جامعة جنوب الوادي
كلية العلوم
قسم النبات والميكروبيولوجي

# الدروس العملية

# لمادة نبات 7

# "جزء فسيولوجيا نبات"

الفرقة الرابعة

شعبة العلوم البيولوجية والجيولوجية

الفصل الدراسي الاول

للعام الجامعي 2023/2022

اعداد/

اعضاء هيئة تدريس قسم النبات والميكروبيولوجي

## Theoretical basis of Vander blank method

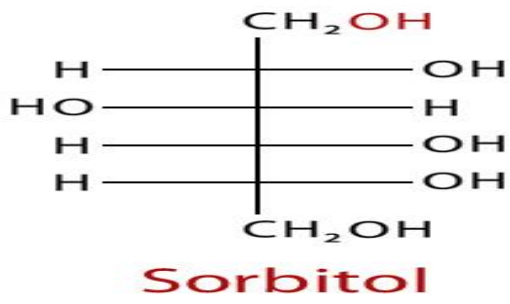
It is a quantitative method used for determination concentration of sugars in solutions.

**Carbohydrates** are often known as sugars, also known as saccharides, in Greek sakcharon mean sweetness.

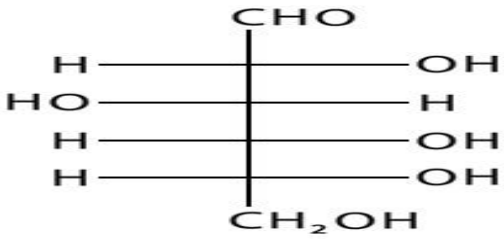
**Carbohydrate** is a organic compound, it comprises of only oxygen, carbon and hydrogen. The oxygen : hydrogen ratio is usually is 2:1. The empirical formula being $C_m(H_2O)n$ (where *m* can be different from *n*). Carbohydrates are hydrates of carbon, technically they are polyhydroxy aldehydes and ketones.

**Note**

**Not all carbohydrates contain carbon, hydrogen and oxygen by 1:**



Sorbitol

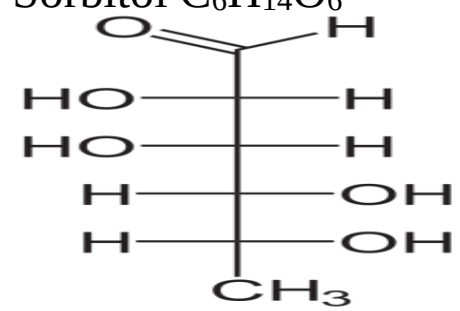


Glucose

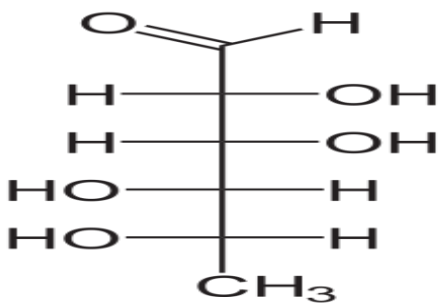


**2: 1**

* Sorbitol $C_6H_{14}O_6$



D-Rhamnose



L-Rhamnose

Rha

mno

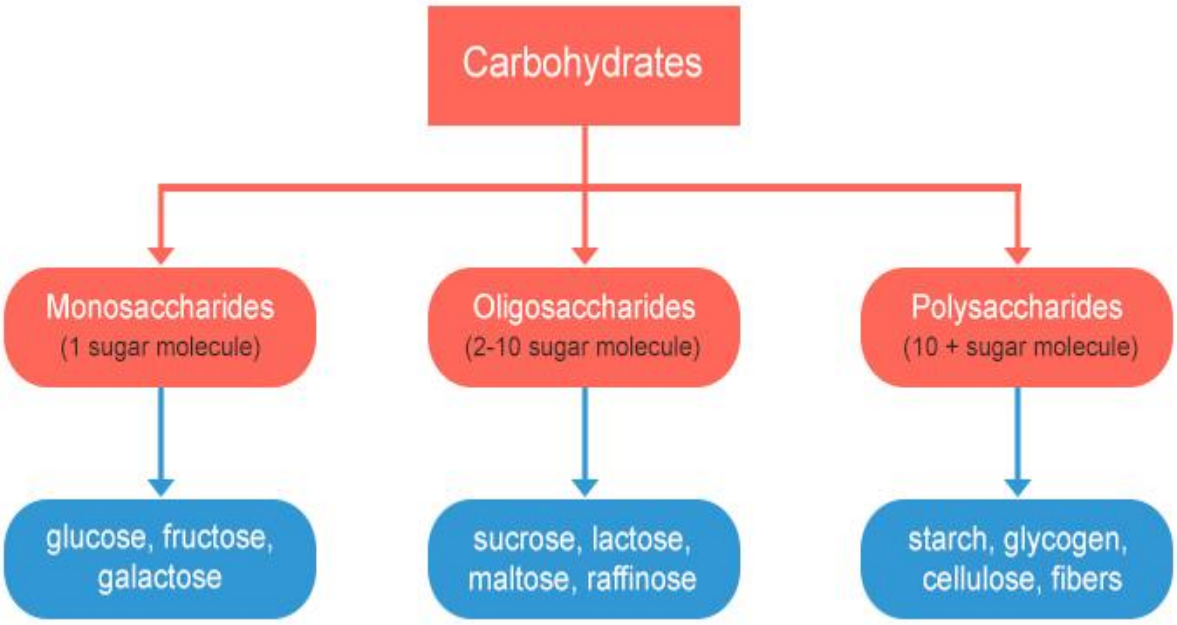
* se $C_6H_{12}O_5$

**Not all compounds containing carbon, hydrogen and oxygen by 1: 2: 1 are carbohydrate.**

***Formaldehyde HCHO**

***Acetic acid $CH_3COOH$**

## Carbohydrates Classification

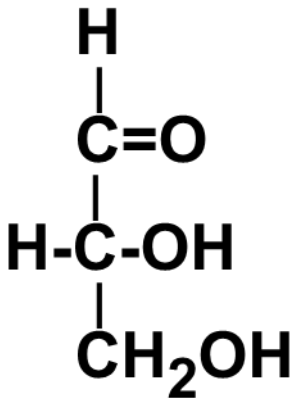



1- Monosaccharides

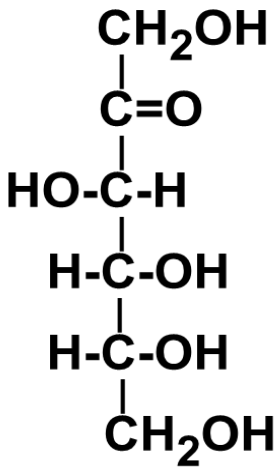
monosaccharaides, commonly known as simple sugars are the simplest units of carbohydrates. Monosaccharaides doesn't need to be broken down further into smaller units so as to get absorbed by the body. For example: glucose, fructose, galactose etc.

The most important types of sugars because it is the structural units of other sugars

They are called reducing sugars because they contain free active groups such as aldehydes(CHO) and ketones( C=O )so they react with some reagent like Fehling and reduced it.




analyzed to one Because simplest




Can not be the simple it is the it sugars.

The monosaccharides are subdivided into trioses, tetroses, pentoses, hexoses, heptoses, etc.. according to the Number of carbon atoms in the chain

**D-glyceraldehyde** **D-fructose**

also as aldoses or ketoses depending upon whether they contain aldehyde or ketone group.

2- **Oligosaccharides**

Oligosaccharides are made up of 2-10 sugar units. It needs to be further broken down into smaller sugar units with the help of the enzymes or chmical substance like HCL. Oligosaccharides containing two monosaccharide units are called dissacharides for example sucrose ,maltose etc whereas, those containing three units are known as trisaccharide for example, raffinose etc.

Do not consider reducing sugars because they do not contain free active groups

3- **Polysaccharides (Complex Carbohydrate)**

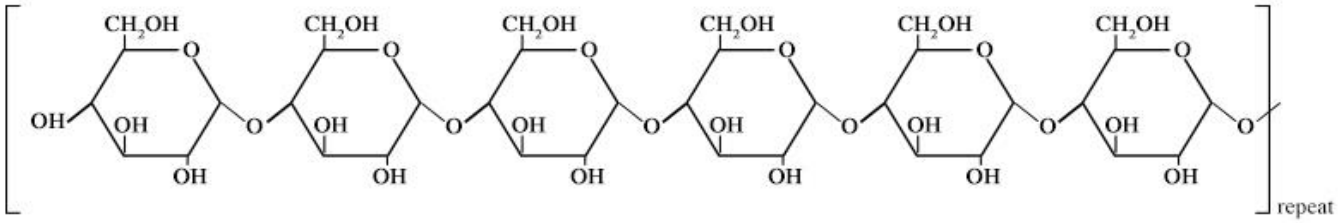
Polysaccharide (starch, glycogen,cellulose, dietary fibers etc.) also known as Complex carbohydrate, have complex chemical structure as they contain thousands of sugar molecules (monosaccharide) connected, to form these polysaccharides and further needs to be broken down into monosaccharides (especially glucose) so as to get digested and absorbed by the body.

They broken down into smaller sugar units with the help of the enzymes or chmical substance like HCL.

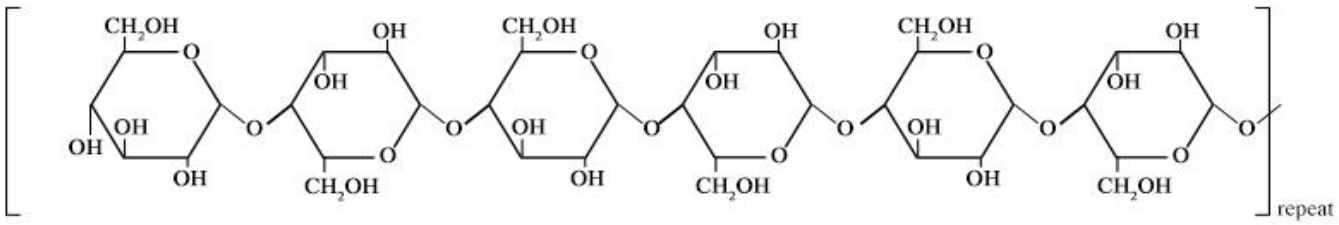
Do not consider reducing sugars because they do not contain free active groups

They may be homopolysaccharides i.e, monosaccharides of the same type or heteropolysaccharides i.e., monosaccharides of different types.

STARCH




CELLULOSE




The general formula is $(C_6H_{10}O_5)_x$

Example of homo polysaccharides are starch, glycogen, cellulose, pectin. Heteropolysaccharides are Hyaluronic acid, Chondrotin.

**Carbohydrates** are group of organic compounds consisting of carbon(C), hydrogen(H), and oxygen(O), usually in the ratio of 1:2:1. Carbohydrates(or carbs), also known as saccharides, are the primary source of energy to carry out all your daily activities

**Conditions of van der blank method**

- **Presence of Monosaccharides**
- **Neutralized medium**

## Reagents of van der blank method

**Sol A** : 23 g. CuS04.5H20 per liter.

**Sol B** : 35 g. anhydrous sodium bicarbonate **$NaHCO_3$**

28 g. anhydrous sodium carbonate **$Na_2CO_3$**

1 g. potassium iodate **$KIO_3$**

**$KNaC_4H_4O_6 \cdot 4H_2O$** Sod. Pot Tartarte (Rochelle salt) Potassium sodium tartrate tetrahydrate

**Sol C**: 10 g. potassium iodide **KI**

19 g. potassium oxalate **$K_2C_2O_4$**

**Sol D**: 1*N* sulphuric acid.**$H_2SO_4$**

**Sol E**: Standardized thiosulphate solution (0·01 *N).***$Na_2S_2O_3$**

*Starch as Indicator*

## Equations of van der blank method

1-first equation


Condition of reaction

1- This reaction depends on the presence of monosaccharides.
2- This oxidation and reduction reaction results in the formation of a wine red ppt. of Copper(I) oxide or cuprous oxide **$Cu_2O$**
3- The amount and color of the precipitate **$Cu_2O$** depends on the concentration of the monosaccharides in the solution

2-second equation

**$KI+5KIO_3+ 3H_2SO_4 \longrightarrow 3\ K_2SO_4 +3\ H_2O+3I_2$**

**Condition of reaction**

1- This reaction does not depends on the presence of monosaccharides it take place in sugar and blank tubes.
2- This reaction results in the formation of oxidizing reagent called molecular iodine or free iodine or total iodine **$3I_2$** .

3-thrid equation

**$Cu_2O +2I_2 \longrightarrow CuO+2I^-$**

**Condition of reaction**

**1-** This reaction depends on the presence of monosaccharides but indirectly it depends on the presence of **$Cu_2O$** that come from first equation

2- This oxidation and reduction reaction where (**cuprous oxide $Cu_2O$** )is oxidized to (**cupric oxide CuO**) and (molecular iodine **$2I_2$** )oxidized to **$2I^-$** which called **consumed iodine or ionic iodine.**

*to calculate sugar conc. **$C_6H_{12}O_6$= $Cu_2O$**=$2I^-$ we need to calculate the amount of consumed iodine

Total iodine divided to two parts

1-consumed iodine that oxidized **$Cu_2O$** to **CuO**

**2-**remained or un consumed iodine

**We titrate remained iodine by using $Na_2S_2O_3$ (E solution)**

**$2\ Na_2S_2O_3 + I_2 \longrightarrow Na_2S_4O_6 + 2NaI$**

**Calculation**

We calculate total iodine from blank tube $V_1$

We calculate remained iodine from sugar tube $V_2$

**$V_3$(consumed iodine)= (total iodine $V_1$)- (remained iodine $V_2$)**

**Concentration of hexoses g/l= $V_3$ consumed*0.27*1000**

**V sample*1000**

$$I_2 + 2\,Na_2S_2O_3 \longrightarrow Na_2S_4O_6 + 2\,NaI$$

Sodium thiosulphate — Sodium tetrathionate

**C o**

**ncentration of hexoses m g/l =** $V_3$ **consumed*0.27*1000**

**V sample**

**1ml of $Na_2S_2O_3$ .01 N=.27 mg of hexoses**

**Concentration of sucrose g/l=** $V_3$ **consumed*0.95*0.27*1000**

**Vsample*1000**

We add 0.95 because consist of glucose and fructose before fructose participate in the reaction a rearrangement take place and it convert to glucose only 0.95 of fructose converted to glucose and other part found in intermediate state

# Determination of glucose by van der blank method

1-take 5ml of glucose and 5 ml of fehling solution( A:B 2:5) in clean test tube it called **sugar tube**

2- prepare **Blank tube** which consist of 5ml dist.water and 5ml fehling solution

3-put the test tubes **(Blank tube+ sugar tube)** in water bath 100 $C^5$ for 15 min.

4- Prepare clean conical flask and trasnsfer the content of **Blank tube** in it and add 5ml of C soln. and 5 ml of **D** soln

5- Add drops of starch indicator titrate with E soln. from blue to faintly blue calculate the amount of E solution which equal the total iodine (**$V_1$ )**

6- Repeat the same steps with **sugar tube** and Titrate blank tube with E soln. from blue to faintly blue calculate the amount of E solution which equal the remained iodine (**$V_2$ )**

**$V_3$(consumed iodine)= (total iodine $V_1$)- (remained iodine $V_2$)**

$$\textbf{Concentration of glucose g/l} = \frac{V_3 \text{ consumed} * 0.27 * 1000}{V \text{ sample} * 1000}$$

**Concentration of glucose m g/l =** $\frac{V_3 \text{ consumed} * 0.27 * 1000}{V \text{ sample}}$

*precautions

1-The sugar tube should be cooled and the blank should be cooled after taking them out of the water bath because they are reacting with solution c and D solution an exothermic

2- The sugar tube should be placed in the conical flask first, then the rest of the precipitation should be washed with solution c and then washed with solution D to ensure that the entire amount of precipitation has been taken.

3- The titration must be done immediately after adding the starch indicator.