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Practical Note
General Biochemistry 2
(BCH 302)

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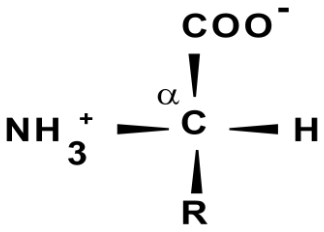
1. Amino Acid

Amino acids play central roles both as building blocks of proteins and as intermediates in metabolism. There are 20 natural amino acids that are found within proteins convey a vast array of chemicals versatility. All of them are L-α amino acids.



All amino acids found in proteins have this basic structure, differing only in the structure of the R-group or the side chain.

The simplest, and smallest, amino acid found in proteins is glycine for which the R-group is hydrogen (H).

All these amino acids are found in solutions in their ionized form (Zwitter ion), i.e. they are polarized and their ionization depends on the pH of the medium where they are located.

According to their ionization (polarity) in water, they are classified into 4 categories:

1- Non-polar.
2- Uncharged polar.
3- Basic polar (positively charged).
4- Acidic polar (negatively charged).

- Polar amino acids are more soluble in water than non-polar, due to presence of amino and carboxyl group which enables amino acids to accept and donate protons to aqueous solution, and therefore, to act as acids and bases. A molecule that functions as such is known as an amphoteric.
- The pH value at which concentration of anionic and cationic groups are equal (i.e the net charge of this molecule equals zero) is known as isoelectric point (pI), a point at which the molecule does not move to either cathode or anode if it is put in electric field and its solubility is minimum.
- Amino acids are able to rotate polarized light either to the left (livo) L. or to the right (dextro) D, since they have an asymmetric C atom (a carbon atom linked to 4 different groups), glycine which lacks asymmetric C atom (has 2 H^+ on α-C) is an exception.

1.1 Qualitative tests of amino acids

1.1.1 Ninhydrin test

- A test often used to detect α-L-amino acids.

Caution:

Ninhydrin is a strong oxidizing agent, it should be handled with care, and applied apart from contact with skin or eyes, gloves and mask is a must, using hood is required, if accidently get in touch with the skin, the resulting stains is a temporarily one, that will be eliminated within 24 hours.

Principle:

- Ninhydrin (triketohydrindene hydrate) degrades amino acids into aldehydes (on pH range 4-8), ammonia and CO_2 though a series of reactions. The net result is ninhydrin in a partially reduced from hydrindantin.
- Ninhydrinthen condenses with ammonia and hydrindantin to produce an intensely blue or purple pigment, sometimes called ruhemann's purple:
- The color varies slightly from acid to acid. Proline and hydroxyproline (amino acids) give yellow color.
- Many substances other than amino acids, such as amines will yield a blue color with ninhydrin, particularly if reaction is carried out on filter paper.

Materials:

- Amino acids (0.1% solution of Glycine, Tyrosine, Tryptophan and Proline).
- 0.1% solution of NH_4OH.
- Distilled water.
- Ninhydrin (0.2% prepared fresh)
- Test tube.

Method:

- You are provided with 6 solutions (A-F). Place 1 ml of each of the solutions in a test tube and ad a few drops of ninhydrin solution.
- Boil the mixture over a water bath for 2 min.
- Allow to cool and observe the blue color formed
- Complete the below table.

Results:

Tube number		Observation after the addition of ninhydrin	Result
A	Glycine		
B	Tyrosine		
C	Tryptophan		
D	Proline		
E	Hydroxyproline		
F	Control		

Table1: Summarize the result after adding ninhydrin reagent to the sample (A-F).

Questions:

1. Depending on its sensitivity to amino acids, when could ninhydrin be used or applied?

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2. Why proline gives a yellow color?

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1.1.2 Xanthoproteic test

- This test is used to differentiate between aromatic amino acids which give positive results and other amino acids.
  Amino acids containing an aromatic nucleus form yellow nitro derivatives on heating with concentrated HNO_3. The salts of these derivatives are orange in color.

Principle:

- Concentrated nitric acid reacts with the aromatic rings that are derivatives of benzene giving the characteristic nitration reaction. Amino acids tyrosine and tryptophan contain activated benzene rings which are easily nitrated to yellow colored compounds. The aromatic ring of phenyl alanine dose not react with nitric acid despite it contains a benzene ring, but it is not activated, therefore it will not react.

Caution:

- Concentrated HNO_3 is a toxic, corrosive substance that can cause severe burns and discolor your skin. Prevent eye, skin and cloth contact. Avoid inhaling vapors and ingesting the compound. Gloves and safety glasses are a must; the test is to be performed in a fume hood.

Material:

- Amino acid solution (1% w/v solution of glycine, 2% of tyrosine, tryptophan and phenyl alanine).
- Phenol solution 0.1% (w/v).
- Concentrated nitric acid (conc. HNO_3).
- Sodium hydroxide, NaOH, solution (10 w/v).

Method:

- Label five tubes (1 - 5), then add 0.5 ml of each amino acid solution and phenol solutions to those test tubes each alone.
- Heat these tubes till boiling
- Add an equal volume of conc. HNO_3.
- Heat over a flame for 2 min and observe the color.
- Compare the color with that given by blank using water instead.
- Now COOL THOROUGHLY under the tap and CAUTIOSLY run in sufficient 40% NaOH to make the solution strongly alkaline.
- Write your observations in following table.

Results:

Tube number	**Observation**	
	+ HNO_3	**+ NaOH**
1% glycine		
1% tyrosine		
2% tryptophan		
2% phenylalanine		
phenol		

Question:

Discuss the reasons of which aromatic amino acids give positive result but not aliphatic ones in Xanthoproteic test.

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1.1.3 Millon's test

This test is specific for tyrosine, the only amino acid containing a phenol group, a hydroxyl group attached to benzene ring.

Principle:

- In Milon's test, the phenol group of tyrosine is first nitrated by nitric acid in the test solution. Then the nitrated tyrosine complexes mercury ions in the solution to form a brick-red solution or precipitate of nitrated tyrosine, in all cases, appearance of red color is positive test.

Note: all phenols (compound having benzene ring and OH attached to it) give positive results in Millon's test.

Material:

- Amino acids solutions (0.1% (w/v) solution of glycine, tyrosine and phenylalanine).
- Millon's reagent (prepared by dissolving 100g $HgNO_3$ then diluted with distilled water (1:2) freshly prepared.
- Phenol 100% (w/v).
- Boiling water bath.
- Test tubes.

Method:

- Label 4 test tubes (1 - 5).
- Add 1 ml of test solutions in separate tubes and the phenol solution in one tube.
- Add to each tube 0.5 ml Millon's reagent and shake it well.
- Place the test tubes in the boiling bath with care, for 10 min.
- Write your observation in the following table.

Results:

Sample	Observation
1. glycine	
2. tyrosine	
3. tryptophan	
4. phenylalanine	
5.Phenol	

Table of results after adding Millon's reagent to the tested samples

Questions:

1. Would phenol give positive results, explain your answer?

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2. Discuss the results of tyrosine comparing it with that of tryptophan and phenyl alanine?

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1.1.4 Detection of amino acids containing sulfhydral group (- SH)- Lead Sulfite Test

This test is specific for –SH containing amino acid (Cystein).

Principle:

- Some of sulfur in cystine, is converted to sodium sulfide by boiling with 40% NaOH.
- The Na_2S can be detected by the precipitation of PbS from an alkaline solution.
- The amino acids containing sulfhydryl group when heated with base, the sulfhydryl group and disulfhydryl are directly converted to inorganic sulfur. Which is confirmed by the black precipitate of PbS (lead sulfide) when adding lead acetate Pb $(CH_3COO)_2$.

Materials:

- Amino acid solution (0.1% (w/v) of cystein, methionine and glycine).
- 10% (w/v) sodium hydroxide (NaOH).
- Pb $(CH_3COO)_2$ 5 % (w/v).
- Boiling water bath.
- Test tubes.

Method:

- Label three test tubes (1 - 3).
- Add 2 ml of amino acid solutions in each tube.
- Add to each test tube 1 ml of sodium hydroxide solution.
- Place the test tube carefully in the boiling bath for 3 min.
- Add 0.5 ml of Pb $(CH_3COO)_2$ to each tube and mix by vortex.
- Finally write your observation in the following table.

Sample	Observation	Result
1.glycine		
2.cystine		
3.methionine		

Questions:

Which of the amino acids contain (-SH) group?

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What is the difference between cystein and cystin ?

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Give an example of a functional protein with a disulfide bridge?

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2. Proteins

2.1 Qualitative chemical reactions of amino acid protein functional groups :

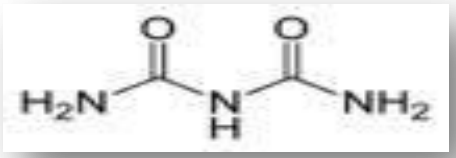
Certain functional groups in proteins can react to produce characteristically colored products. The color intensity of the product formed by a particular group varies among proteins in proportion to the number of reacting functional or free groups present and their accessibility to the reagent. In this part of the experiment we will use various color producing reagents (dyes) to qualitatively detect the presence of certain functional groups in proteins.

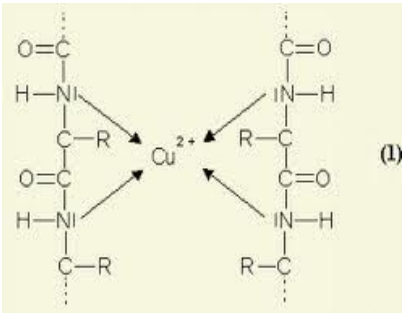
2.1.1 Biuret test :

This test is specific for the peptide bond. Substances containing not less than two peptide linkages give this test. In this reaction, proteins form a pink-purple colored complex with $CuSO_4$ in a strongly alkaline solution.

Principle:

- This test is used to detect the presence of proteins and peptides (i.e peptide bonds) by treating them with an alkaline solution of dilute copper sulfate . A positive test is indicated by the formation of a pink-violet color. The name of the test is derived from a specific compound, biuret which give a positive test with this reagent.





(1)

Materials:

- Proteins solutions [2% gelatin , 2%BSA, or 2% raw egg albumin dissolved in 0.1 NaCl and 1% casein] (casein is to be dissolved in diluted NaOH) .
- NaOH (3M).
- Copper sulfate (1% $CuSO_4 .5H_2O$, Fehling's solution A diluted 1/10 with water).
- Test tube .
-

Method:

- in 3 different test tubes put 2ml of each protein solution.
- to each tube add 1ml 3M NaOH.
- Add 0.5 ml of $CuSO_4 .5H_2O$, and mix well.
- Observe the colors produced and write your observation in the table.

Sample	Observation	Result
1.glycine		
2.cystine		
3.methionine		

Questions:

1. Write the chemical formula of this reaction and the resulting copper complex.

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2. Do you think free amino acids will give a positive result with this reaction? why?

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3. What is the least number of amino acids bonded together by peptide bonds that will respond positively to this test?

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2.1.2 Effect of salt concentration on the protein solubility **:**

This experiment is used to separate different proteins using salting-out theory. Each protein can be precipitated at specific salt concentration.

Principle:
The low salt concentration solutions make protein solubility easier using the attraction of salt ions to the functional groups of the protein. On contrast, high salt concentration or solids dissolved in the reaction medium up till saturation solutions causes the protein to precipitate since salt ions, in this case, compete with the protein molecules in binding water molecules.

Materials :

- Egg albumin and casein.
- Sodium chloride NaCl -0.1M.
- Ammonium sulfate $(NH_4)_2SO_4$-solid.

Method:

- Take the egg albumin dissolve it in 300 ml water.
- The undissolved part (large gelatinous particles) are globulin.
- Separate globulin by centrifugation.
- Chick the ability of precipitated globulin to dissolve in NaCl 0.1 M.
- Add to 10 ml of this globulin solution, 10 ml of saturated ammonium sulfate solution.
- Observe the precipitation of globulin and the degree of saturation caused it.
- Repeat the experiment using the filtrate (supernatant) that contains albumin.
- Note what happens when you add saturated ammonium sulfate to 10 ml dissolved albumin, is there any precipitation occur.
- Add solid $(NH_4)_2SO_4$ till saturation.
- Note what happens, what is the degree of saturation here.
- Brief your results and comment on each step.

Results:

Step	Observation	Comment
1-Globulin ppt.+NaCl		
2-Globulin solution +saturated Ammonium sulfate solution		
3-Albumin solution +saturated Ammonium		
4-Saturated solution addition of solid $(NH_4)_2SO_4$ to steep 3		

Question:

Can we use this method in fractionating mixture of proteins? Explain you result with example..

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2.1.3 Acid precipitation of proteins **:**

This experiment is used to precipitate different proteins using strong acid solution. Each protein can be precipitated at specific acid concentration.

Principle:

- Strong acids cause proteins to precipitate by affecting different bonds of the molecule, there are many application of this test in laboratories, i.e in the detection of small amounts of protein in urea sample, also in the separation and purification of proteins or to stop the enzymatic action of an enzyme.
- 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.

Materials:

- Concentrated nitric acid.
- Trichloroacetic acid (TCA).
- Precric acid.
- BSA (bovine serum albumin) 0.5%, or 2% egg albumin dissolve in 0.1 NaCl solution

Method:

- In a test tube, put 3ml of conc. nitric acid.
- Using a dropper add to each tube the protein solutions you have (albumin, casein, gelatin) drop-wise on the inner wall of the tube to form a layer up the acid.
- Note the white precipitate at the inner face of the protein in contact with the conc. acid.
- To 3 ml of the protein solution add T.C.A drop-wise till a precipitate forms.
- Be careful what happen to the precipitate if a few drops more will added? Dose it dissolve in excess acid?

Brief your notifications in the following table:

Tube	**Observation**			**Comments**
1-H_2NO_3	albumin	casein	gelatin	
2-T.C.A				

Questions:

1. Why albumin is dissolved in 0.1 NaCl?

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2.1.4. Protein denaturation :

Denaturation is destruction of the usual nature of a substance, as by the addition of methanol or acetone. Most globular proteins exhibit complicated three-dimensional folding described as secondary, tertiary, and quarternary structures. These conformations of the protein molecule are rather fragile, and any factor that alters the precise geometry is said to cause denaturation. Extensive unfolding sometimes causes precipitation of the protein from solution. Denaturation is defined as a major change from the original native state without alteration of the molecule's primary structure, i.e., without cleavage of any of the primary chemical bonds that link one amino acid to another.

Principle:

- Treatment of proteins with strong acids or bases, high concentrations of inorganic salts or organic solvents (e.g., alcohol or chloroform), heat, or irradiation all produce denaturation to a variable degree. Loss of three-dimensional structure usually produces a loss of biological activity.
- This test illustrates the importance of week bonds in globular protein's tertiary structure (the functional structure). Acid and heat disrupt ionic bonds and hydrogen bonds, respectively causing loss of the quaternary structure. This leads to denaturation and loss of biological function.

Materials:

- Albumin (2% raw egg albumin in 0.1 NaCl), 1% gelatin ,1% casein,1% globulin in 0.1 NaCl.
- Diluted acetic acid.

Method:

- Add 10 ml of protein solutions in different test tubes
- Add 3 drops of acetic acid to each tube.
- Place them in a boiling water bath for 5-10 minutes;
- Remove aside to cool to room temperature.
- Note the change in each tube.

Brief your results in the following table:

Results:

Tube	Observation	result
Globulin		
Albumin		
gelatin		
casein		

Questions:

Are Albumin and gelatin compact, globular proteins?

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By the end of this test did casein and gelatin coagulated, are they still biological active? Why?

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2.1.5. Precipitation of protein by salts of heavy metals:

Heavy metal salts usually contain Hg^{+2}, Pb^{+2}, Ag^{+1} Tl^{+1}, Cd^{+2} and other metals with high atomic weights. Since salts are ionic they disrupt salt bridges in proteins. The reaction of a heavy metal salt with a protein usually leads to an insoluble metal protein salt.

Principle:

- At pH7 the protein is normally negatively charged. Addition of the heavy metal cation will neutralize those negative charges and will cause the protein to precipitate, but any elevation of pH of the medium to higher than 7 (to basic) will precipitate protein as hydroxides, whereas more of metal cations will dissolve this precipitate.
- The family's application of this technique is to eliminate poisoning by palladium (Pb^{++}) and mercury salts (Hg^{++}).

Materials:

- Albumin (2% dissolved in 0.1 NaOH) ,1% gelatin ,1% casein (dissolved in 0.1 NaOH)
- Pb acetate (Pb OOC-CH_3) or 2% silver nitrate (2g $AgNO_3$) dissolved in 100ml distilled water)
- Mercury chloride 5% $HgCl_2$.

Method:

- In different test tubes take 1ml of protein solutions.
- Add to each tube 0.5ml of $AgNO_3$ (be careful).
- Repeat the process using $HgCl_2$ instead of $AgNO_3$, compare the results.

Brief them in the following table:

Results:

Tube	Observation	Result
Albumin		
gelatin		
casein		

Questions:

1. How can this technique help eliminating poisoning by Pb^{++} from water pipes or accidental poisoning of Hg?

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3. Carbohydrates

3.1 Introduction:

The term carbohydrates are generally applied to the group of polyhydroxy aldehydes or ketones commonly known as sugars. Carbohydrates are produced from carbon dioxide and water by plants through the process of photosynthesis. Carbohydrates are the major food supply and energy source for the people of the world. Depending on the dietary habits, 50-90% of the carbohydrates consumed come from grain, starchy vegetables and legumes. Despite the major utilization of carbohydrates for energy only a small amount is stored in the body. The average adult reserve is about 370 g stored mainly as liver and muscle glycogen.

Carbohydrates can be classified as simple or complex:

1) Simple carbohydrates, often called monosaccharides or simple sugars, contain one saccharide unit.
2) Complex carbohydrates are those containing more than one saccharide group.

Also it can be classified as:

- Monosaccharides contain one monosaccharide unit.
- Disaccharides contain two monosaccharide units.
- Oligosaccharides contain 3-6 monosaccharide units.
- Polysaccharides can contain over 7 or more monosaccharide units. Complex carbohydrates can be broken down into smaller sugar units through a process known as hydrolysis.

Monosaccharides can be classified in a number of ways:

1. They can be classified by the number of carbon atoms they contain; pentoses (5 carbons) and hexoses (6 carbons) are the most common.
2. Monosaccharides can also be classified as ketoses or aldoses. A ketose contains a carbonyl group attached to two R groups having one or more hydroxyl groups. An aldose contains terminal aldehyde group in addition to R group containing -OH.

Objectives:

1) To identify the carbohydrate from other macromolecules lipids and proteins.
2) To distinguish between reducing and non-reducing sugars.
3) To distinguish between mono- , di- and poly saccharides.
4) To distinguish between aldose and ketone sucrose.
5) To distinguish between pentose monosaccharide and hexose monosaccharide

3.2 Physical properties**:**

3.2.1 Solubility**:**

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 soluble.

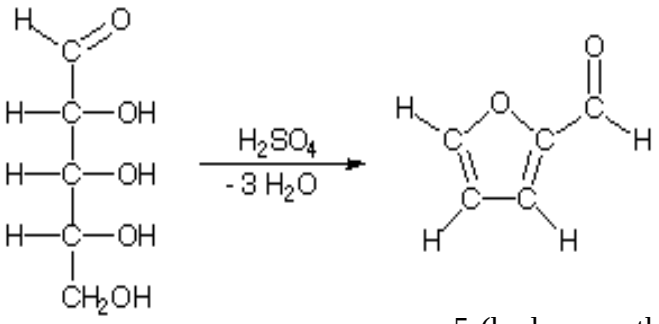
3.3.Chemical properties:

3.3.1. The Molisch Test:

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

Principle:

The test reagent dehydrates pentoses to form furfural and dehydrates hexoses to form 5-hydroxymethyl furfural. The furfurals further react with α-naphthol present in the test reagent to produce a purple product.



Glucose 5-(hydroxymethyl)furfural

Material:

- Naphthol in 95% ethanol
- Different sugar solutions.
- Concentrated sulfuric acid

Method:

- Two ml of a sample solution is placed in a test tube.
- Two drops of the Molisch reagent (a solution of α-napthol in 95% ethanol) is added.
- The solution is then poured slowly into a tube containing two ml of concentrated sulfuric acid so that two layers form, producing violet ring appear as liaison between the surface separations.

Result:

Tubes	Observation	Discussion

Question:

Name the complex formed by the addition of concentrated sulfuric acid to sugar solution and explain the reaction?

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3.3.2. Benedict's Test:

Benedict's reagent is used as a test for the presence of reducing sugars. 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. Starches are also non-reducing sugars.

Principle:

The copper sulfate ($CuSO_4$) present in Benedict's solution reacts with electrons from the aldehyde or ketone group of the reducing sugar. Reducing sugars are oxidized by the copper ion in solution to form a carboxylic acid and a reddish precipitate of copper (I) oxide.

$$CuSO_4 \longrightarrow Cu^{++} + SO_4^{--}$$

$$2\,Cu^{++} + \text{Reducing Sugar} \longrightarrow Cu+$$
(electron donor)

$$Cu^{+} \longrightarrow Cu_2O \text{ (precipitate)}$$

Material:

- Solution of different Carbohydrate (1%)
- Benedict's reagent (a solution of sodium citrate and sodium carbonate mixed with a solution of copper sulfate)
- Water bath

Method:

- One ml of a sample solution is placed in a test tube.
- Two ml of Benedict's reagent is added.
- The solution is then heated in a boiling water bath for three minutes.

A positive test is indicated by: The formation of a reddish precipitate within 3 minutes.

Result:

Tubes	Observation	Discussion
Glucose		
Fructose		
Maltose		
Sucrose		
Starch		

Questions:

Why sucrose gives negative Benedict test?

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Explain, although starch has free hemiacetal bond it gives negative Benedict test?

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Why glucose (monosaccharide) and maltose (disaccharide) give positive Benedict test?

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3.3.3. Barfoed's Test:

This test is performed to distinguish between reducing monosaccharides, reducing disaccharides and non reducing disaccharides.

Principle:

Barfoed's test used copper (II) ions in a slightly acidic medium Reducing monosaccharides are oxidized by the copper ion in solution to form a carboxylic acid and a reddish precipitate of copper (I) oxide within three minutes. Reducing disaccharides undergo the same reaction, but do so at a slower rate. The nonreducing sugars give negative result.

Material:

- Barfoed's reagent (a solution of cupric acetate and acetic acid)
- Glucose, Sucrose
- Water bath.

Method:

- Place one ml of a sample solution in a test tube.
- Add 3 ml of Barfoed's reagent (a solution of cupric acetate and acetic acid.
- Heat the solution in a boiling water bath for three minutes.
- Note your oservation

Result:

Tubes	Observation	Discussion
Glucose		
Fructose		
Maltose		
Sucrose		

Questions:

What is the difference between Benedict and Barfoed's reaction?

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Why should be avoid boiling more than 5 minutes?

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3.3.4. Bial's Test:

This test is used to distinguish between pentose and hexose monosacharides.

Principle:

Bial's test uses concentrated HCl as a dehydrating acid and orcinol + traces of ferric chloride 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.

Material

- Ribose, Fructose
- Bial's reagent (a solution of orcinol, HCl and ferric chloride)
- water bath.

Method:

- Put 2 ml of a sample solution in a test tube.
- Add 2 ml of Bial's reagent (a solution of orcinol, HCl and ferric chloride) to each tube.
- Heat the tubes gently in n a Bunsen Burner or hot water bath.
- If the color is not obvious, more water can be added to the tube.

A positive test is indicated by the formation of a bluish product. All other colors indicate a negative result for pentoses.

Note that hexoses generally react to form green, red, or brown products.

Result:

Tubes	Observation	Discussion
Glucose		
Fructose		
Ribose		

Questions:

What is the principle of Bial's reaction?

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3.3.5. Seliwanoff's Test:

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

Principle:

Seliwanoff's Test uses 6M HCl as dehydrating agent and resoncinol as condensation reagent. The test reagent dehydrates ketohexoses to form 5-hydroxymethylfurfural. 5-hydroxymethylfurfural further condenses with resorcinol present in the test reagent to produce a cherry red product within two minutes. Aldohexoses react to form the same product, but do so more slowly giving yellow to faint pink color.

Material:

- Seliwanoff's reagent (a solution of resorcinol and HCl)
- Glucose, Fructose.
- water bath

Method:

- One half ml of a sample solution is placed in a test tube.
- Two ml of Seliwanoff's reagent (a solution of resorcinol and HCl) is added.
- The solution is then heated in a boiling water bath for two minutes.

Result :

Tubes	Observation	Discussion

Questions:

What are the carbohydrates' that give positive result with Seliwanoff ? why?

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4. Carbohydrate (2): Quantitative analysis of Carbohydrates

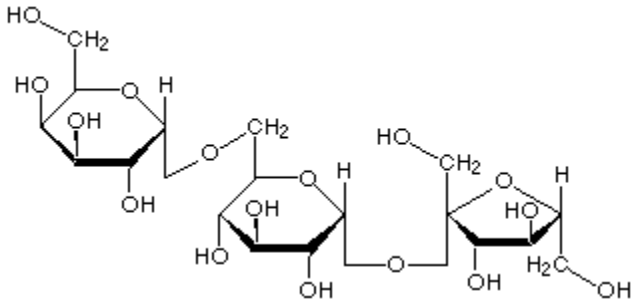
4.1 Introduction:

Structures of common complex carbohydrates

The most abundant carbohydrate molecules found in nature are actually large complex structures consisting of mixtures of monosaccharide derivatives. Carbohydrates consisting of several monosaccharides are called **oligosaccharides**, a designation that could also include disaccharides, whereas, carbohydrates with ~10 or more monosaccharide units are called **polysaccharides**. Polysaccharides can either be homopolymeric (same repeating monosaccharide unit) or heteropolymeric (mixture of monosaccharides).

Disaccharides can be broken into two monosaccharide units by hydrolysis and lost one molecule of water, examples of disaccharides Sucrose, Lactose and Maltose.

Oligosaccharides can be broken into 3-6 monosaccharide units by hydrolysis and lost one or more molecules of water, See below example of oligosaccharides.



Glucose Glucose fructose

Raffinose

Polysaccharides can be broken into7 or more monosaccharide units by hydrolysis and lost one or more molecules of water examples Starch.

Plants and animals store glucose in the form of very large polysaccharide glucose homopolymers that contain both α1-4 and α1-6 glycosidic bonds. The glucose homopolymer produced in plants is called **starch**, while the glucose homopolymer produced in animal cells is called **glycogen**. Plants synthesize two forms of starch, **amylose**, a linear polysaccharide containing about ~100 glucose units linked by α1-4 glycosidic bonds, and **amylopectin**, a branched polysaccharide containing ~100,000 glucose units connected by α1-4 and α1-6 glycosidic bonds.

Objective:

To identify the products of hydrolysis of di- and polysaccharides.

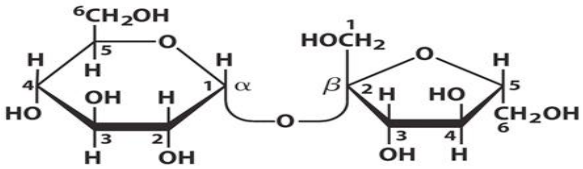
4.2 Practical experiments

4.2.1 Hydrolysis Test:

This test is used to convert sucrose (non-reducing disaccharide) to glucose and fructose (reducing mono saccharides).

Principle:

Sucrose is the only non-reducing disaccharide so it does not reduce the Cu^{++} solution (Bendict's and Fehling's test) because the glycosidic bond is formed between the two hemiacetal bonds. So there is no free aldehydic or ketonic group to give potitive reducing properties. This bond can be hydrolysed and the individual components of sucrose (glucose + fructose) are then able to give positive reducing test.



Sucrose

Glc(α1↔2β)Fru

Materials:

- Sucrose
- Concentrated hydrochloric acid (HCl)

Method:

- 6ml of a sucrose solution is placed in two test tubes.
- Add two drops of concentrated hydrochloric acid (HCl) to only one tube.
- Heat the tubes in boiling water bath for 15 minutes.

A positive test is indicated by the formation of orange color.

Result :

Tubes	Observation	Discussion
Sucrose + HCl		
Sucrose only		

Questions:

How you can convert non-reducing sugar to reducing?

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4.2.2 The Iodine/Potassium Iodide Test**:**

This test used to distinguish between polysaccharides and mono or oligo saccharides.

Principle:

Starch forms deeply blue color complex with iodine. Starch contains α- amylase, a helical saccharide polymer and amylopectin. Iodine forms a large complex with α-amylose helix. This complex absorbs light and reflects the blue light only. Simple oligosaccharides and mono saccharides do not form this complex.

Note that other polysaccharides like glycogen may give other colors (red).

Materials:

- Iodine/potassium iodide solution
- Starch- Glycogen- Dextrin, glucose

Method:

- Two ml of a sample solution is placed in a test tube.
- Add two drops of iodine/potassium iodide solution and one ml of water.
- A positive test is indicated by the formation of a blue-black complex.
- Heat the tubes by exposing them to the flame and write your observation.

Result :

Tubes	**Observation**	**Discussion**
Starch		
Glycogen		
Glucose		

Questions:

Why glucose does not give positive result with iodine test but not starch?

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Explain why the blue color disappears upon heating?

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4.2.3 Hydrolysis of Starch:

This experiment illustrates the conversion of starch (non-reducing sugar) to a reducing sugar by the action of hydrochloric acid at boiling point. The longer the starch is exposed to the acid the further hydrolysis proceeds.

Principle:

Although starch has free hemiacetal in the terminal glucose residue, it has no reducing properties, because the percentage between the free residues is very low in comparison to the whole molecule. Heating starch solution in acid medium hydrolyses the glycosidic bonds giving many free glucose residues. These glucose molecules give reducing properties to the hydrolysis product.

Materials:

- Starch
- Benedict's reagent
- iodide reaction
- Water bath

Method:

- Two ml of starch in large tube
- Add three drops of Hydrochloric acid, heated in boiling water bath for 10 mints. then cold solution
- Add the amount of sodium hydroxide to become the base
- Divided in two tube (a,b)
- In tube (a) add 1 ml of iodine solution and note the result.
- In tube (b) add 1 ml of Benedict reagent, mix and heated for 3 mint and record result.

Result:

Tubes	Observation	Discussion

Questions:

Although starch has free hemiacetal bonds it is non reducing sugar, explain?

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5. Lipids

5.1 Introduction

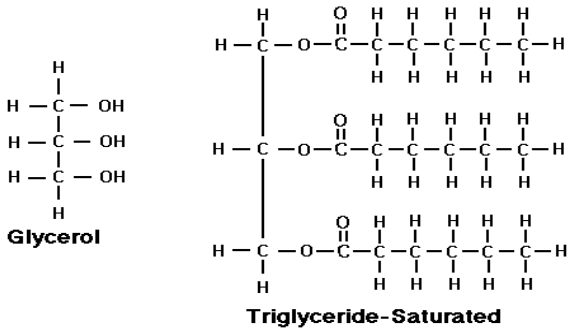
Lipids are found naturally in all living organisms. It represents about 5% of the cell structure and has a structural function in the cell, since it presents in cell membranes, and also it is an essential source of energy in the body. It give more energy than carbohydrate and proteins. It can be defined as nonpolar organic compound insoluble in polar water, but soluble in organic solvents such as benzene,ether, chloroform and boiling alcohol.

Fatty acids:
They are the building blocks of lipid. Fatty acid have a long hydrocarbon chain containing a carboxyl group at the end. They are divided into: saturated fatty acids and unsaturated fatty acids (unsaturated contain double bonds). The general formula for fatty acids $\{CH_3 (CH_2)_n COOH\}$

Fats can be divided according to their chemical composition to:

A- Simple lipids:
Triglycerides are esters of fatty acids with glycerol. It is found in fats and oils. The triacyglycerol is the simplest and most common fat. It is the form in which lipids are stored in the cell.

The general formula of fats and oils



B - Compound (conjugated) lipids:
Lipids are linking with other compounds, such as phospholipids and glycolipids.

C - Derived lipids:
They are substances that are soluble in lipid or derived from the above groups of lipids by hydrolysis; for examples, cholesterol and fat soluble vitamins.

5.2 Qualitative tests of lipids:

5.2.1 Solubility test:

Principle:

Fats are not dissolved in water due to their nature, non-polar (hydrophobic), but it is soluble in organic solvents such as chloroform, benzene, and boiling alcohol. Different lipids have ability to dissolve in different organic solvent. This property enable us to separate a mixture of fat from each other for example, undissolve phosphatide lipid in acetone; undissolve of cerebroside, as well as sphingomyline in the ether.

Materials and tools required:

- Olive oil (or cottonseed oil) - butter - corn oil.
- Solvents: diluted acid - dilute alkaline - ethanol - ether - chloroform - acetone
- Test tubes
- Water bath

Method:

- Place 0.5ml of oil in 6 test tubes clean, dry containing 4ml of different solvents (acetone, chloroform and ether and ethanol, cold ethanol and hot water),
- Shake the tubes thoroughly, then leave the solution for about one minute,
- Note if it separated into two layers , the oil are not dissolve; but if one layer homogeneous transparent formed , oil be dissolved in the solvent.

Results:

Tube	Solvent	Degree of solubility

Questions:

Which solvent is the best for lipid?

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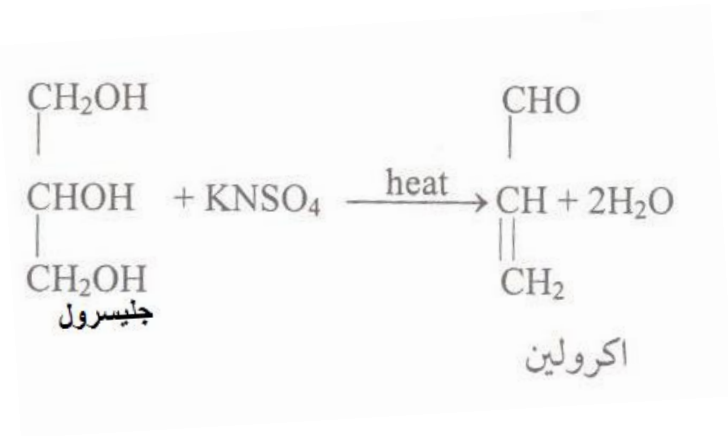
5.2.2 Acrlein test

Most lipid are found in the form of triglycerides, an ester formed from glycerol and fatty acids. When a fat is heated strongly in the presence of a dehydrating agent such as $KHSO_4$, the glycerol portion of the molecule is dehydrated to form the unsaturated aldehyde, acrolein

Principle:

When a fat is heated strongly in the presence of a dehydrating agent such as $KHSO_4$, the glycerol portion of the molecule is dehydrated to form the unsaturated aldehyde, acrolein

CH_2=CH-CHO , which can be distinguished by its irritating acrid smell and as burnt grease.



Lipid can detect by dye Sudan IV (general dye for lipid), which produce red color with lipid.

Materials:

- different types of vegetable oils (such as corn oil, olive oil, butter)
- glycerol
- Solid potassium hydrogen sulfate $KHSO_4$
- test tubes
- a water bath (boiling)

Method:

1. Place an amount of solid potassium hydrogen sulfate $KHSO_4$ to about 0.5ml of glycerol in a test tube, then carefully heat the tube in boiling water bath and observed the emergence of the smell of acrolein (irritating to the membranes).
2. Repeat the previous step using oil from different sources instead of glycerol

Results:

Tube	Observation	Conclusion
Glycerol		
Palm oil		
Sun Flower oil		

Questions:

Why acrolein test is used as a general test for oils and fats?

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Do you expect to get a positive result if you use free fatty acid like oleic acid or palmitic acid and why?

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Do you expect to get a positive result if you use beeswax and why?

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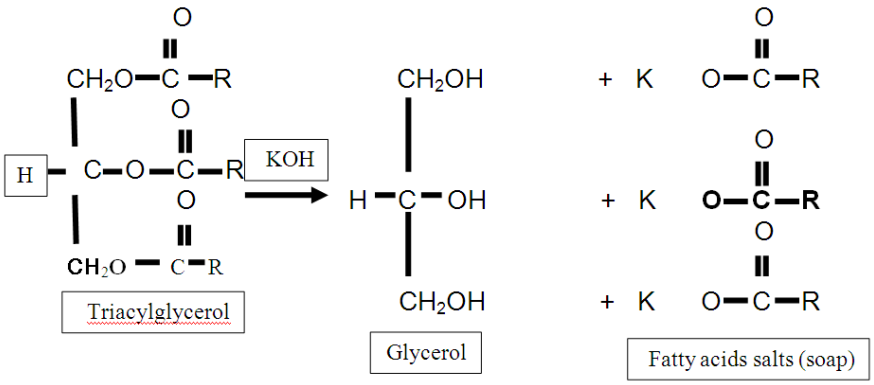
5.2.3 Saponification test:

Triacyl glycerol can be hydrolyzed into their component fatty acids and alcohols. This reaction can also be carried out in the laboratory by a process called **saponification** – where the hydrolysis is carried out in the presence of a strong base (such as NaOH or KOH).

Principle:

Saponification is a process of hydrolysis of oils or fat with alkaline and result in glycerol and salts of fatty acids (soap) and can be used the process of saponification in the separation of saponifiable materials from unsaponified (which are soluble in lipid). The process of saponification as follows :



Soap can be defined as mineral salts of fatty acids. The soap is soluble in water but insoluble in ether. Soap works on emulsification of oils and fats in the water as it works to reduce the attraction surface of the solution.

Materials:

- types of oils like corn oil, butter, olive oil.
- KOH solution in alcohol (2O% KOH)
- a water bath (boiling)

Method:

- Place 2 ml of oil in a large test tube (or flask).
- Add 4 ml of alcoholic potassium hydroxide (preferably add little small pieces of porcelain to regulate the boiling point).
- Boil the solution for 3 minutes. After this period, make sure it is perfectly saponification process, by taking a drop of the solution and mix with the water if oil separated indicates that the non-completion of the saponification. In this case, continued to boil until all the alcohol evaporates.
- Take the remaining solid material (soap) and add about 30 ml of water and keep it for the following tests.
- Shake the solution after it cools and noted to be thick foam.

Results:

Tube	Observation	Conclusion

Questions:

What is the chemical composition of soap?

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Why potassium hydroxide is used in this test?

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If you use cocoa butter, what kind of soap that will get it?

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If you use fatty acids instead of oil do you expect to get the soap?

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5.2.4 Testing the separation of soap from the solution by salting out:

Principle:

To get the soap out of solution by salting out when added solid sodium chloride to the solution until saturation; separated soap in the form of insoluble and floats above the surface.

Materials:

- Soap (which was prepared in the previous experiment)
- Solid sodium chloride NaCl
- A small beaker.

Method:

Place about 10 ml of soap in the beaker, then add small amounts of sodium chloride in batches, stirring until saturated solution.

Tube	Observation	Results: Conclusion

Questions:

Q/Why is a separation layer on the surface of soap when you add salt?

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5.2.5 Test formation insoluble fatty acids salt (insoluble soaps):

Working calcium, magnesium, lead or iron ions to the deposition of soap and make it insoluble in water, where solve these ions replace the sodium or potassium ions are present in soap. Due to the hard water to contain significant quantities of $Ca^{++,}$ Mg^{++} and some Fe^{+++} are difficult to foam.

Potassium soap + calcium sulfate=.> calcium soap + potassium sulfate.

(Consisting of a white precipitate from calciumstearte or oleate).

Materials:

- Soap (which was prepared in the previous experiment)
- Calcium chloride($CaCl_2$) 5%
- Magnesium chloride or sulfate 5%
- Lead acetate .
- Test tubes.

Method:

1 - Add about 4 ml of distilled water to 2 ml of soap in three test tubes

2 – Add to the first tube a few drops of calcium chloride, to second tube MgCl ,and third tube lead acetate.

Results:

Tube	Observation	Conclusion

Questions:

Q1/ Write the equation of reacting calcium chloride with soap?

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Q2 / What happens to the soap when washing with water hardness?

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5.2.6. Copper acetate test:

This test is used to distinguish between oil or neutral fat and fatty acid saturated and unsaturated.

Principle:
The copper acetate solution does not react with the oils (or fats), while saturated and unsaturated fatty acids react with copper acetate to form copper salt.
Copper salt formed in the case of unsaturated fatty acids can only be extracted by petroleum ether.

Materials:
Olive oil - oleic acid (polyunsaturated fatty acid) - stearic acid (saturated fatty acid) - petroleum ether - copper acetate solution (5%)

Method:

- Take three test tubes put 1 / 2 g of each sample and then added 3 ml of petroleum ether and an equal volume of a solution of copper acetate.
- Shake the tube and leave it for some time.
- In the case of olive oil notice that petroleum ether upper layer containing the dissolved oil and appears colorless, aqueous solution remains blue in the bottom.
- In the case of oleic acid the upper layer of petroleum ether becomes green as a result of copper oleate. The lower layer becomes less in blue.
- In the case of stearic acid notice that the petroleum ether upper layer remains colorless, while consists of pale green precipitate of copper stearate at the bottom.

Results:

Tube	Observation	Conclusion

Questions:

Why olive oil does not form green color?

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Q / What do you expect if you used palmitic or linoleic acid?

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5.2.7. Testing lack of saturation (iodine test):

This test is used to identify the nature of fatty acids in the oil or fat, if they saturated or unsaturated.

Principle
Iodine (brown) reacts with unsaturated compounds, The double bond is changed to single bond and iodine is added to both side of the bond.

Materials:
Olive oil, oleic acid, butter, stearic acid (dissolved in chloroform).
Iodine solution in alcohol

Method:
Add to about 2 ml of each of the solutions sample three drops of iodine solution. Note what is happening and interpreted watching.

Results:

Tube	Observation	Conclusion

Questions:
Why stearic acid gave negative result while oleic positive?

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6. Spectrophotometric DNA Quantification

Principle:

If a DNA sample is free of contamination from protein, phenol, agarose, or RNA, its concentration can be measured accurately by determination of the amount of UV radiation that is absorbed by the bases present in an aliquot of the sample.

Materials:

DNA sample
UV spectrophotometer.

Method:

1.Make sure the spectrophotometer's UV light source has been turned on and allow it to warm up for 10 minutes before using.
2.Pipette 50 ml of dH_2O into cuvette (path length: 10 mm) to be used.
3.Set spectrophotometer for DNA measurement, then wavelength to be measured for 260 nm and 280 nm. Insert cuvette.
4.Zero spectrophotometer using cuvette containing dH_2O as a blank, then set reference
5. Pipette 2 ml of sample into an 0.5 ml Eppendorf tube containing 48 ml dH_2O. Mix DNA by vortexing.
6. Remove dH2O from blank cuvette with a Pasteur pipet.
7. Pipet sample into cuvette and record OD260 and OD280 of sample.
8. Wash cuvette by rinsing with dH_2O several times or rinsing with cuvette washing device. Replace cuvette in holder.Repeat process to record all samples' OD.

Calculations

For calculation of DNA concentration of samples free of RNA, the following conversion factor is used: 1 OD260 = 50 ug of DNA/ml.

OD260/OD280 =1.7 -1.8
A value out of this range is not acceptable. It may indicate that the DNA sample is not in solution or that there are contaminants (i.e., protein) in the sample that may inhibit subsequent reactions.

Question:

Can you quantify DNA using photometer?

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7. References:

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