**ESTIMATION OF GLUCOSE – DINITROSALICYLIC ACID**

**AIM**

To estimate the amount of glucose present in the whole of the given solution.

**PRINCIPLE**

Reducing sugars have the property to reduce many of the reagents. One such reagent is

3,5-dinitrosalicylic acid (DNS). This method involves the oxidation of the aldehyde functional group present in, for example, glucose functional group is aldehyde. Simultaneously, 3,5- dinitrosalicylic acid (DNS) is reduced to 3-amino,5-nitrosalicylic acid under alkaline conditions. The glucose present in the sample reacts with dinitrosalysilic acid and reduces it to a red colored product which was read calorimetrically at 570 nm.

aldehyde group - ---------> carboxyl group (Oxidation)

3,5-dinitrosalicylic acid ----------> 3-amino,5-nitrosalicylic acid (Reduction)

**MATERIALS REQUIRED**

**Stock solution:**

10 mg of glucose was dissolved and made up to 100 ml with distilled water with concentration 1 mg/ml.

**Working standard:**

10 ml of the stock was diluted to 100 ml with distilled water in concentration 100µg/ml.

**DNS Reagent:**

Dissolve by stirring 1g dinitrosalysilic acid, 200 mg crystalline phenol and 50 mg sodium sulphite in 100 ml 1% NaOH store at 4oC. since the reagent deuterates due to sodium sulphite if long storage is required, sodium sulphite may be added at the time of use.

**Rochelle salt solution:**

40% potassium sodium tartrate.

**PROCEDURE**

1. Pipette out 0.5, 1.0, 1.5, 2.0, and 3.0 ml of the working standard in the test tube and equalize the volume to 3.0 ml with distilled water in all the test tubes.
2. Pipette out 0.5 ml of unknown solution and made up to 30 ml distilled water.
3. Added 3.0ml of DNS reagent to all the test tubes.
4. Heated the contents in a boiling water bath for 5 minutes.
5. When the contents of the tubes are stirred war, added 1 ml of 40% Rochelle salt solution.
6. Cooled and red the intensity of dark red color at 570 nm.

* A standard graph was drawn by plotting the concentration of reducing sugar on x axis and optical density on y axis.

**STOCK STANDARD Solution:** Concentration: 1 mg/ml. 100 mg of Glucose in 100 ml of distilled water.

**WORKING STANDARD SOLUTION:** Concentration: 100µg/ml. 10ml of the stock made upto 100ml with distilled water.

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| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **S.No** | **PARTICULARS** | **B** | **S1** | **S2** | **S3** | **S4** | **S5** | **T** |
| 1 | Volume of working standard (ml) | - | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 | - |
| 2 | Concentration of working standard (µg) | - | 50 | 100 | 150 | 200 | 250 | - |
| 3 | Volume of Unknown (ml) | - | - | - | - | - | - | 0.5 |
| 4 | Volume of Water  (ml) | 3.0 | 2.5 | 2.0 | 1.5 | 1.0 | 0.5 | 0.5 |
| 5 | Volume of DNS (ml) | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 |
| Heat the contents of the test tube in a boiling water bath for 10 minutes | | | | | | | | |
| 5 | Volume of Rochelle salt solution(ml) | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| After 10 min make up the volume to 10 ml in all test tubes with distilled water | | | | | | | | |
| Kept the test tubes at a room temperature. Blue color Read at 570 nm | | | | | | | | |
| 7 | Optical Density at 570 nm |  |  |  |  |  |  |  |

**CALCULATION:**

Optical density \_\_\_\_\_\_\_\_\_ corresponds to \_\_\_\_\_\_ µg of glucose

0.2 ml of sample corresponds to \_\_\_\_\_ µg of glucose

100ml of sample contains \_\_\_\_\_ x100/1000

= \_\_\_\_\_\_ mg of glucose

**RESULT:** The amount of glucose present in the given sample = \_\_\_\_\_ mg