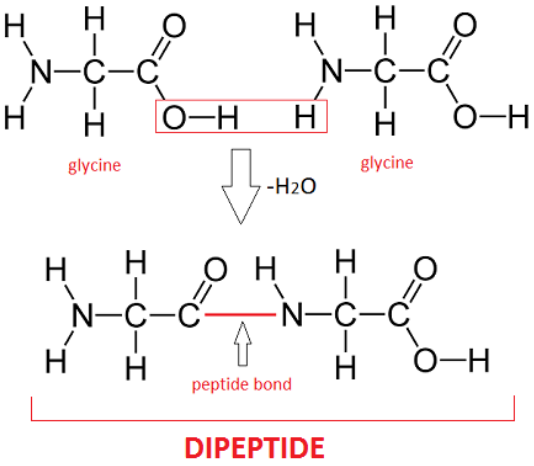
1.

Aim: Preparation of models of amino acids and dipeptides

Requirements: beads, pins, thermocol paper

Principle: Amino acids are organic compound consisting of a H atom, carboxylic group, amino group, and a functional group. There are the 22 α-amino acids incorporated into proteins and only these 22 appear in the genetic code of all life. Peptides are chemically synthesized by the condensation reaction of the carboxyl group of one amino acid to the amino group of another. Two molecules of amino acid joined by peptide bond to form a dipeptide.

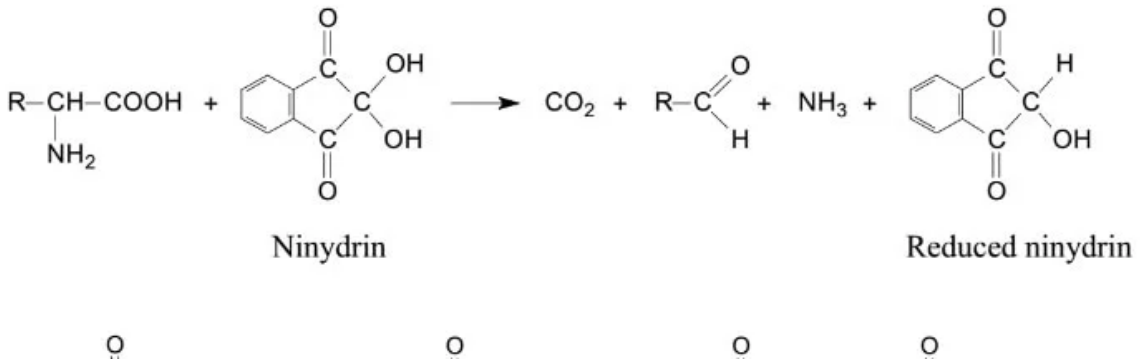


2.

Aim: To identify the presence of amino acid in a sample by Ninhydrin test

Requirements: Ninhydrin reagent: Dissolve 0.35g of ninhydrin in 100 ml ethanol (iso-propanol or 1:1 mixture of acetone/butanol may be used instead of ethanol). Diluent solvent (for the quantitative test): Mix equal volumes of water and n-propanol. Standard solution (1% protein solution), Test tubes, Test tube stand, Water bath

Principle: Ninhydrin test is a chemical test performed to detect the presence of ammonia, primary/secondary amines, or amino acids. This test involves the addition of ninhydrin reagent to the test sample that results in the formation of deep blue color, often termed as Ruhemann's purple, in the presence of an amino group. This assay is based on the fact that two molecules of ninhydrin (2, 2- dihydroxyindane-1, 3- dione) react with a free alpha-amino acid to produce a deep purple or blue color known as Ruhemann's purple. In this reaction, ninhydrin acts as an oxidizing agent and causes the deamination and decarboxylation of the amino acids at an elevated temperature. This reaction is then followed by condensation between the reduced ninhydrin molecules, released ammonia, and the second molecule of ninhydrin. By the end of the reaction, a diketohydrin complex is formed which has a deep purple colour. In amino acids like proline and hydroxyproline, this test yields an iminium salt, which is yellow-orange in color. Similarly, proteins with a free amine group like asparagine, react with the ninhydrin



reagent to form a brown coloured product. The intensity of the formed complex is proportional to the concentration of amino acids in the solution. The colour intensity, in turn, depends on the type of amino acid present.

Procedure

1. Take 1 ml of standard protein solution in one test tube and 1 ml of the test sample in another dry test tube.
2. Add a few drops of ninhydrin reagent to both the test tubes.
3. Place the test tubes in the water bath for 5 minutes and then allow cooling to room temperature.
4. Observe the formation of colour and note down the result.

Result and Interpretation of Ninhydrin Test

• The presence of a purple-colored complex in the tube represents a positive result and indicates the presence of amino acid in the sample.

• The absence of the complex in the tube represents a negative result and indicates the lack of amino acids in the sample

3.

Aim: Determination of pK and pI value of glycine

Requirements:

Standardized sodium hydroxide (0.1 N): dissolve 4 g of NaOH in 1L distilled water

ii) Standardized hydrochloric acid (0.1 N): Pipette out 8.33 mL of concentrated HCl into 1L volumetric flask and make the volume to 1000 mL with distilled water.

iii) Glycine solution (0.1 N): Dissolve 7.5 g of glycine powder in 1000mL distilled water

Glassware: conical flask, pipette, burette, wash bottle, beakers and measuring cylinders

Equipment: pH meter

Principle: Amino acids owing to the presence of amino and acidic groups tend to accept and donate protons in acidic and alkaline conditions. In this process amino acid attains +ve charge (cation) in acidic pH and –ve charge (anion) in alkaline pH. However amino acids exist as Zwitter ion (possess both +ve and –ve charges) at neutral pH. This property of amino acid enables to determine the following:

pKa: Dissociation constant in acidic pH (pK1)

pKb: Dissociation constant in alkaline pH (pK2)

pI: Isoelectric point.

Procedure:

Prepare all the reagents freshly prior to start the titration.

i) Take 20 mL of 0.1 N, glycine solution in a 50 mL beaker and measure the pH and note it in the log book. Take 50 mL burette and fill it upto "0" (zero) mark with 0.1 N HCl and slowly start titrating against glycine in the beaker. Record the change in pH value for every 1 mL addition of acid (Table4.1). Repeat the procedure of amino acid titration with 0.1N NaOH and record the change in pH value for every 1 mL addition of base (Table 4.2).

Plotting the titration Curve:

ii) Plot the curve by taking amount of acid or alkali added on x-axis, against change in the values of pH on the y-axis. The point where half of the volume of acid or alkali consumed in the titration will give pKa and pKb value. pI value of glycine can be calculated by using the following equation, (pI)=1/2 (pKa + pKb).

$$pI = \frac{pK_{a1} + pK_{a2}}{2}$$

Observation table:

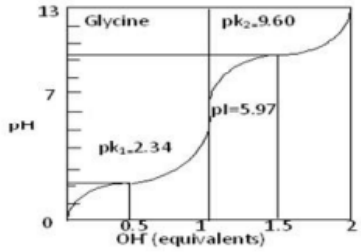
Volume of 0.1 N HCl added (ml)	Observed pH
0	
1.0	
2.0	
3.0	
Continue up to 20 ml	

Observation table 2

Volume of 0.1 N NaOH added (ml)	Observed pH
0	
1.0	
2.0	
3.0	
Continue up to 20 ml	

Results:

The pKa and pKb values obtained by plotting the titration curve are _____ and pI value is _____.



Precaution:

1. Rinse pH electrode with distilled water while changing form acid to alkaline solutions.
2. Note the change in pH regularly after addition of 1 mL of acid/alkali.
3. Immerse the electrode properly in glycine solution and avoid the touching of the electrode bulb with walls and bottom of the beaker.
4. Use electrode clamp to hold and avoid erratic values.

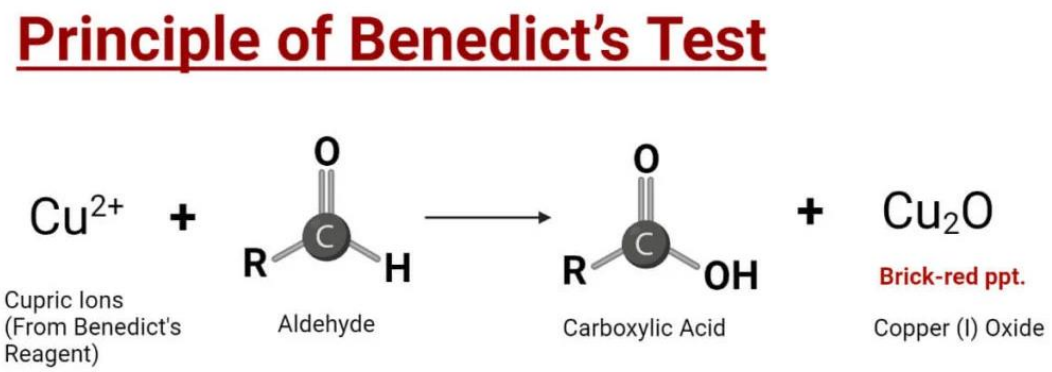
4.

Aim: Identify the presence of sugar molecule by Benedict's test

Requirements: Sample solution of unknown carbohydrate (or urine sample), Test-tubes and test-tube holders, Pipette, Bunsen burner, Benedict's Reagent

Preparation of Benedict's Reagent: Measure 17.3 grams of copper sulfate (CuSO4), 173 grams of sodium citrate ($Na_3C_6H_5O_7$), and 100 grams of anhydrous sodium carbonate (Na_2CO_3) (or 270 grams of sodium carbonate decahydrate ($Na_2CO_3.10H_2O$)). Put all the measured chemicals in a volumetric flask of 1000 mL. Pour distilled water up to 1000 mL marking. Dissolve all the components properly by shaking gently.

Principle: Benedict's Test is a chemical analytical method used for the detection of reducing sugar in a solution. Benedict's Test is a qualitative test often used for the differentiation of carbohydrates (saccharides/sugars) into reducing and non-reducing types. Sodium carbonate in the Benedict reagent increases the pH of the sample-reagent solution mixture. Under warm alkaline conditions reducing sugars are tautomerism to strong reducing agents, enediols. These enediols reduce the cupric ions (Cu^{2+}) (present as Copper Sulphate ($CuSO_4$)) of Benedict reagent into cuprous ions (Cu^{+}). The cuprous particles are present in form of insoluble Copper (I) oxide or cuprous oxide (Cu_2O) which is of red colour. These red-coloured copper oxides get precipitated. The concentration of reducing sugar in the sample differs from the intensity and shade of the colour of the reaction mixture. This shade of colour can be used to estimate the concentration of reducing sugar in the sample. Colour may vary from greenish to yellow to orange-red to brick-red. As the concentration of reducing sugar increases colour gradually changes from greenish to yellowish to orange to brick-red.



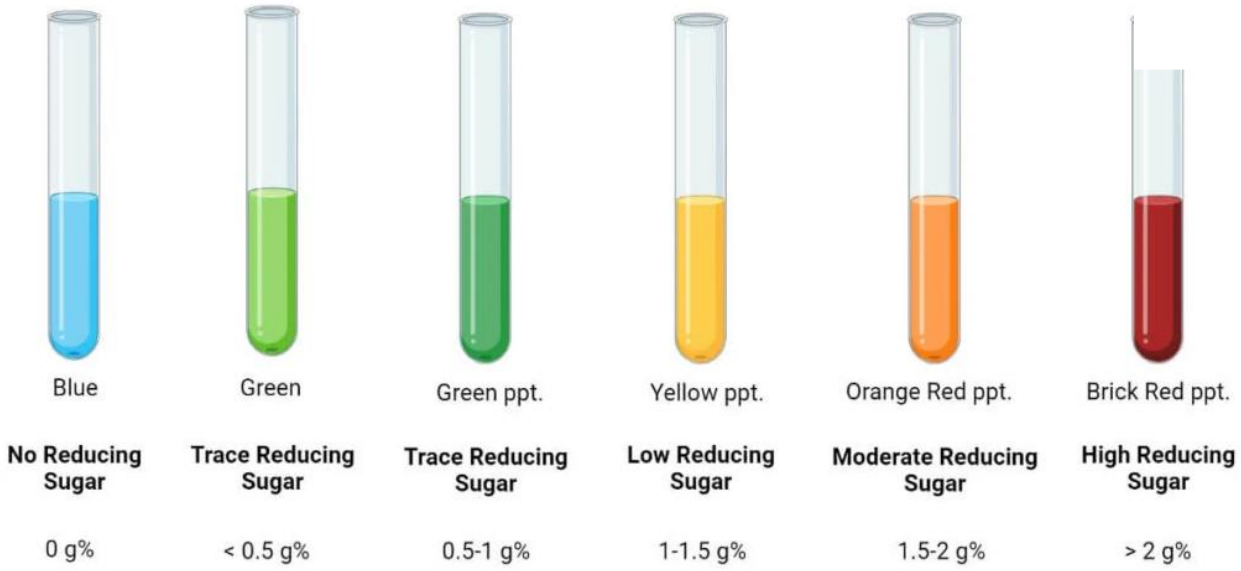
Procedure:

1. In a clean test tube add 1 mL of sample solution (urine or carbohydrate solution).

2. Add 2 mL of Benedict's reagents over the sample.
3. Place the test tube over a boiling water bath and heat for 3–5 minutes or directly heat over a flame.
4. Observe for colour change.

Results:

Any change in colour from blue to green or yellow or orange or red within 3 minutes indicates a positive Benedict test i.e. presence of reducing sugar in the sample.



Precaution:

1. Measurement must be accurate.
2. Do not heat the mixture quickly. It is best to heat over a water bath slowly.
3. During heating the solution, use a test-tube holder.
4. Do not face the test tube towards oneself or others during heating.
5. Heating should be done at least thrice before reporting negative.

5.

Aim: Identify the presence of starch by iodine test

Requirements: Knife, Spatula, Porcelain tile, Iodine solution, Food sample – Potato or any other vegetables or fruits.

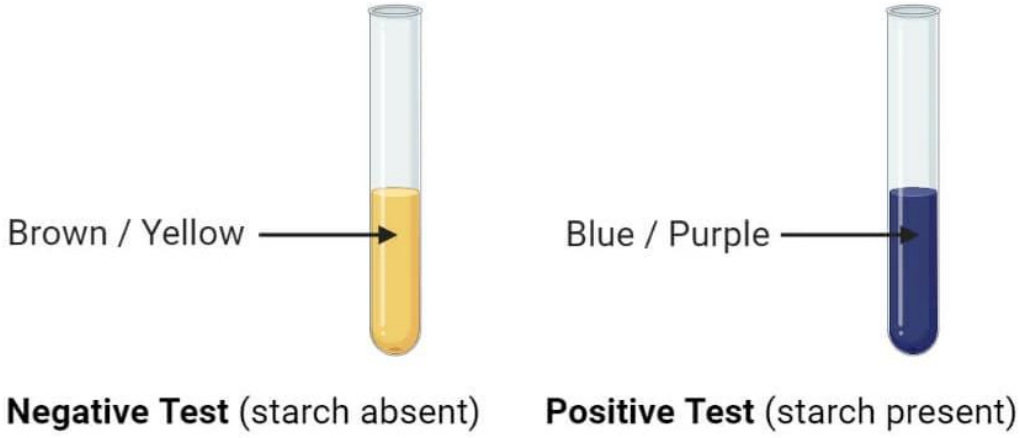
Principle:

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

Procedure:

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

Result:



- The appearance of blue-black or purple colour represents a positive test, indicating the presence of starch.
- If there is no change in colour, the result is negative and indicates the absence of starch

6.

Aim: To determine the acid value of oils

Requirements:

Reagents:

a) Phenolphthalein indicator solution: - Dissolve one gram of phenolphthalein in 100mL of ethyl alcohol.

b) Alkali Blue 6B indicator solution: When testing rice bran oil or rice bran oil based blended oils or fats, which give dark coloured soap solution, the observation of the end point of the titration may be facilitated, by using Alkali Blue 6B in place of Phenolphthalein.

Preparation: (2%) Extract 2gm of alkali blue 6B with rectified spirit in a Soxhlet apparatus at reflux temperature. Filter the solution if necessary and dilute to 100Ml with rectified spirit. Alkali blue 6B indicator to be stored in closed Ambered coloured bottle to avoid oxidation of dye.

c) Ethyl alcohol:

1) Ninety-five percent alcohol or rectified spirit neutral to phenolphthalein indicator.

2) Ninety-five percent alcohol or rectified spirit neutral to Alkali blue 6B indicator in case of rice bran oil or rice bran oil based blended oil or fats.

d) Standard aqueous Potassium hydroxide or sodium hydroxide solution 0.1 or 0.5 N. The solution should be colourless and stored in a brown glass bottle. For refined oils, the strength of the alkali should be fixed to 0.1 N.

Principle: The acid value is determined by directly titrating the oil/fat in an alcoholic medium against standard potassium hydroxide/sodium hydroxide solution.

Procedure:

Mix the oil or melted fat thoroughly before weighing. The mass of the test sample shall be taken based on the colour and expected acid value.

Expected acid value	Mass of test portion	Accuracy of weighing test portion (gm)
<1	20	0.05
1-4	10	0.02
4-15	2.5	0.01
15-75	0.5	0.001
75>	0.1	0.0002

a) Weigh accurately appropriate amount of the cooled oil sample as mentioned in the above table in a 250 mL conical flask.

b) Add 50 mL of freshly neutralised hot ethyl alcohol and about one ml of phenolphthalein indicator solution. In case of rice bran oil or RBO based blends, add about 1mL of Alkali blue indicator.

c) Heat the mixture for about fifteen minutes in water bath (75-80°C) In case of Rice bran oil or RBO based blended oils or fats, add 1mL of Alkali blue indicator after heating.

d) Titrate while hot against standard alkali solution shaking vigorously during the titration.

e) End point using phenolphthalein indicator shall be from colourless to light pink (Persisting for 15 sec.)

f) End point using Alkali blue 6B indicator shall be disappearance of blue colour which developed during addition of indicator.

Calculation:

Acid value = $(56.1\ V \times N)/W$

Where, V = Volume in mL of standard potassium hydroxide or sodium hydroxide used

N = Normality of the potassium hydroxide solution or Sodium hydroxide solution; and

W = Weight in gm of the sample

Results:

The acid value of the given sample is _____.

Precautions:

Noting burette reading after "obtaining dark pink colour OR Orangish red" as end point should be avoided as it will lead to erroneous result