

EXERCISE 3

Carbon Compounds

LEARNING OBJECTIVES

- Perform diagnostic tests to detect the presence of reducing sugars (Benedict's), starch (Lugol's), protein (Biuret), lipid (SudanIV) and sodium chloride (silver nitrate).
- Identify which substances are present in an unknown mixture using these diagnostic tests.

INTRODUCTION

The organic molecules of life are carbohydrates, lipids, proteins and nucleic acids. Each of these groups of molecules is responsible for specific important roles in living cells.

Give 2 functions of each of these compounds in living cells:

Carbohydrates

Lipids

Proteins

Nucleic acids

In this exercise some simple chemical tests will be used to identify the presence of members of these groups of organic compounds. (There are no simple tests for nucleic acids that we can perform in this lab).

CARBOHYDRATES

Three types of carbohydrates are commonly found in living cells: monosaccharides, disaccharides and polysaccharides.

Give an example of each type:

Monosaccharide _____

Disaccharide _____

Polysaccharide _____

Some monosaccharides are called reducing sugars. They react with a blue reagent called Benedict's solution to form a colored precipitate (a solid that settles out of the solution). Sugars that do not react with Benedict's are called non-reducing sugars. Polysaccharides do not react with Benedict's, but will react with another reagent called Lugol's iodine.

IMPORTANT NOTE: Wear gloves. Dispose of Benedict's, Biuret, Silver Nitrate, Lugol's, Sudan IV in appropriate waste jar/tub. Spot plates with Lugol's are to be rinsed with squeeze bottles containing water over the waste tub.

TESTS FOR CARBOHYDRATES

1. Benedict's Test for Reducing Sugars

In the Benedict's test, the sample is heated with the Benedict's reagent. If reducing sugar the precipitate forms. The color of the precipitate can range from green, to yellow, orange, red or brown. The color depends on the amount of reducing sugar present in the sample, with brown being the most concentrated.

Procedure

- Mark a clean test tube to identify the sample being tested.
- Add 2 ml of the sample and 2 ml of Benedict's reagent in the tube. Record the color of the solution in the tube. This is your initial color.
- Heat the tubes in a hot bath. Remove the tubes in which the color changes. If there is no immediate color change, allow the tube to heat for 5 minutes.
- Record results in Table 1 below.

Table 1 – Results of Benedict's for reducing sugars.

	1	2	3	4	5	6	7	8	9	10	11
	Water	Starch	Glucose	Nutra-Sweet	Sucrose	Onion juice	Potato slice	Milk	Fructose solution	Apple juice	Honey
Initial color											
Final											

color											
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Explain in your own words why some of the colors changed after heating the solutions and why others did not.

Why is it important to have a tube with just water in it?

Sucrose is a sugar. Why did it not form a precipitate with Benedict's?

2. Lugol's Iodine Test for Starch

Starch reacts with Lugol's Iodine solution (iodine-potassium iodide, I-KI) to produce a complex of starch and iodine with an intense blue or black color.

Procedure

1. Obtain a clean white spot plate.
2. Place one drop of the sample solution in a depression in the spot plate. Use a small piece if it is a solid. Your initial color is the color **before** you add the Lugol's.
3. Add one drop of Lugol's I-KI solution to the drop of sample.
4. Note any color change.
5. Record results on Table 2 below.

Table 2 – Results of Lugol's test for starch

	1	2	3	4	5	6	7	8	9	10	11
	Water	Starch	Glucose	Rice	Sucrose	Onion juice	Potato slice	Milk	Pasta	Bread	Yuca
Initial color											
Final color											

Why did some of these chemicals react with the Lugol's iodine and others did not?

Which **substance** is your positive control? Negative control?

LIPIDS

Lipids e.g. fats, are compounds that are non-soluble in water (non-polar). They also tend to be less dense than water and so will float on it. Triglycerides are the most common form of fats found in nature and are made up of glycerol and three fatty acids.

What does the term hydrophobic mean? _____

How do saturated lipids differ from unsaturated lipids?

Sudan IV Test for Lipids

Sudan IV is a dye that will dissolve only in non-polar solvents, such as oily hydrocarbons or lipids. If a liquid is mixed with Sudan IV dye and the solution turns red then it can be assumed that the liquid was a lipid or hydrocarbon. Typically this would be an oil, but Sudan IV will also dissolve in non-oily hydrocarbons such as acetone and alcohol. The Sudan IV reagent that you will use has been dissolved in alcohol to make it easy for you to handle. If fat is present in any of the substances you test, the Sudan IV will dye it red and it will be seen floating as a red layer.

Procedure

1. Mark a clean test tube to identify the sample being tested.
2. Add 2 ml of the following substances to each tube.

Water
Vegetable oil
Hamburger juice
Salad dressing

3. Add 2 ml of water to each tube.
4. Add five drops of the Sudan IV solution.
5. Mix each tube by agitating from side to side.
6. Record results in Table 3 below.

Table 3 – Results of Sudan IV test for fats.

	Water	Vegetable Oil	Hamburger juice	Salad dressing
Positive red layer?				

What do your results tell you about each of the samples you have tested?

PROTEINS

Proteins are the most abundant of all the organic molecules found in the cell. They are made up of amino acids which are bonded together by peptide bonds to form chains called polypeptides.

Biuret Test for Proteins

The Biuret reagent reacts with peptide bonds and so is an indication of the presence of proteins. Biuret is sensitive to even a few peptide bonds. Consequently, when Biuret reagent is mixed with a solution containing proteins (with many peptide bonds) a strong reaction occurs that produces a violet color. Because the Biuret reagent is blue, it is sometime necessary to look carefully to make sure that the reaction mixture is actually violet or purple and not just an intense blue. Holding the solution against a white background may be helpful.

Procedure

1. Mark a clean test tube to identify the sample being tested.
2. Add 1 ml of the sample to the tube followed by 2 ml of Biuret reagent.
3. Place a piece of parafilm over the mouth of the test tube with your thumb over it and shake vigorously.

4. Allow the tube to sit at room temperature for 3 minutes.
5. Note any color change.
6. Record results in Table 4 below.

Table 4 – Results of Biuret test for protein.

	Distilled water	Egg albumin	Milk	Hamburger juice	Amino acid	Gelatin
Color present						
Protein present						

Which sample do you think contained the most protein? Why?

What was the final color of the amino acid sample? Explain why.

SODIUM CHLORIDE

Sodium chloride is an inorganic substance and is very important in living cells.

Silver Nitrate Test for Salts

The silver nitrate reagent is a mixture of silver nitrate with dilute nitric acid. Handle this solution with care! If any gets on your skin wash immediately with lots of water. The skin areas contacted by silver nitrate solution will darken but will return to normal in about a day. Minor exposure of this type is not harmful.

Silver test detects the presence of certain chloride ions. As chloride ions are most commonly encountered in water solutions (e.g. sodium chloride is sea salt/table salt), we will be looking mainly for chloride ions as an indication of salt.

The addition of a few drops of silver nitrate reagent to a water solution will immediately produce a milky-white precipitate which will be intense and persistent if the solution has a significant concentration of chloride ions. If no precipitate whatsoever is seen, the solution must have a solute free of chloride ions.

Procedure

1. Mark a clean test tube to identify the sample being tested.
2. Place 1 ml of the sample into the test tube.
3. While carefully observing the tube, add 2 drops of the silver nitrate reagent to the sample. (Note the possible formation of a white precipitate. The precipitate may or may not form and may or may not persist, depending on the concentration of ions. If chloride ions are abundant, the white precipitate will persist and make the sample cloudy).
4. Record results in Table 5 below.

Table 5 – Results of test silver nitrate test for chloride ions.

	Water	Salt solution	Salad dressing	Meat juice	Starch	Albumin	Apple juice
White precipitate?							

Lab Report

CARBON COMPOUNDS LAB DATA SHEET

1. Use this sheet to summarize the results of all your tests. Use the symbols shown. (If you did not perform the test on a substance leave the box blank).

Positive: + Negative: –

Sample	Benedict's test	Biuret test	Lugol's Iodine test	Sudan IV test	Silver Nitrate test
Distilled water					
Albumin					
Amino acid					
Apple juice					
Bread					
Fructose solution					
Gelatin					
Glucose solution					
Honey					
Meat juice					
Milk					
NutraSweet					
Onion juice					
Pasta					
Potato					
Rice					
Salad dressing					
Sodium chloride					
Starch solution					
Sucrose solution					
Vegetable oil					
Yuca					
Unknown # A					
Unknown # B					
Unknown # C					

2. Summarize the tests you performed in the table below:

Test	Reagent used	Description of positive result
Reducing sugar		
Starch		
Lipid		
Protein		

3. Why was a water sample included with each test?
