

Exercise 8

Carbohydrates. Specific reactions of monosaccharides and disaccharides. Quantitative determination of glucose in urine.

Theoretical part

Carbohydrates are named for their characteristic content of carbon, hydrogen, and oxygen (CH_2O). Short chains containing from three to seven carbons form the monosaccharides, the individual building blocks of carbohydrates. Of these, trioses, pentoses and hexoses are most common in cells. All monosaccharides occur in linear form and each carbon atom in the chain, except one, carries an $-\text{OH}$ group. The remaining carbon carries a $-\text{C}=\text{O}$ (carbonyl) group. In monosaccharides all other available binding sites of carbon are occupied by hydrogen atoms. The carbonyl oxygen of a linear sugar may be located at the end of the carbon chain as an aldehyde group or inside the chain as a keton group.

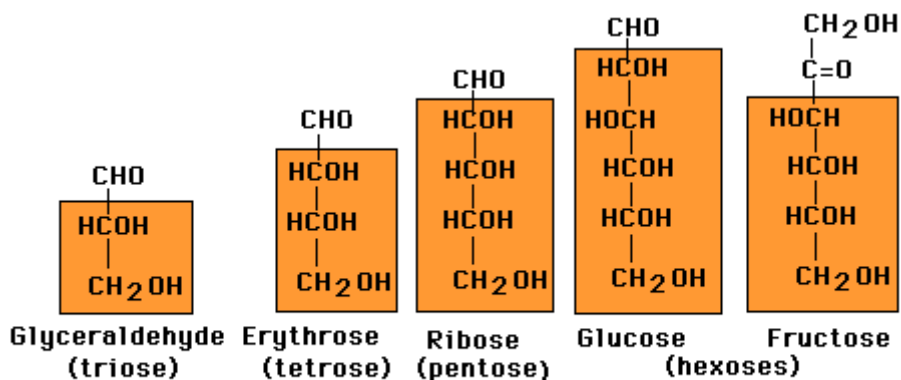
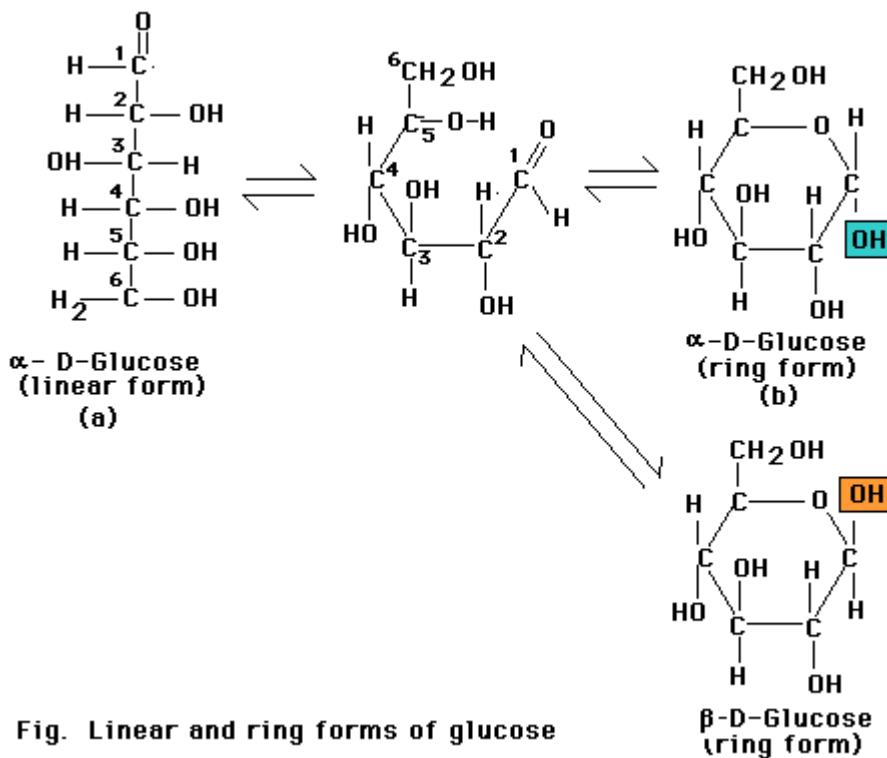


Fig. Monosaccharides with various numbers of carbon atoms.

Monosaccharides with five or more carbons can form a ring as well as a linear configuration. The rings form through a reaction between two functional groups in the same molecule. In the six-carbon monosaccharide (glucose), a covalent bond can form through a reaction between the aldehyde at the 1-carbon and the hydroxyl at the 5-carbon. The reaction produces either of the two closely related glucopyranose ring structures. The aldehyde at the 1-carbon can also react with the hydroxyl at the 4-carbon to produce a glucofuranose ring. Monosaccharide rings such as that of glucose are frequently depicted as a Haworth projection a diagram that suggests the three-dimension orientation of the attached $-\text{H}$, OH , and $-\text{C}_2\text{OH}$ groups in relation to the ring. However, the glucopyranose ring does not actually lie in a flat plane as suggested by this projection. Instead, the ends of the ring are bent up or down, most frequently in the "chair" conformation. More over, the side groups attached to the ring extend at the various angles, not at right angles as depicted in the Haworth projection.



The glucopyranose ring occurs in two forms that differ only in the orientation of the -OH group at the 1-carbon. In a Haworth projection -OH group points downward, in the alpha form of the sugar, as in α -glucose. In the other form, the -OH group points upward from the ring in a beta position of the sugar as in β -glucose.

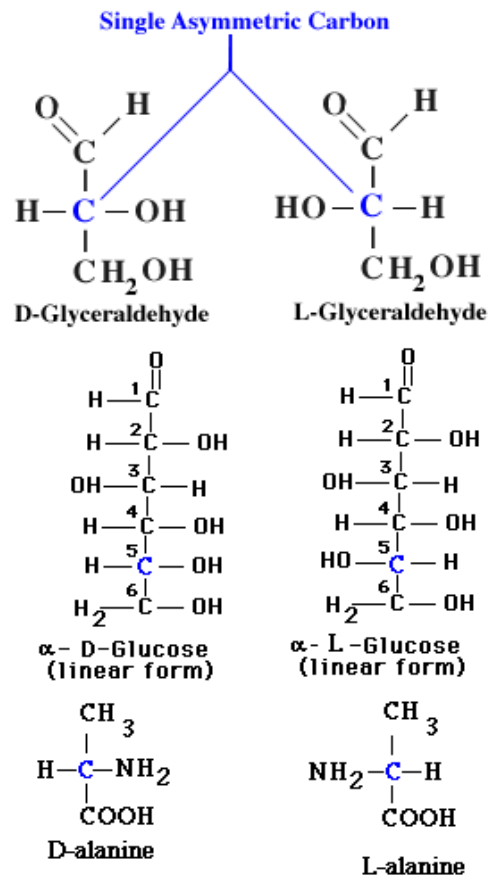
Although the difference between the two ring forms might seem trivial, it has great significance for the chemical properties of polysaccharides assembled from monosaccharide rings. For example, starches, which are assembled from α -glucose units, are soluble and easily digested. Cellulose, synthesized from β -glucose units, is insoluble and cannot be digested as a food source by most animals.

The orientation of other groups attached to the carbon chain of carbohydrates also affects the properties and interactions of monosaccharides. The alternative forms of a molecule in which atoms or groups attached to the carbon chain point in different direction are termed stereoisomers. By convention, stereoisomers are identified as D- or L- depending on the directions pointed by atoms attached to the carbon chain.

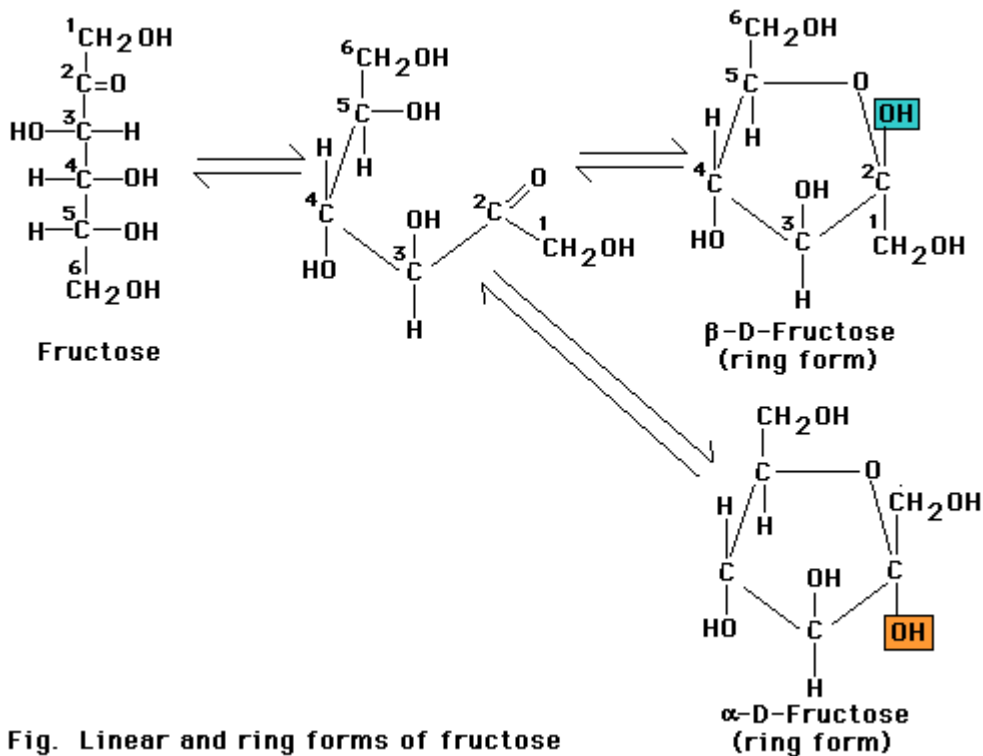
With several exceptions, the different stereoisomers react at significantly different rates with D- and L- forms of other stereoisomers. Because the amino acid subunits of enzymes are stereoisomers, enzymes typically react much more efficiently with one of the two stereoisomers of monosaccharides. In most cases this is the D-form of the monosaccharide, which accounts for the fact that D-forms are much more common than L- forms among cellular carbohydrates.

Many internal carbon atoms of monosaccharides and other organic molecules are asymmetric—each of their four covalent bonds links to a different atom or chemical group. The middle carbon of the triose glyceraldehyde, for example, is asymmetric because it shares electrons in covalent bonds with -H, -OH, -CHO, and -CH₂OH.

The groups attached to asymmetric carbon atoms can take up either of two fixed positions with respect to other carbon atoms in a chain. In glyceraldehyde the -OH group can extend either to the left or to the right of the carbon chain with reference to the -CHO and -CH₂OH groups. By convention the stereoisomer in which the -OH extends to the right is called D-glyceraldehyde (from the Latin dexter= right), and the stereoisomer in which the -OH extends to the left is L-glyceraldehyde (from laevus = left).



Monosaccharides in the ring form can link together to form disaccharides or in greater numbers to form polysaccharides. In some polysaccharides, such as cellulose, the chain is unbranched; in others, such as glycogen, the chain is branched. Some polysaccharides, such as cellulose, contain only a single type of monosaccharide building block.



Disaccharides

Covalent bonds between the anomeric hydroxyl of a cyclic sugar and the hydroxyl of a second sugar (or another alcohol containing compound) are termed **glycosidic bonds**, and the resultant molecules are **glycosides**. The linkage of two monosaccharides to form disaccharides involves a glycosidic bond. Several physiologically important disaccharides are sucrose, lactose and maltose.

Sucrose: prevalent in sugar cane and sugar beets, is composed of glucose and fructose through an α -(1,2) β -glycosidic bond.

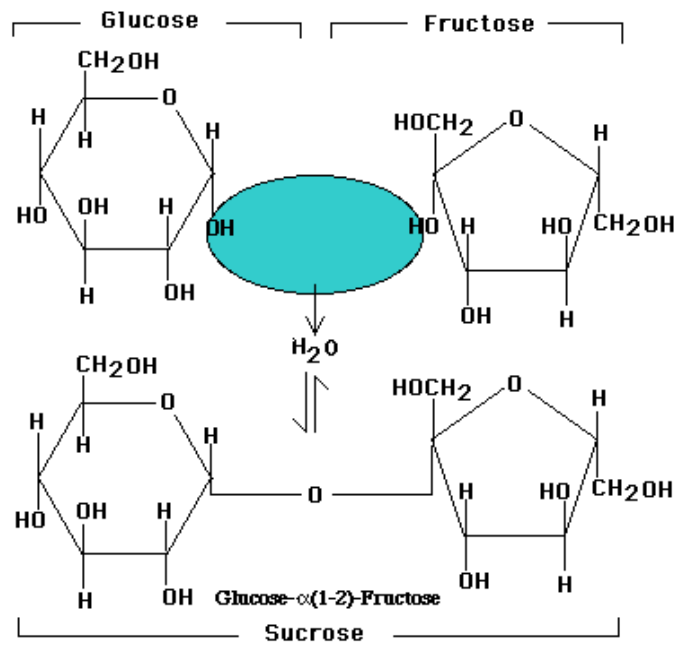
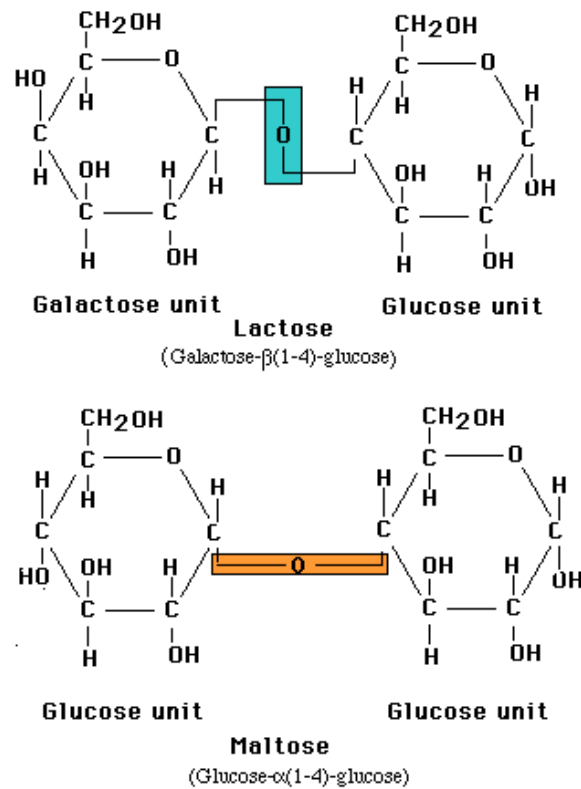


Fig. Dehydration synthesis of a sucrose molecule by the condensation of glucose and fructose

Lactose: is found exclusively in the milk of mammals and consists of galactose and glucose in a $\beta(1,4)$ glycosidic bond.

Maltose: the major degradation product of starch, is composed of 2 glucose monomers in an $\alpha(1,4)$ glycosidic bond.



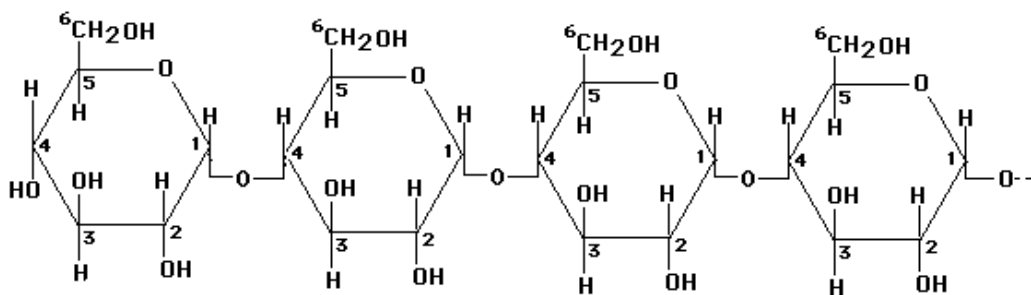
Polysaccharides

Most of the carbohydrates found in nature occur in the form of high molecular weight polymers called **polysaccharides**. The monomeric building blocks used to generate polysaccharides can be varied; in all cases, however, the predominant monosaccharide found in polysaccharides is D-glucose. When polysaccharides are composed of a single monosaccharide building block, they are termed **homopolysaccharides**. Polysaccharides composed of more than one type of monosaccharide are termed **heteropolysaccharides**.

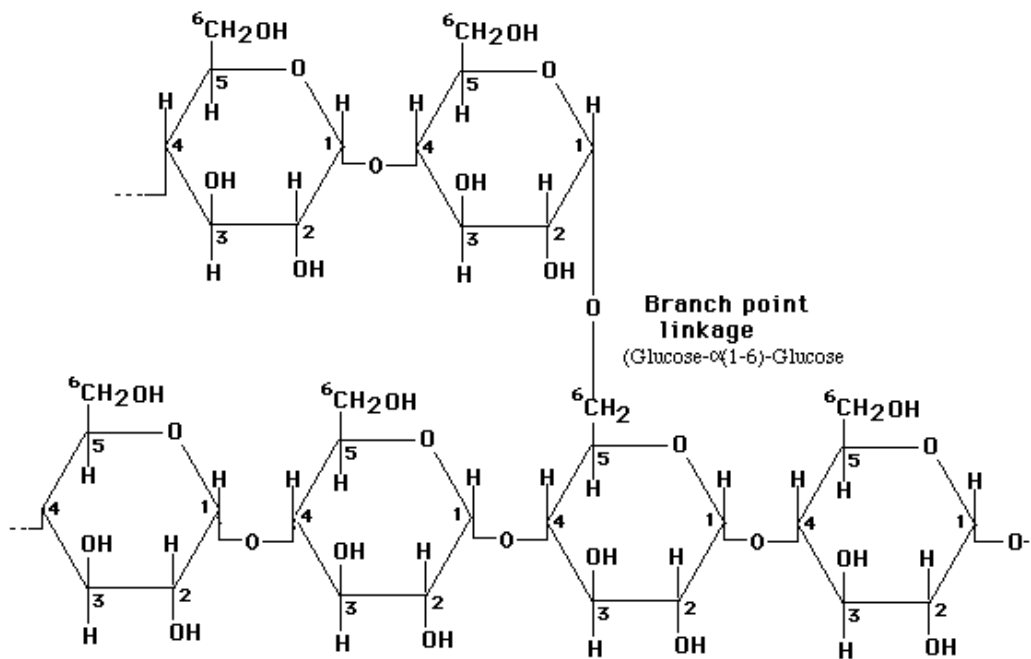
Starch

Starch is the major form of stored carbohydrate in plant cells. Its structure is identical to glycogen, except for a much lower degree of branching (about every 20-30 residues).

Unbranched starch is called **amylose**; branched starch is called **amylopectin**.



(a) Amylose
(Glucose- $\alpha(1-4)$ -Glucose)



(b) Amylopectin

Glycogen

Glycogen is the major form of stored carbohydrate in animals. This crucial molecule is a homopolymer of glucose in $\alpha(1,4)$ linkage; it is also highly branched, with $\alpha(1,6)$ branch linkages occurring every 8-10 residues. Glycogen is a very compact structure that results from

the coiling of the polymer chains. This compactness allows large amounts of carbon energy to be stored in a small volume, with little effect on cellular osmolarity.

Cellulose

Cellulose is a polysaccharide consisting of β -glucose units. It is the chief constituent of plant cells. It is a tough, resistant substance that gives plant tissues considerable rigidity. It thus serves an important an important function in the plant world as a structural or supporting substance. Wood, paper, cotton, flax, and hemp are composed principally of cellulose. Cellulose is not digested by the enzymes of the alimentary tract of man but is a desirable constituent of the diet because it gives bulk to the feces and thus tends to prevent constipation.

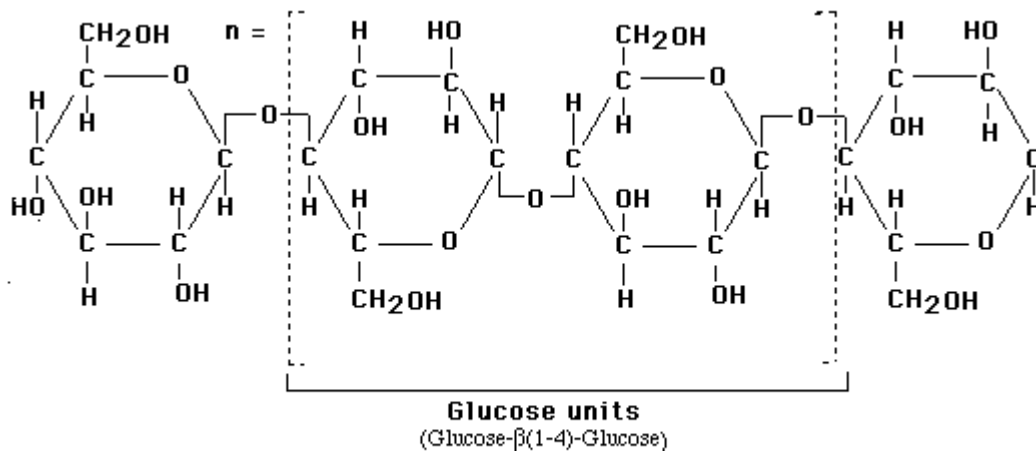


Fig. Structure of a portion of a cellulose molecule in which n = repeating pair of glucose units.

Dextrin

The dextrins are intermediate products of the hydrolysis of starch to maltose. In addition to the methods involving hydrolysis, dextrin may be prepared from starch by heating of temperatures ranging from 170⁰ to 240⁰ C for varying lengths of time. They are colloidal substances, and their chief use is as mucilages for postage stamps, envelopes, and other purposes.

Inulin

Inulin is a polysaccharide consisting of fructose units. It occurs in the tubers of dahlias and in the Jerusalem artichoke. Inulin is not digested by the enzymes of the alimentary tract of man. It is used in clinical laboratory work as a test substance for measuring kidney function (the inulin clearance test).

Agar

Agar is a polysaccharide composed of galactose units. It is prepared from seaweed. Agar is used extensively in microbiology in the preparation of culture media for growing bacteria.

Dextrans

Dextrans are polysaccharides consisting of glucose units. They are synthesized from sucrose by the microorganism *Leuconostoc mesenteroides*. Dextrans differ from other polysaccharides of glucose, such as starch, dextrans, and glycogen, in the way the glucose molecules are linked to each other. Their molecules size differ from each other. Under certain conditions the bacteria synthesize dextrans with molecular weight of probably several million, and other conditions smaller molecules are produced.

Dextran is used in medicine as plasma volume expanders in the treatment of shock. Clinical shock is characterized by a reduced circulating blood volume. When dextran solution is injected intravenously, the dextran remains in the bloodstream for several days, being slowly excreted in the urine. While in the vascular system, dextran increases the osmotic pressure of the blood. This causes water to pass into the blood vessels from the extracellular spaces. The increase in blood volume raises the blood pressure and thus relieves the patient suffering from shock.

The dextran molecules synthesized by *Leuconostoc mesenteroides* are too large for clinical use. To make the bacterially synthesized dextrans suitable for clinical use, the product is submitted to mild, controlled hydrolysis, which shears the molecules down to a smaller size. Dextran suitable for clinical use has an average molecular weight of 70,000.

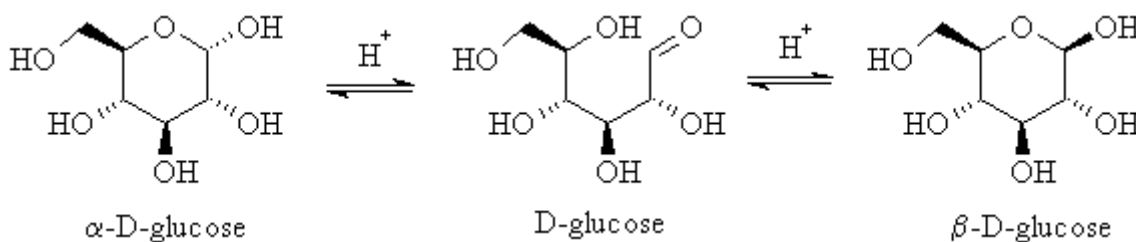
Physical and chemical properties.

The monosaccharides and disaccharides are crystalline solids. They are soluble in water. The polysaccharides are amorphous substances of varying solubilities. The dextrin and glycogen are soluble in cold water; starch, inulin. Agar is soluble in hot water; and cellulose is highly insoluble in water. The polysaccharides that can be dissolved form colloidal solutions.

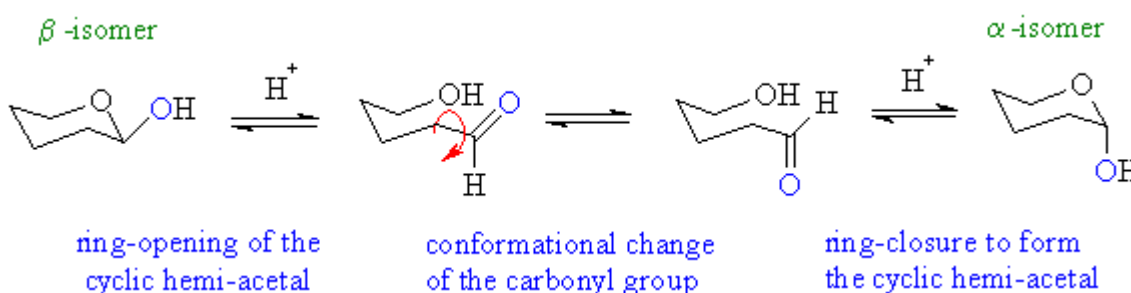
Mutarotation : interconversion of α - and β - anomers

- The α - and β - anomers of carbohydrates are typically stable solids.
- However, in aqueous solution, they quickly equilibrate to an equilibrium mixture of the two forms.

- For example, in aqueous solution, glucose exists as a mixture of 36% α - and 64% β - (>99% of the pyranose forms exist in solution).
- The equilibration occurs via the ring opening of the cyclic sugar at the anomeric center with the acyclic form as the intermediate.

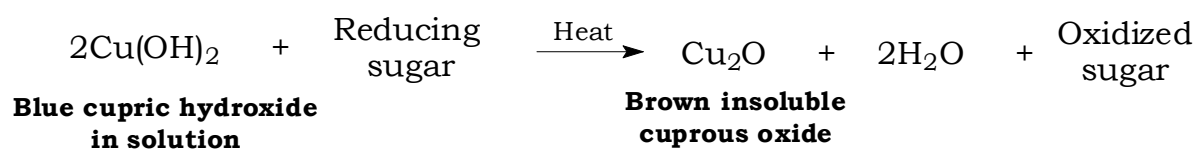


- Or, in a simplified form with all the "extra" hydroxy groups removed:



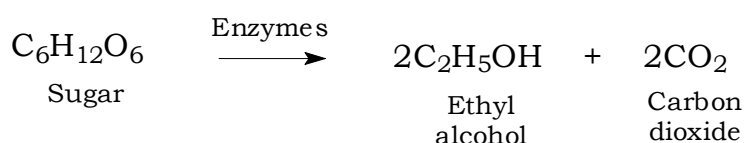
Stability. Carbohydrates show the highest stability in a neutral medium. Sugars (monosaccharides and disaccharides) decompose to some extent in acid and alkaline solution. Polysaccharides (such as glycogen, starch, and cellulose) have a high degree of stability in alkalies.

Reducing substances (Fehling's test). Carbohydrates containing free aldehyde or ketone groups have the power to reduce alkaline metallic oxides, and this property is the basis of Fehling's test for sugars. Fehling's reagents are alkaline copper sulfate solution coloured blue. When these reagents are heated with a few drops of simple sugar solution, the blue color fades or disappears entirely, and a reddish brown precipitate of cuprous oxide is formed. This reaction is illustrated by the following equation:



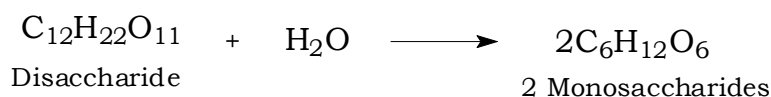
This is the standard test for sugar glucose in urine. The appearance of reddish brown cuprous oxide when a few drops of urine are heated with Fehling's reagents a positive indication of the presence of sugar, since is the only reducing substance that occurs in appreciable amounts in the urine. All monosaccharides and all disaccharides except sucrose reduce Fehling's reagent.

Fermentation. Sugars undergo a chemical change known as fermentation in the presence of enzymes secreted by certain microorganisms.

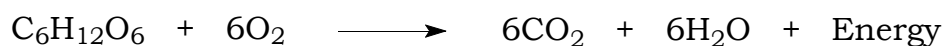


Alcoholic fermentation is commercially used in making wines, beers, and other alcoholic beverages. Lactose is the only sugar that cannot be fermented, since yeast does not contain the proper enzyme.

Hydrolysis. When boiled with dilute acids or treated with certain enzymes, the polysaccharides and the disaccharides are hydrolysed to monosaccharides.

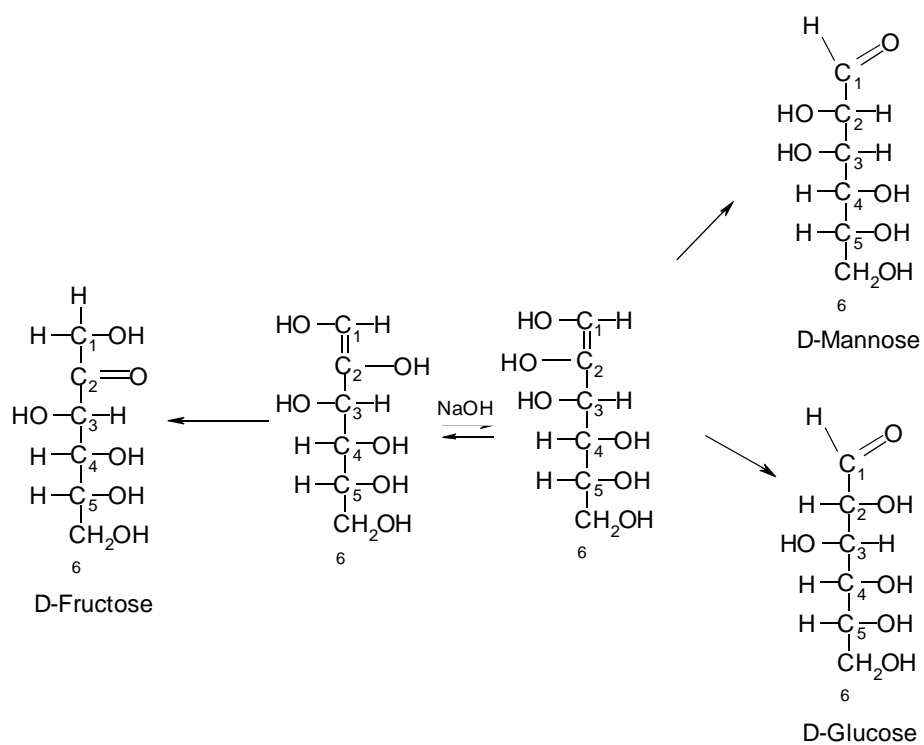


Oxidation. When oxidized completely, carbohydrates yield carbon dioxide and water and liberate considerable energy.

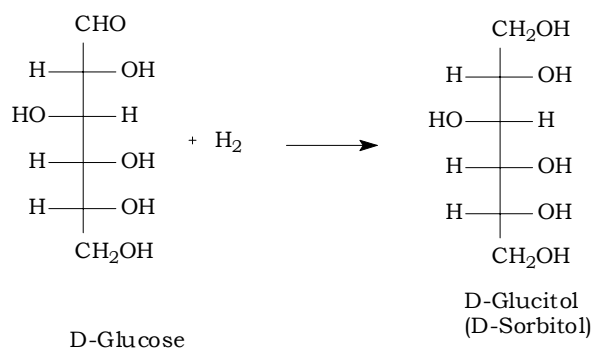


When burned either in the laboratory or in the human body, carbohydrates have an average heat value of 4 Calories per gram.

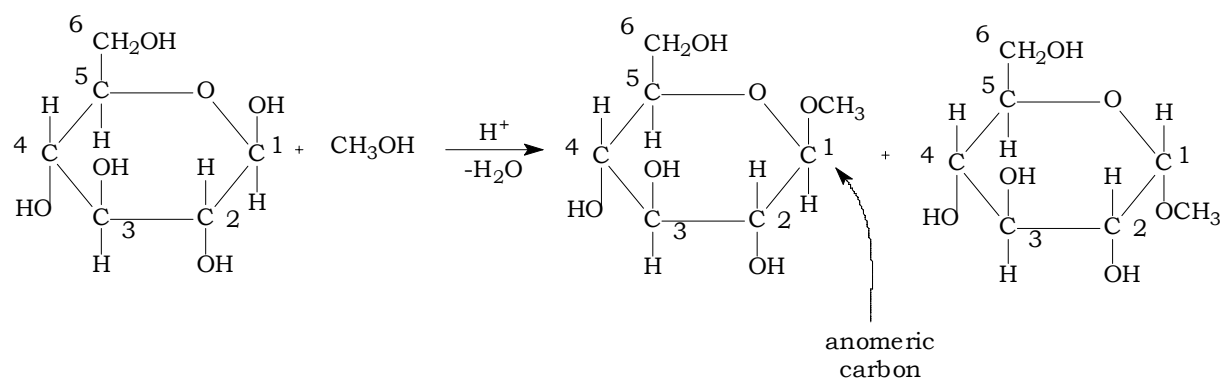
Epimerization of glucose



Reduction to alditols.



Formation of glycosides (Acetals)



α -D-glucopyranose
 lucopyranoside

Methyl β -D-glucopyranoside; Methyl α -D-

Experimental

1. *Molisch's test – detecting sugar*

Add 2 drops of fresh made 10% solution of β -naphthol to few drops of examined solution of carbohydrates and mix. Stratify carefully concentrated sulphuric acid on the bottom of the tube (about 1cm³). In a presence of sugar the reddish-violet ring appears at the juncture of the two liquids.

2. *Antron test.*

Transfer four drops of antron to 1 ml of the examined solution of sugar. Green or blue-green color appears in the case of the presence of sugar. (Antron solution contains 0,2% antron solution in concentrated sulphuric acid).

3. *Differentiation polysaccharides from monosaccharides – Barfoed's test*

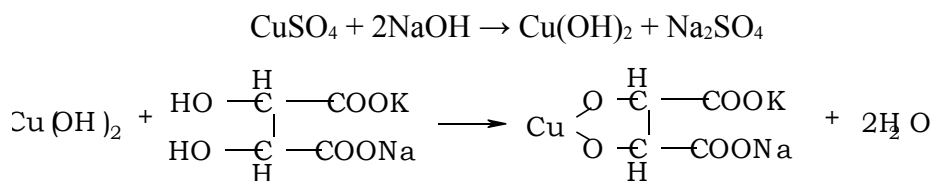
To 1cm³ Barfoed's solution add 1cm³ of detected solution of sugar and heat for 3 minutes. The red precipitate of Cu₂O appears in the presence of monosaccharide. Disaccharides give positive result after longer heating (about 15 minutes). Sucrose does not give this reaction.

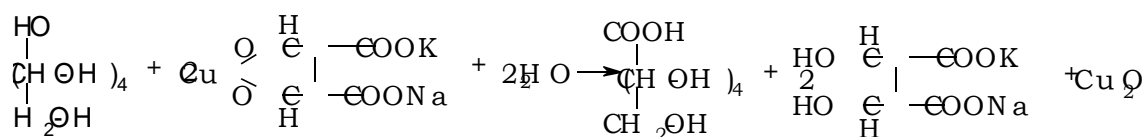
(Barfoed's reagent contains copper acetate with lactic acid).

3. *Reduction tests:*

a) *Fehling's reaction*

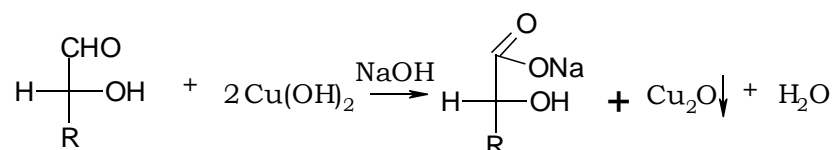
To about 1cm³ of detected solution of sugar add about 1cm³ of Fehling's reagent. After heating red precipitate of cuprous oxide appears in a presence of reducing sugar. (Fehling's reagent contains: Fehling I – copper sulfate in water; Fehling II – Rochelle salt (sodium potassium tartrate) and potassium hydroxide).





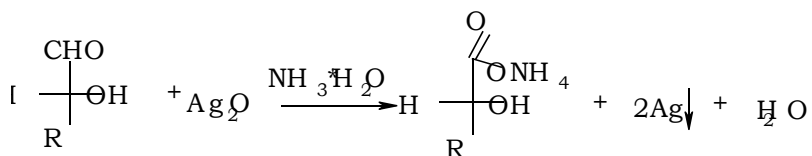
b) *Trommer's reaction*

Add 3 drops of CuSO_4 solution to about 2cm^3 of detected solution of sugar. Mix and then introduce drop by drop 2M solution. Stir incessantly until obtained $\text{Cu}(\text{OH})_2$ dissolve. Heat the blue solution to boiling. Red precipitate appears in the presence of reducing sugar. (Trommer reagent contains copper sulphate with sodium hydroxide).



c) *Tollens's reaction*

Add 1cm^3 of detecting of sugar to about 1cm^3 Tollens reagent. The whole mix well and place the tube in the boiling water bath for 5 minutes. Silver mirror becomes in the presence of reducing sugar. (Tollens reagent contains silver oxide with ammonia).



4. *Detection of fructose –reaction of Selivanoff*

Add to 1cm^3 of detected solution fresh made resorcinol solution in concentrated hydrochloric acid. Place the tube in the boiling water bath for not longer than 30 seconds. A reddish-orange color appears in the presence of fructose.

5. *Differentiation pentoses from hexoses*

a) *Bial reaction with orcine*

Add to 1cm^3 of detected sugar solution 1cm^3 of concentrated HCl, mix and add a few crystals of orcine. Heat to boiling. Blue-violet color and green precipitate appears in the presence of pentose.

b) *Tollens reaction with phloroglucinol*

To 1cm³ of detecting solution add 2cm³ of concentrated hydrochloric acid, mix and add a few crystals of phloroglucinol. Heat the mixture on boiling water bath for 1 minute. In the presence of pentose red color and precipitate appears.

6. Detection of polysaccharides

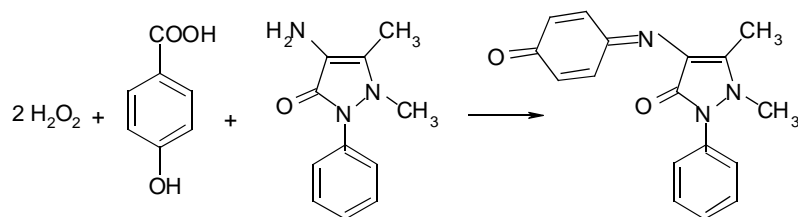
To the examined solution of sugar (about 1cm³) add hydrochloric acid and a few drops of Lugol's reagent. Blue color appears in the presence of starch, red - when the solution contains glycogen or dextrin.

Lugol's reagent contains iodine solution in potassium iodate.

7. Determination of glucose – oxydase method

This is the diagnostic quantitative test for determining of glucose in human blood serum.

Glucose is oxidised by glucose oxydase to yield D-gluconic acid and hydrogen peroxide. H₂O₂ reacts with 4-aminoantipyrine and p-hydroxybenzoic acid to give red dye quinonoimine.



The intensity of red color is proportional to the concentration of glucose.

Procedure: Transfer 5 ml of the reagent GLUCOSE.DST to the dry test tube, and then add 0.1 ml of the tested solution using the automatic pipette. Shake well and keep in water bath (temp. 37 °C) for 5 min. Measure the absorbance value of the tested solution using the reference sample at wavelength 520 nm. The color fades after 30 min.

Use the standard curve to estimate the concentration of cholesterol.