Estimation of DNA and Protein Concentration

XIII.A - Principle

The soluble proteins show its absorbance under ultraviolet light in a range at 280 and 200 nm. The reason for the absorbance peak at 280 nm because of the presence of aromatic rings in Amino acids and the peak shows at 200nm are responsible for peptide bonds. The other stricter of protein like secondary, tertiary, and quaternary are all responsible for different absorbance spectrum. On the other hand, pH, ionic strength can also alter the spectrum ranges.

Equipment

UV lamp with quartz cuvette (for Spectrophotometer study) is required along with the standard liquid for control sample.

Procedure

1. UV lamp is warmed up for 15 min
2. Wavelength is adjusted to 280 nm
3. Calibration has done by using buffer solution to zero absorbance
4. Protein solution absorbance has measured
5. Wavelength is adjusted to 260 nm
6. Calibration has done by using buffer solution to zero absorbance
7. Protein solution absorbance has measured

Result and Analysis

Unknown proteins or protein mixtures: The following formula is used to estimate approx protein concentration. Path length is 1 cm for most spectrometers.

Concentration (mg/ml) = Absorbance at 280 nm/ path length (cm.)

Pure protein of known absorbance coefficient: The following formula is used for a path length of 1 cm. Concentration is in mg/ml, %, or molarity depending on coefficient is used.

Concentration = Absorbance at 280 nm/ absorbance coefficient

To convert units, use these relationships:

Mg protein/ml = % protein divided by 10 = molarity / protein molecular weight

Unknowns with possible nucleic acid contamination*:* The following formula is used to estimate protein concentration

Concentration (mg/ml) = (1.55 x A280) - 0.76 x A260

Table - 45: Protein content of three taxa

Sl.No.	Plant Name	Protein Content mg/ml
1	*Loranthus parasiticus*	**0.042**
2	*Macrosolen cochinchinensis*	**0.408**
3	*Viscum album*	**0.064**

XIII.B - Determination of DNA concentration by Spectrophotometric Estimation

In aqueous solutions, DNA dissolves easily. However, the DNA is viscous when dissolved at high concentrations (10 mg/ml and above). At lower concentrations, the DNA cannot be detected by as well as no viscosity has observed. It is often important to know its concentration of DNA at time of work (the concentration is expressed in units of mass/volume). The determination of DNA concentration is done by means of spectrophotometric analysis. It is obviously observed there is a correlation between concentration of DNA and absorbance spectra as nitrogenous bases absorb UV light, soo more concentrated the DNA showed more UV light absorbance.

Result and Analysis:

The double-stranded DNA concentration of pure condition at A260 is 1.0 is 50 mg/ml. Thus, the following formula can be used to determine the concentration of DNA in solution.

Unknown (mg/ml)/ Measured A260 = 50 (mg/ml)/ 1.0 A260

Unknown mg/ml = 50 mg/ml x Measured A260 x dilution factor

Table - 46: DNA content of three taxa

Sl.No.	Plant Name	DNA Content µg/ml
1	*Loranthus parasiticus*	**248**
2	*Macrosolen cochinchinensis*	**560**
3	*Viscum album*	**284**