Mustansyriah University
College of Medicine
Department of Chemistry &
Biochemistry

MEDICAL CHEMISTRY

LABORATORY MANUAL

FOR FIRST YEAR STUDENTS

2024-2025

Aims of the Chemistry Laboratory

1. To give you the chance to observe first some of the substances and reactions discussed in the lectures and textbooks.
2. To learn some of the skills and techniques used by chemists in their work such as analytical work and chemical synthesis.
3. To offer you training in the careful and complete observation of certain phenomena and in the recording of data about them. Data gathering is followed by analysis of these data, determination of the significance of the data and then your decision of what you can conclude from your data. This is shown in the report written at the end of each experiment.

Safety Rules

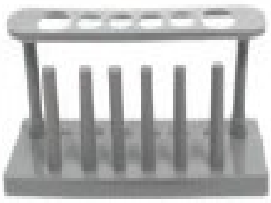
1. Dress properly during lab activity:
   - A lab coat should be worn
   - Gloves should be used
   - Safety glasses or googles must be used when certain procedures are being carried out
   - Long hair must be tied back
   - Dangling jewelry, and loose or baggy clothing must be secured.
   - Shoes must completely cover the foot
2. No drinking, eating or smoking is allowed in the lab.
3. Chemical contact should be washed immediately with copious amounts of water and to notify the instructor.
4. Avoid inhaling any fumes. When necessary, a substance may be smelt by Wafting (fanning) its vapor gently towards your face. Containers emitting noxious fumes should be placed in the hood (fume cupboard).
5. Whenever a reagent is discarded in the sink it should be flushed down the drain with copious amounts of water.
   Noxious gases may evolve from residual reagents in the drain.
6. Never direct the open end of test tube toward yourself or anyone else.
7. Develop the habit of keeping your hands away from your mouth, nose and eyes. This will reduce the possibility of self-contamination.

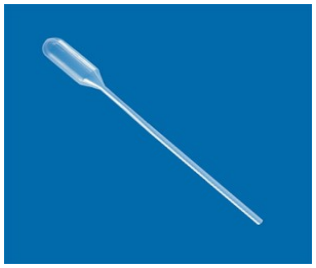
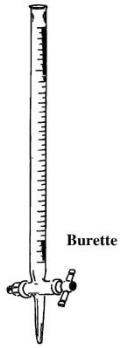
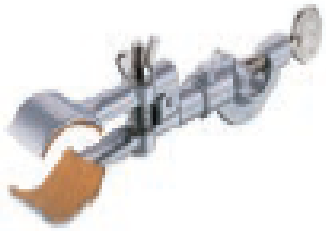
8. Electrical equipment and connections should not be handled with wet hands, nor should electrical equipment be used after a liquid has been spilled on it.
9. Always switch off electrical apparatus at the mains when not on use. Switching off the apparatus by its own switch is not satisfactory.
10. If you see dangers procedures taking place in the lab, please inform your instructor.
11. Hand washing is mandatory on leaving the laboratory.

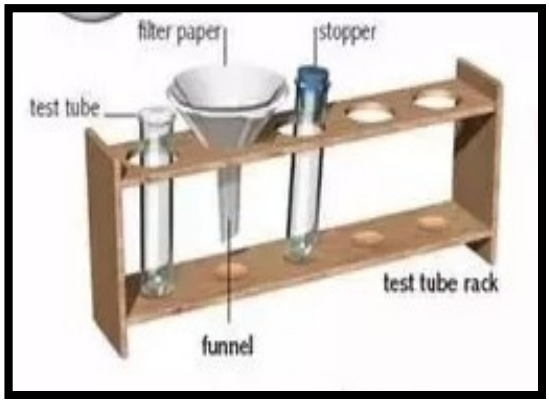
General Instructions

1. It is the responsibility of each student to study the experiment before his lab time and to answer the questions of a pre-lab quiz.
2. Attendance at laboratory is compulsory for there is simply no other way to accomplish this part of the study program.
3. Read the label twice before taking anything from a bottle.
4. Do not lay the stopper of a bottle down. Impurities may be picked up and thus contaminates the solution when the stopper is returned.
5. Leave the reagent bottles at the side benches. Bring test tubes or beakers to the bench for transferring chemicals and carrying them to your bench.
6. Reagent bottles are provided with their own pipettes or droppers. Do not insert your own pipette or dropper into the reagent bottles.
7. Glassware are cleaned by washing carefully with a brush in water and detergent, then rinsed thoroughly with tap water and finally rinsed once again with a small quantity of distilled water. Then allow the glassware to dry. If you must use a piece of glassware while it is still wet, rinse it with the solution to be used.

Basic Glassware Equipments in Chemistry Lab

Equipment Type	Image	Equipment Type	Image
Beaker		Test tube	
Volumetric Flask		Test-tube rack	
Erlenmeyer flask (or conical flask)		Test-tube clamp (or Test-tube holder)	
Graduated cylinder		Test-tube brush	

Pipet (or pipette)		Volumetric Pipet	
Pipet pump		Funnel	
Transfer Pipet		Medicine Dropper	
Burette (or Buret)		Burette Clamp	
Stand		wash bottle	


filter paper
stopper
test tube
test tube rack
funnel


burette (also buret)
clamp
stand

Chemical Reactions of Group I Ions

The group I ions are silver Ag^{+}, mercury Hg_2^{+2} and lead Pb^{+2}. The chloride salts of these ions are insoluble, therefore, to prevent any interference with the cations of group II, hydrochloric acid is added to remove them as the chlorides:

$$Ag^{+} + Cl^{-} \longleftrightarrow AgCl \downarrow$$

White ppt.

$$Hg_2^{+2} + 2Cl^{-} \longleftrightarrow Hg_2Cl_2 \downarrow$$

White ppt.

$$Pb^{+2} + 2Cl^{-} \longleftrightarrow PbCl_2 \downarrow$$

White ppt.

A large excess of chloride ions must be avoided to prevent the formation of soluble silver chloride or lead chloride anions.

The Aim:

Is to teach students how to deal with laboratory glassware's, chemical reagents and observation of common colored products of chemical reactions.

Reactions of the Aqueous Solution of Hg_2^{+2}:

1) HCl (diluted acid):

Put few drops (2-3) of the sample solution in a test tube, add dilute HCl drop by drop till a cloudy white precipitate of **Hg_2Cl_2** is formed.

$$Hg_2(NO_3)_2 + 2HCl \longrightarrow Hg_2Cl_2\downarrow + 2HNO_3$$

This white precipitate of Hg_2Cl_2 is:

a) Insoluble in hot water and cold diluted acids.

b) Turns black when ammonia solution is added.

$$Hg_2Cl_2 + 2NH_3 \longrightarrow Hg(NH_2)Cl + Hg + NH_4Cl$$

Black powder

2) Potassium chromate solution (K_2CrO_4):

Add few drops of K_2CrO_4 to few drops of the sample in a test tube, **Hg_2CrO_4** will formed as a brown precipitate which turns to red crystals on boiling.

3) Ammonia solution:

When ammonia solution is added to aqueous solution of Hg_2^{+2} a black precipitate will be formed which consists of the ammoniacal mercuric salt and fine powder of mercury.

Reactions of the Aqueous Solution of Pb^{+2}

In all of these reactions only few drops are used:

1) HCl (diluted):

On addition of diluted HCl to the aqueous solution of Pb^{+2}, a white precipitate will be formed only when the solution is cold.

$$Pb(NO_3)_2 + 2HCl \longrightarrow PbCl_2\downarrow + 2HNO_3$$

White ppt.

This precipitate dissolves in hot water but separates again as needle-like crystals when the solution is re-cooled

2) Potassium chromate solution:

It forms a yellow precipitate of **$PbCrO_4$** which is insoluble in acetic acid and ammonia solution but soluble in hydroxides and in HNO_3.

3) Sodium hydroxide:

It forms a white precipitate of **$Pb(OH)_2$** which dissolves on adding excess of NaOH to form Na_2PbO_2.

$$Pb(NO_3)_2 + 2NaOH \longrightarrow Pb(OH)_2\downarrow + 2NaNO_3$$

$$Pb(OH)_2 + 2NaOH \longrightarrow Na_2PbO_2 + 2H_2O$$

Reactions of the Aqueous Solution of Ag^+:

1) HCl (diluted):

A white precipitate of **AgCl** is formed, this precipitate is insoluble in water and acids including HNO_3 but soluble in ammonia solution because it forms a complex ion.

2) Potassium chromate:

Red precipitate of **Ag_2CrO_4** is formed, insoluble in acetic acid (diluted), but soluble in diluted HNO_3 and ammonia solution.

3) Sodium hydroxide:

Brown precipitate of **Ag_2O** is formed, insoluble on addition of excess of the precipitant (NaOH solution).

$$AgNO_3 + 2NaOH \longrightarrow Ag_2O\downarrow + 2NaNO_3 + H_2O$$

Qualitative Analysis of A mixture of Group I Ions

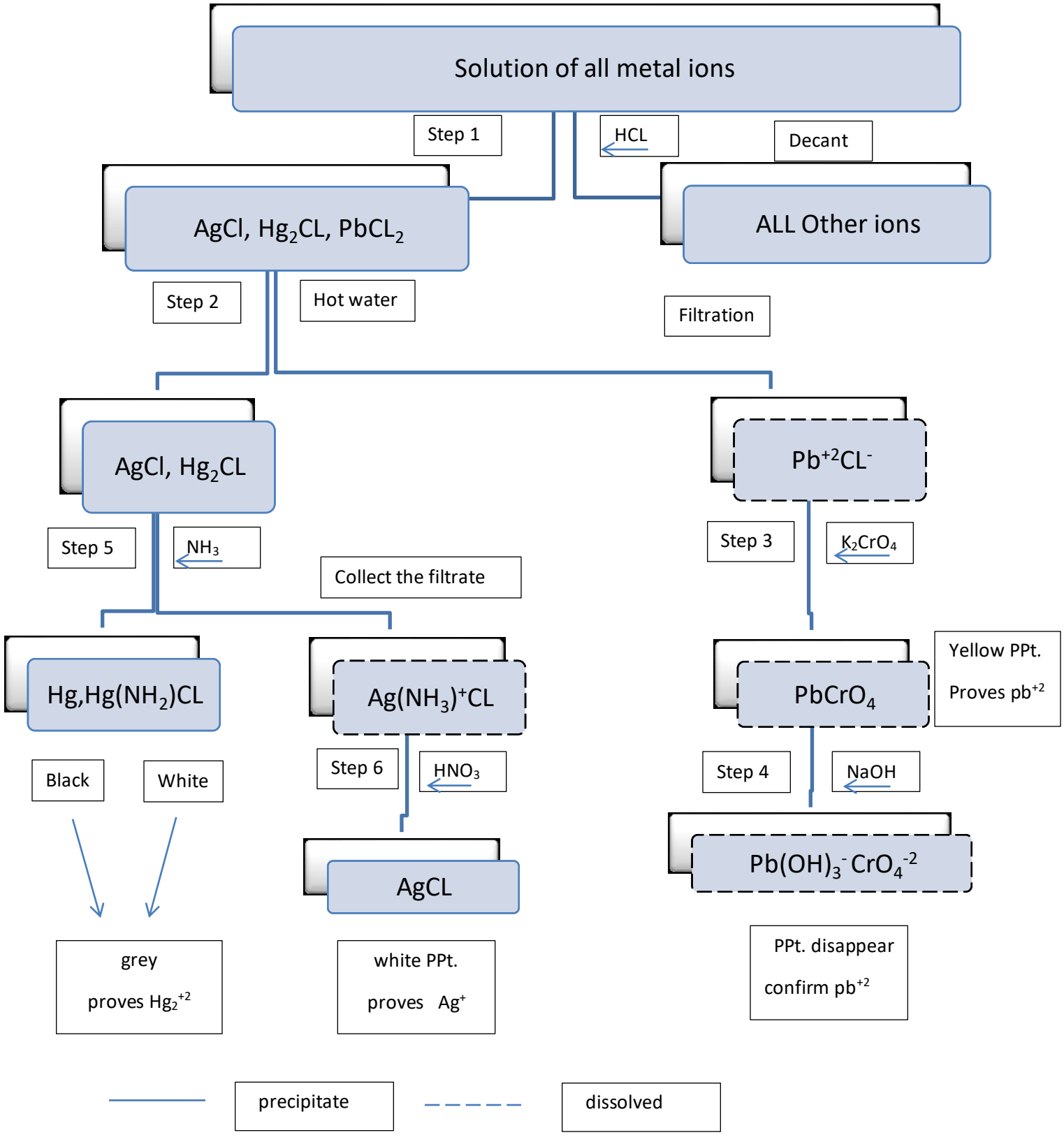
(Ag^{+} , Hg_2^{+2} , Pb^{+2})

Analysis means the separation and identification of a substance in a mixture; Analysis could be either Quantitative or Qualitative.

Quantitative analysis means the determination of the actual amount of the substance in a measured volume of unknown sample. Its units are gm/100ml, meq/L, mmol/L or I.U/L. While **Qualitative analysis** is used to detect whether a particular substance is present in an unknown sample or not, i.e. it is yes or no test.

There are many analytical methods used to separate a substance from a mixture, the simplest one is based on the solubility behavior of that substance in different solutions (which depend on the ionic equilibria). So that by adding a precipitating agent and filtration you can separate a particular substance and by knowing its reactional behavior with a second agent you identify it. The aim of this experiment is to learn a systematic procedure to separate ions and then to identify them.

Analysis Chart for Ag^{+} , Hg_2^{+2} , Pb^{+2}



Procedure

1. Place 20 drops of mixture solution in a test tube, add20 drops of diluted HCL (drop by drop) till a precipitate is formed.

2. Decant the supernatant (clear fluid), then add 5-10 ml. of hot distilled water to the precipitate.

3. Heat the tube in hot water bath nearly to boiling for 2-3minutes, then filter while still warm.

4. To the filtrate add 5 drops of 1 M K_2CrO_4, a yellow precipitate indicates the presence of lead ion, sometimes to confirm it more, add 5 drops of NaOH to the test tube (precipitate will dissolve).

5. Add 5-8 drops of 6 M NH_3 to the precipitate and take the filtrate, a grey precipitate indicates mercury ion.

6. Acidify the filtrate from No.5 with 5-10 drops of 4 M HNO_3, a white precipitate confirms the presence of silver ion.

Acid –Base Titration

Titration is a technique where by one solution is chemically balanced with another solution. In acid –base titration an acid solution is balanced with a base for the purpose of determining the concentration of one or the other.

Neutralization is the complete reaction of an acid with a base to form water and salt.

$$NaOH + HCl \longrightarrow H_2O + NaCl$$

When titration is used to standardize a base solution a carefully measured volume of an acid solution of known concentration (standard solution) is used, a few drops of an indicator are add. acid –base indicators are complex organic compounds that oxist in at least two differant colored forms. The colored form is dependent upon wether they are in acid, base or neutral conditions. As an example the indicator phenolphathalein is colorless under acid condition and pink under basic condition. The first change in color of the indicator is the end of titration (the end point or equivalence point).

The concentrations of solutions used in acid –base titration may be expressed in terms of molarity (M) or normality (N).

Molarity is the number of moles of solute in one liter of solution (M:moles/liter)

Normality is the number of the equivalent weight of a compound in a liter of solution. (N: equivalent weight/ liter).

$$\text{An equivalent weight of a acid} = \frac{\text{gram molecular weight}}{\text{No. of (H) groups that can be replaced}}$$

$$\text{An equivalent weight of a base} = \frac{\text{gram molecular weight}}{\text{No. of (OH) groups that can be replaced}}$$

At end of titration the number of equivalents of acid equals the number of equivalents of base:

$$\mathbf{N_a\ V_a = N_b\ V_b}$$

Procedure:

Standardization of HCl solution

1- In a conical flask of 100 ml. add 5 or 10 ml. of HCl.
2- Rinse and fill a clean 25ml. burette with 0.1 N NaOH, be careful that no air bubbles are in tip of the burette.
3- Record the initial reading to the nearest 0.01 ml. on the data sheet.
4- Add 2 drops of phenolphthalein indicator solution and mix by swirling.

Slowly add NaOH solution from the burette to the titration flask, the first drops yiel

5- d a pink color which quickly disappears upon swirling.
6- Continue to add the NaOH solution until a very slight pink color persists. This is the end point.
7- Record the reading from the burette as the final reading.
8- The difference between the intial and the final readings is the volume.

Note:

A dark pink color indicates an over titration.

Calculation:

Calculate the N of HCl solution using $\mathbf{N_a V_a = N_b V_b}$

Reagest:

1- Phenolphthalein indicator: 2 gm in 50% ethanol in water.
2- 0.1 N NaOH .
3- HCl solution

Quantitative analysis of Vinegars (Weight-Volume Percent)

Most vinegar contains 4-5 % (W/V) acetic acid (CH3COOH). Flavoring and coloring agents may also be added. The most usual way of expressing solution strength is the weight per unit volume percent (W/V %). It refers to a solution prepared by dissolving a measured weight of the solute (gm.) in a solvent to give 100 ml. of the final solution.

$$\text{weight} - \textit{Volume percent} = \frac{\text{Wt solute , gm}}{\text{Vol. solution , ml}} \times 100\%$$

The weight –volume percent of acetic acid in vinegars is determined by titrating a measured volume of vinegar to a phenolphthaline end point with a measured volume of a standard NaOH solution.

$$\mathbf{CH3COOH + NaOH \longrightarrow CH3COONa + H2O}$$

At the end point the number of equivalents of NaOH equals the number of equivalents of CH3COOH. By the end of this experiment the student should know how to calculate the concentration of solution by comparing the weight of solute with the total volume of solution. The units used to express the concentration of solute in blood and urine is milligram per 100 ml.

Procedure:

1- In a conical flask add 5 ml. of vinegars solution.
2- Rinse and fill a clean 25ml. burette with 0.1 N NaOH, be careful that no air bubbles are in tip of the burette.
3- Record the initial reading on the data sheet.
4- Add 2 drops of phenolphthalein indicator and titrate versus standard NaOH until the end point occurs.

Calculation:

Equivalent weight of CH3COOH = 60.05 gm/eq. (GEW).

$$\mathbf{N_a\ V_a = N_b\ V_b}$$

$$\mathbf{Na} = \frac{\mathbf{Nb\ Vb}}{\mathbf{Va}}$$

No. of grams (wt/L) = Na × (GEW) a

$$\text{The percent of CH3COOH in vinegar} = \text{no. of gram} \times \frac{100}{1000}\%$$

Organic Compounds

Functional Group Identification

Many organic compounds contain an atom or group of atoms that substitute for hydrogen or carbon in a basic hydrocarbon. The atom or group of atoms is commonly referred to as **functional group**. Each imparts characteristic chemical properties to the substituted hydrocarbon.

The aim of this experiment is to study the chemical properties of several functional groups: alcohols, carboxylic acids, phenols, aldehydes and ketones.

Alcohols

They are hydrocarbons in which an (-OH) replaces a hydrogen atom. Alcohols are classified as primary (ethanol), secondary (isopropanol), and tertiary (tertiary butanol).

Oxidation:

All alcohols (except tertiary alcohols) are oxidized by potassium permanganate ($KMnO_4$) and potassium dichromate ($K_2Cr_2O_7$) to give aldehydes or ketones.

Procedure:

A. Oxidation by $KMnO_4$:

1) Place 10 drops of sample in 3 separate test tubes.
2) Add 5 drops of 2% $KMnO_4$ to each tube and heat and notice:
    a. In case of primary alcohol a brown precipitate will be formed. Primary alcohol will be oxidized to aldehyde then further into carboxylic acid.
    b. Secondary alcohol will be oxidized to ketone and a brown precipitate will be formed (MnO_2).
    c. No reaction with the tertiary alcohol.

B. Oxidation by $K_2Cr_2O_7$:

2-3g of $K_2Cr_2O_7$ is dissolved in few mls of water then continue the volume to 500 ml with conc. H_2SO_4 with cooling.

1) Place 10 drops of sample in 3 separate test tubes.
2) Add 5 drops of $K_2Cr_2O_7$ solution, heat and notice that:
    a. Primary alcohol: a green solution will be formed.
    b. Secondary alcohol: a green solution will be formed.
    c. Tertiary alcohol: no reaction.

$$\mathbf{R\text{-}CH_2\text{-}OH + K_2Cr_2O_7 \xrightarrow{H2SO4} R\text{-}CHO + CrO_3 + K_2SO_4}$$

Phenols

Are aromatic hydrocarbons consisting of a benzene ring attached to –OH group. They are more reactive than alkanes but less reactive than alkenes and alkynes.

They are soluble in 5% sodium hydroxide, but insoluble in 5% NaHCO3.

They react with a solution of bromine in carbon tetrachloride by substitution and an equivalent quantity of hydrogen bromide is evolved. they yield intense coloration (blue, green, red, or purple) when treated with a solution of ferric –chloride.

Ferric chloride test:

1) To small quantity of phenol add one drop of $FeCL_3$.
2) Complex coloration will be formed according to the type of phenol used.

Carboxylic Acids

Organic acids are prepared by the oxidation of primary alcohols or aldehydes with strong oxidizing agent e.g. $KMnO_4$.

Carboxylic acids are soluble in 5% sodium hydroxide and 5% of sodium bicarbonate ($NaHCO_3$) (The latter reaction is accompanied by the evolution of CO_2)

Carboxylic acids are non-reactive towards a solution of bromine in CCL_4. They give positive test with ferric chloride $FeCL_3$ solution.

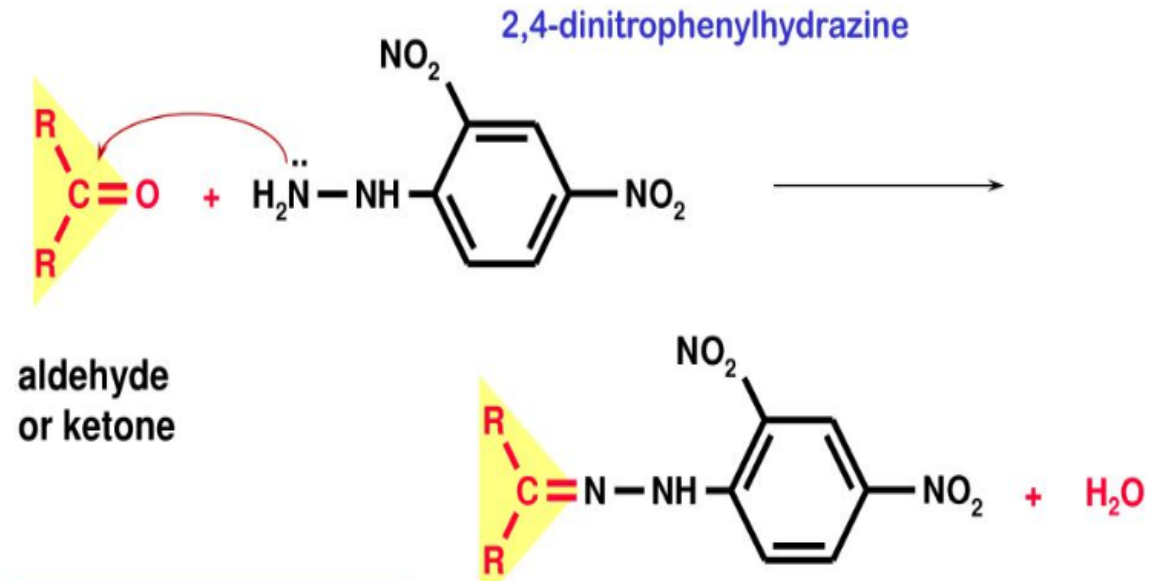
Ferric chloride test:

1)To 10 drops of carboxylic acid add 3 drops of $FeCL_3$.

2)Observe the color change.

Aldehydes and Ketones

Both aldehydes and ketones contain the carbonyl group, hence a general test for carbonyl compounds will immediately characterize both classes of compounds. The preferred reagent is 2.4- dinitrophenyl hydrazine (DNP) which give insoluble phenyl hydrazone with carbonyl compounds.



Test for carbonyl group:

1) To 10 drops of the sample add 5 drops of 2.4- dinitrophenyl hydrazine.
2) Yellow-Orange precipitate will be formed.

Reagent: (2.4- dinitrophenyl hydrazine)

- Dissolve 0.25g of 2.4- dinitrophenyl hydrazine in 42 ml of conc. HCL and 50 ml of water, heat in water bath and complete the volume to 250 ml

Aldehydes can be oxidized to carboxylic acids using oxidizing agents such as Tollen's and Fehling's reagents.

Tollen's Reagent: (freshly prepared)

1) Add few drops of 10% NaOH to $AgNO_3$ (10%), a brown precipitate will be formed (Ag_2O).
2) Then add drop by drop dilute ammonia till all Ag_2O get dissolved

Procedure:

1) Add few drops drops of aldehyde sample to Tollen's reagent.
2) Warm in hot water bath for about (2-5) min.
3) A silver mirror is deposited on the walls of the tube.

$$\mathbf{R\text{-}CHO + 2\ [Ag\ (NH_3)_2]^+ + 3OH^- \longrightarrow R\text{-}COO^- + 2Ag\downarrow + 2H_2O + 4NH_3}$$

(silver mirror)

pH-Metery

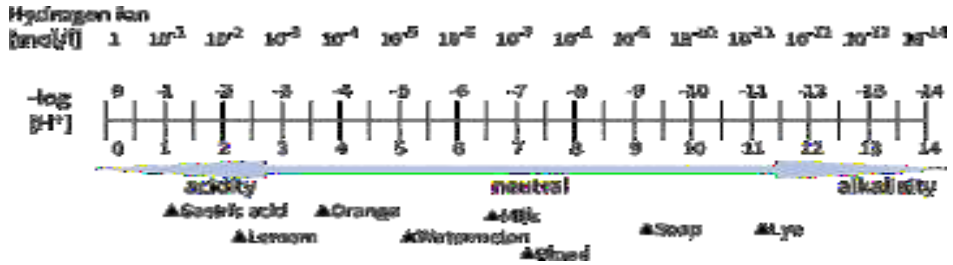
pH is the negative logarithm of the molar concentration of hydrogen ion. pH= -

$$\log (H^+)$$

Water dissociates very slightly producing equal concentration of H^+ and OH^-:

$$H_2O \rightleftarrows (H^+) + (OH^-)$$

At 25 C° the $(H^+) = (OH^-) = 1 \times 10^{-7}$ mole/L so has pH equals to 7. Addition of an acid which either releases H^+ or absorbs OH^- ions increases the (H^+) and produces pH less than 7. A base which either releases OH^- ions or absorbe H^+ decreases the (H^+) in solution and produces pH greater than 7.



pH is measured using either acid-base indicators or a pH-meter. A pH- meter measures the potential difference between two electrodes of an electro-chemical cell. One is a reference electrode usually a calomel electrode which has a fixed potential. The other is the indicating electrode, called the glass electrode and its potential depends on the hydrogen ion concentration in solution.

Buffers

A buffer solution is amixture of weak acid and its conjugate base or a weak base and its conjugate acid in aqueous solution. Such a solution resists a pH change when large amounts of H^+ (from a strong acid) or large amount of OH^- (from a strong base) are added. This solution must be able to consume H^+ and OH^-without undergoing large pH changes.

Examples of buffer mixtures:

CH_3COOH and CH_3COONa

NH_4OH and NH_4C1

Medical applications

The pH of various body fluids is maintained by buffers. There are several different buffer systems in the body such as the phosphate and bicarbonate buffers:

Dihydrogen phosphate HPO_4^- / Monohydrogen phosphate HPO_4^{-2}

Carbonic acid H_2CO_3 / Bicarbonate HCO_3^-

Normally in body fluids such as plasma there is 24 mEq/L of bicarbonate ions to 1.2 mEq/L of carbonic acid. The pH of the blood is within normal range of 7.35-7.45 when this ratio is maintained.

$$CO_2 + H_2O \rightleftarrows H_2CO_3 \rightleftarrows HCO_3^- + (H^+)$$

The acidic condition of the blood signified by pH less than 7.35 is called acidaemia. The alkaline condition of the blood greater than 7.45 is called alkalaemia. Death occurs if the pH of the blood is more acidic than 6.8 or more basic than 7.8

Procedure

1. Prepare 20 ml of 0.1 N acetic acid in 50 ml beaker, Stir gently and measure the pH then record your result.
2. Add 2-3 ml of 0.1 N NaOH from a 25 ml burette, mix for 30 seconds and wait for an additional one minute.
3. Repeat the addition of 2 ml of base from burette, measure the pH after each addition until you notice that a rapid change starts to occur in the pH readings. Now be careful in addition of the base.
4. Now add 1 ml of the base each time and measure the pH after each addition. Get five measurements.
5. Plot the pH values as ordinate against the volume of the base as abscissae on graph paper to get the titration curve.
6. Identify the midpoint of the sharp change in the pH values and take it as the end point.

Carbohydrates

Carbohydrates are a class of organic compounds such as sugar, starch, glycogen, cellulose. Carbohydrates were considered to be hydrates of carbon because they contain hydrogen and oxygen in the ratio of 2:1 just as in water and the general formula of carbohydrates is $C_n (H_2O)_n$.

Carbohydrates are defined as aldehydes or ketones of polyhydroxy alcohols.

Carbohydrates are divided into three major categories:

1- Monosaccharides are simple sugars, that can't be changed into simpler sugars upon hydrolysis (reaction with H_2O) e.g. glucose, fructose, galactose, arabinose and xylose.

2- Disaccharides are double monosaccharides: on hydrolysis they yield two simple sugars. e.g. maltose, sucrose and lactose.

3- Polysaccharides are complex saccharides: on hydrolysis a polysaccharide yields many simple sugars.

Sugars which contain free aldehyde or ketone group have a reducing ability and are known as an aldoses or ketoses respectively.

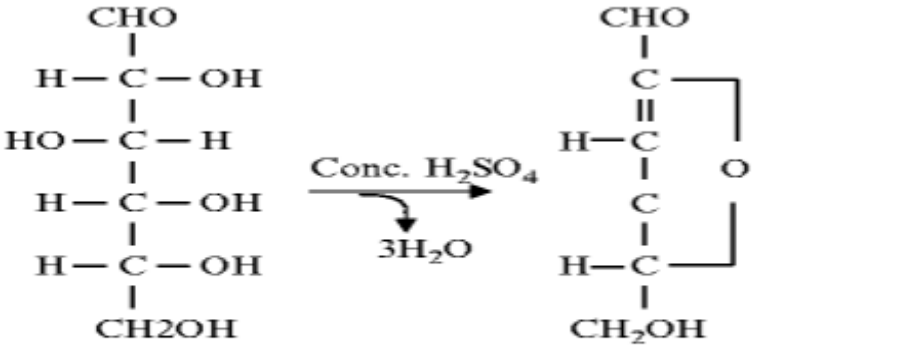
Carbohydrates form first by photosynthesis in plants from CO_2 and H_2O.

Carbohydrates can be a source of energy, stores of energy, structural units in the living body or are part of function molecule such as antibodies and certain hormones.

Qualitative Tests for Carbohydrates:

Molisch test:

Concentrated sulphuric acid H_2SO_4 hydrolyse glycosidic bonds to give monosaccharides which are then dehydrated to give furfural (from pentoses) or its derivative (hydroxy-methyl furfural) from hexoses, which in turn combine with sulphonated naphthol to give purple ring.



D-Glucose Hydroxymethyl furfural

Procedure:

1) Add 2 drops of α- Naphthol to10 drops of sugar solution in a test tube.
2) Carefully add about 20 drops of conc. H_2SO_4 down the side of the tube, two layers will be formed, observe the purple ring at the junction of the two layers.

Benedict's test:

Carbohydrates with a free aldehyde or ketone group have reducing properties in alkaline solution of copper (II) hydroxide $Cu(OH)_2$, forming rust-brown cuprous oxide precipitate.

$$Cu(OH)_2 \longrightarrow CuO + H_2O$$

$$2\,CuO + \text{reducing sugar} \longrightarrow Cu_2O\downarrow + \text{oxidized sugar}$$

Procedure:

1) Add 10 drops of the sugar solution to 15 drops of Benedict's reagent in a test tube.
2) Place the tube in a boiling water bath, observe any change in color or precipitate.

Barfoed's test:

This test is used to distinguish reducing monosaccharides from reducing disaccharides. Since the monosaccharides reduce cupric ions (Cu^{+2}) faster than disaccharides even in slightly acidic solution. The rate of reduction depends upon the concentration of cupric ions and the time of heating.

Procedure:

1) Add 5 drops of the sugar solution to 15 drops of Barfoed's reagent in a test tube.
2) Boil for 3 minutes, and allow to stand. Report your observations.

Seliwanoff's test:

This test is used to distinguish an aldohexose from ketohexose. Heating with HCL dehydrates hexoses to hydroxymethyl furfural (HMF). Ketohexoses yield large amount of HMF and at faster rate than do aldohexoses. HMF form red condensation product with resorcinol.

Procedure:

1) To 15 drops of Seliwanoff's reagent add 10 drops of sugar solution (fructose or glucose).
2) Place the tube in boiling water bath, record the time needed for your result for each sugar used. A red color develops with fructose (a ketohexose) and no such color with glucose (an aldohexose).

Notes:

1) Sucrose also gives a positive test because it is readily hydrolysed during the course of the test yielding fructose as one of the products.
2) The time factor in Seliwanoff's test is very important.

Bial's test:

This is specific for pentoses.

Procedure:

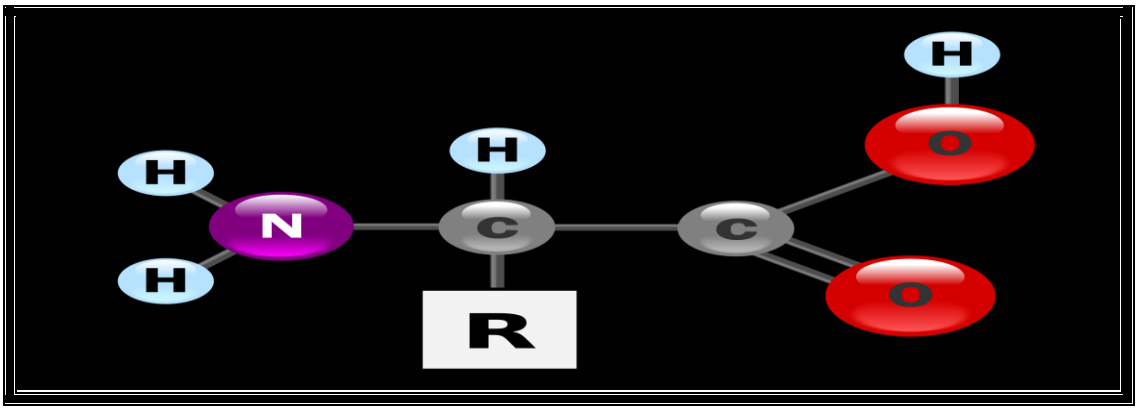
1) To 15 drops of Bial's reagent add 5 drops of pentose solution (Xylose or arabinose) and heat in boiling water bath for 3-4 minutes, to get a blue-green color.

Reagents:

1) **Benedict's reagent:** Dissolve 173 gm of sodium citrate, 100 gm sodium carbonate in 800 ml warm water. filter into a 1000 ml measuring cylinder and make up to 850 ml with distilled water. Meanwhile, Dissolve 17.3 gm of copper sulfate in about 100 ml H_2O and make up to 150 ml pour the first solution into a 2 L beaker and slowly add the copper sulphate solution with stirring.
2) **Barfoed's reagent:** Consists of 6.5% copper acetate in 1% acetic acid.
3) **Bial's reagent:** Is made by dissolving 0.3 g orcinol in 100 ml conc. HCL and then adding 5 drops of 1% solution of ferric chloride.
4) **Seliwanoff's reagent:** Is made by dissolving 0.05 g of resorcinol in 100 ml of dilute (1:2) HCL.

Color Test For Proteins And Amino Acids

Proteins are polymers or macromolecules, the building units or monomers of which are the alpha amino acids. An amino acid contains both a carboxyl group and an amino group, both of which are attached to the alpha carbon atom of the acid.



Amino acid structure

Beta – amino acids and gamma-amino acids also occur in nature but not as components of proteins. With the exception of glycine, all α- amino acids are asymmetric, i.e., four different groups are bonded to the α- carbon atom, so are optically active. Also an α- amino acid can be L-isomers or D- isomer. In natural proteins of higher organisms, only the L-isomer of one or more of approximately 20 amino acids are present.

When an amino group and a carboxyl group of two amino acids combine the bond is called the peptide bond and the constituent amino acids are termed amino acids residues.

A peptide consists of two or more amino acid residues linked by peptide bonds. Peptides of more than ten amino acid residues are termed polypeptides. With the increase in molecular weight the proteins will form. The dividing line between large polypeptides and small proteins is usually taken to be between MW 8000 and 10000.

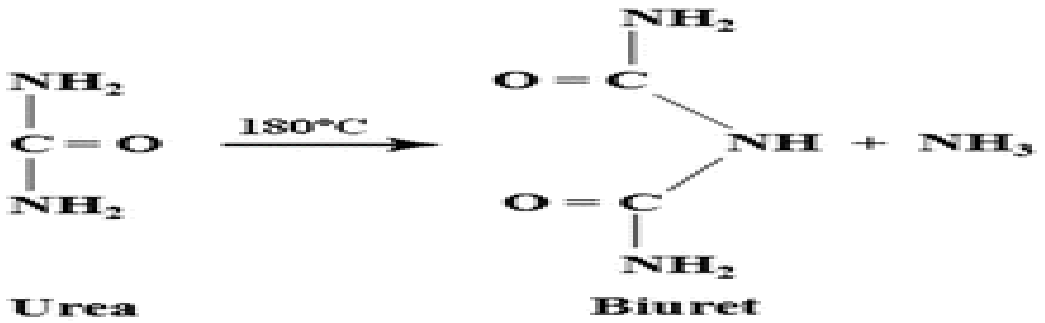
Proteins and amino acids can be analyses qualitatively and quantitatively. Different proteins and amino acids may be separated by chromatography or electrophoresis before individual testing.

Principles of the color test:

1- Biuret test:

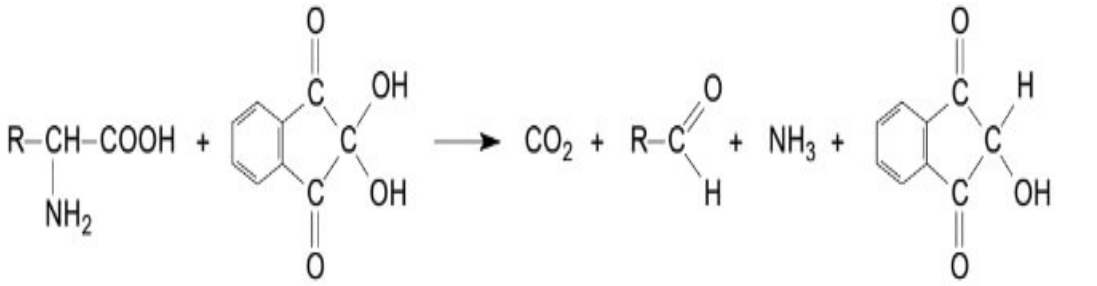
Compounds containing two or more peptide bonds react with cu (II) ions in alkaline solution to form a purple (pink to violet) color. the color is due to coordination complex formation or chelation of the cu (II) ion and the carbonyl oxygen and amid nitrogen atoms of the peptide bond . At least two peptide bonds (tri- peptide) are required for a positive test.

The name of this test comes from the compound biuret, which due to structural similarity to peptide bond also gives a typically positive reaction.

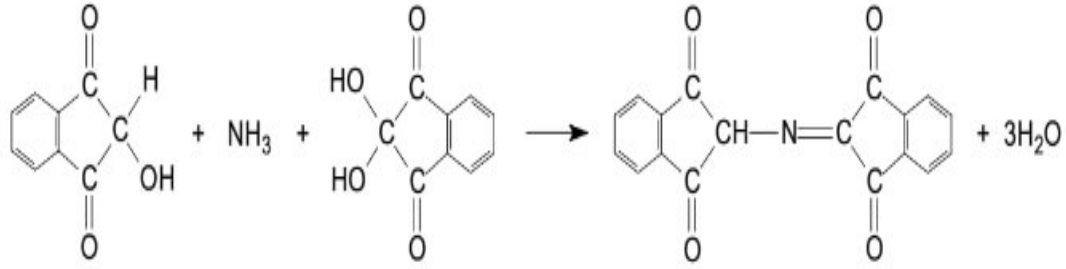


2- Ninhydrin test:

Ninhydrin is a powerful oxidizing agent which causes oxidative decarboxylation of alpha – amino acids producing CO_2, NH_3 and an aldehyde. The liberated ammonia reacts with two equivalents of ninhydrin to produce a blue or a purple colored product.



Ninydrin Reduced ninydrin



Reduced ninhydrin Ninhydrin Violet-blue condensation product

The reaction depends on presence of free amino group so Proline and hydroxyproline which lack a free amino group yield a yellow color with ninhydrin. Peptides and proteins, owing to their free terminal amino groups yield a positive test.

Xanthoproteic test:

Nitration of the aromatic ring of an amino acid by hot concentrated nitric acid produces a yellow color so tyrosine and tryptophan give a positive test.

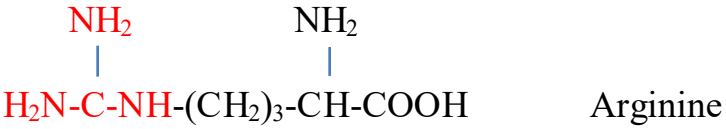
Under the conditions of the test, phenylalanine doesn't produce the color. However, if one adds a small amount of concentrated sulfuric acid together with the nitric acid one will obtain a positive result.

3- **Hopkins-cole test for tryptophane**:

Tryptophan, due to its indole ring, condenses with the aldehyde glyoxylic acid (CHO-COOH) in presence of concentrated H_2SO_4 to produce a purple to blue color.

4- **Sakaguchi test for arginine**:

The only amino acid containing the guanidine group is arginine and this reacts with alpha-naphthol and an oxidizing agent such as bromine to give a red color.



Arginine

Guanidine group

This is a sensitive test for free and comnined arginine.

Procedure:

1- Biuret test:

Samples: albumin, tryptophan.

Mix 1 ml of each sample with (**1 ml**) of 10% NaOH and (**0.5 ml**) of 1% $CuSO_4$. Observe the color produced.

2- Ninhydrin test:

Samples: albumin, tryptophan, Proline.

A- Mix **1 ml** of each sample with **1 ml** of 0.1% aqueous ninhydrin.

B- Heat the tubes in boiling water for 3-4 minutes and observe the color after standing for few minutes.

3- Xanthoproteic test:

Samples: albumin, tryptophan, Proline.

A- Mix **1 ml** of each sample with **1 ml** of concentrated HNO_3 .

B- Heat for 1-2 minutes in boiling water, observe any change in color.

4- Hopkins-cole test:

Samples: albumin, tryptophan,arginine.

A- Mix **1 ml** of each sample with **1 ml** of Hopkins-Cole reagent. Mix thoroughly.

B- Carefully add **1 ml** of concentrated H_2SO_4 along the side of the tube so that the two liquids from separate layers.

C- Notice the ring at the junction.

5- Sakaguchi test:

Samples: Arginine, albumin.

A- Mix **2ml** of each sample with **0.5ml** of 10% NaOH and **0.5 ml** of α- naphthol solution.

B- After 3 minutes

C- add 2-4 drops of NaOBr and observe the color change.

Materials and reagents:

1- 1% solution of alanine, arginine, tryptophan, albumin, gelatin.

2- Reagents for sakaguchi test :

10% NaOH , 0.02 % α- naphthol

Sodium hypobromite(2 grams Br_2 in **100 ml** of 5% NaOH).

3- Reagent for Hopkins-Cole test :

10 gm. of powdered magnesium are covered with shaking by some distilled water. **250 ml** of saturated oxalic acid are adding slowly with cooling under tap water. Filtrate to remove the insoluble magnesium oxalate. Acidify the filtrate with acetic acid to prevent partial precipitation of the magnesium on long standing and make up to a liter with distilled water. This solution contains only the magnesium salt of glyoxylic acid.

Lipids

Lipids a heterogeneous group of compounds which share the property of being relatively insoluble in water and soluble in organic solvents such as alcohols, acetone, benzene, chloroform, carbon tetra chloride and ether. Lipids are classified into three major groups:

Simple lipids: are esters of fatty acids:

$$\textbf{Simple lipid} + \textbf{H}_2\textbf{O} \xrightarrow{Hydrolysis} \textbf{Fatty acids} + \textbf{alcohol}$$

If the alcohol produced is glycerol then, the simple lipid is called a fat

Compound, lipids: On hydrolysis they yield some additional groups in addition to fatty acids and alcohol.

e.g.: phospholipids and glycolipids.

Derived lipids: These are compounds derived from hydrolysis of simple and compound lipids, such as the fatty acids, glycerol and other alcohols, and steroids.

Lipids that are solid at room temperature are called fats, those that are liquid at room temperature is called oils. Lipids serve as the main energy reserve for living systems, form parts of cell membranes, and regulate the activities of cells and tissues.

Tests for lipids

1- Grease stain test: This is a general test given by all lipids and is used in their identification. Fats, and oils have the property of filling the meshes of filter paper causing the paper to be more permeable to light, and unwettable by water.

Procedure:

1-Add one drop of the oily material on a piece of filter paper.
2- Examine the paper in front of a source of light, the paper becomes translucent to light.

2- Iodine test:

Unsaturated fatty acids can add halogens (Iodine) at the double bonds to form halogenated derivatives, while saturated fatty acids cannot. Oils contain a higher percent of unsaturated fatty acids than solid fats, so oils can react with more iodine (Iodine solution is made by dissolving 3 gm. of KI in distilled water and add 1 gm. of iodine, make the volume to: 100 ml.).

Procedure

1- Dissolve 3 drops of the oily material or a little piece of solid fat in 3 ml. chloroform drop by drop.

2- Count the number of iodine drops needed to produce a permanent color.

3- Reaction test: This test can be used to differentiate triacylglycerol (triglycerides, TG) from fatty acids. TG are chemically neutral while fatty acids are acidic due to their free carboxylic group and can decolorize the alkaline red color of phenolphthalein.

Procedure:

1-Dissolve a small amount of a TG and a small amount of a fatty acid, each in about 3 ml. of ether.

2- Add the alkaline alcoholic phenolphathalein solution dropwise (2gm. of phenolphthalein in 50% ethanol in water then add 2-3 ml of NaOH solution).

3-Note that the red color disappears with the fatty acids and remains with the TG.

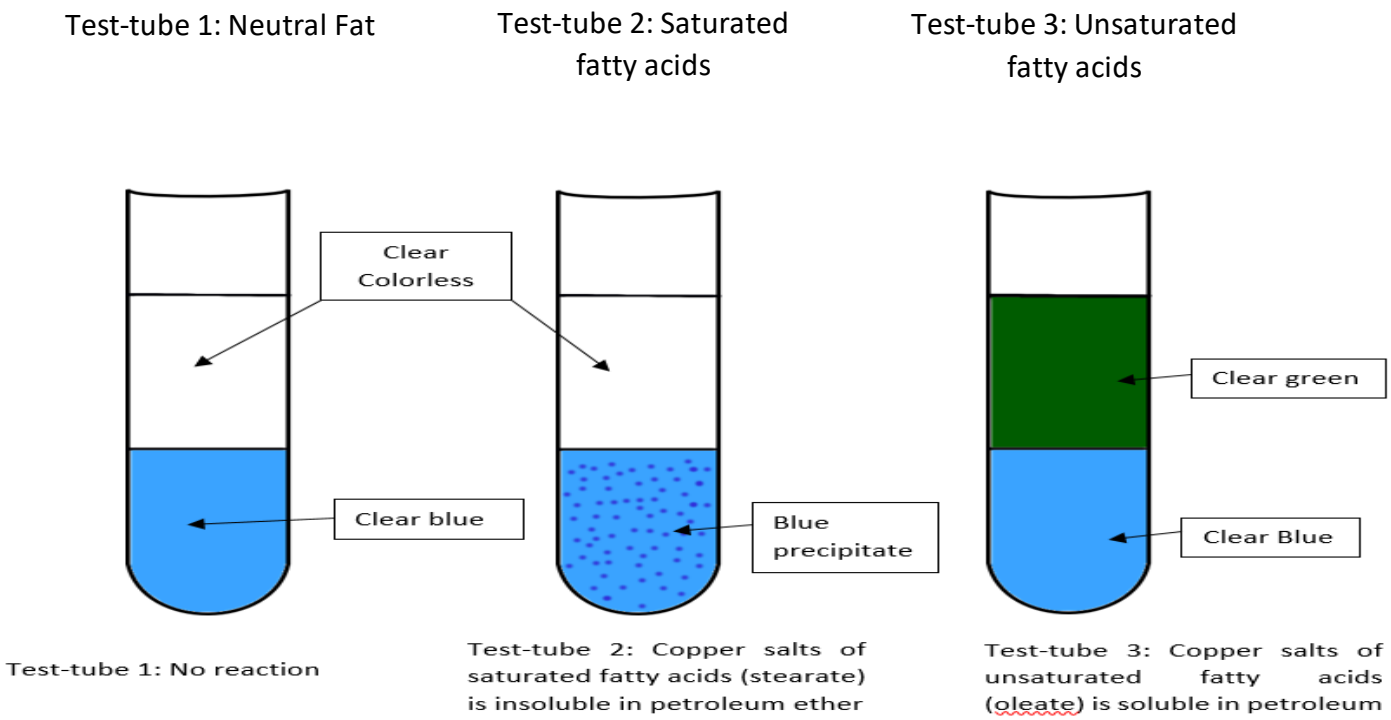
4- Copper acetate test: This test is used to differentiate between TG, saturated fatty acids and unsaturated fatty acid. Neutral TG do not react with a solution of copper acetate (5%), however, saturated fatty acids give rise to blue or green precipitate in the water layer, and unsaturated fatty acids give copper salts that are green in color, soluble petroleum ether.

Procedure:

1-Dissolve few drops of the oily material or a small piece of the solid fat, each in a separate tube, in about 3 ml. petroleum ether.

2- Add about 3 ml of the copper acetate solution and shake.

Allow to stand, notice that there is no change in the two layers in the case of TG (Fats and oils), while a blue precipitate in the water layer in the case of saturated fatty acids (palmitic, stearic) and a green color in the petroleum ether layer. in the case of unsaturated fatty acids (oleic, linoleic).



Note-: avoid shaking the two layers vigorously to avoid the formation of a heavy emulsion, shake the two layers gently, or mix by inversion.

5-Emulsification of Oils: The liquid TG is shaken with water both liquids are broken down to minute particles that give the mixture a milky appearance known as emulsion. The emulsion in this case is temporary because the oil particles will soon get together and become carried above the surface of water. When oils are shaken with a soap solution the fine particles of the oil will remain soluble in the solution. Soap is a sodium salts of fatty acids. The sodium parts become attracted to the water particles while the fatty acid long chains become attached to the oil particles, such emulsion is a permanent one and doesn't separate on standing.

Note: There is a group of chemical substances that can lower the surface tension of water leading to the formation of permanent emulsion when the water is shaken with oils. The most important of these substances are soaps, bile salts, proteins and phospholipids.

Procedure:

1- Shake up few drops of oil with a little water in a test tube.

2- Notice the formation of a temporary emulsion that separates on standing.

3- Add few drops of soap solution and shake again, the emulsion will be permanent.

6- Tests for cholesterol: Cholesterol is a constituent of all animal Cells. It is a colorless substance.

A-Salkowski test

1- Place in a dry test tube 2 ml. of 0.5% cholesterol in chloroform.

2- Add an equal volume of conc. H_2SO_4

3- Mix carefully and allow to stand. The upper (chloroform) layer becomes red, and the lower layer with a green fluorescence.

B-Liebermann-Burchard test:

1- Take 2 ml. of 0.5% cholesterol in chloroform in a dry test tube

2- Add 10 drops of acetic anhydride, then 2 drops of conc.

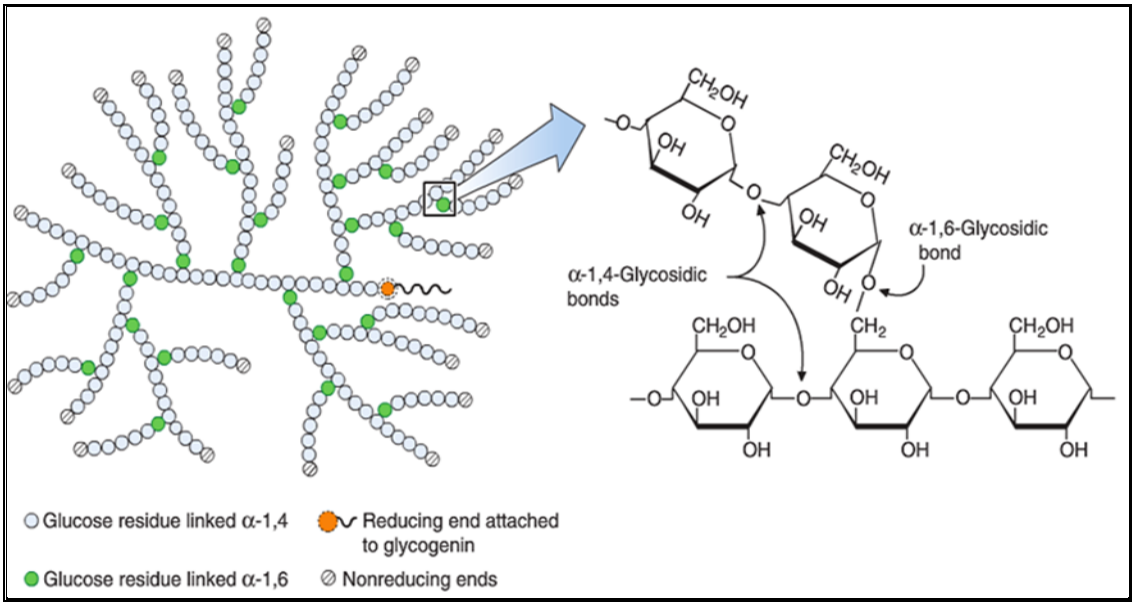
H_2SO_4 and mix.

3- The solution becomes deep blue which gradually turns green.

This test is commonly used in the estimation of blood cholesterol as it is very delicate.

Hydrolysis of starch by salivary amylase

Amylases are digestive enzymes secreted by the salivary glands and the exocrine pancreas for the purpose of breaking down complex polysaccharides (starch and glycogen) into simpler saccharides like glucose, maltose (2glocose molecules) and limit dextran (5-8 glucose molecules, oligosaccharides). The isoenzymes of amylase are the pancreatic amylase which is designated as (p) and appears to be only of pancreatic origin whereas the salivary amylase (s) may also secreted by the fallopian tube and certain tumours. The salivary amylase, ptyalin, begins the digestion in the mouth, continues briefly in the stomach until the pH drops too low. Digestion is then completed in the intestine by the attack of pancreatic amylase. Both types digest polysaccharide by breaking down the α, 1-4 glycosidic linkage between glucose molecules while the 1-6 bond leaved untouched. In this experiment the effect of amylase enzyme in saliva on starch suspension will be studied.



The structure of glycogen

Hydrolysis of starch by salivary amylase

Procedure:

1- Collect 5-10 ml of saliva in a beaker and filter through a wet filter paper.

2- Label 4 test tubes from (1-4).

3- In each tube add 20 drops starch +2 drops iodine solution + 3 ml distilled water and mix gently, and note the color.

4- Repeat the iodine test at 5 min, 10min and 20 min in tube 2, 3 and 4 respectively and note the color.

5- Compare the color produced in the 4 tubes.