Aim 5 To Estimate the Protein in the given sample by Bradford Method

Introduction

The best method for the estimation of proteins is Bradford Method which is very simple, sensitive and fast. It is based on the principle that after addition of protein the absorbance maximum of the dye in acidic solution shifts from 465 to 595 nm which is due to stabilization of the anionic form of the dye by both hydrophobic and ionic interactions. Out of histidine, lysine, arginine, tyrosine, tryptophan and phenylalanine residues; arginine residues reacts with dye with greater extent while all others with lesser extent.

The colour can be developed within 5 minutes and remain the same for about one hour.

With the increase in the concentration of protein, the intensity of the colour also increases. In the estimation of proteins, Interference of SDS, Triton X-100 is observed while there is no interference of metal ions like NH_4, K^+, Mg^+, phenol, sucrose.

Requirements

1. Protein sample
2. Bradford reagent (100 mg of Coomassie Brilliant blue G-250 is dissolved in 50 ml of ethanol Then, add 100 ml of 85% ortho-phosphoric acid. Final volume is raised to 1 L. with deionised water).
3. Standard protein solution (5mg of bovine serum albumin is dissolved in 50 ml of 10 mM phosphate buffer, pH 7.0).

5 mg BSA/50 ml
Or

500 µg/ 50 ml

Or

100 µg/ml

Use after diluting the solution ten times.

4. Spectrophotometer.

Procedure

The different volume of BSA solution is taken into series of test tubes. Mark one tube as blank (no BSA added).

Add phosphate buffer in all test tubes to make final volume to 1ml.

ml and 0.5 ml of the sample is taken in test tube and buffer is added to make the final volume to 1 ml.

Add 5 ml of Bradford reagent to all the tubes and mix properly.

Read the O.D. at 595 nm against the reagent blank.

Plot the standard curve by taking O.D. on Y-axis and concentration of BSA on X-axis.

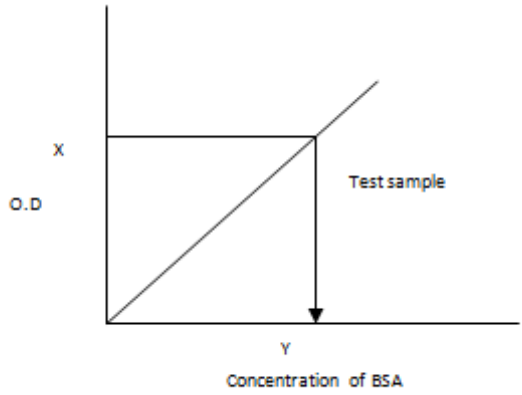
Protein content in the sample can be calculated from the standard curve.

Observation table

S. No.	BSA solution volume (ml)	BSA solution Conc. (mg)	Buffer volume (ml)	Bradford reagent (ml)	O.D.
Blank	-	-	1.0	↑	
1	0.1	1	0.9		
2	0.2	2	0.8		
3	03	3	0.7		
4	0.4	4	0.6		
5	0.5	5	0.5	5.0	
6	O.6	6	0.4		

7	0.7	7	0.3		
8	0.8	8	0.2		
9	0.9	9	0.1		
10	1.0	10	-		
Unknown 1	0.1		0.9		
Unknown 2	0.5		0.5		

Standard curve



Calculations

O.D. of the taken sample - Suppose x

From standard curve

X corresponds to y µg protein

Volume of test sample - 0.5 ml

So 0.5 ml of given sample contains y µg protein

1 ml will contain = (y/0.5)x 1 = 2y µg protein/ ml test sample

Precautions

1. Clean and dry cuvettes may be used for taking O.D.
2. Use alcohol to clean the cuvettes.