

Carbohydrate :-

- ⇒ The carbohydrates are most abundant biomolecules found chiefly in vegetable tissues (about 75% of the dry weight of plant is carbohydrates), many of them form important foods.
- ⇒ Cellulose of wood and paper, starch present in cereals, roots and tubers, cane sugar and milk sugar are examples of carbohydrates.

Chemical nature and Classification:-

$(C_6H_{12}O_6)_n$ may be taken as the general formula to represent carbohydrates. Carbohydrates are classified into following four different groups:-

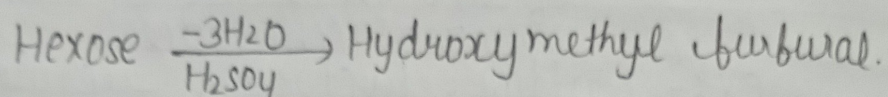
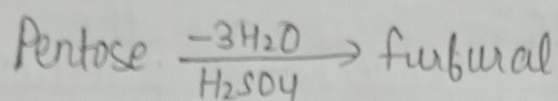
- 1) Monosaccharides
- 2) Disaccharides
- 3) Oligosaccharides
- 4) Polysaccharides.

① Monosaccharides:- They are simple sugar found in nature.
e.g:- glucose, fructose, galactose & mannose.

② Disaccharides :- These are the condensation products of two molecule of monosaccharides.
ex:- Maltose, lactose & sucrose.

③ Oligosaccharides :- Three to nine molecule of monosaccharide units condense to form oligosaccharides.
ex:- Maltose.

④ Polysaccharides :- More than nine molecules of monosaccharides condense to form polysaccharides.
Ex:- starch, glycogen.



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Expt. No. _____

Aim:- Qu

Reference

Requirement

Principle

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Procedure

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Note

Experiment - 1

Aim:- Qualitative analysis of carbohydrates by the Molisch's Test.

Reference:- Sharma Sadhana, Sharma Reema, "Practical book of Biochemistry" First edition - 2016, Published by Medtech, Page No 26.

Requirement:-

a) Glass wares:- Test tube

b) Chemicals:- Molisch's Reagent, Sulphuric Acid.

Principle:- This test is given by all soluble carbohydrates which have five or more carbon atoms and conjugated carbohydrates such as glycoproteins and glycolipids. $\Rightarrow$ Pentoses and hexoses are dehydrated in presence of strong mineral acids to form furfurals and hydroxy methyl furfural.

Procedure:-

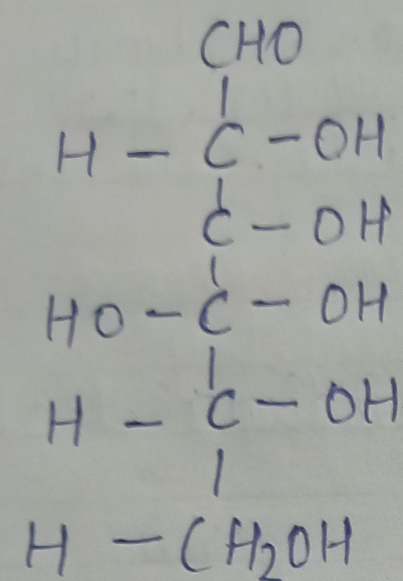
$\Rightarrow$ To 2.0ml of dil. sugar soln (1% w/v) in a test tube, add 2 drops of Molisch's reagent mixed thoroughly.

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- ⇒ Allow about 2ml of Concentration sulphuric acid to flow down the side of the test tube, forming a layer b/w the acid and sugar solution.
- ⇒ formation of a purple ring.

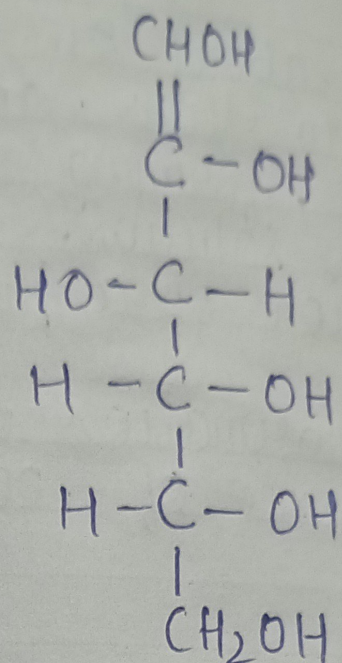
Result :- formation of purple ring indicate the presence of carbohydrates.

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(Glucose)

+ Alkali $\longrightarrow$



Ene-diol.

Expt. No. ...

Aim :-

Reference

Reagent

Principle

Procedure

Result

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Experiment $\Rightarrow$ 2

Aim :- Qualitative analysis of carbohydrates by the Benedict's Test.

Reference :- Same as Exp. 1 (Page no 27).

Requirements :-

(a) Glasswares :- Test tube

(b) Chemicals :- Benedict's Reagent.

Principle :- Benedict's reagent contains a weak alkali sodium carbonate. In the presence of weak alkali, reducing sugar form emediols which are strong reducing agents.

Procedure :- $\Rightarrow$ Boil 8 drops of sugar soln with about 5.0 ml of Benedict's reagent for about 1 minutes.

$\Rightarrow$ The formation of a red colour indicate the precipitate.

Result :- formation of red colour indicate the presence of Reducing sugar.

Experiment ⇒ 3

Aim :- Qualitative analysis of carbohydrate by Barfoed's Test.

Reference :- Same as previous Exp. (Page No. 28)

Requirements :-

Glassware :- Test tube

Chemical :- Barfoed's reagent.

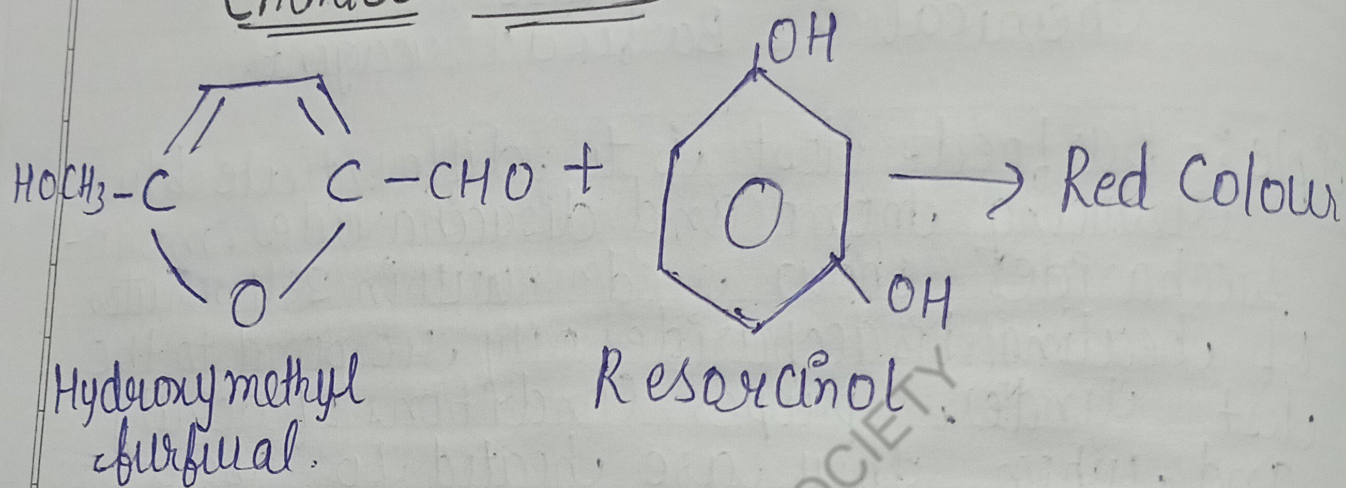
Principle :- Barfoed's test is to differentiate b/w reducing mono and disaccharides. monosaccharides show redⁿ within 3-4 minutes of heating. Disaccharides only respond to the test under proper conditions of acidity and when they are heated for about 10 minutes.

Procedure :- ⇒ Take 3.0 ml of Barfoed's reagent in a test tube, add 0.5 ml of the sample soln & place the tube in beaker of boiling water for 3-4 minutes.
⇒ formation of red precipitate.

Result :- formation of red 'ppt' indicate the presence of monosaccharides.

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Chemical Reaction:-



Experiment $\Rightarrow$ 4:-

Aim:- Qualitative analysis of Carbohydrates by Seliwanoff's Test.

Reference:- Same as Exp. 1 (Page No-28)

Requirement:-

Glassware:- Test tube

Chemical:- Seliwanoff's reagent.

Require Principle:- Seliwanoff's test is to differentiate b/w ketohexoses and aldohexoses.

The test is based on the fact of mesoxyd with hydroxyl methyl furfural which is rapidly produced from ketohexoses and much slowly from aldohexoses. The test is also given by sucrose which is hydrolysed during the course of the test to yield fructose.

Procedure:- $\Rightarrow$ Take 2.0ml of Seliwanoff's reagent, add about 0.5ml of sugar soln in a test tube and heat the tube for 2 minutes in a boiling water bath & red colour appears.

Result:- Appearance of red colour indicates the presence of a ketohexose.

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Experiment $\Rightarrow$ 5 :-

Aim :- Qualitative analysis of carbohydrates by phenyl hydrazine Test (Osazone Test).

Reference :- Same as exp. 1 (Page No-29-30)

Requirements :-

Glassware :- Test tube

Chemicals :- Phenyl Hydrazine

Principle :- An aldose or ketose reacts with phenylhydrazine to form hydrazone. which further reacts with another molecule of phenylhydrazine to form osazone. Each individual sugar in general gives rise to an osazone of a definite crystalline shape which is typical for that sugar. Glucose, fructose and mannose yield the same osazone bcz the osazone formed after reaction with phenylhydrazine masked their structural difference at C₁ and C₂ positions. maltose and lactose being reducing sugars also form osazones. These disaccharides derivatives are soluble in hot water, sucrose does not form any osazone.

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Procedure:-

- ⇒ Plan sufficient amount of dry phenylhydrazine sodium acetate mixture. (1 Part of phenylhydrazine hydrochloride and four parts of sodium acetate, mixed in a mortar just before use).
- ⇒ Add 5ml of 0.1% soln of sugar.
- ⇒ After shaking, heat in the Boiling water Bath for 15 min and allowed to cool slowly.
- ⇒ Examine the crystals under microscope.
- ⇒ Better crystals under may be obtained if the tubes are allowed to cool in the water bath overnight.

Result:- Formation of crystals of different shapes indicates the presence of Reducing sugar.

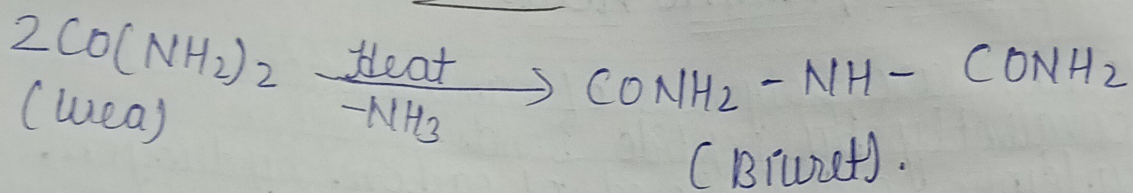
Protein/Amino Acid:-

- ⇒ Proteins are polymers of amino acids linked covalently by peptide bonds.
- ⇒ Proteins are composed of carbon, hydrogen, oxygen, nitrogen and sulphur.
- ⇒ Some proteins contain phosphorus and contain metals.
- ⇒ Proteins possess amphoteric properties, combining with both bases and acids.
- ⇒ Proteins are the main structural elements of cell membrane, intracellular matrix etc.

There are major three classes of proteins:-

- (A) Simple proteins :- Albumin, Histone, sclero-proteins and protamines.
- (B) Conjugated Proteins :- Proteins are conjugated with a non-protein part, known as prosthetic group.
- Metal protein ⇒ Metal ions
 - Chromoprotein ⇒ Respiratory pigments
 - Glycoprotein ⇒ Carbohydrate
 - Lipoprotein ⇒ Lipid
 - Nucleoprotein ⇒ Nucleic acid
 - phosphoprotein ⇒ phosphate.
- (C) Derived proteins :- Degraded proteins like peptones, proteases, peptides.

Chemical Reaction :-



Expt.

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Experiment $\Rightarrow$ 6

Aim:- Qualitative analysis of protein by Biuret Test.

Reference:- Same as exp. 1 (Page No - 35).

Requirement:-

Glassware:- Test

Chemicals:- Dis. water, protein soln, sodium hydroxide, Copper sulphate.

Principle:- Compound containing minimum of two peptide bonds or similar substances respond to Biuret test. Similarly, non-protein substances containing $-\text{CSNH}_2$, $-\text{GNHCNH}_2$ or $-\text{CH}_2\text{NH}_2$ in place of $-\text{CONH}_2$ group also give positive Biuret test.

$\Rightarrow$ Cupric ions in an alkaline medium form a violet colour complex with peptide bond nitrogen. Upon heating forms a compound known as Biuret.

Procedure:- $\Rightarrow$ Take two test tube

$\Rightarrow$ In one, take 1.0 ml of given protein soln and in other, take 1.0 ml of distilled water, as control.

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- ⇒ To both, add 4ml of 10% NaOH and 5 drop of CoSO₄ soln.
- ⇒ Protein gives a purple colour as against blue colour of control.

Result:- formation of purple colour indicate the presence of only peptides and proteins.

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