Laboratory Manual of Biochemistry

for 2nd year

Biotechnology

1st course

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Carbohydrate

Introduction:

The word carbohydrate is formed from the words carbon and hydrogen. Carbohydrates are combinations of the chemical elements carbon and hydrogen plus oxygen. In the natural world, carbohydrates are the most common chemical compounds used for food.

Carbohydrates are the most abundant and diverse class of organic compounds occurring in nature. It played a key role in the establishment and evolution of life on earth by creating a direct link between the sun and chemical energy.

Carbohydrates are the key source of energy used by living things.

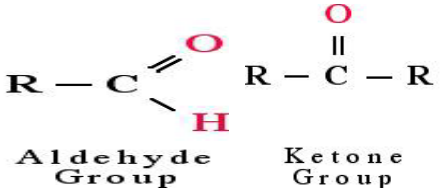
•Also serve as extracellular structural elements as in cell wall of bacteria and plant.

Carbohydrates are defined as the polyhydroxy aldehydes or polyhydroxy ketones.

•Most , but not all carbohydrate have a formula (CH2O)n (hence the name hydrate of carbon)

•In human body, the D-glucose is used.

•Simple sugars ends with –ose.



Several classifications of carbohydrates have proven useful, and are outlined in the following table.

Complexity	**Simple Carbohydrates** monosaccharides			**Complex Carbohydrates** disaccharides, oligosaccharides & polysaccharides	
Size	**Tetrose** C_4 sugars	**Pentose** C_5 sugars	**Hexose** C_6 sugars	**Heptose** C_7 sugars	etc.
C=O Function	**Aldose** sugars having an aldehyde function or an acetal equivalent. **Ketose** sugars having a ketone function or an acetal equivalent.				
Reactivity	**Reducing** sugars oxidized by Tollens' reagent (or Benedict's or Fehling's reagents). **Non-reducing** sugars not oxidized by Tollens' or other reagents.				

Classification :

1-Simple sugar (one unit) :

Monosaccharides contain one monosaccharides unit

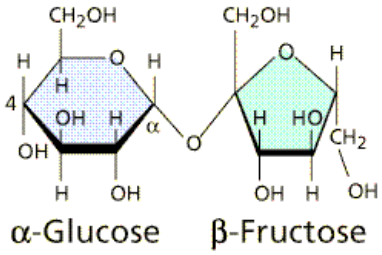
2-Complex sugar (more than one) :

•Disaccharides contain two monosaccharide units.

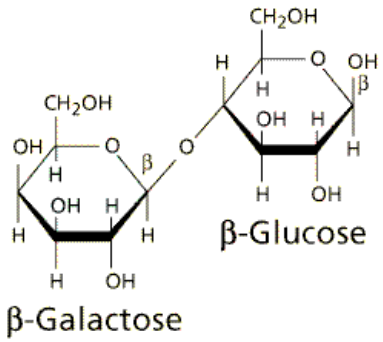
•Oligosaccharides contain 3-9 monosaccharide units.

•Polysaccharides can contain more than 9 monosaccharide units.

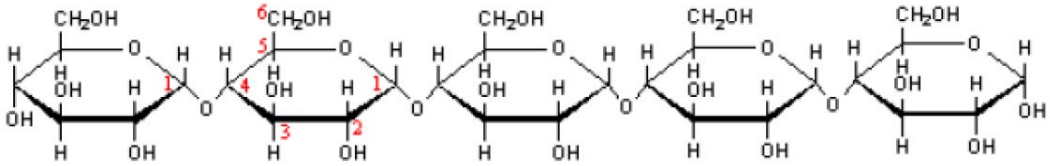
-Complex carbohydrates can be broken down into smaller sugar units through a process known as hydrolysis



Sucrose



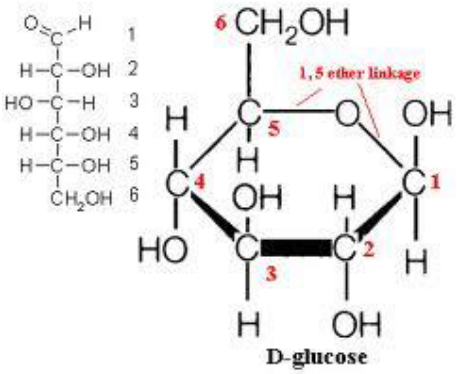
β-Lactose

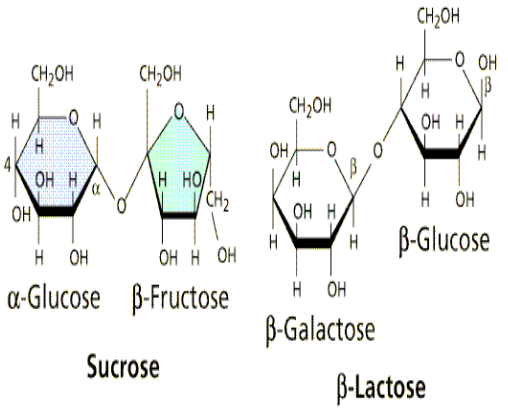


Amylose

Reducing and non reducing sugars

non reducing sugar :If the oxygen on the anomeric carbon of a sugar is not attached to any other structure, that sugar can act as a reducing agent and is termed a reducing sugar





Solubility of sugars [physical property] :

Monosaccharide and disaccharide can be dissolved freely in water because water is a polar substance, while polysaccharide cannot be dissolved easily in water, because, it has high molecular weight , which give colloidal solutions in water

Chemical Properties of Carbohydrates:

The following are the tests to identify the presence of carbohydrates

1- Molisch Test: specific for carbohydrates.
2- Benedict's Test: presence of reducing sugars.
3- Barfoed'sTest: used for detecting the presence of monosaccharides.
4- Bial'sTest: used to detect pentose [5C] monosacharides.
5- Seliwanoff'sTest: distinguish between aldoses
6- Osazone Test: To distinguish different reducing sugars between each other

1. Molisch test:

Molisch's test is a chemical test which is used to check for the presence of carbohydrates in a given analyte. This test is named after Czech-Austrian botanist Hans Molisch, who is credited with its discovery. Molisch's test involves the addition of Molisch's reagent (a solution of ∝-naphthol in ethanol) to the analyte and the subsequent addition of a few drops of concentrated H_2SO_4 (sulphuric acid) to the mixture. The formation of a purple or a purplish-red ring at the point of contact between the H_2SO_4 and the analyte + Molisch's reagent mixture confirms the presence of carbohydrates in the analyte.

This test is specific for all carbohydrates Monosaccharide gives a rapid positive test, Disaccharides and polysaccharides react slower.

Objective: To identify the carbohydrate from other macromolecules, lipids and proteins.

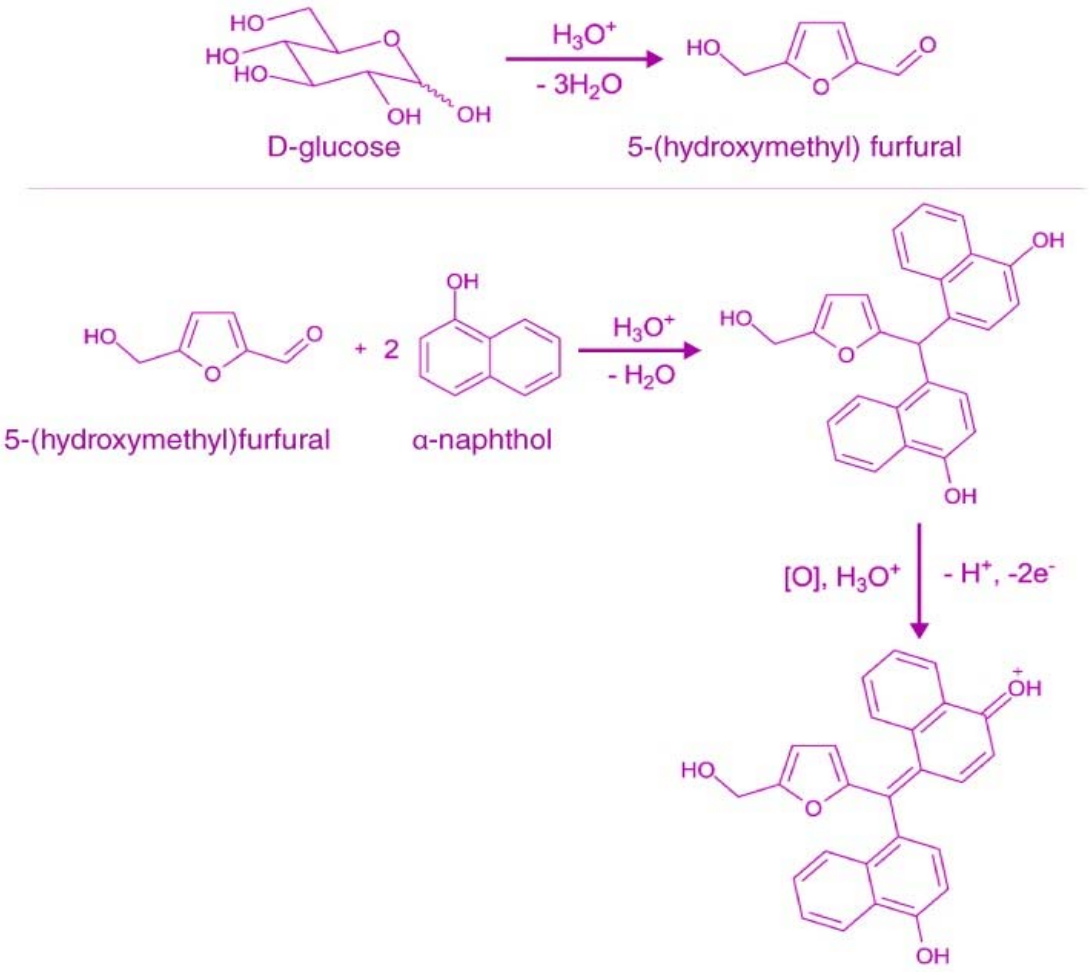
Principle:

In Molisch's test, the carbohydrate (if present) undergoes dehydration upon the introduction of concentrated hydrochloric or sulphuric acid, resulting in the formation of an aldehyde. This aldehyde undergoes condensation along with two phenol-type molecules (such as ∝-naphthol, resorcinol, and thymol), resulting in the formation of a purple or reddish-purple coloured complex.

•The test reagent(H_2SO_4) dehydrates pentose to form furfural and dehydrates hexoses to form 5- hydroxymethyl furfural.

•The furfural and 5- hydroxymethyl furfural further react with α-naphthol present in the test reagent to produce a purple ring.

The formation of a purple ring is a positive indicator for Molisch's Test



Method;

1. 2-3 drops of Molisch's reagent(which α-napthol in 95% ethanol) must be added to a small amount of the analyte in a test tube and mixed well.
2. few drops of concentrated sulphuric acid must be added drop-wise along the walls of the test tube to facilitate the formation of a layer and avoid mixing. The development of a purple ring at the layer formed by the concentrated acid is a positive indicator for Molisch's test. If no purple or reddish-purple colour arises, the given analyte does not contain any carbohydrate.

2. Benedict's test:

Objective: Benedict's test is a chemical test that can be used to check for the presence of reducing sugars in a given analyte.

Therefore, simple carbohydrates containing a free ketone or aldehyde functional group can be identified with this test.

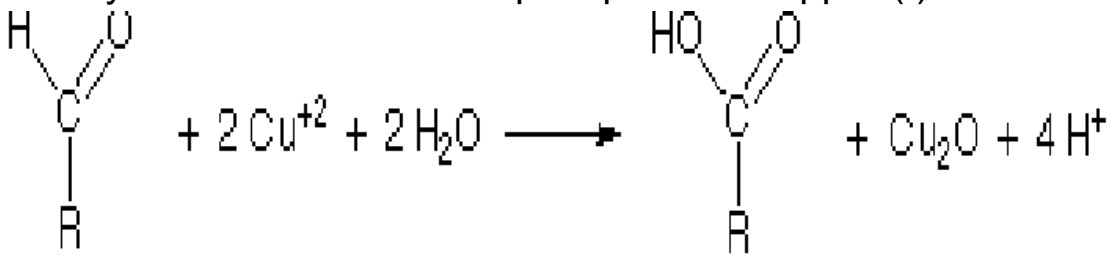
•All monosaccharides are reducing sugars; they all have a free reactive carbonyl group.

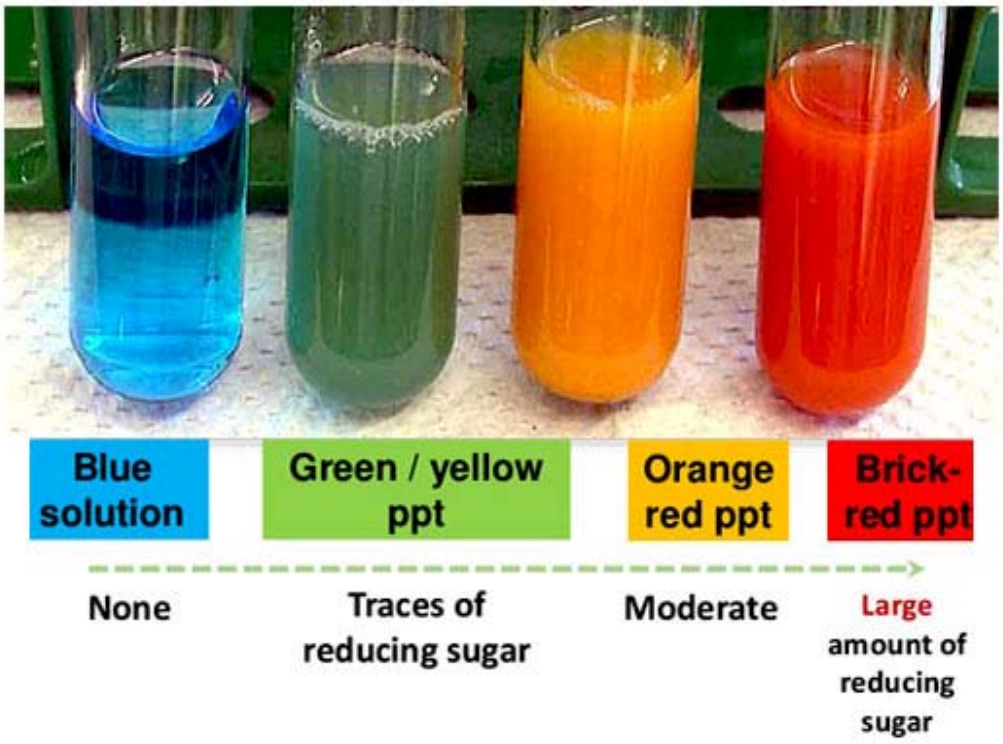
•Some disaccharides have exposed carbonyl groups and are also reducing sugars. Other disaccharides such as sucrose are non-reducing sugars and will not react with Benedict's solution -Large polymers of glucose, such as starch, are not reducing sugars,

Principle:

The copper sulfate (CuSO4) present in Benedict's solution reacts with electrons from the aldehyde or ketone group of the reducing sugar in alkaline medium.

Reducing sugars are oxidized by the copper ion in solution to form a carboxylic acid and a reddish precipitate of copper (I) oxide

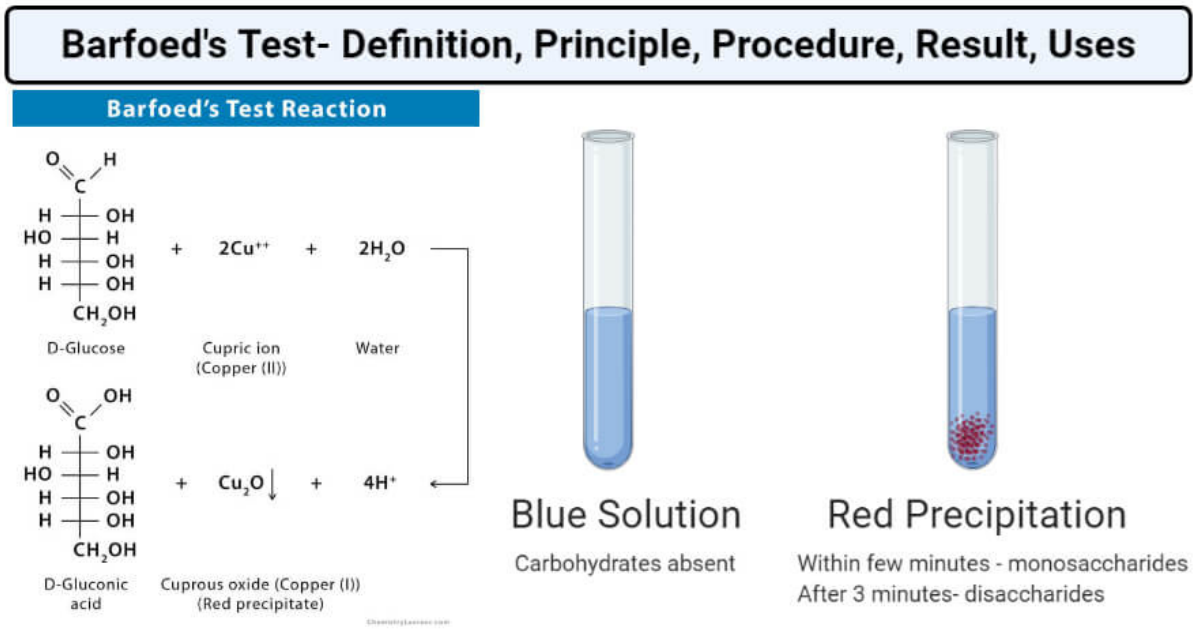




Method;

One millilitre of the analyte sample must be mixed with 2 millilitres of Benedict's reagent and heated in a bath of boiling water for 3 to 5 minutes. The development of a brick-red coloured precipitate of cuprous oxide confirms the presence of reducing sugars in the analyte.

3.Barfoed's Test:



Objective :

- To detect reducing carbohydrates.
- To distinguish reducing monosaccharides from disaccharides.

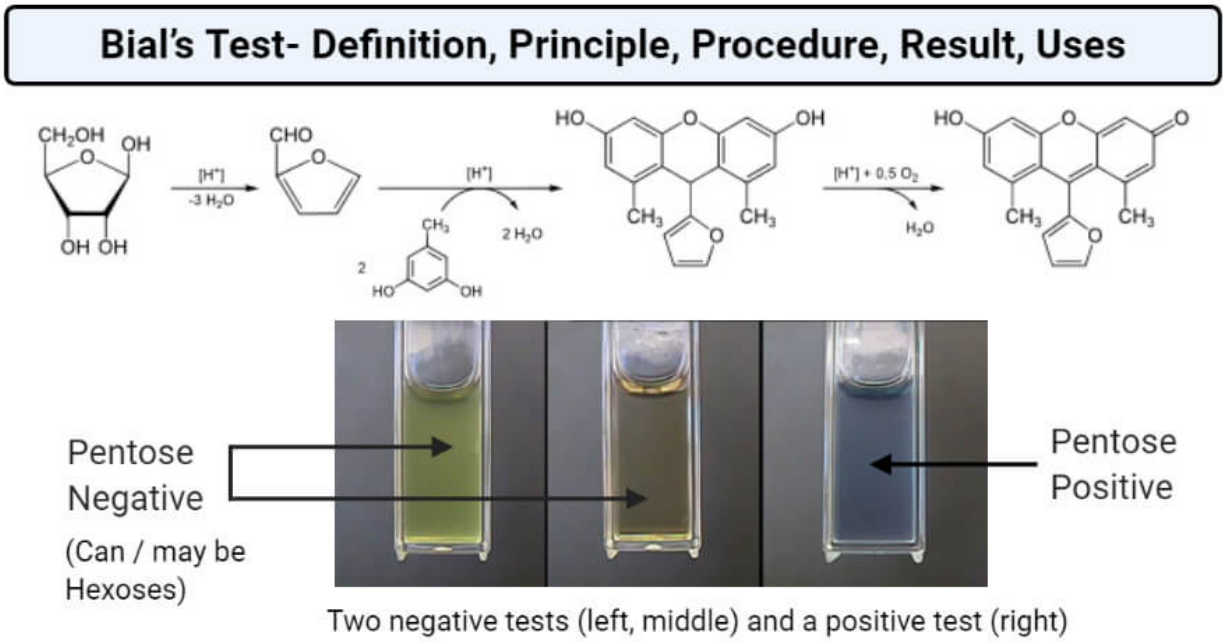
Principle:

The Barfoed reagent is made up of copper acetate in a dilute solution of acetic acid. Since acidic pH is unfavorable for reduction, monosaccharides, which are strong reducing agents, react in about 1-2 min. However, the reducing disaccharides take a longer time of about 7-8 minutes, having first to get hydrolyzed in the acidic solution and then react with the reagent. Once the reaction takes place, thin red precipitate forms at the bottom of the sides of the tube. The difference in the time of appearance of precipitate thus helps distinguish reducing monosaccharides from reducing disaccharide. Extreme Wildfires, Storms Are Here

Method;

1. Take 1 ml of a given sample in a clean, dry test tube.
2. Take control of 1 ml of distilled water in another tube.
3. Add about 2-3 drops of Barfoed's reagent to both the tubes and mix .
4. Keep the test tubes in the water bath for 1-2 minutes. The boiling should not be done for more than 2 minutes as the disaccharides might hydrolyze into monosaccharides and give a positive result.
5. Observe the appearance of color in the test tubes.

4.Bial's Test:



Two negative tests (left, middle) and a positive test (right)

Objectives

To distinguish between the pentoses monosaccharides and monosaccharides hexose

Principle:

Bial's test uses concentrated HCl as a dehydrating acid and orcinol + traces of ferric chloride [FeCl3] as condensation reagent. The test reagent dehydrates pentoses to form furfural. Furfural further reacts with orcinol and the iron ion present in the test reagent to produce a bluish or green product, while hexoses yield muddy-brown to grey condensation product.

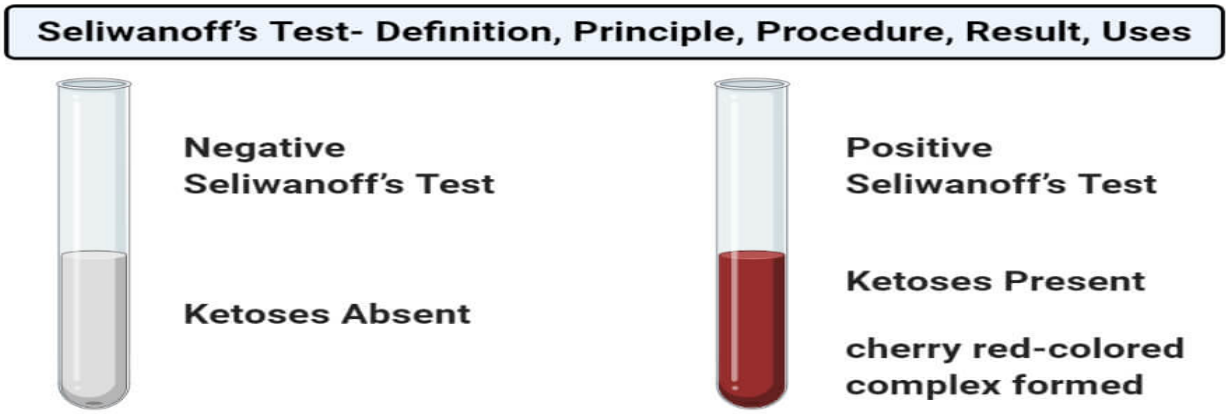
Method

1. •Put 2 ml of a sample solution in a test tube.
2. •Add 2-3 droups of Bial's reagent (a solution of orcinol, HCl and ferric chloride) to each tube.
3. •Heat the tubes gently in biolling water bath.
4. •If the color is not obvious, more water can be added to the tube.

5. Seliwanoff's test

Objective:

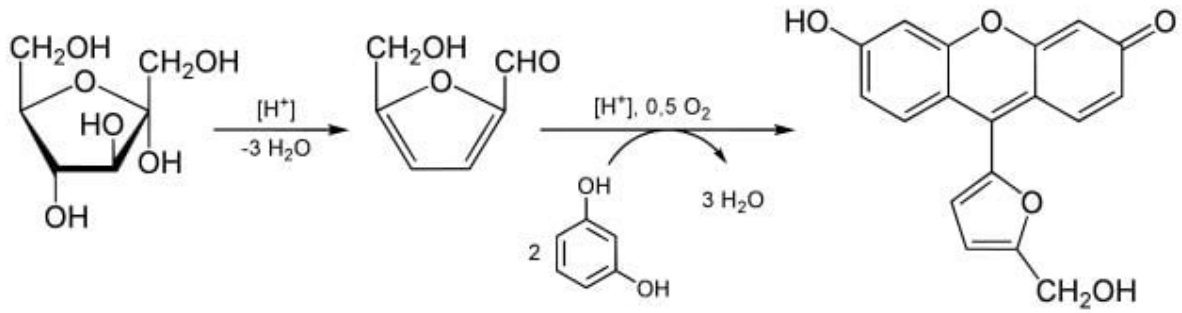
used to distinguish between aldoses (like glucose) and ketoses (like fructose).



Principle:

- The reagent of this test consists of resorcinol and concentrated HCl.
- The acid hydrolysis of polysaccharides and oligosaccharides yields simpler sugars.
- Ketoses are more rapidly dehydrated than aldoses.
- Ketoses undergo dehydration in the presence of concentrated acid to yield 5-hydroxymethyl furfural.
- The dehydrated ketose reacts with two equivalents of resorcinol in a series of condensation reactions to produce a complex (not a precipitate), .

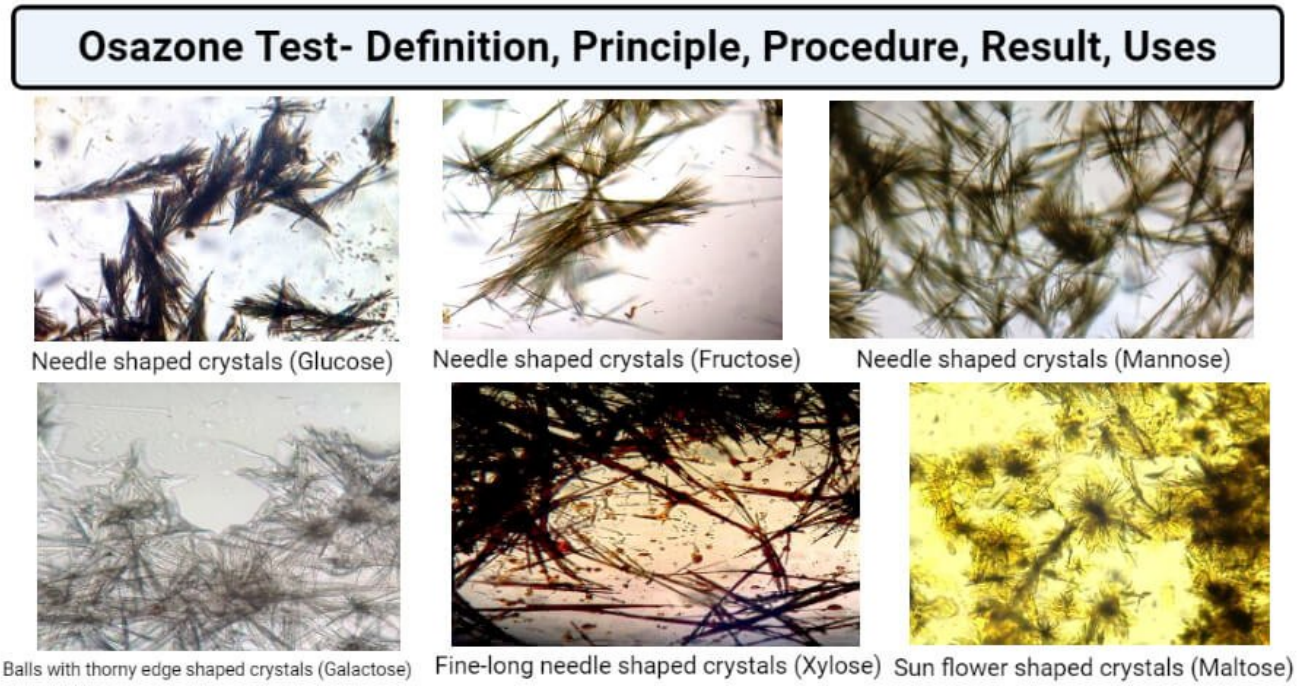
  .



Method

- Take two clean, dry test tubes and add 1 ml of the test sample in one test tube and 1 ml of distilled water in another as blank.
- Add 2-3drops of Seliwanoffs' reagent(6M HCl as dehydrating agent and resoncinol) as condensation reagent to both the test tubes.
- Keep both the test tubes in a water bath for 1 min.
- Observe the formation of color and note it down.

6. Osazone test.

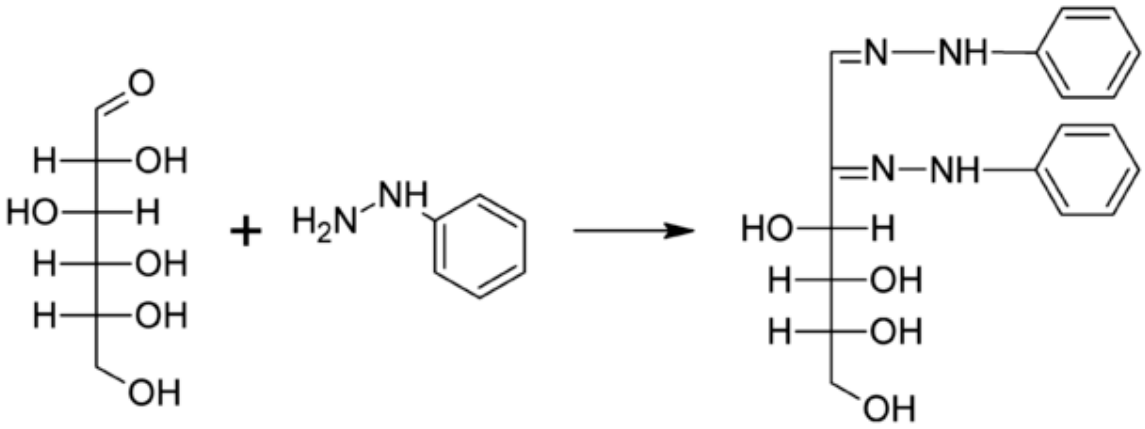


Objectives;

- To detect reducing sugars.
- To differentiate reducing sugars from non-reducing sugars.
- To distinguish different reducing sugars between each other.

Principle

- The reagent for this test consists of phenylhydrazine in acetate buffer.
- This test is based on the fact that carbohydrates with free or potentially free carbonyl groups react with phenylhydrazine to form osazone.
- The condensation-oxidation-condensation reaction between three molecules of phenylhydrazine and carbon one and two of aldoses and ketoses yields 1, 2-diphenyhydrazone, which is known as osazone.



Procedure

1. Take 5 ml of test solution in a clean, dry test tube.
2. Add 0.3 g of osazone mixture and five drops of glacial acetic acid to the test tube.
3. Mix it well and warm the test tube gently in the water bath if required to dissolve all the elements.
4. Keep the test tube in boiling water and observe the formation of crystals at various time points.
5. Observe the shape of the crystal under low magnification under a microscope.

7. Iodine Test:

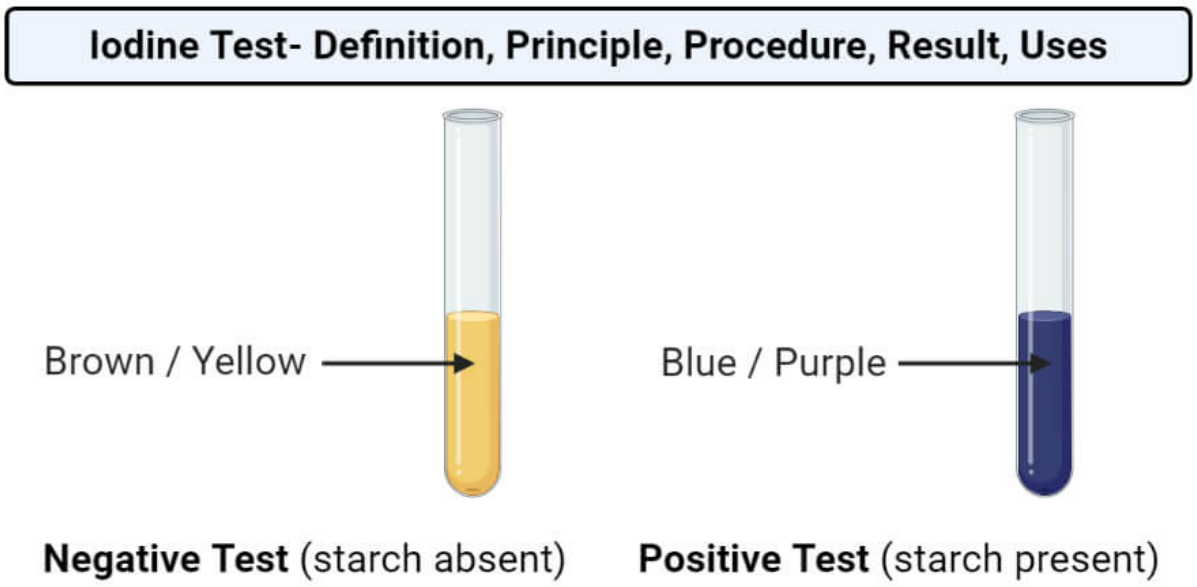
Objectives

To detect the presence of polysaccharides, primarily starch.

Principle

- Iodine test is based on the fact that polyiodide ions form colored adsorption complex with helical chains of glucose residue of amylase (blue-black), dextrin (black), or glycogen (reddish-brown).
- Monosaccharides, disaccharides, and branched polysaccharides like cellulose remain colorless. Amylopectin produces an orange-yellow hue.
- The reagent used in the iodine test is Lugol's iodine, which is an aqueous solution of elemental iodine and potassium iodide.
- Iodine on its own is insoluble in water. Addition of potassium iodine results in a reversible reaction of the iodine ion with iodine to form a triiodide ion, which further reacts with an iodine molecule to form a pentaiodide ion.
- Bench iodine solution appears brown, whereas, the iodide, triiodide, and pentaiodide ion are colorless.
- It is observed that the helix (coil or spring) structure of the glucose chain is the key to this test.
- Further, the resulting color depends on the length of the glucose chains.
- The triiodide and pentaiodide ions formed are linear and slip inside the helix structure.
- It is believed that the transfer of charge between the helix and the polyiodide ions results in changes in the spacing of the energy levels, which can absorb visible light, giving the complex its color.
- The intensity of the color decreases with the increase in temperature and the presence of water-miscible organic compounds like ethanol.
- On heating, the blue color amylase-iodine complex dissociates but is formed again on cooling because the helical structure is disrupted; thereby amylose loses its iodine binding capacity and the blue color.

- The blue color reappears on cooling due to the recovery of iodine binding capacity due to regaining of the helical structure.



1. Take the sample solution to be tested in a clean test tube.
2. Add 2-3 drops of iodine solution.
3. Observe the change in colour.

If there is the appearance of a blue colour then the presence of starch is confirmed. Procedure of Iodine Test

1. Take 1 ml of a given sample in a clean, dry test tube.
2. Take control of 1 ml of distilled water in another tube.
3. Add about 2-3 drops of Lugol's solution to both the tubes and mix them in a vortex.
4. Observe the appearance of color in the test tubes.
5. Heat the test tubes in the water bath until the color disappears.
6. Take the test tubes out for cooling
7. Note down the appearance of color seen in the test tubes.

Precautions:

1. Handle the acids like concentrated sulfuric acid with care.
2. Always use droppers to take reagents from the reagent bottles.
3. While heating the reaction mixture do it carefully.

Starch:

Starch is the most important source of carbohydrates in the human diet and accounts for more than 50% of our carbohydrate intake. It occurs in plants in the form of granules, and these are particularly abundant in seeds (especially the cereal grains) and tubers, where they serve as a storage form of carbohydrates. The breakdown of starch to glucose nourishes the plant during periods of reduced photosynthetic activity. We often think of potatoes as a “starchy” food, yet other plants contain a much greater percentage of starch (potatoes 15%, wheat 55%, corn 65%, and rice 75%). Commercial starch is a white powder.

Starch is a mixture of two polymers: amylose and amylopectin. Natural starches consist of about 10%–30% amylase and 70%–90% amylopectin. Amylose is a linear polysaccharide composed entirely of D-glucose units joined by the α-1,4-glycosidic linkages we saw in maltose (part (a) of Figure 5.1.1). Experimental evidence indicates that amylose is not a straight chain of glucose units but instead is coiled like a spring, with six glucose monomers per turn (part (b) of Figure 5.1.1). When coiled in this fashion, amylose has just enough room in its core to accommodate an iodine molecule. The characteristic blue-violet color that appears when starch is treated with iodine is due to the formation of the amylose-iodine complex. This color test is sensitive enough to detect even minute amounts of starch in solution.

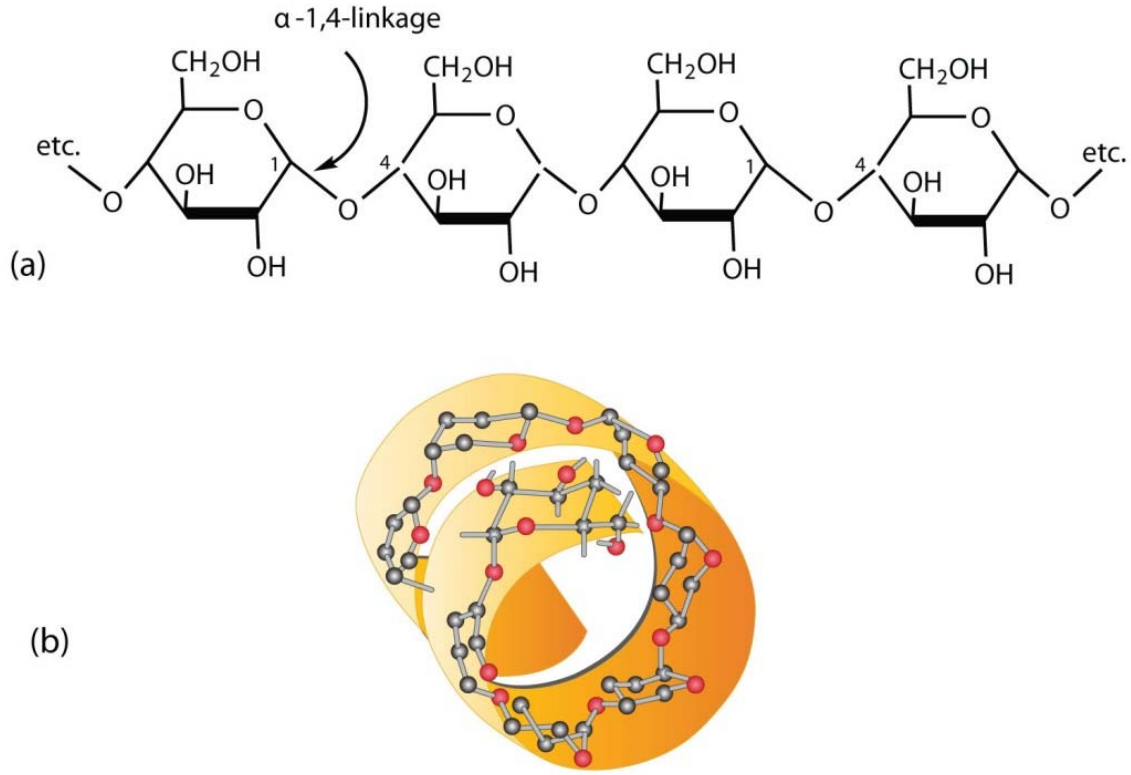


Figure 5.1.1: Amylose. (a) Amylose is a linear chain of α-D-glucose units joined together by α-1,4-glycosidic bonds. (b) Because of hydrogen bonding, amylose acquires a spiral structure that contains six glucose units per turn.

Hydrolysis of Starch

Polysaccharides, such as amylase or amylopectin, are polymers of glucose molecules. Starch can form an intense, brilliant, dark blue or violet colored complex with iodine. The straight chain component of starch (or amylose) gives a blue color, while the branched component (or amylopectin) yields a purple color. In the presence of iodine, amylose forms helixes, where the iodine molecules assemble as long polyiodide chains. The helix– forming branches of amylopectin are much shorter than those of amylose. Therefore, the polyiodide chains are also much shorter in the amylopectin–iodine complex than in the amylose–iodine complex. The result is a different color (purple). When starch is hydrolyzed and broken down to small carbohydrate units, the iodine will not give

a dark blue (or purple) color. The iodine test is used in this experiment to indicate the completion of the hydrolysis.

Amylase is an enzyme found in the saliva as well as the intestines of humans and animals. It begins the breakdown of starch molecules in the mouth as food is chewed and finishes its job in the intestines. Since it is a protein, it will coagulate and be rendered useless if it is treated with high heat.

Purpose:

In this lab two different methods will be applied to break down starch molecules into smaller units containing of 2 to 3 glucose molecules. In the first part of the experiment acid and heat will be used to hydrolyze the starch chains. In the second part, and enzyme will be used to break apart the starch chains.

Materials:

starch solution, 10% iodine solution, 2M hydrochloric acid, 1M sodium hydroxide, amylase, benedicts solution , 3 test tubes, test tube rack, hot water bath, pipettes, 24-well plate, small beaker with rinsing water, small waste water beaker

Safety:

Wear goggles and gloves. Be aware that hot plates stay hot for a long time after they are turned off.

Procedure:

Hydrolysis of Starch with Acid:

1- Start the hot water bath.
2- Place a few drops of the starch solution into a well on the spot plate. Add a couple of drops of the 10% iodine solution and record the color.
3- Add 1.0 mL of dilute hydrochloric acid to the test tube and heat the solution.
4- After two minutes of heating perform the iodine test for starch (step 2).
5- Keep heating the solution and test every two minutes for starch until the solution no longer tests positive for starch. Make sure to rinse the pipette with distilled water after each test.

6- When the solution no longer tests positive for starch, add 10 drops of NaOH to the starch solution until the solution is slightly basic. (Place a drop of the solution onto Litmus paper. When the paper turns blue, the solution is basic.)
7- Perform the Benedict's test on the starch solution.

Hydrolysis of Starch with an Enzyme:

1. Add a tiny amount of amylase to a test tube containing 10.0 mL of starch solution and swirl the test tube to mix.
2. Test the solution for the presence of starch.
3. Place the solution into a warm water bath (body temperature).
4. Every two minutes test the solution for presence of starch.

Observations:

Starch solution and iodine:

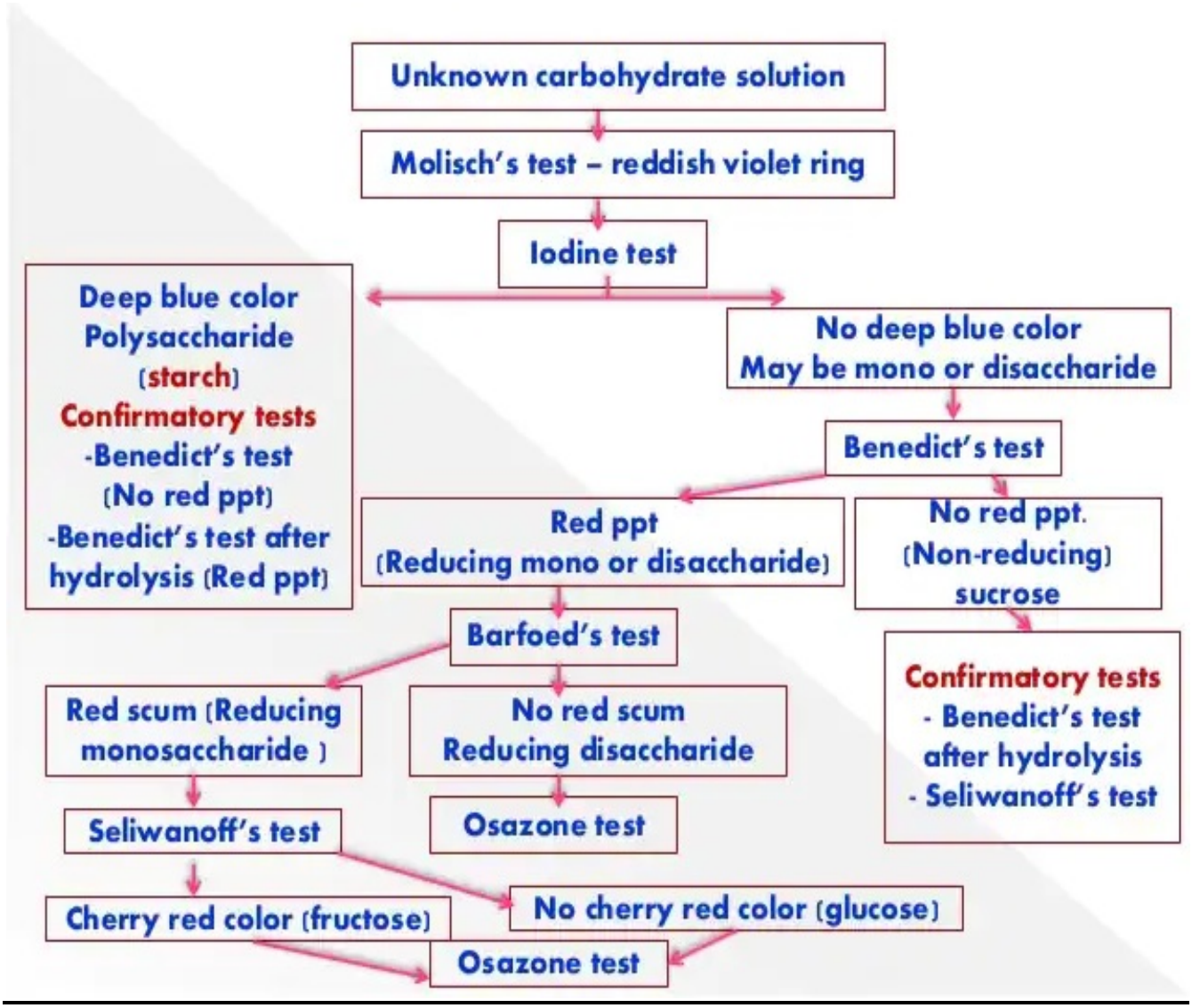
Hydrolysis with acid

Iodine Test

Time	Color
2 min	Dark blue
6 min	blue
9 min	Deep purple
12 min	Muddy brown
15 min	Golden brown

Benedict s Test:

Time	Color
2 min	blue
6 min	blue
9 min	green - yellow
12 min	organe
15 min	Brick red


Unknown carbohydrate solution
Molisch's test – reddish violet ring
Iodine test
Deep blue color
Polysaccharide
(starch)
Confirmatory tests
-Benedict's test
(No red ppt)
-Benedict's test after hydrolysis (Red ppt)
No deep blue color
May be mono or disaccharide
Benedict's test
Red ppt
(Reducing mono or disaccharide)
No red ppt.
(Non-reducing)
sucrose
Barfoed's test
Confirmatory tests
- Benedict's test after hydrolysis
- Seliwanoff's test
Red scum (Reducing monosaccharide)
No red scum
Reducing disaccharide
Seliwanoff's test
Osazone test
No cherry red color (glucose)
Cherry red color (fructose)
Osazone test

Lipids

– Fats
–Oils
–Cholesterols
–Vitamin

Identifying Lipids Using Chemical tests

1. Acrolein Test

Principle

When glycerol is heated with potassium bisulphate or concentrated H2SO4, dehydration occurs and aldehyde Acrolein formed which has characteristic odour. This test responds to glycerol free or linked as an ester

$$\text{Oil or fat} \xrightarrow{\Delta} \text{Glycerol} + \text{Fatty acid}$$

$$\begin{array}{lcl} CH_2OH & & CH_2 \\ | & KHSO_4 & \| \\ CHOH & \xrightarrow{\Delta} & CH + 2H_2O \\ | & & | \\ CH_2OH & & CHO \\ \text{Glycerol} & & \text{Acrolein} \end{array}$$

Materials

1. Test compounds (Oil or fat ,Oleic acid)
2. Potassium bisulphate or conc. H2SO4

Procedure

1. Place 5 drops of test compound in a clean and dry test tube
2. Add 1 ml of conc. H2SO4 carefully. Or 1.0 g of KHSO4
3. Heat the test tube directly.
4. Note the characteristic pungent odour of Acrolein.

2. Test of cupric acetate for detecting fatty acids

This test is used for detecting saturated and unsaturated fatty acids, fatty acids reacts with cupric acetate to form cupric salts of fatty acids. The cupric salts of saturated fatty acids are not dissolved in water or petroleum ether; therefore, it's precipitated at the bottom of

test tube. The cupric salts of unsaturated fatty acids are dissolved in petroleum ether with blue-green color.

Materials

- Saturated fatty acid (stearic acid or palmitic acid)
- Unsaturated fatty acid (Oleic acid)
- 10% aqueous cupric acetate
- Petroleum ether

Procedure

1. Dissolve small mount of fatty acid in petroleum ether.
2. Add appropriate amount of aqueous cupric acetate solution.
3. Shake the test tube carefully and observe green precipitate is appear at lower aqueous layer (cupric acetate layer) where this is happened with saturated fatty acids while at upper organic layer (petroleum ether layer) , blue or green color is appear when unsaturated fatty acid is used.

3 Test for the Degree of Unsaturation of Fatty Acids (Iodine test)

Fatty acids in animal fats are usually saturated, whereas those in vegetable oils are generally unsaturated. Halogens (I, Br) will add across the double bonds and thus the decolorization of an iodine or bromine solution will indicate the presence of fatty acids. Iodine test is used for distinguish between saturated and unsaturated fatty acids as well as between oils and fats.

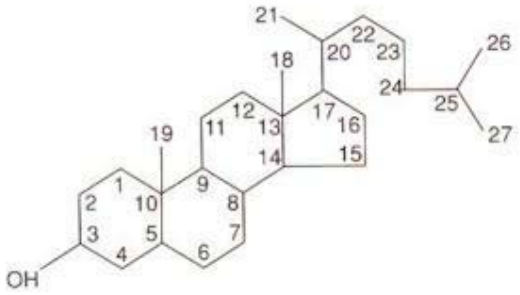
Materials

1. Oil, fat or oleic and stearic acids.
2. Organic solvents (chloroform or ethanol)
3. Hubl's reagent (alcoholic solution of iodine which contains some mercuric chloride)

Procedure

1. Dissolve small amount of unsaturated fatty acid (Oleic acid) in small volume of chloroform.
2. Add Hubl's reagent drop by drop and shaking the tube after each addition. The halogen solution should be added just until it fails to be decolorized.

3. Record the number of drops needed to bring about full decolorization.
4. Repeat the same procedure but by using saturated fatty acid (stearic acid) and Compare your results.
4. Detection of Cholesterol



Precursor and derived lipids Cholesterol is a member of the group of steroid known as sterols which have in common
a hydroxyl group at carbon number 3 and a long saturated eight carbon at carbon number 17. It is the source of steroid hormones.

4. Libermann-Burchard's test

- 2 ml of the provided chloroformic extract of canned food,
- Add 2 mL of acetic anhydride + 5 drops of concentrated sulfuric acid;
- A bluish-green color is formed indicating the presence of cholesterolm

Salkowski test

2 ml of the provided chloroformic extract of canned food,
- Add an equal volume of concentrated sulfuric acid;
- A yellow to brick-red color is formed indicating the presence of cholesterol.

Qualitative tests of lipids

Lipids are a heterogeneous group of compounds, including fats, oils, steroids, waxes, and related compounds, which are related more by their physical than by their chemical properties. Lipids are a

class of compounds distinguished by their insolubility in water and solubility in non polar solvents. Lipids are important in biological systems because they form the cell membrane, a mechanical barrier that divides a cell from the external environment. Non polar lipids, such as triglycerides, are used for energy storage and fuel. Polar lipids, which can form a barrier with an external water environment, are used in membranes. Polar lipids include glycerophospholipids and sphingolipids. Fatty acids are important components of all of these lipids.

Lipids have the common property of being relatively insoluble in water and soluble in nonpolar solvents such as ether and chloroform. They are important dietary constituents not only because of their high energy value but also because of the fat-soluble vitamins and the essential fatty acids contained in the fat of natural foods.

A. Solubility test

1. Principle.

This test is used to know the solubility of lipids in some solvents, according to polarity feature lipids are insoluble in polar solvents because lipids are non polar compounds, thus lipids are soluble in non polar solvents like chloroform, benzene and boiling alcohol.

1. Procedure.

1. In two clean dry test tubes add 1 ml of olive oil for each tube.
2. Add for the first one 1 ml of chloroform.
3. Add for the second tube 1 ml of distilled water.
4. Shake both tubes vigorously for 2 minutes.
5. Allow the tubes to stand and note the formation of homogenous solution with chloroform indicating that the lipid is dissolved and the formation of two layers with water indicating that the lipid is insoluble in water.

B. Saponification test

1. Principle.

This test is used to form soap by saponification process which includes the hydrolysis of fat or oil with alkaline to yield glycerol and salt of fatty acid (soap).

Triglyceride + KOH Glycerol + Soap

2. Procedure.

1. In clean dry large test tubes add 2 ml of olive oil.
2. Add 4 ml of 20% alcoholic potassium hydroxide.
3. Shake well and boil the mixture in boiling water bath for 5 minutes.
4. Make sure after the five minutes that the soap is formed, all of the fat is converted to soap and an alcohol is evaporated. If not continue the boiling.
5. The solid material formed is soap.

C. Iodine test

1. Principle.

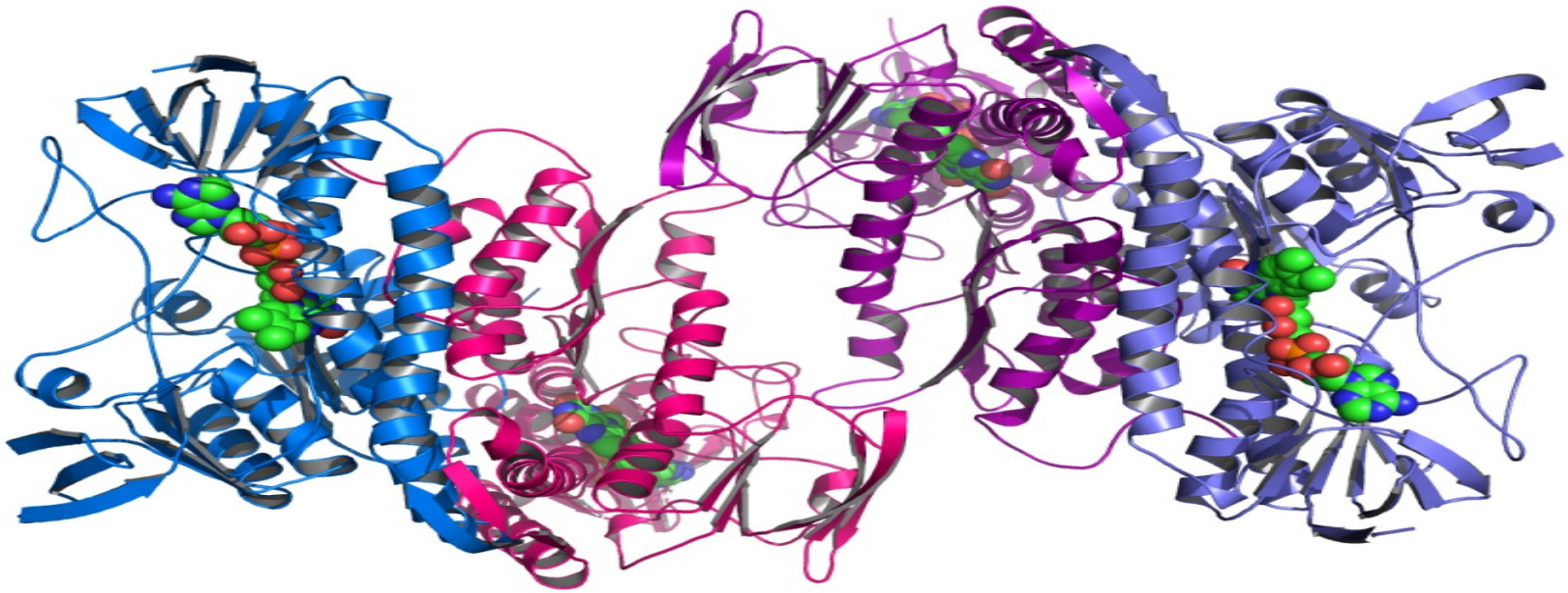
This test is used to test the degree of unsaturation of fatty acids. Fatty acids in animal fats are usually saturated, whereas those in vegetable oils are generally unsaturated. Halogens like iodine or bromine when added to unsaturated fatty acid the double bond will be.

saturated and decolorize the iodine or bromine, the decolorization indicates the presence of unsaturated fatty acids. Iodine test is used for distinguish between saturated and unsaturated fatty acids as well as between oils and fats.

2. Procedure.

1. In clean dry test tube add 1 ml of oleic acid (unsaturated).
2. In other test tube add 1 ml of stearic acid (saturated).
3. Add for each tube 5 drops of hubl's reagent.
4. Observe that the color of halogen in the reagent will be disappeared when it is added to the oleic acid until we reaches a certain point after which the color of the reagent will persist indicating that all of the double bonds are saturated by halogens.
5. Observe that the color of halogen in the reagent does not changed when it is added to stearic acid because this fatty acid is saturated.

Proteins: Precipitation test for protein



Maysoon Khalid Hussein

Protein precipitation

Is widely used in downstream processing of biological products in order to concentrate proteins and purify them from various contaminants.

The solubility of proteins is affected by pH, temperature, salts, heavy metal salts ..etc

Proteins will get denatured while using some factors that lead to precipitation.

Denaturation of Proteins

Denaturation is the disruption of secondary, tertiary and quaternary structure of proteins leading to loss of their biological activity.

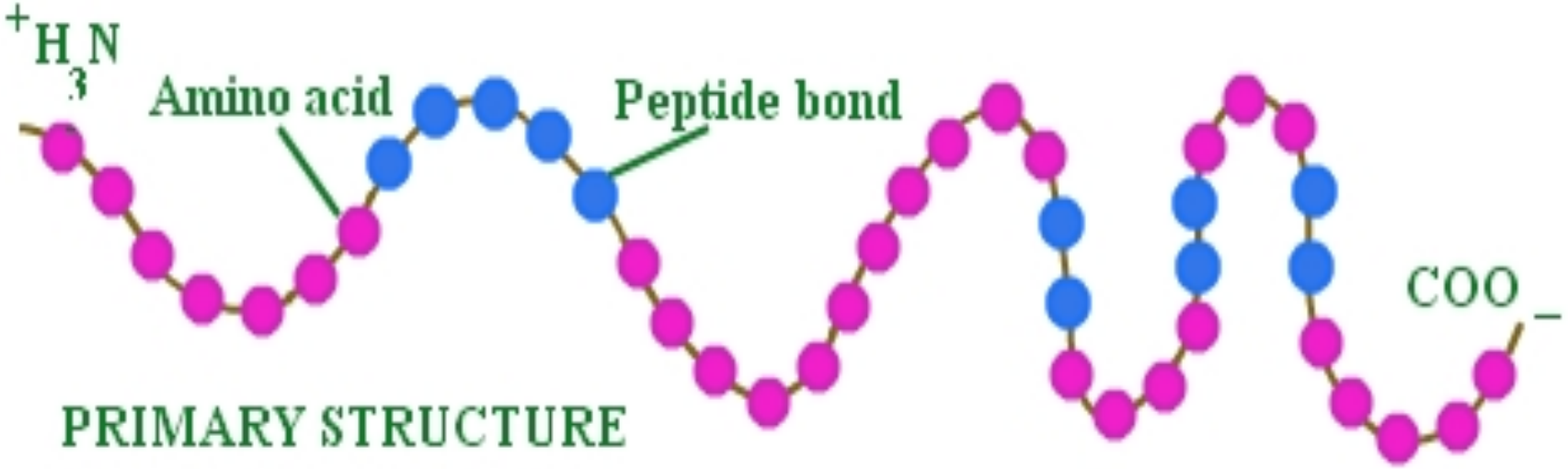
Proteins denature when they lose their three-dimensional structure.

Proteins may be denatured at the secondary, tertiary and quaternary structural levels, but not at the primary structural level.

Levels of Protein Structure:

1- Primary structure:

linear amino acid sequance,strarting from N terminal to C terminal.



Levels of Protein Structure:

2- Secondary structure:

Is the general three-dimensional form of protein fromed by hydrogen bonding of amide groups.

Alpha Helix:

Polypeptide chain is coiled rightly as coil

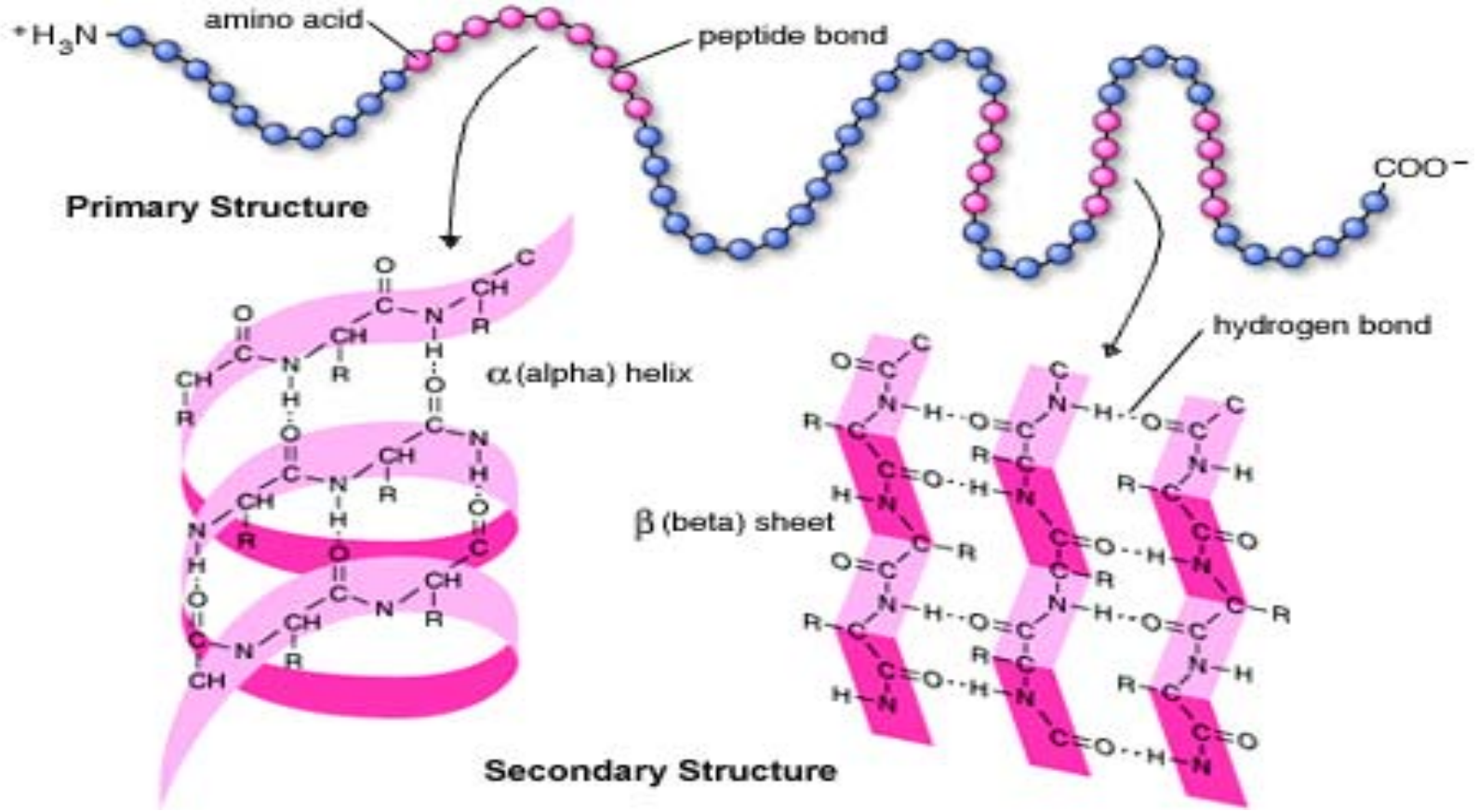
The backbone forms the inner part while the side chains extends outward

Stabilized by hydrogen bonds of one amino acid and the carbonyl gp in the 4^{th} amino acid

Levels of Protein Structure:

Beta Pleated Sheet :

Polypeptide chains are aligned side by side with another chain aligned oppositely



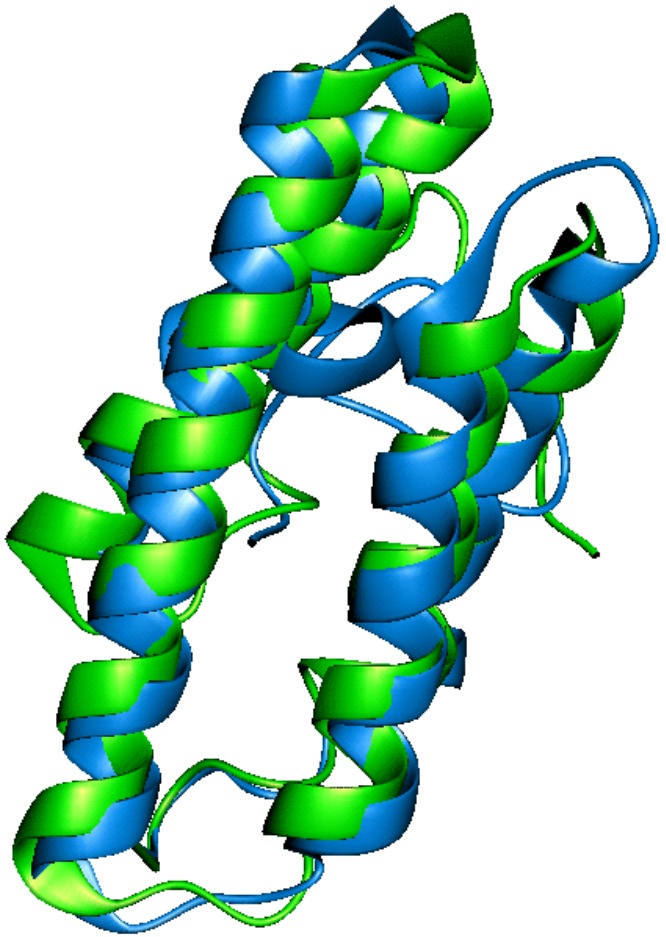
Levels of Protein Structure:

3- Tertiary structure:

The entire three-dimensior shape of the protein. This shape is determined by the sequence of amino acids

Have a single polypeptide chain "backbone" with one more protein secondary structures.

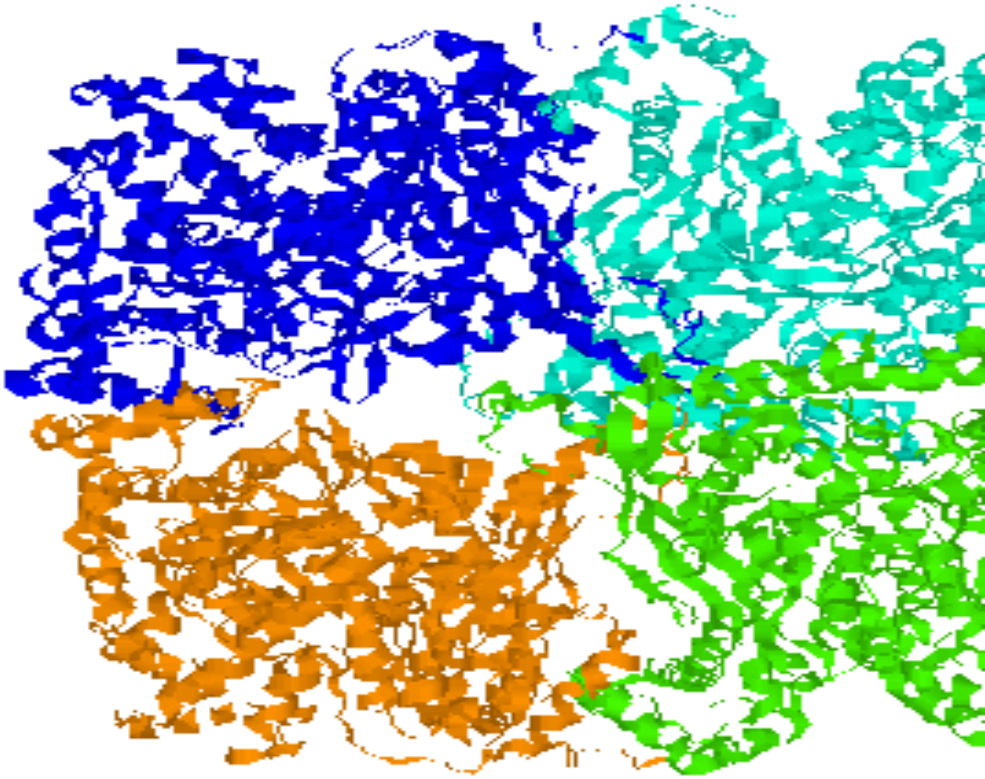
Formed by hydrophobic interactions, but hydrogen bonds, ionic interactions, and disulfide bonds are usually



Levels of Protein Structure:

4- Quaternary structure:

The shape or structure tha
results from the union of m
than one protein molecule,
usually called protein subu
this context, which functior
part of the larger assembly
protein complex



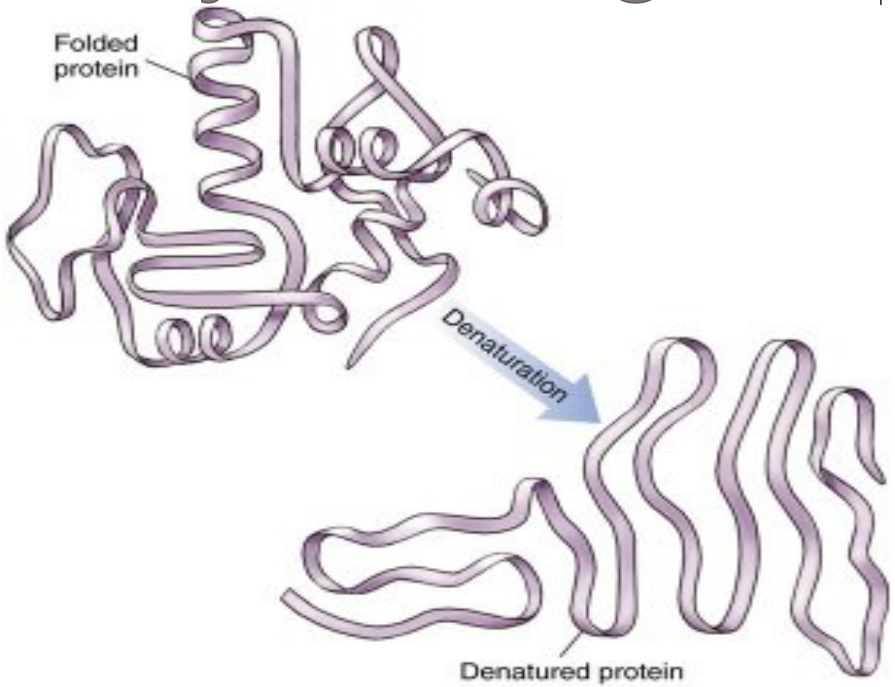
Denaturation of Proteins

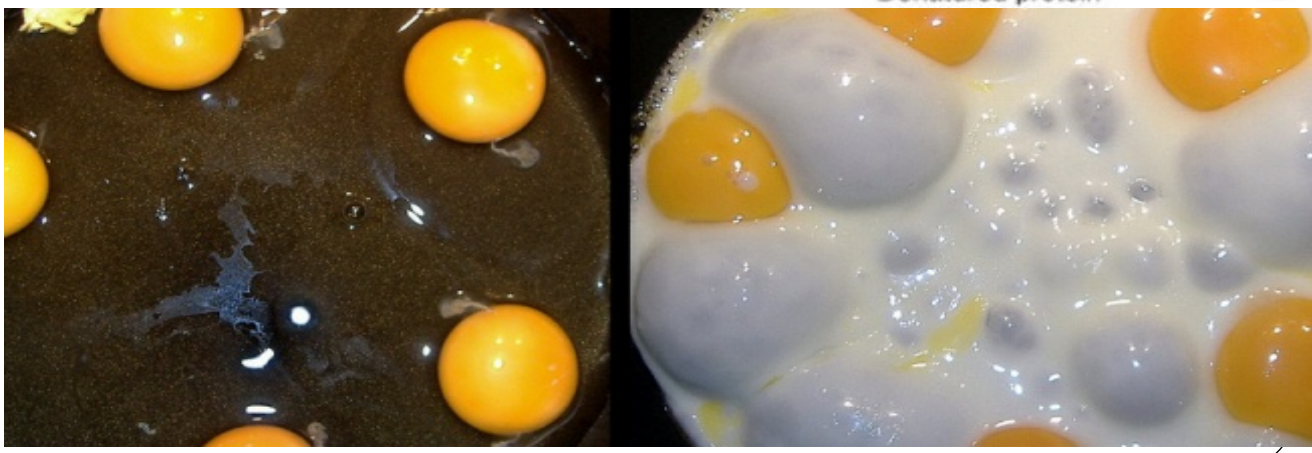
Denaturation is a process in which the proteins losing its quaternary structure, tertiary structure and secondary structure, by application of some external factor or compound such as a strong acid or base, a conc. inorganic salt, an organic solvent (e.g., alcohol or chloroform), or heat.

Denatured proteins can exhibit a wide range of characteristics, from loss of solubility to aggregation.

proteins denaturation by heating

Non-covalent bond can be broken by heating, leading to protein denaturation and the precepitation





Chemical factors of Protein precipitate

- Effect of salt concentration on the protein solubility
- precipitation of proteins by mineral acid
- Precipitation of protein by salts of heavy metals
- Precipitation of protein by organic acid

Effect of salt concentration on the protein solubility :

to investigate the effect of different salt concentration on protein solubility.

When low concentrations of salt is added to a protein solution the solubility increases (This is called salting in)

At some point, solubility begins to decrease as salt increases-"salting out"

It is Reverse process, the protein can again become soluble when we add water

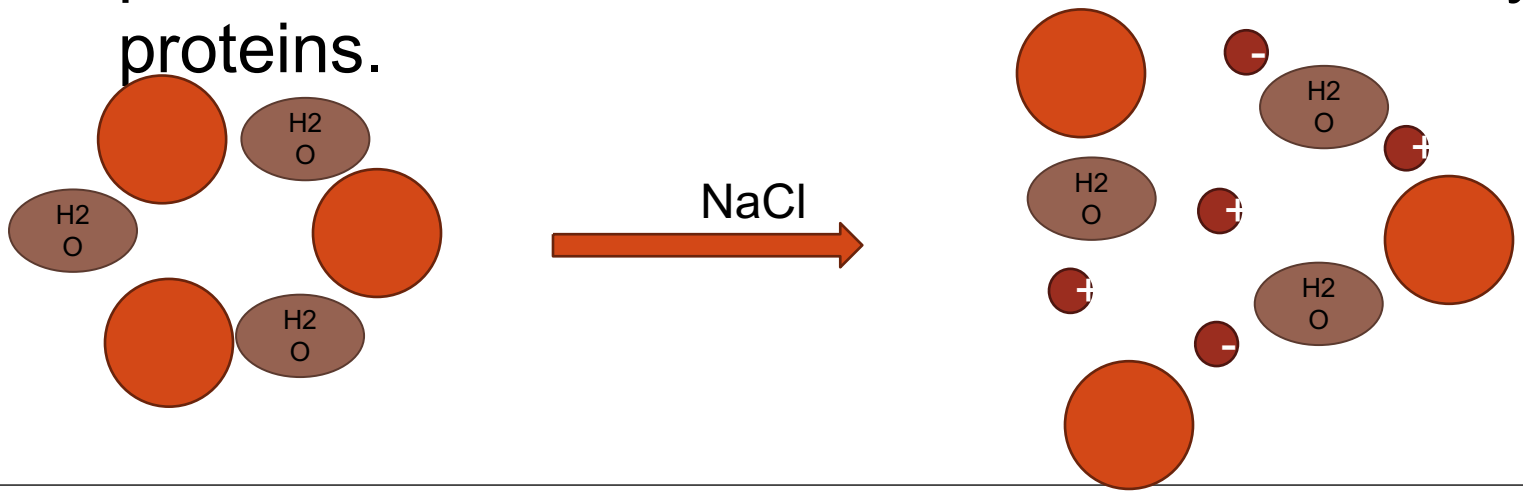
Each protein can be precipitated at specific salt concentration.

Principle: Salting In

Low concentrations of salt → the solubility increases. This could be explained by the following:

Salt molecules stabilize protein molecules by :

Decreasing the electrostatic energy between the protein molecules which increase the solubility of proteins.

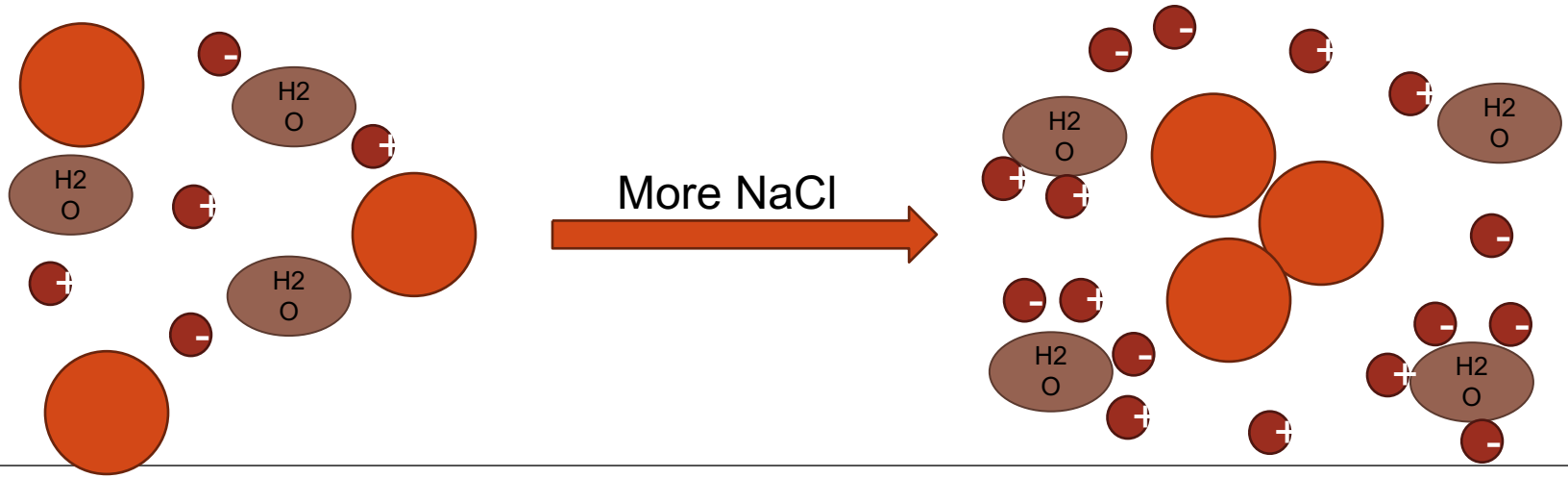


Principle: Salting out

High concentration of salts the solubility decreases, and protein precipitates.

This could be explained by the following:

because the excess ions (not bound to the protein) compete with proteins for the solvent. The decrease in solvation allows the proteins to aggregate and precipitate.



Method:

T3	T2	T1
Take 1 ml of 0.5% albumin sample	Take 1 ml of 0.5% albumin sample	Take 1 ml of 0.5% albumin sample
Slightly add of saturated Mg SO4 solution	Slightly add of saturated (NH4)2SO4 solution	Slightly add of 3% NaCl solution
Concentrate your vision on the tube while adding	Concentrate your vision on the tube while adding Shake gently	Shake it well and write your observation

Results:

Comment	Observation	Tube
		(Albumin+ NaCl)
		Albumin+50% saturated 2SO4(NH4)
		Albumin+50% saturated (MgSO4)

Discusses each result and Compare between them what and why you obtain it …

Acid precipitation of proteins

To investigate the effects of mineral acids and organic acids on the protein solubility.

Applications:

-Separation and purification

-Detection of small amount of protein in urea sample

Stop the enzyme reaction

Principle:

This test depend on affecting solubility of the protein as a function of changes in pH in highly acidic media, the protein will be positively changed, which is attracted to the acid anions that cause them to precipitate.

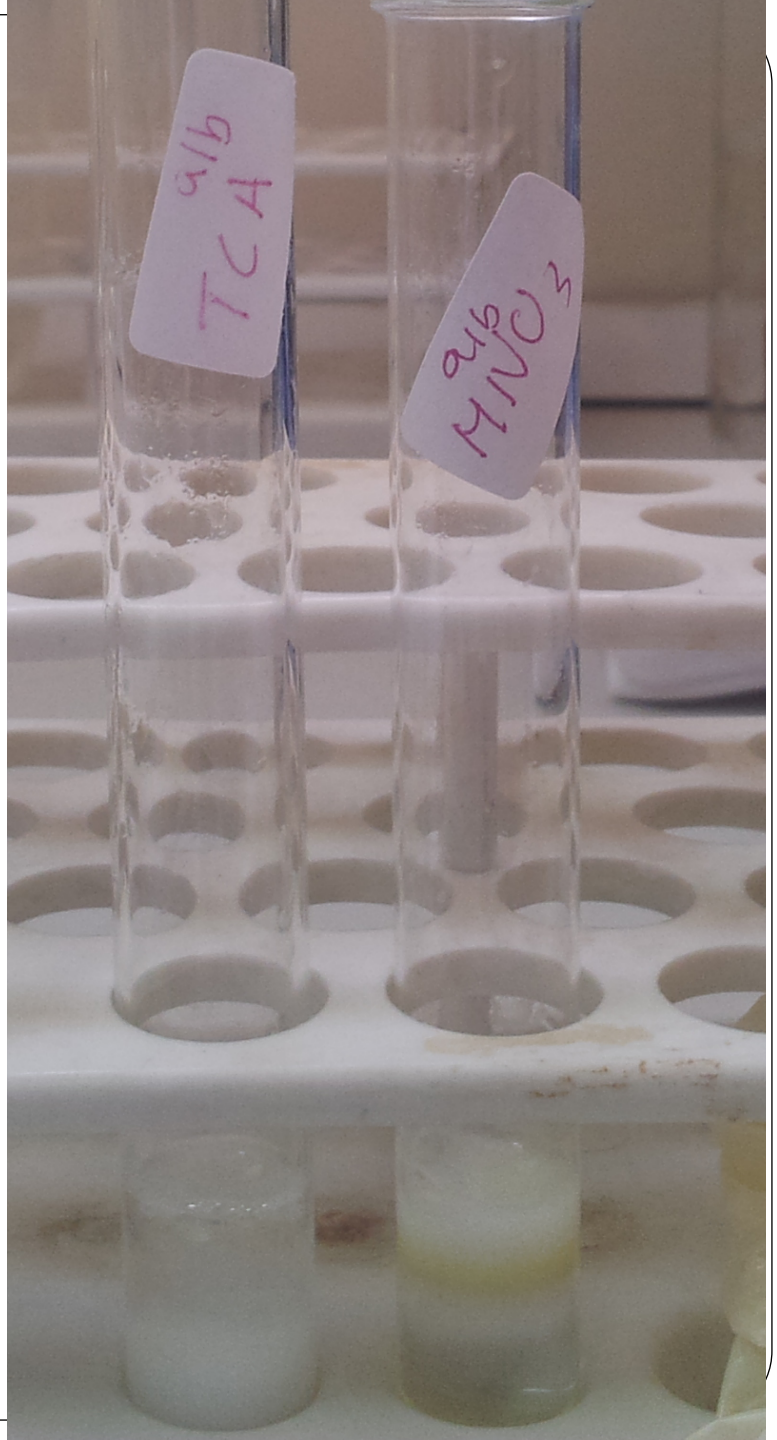
Method

B	A
Put 1 ml of the albumin solution	Put 1 ml of the albumin solution
add 5-7 drops of T.C.A solution carefully	add 5-7 drops of HNO_3 solution carefully
Record your observation	Record your observation

Results:

Comment	Observation	Tube
		+ HNO3 Albumin
		+ Albumin TCA

Discusses each result what and why you obtain it …



Precipitation of proteins by heavy metals:

Proteins are precipitated in alkaline medium with heavy metals due to the direct union of cation (Cu^{++}, Ag^{+}, Ba^{++}, Pb^{++}) with anionic groups of proteins, which are formed in basic medium

At alkaline pH 7 and above, proteins are usually negatively charged so the addition of positively charged ions will neutralize this charge and the proteins come out of solution (i.e. heavy metals combine with proteins forming insoluble

At PH > 7

Method

B	A
Put 1 ml of the albumin solution	Put ml of the albumin solution 1
Add 5-7 drops of Pb(CH3COO)2 solution carefully	add 5-7 drops of AgNO3 solution carefully
Record your observation	Record your observation

Results:

Comment	Observation	Tube
		+ AgNO3 Albumin
		+ Albumin Pb(CH3COO)2

Discusses each result what and why you obtain it …