

# Estimation of reducing sugars by dinitrosalicylic acid method

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## - Carbohydrate in milk:

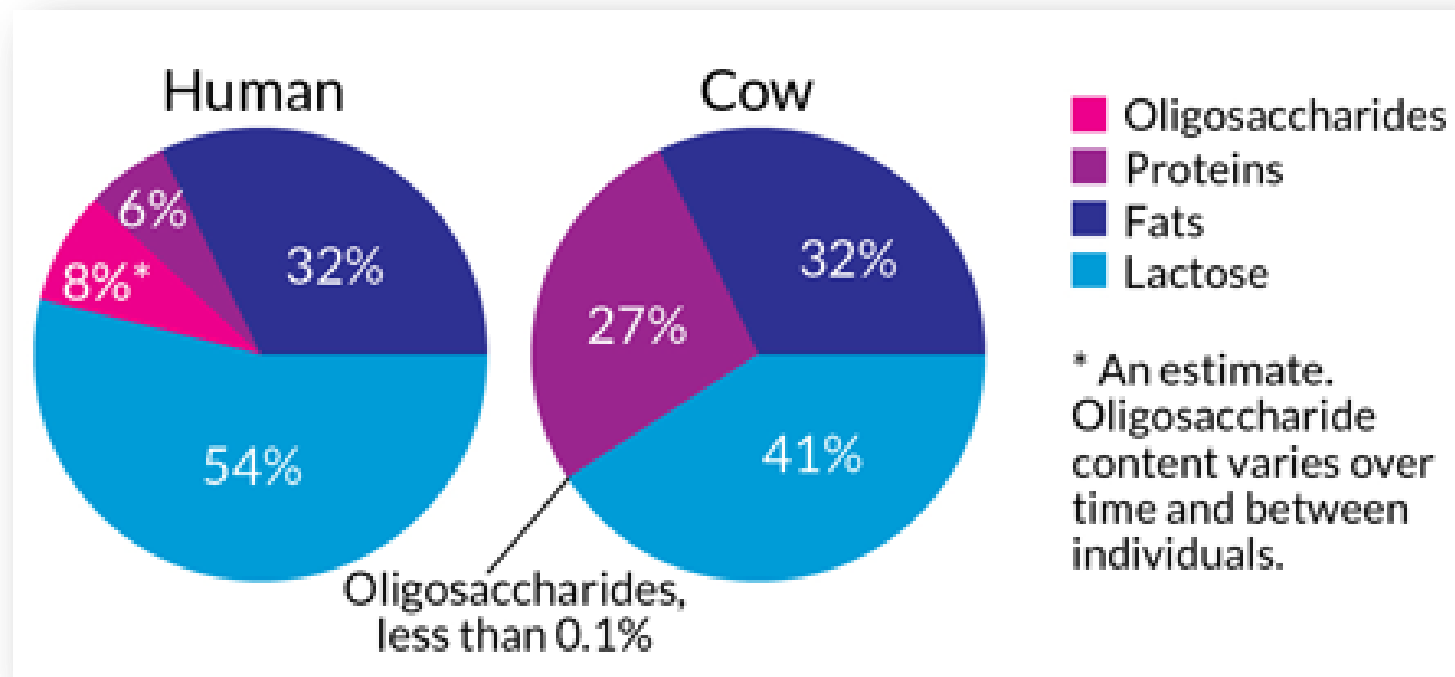
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- The major constituents of milk are **lactose**, **fats** and **proteins** .
- Other **free** carbohydrates found in milk but at **low concentrations**, including **glucose** , **galactose** and **others**.
- As **lactose** is the main carbohydrate in commercial milk, its determination is a basic indicator of **quality control and detection of abnormal milk**.

# - Human vs cow milk:

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- Human milk contain more lactose than cow's milk.



# Practical Part

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# Objective:

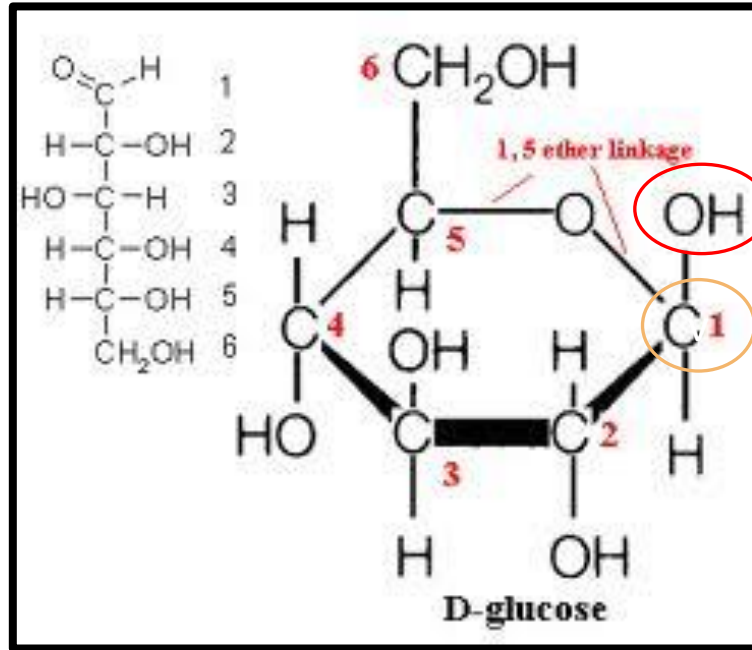
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- Estimation of reducing sugars by dinitrosalicylic acid method in milk sample.

# DNS method:

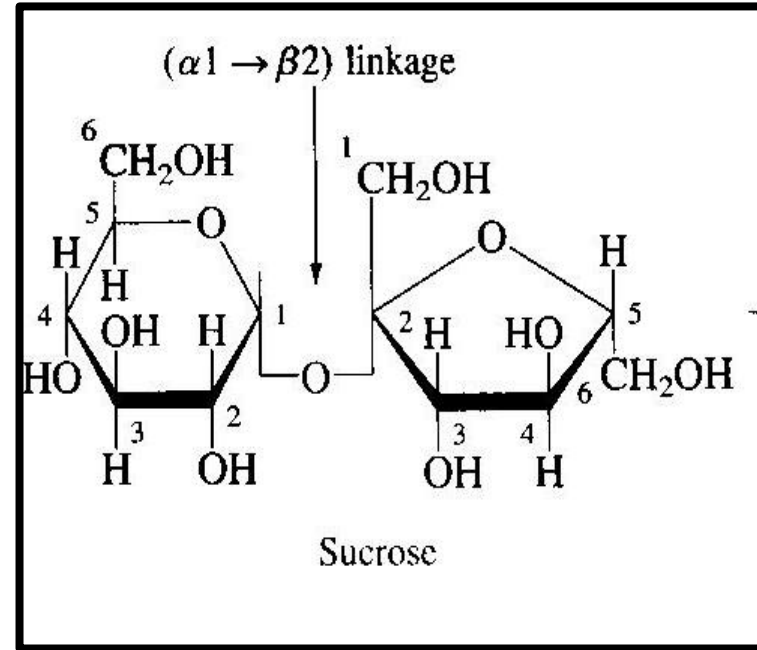
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- The dinitrosalicylic acid (DNS) method for estimating the **concentration** of **reducing sugars** in a sample.
- Not specific.
- Reducing sugars contain free carbonyl group, have the property to **reduce** many of the reagents.
- **All** monosaccharide and **some** disaccharide are reducing sugars (sucrose?).



Free carbonyl group

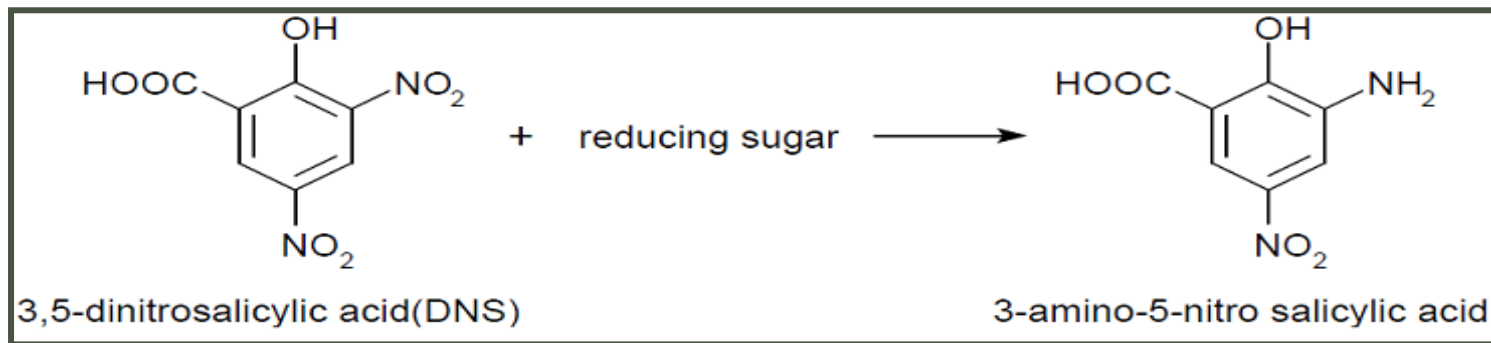
Reducing



Non-reducing

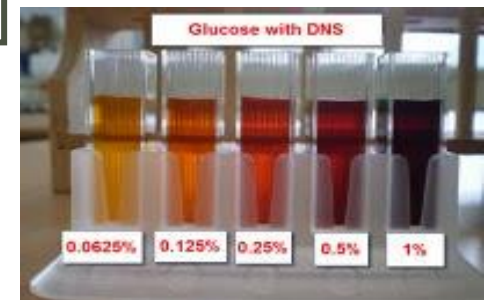
# Principle:

- 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 which absorbs at 540nm)



**orange**

- Intensity of the colour is an index of **reducing sugar**.





# - Method:

	Glucose solution (100mg/dl) (ml)	Sample (ml)	Water (ml)	DNS reagent (ml)		Sodium potassium tartrate (ml)
B	--	--	1	3	<b>Cover the tubes (with aluminum foil) And heat for 5 min. in a boiling water bath</b>	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
<b>S1 (Milk)</b>	--	1	--	3		1
<b>S2 (Milk)</b>	--	1	--	3		1

## - Method:

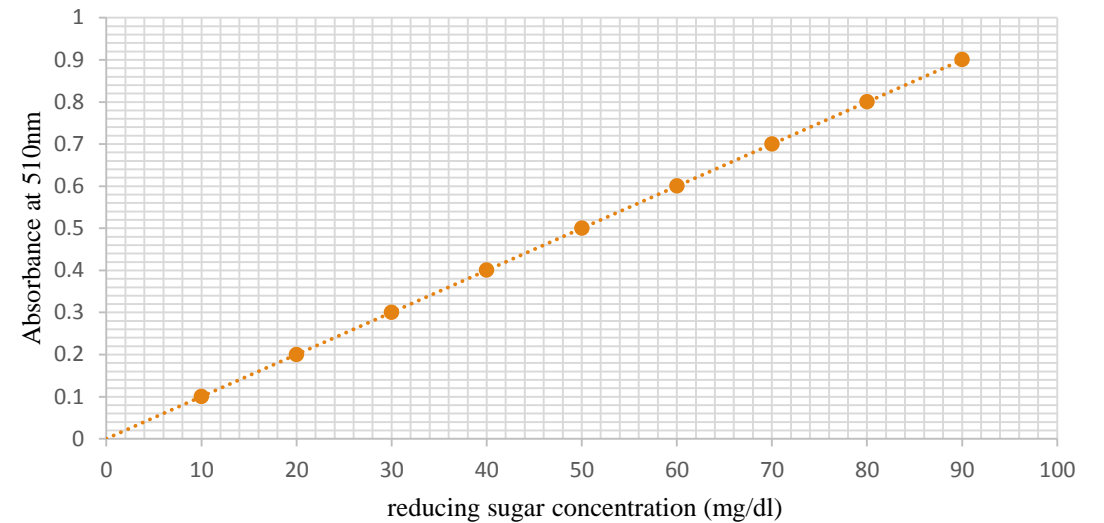
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- Mix the contents.
- Cool by immersing in cold water and read at 510 nm.
- Plot the standard curve and calculate the amount in the sample from standard curve and calculate the contents.

# Results:

Tube	Absorbance at 510nm	CHO content (mg/dl)
B	--	--
1		$C_1 \times V_1 = C_2 \times V_2$ $100 \times 0.1 = C_2 \times 1 \longrightarrow C_2 = 10$
2		
3		
4		
5		
6		
7		
8		
9		
10		
S1		
S2		

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# Calculations:

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- Dilution factor (DF) = final volume / aliquot volume
- The amount of carbohydrate in the sample = .....mg/dl x DF