Estimation of reducing sugars In milk

by dinitrosalicylic acid method

Objective

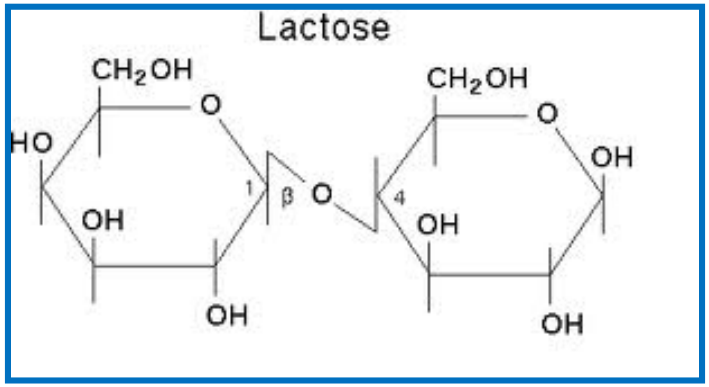
- Estimation of reducing sugars by dinitrosalicylic acid method in milk sample

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

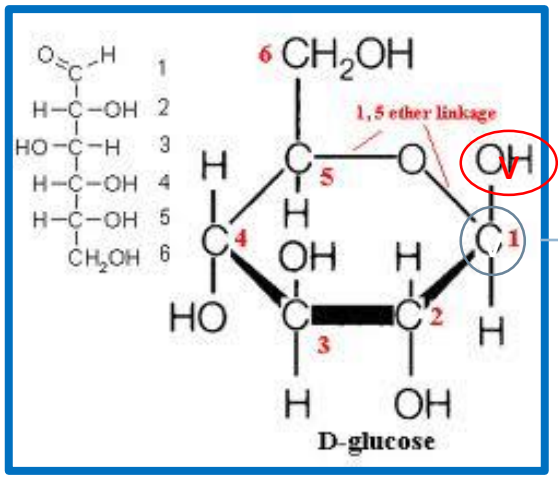
Lactose in milk

- **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)



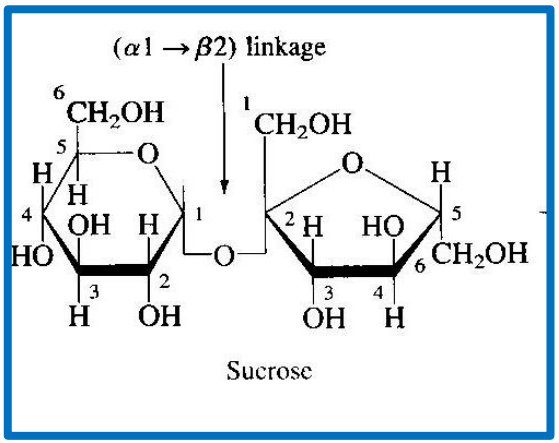
DNS method

- 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



reducing

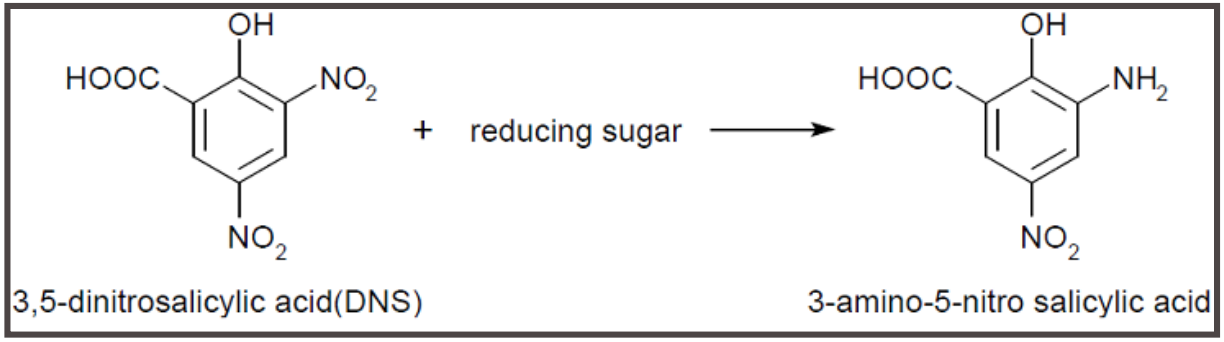
Free carbonyl group



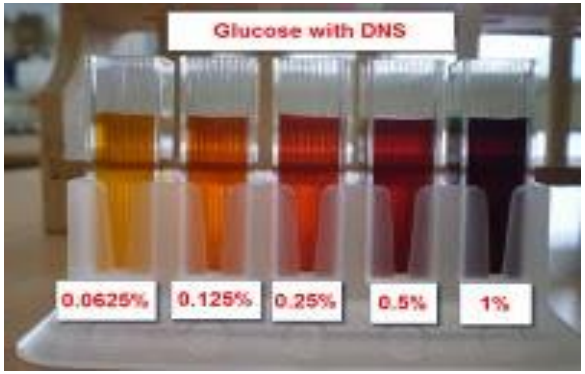
Non-reducing

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.



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	--	1	---	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		
3		
4		
5		
6		
7		
8		
9		
10		
S1		
S2		

- Calculation:

-Dilution factor= final volume

aliquot volume

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