CONCEPT: SPECTROPHOTOMETRY

●Proteins can be quantified by measuring their _____ *absorbance*.

□ _____ acquire light absorbance values, which can be used to determine [solute].

●Lambert-Beer Law (_____ *Law*): expresses relationship between light absorbance & absorbing [solute].

□ A : _____ of the solute.

□ I_0 : _____ intensity of light (Incident light).

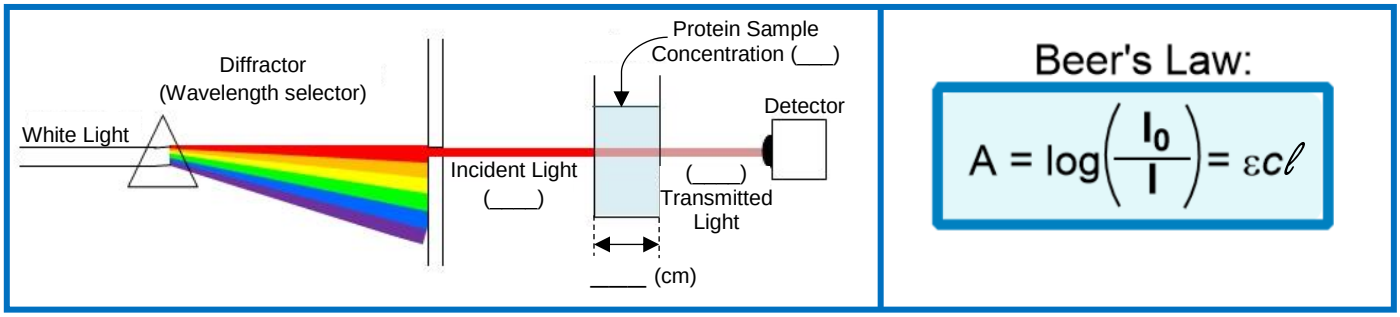
□ I : _____ intensity of light.

□ *c* : _____ of the absorbing solute.

□ ℓ : _____ of light path (cm).

□ ε : _____ coefficient (or absorptivity) of a solute.

EXAMPLE: Spectrophotometer & Beer's Law.



PRACTICE: What is the relationship between light absorbance (A) & the amount of light transmitted through a sample?

a) Increased transmitted light results in increased A.

b) Decreased transmitted light results in decreased A.

c) Decreased transmitted light results in increased A.

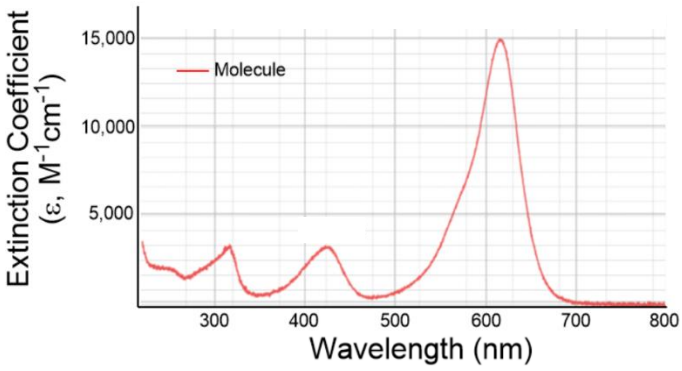
d) Transmitted light & A should always remain equal.

Extinction Coefficient or Absorptivity (ε)

●Extinction coefficient (ε): a property measuring how strongly a chemical absorbs light at a particular _____ (λ).

□ The greater the ε, the _____ the absorbance.

□ ε of a solute varies with wavelength of light & has units of _____.



PRACTICE: Which of the following options is false for Beer's Law?

a) Absorbance increases as concentration increases.

b) Absorbance decreases as path length increases.

c) Absorptivity is wavelength specific.

d) Absorbance spectrums plot absorbance and wavelength.

CONCEPT: SPECTROPHOTOMETRY

Trp & Tyr Absorb Light at 280 nm Wavelength

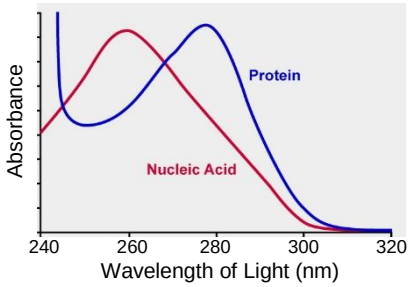
●Spectrophotometry data is plotted on an absorbance _____ (absorbance values vs. wavelength of light).

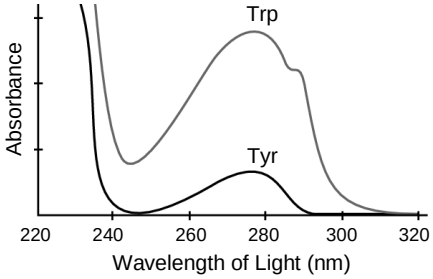
●A peak light absorbance at _____ nm wavelength (λ) is a characteristic property of most proteins.

□ Trp _____ absorbs light at a wavelength of 280 nm.

□ Tyr _____ absorbs light at a wavelength of 280 nm.

EXAMPLE: Protein Absorbance Spectrums.





PRACTICE: Which amino acid strongly absorbs light at 280 nm λ?

a) Y b) W c) T d) Tyr

PRACTICE: A) Suppose myoglobin's molecular weight is 17,800 g/mole and its extinction coefficient at 280 nm wavelength is 15,000 M-1 cm-1. What is the absorbance of a myoglobin solution (concentration = 1 mg/mL) across a 1-cm path?
Hint: Use Beer's law.

a) 0.49 b) 0.73 c) 0.36 d) 0.84

B) What is the percentage of the incident light that is transmitted through this solution?

a) 14% b) 6% c) 21% d) 58%

CONCEPT: SPECTROPHOTOMETRY

PRACTICE: A protein solution has an absorbance of 0.1 at 280 nm with a path length of 1 cm. If the protein sequence includes 3 Trp residues but no other aromatic residues, what is the concentration of the protein? (Trp $\varepsilon = 3{,}400\ M^{-1}\ cm^{-1}$).

a) 29.4 µM b) 5.32 µM c) 9.8 µM d) 0.7036 µM

PRACTICE: An unknown protein has been isolated in your laboratory and determined to have 172 amino acids but does not have tryptophan. You have been asked to determine the possible tyrosine content of this protein. You know from your study of this lesson that there is a relatively easy way to do this. You prepare a pure 50 µM solution of the protein, and you place it in a sample cell with a 1-cm path length, and you measure the absorbance of this sample at 280 nm in a UV-visible spectrophotometer. The absorbance of the solution is 0.398. How many tyrosine residues are there in this protein? (Tyr $\varepsilon \approx 1{,}000\ M^{-1}\ cm^{-1}$).