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Date: September 8, 2026

Title: Identification and Detection of Important Macromolecules

Objective(s)/Aim(s): To identify the presence of major macromolecules including carbohydrates, lipids, proteins, and nucleic acids in various test solutions. Done by performing biochemical tests including Benedict's test, Iodine test, Biuret test, Sudan test, and Dische diphenylamine test.

Introduction: All living organisms are composed of four groups of large, complex molecules made up of smaller organic polymers held together by covalent bonds, called macromolecules. These molecules consist of monomers: Carbohydrates, Lipids, Proteins, and Nucleic Acids. Carbohydrates are simple sugars used for quick energy; lipids are fatty acids that serve as long-term energy, proteins are made up of amino acids and function in catalysis and regulation, and nucleic acids are made up of nucleotides and store and transmit genetic.

Chemical tests are a useful tool to detect the presence of these macromolecules. For sugars (Carbohydrates), the Benedict's reagent is used, the iodine solution identifies starch (complex carbs), Biuret's reagent detects the peptide bonds in proteins, Sudan dye detects lipids, and the Dische diphenylamine test detects DNA. The goal of this lab is to use these indicator tests to determine which macromolecules were present in the given set of samples.

Materials and Methods

- Benedict's Test for Reducing Sugars: 0.5 mL of each sample solution (Water, Glucose, Sucrose, Starch) mixed with 0.5 mL of Benedict's reagent and boiled. A color change from blue to green to reddish orange indicated reducing sugars.
- Iodine Test for Starch: 0.5 mL of each sample (Water, Glucose, Sucrose, Starch) with 0.5 drops of iodine solution. A blue-black color indicated starch.

- Biuret Test for Proteins: 1mL of each sample (Egg albumen, Starch, Amino acid solution, Distilled water) mixed with Biuret's reagent. A violet color indicated proteins.
- Dische Diphenylamine Test for Nucleic Acids: 0.5 mL of one DNA solution, RNA solution, and distilled water. 0.25 mL of a second DNA solution, and distilled water. All mixed with 1 drop of diphenylamine reagent and heated. A blue color indicated DNA.
- Sudan III Test for Lipids: 0.5 mL of each sample (Salad oil, Honey, Distilled water) with 2 drops Sudan dye. A red-stained oil layer indicated lipids.

Data and Results:

Results: Benedict's Test for Reducing Sugars

TABLE 4.2					
Tube Number	Sample				Glucose Present/Absent
	Name	Volume	Benedict's Reagent	Color after Heating	
1	Water	0.5 mL	0.5 mL	blue	Absent
2	Glucose	0.5 mL	0.5 mL	red	Present
3	Sucrose	0.5 mL	0.5 mL	blue	Absent
4	Starch	0.5 mL	0.5 mL	blue	Absent
5	Stomach Contents	0.5 mL	0.5 mL	N/A	N/A

Results: Iodine Test for Starch

TABLE 4.3					
Tube Number	Sample				Starch Present/Absent
	Name	Volume	Iodine Solution*	Color	
1	Water	0.5 mL	1 drops	Yellow	Absent
2	Glucose	0.5 mL	1 drops	Yellow	Absent
3	Sucrose	0.5 mL	1 drops	Yellow	Absent
4	Starch	0.5 mL	1 drops	Black	Present
5	Stomach Contents	0.5 mL	1 drops	N/A	N/A

*Add dropwise until characteristic color forms (blue-black)

Results: Biuret Test for Protein

TABLE 4.4					
Tube Number	Sample Biuret's Reagent*				Protein Present/Absent
	Name	Volume	Volume	Color	
1	Egg Albumen	1 mL	1 drop	Light purple	Present
2	Starch	1 mL	1 drop	light blue	Absent
3	Amino Acid Solution	1 mL	1 drop	light blue	Absent
4	Distilled Water	1 mL	1 drop	light blue	Absent
5	Stomach Contents	1 mL	1 drop	N/A	N/A

*Add dropwise until characteristic color forms (Lilac)

Results:Diphenylamine Test for Nucleic Acids

TABLE 4.5				
Tube Number	Sample			
	Name	Volume	Diphenylamine Reagent*	Color
1	DNA Solution	0.5 drop	1 dropwise	very cloudy
2	DNA Solution	0.25 drop	1 dropwise	very cloudy
3	Distilled Water	0.25 drop	1 drop wise	Clear/cloudy
4	RNA Solution	0.5 drop	1 dropwise	cloudy
5	Distilled Water	0.5 drop	1 dropwise	cloudy

*Add dropwise. The solution will become cloudy. Continue adding dropwise until color becomes clear.

Results Sudan Dye Test for Lipids

TABLE 4.6					
Tube Number	Sample				Lipids Present/Absent
	Name	Volume	Name	Volume	
1	Salad Oil	0.5 mL	Sudan Dye*	2 drops	Absent
2	Honey	0.5 mL	Sudan Dye*	2 drops	Absent
3	Distilled Water	0.5 mL	Sudan Dye*	2 drops	Present
4	Stomach Contents	0.5 mL	Sudan Dye*	2 drops	N/A

*Sudan Dye is Sudan III or Sudan IV.

Conclusion: This experiment was successful overall and showed how qualitative chemical indicator tests can identify the major macromolecules. Supported the expected outcomes of each biochemical test except for the Diphenylamine Test for Nucleic acids due to the lack of drops of diphenylamine reagent. One of the reducing sugar, Glucose, tested positive with Benedict's reagent while sucrose, water (control), starch did not, confirming that only free aldehyde or ketone groups reduce copper ions leading to the dark reddish orange color that was presented in glucose. Starch tested positive with iodine, producing a characteristic dark purple or black color while sucrose, water, and glucose did not react and remained yellow. Egg albumen produced a very light lilac color with Biuret's reagent, after swirling the test tubes confirming the presence of proteins with peptide bonds which means that there were less peptides present in the solution due to the light color. Salad oil mixed in with Sudan dye, indicating lipids while the other solutions were layered with the Sudan Dye on the bottom. The correct DNA solution reacted with diphenylamine and would confirm the presence of nucleic acids, but our test did not react. It was interesting to learn how these indicator tests translate into real-life applications including urine diagnostic strips which test for glucosuria in diabetes, kidney diseases, or for substances such as marijuana.

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