Estimation of carbohydrate by Nelsons's Method II

Principle:

Cupric ions are reduced by sugars to cuprous ions, which quantitatively reduce phosphomolybdic acid to a blue complex.

Materials:

1. Alkaline copper reagent.
2. Phosphomolybdic acid reagent.
3. Standard glucose solution containing 100μg/ml.
4. Glucose solution of unknown strength(30-70 μg/ml).
5. Colorimeter or spectrophotometer.

Procedure:

1. Pipette in duplicate 0.25, 0.5, 0.75, 1.0, 1.5 and 2ml of standard glucose solution into a series of test tubes.

2. Make the volumes up to 2ml with water.also prepare a blank containing 2ml of water

3. Prepare two tubes containing 2ml of unknown glucose solution.

4. Add 2ml of alkaline copper reagent to each tube, mix and plug each tube with a small piece of cotton wool, this prevents reoxidation of Cu+ ions.

5. Heat the tubes in a boiling water bath for exactly 8 minutes.

6. allow the tubes to cool in cold water.

7. Add 2ml of phosphmolybdic reagent to each tube and shake well.

8. After about 2 min dilute each tube to 10ml with water.

9. Read absorbance against the reagent blank at 630nm .

10. Plot standard cure for glucose. read the concentration of the unknown from the standard curve.

Name: *No.*

Experiment 3:

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 630 nm)
1		
2		
3		
4		
5		
6		
7		
8		

- 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 3:

<u>Results Sheet</u>