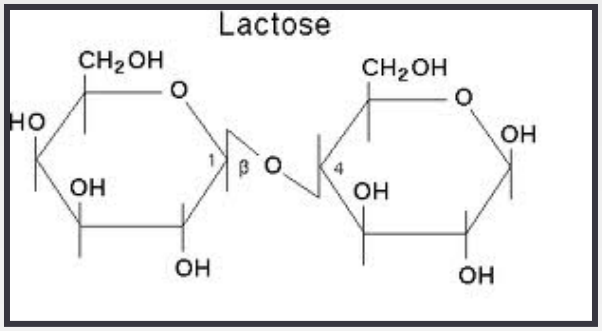
Estimation of reducing sugars by dinitrosalicylic acid method

- Determining the sugar concentration of food samples is very important especially in industries where quality control is monitored.

- There are different method for sugar estimation, such as pheno-sulfuric acid method, somogyi Nelson method, dinitrosalicalic acid method.

Carbohydrate in milk

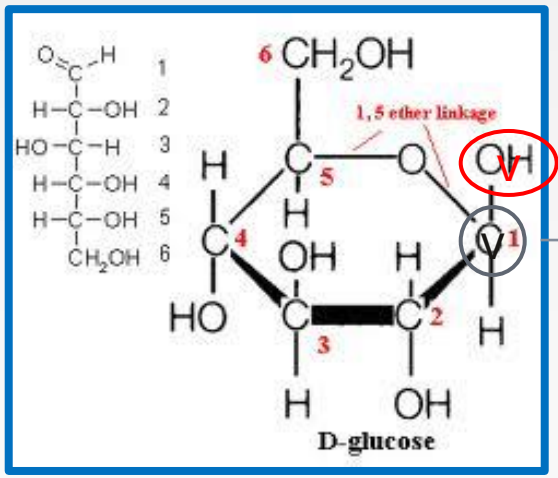
- The major constituents of milk are lactose, fats and proteins.



- The determination of lactose in dairy product is important and there are many methods are available.
- Theses methods are based on the assumption that lactose is the only reducing sugars in milk.
- In this experiment, DNS method will be used.

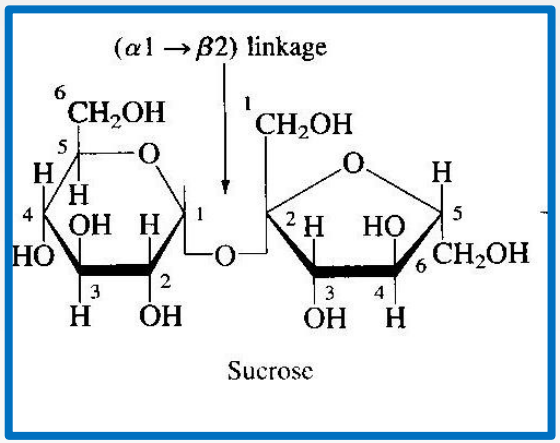
DNS method

- The DNS method for estimating the concentration of reducing sugars in a sample
- Reducing sugars contain free carbonyl group, have the property to reduce many of the reagents.
- All monosaccaride and some disaccaride are reducing sugars



Free carbonyl group

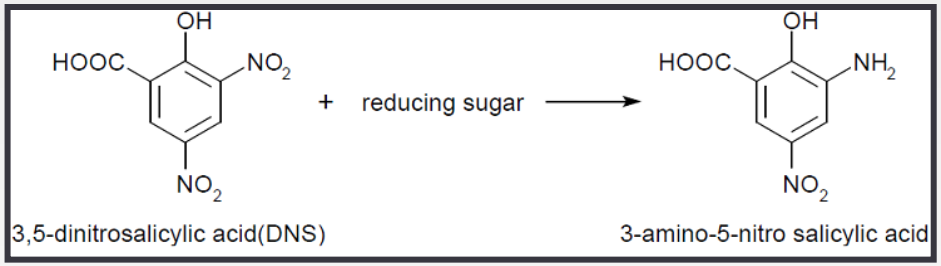
reducing



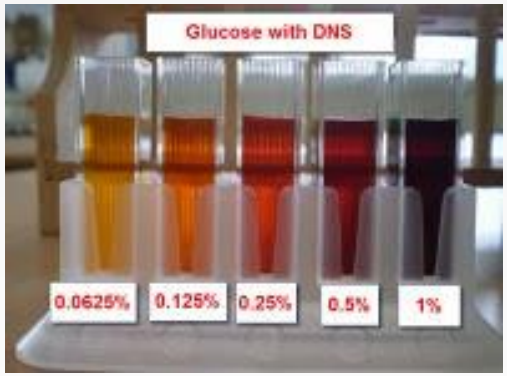
Non-reducing

Principle

- When **alkaline** solution of 3,5-dinitrosalicylic acid reacts with reducing sugars(eg. Glucose, lactose..) it is converted into 3-amino-5-nitrosalicylic acid with orange color.



Intensity of the colour is an index of reducing sugar.



Objective

- **Estimation of reducing sugars by dinitrosalicylic acid method in milk sample**

Method

	Glucose solution	sample	water	DNS reagent		Soduim potasuim tartarate
B	--	--	1	3	Cover the tubes (with aluminuim foil) And heat for 5 min. in a boiling water bath	1
1	0.1	--	0.9	3		1
2	0.2	--	0.8	3		1
3	0.3	--	0.7	3		1
4	0.4	--	0.6	3		1
5	0.5	--	0.5	3		1
6	0.6	--	0.4	3		1
7	0.7	--	0.3	3		1
8	0.8	--	0.2	3		1
9	0.9	--	0.1	3		1
10	1	--	--	3		1
S1	--	0.4	0.6	3		1
S2	--	0.6	0.4	3		1

Method

- Mix the contents.
- Cool by immersing in cold water and read at 510 nm.
- Plot the standard curve and calculate the amount in the sample from standard curve and calculate the contents.

Result:

Tube	Absorbance	CHO content (mg/dl)
B	--	--
1		2
2		4
3		6
4		8
5		10
6		12
7		14
8		16
9		18
10		20
S1		
S2		

- Calculation:

-Dilution factor= final volume / aliquot volume

- **D.F Sample 1=** 1 / 0.4 = 2.5
- **D.F Sample 2 =** 1 / 0.6 = 1.7
- **The amount of carbohydrate in 1 gram of sample= ----------mg/dl x dilution factor x 100**
- **Normal range**= 4-5 gm