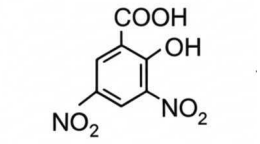
Jun 08, 2025

To Perform Quantitative estimation of carbohydrates using Dinitro Salicylic Acid (DNS Method)



3,5-dinitrosalicylic acid (yellow)

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Protocol status: Working
We use this protocol and it's working

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Keywords: Quantitative Estimation, DNS Method, quantitative estimation of carbohydrate, quantitative estimation of sugar, quantitative analysis of carbohydrate, measurement of total carbohydrate, concentration of an unknown sugar sample, unknown sugar sample, reducing sugar, carbohydrate, dinitro salicylic acid, using dinitro salicylic acid, nutritional labelling, total carbohydrate, sugar, glucose, salicylic acid, functional groups of glucose, caloric content, known c6h12o6 concentration, concentrations of unknown sample, c6h12o6 concentration, quantitative estimation, quantitative analysis, concentration, given sample, acid, reagent

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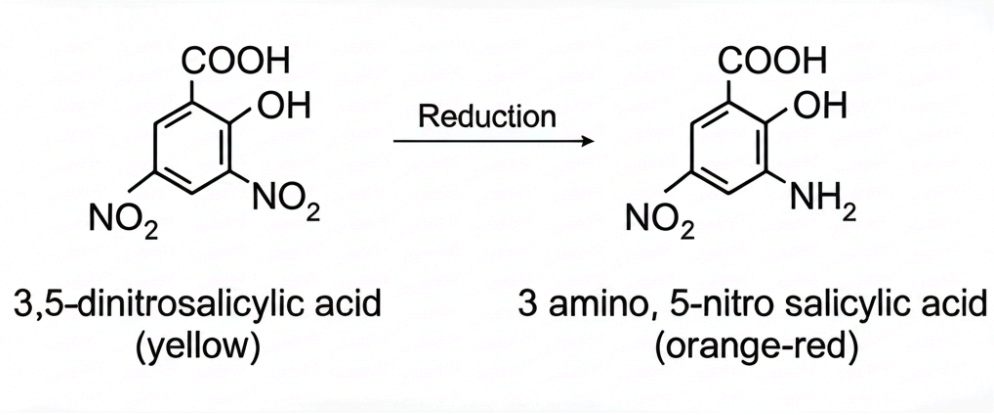
Abstract

This method is used for the measurement of total carbohydrates present in the given sample. Quantitative analysis of carbohydrates is important for a variety of reasons, like nutritional labelling, quality control, authenticity, caloric content, and energy evaluation. We use Dinitro salicylic acid,which is a low carcinogenic reagent and light sensitive and also used to bind functional groups of glucose in alkaline conditions. Here we are doing a quantitative estimation of sugar or carbohydrate. DNS is used only in alkaline conditions. We can also use this method to determine the concentration of an unknown sugar sample by preparing a set of solutions with known C6H12O6 concentration. Then, plot a standard curve and use it to derive the concentrations of unknown samples. There are some properties of reducing sugars that reduce so many reagents DNS is also one of them, so we used to perform quantitative estimation, which is used to determine the mass, percentage composition or concentration of a substance.

Image Attribution



Reduction of DNS (3,5-dinitrosalicylic acid) to 3 amino, 5-nitro salicylic acid.

Guidelines

The results demonstrate a clear inverse relationship between sugar concentration and absorbance, with higher sugar levels yielding stronger colourimetric responses. Tube 1's extreme absorbance suggests potential reagent saturation, which may require sample dilution for accurate quantification in future assays. The data confirms the DNS method's effectiveness in detecting a wide range of reducing sugar concentrations. The results clearly demonstrate that higher sugar concentrations lead to greater absorbance at 540 nm. The extreme value in Tube 1 may require dilution for accurate quantification, as absorbance values above 1.5 often exceed the linear detection range of spectrophotometers.

Materials

	Component	Description
	Sodium sulphate solution	Use to make DNS reagent
	Distilled water	Use to make DNS reagent
	3,5-dinitrosalicylic acid	Use to make DNS reagent
	Sodium hydroxide	Use to make DNS reagent
	Sodium potassium titrate	Use to make DNS reagent

Materials and components required for this experiment

	A	B
	Glassware	**Description**
	Test tube	Used to store a solution.
	Pipette	Used to measure the volume of a solution.
	Boiling water bath	To keep the solution at a constant temperature.

Glassware Required for this experiment

Safety warnings

Wear a Lab Coat & Purple Nitrile Gloves while handling solutions

DNS Solution & Quantitative estimation using sugar as a Source

1 Take 7 clean, dry test tubes

2 Pipette out standard sugar solution in different test tubes and make up the volume of all test tubes to 1 mL with distilled water.

3 Add 2 mL DNS reagent to all the test tubes. Mix the plug in the test tube with cotton or marble and keep the test tube in a boiling water bath for 5 minutes.

4 Take the tubes and cool them to Room temperature

5 Add 7 ml of water after cooling the solution completely.

6 Read extinction at 540 nm against the blank.
Take Blank Water

7 All the tubes must be cooled to room temperature before reading since the absorbance is sensitive to temperature.

8 we use carbohydrates of different concentrations to measure absorbance of sugar

Test tube	*Wavelength*	*Absorbance*
1	540nm	4
2	540nm	1.661
3	540nm	0.151
4	540nm	0.028
5	540nm	0.009
6	540nm	0.015

Observation Table

9

Test Tube 1

Test Tube 2

Figure (4.2): Test Tube 1 showed an exceptionally high absorbance (**4.000**), indicating a **very concentrated sugar solution**. This suggests either an undiluted sample or possible saturation of the DNS reagent. **Test Tube 2** exhibited a lower but still significant absorbance (**1.661**), confirming a **high sugar concentration**, though reduced compared to Tube 1.

Results

Protocol references

To Learn more about this method, read this paper

(PDF) Comprehensive Review of Fundamental Biochemical Techniques: Integration of Quantification, Separation, and Analytical Methods in Biomolecular Studies

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