Questions:

1. A globular protein eluted from a gel filtration column showed molecular weight of 100 kDa. In a Non-reducing SDS-PAGE, the protein was separated as two distinct bands of 40 kDa and 60 kDa each. Further, when the same protein is resolved in SDS-PAGE in presence of β-mercaptoethanol, two distinct bands of 20 kDa and 30 kDa were found. Discuss, how you would infer the data with proper justification and diagram.

2. A solution present in a 1 cm cuvette transmits 40% incident light. Calculate the concentration of the solution given that $\varepsilon = 6000\ M^{-1}\ cm^{-1.}$

3. Define Beer-Lambert Law. Suppose you are given a solution containing unknown concentration of a protein. A sample of this solution is placed in a UV/Visible spectrophotometer and the absorbance is measured at 280 nm where ∑ (epsilon) for that protein is known to be 20 $M^{-1}cm^{-1}$. The absorbance (A) is found to be 0.135 after 10 times dilution of stock. The width of the sample tube (cuvette) is 1.0 cm. Determine the concentration of the protein in the stock solution.

4. A monochromatic radiation is incident on a solution of 0.06 molar concentration of an absorbing substance. The intensity of the radiation is reduced to one fourth of the initial value after passing through 8 cm length of the solution. Calculate the molar extinction coefficient of the substance.

5. A solution containing NAD^+ and NADH had an optical density (i.e. absorbance) of 0.311 at 340 nm and 1.2 at 260 nm in a 1 cm cuvette. Calculate the concentrations of the NAD^+ in the solution. Both NAD^+ and NADH absorb at 260 nm, but only NADH absorbs at 340 nm. The extinction coefficients are given below.

Compound	**260 nm**	**340 nm**
NAD^+	18,000	~0
NADH	15,000	6220

6. A protein has one tryptophan and one tyrosine in its sequence. Assume molar extinction coefficients at 280 nm of Trp and Tyr as 3000 and 1500 $M^{-1}cm^{-1}$, respectively. What would be the molar concentration of that protein if its absorbance at 280 nm is 0.90 in a 1 cm cuvette?