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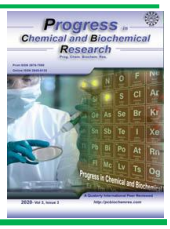

Original Research Article

Practical Biochemistry Principles and Techniques Approach

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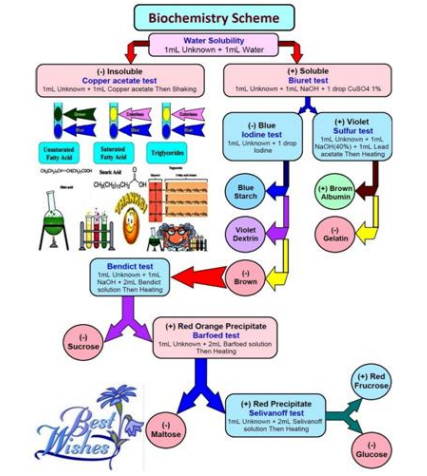
GRAPHICAL ABSTRACT



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ABSTRACT

Biochemistry, sometimes called biological chemistry, is the study of chemical processes within and relating to living organisms. Biochemical processes give rise to the complexity of life. A sub-discipline of both biology and chemistry, biochemistry can be divided into three fields; structural biology, enzymology, and metabolism. Over the last decades of the 20th century, biochemistry has through these three disciplines become successful at explaining living processes. Almost all areas of the life sciences are being uncovered and developed by biochemical methodology and research. Biochemistry focuses on understanding the chemical basis which allows biological molecules to give rise to the processes that occur within living cells and between cells, which in turn relates greatly to the study and understanding of tissues, organs, and organism structure and function. Biochemistry is closely related to molecular biology, the study of the molecular mechanisms of biological phenomena. Much of biochemistry deals with the structures, functions, and interactions of biological macromolecules, such as proteins, nucleic acids, carbohydrates, and lipids, which provide the structure of cells and perform many of the functions associated with life. Metabolism is the mechanism by which cells harness energy via chemical reactions. The findings of biochemistry are applied primarily in medicine, nutrition, and agriculture. In medicine, biochemists investigate the causes and cures of diseases. In nutrition, they study how to maintain health wellness and study the effects of nutritional deficiencies. In agriculture, biochemists investigate soil and fertilizers and try to discover ways to improve crop cultivation, crop storage, and pest control.

INTRODUCTION

Biochemistry studies the quality of biomaterials and estimates their quantity and position in the organism's biological systems. From the many information derived from the bio-studies, it was possible to determine the location and functioning of most of the vital compounds in the body and the specific information of each compound. Based on laboratory studies of compounds in the organism, biochemistry was divided into three main sections: Structural Biochemistry, Dynamic Biochemistry and Functional Biochemistry [1].

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Biochemistry possesses specialized methods of research and study. Accordingly, many biochemical compounds have been continuously discovered and their quantitative and biological properties, as well as their biological and physiological and energy transformations, have been estimated. A detailed study of the biological systems on the organism as a whole or certain organs or their tissues or extracts or leachate from these tissues. Electron microscopy was able to obtain new and important information concerning the study of the internal structure of the living cell [2].

1. Carbohydrates:

Carbohydrates (**Fig. 1**) are found in the plant and animal kingdoms. The general symbol of carbohydrates is ($C_nH_{2n}O_n$) is a polyhydroxyl aldehydes or ketones, or compounds produced by the hydrolysis of polyhydroxyl aldehydes or ketones. Carbohydrates are divided into the following sections: Monosaccharides, it is the simplest unit of carbohydrates and does not decompose into simpler units of which all reductive sugars such as arabinose, glucose, fructose, etc. Oligosaccharides, containing two to ten units of monosaccharide units, such as sucrose, maltose, and lactose, which are double sugars, raffinose (triple sugar). Polysaccharides, this group consists of the binding of a large number of monosaccharide units together with glycoside bonds, such as starch, cellulose, dextrin, and glycogen and is widely distributed in the plant and animal kingdoms, and is represented by the starch of various types, glycogen, dextrin, cellulose, etc. The microscopic shape of the starch granule varies depending on the plant source. For example, potato starch differs in shape and size from rice and wheat starch. The starch granule is composed of two substances, amylopectin, and the outer shell and contains amylose. Amylose is relatively soluble in water and gives a blue color with iodine - while amylopectin is insoluble in water but swells in hot water. Starch is formed in plants by carbon representation, and is abundant in grains such as rice, wheat, maize, barley, cucumber, chickpeas, beans, etc., in tubers such as potatoes, in roots such as potatoes, and in general almost no plant species [3].

1.1. Moore's test:

Add to 2 milliliters of sugar solution (1%) equal amount of NaOH solution (40%) and heated to boil and notice the yellowish color of the solution and continuously heating turns brown - this test is positive with all sugars. Add to another part of the sugar solution a similar volume of NaOH (40%) and boil for a minute and then add 2 milliliters of Benedict's solution and boiling. It is observed that no yellow or red precipitate is present, which is evidence that glucose boiling with NaOH loses the reductive trait (**Fig. 2**) [4].

1.2. Molish test:

Molish reagent is composed of 2% alpha naphthol in 95% alcohol + concentrated sulfuric acid. 2 ml of sugar solution + 2 drops of alpha naphthol solution then shake the tube to mix the two solutions and then add concentrated sulfuric acid with caution on the wall of the tube. A purple ring is formed at the surface of the separation. The reaction is done first by concentrating the concentrated sulfuric acid directly with the mono sugar molecules or analyzing many sugar into its mono-sugar units and then removing three water molecules from each mono-sugar molecule, forming the furfural compound (produced from Pentose) or hydroxymethylfurfural (resulting from Hexose) (**Fig. 3**). The compounds combine with alpha naphthol forming the purple ring (**Fig. 2**). Note: When performing this test a green color may appear beneath the purple ring. This color is the result of the reaction of the increase of alpha naphthol with concentrated sulfuric acid [5].

1.3. Tolen test:

The ammonium silver hydroxide solution is prepared by placing 5 ml of silver nitrate solution in a test tube and adding two drops of NaOH solution. A gray precipitate of silver hydroxide is formed and then gradually adding aluminum hydroxide until the precipitate is dissolved. Take 3 ml of ammoniac silver hydroxide solution + 1 ml of reduced sugar solution and then heat in a boiling water bath. A black precipitate or a shiny silver mirror of silver is observed on the inner wall of the tube (**Fig. 2 and 4**) [6].

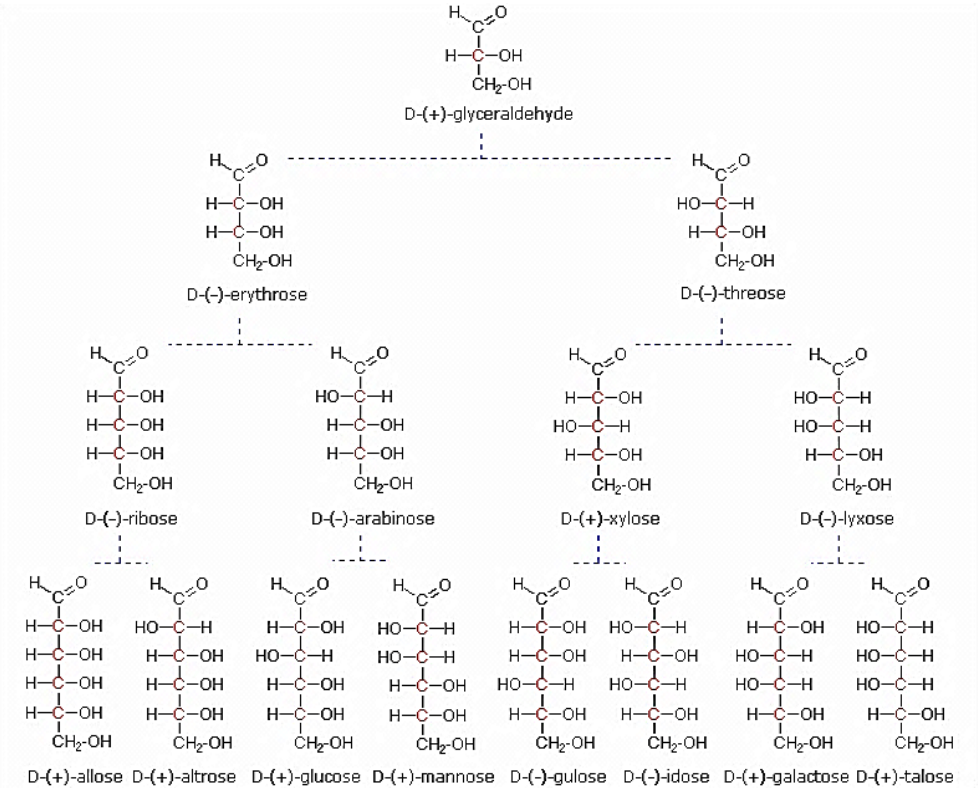

Fig. 1. Structures of different carbohydrates.

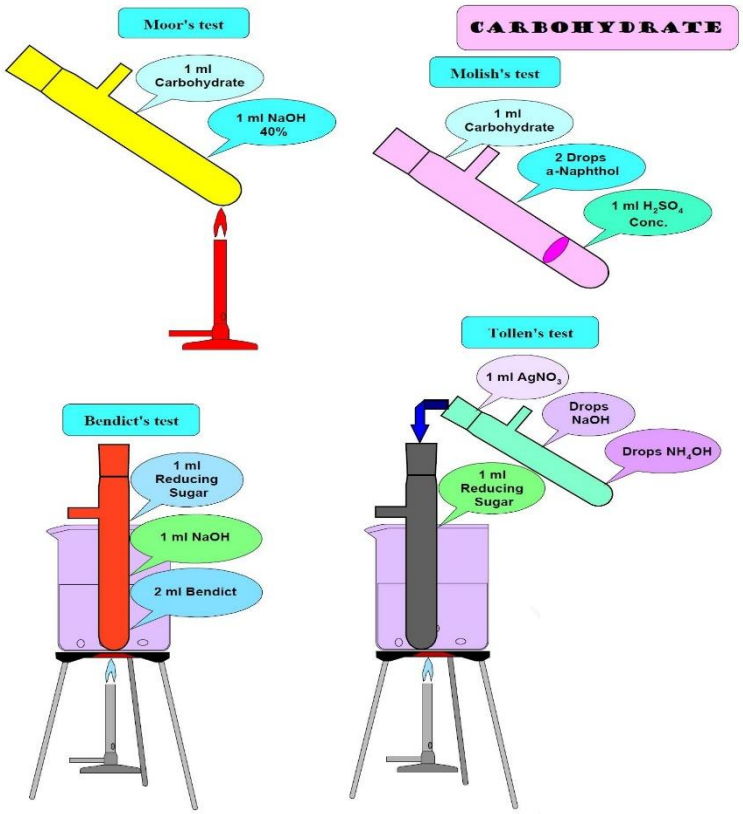

Fig. 2. Carbohydrate detection tests

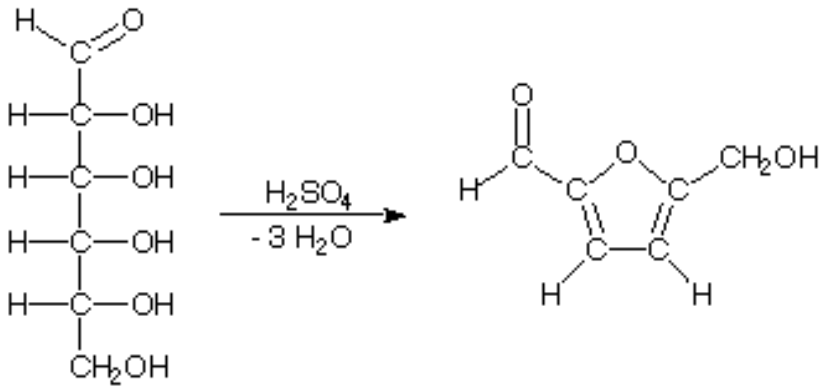

Fig. 3. The reaction involved Molish test.

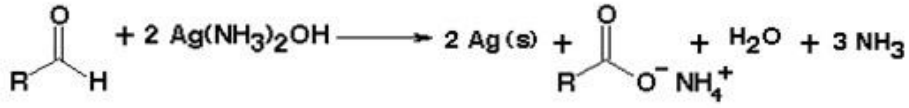

Fig. 4. The reaction involved Tolen test.

1.4. Trommer test:

Reduced sugars have a free aldehyde group within their composition and these groups have the ability to reduce oxides and hydroxides of metals such as silver and copper (Fig. 5).

This test is based on the effect of diluted alkalis on the reduced sugars using NaOH. By heating part of the copper hydroxide, a black color of copper oxide is observed. Add another part of $Cu(OH)_2$, which is a bit of reduced sugar solution and heated to boil. Note the appearance of a precipitate ranging from yellow to orange to red from Cu_2O [6].

1.5. Benedict test:

Why is the alkaline medium used in reductive tests? Because in the alkali medium, the ene-di-ol form is made easier in the reduction process, which is oxidized to the corresponding acid while copper is reduced to copper oxide (Fig. 2 and 6) [6].

1.6. Picric acid test:

Add 5 ml of sugar solution to 2-3 ml of saturated picric acid + 1 ml of sodium hydroxide solution and heat it softly. Note that the solution turns yellow to red in the case of reduced sugars due to the reduction of picric acid to picramic acid [6].

1.7. Osazone crystals formation test:

Place three test tubes of approximately 5 mL of 1% glucose, fructose, and maltose solution, then add to each tube 100 drops of concentrated acetic acid and then add about 1 g of a newly prepared mixture of phenylhydrazine hydrochloride and sodium acetate. Mix well and then heat in a water bath for five min. Filter in clean test tubes and then heat again in a water bath for 45-60 minutes. Let the tubes cool gradually. Note the formation of yellow crystals hanging in the tubes - taken from them on a glass slide and examined under the microscope and draw a crystal shape. 3 molecules of vinyl hydrazine hydrochloride react with a molecule of reduced sugars and form crystals with a distinctive crystalline form known as osazone. The following equations illustrate the osazone test [7].

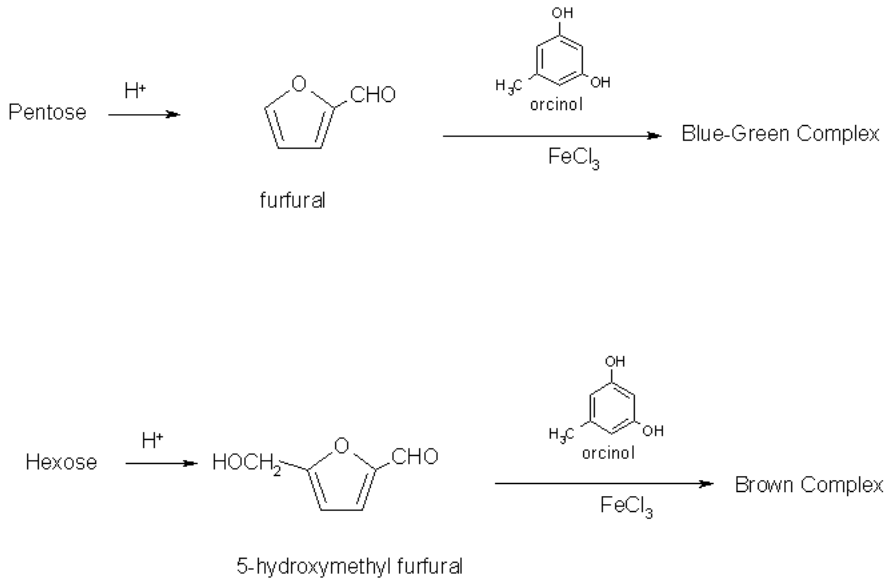

Fig.5. The reaction involved Trommer test.

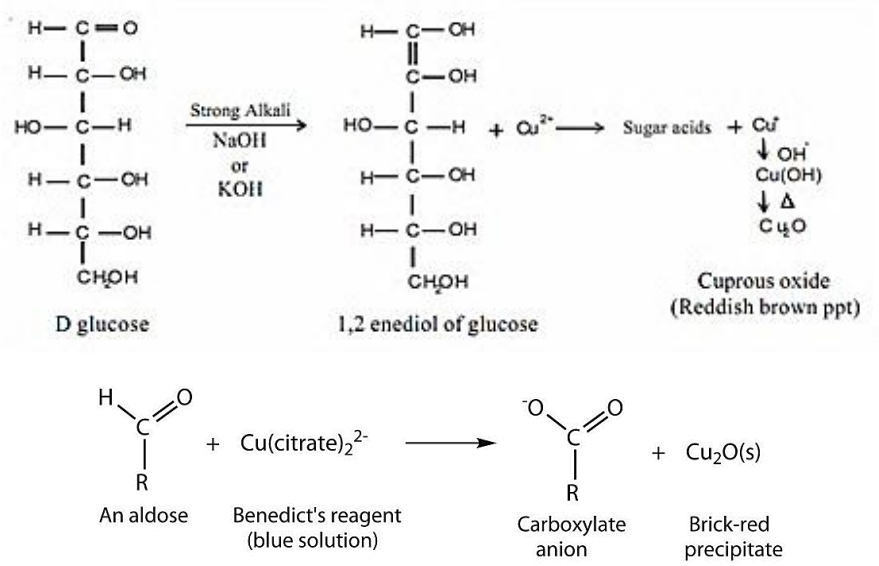

Fig. 6. The reaction involved Benedict test.

1.8. Barfoed test:

Barfoed reagent is prepared by dissolving copper acetate in acetic acid (13.3 g copper acetate + 200 ml of 1% acetic acid). When treated with mono-sugar in this solution, the aldehyde group can reduce the said copper to a red copper oxide precipitate (**Fig. 7**). This test works only with monosaccharides, which are heated for a short time, but if the binary sugar is heated with a solution of boiling Barfoed for a long time, the acidic medium of the solution often occurs hydrolysis of binary sugars and decomposition to its simple sugary components so you should not heat for a long time and remove the tube from the water bath to cool. Take 1 ml of mono sugar solution in a test tube and add 2-3 ml Barfoed solution and placed in a water bath for a simple time and then leave to cool.

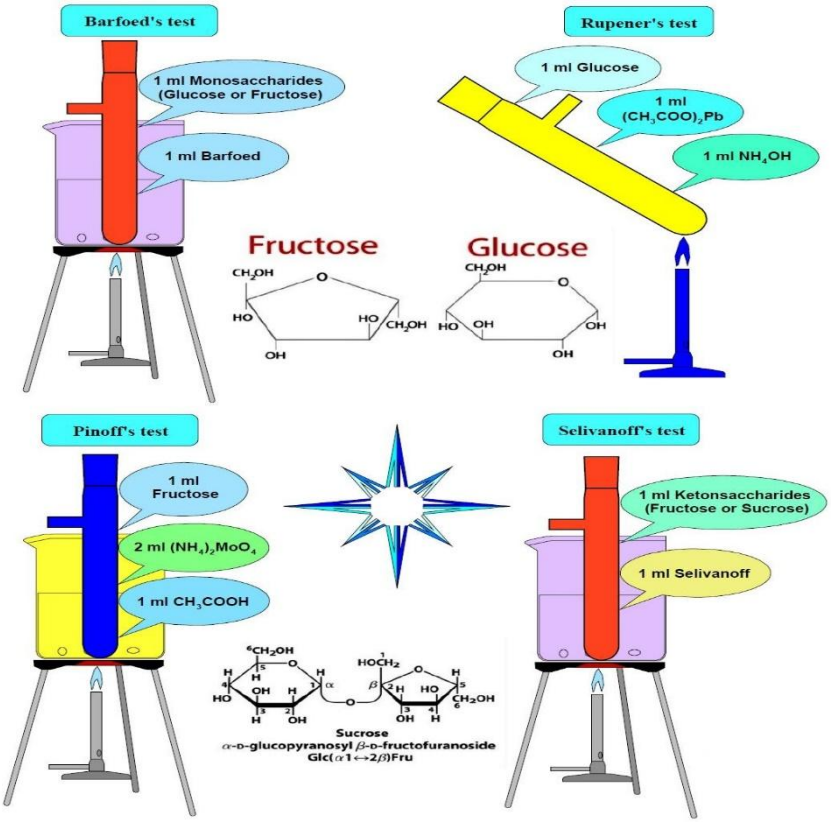

Fig. 7. Some specific saccharides detection tests

This test was conducted on glucose-fructose - lactose - sucrose [7].

1.9. Rupener test:

It is a special and unique test for glucose sugar only, which is a reduction of lead hydroxide to red lead oxide and test solution is a lead acetate added to the glucose sugar solution and then add the solution of NH_4OH and then boiling until the color of the precipitate to red or leather. This test was performed on glucose and then another mono-sugar solution (Fig. 7) [8].

1.10. Pinoff's test:

The research substance in the Pinoff test is 4% newly prepared ammonium molybdate + a few drops of ice acetic acid. Take 1 gram fructose + 5 ml water + 5 ml ammonium molybdate + a few drops of acetic acid. The tube contents are heated in a boiling water bath for 5 minutes and the color is dark blue. This test is characteristic of fructose and in the case of the presence of mineral acids does not give this reagent any success (Fig. 7) [8].

1.11. Selivanoff test

This test works with ketones or ketones, such as fructose or sucrose, which, when hydrolyzed, give fructose. Selivanoff solution is crystals resorcinol + HCl acid and prepared by dissolving 1 g resorcinol in 100 ml 12% HCl acid (2: 1) (1 part HCl + 2 part water). Add 3-5 cm 3 of the solution of Selivanoff to 1 cm 3 of the solution of sugar of ketone origin such as fructose (in a water bath) notice the appearance of a red color and then a precipitate. The cause of the appearance of red color is due to the production of hydroxymethylfurfural resulting from the interaction of fructose with HCl with the compound resorcinol (Fig. 7) [8].

1.12. Furfural test:

Place in a test tube about 1.5 milliliters of transformed fructose solution or sugar, add 5 milliliters of HCl acid (1:1) and then boil for a minute, display a paper moistened with aniline acetate solution to the vapors during boiling. Note the color of the paper in red. Note: Aniline acetate is prepared by mixing two equal volumes of glacial acetic acid + aniline [8].

1.13. Microscopic examination:

Examine the microscopy of starch granules from different plant sources such as potato starch, rice, and corn by placing them on a glass slide, adding drops of water and drawing their distinctive shape. Add to the slide the starch grains with a drop of diluted iodine solution and note that the grains take a clear blue color [9].

1.14. Iodine test:

Add to the part of the starch paste a little solution of iodine dissolved in potassium iodide and note the appearance of blue color. Sedimentation tests: Add about 2 milliliters of starch paste equal to the amount of ammonium sulfate solution or basal lead acetate with shaking well. Note the starch precipitation and prove that the solution is filtered and added to the filtrate point to the iodine does not form blue (**Fig. 8**). Add a drop of diluted iodine solution to a cotton swab or filter paper and note that the cellulose is not discolored in blue as the starch. Place a piece of filter paper with a drop of iodine in an hour bottle, then add a little diluted sulfuric acid (65%) and note the appearance of a blue color in the iodized areas. The reason for this is that cellulose is produced by some concentrated acids such as sulfuric acid or some concentrated salt solutions such as zinc chloride [9].

1.15. Schweizer test:

Prepare a Schweizer solution: Pass by passing a stream of air for a few hours in a bottle with ammonia and a copper lathing. Place a piece of filter paper or cotton in a test tube and add a little Schweizer solution. Note the dissolution of cellulose and the formation of a colloidal system. Cellulose can be deposition in this system by adding a dilute acetic acid solution [9].

1.16. Bevan test:

Add a piece of filter paper slightly from the Bevan solution and invert the solution with a glass mold. Note that cellulose dissolves in this solution. Cellulose can be re-deposited in this solution by ethyl alcohol [9].

2. Proteins

Proteins contain several elements in addition to carbon, hydrogen, oxygen, nitrogen including sulfur, phosphorus, chlorine, bromine as well as iodine. These elements cannot be detected directly by inorganic chemistry, as this method requires that these compounds have ionic bonds. In general, the bond is of the covalent type, so it is necessary to convert the elements in organic compounds to ionic compounds containing these elements. This can be heated by the organic compound with the element sodium at high temperature, thus forming sodium salts The elements of nitrogen, chlorine, sulfur, bromine, and iodine were present in the organic matter as cyanide - chloride - bromide - iodide [10].

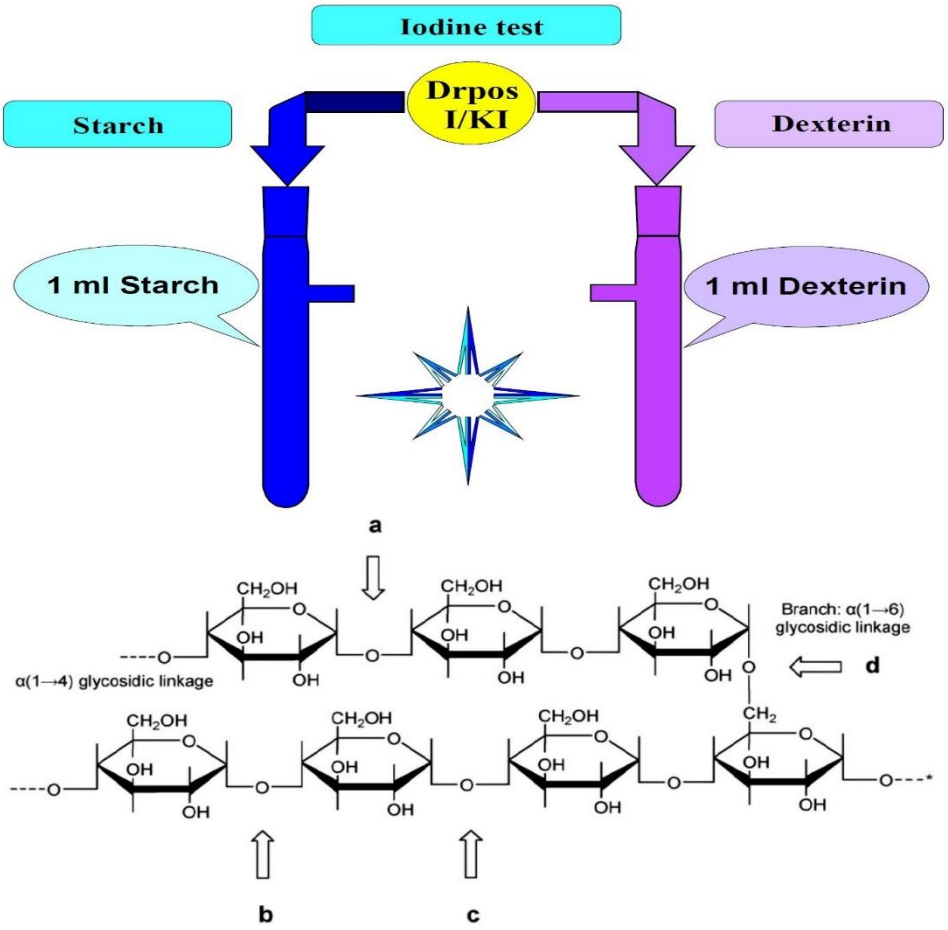

Fig. 8. Polysaccharides detection tests

2.1. Biuret test:

This test works with compounds containing two peptide bonds and more. When a copper sulfate solution is added to a protein alkaline solution or a solution containing compounds with peptide bonds, a violet color is formed and the intensity of this color depends on the number of these peptide bonds. Copper and sodium sludge is composed of the copper complex with peptide bond nitrogen atoms. Preheat a little urea crystal in a clean dry test tube and note that they are fused and ammonia is rising - keep warming until a yellow material remains at the bottom of the tube known as a Biuret compound. Add the caustic sulfate compound and then one drop of 1% copper sulfate solution. 1 ml protein solution + 1 ml dilute NaOH solution and mix the two solutions well. Add only one drop of 1% copper sulfate solution and shake. Note the formation of reddish-purple or violet color as a positive result of this test (Fig. 9) [11].

2.2. Xanthobrotic test:

Characteristic for amino acids containing an aromatic ring such as tyrosine, tryptophan and phenylalanine. When heating the protein content of these acids with concentrated nitric acid shows a yellow color turns to orange by adding alkali with cooling. Add to about 3 ml dilute protein solution about 1 ml concentrated nitric acid notice the emergence of a white precipitate turns warming to boiling to yellow as a result of the formation of compound xanthoprotein. Repeat the same test using a 1% phenol solution instead of a protein solution and note that it gives a positive result as well [12].

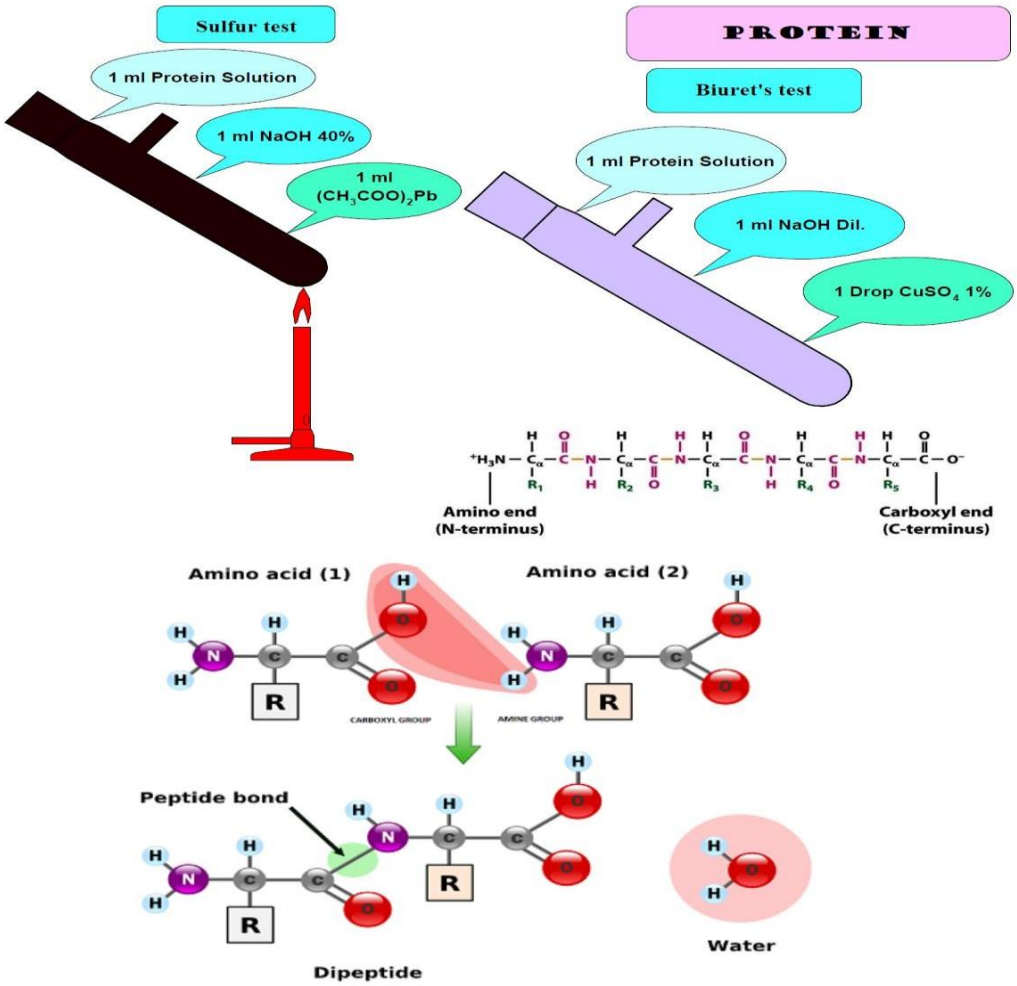

Fig. 9. Protein detection tests

2.3. Millon test:

Characteristics of amino acid tyrosine as well as any protein substance containing this acid. Add about 1 ml of protein solution, about 5 drops of Millon's solution, shake the tube well and then heat to boil on a quiet flame and form a white precipitate that turns into red brick continuously heating. This test was done using a phenol solution or salicylic acid instead of protein. Note that it gives a positive result. Compounds containing hydroxy benzene radicals react with the essence of the Mellon searchlight, forming red complexes. Millon's solution is composed of nitrate, mercury nitrate and mercury dissolved in concentrated nitric acid [13].

2.4. Sulfur test:

This test works with sulfur amino acids such as cysteine, cysteine, and methionine. Add to a portion of the protein solution an equal volume of diluted sodium hydroxide solution and heat to boil. Then add the lead acetate solution and keep warming. Note the formation of a precipitate indicating the presence of sulfur within the protein composition and thus prove the presence of amino acid cysteine/cysteine and methionine. The sulfur contained in the synthesis of methionine acid is highly binding to the parts of the compound and therefore add alkali The sulfur amino acid cysteine facilitates the separation and give sulfur test quickly. If HCl is added to the black precipitate, a

white precipitate is formed of lead chloride (Fig. 9) [14].

2.5. Molish test:

Give a positive result (purple ring) with proteins associated with carbohydrate cracks such as Nucleoprotein, glycoprotein. 2 ml of protein solution + 2 drops of alpha naphthol solution then shake the tube to mix the two solutions and then add concentrated sulfuric acid with caution on the wall of the tube. A purple ring is formed at the surface of the separation [15].

2.6. Sakagosh test:

Characteristics of the amino acid arginine (guanidine group) by adding a solution of alpha naphthol and bromine water to the protein solution shows a red color characteristic of the group of guanidine in arginine and red color due to the condensation of alpha naphthol with arginine. The bromine action here as an oxidizer and may enter the intermediate reactions [16].

2.7. Hopkin Col test:

This test is characteristic of the indole group and thus distinguishes the amino acid tryptophan because it contains indole where condensation of the indole molecule occurs with the glyoxalic acid molecule forming a complex by adding a concentrated sulfuric acid on the tube wall forming a scarlet ring [17].

2.8. Rosenheim formaldehyde test:

Characteristic for indole and amino acid tryptophan. Rosenheim's solution is a ferric chloride solution with formaldehyde. Add 2 drops of Rosenheim solution to 2 ml of protein solution, mix thoroughly and then carefully add to the wall of the concentrated sulfuric acid tube, showing a scarlet ring on the surface of the separation characteristic of the indole group [18].

3. Lipids:

Lipids are substances that are very similar in their natural properties but differ in chemical composition and share that they are not all soluble in water but soluble in organic solvents such as ether, chloroform, carbon tetrachloride, benzene, hot ethanol, acetone, etc. Lipids are found in all cells and tissues, whether plant or animal, such as oils, fats, candles, phospholipids, sterols, and other compounds. They are also found in compounds associated with proteins and carbohydrates in the formation of cell protoplasm. Oils and fats are the largest and most prevalent group of lipids, where the bulk of the food stored in the body tissues of humans and animals and seeds and fruits of plants [19].

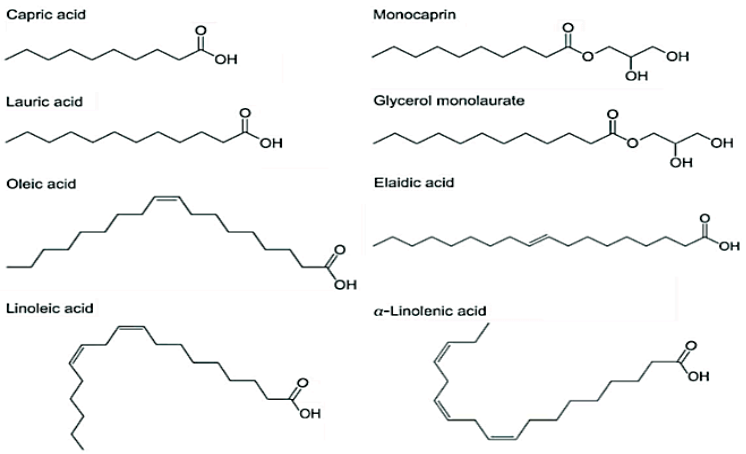

Fig. 10. The structures of various fatty acids.

They are esters of fatty acids (Fig. 10) with glycerol. The difference between them is that: Naturally: oils are liquid at room temperature while fat is solid at room temperature. Chemically, oils contain more unsaturated fatty acids than saturated fatty acids, while fats contain more saturated fatty acids than unsaturated fatty acids [20].

3.1. Melting test:

Add a small portion of the oil sample in front of you with a little water in a test tube and shake well and then heat up softly - note that the oil does not mix with water. It is also noted that the oil is insoluble in dilute acids and alkalis such as dilute HCl acid, dilute NaOH, but dissolved in organic solvents such as ether, chloroform, carbon tetrachloride, benzene and acetone [21].

3.2. Spot test:

Place a drop of oil on a filter paper. Note that the position of this point becomes permanently translucent [22].

3.3. Acrolein test:

Succeed with glycerol and therefore it succeeds with glycerides (oils and fats) and this evidence that oils or fats contain glycerol (esters fatty acids with glycerol), and produce acrolein as a result of the removal of two molecules of water from each molecule of glycerol, and does not succeed with individual fatty acids. Put in a test tube 3.2 grams of sodium or potassium sulfur ($KHSO_3$ or $NaHS_3O$) and add 8 drops of oil ... heat the tube heated and then strongly note the rise of suffocating vapors if offered a filter paper moistened with a transparent solution, they red, these vapors of acroline [23].

3.4. Emulsion composition test:

Shake a bit of oil with water. Shake well in a test tube. Note that the oil disperses into small particles in the water and is an emulsion that only lasts a little time once the shaking is finished. Put in a test tube about 5 milliliters of water and then add 3.2 milliliters of 0.5% sodium carbonate solution and shake well and add to this solution a few drops of oil and continue to shake. Note that the emulsion stays the same for longer than in the case of water without Any oil separation [24].

3.5. Saponification test:

Soap is defined as sodium or potassium salt of fatty acid and is produced from basal analysis of oil or fat. Place in a jar of about 5 grams of oil or fat and add 50 ml of a report of an alcoholic KOH solution (dissolve potassium hydroxide in the least possible amount of water and then complete the volume with ethyl alcohol) Heat on a water bath until saponification and can be sure that the solution becomes Completely. When shaking a point of it with warm water is not separated into drops of oil or fat. The soap formed is a potassium salt of fatty acids in oil or fat and divided the resulting soap into the following sections: Add to the first section sulfuric acid and note the solubility of fatty acids on the surface.

Add to the second section of the soap sodium chloride solution and shake the tube thoroughly. Note that the soap does not melt and detaches on the surface. This process is called salting out and is used to separate the soap as it floats on the surface of the solution in oil and soap factories. Add to the third section a small amount of calcium chloride solution or magnesium sulfate. The insoluble precipitate is composed of calcium or magnesium soap for fatty acids [25].

3.6. Iodine test:

It is used to distinguish between saturated and unsaturated fatty acids. Dissolve 7 drops of oleic acid in about 5 mL of chloroform and then add drops of iodine solution (iodine/alcohol). Shake well. Note the disappearance of iodine color due to absorption of unsaturated oleic acid with added iodine. Do the same test on linoleic acid ... Note that you get the same result because both are also unsaturated acid. Repeat this test on stearic and palmitic acid and note that iodine does not disappear because both are saturated [26].

3.7. Copper acetate test:

It is used to distinguish between neutral fats (esters) and saturated and unsaturated fatty acids. Dissolve the fatty substance in an organic solvent (ether), add to it an equal volume of copper acetate solution and shake well, then leave the tube on the holder and separate the solution to two layers. In the case of oil (neutral glyceride) two clear layers

are formed colorless upper layer (ether layer) and the bottom layer (copper acetate) blue color. In the case of saturated fatty acid (such as stearic or palmitic acid) two clear layers form the upper layer (ether layer) colorless and the bottom layer (copper acetate) blue color with the appearance of a deposit between the two layers. In the case of unsaturated fatty acid (eg oleic acid) the top layer (ether layer) is colored green and the bottom layer (copper acetate) is blue (**Fig. 11**)

The reason for this is that copper does not react (not copper soap) with neutral glycerides, ie oil and fat, while copper reacts with individual fatty acids to form copper soap, and copper soap for unsaturated fatty acids (such as copper primers) is soluble in ether. The ether layer is green while the copper soap for saturated fatty acids (with copper palmitate) is insoluble in ether and therefore remains deposited between the two layers [26]..

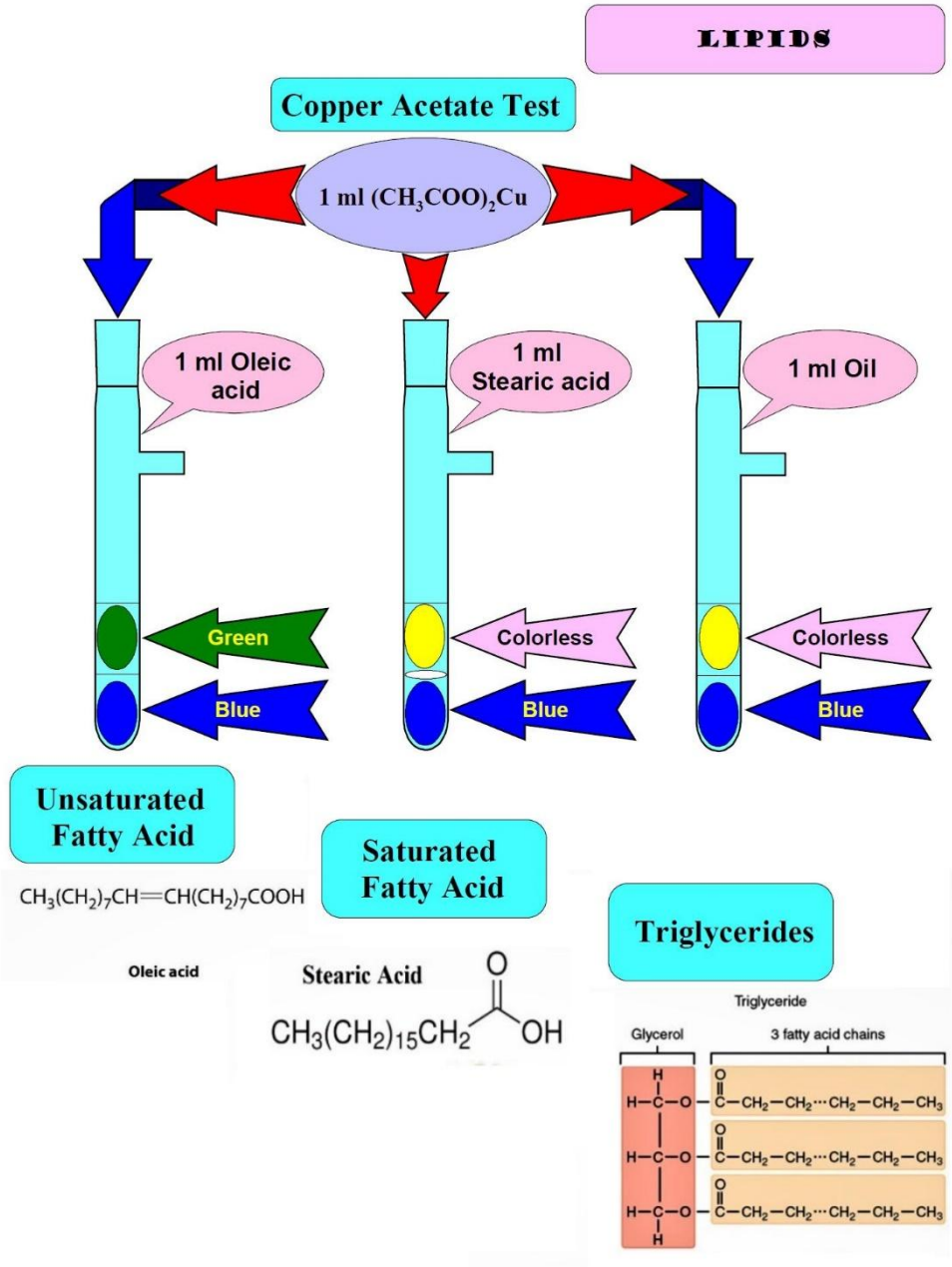

Fig. 11. Lipids detection tests

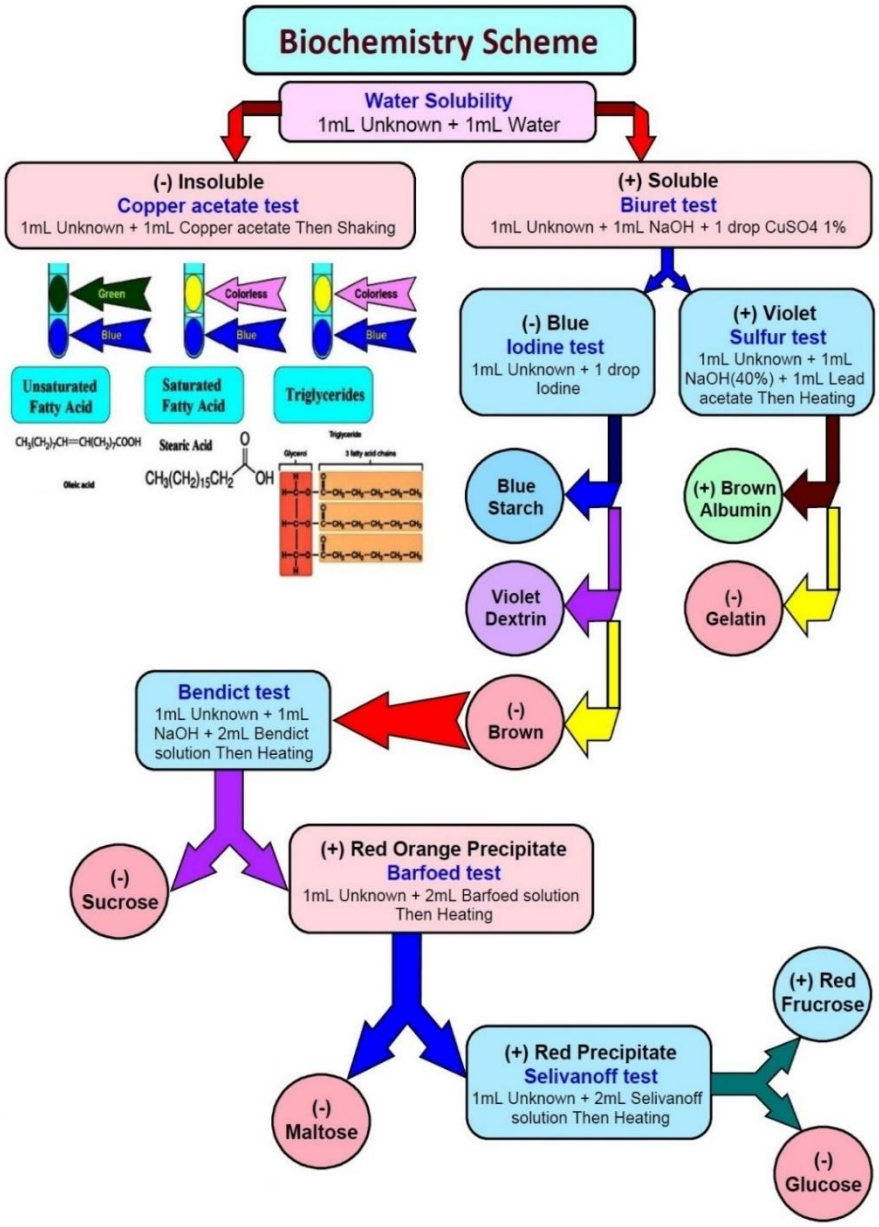

Fig. 12. Detection of unknown biochemical compound

CONCLUSION

The purpose of this research is to serve as a resource to enhance student learning of theories, techniques, and methodologies practiced in the biochemistry teaching and research lab. The extensive availability of laboratory experiments published in journals and the desire of instructors to design their projects and teaching styles have lessened the need for laboratory manuals. Laboratory instructors are especially eager to introduce new student-centered education methods such as Problem Based Learning (PBL), Research-Based Learning (RBL), Process Oriented Guided Inquiry Learning (POGIL), and other "active learning" styles into their labs. However, because published experiments and laboratory manuals usually contain only procedures, there is an increased need for a companion text like this one to explain the theories and principles that under pin laboratory activities.

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