Estimation of reducing sugars in milk

by dinitrosalicylic acid method

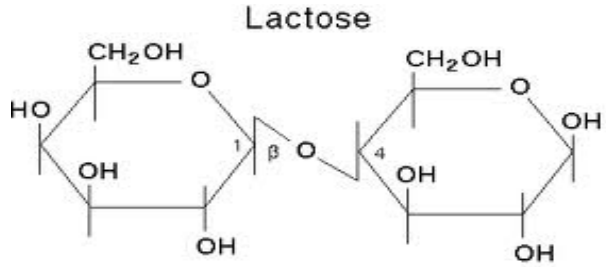
Carbohydrate in milk

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

Other free carbohydrates found in milk but at low concentrations, including glucose , galactose and others

As lactose is the main carbohydrate in commercial milk, its determination is a basic indicator of quality control and detection of abnormal milk

Lactose in milk



- **Lactose** is a disaccharide sugar derived from galactose and glucose.
- It is a **reducing sugar**.
- Some of methods for lactose detection in milk are based on the assumption that lactose is the only reducing sugars in milk.
- In this experiment, **DNS method** will be used, which based on the detection of reducing sugar (which will give a general estimation for lactose not an accurate one, because in milk there are also other reducing sugars)

Objective

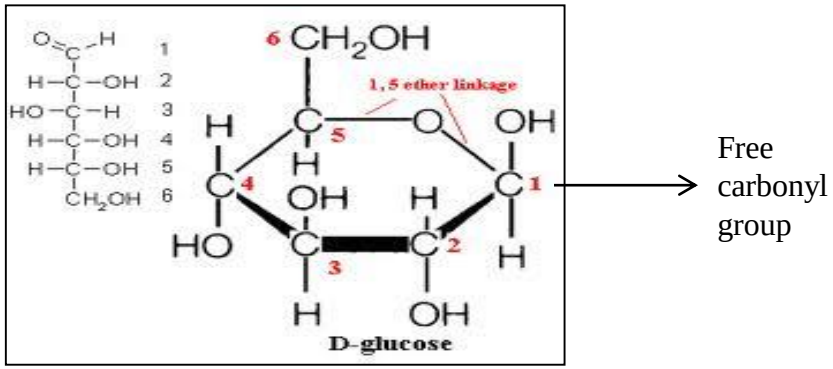
Estimation of reducing sugars by dinitrosalicylic acid method in milk sample

DNS method

- The DNS method for estimating the concentration of reducing sugars in a sample.

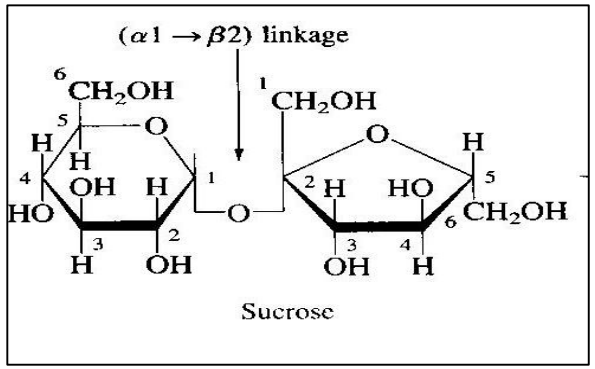
- Reducing sugars contain **free carbonyl group**, have the property to reduce many of the reagents.

- All monosaccharide and some disaccharide are reducing sugars



Free carbonyl group

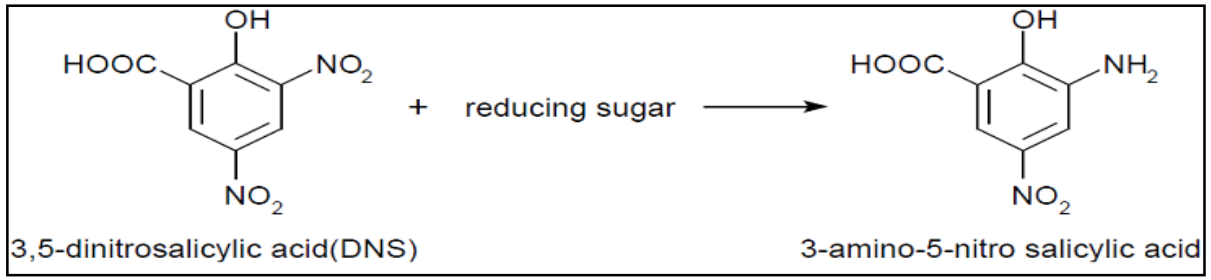
reducing



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 color is an index of reducing sugar.

Method

	Glucose Solution 100mg/dl	sample	water	DNS reagent		Sodium potassium tartarate
B	--	--	1	3	Cover the tubes (with aluminum 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
Sample	--	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		
3		
4		
5		
6		
7		
8		
9		
10		
Sample		

Calculation:

-Dilution factor = $\frac{\text{final volume}}{\text{aliquot volume}}$

- The amount of carbohydrate in the sample = ----------mg/dl x dilution factor

Note:

***Milk was diluted in sample preparation (1:100)**

[Two dilution factor]