

# Estimation of dextrose by Colorimetry

**AIM-** Aim of the experiment is to estimate sucrose by colorimeter.

## BACKGROUND-

Colorimetry is the measurement of concentration of analyte in a solution by determining absorption of a particular wavelength. The technique is based upon Beer-Lambert's law. Overall the process is accomplished with the measurement of analyte in a colored solution or development of color that appears in the solution following reaction with specific reagent.

In alkaline medium p-hydroxybenzoic acid hydrazide (PHBAH) reacts with dextrose that give products which can be assayed by colorimetry. Dextrose reacts with p-hydroxybenzoic acid hydrazide (PHBAH) reagent to give coloured product; the absorbance noted is proportional to concentration of sugar.

## REQUIREMENTS-

Dextrose: 5mg

Sodium hydroxide: 3-5 pellets

p- hydroxybenzoic acid hydrazide (PHBAH) : 50 ml

Test tubes

Burette

Colorimeter

Water bath

## PROCEDURE-

### Alkaline solution

Put 3-5 pellets of sodium hydroxide in about 50 ml of water, stir to mix well.

### Diluent

Add 10.0 ml of p-hydroxybenzoic acid hydrazide (PHBAH) solution with 30.0ml of water along with few drops of Sodium hydroxide.

### Standard stock solution preparation

Weigh 5.0 mg of dextrose; add in a test tube containing 15.0 ml of water, 2-3 drops of sodium hydroxide and 5.0 ml of p-hydroxybenzoic acid hydrazide (PHBAH) solution, and mix well.

### Working standard solution preparation

Dilute 5.0ml of stock solution with 15.0 ml of diluent.

### Sample solution preparation

Take a test tube, add 1.0 ml of sample with burette, add 4.0 ml of water to it, and shake gently to mix. Keep the above sample solution for 30 -35 minutes at normal temperature. Again shake gently, then add 5.0 ml of p-hydroxybenzoic acid hydrazide (PHBAH) coloured solution and few drops of sodium hydroxide,

mix well and keep the test tube in water bath in boiling mode. After about 5 minutes withdraw test tube from water bath, allow to cool, then add 10 ml of water and mix well.

### **Instrumentation**

Before measuring absorbance by colorimeter clean the glass tube with water, and ensure zero absorbance with diluent.

To finalize the light source, take sample solution in glass tube and keep first in blue light source and repeat with the available light source simultaneously recording absorbance. Use the light source with highest absorbance and record the absorbance for the followings.

1. Standard stock
2. Working standard solution
3. Sample of unknown concentration.

Note the above three different absorbance. Now plot the graph of two known solution (1 is standard stock, 2 is working standard) i.e. absorbance (Y-axis) against concentration (X-axis), plot a straight line in between two points and find the concentration of unknown sample with the sample absorbance.

### **CONCLUSION-**

The chelate formed from the reaction of sucrose and p-hydroxybenzoic acid hydrazide (PHBAH) in alkaline medium is measured by colorimeter which is the basis of quantifying the amount of sucrose present in sample.

### **REFERENCES-**

1. <https://www.vernier.com/products/sensors/colorimeters/col-bta>. Accessed on 5 August 2017.
2. Lever M. Colorimetric and Fluorometric Carbohydrate Determination with p-Hydroxybenzoic Acid Hydrazide. *Biochem Med.* 1973;7: 274-81