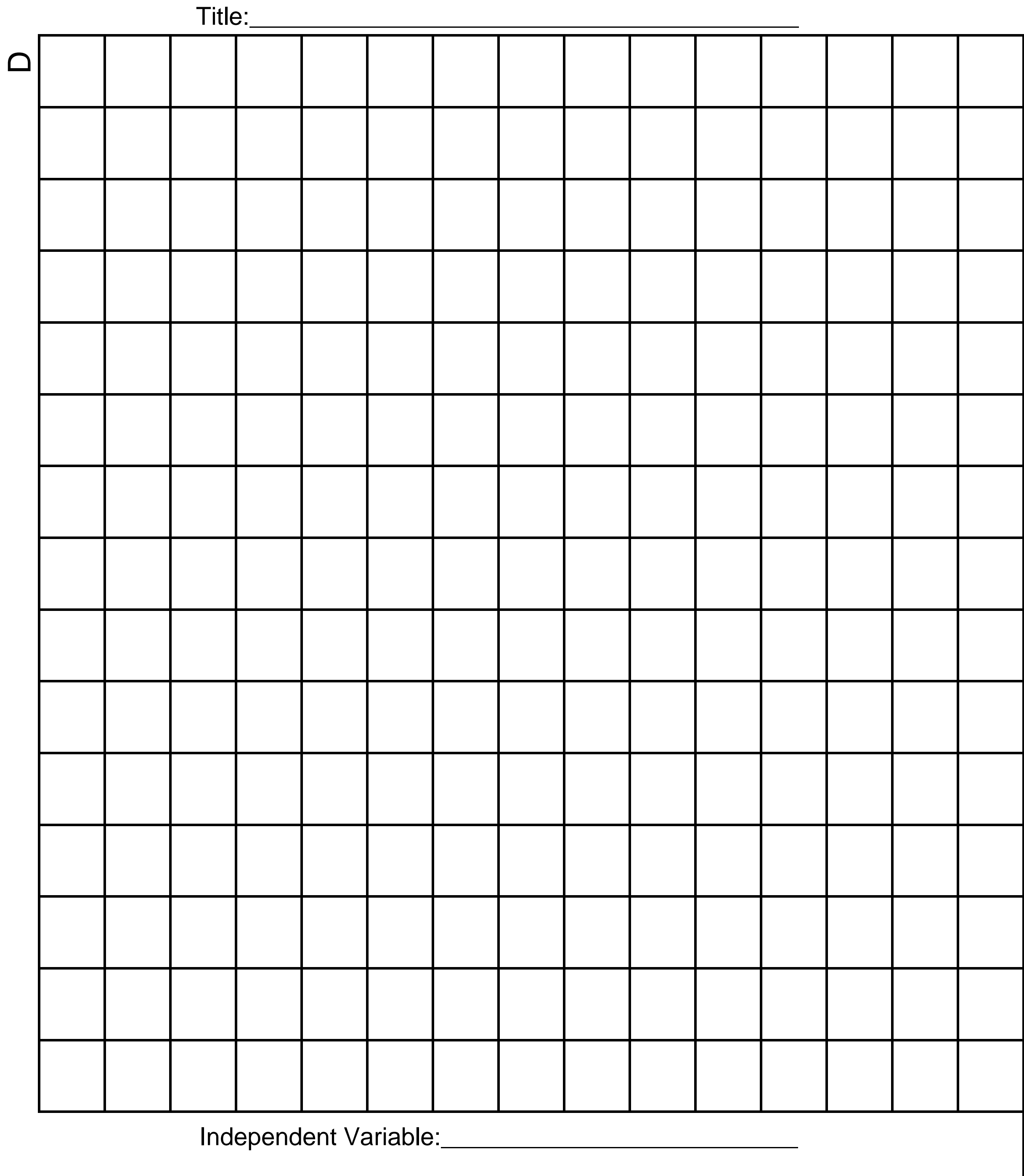
1. To begin the calibration curve, first take 2 ml of albumin solution using a pipette and add it to test tube **A**. Then add 18 ml of H2O to test tube **A**. Swirl to mix.
2. Next, add 8 ml of H2O each to test tubes **C, D, and E**.
3. Take 2 ml from test tube **A** and add to test tube **C**. Swirl solution.
4. Take 2 ml from test tube **C** and add to test tube **D**. Swirl solution.
5. Take 2 ml from test tube **D** and add to test tube **E** then swirl.
6. For test tube **B**, take 5 ml from test tube **B** and add 5 ml of water. Swirl to mix
7. Add 10 ml of H2O to test tube **F**. This will be your control test tube.
8. After the test tube solutions are prepared, add 8 drops of Biuret solution to each test tube and record observations of color in the chart on page 20.

9. Get a clean pipette and pipette out a sample from test tube A into a clean plastic cuvette until the cuvette is filled ⅔ of its total volume. Give cuvette with sample to the lead so they can insert your cuvette into the spectrophotometer. (590 nm)
10. Click "**Collect**" to measure the absorbance of the sample. Wait for the Absorbance Value to stop changing after a few seconds and click "**Keep**." Type in the concentration of the sample you're testing. For example, if you're testing test tube A, name the concentration as "20 mg/ml." Label your tables well so you'll be able to keep track of all the corresponding absorbance values for each sample.
11. Record the absorbance value for test tube A in your notebooks. After you saved the absorbance value and the concentration, click "**Stop**" to reset for measuring absorbance for another sample.
12. After your lead gives you back your cuvette, dump the sample into a waste beaker. Add water to the cuvette and pipette up and down to flush out any remaining sample. Rinse again with water to clean the cuvette.
13. Repeat steps 9-11 for each remaining test tube to get absorbance values for all your samples until you generated an Absorbance vs. Concentration graph from all the data points you recorded.
14. After getting absorbance values for all your test tube samples, report your data to the lead at the front to be plotted.

	Test Tube A	**Test Tube B**	**Test Tube C**	**Test Tube D**	**Test Tube E**	**Test Tube F**
Protein Concentration	20 mg/mL	10 mg/mL	4 mg/mL	0.8 mg/mL	0.16 mg/mL	0 mg/mL (Control)
Biuret Amount	8 drops	8 drops	8 drops	8 drops	8 drops	8 drops
Color Display						
Absorbance @ 590 nm						

Record the data from the spectrophotometer and send one person from the group up to the lead to relay the information. As you collect data, graph your values below, making sure to

properly label everything. Compare your graph to the class average. What differences can you see?



Questions:

1. What happens to the color of the solution as the protein/ sugar/ complex carbohydrate concentration increases? (*Three different answers)*

2. Why do we need a control such as a sample of water?

3. Why is it important to only test one variable at a time?

4. What is the purpose of a spectrophotometer?

5. What type of interaction is the Biuret solution causing to the protein in the sample to make a color change? (Refer to the background information behind the Biuret reagent)

6. What is the purpose of making calibration curves?

Linear Regression: Making the Best of Everyone's Data

Take the data you and your group have collected and add it to the Google Sheets for the class found here: https://tinyurl.com/y2wav293

Paste or redraw images of the combined class data graphs below and use it to answer the questions on the next page.

Compare the R-squared value of your group's data to the R-squared value of the overall class data. What happened to the tightness of the linear fit as more groups added data to the scatterplot? Which line do you think is more accurate to use, your original line or the overall class line?

____

____

____

____

On the graph, notice the vertical lines passing through each data point. These are called **error bars,** and they represent the uncertainty or deviation from a given data point.

Are there some points that don't fit in with the rest of the data? If so, what familiar term to we regard these points as. How do they affect the overall average of the graph?

____

____

____

____

Below, please record all of the linear equations of the class average for each of the different macromolecule concentration curves. These equations will be very helpful for us in the future!

Class Average Equations	Simple Sugars	Complex Carbohydrates	Proteins
Equation			
R Squared value			

DAY 4 The Ripening Finale

In front of you are two samples which were put together by SciTrek volunteers. Each sample contains three test tubes with different a different amount of sugar, protein, or

complex carbohydrate. How would you go about testing the concentration of these molecules? Discuss with your group and come up with an accurate way to measure the concentration of you synthetic samples. Record all data below:

Materials:

- Two synthetic samples
- 6 test tubes
- 6 pipettes
- 6 cuvettes
- 1 spectrophotometer
- 1 hot plate
- 1 beaker (400 mL)
- 1 stir bar
- 20 mL of each indicator
- 100 mL of DI water

Sample #1

	Color	Absorbance Value	Concentration
Protein			
Sugar			
Complex Carb			

Identity of Unknown Solution:

Sample #2

	Color	Absorbance Value	Concentration
Protein			
Sugar			
Complex Carb			

Identity of Unknown Synthetic:

That didn't seem too difficult, right? Now let's take a look at a real biological sample that you have worked with before, and see if you can spot any differences in the macromolecule concentration as milk transitions to yogurt.

Materials:

- 2 biological samples
- 6 test tubes
- 6 cuvettes
- 6 pipettes
- 1 spectrophotometer
- 1 hot plate
- 1 beaker (400 mL)
- 100 mL of DI water

Milk

	Color	Absorbance Value	Concentration
Protein			
Sugar			
Complex Carb			

Yogurt

	Color	Absorbance Value	Concentration
Protein			
Sugar			
Complex Carb			

Place to do Math!

Did you notice anything different between your two biological samples? If so, why do you think there was a difference in any of the three macromolecules? *Hint: What did we add to*

the milk on day 2 to force it to coagulate? Summarize your findings and make a conclusion. Support your conclusion with numerical data from the data above

____

____

____

____

____

____

____

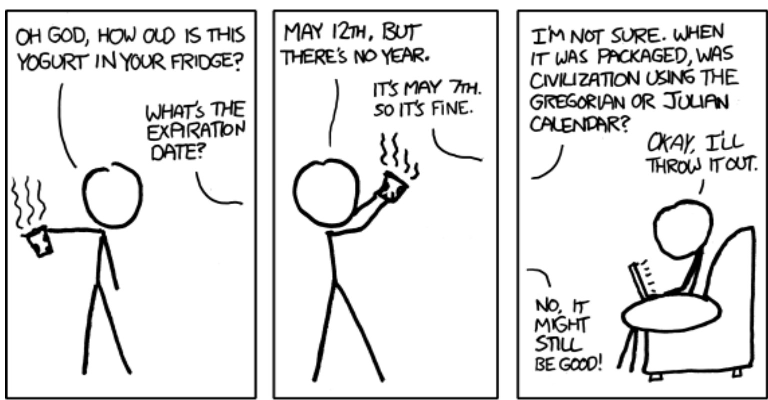
____

____

____

____

____



Feedback for SciTrek!

Please tear this page out and turn it into the lead at the end of the day.

1. What was your favorite part about this week's module?

2. Have you done a SciTrek module before in elementary or middle school? If so how does this experience compare to other modules you have done with us?

3. After participating in this module, I feel that I can construct and revise an explanation based on evidence for how carbon, hydrogen, and oxygen from sugar molecules may combine with other elements to form amino acids and/or other large carbon-based molecules.

Strongly Disagree **Disagree** **Neutral** **Agree** **Strongly Agree**

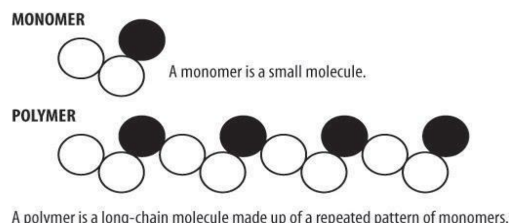
Please explain your response to the prompt above.

4. What was something you did in this module that you think scientists do regularly?

5. Do you have any suggestions on how we can improve this module?

Thank you for inviting us to your classroom! We hope you enjoyed this experience and wish you the best throughout the school year. ~ The SciTrek Team

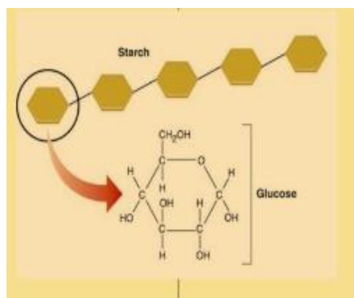
GLOSSARY



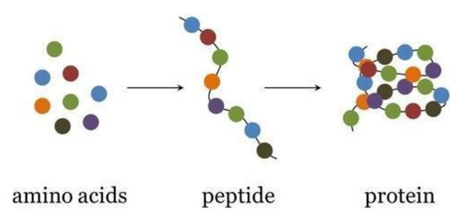
Figure

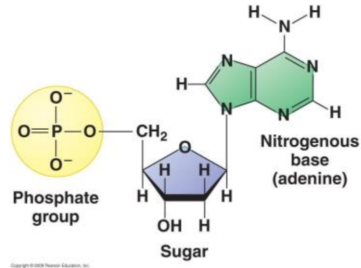
- **Macromolecules -** A macromolecule is a very large molecule made from smaller molecules. Some common macromolecules in biochemistry are nucleic acids, proteins, and carbohydrates.
- **Monomers -** Single units that can be strung together to make large molecules (polymers). Monomers do not need to be identical, but they must have a similar structure. (Figure 1)
- **Polymers:** A chain made of monomers. The common polymers of life (biopolymers) are *nucleic acids, proteins, and carbohydrates*.

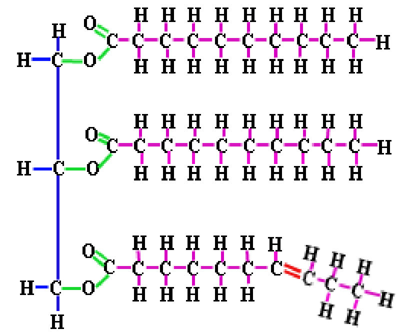
Figure 2



- **Carbohydrates:** Organic compounds with the molecular formula $(CH_2O)_n$ composed of small subunits called monosaccharides (ex. glucose). These combine in different ways to form chains of polysaccharides with different properties and functions. (Carbs give us energy to do science!) (Figure 2)
- **Starch:** A large and complex type of carbohydrate. Starch is a polymer of many sugars bonded together. (As an aside, we give a carbohydrate a different name based on the sugars and types of bond linkages that it has. Starch is only one of thousands of carbohydrates!). (Figure 2)
- **Proteins:** Polymers made of amino acids. (Proteins build and repair muscles; they're amazing!)
- **Lipids:** Can be a fat or oil depending on whether they are solid or liquid at room temperature, respectively. Lipids aren't polymers, but they can interact with to form cell membranes
- **Nucleic Acids:** Biomolecules with subunits that are composed of a phosphate group, a sugar, and an identifying molecule. DNA is responsible for genetic inheritance and RNA helps to translate DNA into protein, which is responsible for genetic expression.







Proteins