

# Aim 23

## To Estimate the Protein Content by Lowry Method

### Introduction

The base of the reaction is mainly the presence of aromatic amino acid (tyrosine and tryptophan) in the protein. Folin-Ciocalteu reagent reacts with protein to form a colored complex. The combination of biuret reaction and the reduction of phosphomolybdate and phosphotungstate components of the folin's reagent by aromatic amino acids are mainly responsible for the production of colored complex. The amount of aromatic amino acid determine intensity of the colour produced and intensity vary with different proteins. Ions like  $K^+$ ,  $NH_4^+$ ,  $Mg^{2+}$ , mercaptoethanol, detergents, EDTA, Tris, phenol, sucrose, interfere in the assay.

### Reagents

1. 2% sodium carbonate in 0.1 N NaOH
2. Bovine serum albumin (BSA) - 1%
3. Sodium potassium tartarate - 2%
4. Folin's reagents – Add distilled water in ratio 1:1 before use.
5.  $CuSO_4$  - 1%
6. Alkaline reagent – Prepare fresh solution by mixing 0.5 ml of sodium potassium tartarate (2%) and Folin's reagents with 49 ml of  $CuSO_4$  (1%).

### Procedure

1. Different concentrations of BSA solution are pipetted out into series of test tubes.

2. Take one tube as blank.
3. Add distilled water in each test tube to make final volume to 0.5 ml.
4. 5 ml of alkaline reagent is added.
5. Mix properly and keep it for 10 min.
6. 0.5 ml of folin's reagent is added.
7. Shake the contents vigorously and leave it for 30 min for maximum development of colour.
8. Measure the O.D. at 660 nm.
9. Plot a standard curve for protein estimation by taking varying concentration (10-100 $\mu$ g) of standard protein (BSA).

Prepare 1% BSA solution

Or

1 g/100 ml

Or

1000 mg/ 100 ml

Or

10 mg/ml

Dilute 100 X

10 mg/100 ml

Or

1 mg/10 ml

Or

500  $\mu$ g/5 ml

Diluted sample (ii) is used for preparing standard curve.

10. Extrapolate the results from the curve.

11. By using the formula estimation can be done for the unknown sample.

### **Calculations**

O.D. of the test sample - x

From standard curve

Suppose x O.D. corresponds to y  $\mu$ g of protein.

Test sample taken - 0.1 ml

So 0.1 ml of unknown sample contains y µg of protein

$$\text{Protein (mg \%)} = (y/0.1) \times (100/1000)$$

Alternatively concentration can be calculated by the formula

O.D test-

O.D. standard-

$$\text{Protein (mg \%)} = \left\{ \frac{\text{O.D test}}{\text{O.D. standard}} \right\} \times (\text{concentration of standard in mg/volume of sample in ml}) \times 100$$

### **Precautions**

1. All glassware should be clean and dry.
2. Use distilled water only.
3. Solutions should be accurately pipette out.
4. BSA should be weighed accurately for preparing standard curve.
5. BSA solution should be prepared gently and frothing should be avoided.
6. Shake well after addition of folin's reagent.