Organic Chemistry: Molecules of life

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Introduction

Molecules are composed of atoms bonded by means of ionic or covalent bonds. Organic molecules always have covalent bonds, and are also called molecules of life because all living things are mostly composed of them. Organic molecules receive that name because they always have a backbone of carbon (C), and other atoms as oxygen (O), hydrogen (H), nitrogen (N), and phosphorus (P). The four types of molecules of life are carbohydrates, lipids, proteins, and nucleic acids (DNA and RNA). Many organic molecules are composed of hundred or thousand of molecules that act as units, and because the size of so many units (called monomers, mono=one) is so big, these organic molecules are called macromolecules (macro=large). Proteins, DNA, and RNA only exist in the form of macromolecules, while several carbohydrates are present in that form. Lipids constitute the only type of organic molecule that, even if sometimes big as a molecule, do not make macromolecules.

Organic molecules have a backbone of carbon and functional groups, meaning specific arrangements of certain atoms (Nitrogen, Phosphorus, Oxygen, etc.) that give to the molecule specific properties. The presence of each one of these molecules and some functional groups can be tested in the lab by means of simple chemical tests in which the reagent reacts with the molecules, producing an evident change in color in the solution when compared to the original color of the reagent. During this lab we will analyze the presence of these four types of molecules.

Carbohydrates

TASK 1: TEST FOR SIMPLE SUGARS

The molecules that constitute the units of carbohydrates are called monosaccharides, and the joining of two of these molecules can give a disaccharide. We commonly call these types of molecules “simple sugars”. The number of carbon (C) atoms may vary in different monosaccharides, and they also may differ depending on the presence of an aldehyde group (terminal) or a ketone group (internal), as shown in the following picture,

Carbohydrates can also exist naturally in two forms: the one shown above is called the chain form, but they can also be present in ring form, as shown below,

The test we will use in this lab to test the presence of simple sugars is called Benedict’s reagent, and has a light blue color. In solution, many simple sugars exist in the ring form, which modifies the original ketone and aldehyde groups (double bonds oxygen-carbon). If the molecules are present in the ring form, they will not react with the Benedict’s reagent. If at least one of aldehyde or ketone groups is present, then the reaction will be positive and a change in color will be observed. In addition, simple sugars in the ring form can convert to the chain form when heated; this is why the experiment we will test for sugars in both cold and hot conditions.
How do we know how much sugar we have in the solution? The color we get in positive reaction depends on the amount of sugars present in the solution, the more we move far from the blue, the more sugars the solution has. This test is used routinely in diagnostic medicine to quantify reducing sugar (glucose) in the urine or blood.

<table>
<thead>
<tr>
<th>BLUE</th>
<th>GREEN</th>
<th>YELLOW</th>
<th>ORANGE</th>
<th>RED</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A little sugar</td>
<td>Positive Results</td>
<td>A lot of sugar</td>
<td></td>
</tr>
<tr>
<td><strong>Negative Result</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Procedure

1. Label five (5) test tubes 1 to 5
2. Add 1.5 ml of each of the solutions indicated above to each of the tubes with the corresponding number
3. Add 1.5 ml of Benedict’s reagent to each of the tubes
4. Agitate each tube to mix the reagent and the solution
5. Record the results in the table below
6. Heat the test tubes in boiling water for 3 minutes and record the colors in the table below

1) Actual results (circle one and write down the color)

<table>
<thead>
<tr>
<th>Solution</th>
<th>COLD</th>
<th>HEAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Water</td>
<td>(+) or (-) ?</td>
<td>(+) or (-) ?</td>
</tr>
<tr>
<td>(2) Glucose</td>
<td>(+) or (-) ?</td>
<td>(+) or (-) ?</td>
</tr>
<tr>
<td>(3) Soda</td>
<td>(+) or (-) ?</td>
<td>(+) or (-) ?</td>
</tr>
<tr>
<td>(4) Starch</td>
<td>(+) or (-) ?</td>
<td>(+) or (-) ?</td>
</tr>
<tr>
<td>(5) Whole milk</td>
<td>(+) or (-) ?</td>
<td>(+) or (-) ?</td>
</tr>
</tbody>
</table>

2) Which tube served as a control in this experiment? Why is that?

3) You are on a diet and a friend offers you something to eat. You have your doubts because of the sugar contents of the food, but your friend says that is sugar free because he tested a sample with Benedict’s reagent in cold and got a negative result. Is that right? Explain

4) Assume you heat the tubes and the result is still negative, can you be sure this food is CARBOHYDRATES free? Explain
**TASK 2: TEST FOR STARCH**

Starch is a complex carbohydrate composed of a chain of many glucose molecules held together by specific chemical bonds called glycosidic bonds. Because a molecule of starch actually has hundred of units of glucose molecules, it is called a macromolecule (macro=large). The presence of glycosidic bonds allows Lugol’s reagent to react with the starch. This reagent contains iodine, which will change from yellowish-brown to dark blue / black if starch is present.

5) If simple sugars are named monosaccharides (one) or disaccharides (two), what would the name for a molecule like starch, composed of many, many units of glucose?

6) What living things we mentioned in the lecture store energy in the form of starch?

   (PLANTS / ANIMALS / BOTH)

**Procedure**

1. Label 3 test tubes 1 to 3
2. Add 1.5 ml of each of the solutions listed below to the tube with the corresponding number
3. Record the original color of each of the solutions
4. Add 1 ml of lugol’s iodine reagent to each of the tubes; agitate the tubes to mix the reagent with the solution, and record below the final color.

**7) Results for starch test**

<table>
<thead>
<tr>
<th>Solution</th>
<th>INITIAL COLOR (solution)</th>
<th>RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Water</td>
<td>( + ) or ( - ) ? color:........................................</td>
<td></td>
</tr>
<tr>
<td>(2) Whole milk</td>
<td>( + ) or ( - ) ? color:........................................</td>
<td></td>
</tr>
<tr>
<td>(3) Starch</td>
<td>( + ) or ( - ) ? color:........................................</td>
<td></td>
</tr>
</tbody>
</table>

8) Was this a qualitative test or quantitative test?

**Proteins**

Proteins are macromolecules, meaning that they are formed by a chain of the same type of molecule acting as unit or monomer. In proteins, these units are called aminoacids. Two aminoacids are held together by a peptide bond formed between the amino group of one aminoacid and the acid group of another.

9) Which of the other three main types of organic molecules mentioned in the introduction also form macromolecules?

**TASK 3: TEST FOR PROTEINS**

The peptide bonds react with Biuret reagent. This reagent does not react with free aminoacids; we need then an entire protein or at least fragments of one, these fragments are called “peptides”. The Biuret reagent is light blue and will turn to light purple if proteins are present in the solution.
Procedure
1. Label three (3) tubes 1 to 3
2. Add 1.5 ml of each of the solutions listed below to the tube with the corresponding number
3. Add 1.5 ml of Biuret reagent to each tube
4. Mix and incubate (leave the tubes in the rack) for two minutes
5. Observe and record the results in table below

10) Results for the proteins test

<table>
<thead>
<tr>
<th>Solution</th>
<th>INITIAL COLOR (solution)</th>
<th>RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Whole milk</td>
<td>( + ) / ( - ) color: ..................</td>
<td></td>
</tr>
<tr>
<td>(2) Egg whites (albumin)</td>
<td>( + ) / ( - ) color: ..................</td>
<td></td>
</tr>
<tr>
<td>(3) Starch</td>
<td>( + ) / ( - ) color: ..................</td>
<td></td>
</tr>
</tbody>
</table>

Lipids

11) Lipids are molecules that have the same kind of atoms than carbohydrates. Name these atoms

Carbohydrates are polar molecules though, and they dissolve well in water if they are not macromolecules, while lipids are non-polar and they do not dissolve in water at all. They are made of the same atoms though.

Consider the following molecule of what is called a fatty acid (a lipid),

12) Compare the both molecules shown above. How do they compare in size?

13) What is the amount of C, O, and H in both types of molecules? (How many of each?)

Carbohydrates: C ....... H ....... O .......
True fats: C ....... H ....... O .......

14) How many water molecules (H2O) can you form with the H and O present in the sugar? .........
15) How many carbon atoms you have in the same sugar molecule? ..........
16) What is the ratio between carbon and water for simple sugar molecules? ...... / .......
17) How many water molecules (H2O) can you form with the H and O present in a fatty acid? ..........
18) How many carbon atoms you have in the same fatty acid molecule? ..........
19) What is the ratio between carbon and water for fatty acid molecules? ...... / .......

20) Based on the previous questions, which organic molecule, sugar or fat, would be more soluble in water? This explains why one dissolves in water and the other not!
TASK 4: BROWN PAPER TEST FOR LIPIDS

Procedure
1. Label 4 pieces of brown paper as “oil”, “milk”, and “water”, and “lemon-lime soda”
2. Place a drop of vegetable oil in the corresponding brown paper and let it soak in.
3. Place a drop of milk in the corresponding brown paper and let it soak in.
4. Place a drop of water in the corresponding brown paper and let it evaporate.
5. When the water has evaporated, hold the three pieces of paper against the light (one at a time!). Fats do not evaporate from brown paper; instead, they leave an oily (translucent) spot that is interpreted as a positive result for the test.
6. Record your observations in the table below

21) Results for the lipids tests (circle one)

<table>
<thead>
<tr>
<th>Solution</th>
<th>BROWN PAPER TEST</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Vegetable oil</td>
<td>(+) / (-)</td>
</tr>
<tr>
<td>(2) Whole milk</td>
<td>(+) / (-)</td>
</tr>
<tr>
<td>(3) Water</td>
<td>(+) / (-)</td>
</tr>
<tr>
<td>(4) Lemon-Lime soda</td>
<td>(+) / (-)</td>
</tr>
</tbody>
</table>

22) Does whole milk contain lipids? How much? (what % approximately) Look at the label in the milk bottle/carton

23) Based on the previous, what does this tell you about the “brown paper” test?

Testing for biological molecules in unknown solutions

TASK 5: UNKNOWN SOLUTIONS

Your lab instructor has prepared three (3) new solutions that are a combination of different solutions containing different organic molecules, hence the name “UNKNOWN” solutions, because you do not now in advance what they contain. Your task now is to find out which organic molecules are present in each one.

Procedure
1. Label nine (9) tubes, three with the number 1, three with the number 2, and three with the number 3
2. Add 1.5 ml of Unknown solution 1 to the three tubes labeled with the number 1
3. Add 1.5 ml of Unknown solution 2 to the three tubes labeled with the number 2
4. Add 1.5 ml of Unknown solution 3 to the three tubes labeled with the number 3
5. Pick one tube with each of the unknown solutions (1,2,3) and perform the Benedict’s test for simple sugar (yes! Heat the tubes)
6. Pick one tube with each of the unknown solutions (1,2,3) and perform the Lugol’s iodine test for starch
7. Pick one tube with each of the unknown solutions (1,2,3) and perform the Biuret test for proteins
8. Place a drop of each unknown solution (1,2,3) in different brown papers and perform the test for lipids
9. Observe and record the results for each test in the table below
24) Results for unknown solutions

<table>
<thead>
<tr>
<th>Solution</th>
<th>Reducing sugars (BENEDICT’S)</th>
<th>Starch (LUGOL’S)</th>
<th>Proteins (BIURET)</th>
<th>Lipids (BROWN PAPER)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( + ) / ( - ) color:……………</td>
<td>( + ) / ( - )</td>
<td>( + ) / ( - )</td>
<td>( + ) / ( - )</td>
</tr>
<tr>
<td>(1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

25) Based on your results, what organic molecules are probably present in solution (1)?

26) Based on your results, what organic molecules are probably present in solution (2)?

27) Based on your results, what organic molecules are probably present in solution (3)?

28) Name different types of food that are good sources of simple sugars

29) Name different types of food that are good sources of lipids

30) Name different types of food that are good sources of proteins

31) Name different types of food that are good sources of starch

Control versus Experimental treatments in an experiment

Control Treatments

Experiments should ideally include, always, a control treatment, which has the aim of reproducing exactly the same conditions that are present in the normal or natural situation. This means that nothing is different compared to the normal situation for the variable Y; in other words, the X variable is not controlled or manipulated by the researcher for this group. The results of all the other treatments in the experiment are compared against the control one to see whether or not the different treatments used had a significant effect on the Y variable.

32) What control treatment was used in today’s lab?

33) If you want to see whether caffeine affects the heart rate, what would be your control in this experiment?
Negative and Positive Control Treatments

When testing for reducing sugars and other organic molecules, we used water as one of the solutions even knowing that the result would be negative because water does not contain simple sugars. This is what is called a negative control treatment or group in an experiment. What is the difference between a control treatment and a negative or positive control? In negative or positive controls we expect a negative or positive result respectively because we know the reagent should not produce a reaction with that particular solution.

34) In the experiment presented in question 33, is the control you proposed a positive or negative one?

In a positive control, we have the opposite situation: the researcher knows in advance that the outcome of one of the groups (one tube in today’s lab) will be positive. The advantage of a positive control is that, depending on the type of experiment, the researcher may use the control as a point of reference to also quantify the amount of reaction present in all the other treatments.

35) What solutions we used today would you say may work as positive controls?

Test for simple sugars: possible positive control: .................................................................
Test for starch: possible positive control: .................................................................
Test for proteins: possible positive control: .................................................................
Test for lipids: possible positive control: .................................................................

Experimental Treatments

Experimental treatments are simply all the other treatments (tubes in today’s lab) in which we really do not know what the outcome is going to be, these treatments are the reason why the experiment is performed. In our exercise, a real experimental treatment is ANY for which we do not know if the molecule we want is present or not. Example: coke in a test for starch.

36) Name three experimental treatments (tubes) used in today’s lab for three different tests we performed

1. .............................................................. for test: ..............................................................
2. .............................................................. for test: ..............................................................
3.............................................................. for test: ..............................................................