QUALITATIVE ANALYSIS OF BIOLOGICAL MOLECULES

Objective

To qualitatively analyze different samples to detect the biomolecules present in them.

Background

Macromolecules are large molecules formed from aggregates of smaller ones. Biological macromolecules are typically classified as carbohydrates, lipids, proteins, and nucleic acids. It is possible to identify macromolecules and monomers by using chemical indicators.

Reagents used as chemical indicators express their results either qualitatively or quantitatively by determining the presence or relative amount of a substance in a solution. The example in Table 1 should help you understand the basic difference between qualitative and quantitative analyses.

Table 2: Case Study Illustrating the Difference between Qualitative and Quantitative Analyses

Case Study	You are given a beaker containing 100 ml of an aqueous solution	
	A	**B**
Question	Are proteins present in this solution?	How many mg of protein are dissolved in this 100 ml solution?
Thinking	Would smelling, tasting, or touching the solution help determining if it has proteins or not? *(not a good idea in lab)* **The best thing to do is add a protein indicator. If the solution changes color, then proteins are present.**	Changing the solution's color indicated proteins is present, but it does not detect exactly how much protein is present. **An analytical test giving the answer in numbers, not just by presence or absence, needs to be done.**
Response	A **qualitative** analysis must be performed.	A **quantitative** analysis must be performed.

Materials

- Test Tubes and Racks
- 10 ml Pipettes
- Pipette Pumps

- 10 ml Graduated Cylinders
- Marking Pencils (Sharpie)
- Filter Paper Disks
- Petri Dish
- Water Baths at 95^{o}C
- 1% Dextrose (Glucose)
- 6% Starch (Amylose)
- 1 M NaOH
- Apple Juice
- Chicken Broth
- Egg White
- Whole Milk
- Vegetable Oil
- Distilled Water
- Benedict 's reagent
- IKI
- Biuret Reagent
- Sudan IV Reagent

Procedure:

Part A: Detection of Carbohydrates

Carbohydrates are molecules consisting of one (**monosaccharide**), two (**disaccharide**), or many (**polysaccharide**) simple sugars. Examples of carbohydrates include glucose, sucrose, glycogen, maltose, and starch (amylose).

In this exercise, you will experiment with two carbohydrate reagents:

1. **Benedict's reagent** – usually light blue in color, forms a yellow-green, orange, or red precipitate when boiled in the presence of reducing sugars such as simple sugars (e.g. glucose)
2. **Iodine-Potassium Iodide (IKI)** – amber colored, forms a dark purple or black precipitate in the presence of starch.

Read the information on the following pages (Parts A1, A2, and A3) and fill in the **first three columns** of Table 2 **before** performing the experiments.

Part A1: Detection of Simple Sugars

1. Obtain a test tube rack and **six** test tubes per group.

2. Label the test tubes 1 through 6. #1 and #2 will be used in this part.

3. Use a 10 ml pipette to transfer 1 ml of the dextrose (glucose) solution to test tube #1.

4. Use a **different** (why?) pipette to transfer 1 ml of the starch solution (swirl to mix before transferring) to test #2.

5. Use a 10 ml graduated cylinder to measure and transfer 1 ml of Benedict's reagent to each test tube. Swirl to mix.

6. Note the color of each solution.

7. Gently heat the contents of each test tube in a 95□C water bath for **two minutes.**

8. Observe and record any color change in Table 3.

Part A2: Detection of Starch

1. Use a pipette to transfer 1 ml of dextrose solution to test tube #3.

2. Use a **different** pipette to transfer 1 ml of starch solution (swirl to mix before transferring) to test tube #4.

3. Add one drop of IKI reagent to each test tube and swirl gently.

4. Observe and record any color change in Table 3.

Part A3: Identification of a Carbohydrate Unknown

If you were given an unknown solution and had to perform both the simple sugar (Part A1) and the starch (Part A2) tests in the same test tube, which test would you perform first? The following experiment will help to answer this question.

1. Use a pipette to transfer 1 ml of dextrose to both test tubes #5 and #6.

2. Use a **different** pipette to transfer 1 ml of starch to both test tubes #5 and #6.

3. In test tube #5, perform the Benedict's test **first.**

4. Make note of any color changes.

5. After the Benedict's test perform the IKI test in test tube #5.

6. In test tube #6, perform the IKI test **first.**

7. Make note of any color changes.

8. After the IKI test perform the Benedict's test in test tube #6.

9. Make note of any color changes.

10. Record your observation in Table 3.

11. From the results of test tubes #5 and #6, determine which test you should run **first** if you were limited to using just one test tube and had to test for both simple sugars and starch. **Only one of these two test tubes will allow you to see the results of both tests correctly.**

Which test would you perform ***first*** *and why?*
12. Obtain a simple sugar / starch unknown (labeled A, B, C, and D) and test it using the proper sequence of Benedict's and IKI reagent
13. Record the letter of your unknown and any color changes in Table 3.

What (water, glucose, starch, or both) was in your unknown?

Table 3: Qualitative Analysis of Simple Sugars, Starch, and a Carbohydrate Unknown

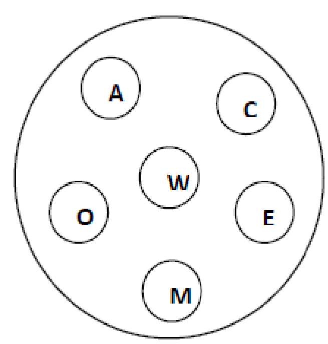
Test Tube	**Test Solution**	**Reagent**	**Hypothesis**	**Results**
1				
2				
3				
4				
5		Benedict's 1st IKI 2nd		
6		IKI 1st Benedict's 2nd		
Unknown				

Part B: Detection of Lipids
A lipid is a non-polar (hydrophobic) organic molecule which is insoluble in water. One type of lipid is fats, also called **triglycerides** or **triacylglycerols**. A fat molecule is composed on one glycerol and three fatty (palmitic) acid molecules. **Sudan IV-lipid complex** will produce an orange spot on filter paper to which lipid has been added.

1. Obtain a blank filter paper disk.

2. Mark the disk with a pencil following the pattern as shown in this figure alongside.

A – Apple Juice
C – Chicken Broth
E – Egg White
M – Whole Milk
O – Vegetable Oil
W – Distilled Water (control)



3. Make a hypothesis as to which of the above substances you would expect to contain lipids.
4. Record this hypothesis in Table 4.

5. Transfer a small drop of each substance to the appropriate circle on the filter paper

6. Allow the filter paper to dry

7. Once dry, soak the filter paper for **3 minutes** in a petri dish containing Sudan IV reagent. Leave the dish on the counter where it was originally to avoid spillage

8. Remove the filter paper disk with forceps and **gently** rinse with distilled water over the sink for **one minute**

9. Hold the filter paper over something white for contrast and observe the results

10. Examine the color for the six spots and indicate whether the substances contained lipid using the by indicating "-"for negative (no color change; no lipid) and "+" for positive (color change; lipid)

11. Record your results in Table 4

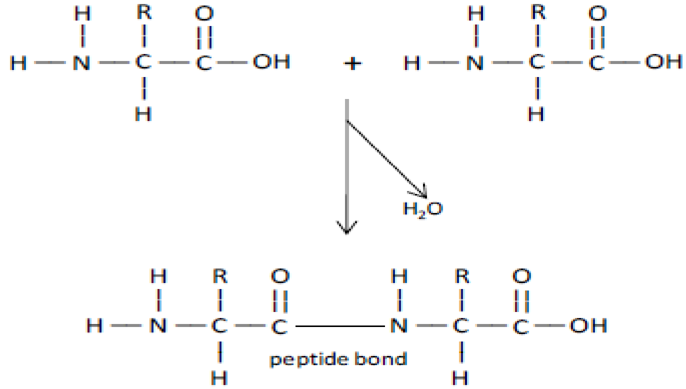
12. Compare your results to your hypothesis

Table 4: Sudan IV Test for Lipids

Substance Tested	Hypothesis	Result
Apple Juice		
Chicken Broth		
Egg White (Albumin)		
Whole Milk		
Vegetable Oil		
Distilled Water		

Part C: Detection of Proteins

Proteins are polymers of **amino acids** in which the carboxyl functional group of one amino acid forms a **peptide bond** with the amine functional group of another amino acid.



Biuret reagent, which is pale blue, contains copper sulfate ($CuSO_4$). The Biuret reaction is based on the complex formation of cupric ions with proteins. In this reaction, copper sulfate is added to a protein solution in strong alkaline solution. A purplish-violet color is produced, resulting from the complex formation between the cupric ions and the peptide bond.

1. Obtain a test tube and rack and **six** clean test tubes per group.

2. Mark the test tubes with the same symbols used in the lipid experiment (Part C).

3. Make a hypothesis as to which of the above substances you would expect to contain proteins.

4. Record this hypothesis in Table 5.

5. Transfer 1 ml (approximately 20 drops) of the appropriate solution to properly marked test tube.

6. Dispense 1 ml of 1M NaOH into each test tube.

7. Swirl gently to mix.

8. Add 0.5 ml of 1% Biuret reagent to each test tube.

9. Swirl gently to mix.

10. Look for any instant change in color from blue to violet. This is the positive test for proteins.

11. Record your results in Table 5 using the same symbols (- and +) as described in Part C.

12. Compare your results to your hypothesis.

Table 5: Biuret Test for Proteins

Substance Tested	**Hypothesis**	**Result**
Apple Juice		
Chicken Broth		
Egg White (Albumin)		
Whole Milk		
Vegetable Oil		
Distilled Water		

Questions/Analysis

1. Explain the difference between a qualitative and quantitative analysis test.

2. What substance is used as a control in the Sudan IV test and Biuret test?

3. In which order must the sugar and starch test be run? Why?

4. What are the differences among polysaccharides, oligosaccharides, disaccharides, and monosaccharides?

5. What are the two primary components of a triglyceride?

6. What are the monomers that make up proteins?

7. List and briefly describe the four levels of protein structure.

8. How do proteins of foods differ from those of the organism consuming them?

9. Name a molecule of living systems other than protein which contains nitrogen.

10. What is hydrolysis?