CHAPTER

1

Reactions of Carbohydrates

INTRODUCTION

Carbohydrates are aldehyde or ketone derivatives of polyhydric alcohols. They are widely distributed in plants and animals. Plants synthesize glucose by photosynthesis and it is converted mainly to storage form, the starch and structural framework form, the cellulose.

Animals largely depend on plant sources to obtain carbohydrates though they can synthesize carbohydrates from non-carbohydrate sources like lactate, glycerol and glucogenic amino acids in their body by a pathway called gluconeogenesis.

The glucose is the major form of carbohydrate absorbed from the gut in humans.

According to the metabolic status of the body, glucose has different fates:

- Catabolized to release energy
- Polymerized to form the storage fuel, the glycogen
- Sometimes converted to other sugars like fructose and galactose.

Different types of carbohydrates are present in intracellular and extracellular fluids and are excreted in urine when the concentrations of them rise in the blood in certain diseases, e.g.:

- Diabetes mellitus → glucose in urine
- Fructosuria → fructose in urine
- Galactosemia → galactose in urine.

Hence, it is essential to understand the tests for their detection.

The classification **of carbohydrates** will be useful for the detection of various types of carbohydrates by different chemical tests.

CLASSIFICATION

- **Monosaccharides:** Cannot be hydrolyzed into simpler carbohydrates. They are classified into trioses, tetroses, pentoses, hexoses, heptoses based on the number of carbon atoms present in them. They are again divided into aldoses and ketoses based on the functional group present in them (Table 1.1).
- **Disaccharides:** Give rise to two monosaccharide units upon hydrolysis, e.g.:
  - Sucrose (glucose + fructose)
  - Lactose (glucose + galactose)
  - Maltose (glucose + glucose).
- **Oligosaccharides:** Yield less than ten monosaccharides upon hydrolysis, e.g.:
  - Maltotriose (3 glucose units)
  - Raffinose (glucose + fructose + galactose).

TABLE 1.1 Classification of monosaccharides

Monosaccharides	*Aldoses*	*Ketoses*
Trioses ($C_3H_6O_3$)	Glycerose	Dihydroxyacetone
Tetroses ($C_4H_8O_4$)	Erythrose	Erythrulose
Pentoses ($C_5H_{10}O_5$)	Ribose	Ribulose
Hexoses ($C_6H_{12}O_6$)	Glucose	Fructose

- **Polysaccharides:** Contain more than ten monosaccharide units
  - *Homopolysaccharides* (consisting of same type of monomeric units), e.g.:
    - Polymer of glucose: Starch, glycogen, cellulose
    - Polymer of fructose: Inulin.
  - *Heteropolysaccharides* (consisting of different types of monomeric units).

Glycosaminoglycans (Proteoglycans) e.g.:
- Heparin (sulfated glucosamine + sulfated iduronic acid)
- Hyaluronic acid (β glucuronic acid + N-acetylglucosamine).

REACTIONS OF MONOSACCHARIDES

Monosaccharides possess one or more hydroxyl groups and an aldehyde or keto group. Therefore, many reactions of monosaccharides are the known reactions of alcohols, aldehydes or ketones. Many of the reactions shown by monosaccharides are exhibited by higher carbohydrates also. Differences in the structures of sugars often affect the rate of a reaction and sometimes the ability to react.

The reactions described below, are applied in the identification of sugars.

The reactions due to hydroxyl group: Dehydration (e.g. Molisch test, Rapid furfural test, Seliwanoff's test).

The reactions due to carbonyl group: Reduction (e.g. Benedict's test, Barfoed's test).

Molisch Test (α-naphthol Reaction) (Fig. 1.1)

Procedure: To 3 mL of sugar solution in a test tube, add two drops of Molisch reagent. Mix thoroughly. Add 3 mL of concentrated sulfuric acid along the sides of the test tube by slightly inclining the tube, thus forming a layer of acid (acid being heavier goes down beneath the sugar solution) in the lower part.

Observation: A **violet/purple colored ring** appears at the junction of two liquids.

Principle: Concentrated acid dehydrates the sugar to form furfural (in the case of pentoses) or furfural derivatives (in the case of hexoses and heptoses), which then condense with α-naphthol to give a **reddish violet** colored complex.

Inference: Indicates presence of a carbohydrate and hence the presence of a monosaccharide.

Application of the test: Used as a general test to detect carbohydrate.

Aberrant Observations

- Instead of a violet ring in the Molisch test, appearance of a **dark brown color** indicates **charring of sugar** due to the **heat generated during the addition of acid** (acid water interaction generates heat). It will become obvious when the concentration of the sugar solution is high. To avoid charring, dilute the sugar sample solution with water as illustrated in Figure 1.2 and repeat the Molisch test.
- Appearance of a **green color** while doing the test, which persist even after completion

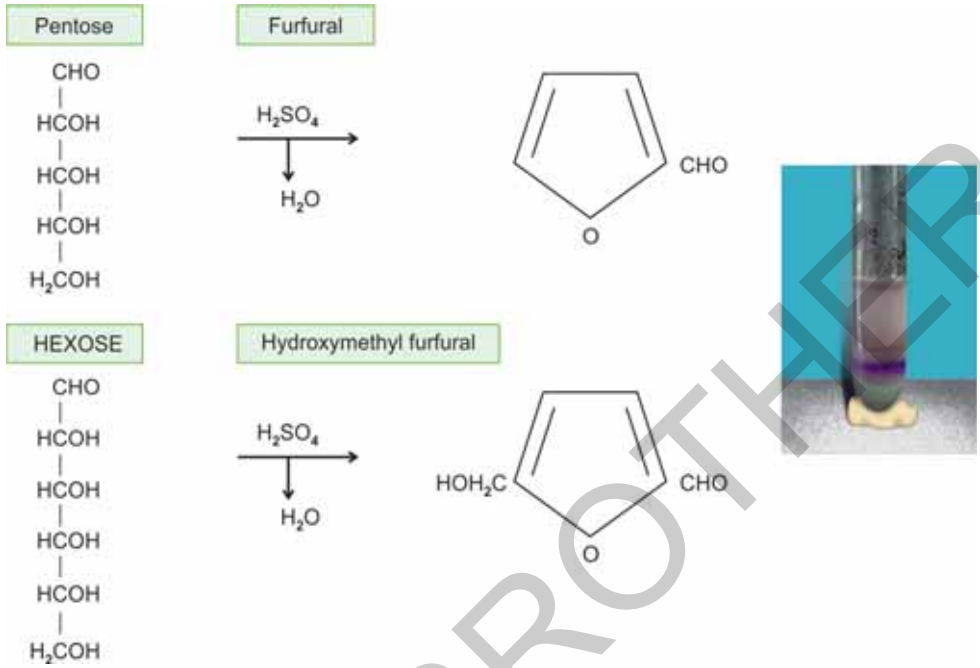

Figure 1.1 Chemistry of Molisch test

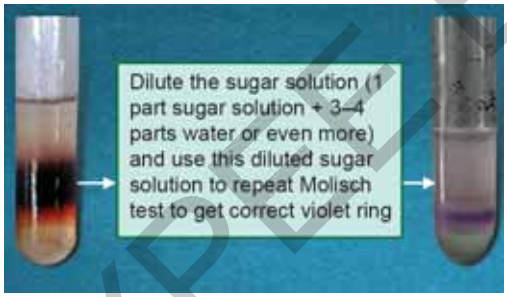

Figure 1.2 Method to avoid charring (Molisch test)

of the test suggest excess use of Molisch reagent than required or due to the presence impurities in the acid.

Benedict's Test (Fig. 1.3)

Procedure: To 5 mL of Benedict's reagent in a test tube add exactly 8 drops of the sugar solution. Mix well. Boil the solution vigorously for two minutes or place in a boiling water bath for three minutes. Allow the contents to cool by keeping in a test tube rack. Do not hasten cooling by immersion in cold water.

Observation: The entire body of the solution will be filled with a precipitate, the color of which varies with the concentration of the sugar solution—green, yellow, orange or brick red.

In the absence of a reducing substance, blue color of the Benedict's reagent remains as such. **The test is sensitive up to 0.1–0.15 g % of sugar in solution (that is Benedict's will not be positive with solutions containing less than 0.1– 0.15 g % of sugar)**.

Inference: Reducing monosaccharides glucose, fructose, galactose and mannose give a positive reaction with Benedict's reagent.

The color of the precipitate gives an idea about the concentration of the sugar solution as shown in Figure 1.3. Thus, Benedict's test is described as a semi-quantitative test.

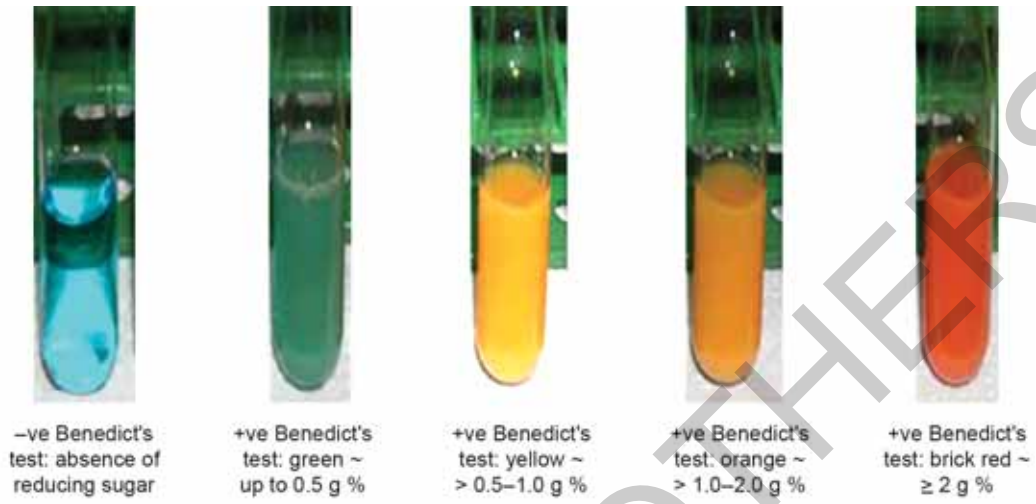

Figure 1.3 Benedict's test at different sugar concentrations

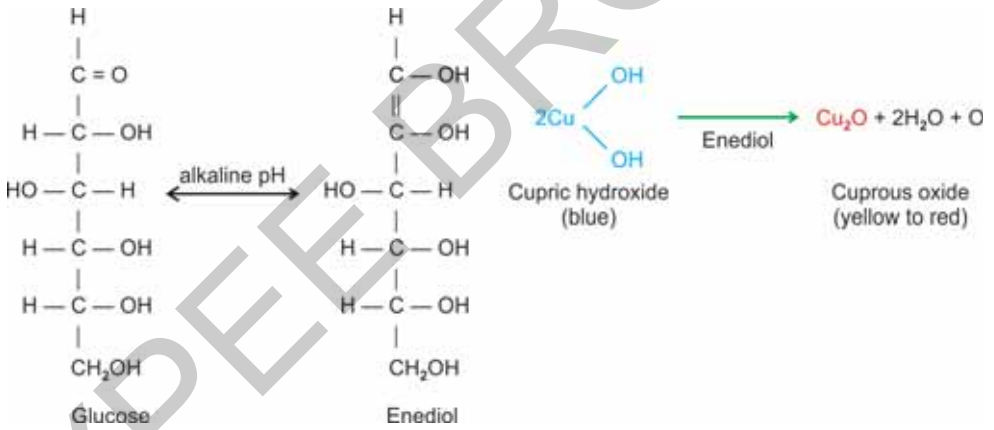

Figure 1.4 Chemistry of Benedict's test

Principle: (Fig. 1.4) Carbohydrates with a free aldehyde or keto group have the ability to reduce various metallic ions. In this test, cupric ions are reduced to cuprous ions by the enediols formed from sugars in the alkaline medium of Benedict's reagent.

Benedict's reagent contains copper sulfate, sodium citrate and sodium carbonate.

Copper sulfate dissociates to give sufficient cupric ions (in the form of cupric hydroxide) for the reduction reactions to occur.

Sodium citrate keeps the cupric hydroxide in solution without getting precipitated (stabilizer).

Sodium carbonate (Na_2CO_3) makes the pH of the medium alkaline.

In the alkaline medium, sugars form enediols, which are powerful reducing agents. They reduce blue cupric hydroxide to insoluble red cuprous oxide.

Application of the test: To detect reducing sugars. It is widely used in detecting glucose in urine even though not specific for glucose.

Barfoed's Test (Fig. 1.5)

Procedure: To 5 mL of Barfoed's reagent in a test tube add 0.5 mL of sugar solution. Mix well. Keep in a boiling water bath for **2 minutes**. Keep the tube in a test tube rack and examine for precipitate.

Observation: A **red** precipitate clinging to the bottom most part of the test tube indicates presence of a monosaccharide**.**

Inference: The test is answered by monosaccharides only, e.g.: glucose, fructose, galactose, mannose.

Principle: It is a reduction test. Reducing property is due to the carbonyl group (aldehyde or keto group). Barfoed's reagent is copper acetate in acetic acid**.**

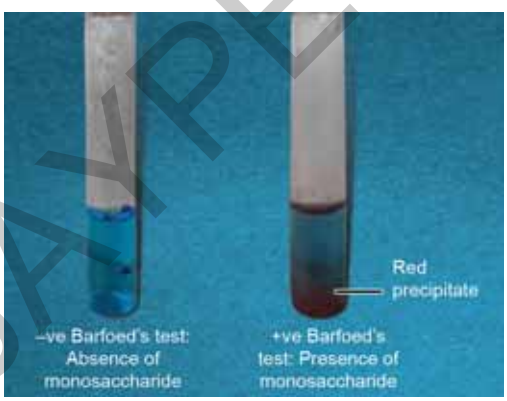

Figure 1.5 Barfoed's test

Difference between Barfoed's test and Benedict's test: Barfoed's test differs from Benedict's test with respect to the pH of the medium. It is alkaline in the case of Benedict's where as acidic in the case of Barfoed's. In the acid medium, monosaccharides enolize much more readily than disaccharides and these enediols reduce cupric ions released by copper acetate of Barfoed's reagent.

Points to Ponder

- It is important to keep the time limit (2 minutes) prescribed for Barfoed's test otherwise disaccharides will also respond to the test positively.
- Disaccharides when present in high concentrations (> 5 g%) also will give positive response.
- Unlike the Benedict's test, Barfoed's test is unsuitable for testing sugars in urine or any fluids containing chloride.
- The red precipitate is formed at the bottom of the tube. To see the precipitate, lift the tube to the eye level, otherwise the red precipitate adhering to the bottom most part of the tube may escape notice.

Application of the test: Useful to distinguish between monosaccharides and disaccharides.

Chemistry of the test: Reduction reaction as shown under Benedict's test.

Rapid Furfural Test

Procedure: To 2 mL of concentrated HCl, add 8 drops of sugar solution and 1–2 drops of Molisch reagent. Mix well and heat just to boil.

Observation: Positive reaction is indicated by the development of dark **violet** color (Fig. 1.6).

Figure 1.6 Rapid furfural test

Figure 1.7 Method to avoid charring in rapid furfural test

Inference: Development of violet color within 30 seconds of boiling indicates presence of a keto sugar, e.g. fructose.

Principle: A dehydration reaction due to the hydroxyl groups of the sugar. Concentrated HCl being weaker than concentrated sulfuric acid, dehydrates ketoses (e.g. fructose) more readily than aldoses (e.g. glucose) to form hydroxymethyl furfural, which then condenses with α-naphthol to form a violet colored complex.

Chemistry of the test: Dehydration reaction as shown under Molisch test.

Aberrant reaction: If red color develops instead of violet color due to charring action of acid, dilute the sugar sample with water and conduct the test with diluted sugar solution (Fig. 1.7).

Application of the Test

- For the detection of ketoses
- Useful for differentiating ketoses from aldoses.

Seliwanoff's Test

Procedure: To 3 mL of Seliwanoff's reagent in a test tube add 5 drops of fructose solution and heat the contents to **just boiling**.

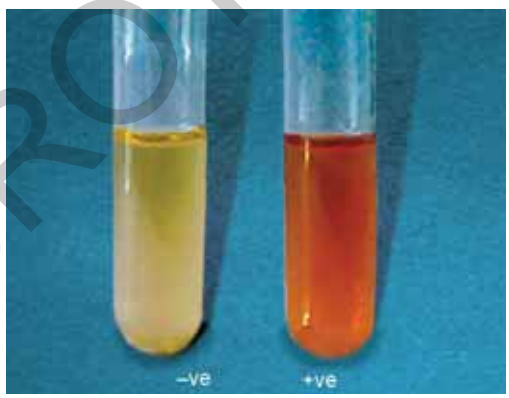

Figure 1.8 Seliwanoff's test

Observation: Positive reaction gives a **red color** within 30 seconds (Fig. 1.8).

Inference: This test is given by ketoses, e.g. fructose.

Principle: A dehydration reaction due to the hydroxyl groups of the sugar. Seliwanoff's reagent is resorcinol in dilute hydrochloric acid. Ketoses (e.g. fructose) are more readily dehydrated by HCl than the aldoses to form hydroxymethyl furfural, which then condenses with resorcinol of Seliwanoff's reagent to form a **red colored** complex.

Points to Ponder

- The test is sensitive up to 0.1 g % of fructose in the absence of glucose.
- In the presence of glucose, the test becomes less sensitive to fructose.
- Large amounts of glucose give the same (red) color.
- If the boiling is prolonged, a positive reaction may occur with glucose because of Lobry de Bruyn–van Ekenstein transformation of glucose into fructose, in the presence of acid.

The precautions to be followed to get a positive test for fructose are given below:

- Concentration of HCl used must be less than 12%
- The reaction must be observed ***within 20–30 seconds of performing the test***
- Those reactions occurring after 20–30 minutes of boiling must not be considered positive
- Glucose must not be present in amounts more than 2 g% or else it will interfere with the test.

Foulger's Test

Procedure: To 3 mL of Foulger's reagent add 8 drops of sugar solution. Boil for 45 seconds directly on a flame. Allow to cool slowly.

Figure 1.9 Foulger's test

Observation: Deep blue color develops (Fig. 1.9).

Inference: This test is given by ketoses, e.g. fructose.

Principle: A dehydration reaction due to the hydroxyl groups of the sugar. Foulger's reagent contains stannous chloride, urea and 40% H_2SO_4. Ketohexoses form hydroxymethyl furfural with acid which then condenses with stannous chloride and urea to form deep blue color.

Osazone Test

Procedure: To 5 mL of sugar solution in a test tube add 300 mg (one or two scoopfuls) of phenyl hydrazine mixture. Shake well. Heat in a boiling water bath for 15–45 minutes (duration of keeping time in water bath depends on the type of sugar). Then take the tube out of the water bath and allow cooling at room temperature by placing in the test tube rack. Avoid showing under the tap water because rapid cooling disturbs crystallization whereas slow cooling ensures crystallization.

Observation: Crystals are formed readily (within 1–5 minutes) at room temperature in the case of mannose. For other sugars, the minimum incubation time required in minutes in the boiling water bath for the formation of insoluble yellow osazone is given in Table 1.2. Look under the microscope to view the crystals (Fig. 1.10).

TABLE 1.2 Time taken by different monosaccharides for osazone formation

Monosaccharides	*Time (Minutes)*
Glucose	5
Fructose	2
Galactose	20

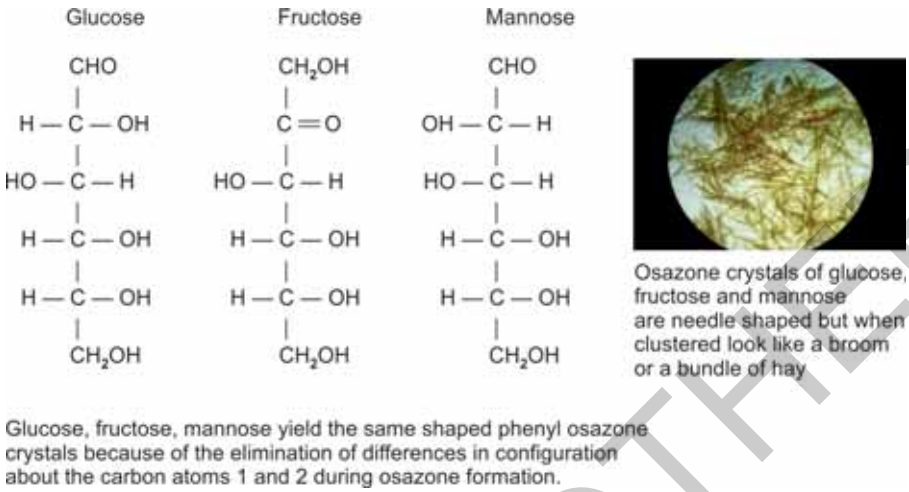

Figure 1.10 Reason for getting the same shaped osazone crystals for glucose, fructose and mannose

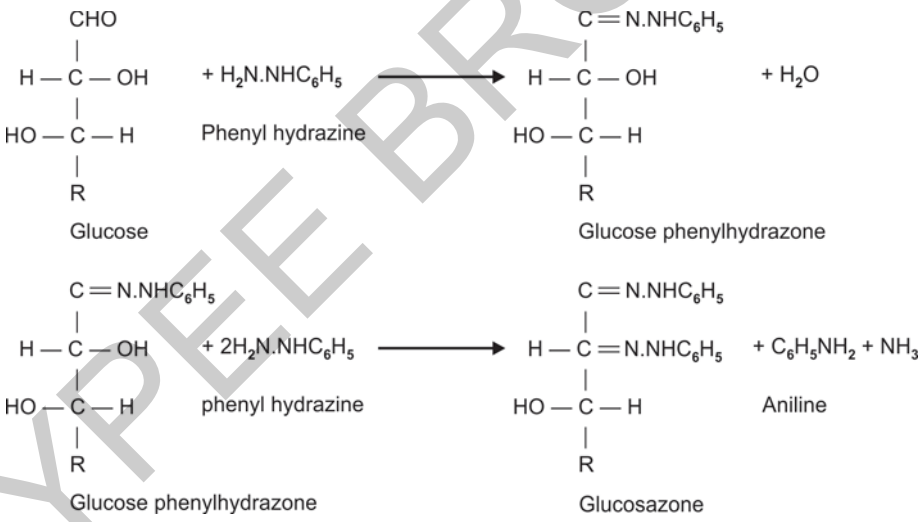

Figure 1.11 Chemistry of osazone test

Inference: Glucose, fructose, mannose yield the same shaped phenyl osazone crystals because of the elimination of differences in configuration about the carbon atoms 1 and 2 during osazone formation (Fig. 1.11).

Principle: The reaction involves the carbonyl carbon (either aldehyde or ketone as the case may be) and the adjacent carbon. One molecule of sugar reacts with one molecule of phenyl hydrazine initially, to form phenylhydrazone