Aim 6 To Estimate the Quality of Protein by Ultraviolet Absorption Method

Introduction

UV absorption method is very easy and rapid with several advantages.

1. Without the addition of reagents, this method can be performed directly by taking the sample.
2. In this no incubation step is required.
3. The protein concentration and absorbance relationship is a linear graph.
4. At 280nm protein show maximum absorbance.
5. Nucleic acids absorb light at 280 nm but they show maximum absorbance at 260 nm. So sample containing RNA and/or DNA will produce speciously high protein estimates. Thus protein concentration can be calculated by using this formula.

Protein concentration (mg/ml) = $1.55A_{280}$-$0.76\ A_{260}$

Reagents

1. Buffer
2. Protein solution in buffer
3. Spectrophotometer

Procedure

1. UV lamp of the spectrophotometer is turn on and warm up the machine (generally for 20 min.)
2. Set the wavelength to 280 nm.

3. Consider buffer as a blank and adjust the O.D. to zero.
4. Note down the absorbance of the protein solution.
5. Repeat the same steps for 260 nm wavelength.
6. Put the sample solution containing the protein in the cuvette and take the O.D.
7. Calculate the protein concentration (mg/ml) from the following formula.

$$\text{Protein (mg/ml)} = 1.55A_{280} - 0.76\ A_{260}$$

Precautions

1. The protein sample is diluted by the same buffer used in original solution if the absorbance of the protein solution is greater than 2.
2. When the protein solution is cold, the outside of the cuvette should be wiped and note down the reading after placing the cold solution into the cuvette because the condensation of moisture on the cuvette produces high reading.