Mosul University College of Dentistry

Practical Medical Chemistry
for
First Year Dental Students

By

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SAFETY IN THE LABORATORY

The laboratory is a dangerous place. To avoid injury to yourself or others, some rules must be made and followed. Furthermore you must keep in mind some of these potential danger when you are working in the laboratory.

1. All students are asked to take good precaution for their eyes. If a chemical should splash into your eye, wash the eye immediately with a large amount of water & the accident should be reported to the instructor.
2. If chemicals are spilled on your body, wash them off immediately with a large amount of water. You have to follow experimental instruction very carefully. Always be sure that you are using the wright reagent bottle. A serious accident, possibly an explosion, could result from using the wrong reagent.
3. Most chemicals are poisonous to some degree. Do not taste or smell chemicals unless you are instructed to do so, and even then do so cautiously. Also it is risky to drink from laboratory beaker. It is advisable to wash your hands after the laboratory class.
4. Inserting a glass tubing or a thermometer into rubber stoppers can be dangerous. Always lubricate the stopper hole with glycerol or water. Always wrap the glass tube with a cloth so that if it breaks, the tube is less likely to gouge your hands. The tube is less likely to break if grasped close to the stopper, and if it is inserted with a twisting motion.
5. Do not add water to small volume of concentrated (conc.) sulphuric acid. Also, do not add conc. Sulphuric acid to small volume of water. If this is done, the amount of heat generated will be very large, and may cause boiling with a consequent spattering. It is safe to add conc. Sulphuric acid to large amount of water.
6. Most heating in the first year course is done with a gas burner known as Bunsen burner, although more advance laboratory procedure may require electric heating mantles, hot plates, electrically heated baths and so on. The Bunsen burner usually has no gas needle valve and has an air vent that is adjusted by rotating a sleeve.

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7. If the air inlet is closed and the gas is lit, the flame will be large and luminous. The light is the radiation given off by hot carbon particles that are burned only partially. This flame is not very hot. If the air control is adjusted so that air is mixed with the gas before it gets in the flame, the flame will become less luminous and finally blue.

Precaution has to be taken when the test tube is heated. Hold the test tube with a test tube holder and heat just below the surface of the liquid, not at the bottom. Also, jiggle the test tube a little. If the tube is heated on the bottom, a bubble may form violently eject the entire content of the test tube. This is called bumping and can cause accident if the test tube is pointed at you or someone else. Therefore, when you are heating a test tube over a burner, do not point it at anyone, if the test tube must be heated just to boiling, it may be more convenient to heat it in a break of boiling water.

Filtration is the method of separating a solid from a liquid by passing the liquid through porous material. Decantation and centrifugation followed by Decantation are two other methods of separating solids from liquids.

In filtration, the porous filtering material can be paper, sintered glass or porcelain and so on. If the filter has larger pores, the liquid pass through it more easily and filtration will be faster. However, the larger the size of the pores in the filter, the larger the porticles of the solid that can pass through. The choice of method of filtration and the filtering material depend on the application in question.

In synthetic work, suction filtration using filter on Buchner funnel is very common. Buchner funnels usually are made of porcelain. The inside bottom of the funnel is flat plate with holes in it that supports the filter paper.

When using this type of funnel, place the appropriate size filter paper circle in place and turn on the vacuum, then wet the filter paper with liquid to be filtered so that the paper is held tightly to the funnel, and solid cannot possibly got under the edge of the filter paper, then filter.

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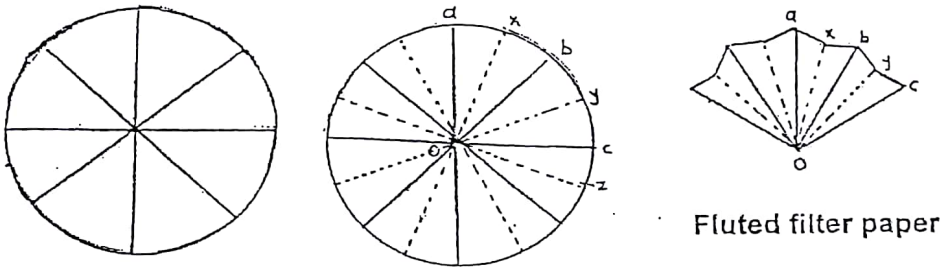
Solution of very volatile liquids, such as ether or hot solution, are not filtered very conveniently with suction, because the suction may cause excessive evaporation of the solvent which may cool the solution enough to cause precipitation of the solute. A convenient method is the use of fluted filter paper in a short stem conical funnel. The fluted filter paper has advantage of large surface area for filtering and makes it filter faster. The fluted filter often is used for filtering volatile solvent such as ether. To fold the filter paper, fold it in half, and then fold it half again to find the middle. Unfold to half-folded, and fold half-circle in accordant luck. Open the filter and place it in the funnel.



Fluted filter paper

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Laboratory No. 1:

RECRYSTALLATION & MELTING POINT DETERMINATION OF MIXTURE MELTING POINT

Solid organic compounds isolated from chemical reactions are seldom pure; they are usually contaminated with small amount of other compounds (impurities) which are produced along with the desired product. The purification of impure crystalline compound is usually effected by crystallization from a suitable solvent or mixture of solvents.

The purification of solids by crystallization is based upon differences in their solubility in a given solvent or mixture of solvents. In its simplest form, the crystallization process consist of:

(1) Dissolving the pure substance in some suitable solvent at or near its boiling point.

(2) Filtering the hot solution from the particles of insoluble material and dust.

(3) Allowing the hot solution to cool thus causing the dissolved substance to crystallize out.

(4) Separating the crystals from the supernatant solution "or mother-liquor".

The resulting solid, after drying, is tested for purity by melting point (m. p.) determination.

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The theory underlying the removal of impurities by crystallization may be understood from the following consideration. It is assumed that the impurities are present in comparatively small portion (usually less than 5%). Let the pure substance be denoted as "A" and the impurities by "B", and let the latter be assumed to be (5%). In most instances the solubility of A (S_A) and of B (S_B) are different in a particular solvent. Two cases will arise for any particular solvent:

(1) The impurity is more soluble than the compound which is being purified ($S_B > S_A$).

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(II) The impurity is less soluble than the compound ($S_B < S_A$).

It is evident that in case (I) several recrystallisations will give a pure sample of A, and B will remain in the mother-liquor. Case (II) can be more clearly illustrated by a specific example. Let us assume that the solubility of A and B in a given solvent at the temperature of the laboratory (25°C) are (10 gm) and (3 gm) per (100 ml) of solvent respectively. If (50 gm) of the crude material (containing 47.5 gm of A and 2.5 gm) of B; are dissolved in (100 ml) of the hot solvent and the solution allowed to cool to (20°C), the mother-liquor will contain (10 gm) of A and (2.5 gm) of B; (37.5 gm) of pure crystals of A will be obtained.

The most desirable characteristic of a solvent for re-crystallization are:

(a) A high solvent power for the substance to be purified at elevated temperature and a comparatively low solvent power at the laboratory temperature or below.

(b) It should dissolve the impurities readily to only a very small extent.

(c) It should yield (well-formed) crystals of the purified compound.

(d) It must be easily removed from the crystals of the purified compound, i.e. it posses a relatively low boiling point.

The student will have an impure compound which he or she must decide on a suitable solvent for crystallization & determine the melting point of the pure crystals.

Procedure A:

Solvent Evaluation

(1) In a small test tube place 0.5 ml (6-10 drops) of one of these solvents: water, ethanol, acetone, light petroleum, benzene or other solvents approved by your instructor.

(2) Add 2-3 mg of the impure compound and heat the solution carefully in a boiling water bath. All these solvents except water are inflammable (you must

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ensure that the vapour are kept away well from any free flame). Allow the solution to cool to room temperature (R.T.) and look for the growth of any crystal as the solution cools. Try to evaluate qualitatively the loss of material (is the compound too soluble) and the crystalline form (are well shaped?). Confirm your choice with your instructor.

Procedure B:

Purification of Benzoic Acid

(1) First of all determine the m.p. of the impure benzoic acid.

(2) Weigh out a sample of the impure acid (2 gm) into a 100 -ml conical flask (never carryout recrystallisation in a beaker).

(3) Add 5 ml (in this case water). And heat to boiling. Keep the solution gently boiling, and if all the solid does not dissolve add more solvent (water) a little at a time, always keeping the solution boiling, until all solid dissolve.

(4) Add an additional small amount of water to prevent crystal formation during the subsequent filtration.

(5) Filter off the hot solution through a fluted filter paper. Wash filter paper after filtration with small amount of hot water. Transfer the slurry of benzoic acid in warm water to aclean conical flask (do not heat the thick-walled filter flask).

(6) Heat the solution until boiling and the acid redissolve.

(7) Cover the mouth of the flask with watch glass, and stand it undisturbed until R.T. is reached and crystallization is completed. If not try to scratch the flask wall with a glass rod in order to commence crystallization.

(8) Filter off the pure crystals of benzoic acid and dry it. Weight the dried crystals and determine its melting point in the usual way, then submit your material for inspection in a sample tube with a label bearing your name.

In your report, calculate the recovery percentage of benzoic acid during crystallization.

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Procedure c:

Determination of Melting Point And Mixed Melting Points

Determine the melting points of one the following pure compounds:

(a) Salicylic acid (159°C) or phenyl urea (mono) (148°C).

(b) Succinic acid (185°C) or P-toly urea (mono) (180°C).

In order to gain experience in the determination of mixed melting points the following simple experiment should be carried out.

(1) Determine the melting point of pure cinnamic acid and pure urea.

(2) Approximately equal weight (Ca. 50 mg) of the two compounds are placed on a clean porous porcelain tile, these are now ground together and intimately mixed with the aid of the flat side of a micro-spatula.

(3) The melting point tube filled with this mixture is placed in the melting point apparatus alongside melting point tubes filled with each of the two components. In this way careful observation of the melting behavior of the mixture and of the pure components will clearly show the considerable depression of melting point.

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Laboratory No. 2:

PURIFICATION BY DISTILLATION AND BIOLING POINT DETERMINATION

The separation of organic compounds is one of the most important tasks for the organic chemist. Organic compounds seldom occur in pure form in nature or as products of a laboratory synthesis. The most common used method for purification of liquids is by distillation, a process by which one liquid can be separated from another liquid or from a nonvolatile solid.

When water is heated with a flame in a simple distillation apparatus (fig. 1), the vapor pressure of the liquid, or the tendency of the molecules to escape from the surface, increases until it becomes equal to the atmospheric pressure, at which point the liquid begins to boil. Addition of more heat will supply the heat of vaporization required for conversion of the liquid water to gas (steam), which rise in the apparatus, warms the distillation heat and thermometer, and flows down the condenser. The cool walls of the condenser remove heat from the vapor and the vapor condenses to the liquid form. Distillation should be conducted slowly and steadily and at a rate such that the thermometer bulb always carries a drop of condensate and its bathed in a flow of vapor. (Liquid and vapor are in equilibrium, and the temperature recorded is the true boiling point). If excessive heat is applied to the walls of the distilling flask above the liquid level, the vapor can become superheated, the drop will disappear from the thermometer, the liquid-vapor equilibrium is upset, and the temperature of the vapor rises above the boiling point.

Consider the separation of cyclohexane and toluene. When distilled in a simple distilling apparatus mixture of these two miscible liquids starts to distil somewhat above the boiling point of cyclohexane and stop distilling somewhat below the boiling point of toluene. All fractions of the distillate are mixtures and little separation of the two components is achieved. A better separation could be

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obtained by redistillation of each fraction, if redistilation is repeated the two components of the mixture will eventually be separated.

Micro-scale Determination of Boiling-Point:

The following alternative methods are recommended:

(a) Draw one drop of the liquid in to a capillary tube so that the drop is about (1 cm) from one end. Hold the tube horizontally and quickly seal this end in burner. Attach the tube (with the open end upwards) to a thermometer in the melting point apparatus so that the trapped bubble of air in the capillary tube is below the surface of bath-liquid (fig. 2) Now heat the bath, and take as the b.p. of the liquid that temperature of which the upper level of the bubble reaches the level of the surface of the bath liquid.

(b) Prepare a fairly wide capillary tube A (fig. 3) (ca. 4 mm x 8 cm). Using a fine pipette insert about (1 mm). Length of the liquid into the bottom of the tube. Now place in the tube A a fine inverted melting-point tube B of about (1 mm) diameter, sealed at the upper end. Fasten the capillary tube to the thermometer by means of rubber band and place in a melting-point apparatus. Heat slowly until a stream of bubbles rise from the bottom of the capillary tube, then remove the source of heat and, as bath cools, take as the b.p. that temperature at which the liquid recedes into the inverted tube B.

Fractional Distillation:

Unless the boiling points of the components of a mixture are widely different it is usual to employ a fractionating column to attempt the separation of liquid mixtures by distillation (fig. 4).

A fractionating column consists essentially of a long vertical tube through which the vapour passes upward and is partially condensed; the condensate flows down the column and is returned eventually to the flask. Inside the column the returning liquid is brought into intimate contact with the ascending more volatile component at the expense of the liquid, in an attempt to reach

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equilibrium within the liquid-vapour system. The conditions necessary for a good separation are:

(a) Comparatively large amounts of liquid continually returning through the column.

(b) Thorough mixing of liquid and vapour.

(c) a large active surface of contact between liquid and vapour.

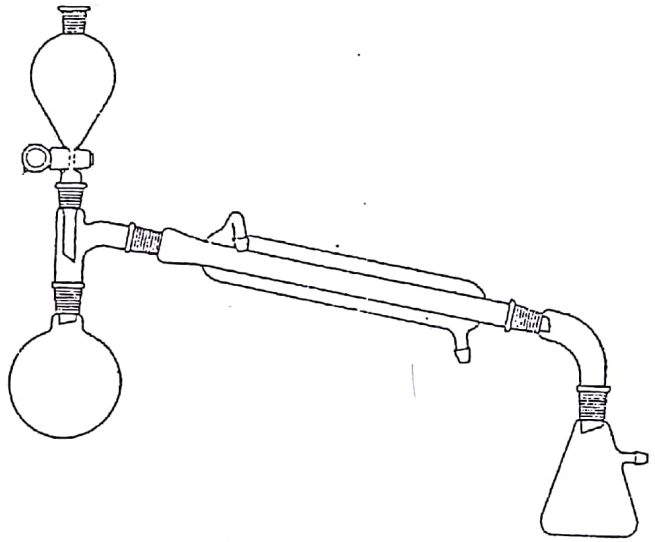

Fig. 1

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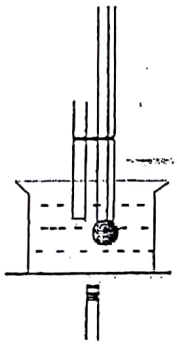

Fig. 2

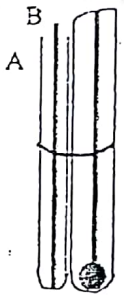

Fig. 3

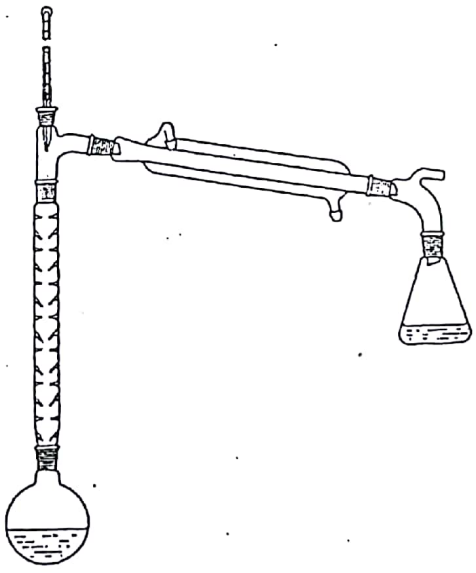

Fig. 4

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3:

REACTIONS OF HYDROCARBONS

Tests for unsaturation:

The following reactions are used as the general tests for the presence of double or triple bonds joining carbon atoms in an organic compound:

1. Bromine test:

The bromine test is applied first. The organic compound if liquid is either treated with 2-3 drops of bromine water or 2-3 drops of bromine in carbon tetrachloride; if the organic compound is a solid, it should be first dissolved in cold carbon tetrachloride. The rapid absorption of bromine (and consequent disappearance of the red colour) is a strong indication that the compound is unsaturated and is therefore undergoing direct addition reaction of bromine:

$$>C=C< \xrightarrow{Br_2 / H_2O} -\overset{Br}{\underset{|}{C}}-\overset{|}{\underset{OH}{C}}-$$

$$\xrightarrow{Br_2 / CCl_4} -\overset{Br}{\underset{|}{C}}-\overset{|}{\underset{Br}{C}}-$$

N.B. Since, however, some compounds, such as aniline and phenol react very rapidly with bromine by substitution, the bromine test should whenever possible be confirmed by the alkaline permanganate test.

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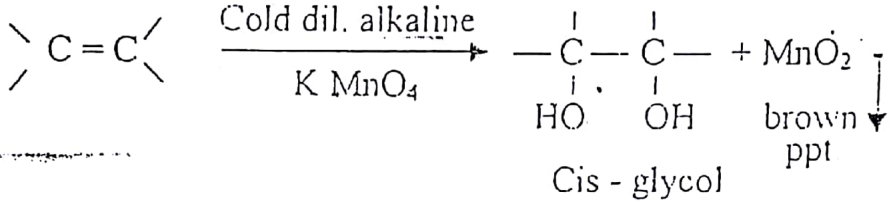
2. Alkaline permanganate test:

If the compound is soluble in cold water, its aqueous solution (after neutralization if acidic) is shaken with adilute solution of potassium permanganate containing sodium carbonate. The conversion of the purple permanganate to the green manganese dioxide confirms the results of bromine test, since the alkaline permanganate is unaffected by possible substitution reaction:



Cis - glycol

N.B. Many readily oxidisable compounds (e.g. aldehydes) will also decolourise cold alkaline permanganate, so both are tests necessary to confirm the presence of double or triple bond.

Preparation of Acetylene:

Acetylene C_2H_2 gas can be readily obtained by the action of water on calcium carbide CaC_2; since, however, commercial calcium carbide contains traces of calcium sulfide, phosphide and nitrite, the acetylene gas obtained should be purified before being used:

$$CaC_2 + 2\ H_2O \longrightarrow Ca(OH)_2 + C_2H_2 \uparrow$$

Procedure:

Set up the apparatus shown in (fig. 1) Put about (5 g) of calcium carbide (calcium carbide always stored in kerosene, explain why?) in round – bottomed

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flask (RBF) and add water dropwise from the separatory funnel into the RBF. Collect a sample of the gas in a small test tube. React the gas with the following solutions:

1. Alkaline potassium permanganate solution:

$$HC \equiv CH + KMnO_4 \xrightarrow{-OH} NaO_2C - CO_2Na$$

sodium oxalate

2. Bromine water solution:

$$HC \equiv CH + Br_2 / H_2O \longrightarrow BrH_2C - CHO$$

bromoethanal

3. Ammoniacal silver nitrate solution:

$$HC \equiv CH + Ag\,NO_3 \longrightarrow Ag^+ {}^-C \equiv C^- Ag^+ \downarrow$$

White ppt

4. Burn the gas:

$$2HC \equiv CH + 5\,O_2 \longrightarrow 4\,CO_2 + 2\,H_2O$$

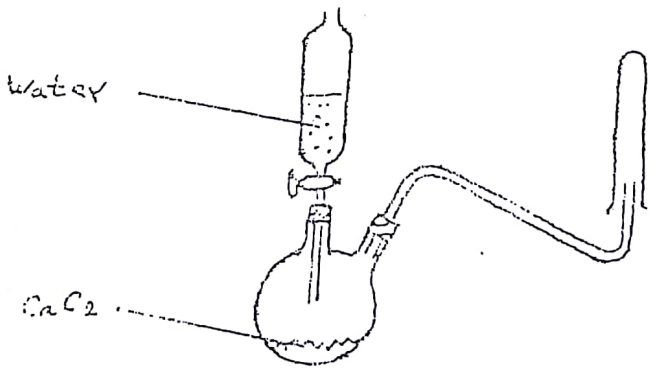

Fig. 1

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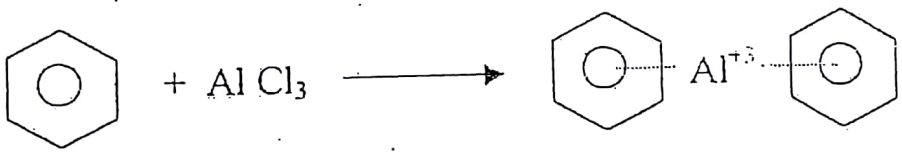
Friedle – Crafts Test:

The test used to distinguish between aromatic hydrocarbons and aliphatic hydrocarbons.

Procedure:

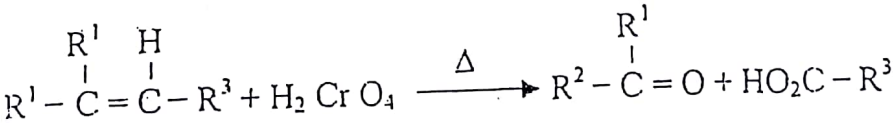
Heat a clean and dry test tube containing about (0.1 g) of anhydrous aluminum chloride in a hot flame to sublime the salt up on the side of the tube. Add a solution of about (20 mg) of unknown dissolved in (0.1 ml) (2-3 drops) of chloroform to the cool tube in such a way that it comes into contact with the sublimed aluminum chloride. Note the colour that appears.

Nonaromatic compounds fail to give a colour with aluminum chloride, benzene and its derivatives give orange or red colour, naphthalene a blue or purple colour, phenanthrene a purple colour and anthracene a green colour.



Oxidiation with Chromic Acid:

The double or triple bond in unsaturated hydrocarbons could be also detected by the reaction of alkene or alkyne with chromic acid (prepared from sodium dichromate $Na_2Cr_2O_7$ and conc . sulfuric acid in which the Cr^{+6} in chromic acid is reduced to Cr^{+3}) and the colour of solution is changed from yellow- orange to green whereas the alkene is oxidized to carboxylic acid or ketone depending one alkene structure:



Procedure:

Add 2-3 drops of unknown compound to (0.5 ml) of chromic acid, heat and watch colour change.

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Laboratory No. 4:

SOME QUALITATIVE TESTS FOR ALCOHOLS & PHENOLS

1- Lucas Test used to differentiate between primary, secondary and tertiary alcohols (Lucas test).

The test depends upon the different rates of formation of the alkyl chlorides upon treatment with Hydrochloric acid- Zinc chloride reagent (Lucas reagent).

Procedure:

To 0.5 ml (10 drops) of the alcohol in a small test tube add quickly 3ml of Lucas reagent at 26-27°C, close the tube with a cork, shake and allow to stand Observe the mixture during 5 minutes. The following results may be obtained:

(a) Primary alcohol lowers than 1- hexanol, dissolve, there may be some darkening, but the solution remain clear.

(b) Primary alcohol's, 1- hexanol and higher do not dissolve appreciably; the aqueous phase remain clear.

(c) Secondary alcohols: the clear solution becomes cloudy owing to the separation of finely divided drops of the chloride. Allyl alcohol (CH_2=CH-CH_2OH) behaves like a secondary alcohol and react within 7 minutes.

(d) Tertiary alcohols: two phases separate almost immediately owing to the formation of the tertiary chloride.

N.B. Lucas reagent is prepared by dissolving 68 g (0.5 mol) of anhydrous zinc chloride (fused, sticks, powder, etc) in 52.2 g (0.5 mol) of concentrated hydrochloric acid with cooling to avoid loss of hydrogen chloride.

Chemistry:

The principle of Lucas test depend on the relative stability of carbon cation intermediate formed during the reaction:

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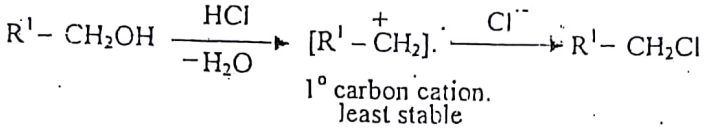
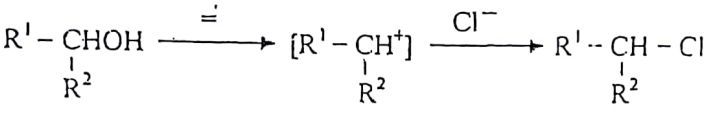
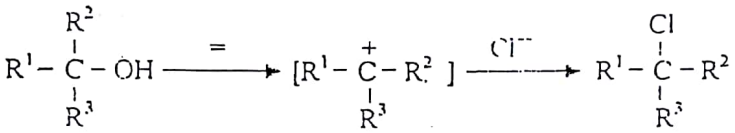


1° carbon cation.
least stable



2° carbon cation.
Intermediate stability

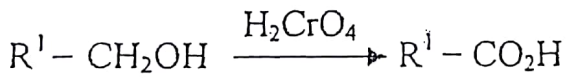


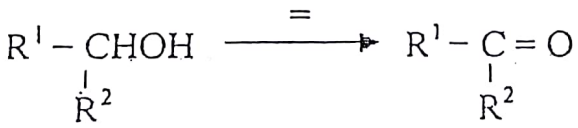
3° carbon cation.
Most stable

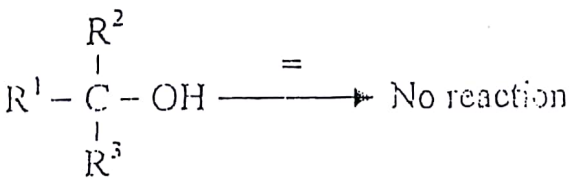
2- Oxidation of alcohols:

Add 2-3 drops of the alchohol (primary, secondary and tertiary) to 1 ml of chromic acid solution, warm in boiling water bath and notice the colour change For primary and secondary alcohols the colour changed to green whereas with tertiary alcohols no change is observed.

N.B. chromic acid is prepared by dissolving 5 g of potassium dichromate in 20 ml of dil. H_2SO_4.







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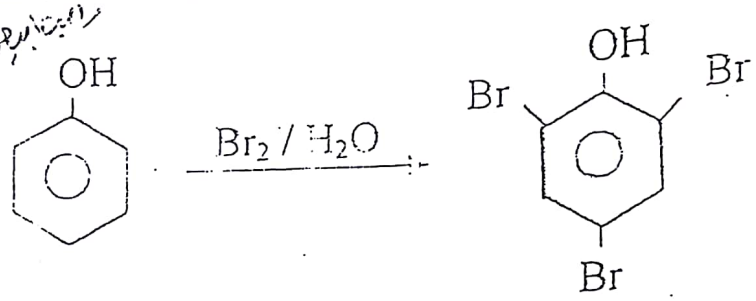
3- Action of bromine water phenols:

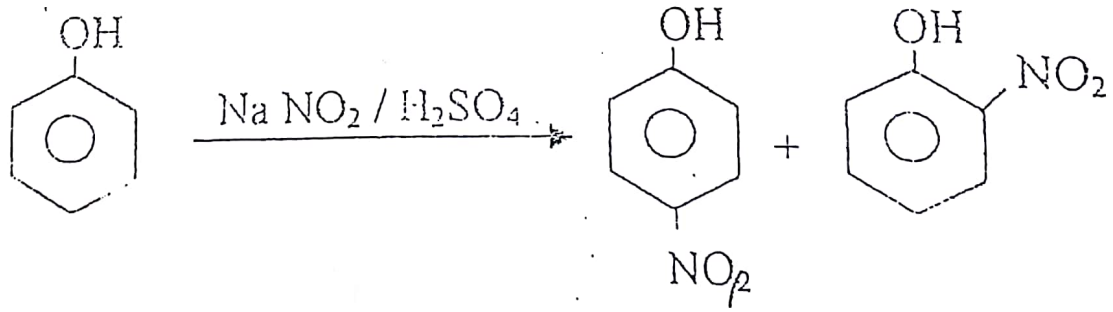
To a concentrated aqueous solution of the phenol or to the phenol itself , add bromine water gradually. At first the bromine is decolourized and then on adding an excess a white or yellowish – white precipitate is formed.



2, 4, 6- tribromophenol
white ppt.

4- Liebermann reaction of phenol:

To one minute crystal of sodium nitrite in a clear dry test tube add (0.5 g) of phenol & heat very gently for approximately 20 seconds, allow to cool and add twice the volume of conc. Sulfuric acid. On rotation the tube slowly mix the contents and adeep green or deep blue colouration develops(sometimes after 1-2 minutes). Dilute casutiously with water. The solution turns red. Now add an excess of NaOH solution, the green or blue colour reappear.



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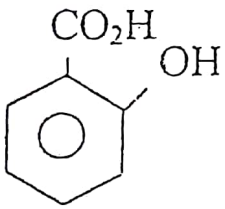
5- Reactions of alcohols and phenols with ferric choride solution:

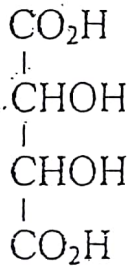
Ferric chloride is an oxidizing agent. Its solution sometimes contain a large excess of HCl which would interfere with the following reactions. If it is markedly acidic add dilute NaOH solution ,drop by drop, to the ferric chloride solution until a small but permenant precipitate of ferric hydroxide is obtained, filter this through a samll fluted filter paper and use the clear solution.

(a) To 2 ml of salycilic acid solution add 3-4 drops of ferric chloride solution, mix and observe the colour change.

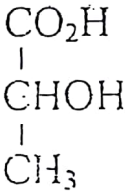
(b) Take two test tubes A&B; in A place about 5 ml of neutralized trataric acid and in B place 5 ml distilled water. To each solution add 3-4 drops of ferric chloride solution. Place a piece of white paper under the tubes, look down their length and note that A is definitely yellow compared to control tube B. this yellow colour is given by α- hydroxycarbxylic acid e.g. tartaric acid, lactic acid and lactic acid.



Salycilic acid



Tartaric acid



lactic acid

(c) Dissolve a few crystals of phenol in water and add ferric chloride solution, a violet colouration is produced, m-cresol again violet colour and catechol gives a green colour.

6- Iodoform reaction :

To 0.5 ml (~ 10 drops) of ethanol add 3 ml of 10 % kI solution and 10 ml of NaOCl solution. Warm gently: fine yellow crystalls of CHI_3 in the cold. Pure methanol and the other alcohols do not give the reaction.

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Laboratory No. 5:

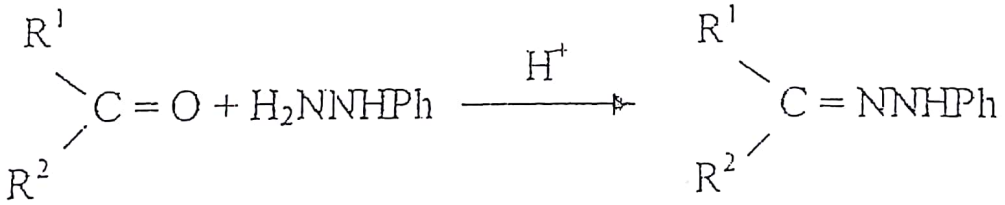
SOME QUALITATIVE TESTS FOR ALDEHYDES & KETONES

Aldehydes and ketones are compounds characterized by the presence of carbonyl group. The following tests aims to distinguish aldehydes and ketones from other functional groups containing compounds and how we can differentiate an aldehyde from ketone:

1. Hydrazones of aldehydes and ketones:

Use benzaldehyde, salicylaldehyde and acetone for the following two experiments:

(a) Phenylhydrazone: To 5 ml of water add approximately 0.5 ml of glacial acetic acid and 0.5 ml of phenylhydrazine solution, then add 3 drops of the aldehyde or ketone and shake the mixture After 1-2 minutes a floculent precipitate of the phenylhydrazone is produced.



phenylhydrazone

(b) 2,4- dinitrophenylhydrazone: To a few drops of formalin or acetone add afew drops of 2,4- dinitrophenylhdrazine reagent A, a yellow precipitate is produced in the cold. Acetaldehyde gives an orange- coloured ppt. Dissolve a few drops of benzaldehyde or salicylaldehyde in 2 ml of methanol and then add a few drops of the reagent B. an orange coloured ppt is obtained.

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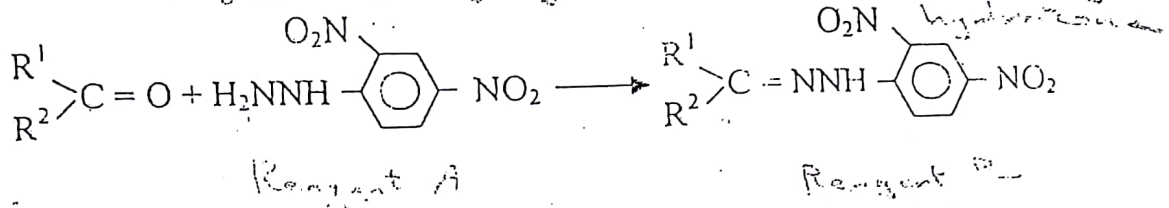


N.B. 2,4-Dinitrophenylhydrazine is much more reactive than phenylhydrazine towards aldehydes and ketones. Furthermore 2,4- dinitrophenylhydrazones usually have lower solubility in water and higher melting point & therefore separate from solution & crystallize more readily.

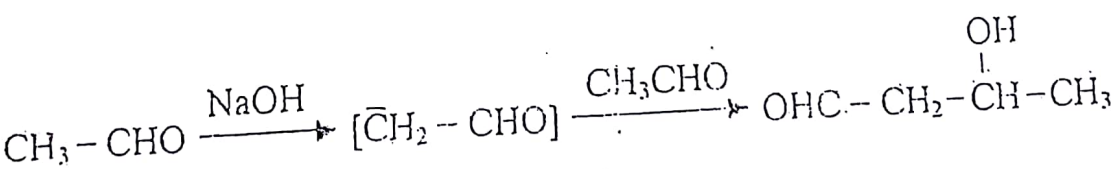
Reagent A is particularly useful for lower aliphatic aldehydes and ketones which are soluble in water whereas reagent B is suitable for aldehydes & ketones not soluble in water.

Reagent A: Dissolve 0.5 g of the powder 2, 4-dinitrophenylhydrazine in a mixture of 80 ml conc. HCl and 100 ml of distilled water by gently heating the mixture in a water bath. Cool the solution & add 120 ml of distilled water, if necessary, filter the pale yellow solution.

Reagent B: Suspend 1g of the powdered 2, 4-dinitrophenylhydrazine in 30 ml of stirred methanol and cautiously add 2 ml of conc. H_2SO_4, if necessary, filter the solution whilst it is still warm & cool the filtrate.

2. Action of sodium hydroxide:

(a) Warm 1 ml of acetaldehyde or acetone with few mls of concentrated (e.g. 30%) NaOH solution. A yellow resin, having a characteristic odour of bad apple is formed.



(b) Cannizaro's reaction: place 0.5 ml of benzaldehyde and 2 ml of 30% aqueous NaOH in a test tube, warm very gently and stir the mixture well

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with a glass rod for 5 minutes. Then add sufficient water to dissolve the sodium benzoate which has been formed. Decant the solution from any unchanged benzaldehyde, and add conc. HCl, a white ppt of benzoic acid is obtained on cooling. Salicylaldehyde give yellow colouration. Also formaldehyde give the reaction:

$$2PhCHO + NaOH \longrightarrow PhCO_2Na + PhCH_2OH$$

sod. Benzoate benzylalcohol

N.B: Aldehydes and ketones with free α-hydrogen gives aldol condensation reaction A) whereas aromatic aldehydes with no α-hydrogen give Canizaro's reaction.

3. Fehling's test for aldehydes and ketones.

Use acetaldehyde and acetone solution:

Aqueous solution of aliphatic aldehydes are almost invariably acidic owing to atmospheric oxidation, and therefore fail to reduce Fehling's solution since the alkali of the latter is nuetralised by the acid present, on the other hand, an excess of Fehling's solution is not recommended, as the blue colour may then mask the ensuing reduction. Therefore proceed thus: To 1 ml of the aldehyde or aldehyde solution add 1 ml of 10% Na_2CO_3 solution and then a few drops of Fehling's solution, and boil the mixture gently for 1 min., the solution usually turns green and on standing a fine yellow or red ppt. of Cu_2O slowly separate. A control experiment using Fehling's solution alone should always be carried out to ensure that no reduction takes place on boiling.

N.B. the colour of the ppt. depends upon the size of the cuprous oxide particles and this in turn upon the rate of reaction and conc. of the solution.

Fehling's solution: Solution A, dissolve (69.28 gm) of $CuSO_4.5H_2O$ in water and make up to one liter. Solution B, dissolve (346gm) of sodium potassium

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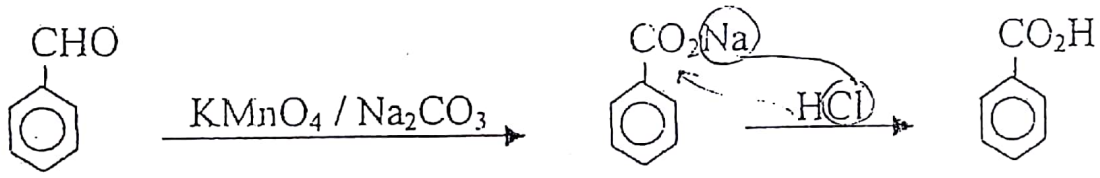
tartarate (Rochelle salt) and (120 gm) of NaOH in water and make to one liter. To prepare Fehling's solution mix equal volumes of solution A & B.

$$R^1 - CHO + Cu^{+2} \xrightarrow{\text{alkaline}} R^1CO_2Na + Cu_2O\downarrow$$

$$R^1 - CO - R^2 + Cu^{+2} \longrightarrow \text{No Reaction}$$

4. Oxidation to acids:

Warm together in a small conical flask on a water bath for 10 minutes a mixture of (0.5 ml) benzaldehyde, (15 ml) of saturated $KMnO_4$ solution & (0.5 g) of Na_2CO_3. Filter and acidify the filtration with conc. HCl on cooling benzoic acid crystallize out.



5. Reduction of ammoniacal silver nitrate:

Place about (5 ml) of $AgNO_3$ solution in a thoroughly clean test tube, add 2-3 drops of dil. NaOH solution. Add dil. Ammonia solution drop by drop, until the ppt of silver oxide is almost redissolved, then add 2-3 drops of formaldehyde or acetaldehyde. A silver mirror is formed. Ketones does not give the test.

$$R-CHO + Ag^+(NH_3) \longrightarrow R-CO_2NH_4 + Ag\downarrow$$

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6. m-Dinitrobenzene test:

To (1 ml) of acetone or acetophenone add about (0.1 gm) of finely powdered m-Dinitrobenzene and then excess of dil. NaOH solution and shake. A violet coloration is produced, but slowly fade. Not given by benzophenone.

N.B.1 more delicate result is obtained if we use 3, 5-Dinitrobenzoic acid instead of m-Dinirobenzene.

extraction solid to solid

* الاستخلاص نوعين [extraction liquid to liquid] تخرج للتقطير distiallation

١- نستخلص زيوت من نباتات نستخدم simple distalation لان درجة غليانها عالية 250c

ولا الماء 100c . (eugenol هو cloves spin).

نفصل الخليط / Crude] ناخذ crude هو خليط من Eugenol + Eugenol acetate] نفصل

القاعدة NaOH لا حيث يتحول الايكنول الى ملح الصوديوم) ثم نضيف الكلوروفورم CHCl4

١- نضيف NaOH منتظم يتحول الايكنول الى فينوكسايد ٢- نضيف CHCl3 من اجل ازالة الـ

عضوية ٢- رطبت المادة بالماء ثم نضيف حامض نرجع فينول امامي ٢- نضيف CHCl3

~~...~~ يذوب الفينول مع المذيب

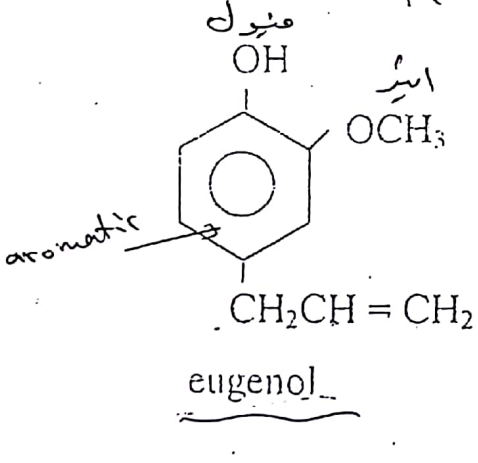
العضويات الزيت)

Laboratory No. 6:

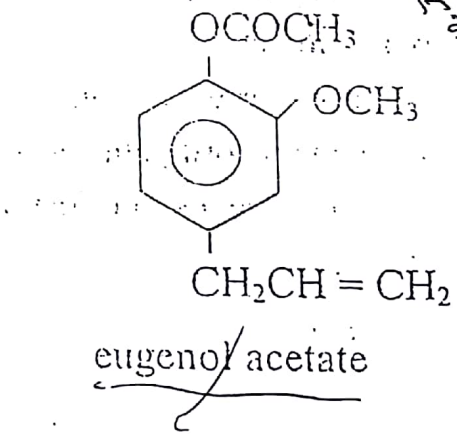
ISOLATION OF EUGENOL FROM CLOVES

This experiment explain how eugenol and other naturally occurring volatile oils can be isolated from plants through simple distillation process in which eugenol can be distilled along with water at temperature far below the boiling point of pure eugenol.

Oil of cloves which consist almost entirely of eugenol and eugenol acetate is a food flavoring agent beside its various application in dentistry. Administration of clove oil as food or drug is declared recently as the most effective nonprescriptive remedy for toothache since eugenol is known for its sedative effect. Also eugenol participate as one essential composition of zinc oxide-eugonol temporary filling, zinc oxide-eugenol impression paste and root canal sealant.



eugenol



eugenol acetate

Procedure:

Place (12.5 g) of whole cloves in a (250 ml) round bottomed flask, add (50 ml) of distilled water and set up an apparatus for simple distillation. Heat the flask strongly until boiling point starts, then reduce the flame just enough to prevent foam from being carried over into the receiver. Instead of using a

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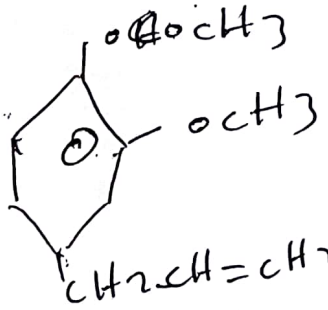
graduated cylinder as a receiver, use an Erlenmeyer flask and transfer the distillate periodically to the graduated cylinder, then if any material does foam over, the entire distillated will not be contaminated. Collect (30 ml) of distillate, remove the flame and add (30 ml) of water to the flask. Resume distillation & collect an additional (30 ml) of distillate.

Eugenol is very sparingly soluble in water and is easily extracted from the distillate with chloroform. Place the (60 ml) of distillate in a (250 ml) separatory funnel and esxtract with three (7 ml) portions of chloroform. In this extraction very gentle shaking will fail to remove all off the product, and long and vigorous shaking will produce on emulsion of the organic layer and water. The separatory funnel will appear to have three layers in it. Let the separatory funnel stand and collect the clear lower layer.

Combine the chloroform extracts (the aqueous layer can be discarded and add just enough anhydrous magnesium sulphate or calcium sulphate s.. that the drying agent no longer clumps together, but appears to be a dry powder as it settle in the solution. This may require as little as one gram of drying agent and then decant the chloroform from the drying agent, and therefore it will not be necessary to setup a filtration apparatus to make this separation.Evaporate the chloroform over water bath and weight residual oil. Perform the following tests to the oil and record your results:

1. Ferric chloride test for phenol:



+fecl3 → N.R

Eguenol acetate

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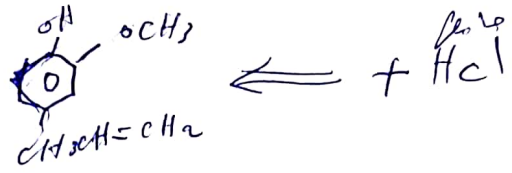
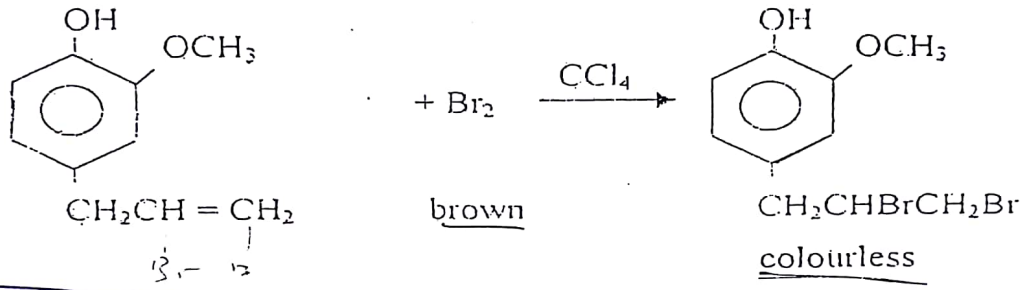
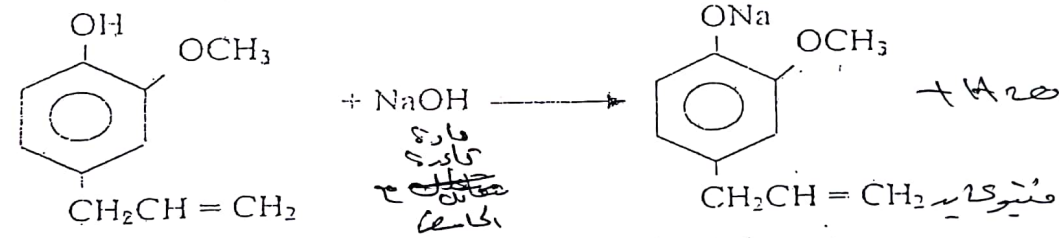


2. Br_2/ CCl_4 test:



3. NaOH test:

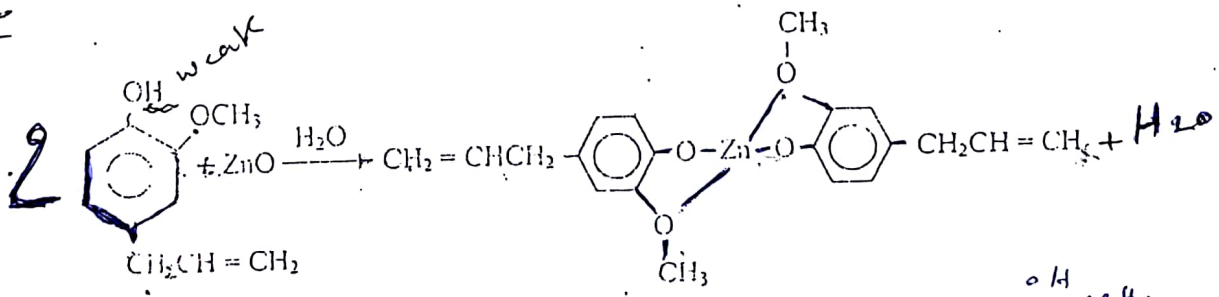
clear solution

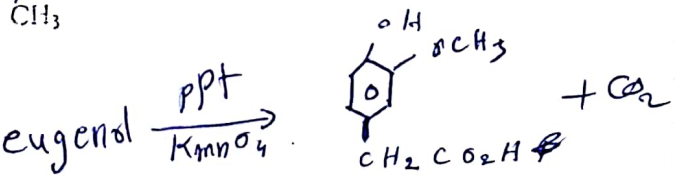


sparingly soluble in water

soluble in water

4. Mix four portions of ZnO with one portion of eugenol and notice the formation of viscous paste that solidified gradually on exposure to water. This mixture forms the basis of temporary filling. Other materials may be added to accelerate or retard the reaction:





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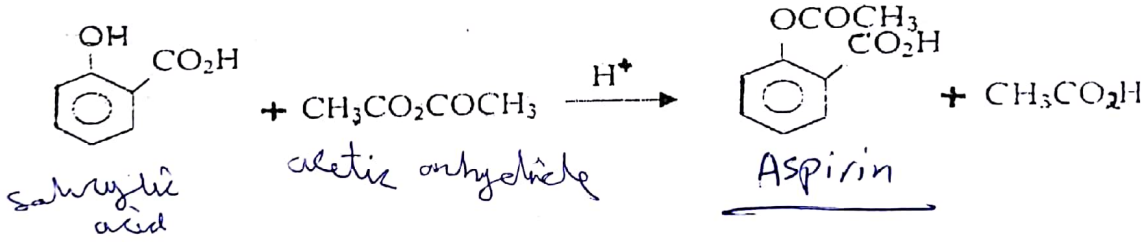
Laboratory No. 7:

PREPARATION OF ASPIRIN

Aspirin or acetyl salicylic acid is largely used in medicine as an analgesic (i.e. for removing pain) and as an antipyretic (i.e. for reducing the body temperature). Aspirin is safe & well tolerated. However it dose have side effects. Because of its relative insolubility in water & acidity, it can irritate the stomach wall. These effects can be partially overcome by using its more soluble sodium salt instead. Acetyl salicylic acid is industrially prepared by acetylation of salicylic (o-hydroxybenzoic acid) with acetic anhydride or acetyl chloride in the presence of sulfuric acid as a catalyst:



Procedure:

Place (5 g) of dry salicylic acid and (7.5 g) of acetic anhydride in a small conical flask, add (5 drops) of concentrated sulphuric acid and rotate the flask in order to secure thorough mixing. Warm on a water bath to about (50-60 ◦C), stirring with a thermometer for about (15 minutes). Allow the mixture to cool and stir occasionally. Add (150 ml) of water, stir well and filter. Dissolve the solid in about (30 ml) of hot ethanol and pour the solution into about (37.5 ml) of warm water; if a solid separates at this point, warm the mixture, until the solution is completely dissolved and then allow the clear solution to cool slowly. Beautiful needle-like crystals will separate. Filter and leave the residue to air-dried and weight it. Calculate the yield percentage. Measure its melting point and compare it with the literature.

$$\text{Yield percentage} = \frac{\text{wt. of product x m.wt. of starting compd}}{\text{wt. of starting compd x m.wt. of product}} \times 100$$

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Reactions of Aspirin:

Distinction from salicylic acid. Shake up with water in two clean test tubes a few crystals of: (a) salicylic acid, (b) aspirin, a very dilute aqueous solution of each substance being thus obtained. Note that the addition of 1 drop of ferric chloride solution to: (a) gives an immediate purple colouration, due to the presence of free hydroxyl group, whereas, (b) gives no colouration if the aspirin is pure.

N.B. 1. All glass wears must be absolutely clean and dry before being used, moisture may hydrolyze the acetic anhydride and no reaction occur:

$$CH_3CO_2COCH_3 + H_2O \longrightarrow 2\ CH_3CO_2H$$

2. Conc. hydrochloric acid cannot be used as a catalyst for the above reaction since conc. HCl contain water and may hydrolyze acetic anhydride as above.
3. The aim of addition of water after completion of reaction is to destroy what's left of unreacted acetic anhydride.

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Laboratory No. 8:

ESTIMATION OF THE NUMBER OF HYDROXYL GROUPS IN A GIVEN POLYHYDRIC ALCOHOL OR PHENOL

A known weight of the alcohol is heated with a definite volume of a mixture of acetic anhydride and pyridine until acetylation is complete:

$$R(OH)_n + n\ CH_3CO_2COCH_3 = R(OCOCH_3)_n + n\ CH_3CO_2H$$

The excess of unchanged acetic anhydride is then hydrolyzed by the addition of water, and the total free acetic acid estimated by titration with standard NaOH solution. Simultaneously a control experiment is performed identical with the above except that the alcohol is omitted. The difference in the volume of NaOH solution required in the two experiments is equivalent to the difference in the amount of acetic acid formed i.e. to the acetic acid used in the actual acetylation. If the molecular weight of alcohol is known, the number of hydroxyl groups can be calculated.

Procedure: Determination of number of hydroxyl groups in phenol m.wt 94.

Prepare the acetylating mixture by adding 1 volume of acetic anhydride to 4 volumes of pure pyridine, and shaken thoroughly, immediately before use, transfer the mixture to a clean dry burette having a well, fitting glass tap, & then close the top of the burette by means of soda-lime tube or piece of cotton.

Fit two similar (100 ml) flasks A and B with reflux water-condensers in series. Weight the flask A and add about (1 g) of pure dry powdered of phenol and weight again. Now add (10 ml) of the acetylating mixture to the flask A & also to the control flask B. Connect the flasks to the reflux condensers and heat

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both A & B on boiling water both for 30 minutes. Then remove the water baths and pour (20 ml) of distilled water down each condenser. Shaking the contents of each flask gently to ensure completes hydrolysis of unchanged acetic anhydride. Finally cool each flask thoroughly in cold water and allow to stand for (10 minutes). Then titrate the contents of each flask with N. NaOH solution using phenolphthalein as an indicator.

Example of calculation:

Weight of phenol used	0.956 g.
Flask A required	33.9 ml N. NaOH solution.
Flask B required	43.85 ml N.NaOH solution.

Difference in N.NaOH solution required for A & B = 9.95 ml.

1000 ml of N.NaOH = 1 g. Mol.NaOH = 1g Mol.CH_3CO_2H = 1 hydroxyl group.

∴ 9.95 ml of N.NaOH = (9.95/ 1000) Hydroxy group.

∴ 9.956 g of phenol contain (9.95/ 1000) Hydroxyl group.

And 94 (1 G. Mol) phenol contain (9.95/ 1000) x (94/ 0.956) Hydroxyl group = 0.97 hydroxyl group.

Determination of the number of hydroxyl group in Glycerol m. wt 92.

Use approximately (0.5 ml) of glycerol in flask A. Heat on water both for 6 minutes instead of 30 minutes and follow the procedure for phenol.

- You can determine the number of hydroxyl groups in the following compounds: Manitol m.wt 182, Glucose 180, pentose 150.

N.B. Control or blank experiments have frequently to be employed in organic estimation. Their value is two fold:

(1) The absolute concentration of a reagent need not to determine since the difference between control and experiment gives at once actual amount.

(2) Adventitious losses of reagent, due e.g. to the chemical action of the alkaline glass vessels... etc. are almost identical for the actual and the actual experiments and therefore not affect the difference in result.

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(3) The use of reflux condensers during the acetylation is not absolutely essential. Since very little evaporation of the acetylating mixture would occur.

(4) Following exactly the same procedure, the number of amino groups in aniline could be estimated according to the following reaction:

$$C_6H_5NH_2 + CH_3CO_2COCH_3 \longrightarrow C_6H_5NHCOCH_3 + CH_3CO_2H$$

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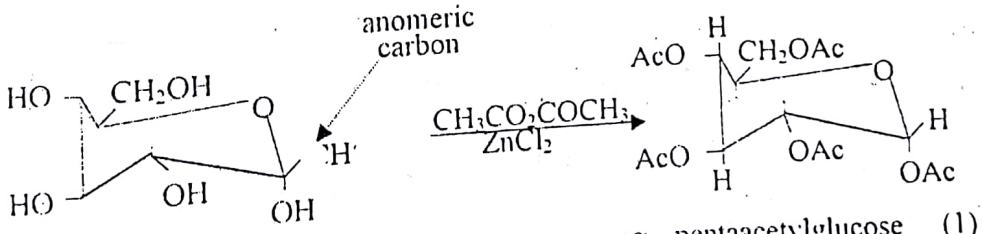
Laboratory No. 9:

PREPARATION OF a AND β- PENTACETYL GLUCOSE

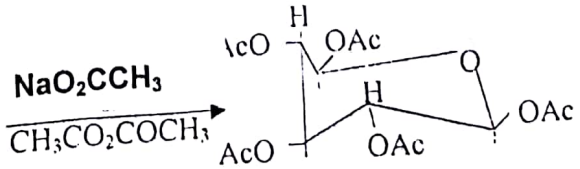
The aim of this experiment is to show how by changing the experimental condition we can control the stereo specificity of product. Glucose is present as an equilibrium mixture of *a*- and *β* - anomers depending on the position of C_1-hydroxyl group, if axial it is *a*- anomer & if equatorial it is *β* - anomer. Notice that all other groups are in equatorial position giving maximum stability for glucose.

When glucose is heated with an excess of acetic anhydride in the presence of a catalyst, all five hydroxyl groups are acetylated, and the resulting pentacetyl glucose can clearly exist in two isomeric forms, corresponding to *a* and *β* forms of glucose itself (structure 1 and 2) When zinc chloride is used as a catalyst the *a*- pentacetyl glucose is formed, and when sodium acetate is used, the *β*- pentacetyl is produced. It is probable that the product in each case contains traces of the other ismer, which however can be readily eliminated by recrystallisation:



Mixture of α and β

α - pentaacetylglucose (1)



β - pentaacetylglucose (2)

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Procedure:

a- PENTACETYL – GLUCOSE

From a clean dry stick of zinc chloride, break of a piece weighing roughly (2.0 g) crush it quickly into coarse fragments in a mortar, and then add it to (12.5 ml) of acetic anhydride contained in a (100 ml). Round-bottomed or conical flask (owing to the very deliquescent nature of zinc chloride, this manipulation must be carried out as rapidly as possible: hence , have both the acetic anhydride in the flask, and the mortar available near the balance, before the zinc chloride stick is withdrawn from the stock bottle). Fit the flask with a reflux water condenser and heat the mixture of zinc chloride and acetic anhydride on a vigorously boiling water-bath for about 5 minutes until the maximum temperature is reached and nearly all the chloride has dissolved. Now remove the condenser and add (5 g) of powdered glucose (anhydrous or monohydrate) cautiously to the acetic anhydride, shaking the mixture around occasionally during the addition, in order to control the vigorous reaction which follows. Finally replace the condenser and heat the mixture for 1 hour on the water-bath, and then pour the liquid into about (125 ml) of cold water, the latter should preferably be cooled externally with ice-water, and be stirred vigorously during the addition of the acetylated product. A viscous oil separated at the bottom of the beaker, and if occasionally stirred will crystallize within about 10 minutes. After solidification of the oil, allow the mixture to stand for a further 15 minutes and then filter at the pump. If any large lumps of the crude acetyl compound have formed, transfer them to a mortar, pulverize, and then wash them back into the Buchner funnel. Finally wash the product on the filter thoroughly with water and drain. Recrystallise the product from methylated spirit until the *a*- pentacetylglucose is obtained as colourless crystals, read melting point. Test the product with Fehling test and compare it with starting material.

N.B. All glass wares used for this experiment must be absolutely clean and dry.

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Laboratory No. 10:

SOME QUALITATIVE TESTS FOR CARBOXYLIC ACIDS

Use one or more of the following carboxylic acids: Formic, acetic, oxalic. Succinic, lactic, tartaric, citric, benzoic, and salicyxlic acid. All carboxlic acids are colourless crystalline solids except formic acid, acetic (m.p. 18° C). Also formic and acetic are readily volatile with characterist pungent odour, cinnamic acid has a faint, pleasant and characteristic odour.

1. Solubility in sodium hydroxide solution:

Place about (0.1 g) of the acid in a test tube and add about (5 ml) of (10%) aqueous NaOH solution. Record your observation. Note that also phenols dissolve in NaOH to give phenoxides.

2. Solubility in sodium carbonate solution:

Place about (0.1g) of the acid in a test tube and add (5ml) of soduim carbonate (10%) solution and observe the evolution of CO_2 gas. Phenols are soluble in water and also in soduim carbonate solution but without evolution of CO_2 gas, i.e. without the formation of soduim derivatives. This reaction can therefore be used to distinguish between carboxylic acids and most phenols.

$$2\ R\text{-}CO_2H + 2\ Na_2CO_3 \longrightarrow 2\ RCO_2Na + CO_2 + H_2O$$

$$PhOH + = \longrightarrow N.R.$$

N.B. Some substituted phenols, particularly nitrophenols, are sufficiently acidic to liberate CO_2 from Na_2CO_3, nitrophenols, however, all give yellow or red solution with Na_2CO_3.

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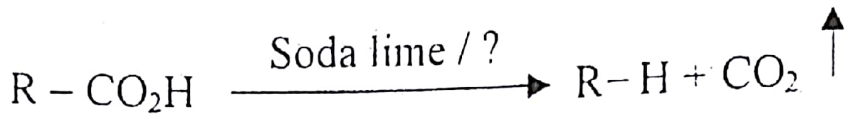
3. Heating with soda-lime:

Carboxylic acids when heated with solid soda-lime undergo decarboxylation reaction:



Mix about (0.2 g) of the acid powder or pure liquid with about (1 g) of powdered soda-lime in a clean and dry test tube. Incline the test tube and heat it very gently and then more strongly and observe the gases evolved, which can be collected in other dry tube.

Note that this test is particularly useful for providing evidence for:

a- Benzoic acid: Gives benzene, detected by odour and by burning with a very smoky flame.

b- Salicylic acid: Gives phenol, detected by odour & by violet colouration with ferric chloride.

Oxalic and formic acid: Hydrogen gas evolved.

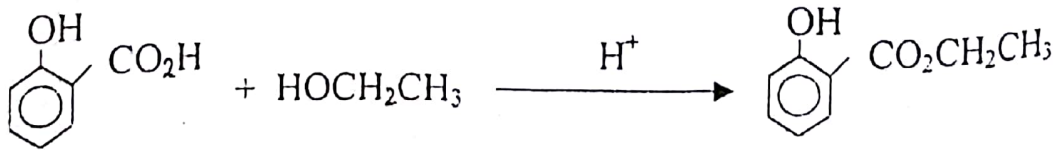
4. Ester formation:

Heat gently (1 ml) of ethanol or propanol with (0.5 g) of the acid or one of its salts and a few drops of conc. H_2SO_4 for about 1 minute. Cool and pour into a few ml of water in a test tube and note the odour.

The test is particularly useful for identifying:

a- Acetic acid: The ester has strong odour of apples.

b- Salicylic acid: The ester has strong odour of wintergreen.



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5. Ferric chloride reaction:

For the success of this reaction it is important that the solution should be neutral. Excess of acid usually inhibits the production of colour or precipitate, and excess of alkali gives a redish-brown precipitate of ferric hydroxide. A neutral solution may be made as follows:

Place about (0.5 g) of the acid in a boiling tube and add slight excess of ammonia solution until just alkaline to litmus-paper. Add a piece of unglazed porcelain and boil until the odour of ammonia is completely removed. To the cold neutral solution add a few drops of neutral $FeCl_3$ solution.

(a) Formate and acetate gives a deep red colouration which changes on boiling to a reddish-brown ppt of the basic ferric salt.

(b) Succinate, benzoate and cinnamate give buff or brownish coloured ppt of the basic ferric salts in the cold. Add dil. H_2SO_4. The basic ferric succinate dissolve giving a clear solution: The other basic ferric salts also dissolve, but simultaneously a white ppt of the free acid is also formed.

(c) A violet coloration denotes salicylate. Note however that a dilute solution of salicylic acid will give this coloration without preliminary neutralization. No colour is formed in the presence of mineral acid.

Special reactions:

Acetic acid:

1- Dose not reduce $KMnO_4$, $HgCl_2$ and ammoniacal $AgNO_3$.

2- The odours of methyl and ethyl acetate are characterstic.

Oxalic acid: $HO_2C\text{-}CO_2H$

1- H_2SO_4 Test: To (0.5 g) of oxalic acid or of an oxalate, add (1 ml) of conc. H_2SO_4 & warm: CO and CO_2 are evolved. The CO burns with a blue flame. Detect the CO_2 by passing through lime-water.

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2- Reduction of acid permanganate: Add few drops of dil. H_2SO_4 to (1 ml) solution of oxalic acid or of an oxalate. Warm gently and add a dil. Solution of $KMnO_4$ drop by drop and note the decolorisation.

Succinic Acid $(CH_2CO_2H)_2$

Fluorescein test: Fuse together carefully in a dry test tube for about (1 minute) a few crystals of resorcinol and an equal quantity succinic acid or a succinate, moistened with a drop of conc. H_2SO_4. Cool. Dissolve in water and add NaOH solution in excess. A red solution is produced which exhibits an intense green fluorescence.

Tartaric Acid:

1. Sulphuric acid test. Warm (0.5 g) of tartaric acid or tartarate with (1 ml) conc. H_2SO_4. Does charring occur, CO and SO_2 evolved?
2. Fenton's reagent. To a solution of tartaric or tartarate add (1 drop) of freshly prepared ferrous sulphate solution. (1 drop) of hydrogen peroxide solution and then excess of NaOH solution; an intense videt coloration is produced, due to the ferric salt of dihydroxyfumaric acid HOOC. C(OH): C(OH) COOH.

Citric Acid: HOOC. CH_2. C(OH) (COOH). CH_2CO_2H

Sulphuric acid test. Heat (0.5 g) of citric acid or citrate with (1 ml) of H_2SO_4: CO and CO_2 are evolved and the mixture turns yellow, but does not char. Acetone di-carboxylic acid (3-Oxopentanedioic acid) is produced. This compound is confirmed by making the solution alkaline with NaOH solution, then addition of a few drops of a freshly prepared sodium nitropruside produce an intense red coloration.

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Laboratory No. 11:

SOME QUALITATIVE TESTS FOR AMINES

Amines are classified into primary, secondary and tertiary amines according to the number of alkyl or aryl groups attached to nitrogen.

Primary amines: use one of these compounds for the following tests:
Aniline, o-, m- and p- toluidine and other substituted anilines, 1- and 2-naphthylamine, benzylamine Ph CH_2NH_2 and ethylene diamine $H_2NCH_2CH_2NH_2$.

1. Isocyanide reaction: Add a few drops of chloroform to about (0.2 g) of the substance, and then add (2-3 ml) of alcoholic NaOH solution. Mix well and warm gently: the odour of isocyanide (carbylamine) is produced:

$$RNH_2 + CHCl_3 + 3NaOH \longrightarrow RN = C: + 3NaCl + 3H_2O$$

Immediately the odour of isocyanide is detected, cool the tube and add carefully an excess of conc. HC1: the isocyanide is thus hydrolyzed to the odourless amine.

2. Acetylation: place (1 ml) of substance in a small clean and dry round bottom flak fitted with a condenser add (5 ml) of an acetic anhydride acetic acid mixture (1:1) and reflux gently for 15 minutes. Pour into water; the solid anilide separates. Filter off, wash with water and recrystallise from water or diluted methylated spirit. Note the m.p.

$$R-NH_2 + CH_3CO_2COCH_3 \xrightarrow{H^+} R-NH-COCH_3 + CH_3CO_2H$$

acetanilide

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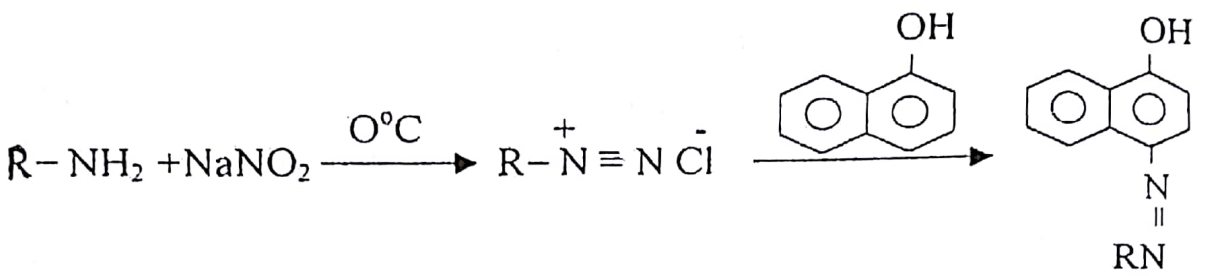
3. Diazotisation: Dissolve (0.2 g) of the substance in (1 ml) of conc. HCl; dilute with about (3 ml) of water, cool in ice and add a few drops of sodium nitrite solution. Now add this cold diazonium solution slowly to a cold solution of 2-naphthol in a considerable excess of (10%) NaOH solution; a brilliant red dye is produced.



red dye.

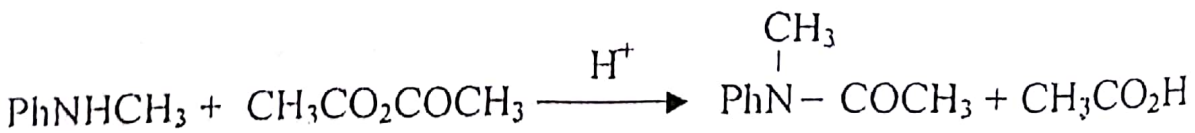
4. (a) Action of bleaching powder: Shake 1-2 drops of aniline with (10 ml) of water and add a few drops of bleaching powder solution. A transient purple coloration is produced which soon turns brown.

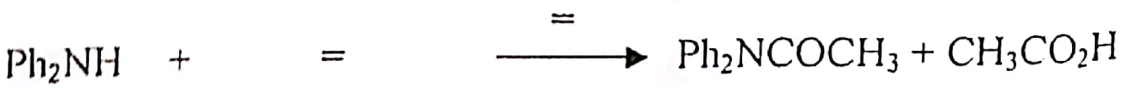
(b) Colour reactions with the enzyme: peroxidase and H_2O_2 permit all the above amines to be distinguished from one another in very dilute solution.

Secondary Amines: Use one of these compounds for the next eperiments; monomethylaniline, monoethylaniline, diphenylamine, dimethylamine solution or diethylamine solution, pipyridine or piperazine.

1. Isocyanide reaction: No reaction occurs.

2. Acetylation: Proceed as in 2. Pour the final acetylation mixture into (10 ml) of water and add (10%) NaOH solution, with stirring until no more aniline is precipitated (acetyl monomethyl aniline is very soluble even in dilute acetic acid; acetyl diphenylamine readily separates without the addition of alkali). Filter, wash with water & recrystallise.





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3. Formation of nitrosamine $R_2N.NO$:

(a) From monomethylaniline. Dissolve (1 ml) of monomethylaniline in about (3 ml) of dilute HCl and add sodium nitrite solution gradually with shaking until the yellow oil separates out at the bottom of the solution.

(b) From diphenylamine. Prepare two solutions, one containing (1 g) of diphenylamine in (8 ml) warm ethanol & the other containing (0.5 g) sodium nitrite in (1 ml) water, and cool both in ice water until the temperature fails to 5°C. Now add (0.8 ml) of conc. HCl steadily with stirring to the diphenyl amine solution & then without delay (otherwise diphenylamine hydrochloride may crystallize out) pour the sodium rapidly into the well-stirred mixture. The temperature rises at once and the diphenyl-nitrosamine rapidly crystallizes out as a pale yellow ppt.

$$Ph_2NH + HONO \xrightarrow{5^\circ C} Ph_2N.NO$$

4. Coloured oxidation products:

(a) Dissolve a few small crystals of diphenylamine in (1 ml) of conc. H_2SO_4. Add (2 drops) of conc. HNO_3 to about (10ml) of water, shake, and add (1 drop) of this diluted HNO_3 to the diphenylamine solution, an intense purple-blue coloration is produced. Monomethylanilire merely turns a dirty brown when treated in this way.

(b) Neither monomethylaniline & diphenylamine when pure gives coloration with bleaching powder.

Tartiary Amines:

Use dimethylaniline, diethylaniline, triethylamine, trimethylamine.

1. Isocyanider reaction: no reaction occur.

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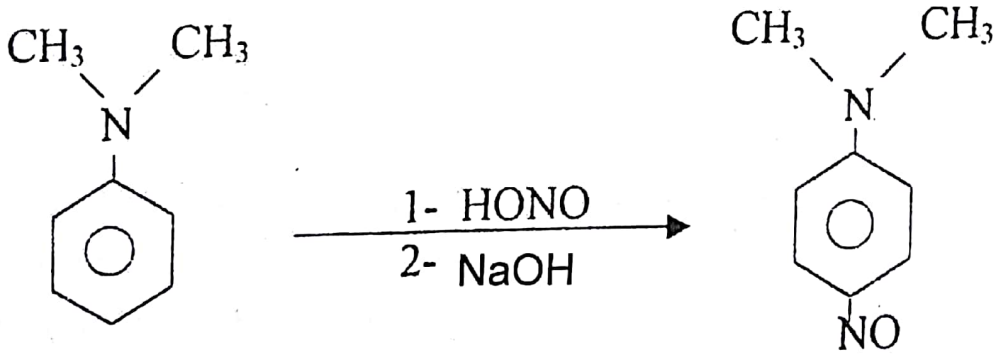
2. Methiodide formation: place (2 drops) of dry pyridine in a dry testtube, add 4-5 drops of methanol, and 2-3 drops methyl iodide. The mixture becomes, hot, & on cooling the colourless crystals of the methiodide separate:

3. p-Nitroso derivative: Dissolve (0.5 ml) of dimethylaniline in about (4 ml) of dil. HCl, chill in ice and then add carefully drop by drop about (2 ml) of (20%) sodium nitrite solution. A reddish solution is obtained, but no oil separate. Allow to stand for (5 minutes) in the cold and then add NaOH solution. A green precipitate of p-nitrosodimethylaniline is obtained.



4. Coloured oxidation products: Neither dimethylaniline nor triphenylamine gives coloration with bleaching powder.

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Laboratory No. 12:

ACID – BASE TITRATION

The following terms are going to be used in this experiment:

Normality: is the number of equivalents of solute per liter of solution. It is usually designated by a capital N.

One **equivalent** of an acid is the quantity of acid required to furnish one mole of H^+ ; one equivalent of a base is the quantity of base required to furnish one mole of OH^- or accept one mole of H^+ . So one mole of H_2SO_4 furnishes two equivalents of H^+ .

pH is the negative logarithm of hydrogen ion concentration.

Indicators are proton-containing organic molecules, which change colour when protons are transferred between them, and the solvent. The pH at which proton transfer and hence colour change occur is different for different indicators.

The aim of this experiment is to show the student how we can evaluate qualitatively and quantitatively the normality of unknown acid or base solution by simple titration using an indicator or pH meter to determine the end point or equivalent point.

Standardization of HCl solution:

We can not prepare HCl solution of accurate normality simply because HCl is a gas and can not be weighed accurately. On the other hand preparation of NaOH solution of accurate normality is also difficult since sodium hydroxide is hygroscopic and absorb atmospheric moisture and cannot be weighed accurately.

The solution is to use standard sodium carbonate solution since anhydrous sodium carbonate can be weighed accurately, and then titrated with hydrochloric acid solution to determine its normality.

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Procedure:

To (10 ml) of N. Sodium carbonate solution in small conical flask add (2 drops) of phenolphthalein indicator and titrate with HCl solution until the pink colour disappear completely. Measure the volume of HCl solution required for neutralization. Repeat the experiment to obtain more accurate results and calculate the normality of HCl solution according to the equation:

$$N_1 \times V_1 = N_2 \times V_2$$

Determination of NaOH solution normality:

This can be achieved by two ways:

a. By titration of (10 ml) NaOH solution containing (2 drops) of phenolphthalein indicator with HCl solution of known normality.

b. By using pH meter titrate (10 ml) of NaOH solution with HCl solution of known measure the pH of NaOH solution to obtain a curve similar to that shown in (fig. 1) from which you can measure the end point.

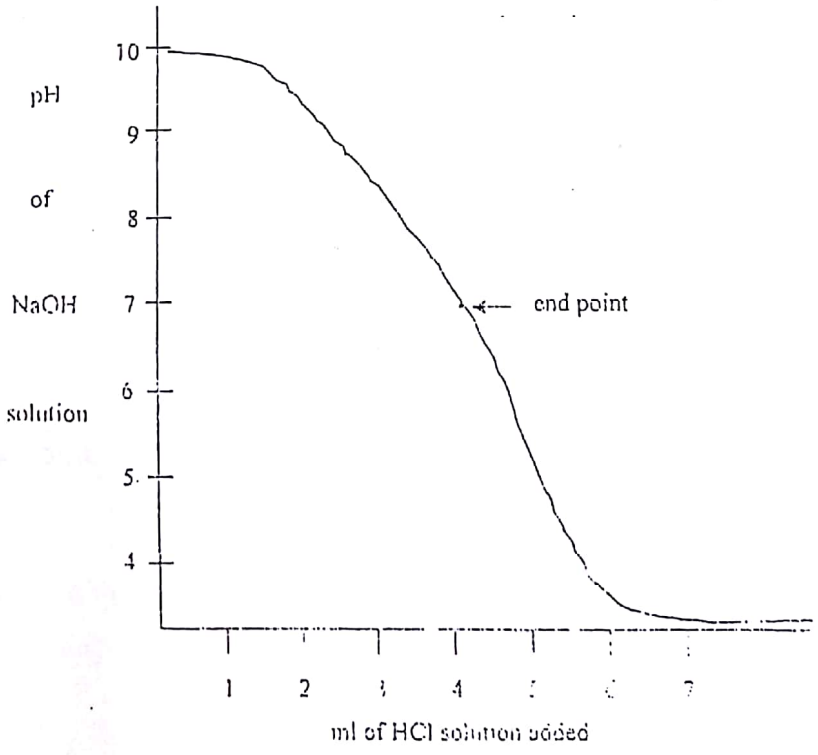

Fig.1.

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Laboratory No. 13 and 14:

SOME QUALITATIVE TESTS FOR CARBOHYDRATE

Introduction:

The word carbohydrate originally signified a group of compounds, which contained carbon, hydrogen and oxygen, the later two elements being present in the same ratio as in water.

Carbohydrates are divided into three main groups: monosaccharides, oligosaccharides and polysaccharides. Monosaccharides are the building blocks of oligo and polysaccharides. Oligosaccharides are compound sugar, which yield between 2-6 units of monosaccharides on hydrolysis. Polysaccharides are macromolecules composed of very large number of monosaccharides.

Monosaccharides are polyhydroxy compounds characterized by the presence of an aldehyde or ketone group. They are classified into triose, tetrose, pentose and hexose depending on the number of carbon atoms. Hexoses are the important sugars in carbohydrate chemistry, the most common naturally occurring are D-glucose, D-mannose, D-galactose and D-fructose.

Oligosaccharides consist of monosccharide units condensed together through glycosidic or acetal linkage. The most important oligosaccharides are the disaccharide's: maltose, lactose, sucrose and cellobiose.

For polysaccharides, the most important is starch, which is the storage form of carbohydrate in plants and glycogen is the storage form of carbohydrate in animals.

Molisch's Test:

Is a general test for all carbohydrates and based on the principle that sulphuric acid act first to hydrolyze all the glycosidic bonds, then dehydrate the monosaccharide to give hydroxylmethylfurfural in case of hexoses and furfural

- Sulphuric acid
hydrolyze ⟶ all the glycosidic bonds
dehydrate the mono.
↳ hydroxylmethylfurfural (hexoses)
↳ furfural (pentoses)

combine with α-naphthol → purple or violet ring

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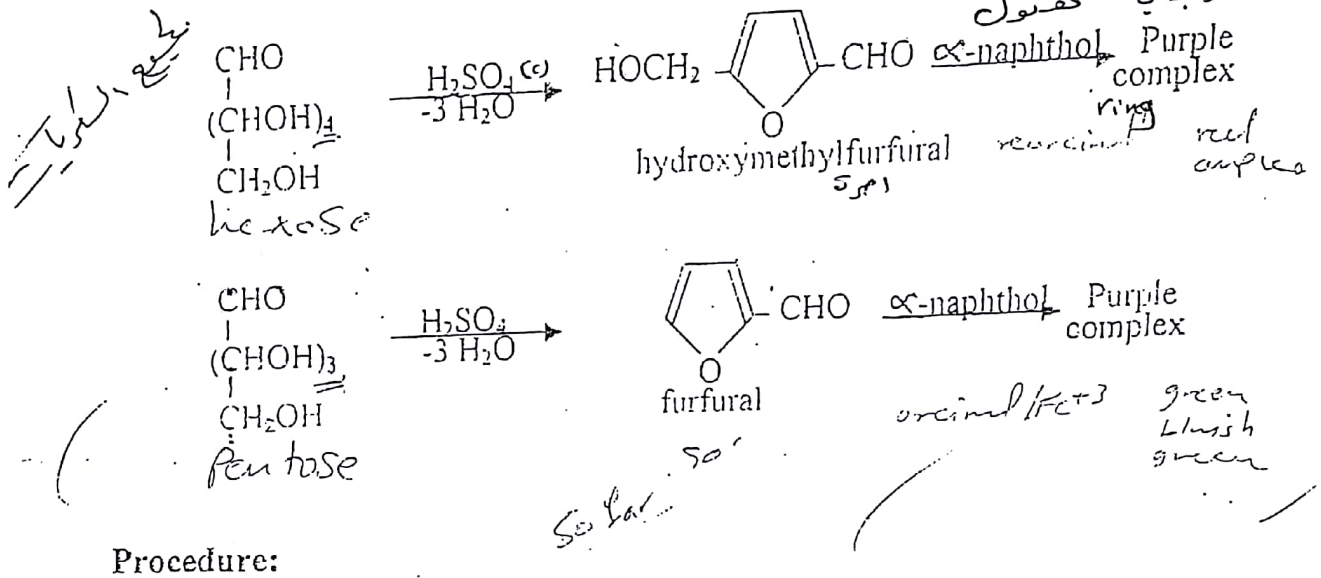
in case of pentoses. The furfural or hydroxmethylfurfural combined with the α-naphthol to give a purple complex, which appeared as a purple or violet ring:



Procedure:

To a test solution (2-ml) of carbohydrate add (4 drops) of α-naphthol alcoholic solution (1%). Then carefully add conc. Sulphuric acid (2 ml) side by side with the reaction test tube, so that two layers should be formed, the upper layer for carbohydrate solution and the lower layer for conc. H_2SO_4. Observe the purple ring.

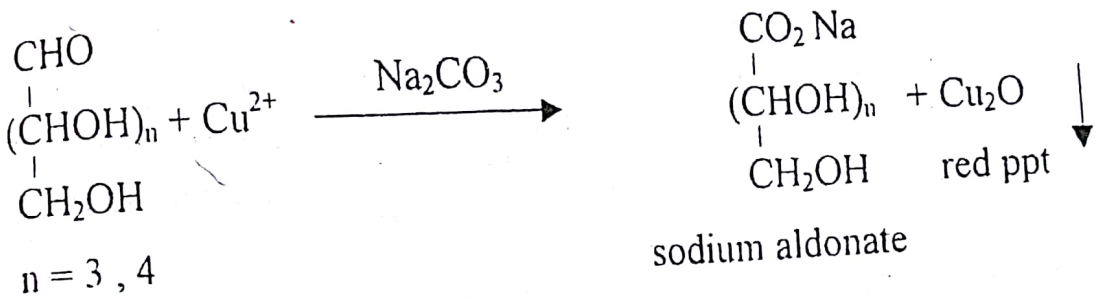
Apply this test on mono-, di- and polysaccharides compare your results with a blank solution using (2 ml) of distilled water as the test solution.

N.B. you may observe the formation of a green ring beside the purple ring. This is due to the reaction of α-naphthol and sulphuric acid & not to the presence of carbohydrate.

(2) **Benedict's Test:**

Some carbohydrate with a free aldo or α-hydroxyketo group processes a reducing power, So they are known as reducing sugars. Benedict's test is a test used to distinguish reducing sugar from nonreducing sugars and is based on the principle that the reducing sugar will reduce the cuppric ions (Cu^{2+}) present in

the reagent to the cupprous ion and precipitated as cupprous oxide Cu_2O in alkaline medium. The test can be used for the detection of glucose in urine:



Procedure:

To the Benedict's solution (2 ml) add (4 drops) of the test solution, then put the reaction test tube in boiling water bath for (5 minutes). Then take it out of the water-bath and let it cool, Observe & record the change in colour (yellow, orange, green or red ppt). This change in colour from blue to the colours mentioned above is considered as positive Benedict's test and the degree of change depends on the conc. of reducing sugar.

N.B. 1. The following carbohydrate should be tested: glucose, fructose, ribose or arabinose, maltose or lactose, sucrose and starch.

2. Benedict's reagent is a solution of copper II sulphate (17.3 g) mixed with sodium citrate (173 g) and sodium carbonate (100 g) in one litter of distilled water.

Barfoed's Test:

Is a test used to distinguish monosaccharide from disaccharide and polysaccharide. The test based on the principle that only monosaccharides are capable of reducing cuppric ions in weakly acidic solution to the corresponding cupprous ions (ppted as Cu_2O). Whereas disaccharide give negative result (solution remain blue) unless they present in high concentration or hydrolyzed to their main constituents of monosaccharide due to prolonged heating of the reaction mixture.

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Benedict } can be used with urine → used to detect glucose in urine
Millon } Barfoed } can not be used with urine (cannot be used to detect glucose in urine)

The test cannot be used for the detection of glucose in urine due to the presence of chloride ions in the urine, which interfere with the test.

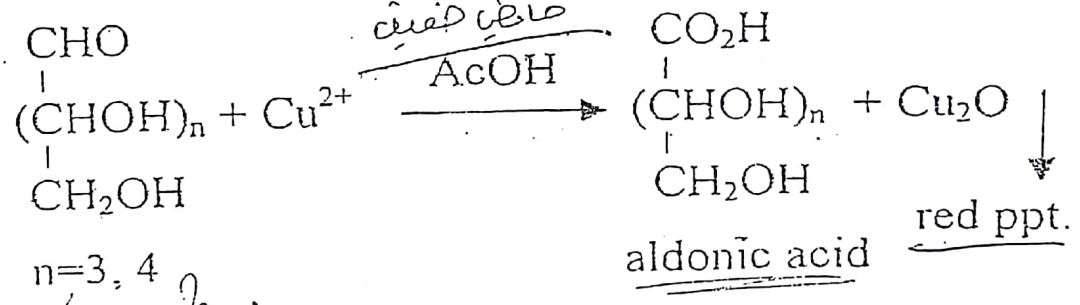
Procedure:

To the test solution (1 ml) add Barfoed's reagent (2 ml), mix and place the test tube in boiling water bath for exactly (3 minutes). Then remove the test tube and allow cooling, gradually a red precipitate of Cu_2O is formed in case of the presence of monosaccharide.

N.B. 1. The following carbohydrates should be tested: glucose, fructose, ribose or arabinose, maltose or lactose, sucrose, and starch.

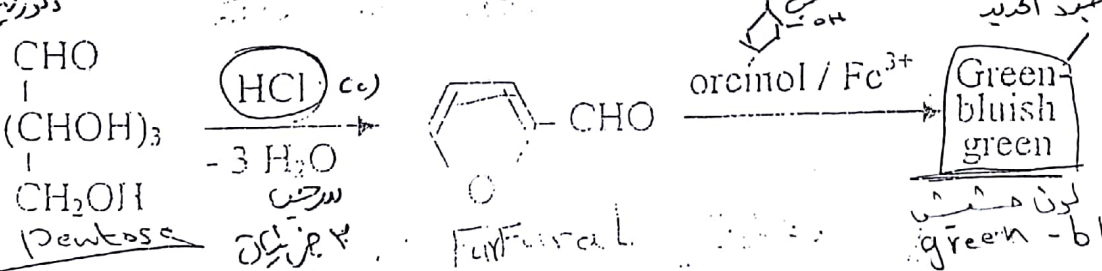
2. Time factor is very important since prolong heating of di- or polysaccharide may hydrolyse it and give positive test.

3. Barfoed's reagent is prepared by dissolving (13.3 g) of crystallized neutral copper (II) acetate in (200 ml) of (1 %) acetic acid solution.



Bial's Test:

Is a test for distinguishing pentose sugar (e.g. ribose, arabinose or xylose) from hexose sugar (e.g. glucose, fructose and galactose) and other di- or polysaccharides. The test is based on the principle that the furfural produced from the dehydration of pentose will condense with orcinol in the presence of ferric chloride to give green-bluish green colour with a precipitate:



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Bials test ← Pentose و hexose التفريق بين

ribose arabinose xylose → + Green-bluish green

Glucose fructose galactose → − brown coloration

Procedure:

To Bial's reagent (3 ml) add the test solution (0.5 ml, approx. 10 drops), mix and heat gently until boiling. The reaction mixture turned green or bluish-green in case of the presence of pentose sugar.

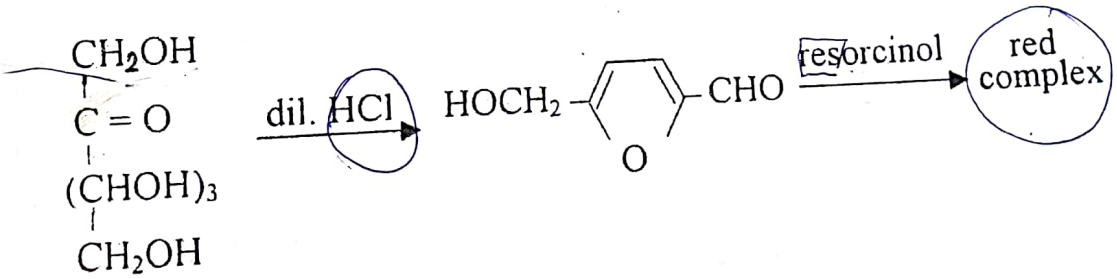
N.B. 1. Any other colour is considered as a negative result, e.g. Glucose give a brown coloration.

2. Bial's reagent is prepared by dissolving orcinol (1 g) in (100 ml) of concentrated hydrochloric acid containing a few drops of ferric chloride solution.

orcinol HCl Fe

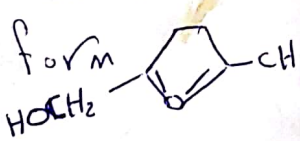
Selivanoff's test:

Used to distinguish ketohexose sugar from aldohexose or aldopentose sugars, and is based on the principle that ketohexoses are dehydrated more easily than aldohexoses and aldopentoses to give hydroxymethyl furfural which condenses with resorcinol to form a red complex:



Ketohexose

Hence prolonged heating should be avoided since aldohexoses may be dehydrated also to give hydroxymethylfurfural or disaccharide and specially sucrose is hydrolysed by the action of acid to give glucose and fructose which in turn react with Selivanoff's reagent to give the pink red colour.

because HCl cause hydrolosis aldohexose and alldopentose and will form 

, and  react with resorcinol and give red complex

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Procedure:

To Selivanoff's reagent (3 ml) add (4 drops) of the test solution, mix and place in boiling water-bath for one minute exactly. The development of pink to deep red colour indicate the presence of ketohexose.

Iodine test:

Used to distinguish polysaccharide (starch, dextrin or glycogen) from mono and disaccharides and is based on the principle that iodine can form an adsorption complex with the helical structure (coil-like) of the polysaccharide. So it give a blue colour with starch, red with dextrin and brown-red with glycogen depending on coil size and molecular weight.

In order to achieve a good result the test solution should be either ~~neutral~~ or slightly acidic but never alkaline, since alkaline medium react with iodine molecule and dissociate it:

$$I_2 + NaOH \longrightarrow NaOI + NaI$$

Sodium hypoiodide

Procedure:

To the test solution (1 ml) add one drop of dilute HCl and one drop of iodine solution. Observe and record your results. Now place the test tube containing the starch solution & iodine in boiling water-bath for one minute and observe the colour change. Then allow it to cool under tap water and notice reappearance of colour.

N.B. 1. On heating the coil shape of starch expand & the adsorption complex dissociated into iodine and starch. On cooling the original coil shape return back and the adsorption complex formed again.

2. Treatment of the adsorption complex with a base. e.g NaOH solution also disappear the colour complex due to the reaction of iodine with base to give sodium hypoidide, Reacidification resume the colour complex.

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3. Iodine solution is prepared by dissolving iodine in potassium iodide solution (5 %) until the colour become yellow.

Hydrolysis of Sucrose:

Sucrose (or sugarcane or sugar beets) is a disaccharide composed of two monosaccharides namely glucose and fructose joined by $\beta_1 \rightarrow \alpha_2$ glycosidic bond, thus lack the presence of free aldehyde or α- hydroxylketo group. The glycosidic bond can be hydrolyzed by the action of acid and heat to give the two monosaccharides.

Procedure:

To (3 ml) of sucrose solution in a clean test tube add (5 drops of dilute acetic acid (10 %) and boil for (2 minutes), cool and neutralize with dilute NaOH solution using litmus paper as an indicator. Perform the following tests for solution of sucrose before and after hydrolysis:

Test	Sucrose solution	
	Before Hydrolysis	After Hydrolysis
Benedict's		
Barfoed's		
Selivanoff's		

N.B. 1. Hydrochloric acid is prefered not to be used for the hydrolysis of sucrose because of the interference of chloride ions with the subsequent Barfoed's test.
2. Neutralization is a very important step since Benedict's reagent work only in neutral or slightly alkaline solution.

Hydrolysis of Starch by Hydrochloric Acid:

Principle:

Starch is a polysaccharide contain only one reducing group for several hundred or more of monosaccharide residues, so that they are effectively non reducing. Acid hydrolysis of starch gives the constituent monosaccharide

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(glucose) which have a reducing property. Therefore the reducing power of starch increase gradually with the progress of hydrolysis; while the ability of starch to react with iodine and form adsorption complex disappear gradually. Such process is done within seconds by the amylase enzyme present in saliva.

Procedure:

Arrange in a rack six or more of clean test tubes, to each is added (1ml) of Bendicit'c reagent. Beside it prepare a clean porcelain tile and place one drop of iodine solution into each spot and number them.

Now take (10 ml) of starch solution in a small beaker or big test tub. Transfer one drop of this solution to spot (no. 1) and 0.5 ml to t.t. (no. 1). To the starch solution add (10 drops) of conc. HCl, shake quickly & take one drop for spot (no. 2) & 0.5 ml for t.t.(no. 2). Place the starch solution in a boiling water bath and take samples for iodine test and Benedict's reagent each two minutes until the starch solution fail to give iodine test. Stop the heating and record the time required for complete hydrolysis. Now place the test tubes containing Benedict's reagent and test solution in a boiling water bath for (5 minutes) and observed and record the graduation in colour change.

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Laboratory No. 17 and 18:

SOME QUALITITATIVE TESTS FOR PROTEINS AND AMINO ACIDS

Introduction:

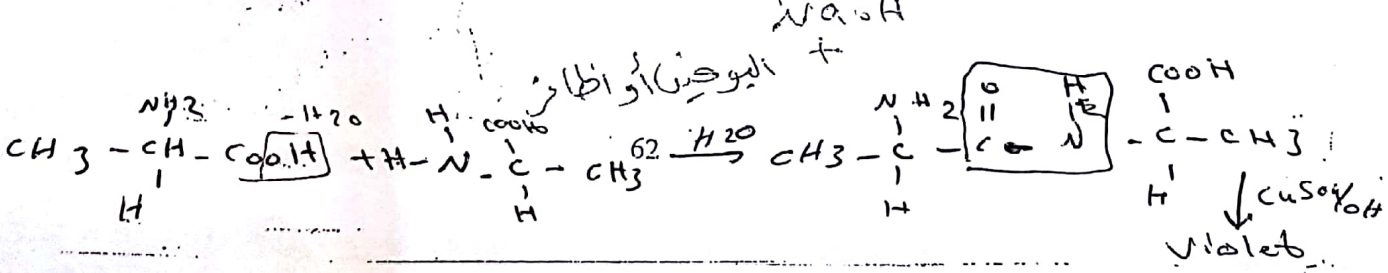
Proteins are macromolecules of great biological important. They are composed essentially of 20 α-amino acid residues. All microorganisms, plants and animals contain the same 20 α-amino acids. Protein may act as an enzymes, hormones, muscles, part of cell wall... etc. The following are simple qualitative tests to distinguish amino acids and proteins.

1. Elementary Composition of Proteins:

(a) Select a narrow Pyrex test tube, which is clean and perfectly dry, and introduce into it a small quantity of powder albumin. Place a piece of moistened red litmus paper across the mouth of test tube and heat until you get the smell of burning of hair. The red litmus paper turns blue. The characteristic smell and the effect on red litmus paper are due to the evolution of ammonia from the heated protein. This indicates the presence of nitrogen and hydrogen in the protein molecule.

Also notice that the upper part of the test tube is moist due to the condensation of water. This is due to the presence of oxygen and hydrogen in protein. The charring of the heated protein demonstrate the presence of carbon in protein.

(b) Introduce a small quantity of powdered albumin or nail into dry and narrow test tube. Add about (1 ml) of (40%) NaOH solution and heat gently for (2 minutes). Then add a drop of lead acetate solution. A black or brown colour indicates the presence of sulphur in the protein molecule.



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N.B. On heating the protein with NaOH some of the organically combined sulphur of protein is converted to sodium sulphide. This reacts with lead acetate to produce a black ppt of lead sulphide:

$$Na_2S + Pb(OCOCH_3)_2 \longrightarrow PbS + 2NaO_2CCH_3$$

(black ppt.)

2. Biuret Test:

Biuret test is a specific test for peptide bond or linkage. So all proteins and most of protein hydrolysis (tripeptide and above) or other compounds containing peptide bond are expected to give Biuret reaction.

(a) Urea:

Formation of Biuret and Biuret reaction. Place (0.2 g) of urea in a dry test tube, heat very gently just above the m.p. and note the production of ammonia. After 1-2 minutes the liquid suddenly solidifies with the formation of Biuret.

$$2\,NH_2CONH_2 \xrightarrow{\Delta} NH_2CONHCONH_2 + NH_3$$

Dissolve the solid residue in few ml. of warm (10%) NaOH solution, cool and add (1 drop) of very dilute copper sulphate solution. A purple coloration is obtained due to the formation of a complex between copper and two-CONH- groups.

(b) With Proteins.

To (2 ml) of a dilute protein solution add an equal volume of (10%) NaOH and mix. Then add, drop by drop a very dilute solution (0.5%) of copper sulphate till a violet or pink colour is obtained.

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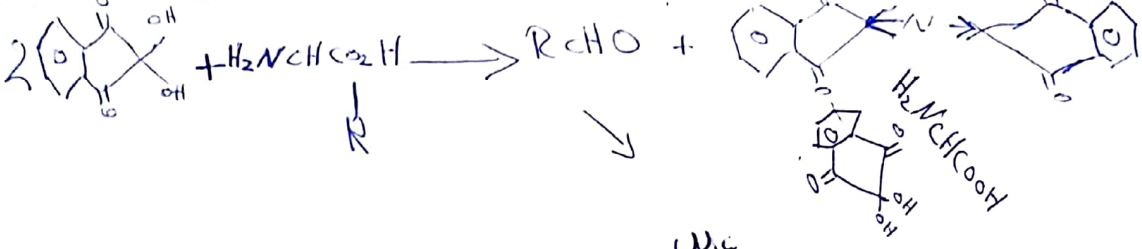


N.B. 1. Avoid adding an excess of copper sulphate, so that the blue colour of the cupric hydroxide formed, may not mask the violet or pink colour of the Biuret reaction.

2. Most proteins produce a violet colour. Protease and peptones give a decidedly pink colour. Gelatin gives a bluish violet colour.

3. Copper Salt of Amino Acids:

Dissolve about (0.5 g) of glycine in about (40 ml) of water and add an excess of freshly precipitated well-washed cupric hydroxide. Boil for (5 minutes) and filter while hot into a small evaporating-basin. Concentrate to about half-bulk on a boiling water-bath, and then allow to cool; fine blue needles of the copper salt $(NH_2CH_2CO_2)_2Cu$ crystallize out.

Prepare the cupric hydroxide by dissolving (2 g) copper sulphate in about (100 ml) of water, and add with stirring (16 ml) N.NaOH solution. Filter off the precipitate and wash thoroughly with water.

4. Ninhydrin Reaction:

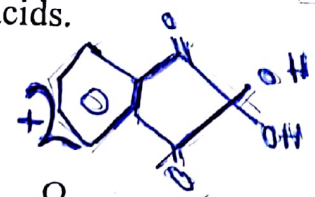
To (1 ml) of the dilute protein or amino acid solution (which must be neutral or at the most faintly acidic) add (2 drops) of a freshly prepared (0.2 %) solution of ninhydrin. Boil for 1-2 minutes and then allow cool. A blue colour is produced.

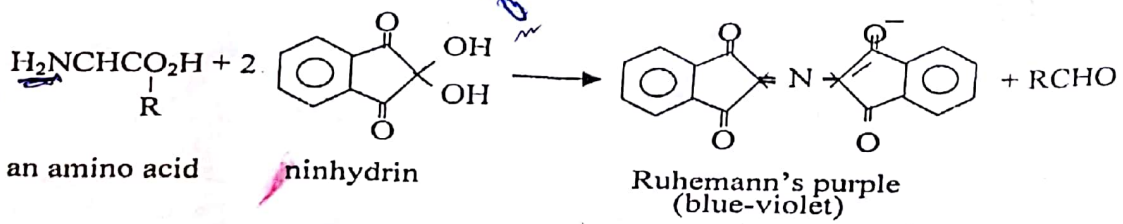
N.B. 1. The solution to be tested must be between pH 5-7.

2. Ninhydrin is readily soluble in water but its solution is not stable for more than two days.

3. This test is positive for proteins and for all products of protein hydrolysis including amino acids.

Reaction:





an amino acid — ninhydrin — Ruhemann's purple (blue-violet)

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5. General Tests for Amino Acids:

a. Action of nitrous acid:

This is a test amino acids in which the α-amino acid react with nitrous acid to form α-hydroxycarboxylic acid and evolution of nitrogen gas:

$$H_2NCHRCO_2H + HONO \longrightarrow HOCHRCO_2H + N_2 + H_2O$$

Procedure:

To a few ml of (20 %) $NaNO_2$ solution add a few drops of cold dilute acetic acid. Pour the mixture into a cold aqueous solution of glycine, and note the brisk evolution of nitrogen. In each case care must be taken not to confuse the evolution of nitrogen with any possible thermal decomposition of nitrous acid.

b. Solid amino acids:

Solid amino acids are soluble in Na_2CO_3 solution, with very slow evolution of CO_2 on heating.

c. Sorensen's reaction:

Formaldehyde converts the basic amino group into neutral $CH_2 = N^-$ group.

Procedure:

Dissolve (0.2 g) of glycine in a few ml. of water in a test tube A, add (2 drops) of phenolphthalein and then very dilute NaOH solution drop by drop until the solution just turn pink. In a second test tube B place (2 ml) of (40 %) formalin solution, add (2 drops) of phenolphthalein solution and then the dil. NaOH solution until the solution just turn pink. Pour contents of B into A and note the immediate decolourisation of the phenophthalein, the solution now being acid. Observe also that several drops of NaOH solution can now be added before the pink colour is restored.

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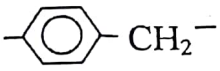
6. Specific Tests for Amino Acids:

A- Tyrosine $H_2NCHRCO_2H$, where R = HO  CH_2^-

The following two tests are specific for tyrosine or a protein containing tyrosine:

(1) Millon's reaction:

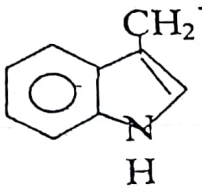
To (2 ml) of the protein or amino acid solution add (2 drops) of Millon's reagent and mix. Heat gradually, to the boiling point. The precipitate or the solution or both turn red in case of the presence of tyrosine.

N.B. 1. Millon's reagent is a solution of mercuric nitrite in acidic medium.

2. This test can be easily performed on solid proteins also. Suspend the solid protein in a little water and then carry out the test as usual. The solid particles will turn red.

3. Excess of chlorides interferes with this test by combining with the mercury of the reagent and thus rendering it inert. This test is therefore never used for the detection of proteins in urine.

(2) Formalin coloration: To a small crystals of tyrosine, add (1 drop) of (40 %) formalin, (1 ml) of water and (1 ml) of conc. H_2SO_4. Boil gently: a deep green coloration is developed.

Tryptophan $H_2NCHRCO_2H$, where R = 

Glyoxylic acid reaction: To (1 ml) of the protein or amino solution add equal volume of glacial acetic acid and mix. Incline the test tube and slide (2 ml) of conc. H_2SO_4 down its side so that the sulfuric acid forms a distinct layer at the bottom of the test tube.

A purple ring slowly develops at the junction of the two layers of fluid. On shaking, the entire fluid may become purple, and if there are any solid particles of protein in suspension, they may also acquire a vivid purple colour.

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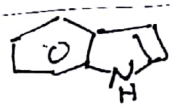
N.B. 1. Glacial acetic acid usually contains some glyoxylic acid as an impurity and is therefore suitable source of glyoxylic acid in this test.

2. This test is due to the presence of indole ring  in tryptophan.

3. Sulphuric acid used in this test must be pure.

C. Arginine ne H_2NCRCO_2H, where $R = H_2N - \underset{\underset{NH}{\|}}{C} - NHCH_2CH_2 -$

Sakaguchi's test:

To about (2 ml) of the protein or amino acid solution add (8 drops) of (10 %) NaOH, (2 drops) of freshly prepared ethanolic α-naphthol solution and mix. Add bromine water, one drop at a time, mixing after each addition. After few drops (usually 3-6 drops) of added bromine water, a bright red colour develops.

N.B. 1. As the reagents themselves give a certain amount of colour, a control is advisable using (2 ml) of distilled water.

2. Excess of bromine water should be avoided, as it may cause the red colour to fade away.

3. This is a very sensitive test for free or combined arginine. Since all known proteins contain sufficient amount of arginine to give a positive Sakaguchi's reaction this test may well be used as a general test for proteins.

D- Cysteine $H_2NCHRCO_2H$ where $R = HSCH_2 -$ and cystine where $R - CH_2 - S - S - CH_2 -$

To (1 ml) protein (undiluted egg albumin) or amino acid solution add (1 ml 40%) NaOH and boil for not less than one minute. This will convert the organically combined sulfur of cysteine or cystine into Na_2S. now add a drop of lead acetate solution. A black or brown colour appears due to the formation of lead sulphide.

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N.B. Wool, hair and fingernails give a marked reaction. Albumins from eggs, milk or blood serum also give a fairly good test. Casein of milk contains less of cystein and cystine and therefore does not give a marked reaction.

7. Specific Tests for proteins:

The following are some tests in which we can distinguish certain types of protein. These tests are of great value in clinical biochemistry for isolation characterization and determination of certain protein related to certain diseases.

A. Heat Coagulation Test for Albumin and Globumins:

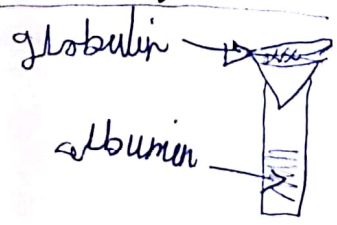
Albumins and globulins often occur together, as in blood serum, in egg white and in milk.

Procedure:

To (5 ml) of the diluted egg white solution add (2 drops) of the indicator chlorophenol red. The solution becomes pink. Now add (2%) acetic acid, drop by drop, till a barely visible pink colour remains. This corresponds to a pH of about (5.4), which is close to the isoelectric point of these proteins. Boil, a coagulum is produced. Filter and perform the Millon's test on a portion of the coagulum and also on filtrate.

B. Separation of Albumin and Globulin:

Measure (5 ml) of undiluted blood serum into a small beaker. Add (2%) acetic acid, drop by drop, until the reaction is practically neutral to litmus. Now add (5 ml) of saturated ammonium sulphate and mix well. The solution is now half saturated with respect to ammonium sulphate and a bulky ppt of serum globulin is produced. Allow to filter through a dry filter paper into a clean test tube (if the first portion cloudy refilter through the same filter paper). The residue on filter paper contains globulin whereas the filtrate contains albumin. To a portion of the filtrate add solid ammonium sulphate in a small portion with shaking till the solution is fully saturated. A precipitate of albumin is obtained.



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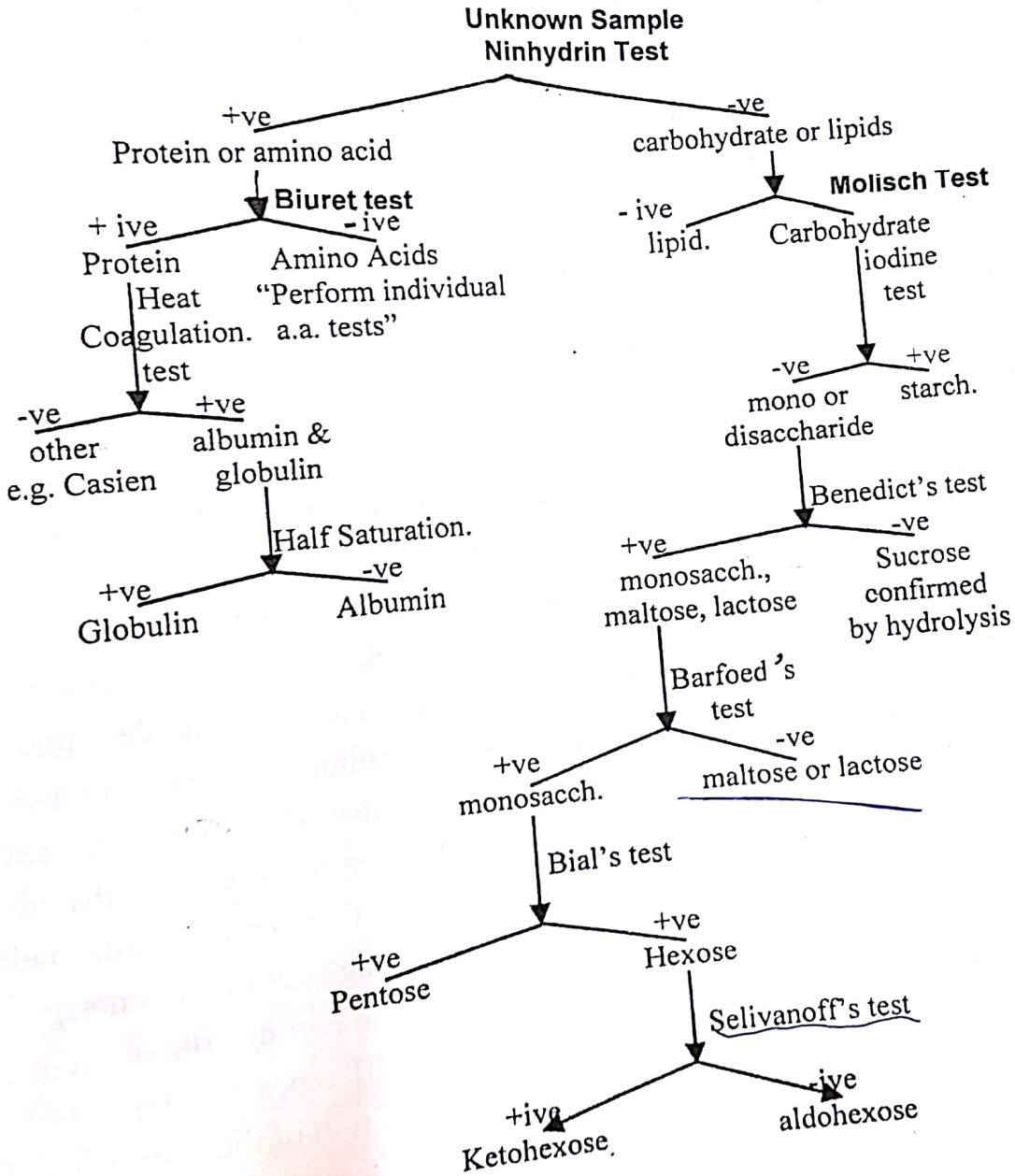
Laboratory No. 19:

IDENTIFICATION OF UNKNOWN SAMPLE

The following scheme is a simple diagram in which the student can distinguish between carbohydrates, lipids and protein.



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