Copper Reduction Method (Somogyi-Nelson Method)

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Principle:

- Cu^{+2} + glucos $\longrightarrow$ Cu^{+1} + oxidation products of glucose
- Cu^{+1} + Phosphomolybdic acid(MO^{+6}) $\longrightarrow$ MO^{+4} (blue color)
- Then the concentration of MO^{+4} is measured using spectrophotometer, at 520 nm,
- N.B. the 1st test is done under alkaline ph and heat conditions.

Procedure:

unknown	**Blank**	**St. 1**	**St. 2**	**St. 3**	**St. 4**	**St. 5**
Distalled water						
x	1 ml	0.8 ml	0.7 ml	0.5 ml	0.3 ml	x
Diluted Glucose standard (0.2 mg/ml)						
x	x	0.2 ml	0.3 ml	0.5 ml	0.7 ml	1.0 ml
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.						
Arseno molybdate 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

N.B.

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

N.B.

- 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

Just for your information

Sulphydryl compounds as Zinc salts and the remaining zinc and barium ions as **Zinc hydroxide** and **Barium sulphate.**

$ZnSO_4 + Ba(OH)_2 \longrightarrow Zn(OH)_2 + BaSO_4$

N.B.

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

Calculations:

$C_1 \times V_1 = C_2 X V_2$