T. NO.

Estimation of Protein by Folins and Ciocalteus Reagent

Theary and principle :-

Protein is a complex, nitrogenous substance (biopolymers of amino acid) and is the principle constituents of protoplasm. No living cell is without proteins Berzelius suggested the name in 1838. Animals have higher contain of protein than plant. Emil Fisher (1880-1918) showed that amino acid are linked through amino group and carboxyl group (Peptide bonds) molecules are D amino acid united by peptide linkage.

Protein react Folin's phenal reagent produce a colour complex. The colour coformed is due to reaction of alkaline copper with proteins as in the biurate test and the reduction of Phosphomalebdate by tyrosine and triptophane present in the protein. The intensity of colour depends upon the amount of this aromatic amino acid present will last varied from protein to protein.

Requirment: A) Chemical and glass wares

i) 0.01 (N) NaOH (4g of NaOH dissolve in 100 ml of distilled water)

ii) Reagent - A (Alkaline Na_2CO_3 solution) - 2g of Na_2CO_3 dissolved in 100 ml of 0.1 (N) NaOH solution with constant stearing.

iii) Reagent - B ($CuSO_4$ - Potasium sodium tertarate)
a) 1% $CuSO_4$ solution (1g of $CuSO_4$ dissolved in 100 ml of distilled water.
b) 2% Potasium sodium tertarate (rochelle salt) 2g dissolved in 100 ml of distilled water.
c) Then mixed the above two solution in 1:1 ratio

iv) Reagent - C (Alkaline copper solution) 50 ml of reagent A mixed with 1ml of freshly prepared reagent B (that is in 50:1 ratio)

v) Standerd Protein solution (stock solution)
Bovine serum albumin (BSA) Protein use as standerd protein. The solution was made with 100mg of BSA dissolved in 100ml of 0.1 (N) NaOH solution. The concentration will be 100 ug.

vi) Folin's and ciocalteu's reagent.

a) It is available in the market commercially This commercial phenal to be mixed with equal volume of double distilled water. This ratio will be 1:1

b) It is also can be prepared in the laboratory. It is a mixture of 100g of sodium tungstame 25g of sodium malibdet in 700ml of distilled water.

50 ml of 85% phosphoric acid and 100ml concentrated HCl will be taken in 1.5 liter of volumetric flusk and were for 10 hours.

Then 150g of lithium sulphate and 50 ml of water and few drop of bromine water were added. The above mixture were boiled fo 15 minutes without condenses to remove the exuss bromine. Then it was cool and diluted to 100ml and filtred The reagent must not have any gremish limit.

vii) Glass wares- Clean testtube, Pipette, Beakar.

B- Test Materials:-

The unknown protein solution concentration Bovine serum Albumin (BSA)

Procedure:-

1) Making a standerd curve:-

a) A 5ml of 100 ug/ml concentration of BSA solution which is dissolved in 0.1 (N) NaOH solution (stock solution) was taken.

b) There after make a seres of grade of 20, 40, 60, 80 and 100 ug/ml concentrate of the said solution to the addition of distilled water or 0.1 NaOH solution in different testtube ultimate volume.

c) Prepare a blank - In a separate testtube taken only 1 ml of distilled water.

Then with all the above solution at 5 ml of reagent @ alkaline copper solution and mixed throughly with vigorous shaking. Then all were allowed to stand - 10 - 15 minutes of room temparature in dark place. After taken from the dark 0.5 ml of Folin's phenal in each testtube immediately mixed and it was kept another for 45 minutes to 1 hours in the

same place. After passing the required time it will be observed a blue colour is developed which mesure in a colorimetre (ERMA) at 620 nm absorption spectrume.

ii) Preparation of test materials 1ml of protein solution of unknown concentration was taken in a test tube and the above procedure was followed.

iii) a) Table :-

Concentration gradient of Protein µg/ml	Stock Protein (ml)	Water in (ml)	Reagent 'c' (ml)	Folin's Phenol (ml)		Optical density
Blank	0	1	5	0·5		
20	0·2	0·8	5	0·5		0·03
40	0·4	0·6	5	0·5		0·06
60	0·6	0·4	5	0·5		0·11
80	0·8	0·2	5	0·5		0·12
100	1	0	5	0·5		0·15
unknown	1	0	5	0·5		0·07
Solution						

46µg

Result :- The unknown protein solution contain of protein as measured against the standard curve.

46 mg/ml

30/11/22

Precautions:-

a) Reagent must be freshly prepared.

b) Reagent bottle, pippete, test tube should be perfectly cleaned.

c) Contaminated pippete should be avaided.