



Unit 2 - Macromolecules: Chemical Testing

INSTRUCTOR:

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Cells, the fundamental units of life, are composed of various combinations of organic macromolecules: carbohydrates, lipids, proteins, and nucleic acids. This lab exercise is designed to show you the qualitative tests commonly used to detect their presence.

Objectives

- Test for the presence of monosaccharides by using the Benedict's test
- Test for the presence of starch, a polysaccharide, by using the Lugol's iodine test
- Test for the presence of lipids by using the Sudan test
- Test for the presence of proteins by using the Biuret reagent test
- Determine the identity of an unknown substance by detecting the presence of organic macromolecules using the above tests

Introduction

Biological macromolecules are defined as large molecules made up of smaller organic molecules. There are four classes of macromolecules: carbohydrates, lipids, proteins, and nucleic acids. The base elements of carbohydrates and lipids are carbon (C), hydrogen (H), and oxygen (O). Proteins are also made up of these base elements but it also contains nitrogen (N).

Carbohydrates have the general formula $[\text{CH}_2\text{O}]_n$ (where n is the number of repeating units). The main function of carbohydrates is short-term energy storage (such as glucose). A secondary function is intermediate-term energy storage (as in starch for plants and glycogen for animals). In addition, some carbohydrates are involved as structural components in cells, such as cellulose in the cell walls of plants and chitin in the exoskeleton of arthropods.

Sugars are structurally the simplest carbohydrates. They are the structural units which make up the other classes of carbohydrates. **Monosaccharides** are single (mono) sugars. Important monosaccharides include ribose ($\text{C}_5\text{H}_{10}\text{O}_5$), glucose ($\text{C}_6\text{H}_{12}\text{O}_6$), and fructose (an isomer of glucose). **Disaccharides** are formed when two monosaccharides are chemically bonded together. Monosaccharides are linked into disaccharides and polysaccharides by a reaction that requires the loss of one water molecule is called *dehydration synthesis*. Sucrose, a common plant disaccharide, is composed of the monosaccharides glucose and fructose. Lactose, which is a sugar found in milk, is a disaccharide composed of glucose and the monosaccharide galactose. **Polysaccharides** are large molecules composed of individual monosaccharide units. A common plant polysaccharide is starch, which is made up of many glucose subunits. Glycogen is another polysaccharide that accumulates in the vertebrate liver and



serves as a storage product. Cellulose is a polysaccharide found in plant cell walls that forms the fibrous and rigid part of the cell wall. In terms of human diets, cellulose is indigestible and thus forms an important part of dietary fiber.

Lipids are primarily involved in long-term energy storage. They are generally insoluble in polar substances such as water. Additionally, lipids serve as structural components (as in the case of phospholipids which are the major building blocks in cell membranes) and as “messengers” (hormones) that play roles in communications within and between cells.

Fats and oils function as an energy source. Animals convert excess sugars (beyond their glycogen storage capacities) into fats. Most plants store excess sugars as starch, although some seeds and fruits have energy stored as oils (for example, corn oil, peanut oil, palm oil, canola oil, and sunflower oil). Fat molecules consist of a **glycerol** molecule (a 3-carbon alcohol) and three **fatty acids** (of varying lengths).

Another use of fats is as insulators and cushions. The human body naturally accumulates some fats in the “posterior” area. Sub-dermal (under the skin) fat plays an important role in insulation. Waxes are an important structural component for many organisms, such as the cuticle, a waxy layer covering the leaves and stems of many land plants, as well as protective coverings on skin and fur of animals.

Phospholipids and glycolipids are important structural components of cell membranes. Phospholipids are modified so that a phosphate group (PO_4^-) replaces one of the long fatty acid tails. The addition of this group makes the phospholipid “head” polar and the two “tails” nonpolar.

Proteins are very important in biological systems as control and structural elements. Two types of proteins, enzymes, and proteinaceous (having to do with proteins) hormones, carry out a number of biological control functions. Enzymes are chemicals that act as organic catalysts (a catalyst is a chemical that speeds up, but is not changed by, a chemical reaction). Structural proteins function in the cell membrane, muscle tissues, etc. The building block of any protein is the *amino acid*, which has an amino end (NH_2) and a carboxylic acid end (COOH). The R indicates the variable or differing component (also known as the “R-group” of each amino acid. Alanine and valine, for example, are both nonpolar amino acids, but they differ, as do all **amino acids**, by the composition of their R-groups. All living things (and even viruses) use various combinations of the same twenty amino acids, which is a powerful piece of evidence for the phylogenetic connection between all living things.

Amino acids are linked together by joining the amino end of one molecule to the carboxyl end of another. Removal of a water molecule, which is known as *dehydration synthesis*, allows the formation of a specialized type of covalent bond known as a peptide bond.

Draw the structures below:

Monosaccharide	Polysaccharide	Lipid	Protein
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Procedures

Part A. Testing for Monosaccharides & Disaccharides

To test for the presence of monosaccharides and disaccharides, we will use the **Benedict's test** for reducing sugars (monosaccharides). Benedict's reagent is clear blue (from the presence of *cupric copper ions*, Cu^{++}), but when combined and heated to boiling with a substance containing glucose in a chain form, the cupric ions are reduced to a cuprous form (Cu^+) and then oxidized to form copper oxide (Cu_2O). Copper oxide is a brownish-orange substance that is insoluble in water. Therefore, a positive reaction in a Benedict's test is the change of the clear light blue solution to an opaque orange-brown solution in a boiling water bath. This color change indicates the presence of glucose in a given solution.

Materials:

- Electric hot plate
- Sucrose solution
- 250-400mL beaker
- Potato juice (filtered)
- Test tubes
- Onion juice (filtered)
- Test tube holder
- Starch solution
- Test tube rack
- Glucose solution
- Benedict's Reagent dropper bottle
- Distilled water

● Part I: Testing for Glucose

1. Prepare a water bath by filling a beaker slightly over halfway with tap water.
2. Place the water-filled beaker on the electric hot plate, turn it on high, and bring the water to a boil.
3. Number 6 medium-sized test tubes using the wax pencil.
4. Place 10 drops of Benedict's reagent in each tube.
5. In the tube labeled 1, place 10 drops of distilled water; in the tube labeled 2, place 10 drops of glucose solution; in the tube labeled 3, place 10 drops of sucrose solution; in the tube labeled 4, place 10 drops of starch solution.
6. Record the color of each tube before heating in the space provided in Table 1.
7. Place the four tubes into the boiling water, being careful not to burn yourself.



8. Allow the test tubes to stay in the boiling water for five minutes. Carefully use the test tube holder to remove each tube and place it in the test tube rack. Record your observations in Table 1. Note: reducing sugars cause a color change in Benedict's solution from its original color to green, yellow, orange, or red.
9. In the last column of the data table, indicate presence with a +, and absence with a -.

Table 1: Results of the Benedict's Test (Part 1)

Tube #	Substrate	Color at Beginning	Color at End	(+) or (-)
1	Distilled water			
2	Glucose solution			
3	Sucrose solution			
4	Starch solution			

● **Part II:**

1. Prepare and label two tubes (labeled 5 and 6 – already containing 10 drops of **Benedict's solution**) with potato juice (tube 5) and onion juice (tube 6) and place them in the boiling water bath for 5 minutes.
2. Record your beginning and end observations in Table 2 below.

Table 2. Results of the Benedict's Test (Part 2)

Tube #	Substrate	Color at Beginning	Color at End	(+) or (-)
5	Potato Juice			
6	Onion Juice			

Part B. Testing for Starch

Potassium iodine solution reacts with starch to produce a purple or black color change.

Materials:

- Lugol's potassium iodine
- Sucrose solution
- Test tubes
- Potato juice (filtered)
- Test tube rack
- Onion juice (filtered)
- Glucose solution
- Wax pencil
- Sucrose solution

Procedure:

1. Place 15 drops of distilled water in another test tube and label it with the letter W.
2. Place 15 drops of glucose solution in another test tube and label it with the letter G.
3. Place 15 drops of sucrose solution in another test tube and label it with the letter S.
4. Place 15 drops of starch solution in another test tube and label it with the letter T.
5. Place 15 drops of potato juice in another test tube and label it with the letter P.
6. Place 15 drops of onion juice in another test tube and label it with the letter O.
7. In each of the test tubes, place 2 drops of Lugol's iodine. Record the results in Table 3.
8. In the last column of the table, indicate the presence or absence of starch in each tube, using a (+) symbol to indicate presence, and a (-) symbol to indicate absence.

Table 3. Results of the Iodine Test for Starch

	Material Tested	Color at Beginning	Color at End	(+) or (-)
1	Distilled water (W)			
2	Glucose solution (G)			
3	Sucrose solution (S)			
4	Starch solution (T)			
5	Potato Juice (P)			
6	Onion Juice (O)			

Part C. Testing for Lipids

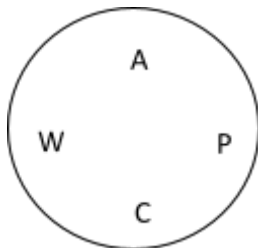
Lipids may be detected in a number of ways. The application of a fat-soluble dye known as **Sudan III** will allow lipids to be detected. The dye sticks to fats and can be seen in a simple test.

Materials:

- Sudan III dye
- Distilled water
- Pencil
- Canola or vegetable oil
- One small box of filter paper
- Almond extract
- Two Petri dish lids
- Peppermint extract
- Forceps

Procedure:

1. Mark a small dry piece of filter paper with a pencil as shown below (NOTE: do not use pen or wax pencil):



2. Place one or two drops of almond extract in the circle next to the letter A.
3. Place one or two drops of peppermint extract in the circle next to the letter P.
4. Place one or two drops of Canola oil in the circle next to the letter C.
5. Place one or two drops of distilled water in the circle next to W.
6. Dry the extracts and water using a hair dryer. Place letter side up in petri dish lid.
7. Drop some of the Sudan III dye solution into the filter paper you have marked. Pour enough Sudan III to just barely cover the paper – you don't need very much of this stain.
8. Gently agitate the dish by sliding it back and forth on the lab bench for three minutes.
9. Use forceps to remove the filter paper from the dye and briefly wash it in distilled water in another Petri dish lid. Agitate the dish by sliding it back and forth. Do a second wash if the paper is still pink after the first wash.
10. Record the color of the spots in the table below. NOTE: Sudan III will stain lipids reddish-orange.
11. In the last column of the data table, indicate the presence of lipids with a (+) and the absence of lipids with a (-).

Table 4. Results of the Sudan III Test

Material Tested	Color at Beginning	Color at End	(+) or (-)
Almond extract (A)			
Peppermint extract (P)			
Canola oil (C)			
Distilled water (W)			

Part D. Testing for Proteins

Biuret is a reagent that will change color from blue to violet when it detects peptide bonds between **amino acids of a protein**. Violet is a positive test, while blue is a negative test.

Materials:

- Biuret reagent (in a dropper bottle)
- Potato juice (filtered)
- Test tubes
- Albumin solution
- Test tube rack
- Chicken broth solution
- Glucose solution
- Distilled water
- Sucrose solution
- Wax pencil
- Onion juice (filtered)

Procedure:

1. Label eight clean small test tubes 1- 8, as indicated in Table 5.
2. Place 5 drops of the indicated material in each of the labeled test tubes.
3. Place 5 drops of Biuret Reagent into each of the labeled test tubes.
4. Record the color and other observations in Table 5.
5. In the last column of the data table, indicate the presence of proteins with a (+) and the absence of proteins with a (-).

Table 5. Results of the Biuret Test

	Material Tested	Color at Beginning	Color at End	(+) or (-)
1	Distilled water			
2	Glucose solution			
3	Sucrose solution			
4	Starch solution			
5	Potato juice			
6	Onion juice			
7	Albumin			
8	Chicken broth			

Part E. Testing an Unknown Substance

There are 3 sets (A, B, and C) of bottles, each containing a different unknown substance. Your challenge is to select one bottle of unknown from 2 different sets and perform the various tests you've just used to identify the unknown. Indicate the presence of a type of macromolecule with a (+) and the absence of that type of macromolecule with a (-). Use Table 6 to help you identify the unknown substances.

Unknown 1

The sample labeled _____ is identified by the following tests as _____

Benedict's Test	Lugol's Iodine Test	Sudan III Test	Biuret Test

Unknown 2

The sample labeled _____ is identified by the following tests as _____

Benedict's Test	Lugol's Iodine Test	Sudan III Test	Biuret Test

Note: Values in parentheses () will not be detected with our tests. Value with an asterisk (*) are at the lower end of our detection capacity and therefore may or may not test positive. Information provided is measured in mgs per 100g of edible portion.

Table 6: Value of Organic Compounds in Various Foods

Food	Protein	Lipids	Starch	Reducing Sugars (Mono- and Disaccharides)
Egg white	720	(trace)	(0)	(54)
1% gelatin (in Jello)	860	(trace)	(0)	(0)
1% glucose	(0)	(0)	(0)	910
1% nonfat milk	3580	(7)	(0)	515
1% corn syrup	(trace)	(0)	(0)	
Oats	12500	7500	10000	(trace)
1% vegetable starch	(trace)	7500	850	0
White flour	210*	(trace)	1522	100*
Table salt	(0)	(0)	(0)	(0)

Analysis Questions

1. What purpose did the tubes containing only distilled water serve?
2. What do you believe your two unknown substances are? Why do you think this?

NOTE: Your final answer may not be certain...you may only be able to narrow one of your unknowns down to two possible substances. If this is the case, be sure to explain why you cannot tell definitively what you had. At all points, explain your thinking and logic.