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Macromolecules

Objectives

To identify the presence of carbohydrates, lipidss, protei,ns and nucleic acids in various solutions by running a series of tests, including Benedict's test, Iodine test, Biuret test, Sudan IV t,est, and Diphenylamine test.

Introduction

In lectulecturearned that living organisms are made up of four main organic macromomacromoleculesbydrates, proteins, lipids, and nucleic acids. Each macromolecule hasa unique role in maintaining the biological structure and function of organisms. Carbohydrates provide energy and structural components, proteins act as catalysts and regulators, lipids provide long-term energy storage and membrane components, and nucleic acids store and transport genetic information.

Because these are organic macromolecules, specific chemical tests can detect them in solutions. Benedict's reagent detects reducing sugars, iodinesolutions identify starches, Biuret's reagent dedetectseptide bonds in proteins, Sudan IV dye ddetectslipids, and the diphenylamine test detects DNA. The main goal of this lab is to use these tests to determine which macromolecules are present in which sample solutions.

Materials & Methods

- ★ Benedict's Test: 1.2 mL sample mixed with 2 mL of Benedict's reagent and boiled. Results in a color change from blue to green/orange/red, indicating thepresences of reducing sugars.
- ★ Iodine Test: 1.2 mL of sample with 3 drops of Iodine solution. A blue-black color indicates starch.

★ Biuret Test: 2 mL of sample mixed with Biuret's reagent; a purple/violet color indicates proteins.

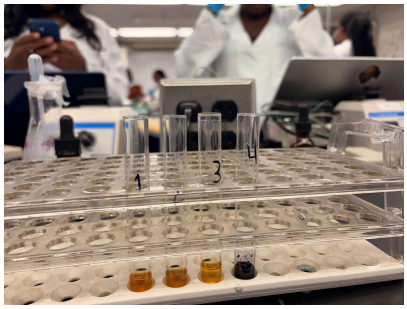
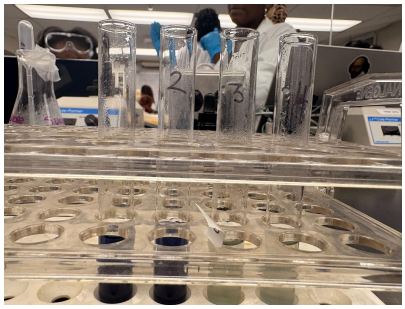
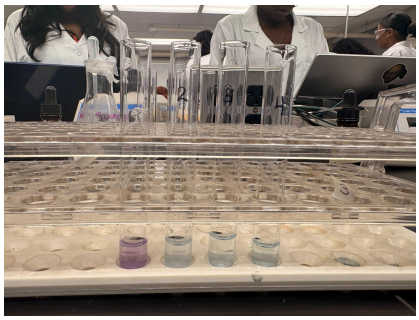
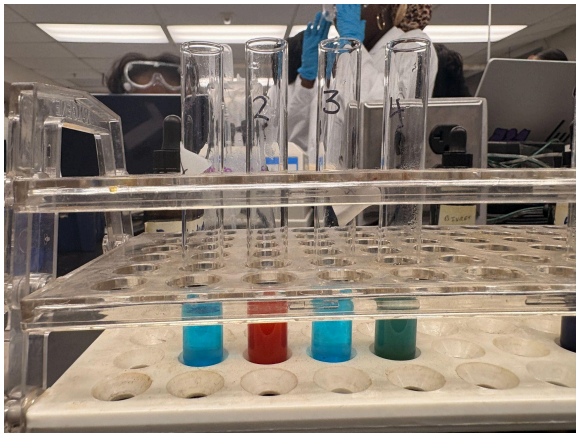
★ Sudan IV Test: 1.2 mL of sample with 5 drops of Sudan dye;; a red-stained oil layer indicates lipids.

★ Diphenylamine Test: Add 2 mL sample to diphenylamine reagent and heat; a blue color indicates DNA.

Results

Sample	Benedict's Test	Iodine Test	Biuret Test	Sudan IV Test	Diphenylamine
Water	Blue	Yellow		on top/insoluble on top/insoluble	Grayish Blue
Glucose	Red(+)	Yellow			
Sucrose	Blue	Yellow			
Starch	Blue	Dark blue	Light Blue		
Albumin			Violet		
DNA			Light Blue		
Salad Oil			Light Blue	Orange	
DNA (2mL)					Dark Blue
DNA (1mL)					Dark Blue
RNA					Grayish Blue
Honey				Reddish	

				orange on top/insoluble	

Discussion/Conclusion:

The purpose of this lab experiment was to identify the four major macromolecules using specific biochemical tests. Water served as a negative control across all tests, showing no characteristic color changes and no reaction with the solvent.

Analysis & Findings

- **Carbohydrates (Reducing Sugars & Polysaccharides):** Benedict's test detects free aldehyde or ketone groups in reducing sugars upon heating. Glucose yielded an expected red precipitate. At the same time, sucrose and starch remained blue, indicating that glycosidic linkages prevent Benedict's reagent from reducing without free carbonyl

groups. The Iodine test successfully differentiated polysaccharides from simple sugars: starch produced a dark blue complex due to triiodide ions lodging within the coiled amylose helix, whereas glucose and sucrose remained yellow.

- **Proteins:** The Biuret test detects peptide bonds under alkaline conditions using copper(II) ions. Albumin tested positive, yielding a distinct violet solution indicative of copper coordination with peptide backbones. In contrast, starch, DNA, and salad oil remained light blue, demonstrating the Biuret reagent's specificity for amino acid polymers.
- **Lipids:** Sudan IV is a nonpolar, hydrophobic dye that dissolves preferentially in hydrocarbons. Salad oil demonstrated clear lipid content by concentrating the dye into an orange/red-stained organic layer. For honey and water, the dye remained insoluble and collected near the surface as a reddish-orange layer rather than dispersing into a distinct lipid phase, reflecting honey's primary composition of dissolved sugars and water rather than fatty acids.
- **Nucleic Acids:** The Dische diphenylamine test specifically identifies deoxyribose sugars in DNA under acidic, heated conditions. Both DNA sample volumes (1 mL and 2 mL) produced dark blue solutions, confirming the presence of DNA. RNA and water produced grayish-blue negative results, showing the assay's ability to distinguish deoxyribose from ribose sugars.

Experimental Limitations & Improvements

Potential sources of error include subjective color interpretation at boundary concentrations, cross-contamination between reagent wells, or insufficient heating time during the Benedict’s and Diphenylamine boiling steps. Future iterations would benefit from spectrophotometric absorbance readings to quantify concentrations rather than relying solely on visual colorimetric endpoints.