**Experiment:8**

**Copper Reduction Method (Somogyi-Nelson Method)**

-In hot alkaline solution, glucose reduces cupric ion to cuprous ion with formation of cuprous oxide.

Cu +2 + glucose Cu2O + oxidation products of glucose

-The reaction depends on the alkalinity, the time and temperature of heating, and the concentration of reagents.

-Reoxidation of cuprous ion by oxygen from the air is prevented by adding Sodium Sulphate in the reagent to decrease the solubility of oxygen.

-Added Phosphomolybdic (or Arsenomolybdic) acid (MO+6) is reduced by the cuprous ion to form compounds with lower oxidation states of molybdenum, which have a blue color and suitable for photometric measurement.

-Due to the interference of proteins in this method, the proteins are precipitated by the addition of Barium hydroxide and Zinc sulphate.

-Protein is removed as Zinc proteinate, Sulphydryl compounds as Zinc salts and the remaining zinc and barium ions as Zinc hydroxide and Barium sulphate.

ZnSO4 + Ba(OH)2 Zn(OH)2 + BaSO4

-Recently, rapid colorimetric procedures using O-toluidine or enzymes (such as glucose oxidase and peroxidase) have replaced the Somogyi-Nelson method.

**Procedure:**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **unknown** | **St. 5** | **St. 4** | **St. 3** | **St. 2** | **St. 1** | **Blank** |
| Distalled water |
| x | x | 0.3 ml | 0.5 ml | 0.7 ml | 0.8 ml | 1 ml |
| Diluted Glucose standard (0.2 mg/ml) |
| x | 1.0 ml | 0.7 ml | 0.5 ml | 0.3 ml | 0.2 ml | x |
| Protein-free filtrate |
| 1 ml | x | x | x | x | x | x |
| Copper reagent |
| 1 ml | 1 ml | 1 ml | 1 ml | 1 ml | 1 ml | 1 ml |
| Incubate in boiling water for 20 min., then cool. |
| Arsenomolybdate Reagent |
| 1 ml | 1 ml | 1 ml | 1 ml | 1 ml | 1 ml | 1 ml |
| Let it stand for 1 min. |
| Distalled water |
| 7 ml | 7 ml | 7 ml | 7 ml | 7 ml | 7 ml | 7 ml |

-Mix , read glucose standard and the unknown .

-With spectrophotometer, at 520 nm, against blank, plot standard curve, find out the concentration of your unknown.