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.

Answer:

The MW obtained from Gel Filtration Chromatography = 100 kDa (Single peak in Elution Profile)

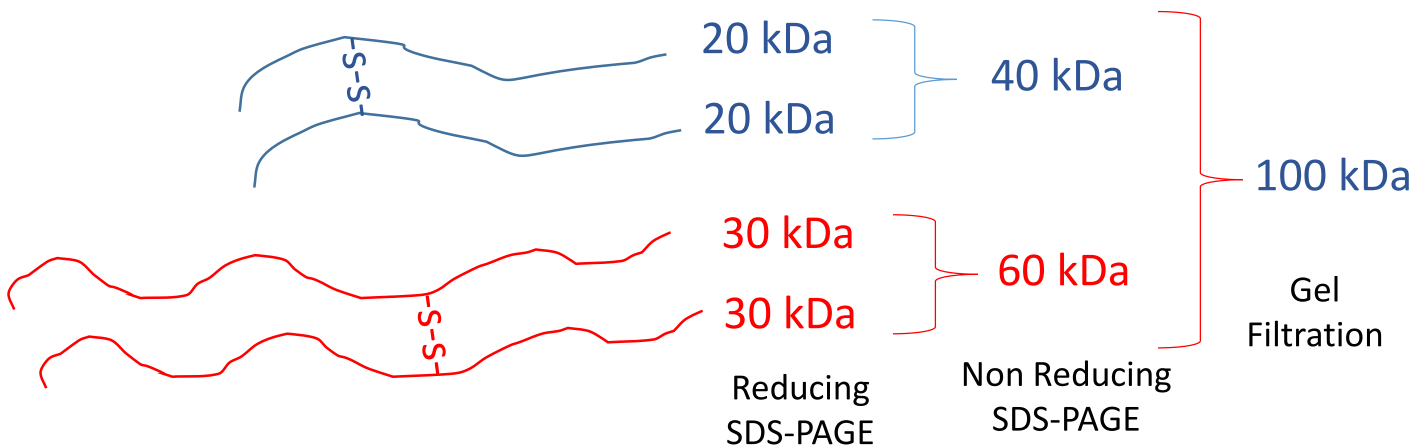
The MW obtained from Non-Reducing SDS-PAGE = One 40 kDa and one 60 kDa Band

The MW obtained from Reducing (in presence of BME) SDS-PAGE = One 20 kDa and one 30 kDa Band

The Protein contains four polypeptide chains: Two twenty kDa chains connected by disulphide bonds and Two 30 kDa chains connected by disulphide bonds. So, we saw in the Reducing SDS-PAGE only two bands of 20 kDa and 30 kDa

In the Non-Reducing SDS-PAGE, the disulphide bonds remain intact, so two bands of 40 kDa (total of two chains of 20 kDa) and 60 kDa (total of two chains of 30 kDa) were observed. But the presence of SDS (denaturing agent) removed al the non-covalent interactions among the chains.

The gel filtration chromatography was performed in native conditions (intact noncovalent interactions) and hence a total of all the chains {(2X20=40) + (2X30=60)} = 100 kDa was observed. Here the whole protein in its native (quaternary) structure was eluted from the column.



2. A solution present in a 1 cm cuvette transmits 40% incident light. Calculate the concentration of the solution given that Σ = 6000 M-1 cm-1

Answer:

Transmittance, T= 40%   i.e. 0.4

Molar extinction coefficient, $\Sigma = 6000\ M^{-1}cm^{-1}$

Path length, $l = 1$ cm

Absorbance, A = -logT

= -log (0.40)

= 0.398

As per Beer-Lambert Law, $A = \Sigma.c.l$

$c = A/\ \Sigma.l = 0.398/(6000 \times 1) =$ **6.63×10^{-5} M**

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.

Answer:

(Refer to the Study materials for the definition of Beer Lambert law part)

Molar extinction coefficient, $\Sigma = 20\ M^{-1}cm^{-1}$

Pathength, $l = 1$ cm

Absorbance, A = 0.135 **(at a dilution of 10 times), DF=10**

According to Beer-Lambert Law, $A = \Sigma.c.l$

$$c = A/\ \Sigma.l = 0.135/(20 \times 1) = 0.00675 = 6.75 \times 10^{-3}\ M$$

So, the concentration after dilution is 6.75×10^{-3} M or **6.75 mM**

The concentration of the Stock solution will be = **10** x 6.75 M = **67.5 mM**

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.

Answer:

Given, $I/I_0 = 1/4 \Rightarrow I = 1/4\ I_0$

According to Beer-Lambert Law, $\mathbf{A = \log(I_0/I) = \Sigma.c.}\boldsymbol{l}$

$\Sigma = \{\log(I_0/I)\}/\ c.l$

$\Sigma = (\log 4)\ /\ 0.06 \times 8$

$\Sigma = 0.6/\ (0.06 \times 8) =$ $\mathbf{1.254\ M^{-1}cm^{-1}}$

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	Σ at **260 nm**	Σ at **340 nm**
NAD+	**18,000**	**~0**
NADH	**15,000**	**6220**

Answer:

The concentration of NADH from its absorbance at 340 nm, (The absorbance at 340 nm is entirely due to that of NADH)

$A = \Sigma.c.l$

$c = A/\Sigma.l = 0.311 / 6220 \times 1$

c (for NADH) = $\mathbf{5 \times 10^{-5}\ M}$

Now, the absorbance at 260 nm resulting from the NADH,

$A = \Sigma.c.l$

$A = 15000 \times (5 \times 10^{-5}\ M) \times 1 = 75 \times 10^{-2} = 0.75$

Now, the absorbance at 260 nm from NAD+

= Total absorbance at 260 nm – Absorbance of NADH at 260 nm

$=1.20 - 0.75 = \mathbf{0.45}$

Finally, from the absorbance of NAD+ at 260 nm,, we can calculate the concentration of NAD+.

$A = \Sigma.c.l = 18000 \times c \times 1$

c (of NAD+) = $0.45 / 18000 = \mathbf{2.5 \times 10^{-5}\ M}$

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?

Answer:

Molar extinction coefficient of Trp = 3000 $M^{-1}cm^{-1}$

Molar extinction coefficient of Tyr = 1500 $M^{-1}cm^{-1}$

Total extinction coefficient = (Number of Trp residues x 3000 $M^{-1}cm^{-1}$) + (Number of Tyr residues x 1500 $M^{-1}cm^{-1}$) = 4500 $M^{-1}cm^{-1}$

According to Beer-Lambert Law, $A = \Sigma.c.l$

$$c = A/\Sigma.l = 0.90/4500 = \mathbf{0.2 \times 10^{-3}\ M}$$