Assay of Glucose using the Nelson-Somogyi method I

Principle:

The sugar is heated with an alkaline solution of copper tartarate and cuprous oxide is produced, which reacts with arsenomolybdate to give molybdenum blue, the intense blue color is then measured in the colorimeter. Sodium sulphate is included in the reaction mixture to minimize the entry of atmospheric oxygen into the solution, which would cause reoxidation of cuprous oxide.

Materials:

1. Nelson's A reagent: 12.5 g Na_2CO_3 (anhydrous), 12.5 g potassium-sodium tartarate, 10 g $NaHCO_3$ and 100 g Na_2CO_3 (anhydrous) dissolved in 350 ml of H2O and diluted to 500 ml with distilled water.

2. Nelson's B reagent: 7.5 g $CuSO_4$. $5H_2O$ dissolved in 50 ml of water and 1 drop of conc. H_2SO_4 is added.

3. Arsenomolebdate reagent: 25 g $(NH_4)_6$ MO_7O_{24}. $4H_2O$ dissolved in 450 ml H_2O. 21ml conc. H_2SO_4 and $3Na_2HASO_4.7H_2O$. dissolved in 25 ml H_2O is added, then stored in brown bottle for 24 hours, at 37oC unstable in light and air.

4. Stock sugar standards (glucose, fructose, and maltose 2g/l solutions in saturated benzoic acid).

5. Some "unknown " sugar solutions.

6. Boiling water bath.

Procedure:

1- Add 19 ml of distilled water to 1ml of standard Solution and mix well.

2- Prepare Nelson's alkaline copper reagent by mixing 12.5 ml of Nelson's A reagent with 0.5 ml of Nelson's B reagent.

3- Set up six test tubes as follows:

Tube No.	Dilute standard glucose (ml) .	Distilled H_2O (ml)
1(blank)	--	1
2	0.2	0.8
3	0.5	0.5
4	0.8	0.2
5	1.0	--

3- Add 1 ml of Nelson's reagent solution to each tube and shake well.

4- Place the tubes in a boiling water bath , and heat for 20 minutes. Remove the tube and cool with cold water.

5- After cooling, add 1ml of arsenomolybdate reagent to each and shake occasionally over a five-minute period to dissolve the Cu_2O and reduce the arsenomolybdate.

6- Add 7.0 ml of distilled water to each tube, and mix.

7- Read the absorbance of standard against the blank at 540 nm.

8- Plot the standard curve with the absorbance (Y axis) against concentration (mg of glucose) (X axis). Draw the best straight line through the origin and points.

10- The concentration of unknown glucose solution can be determined from the curve.

Name: ***No.***

Experiment 2:

Results Sheet

The concentration of standard glucose solution : mg/ml

- After conducting your test, fill the following table :

Tube No.	Concentration (Mg/ml)	Absorbance (At 540 nm)
1		
2		
3		
4		
5		
6		

- Plot the standard curve of the absorbance (y- axis) against the concentration (x-axis)

- Use this plot to estimate the concentration of your unknown glucose sample.

- Express your results in mg/dl , mg% , µg/ml and g/l.

Name: ***No.***

Experiment 2:

Results Sheet