Hemoglobin:

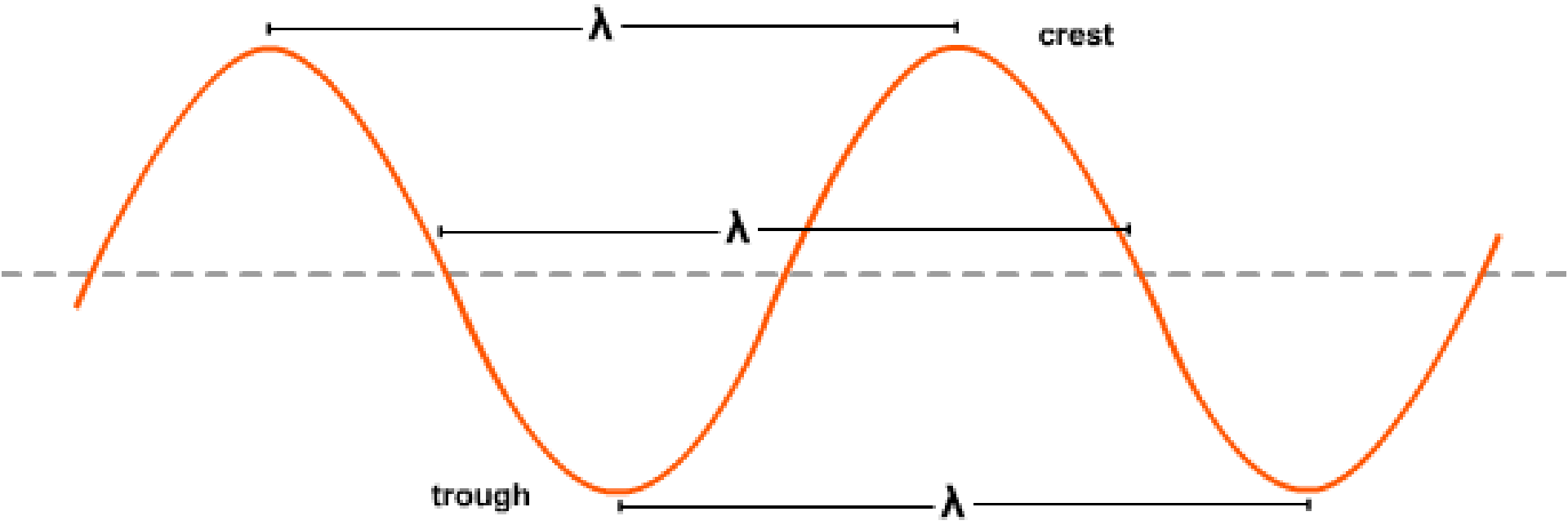
Goal of experiment is to determine the concentration of an unknown hemoglobin solution using spectrophotometry.

Spectroscopy is the study of the interaction between EM radiation and matter.

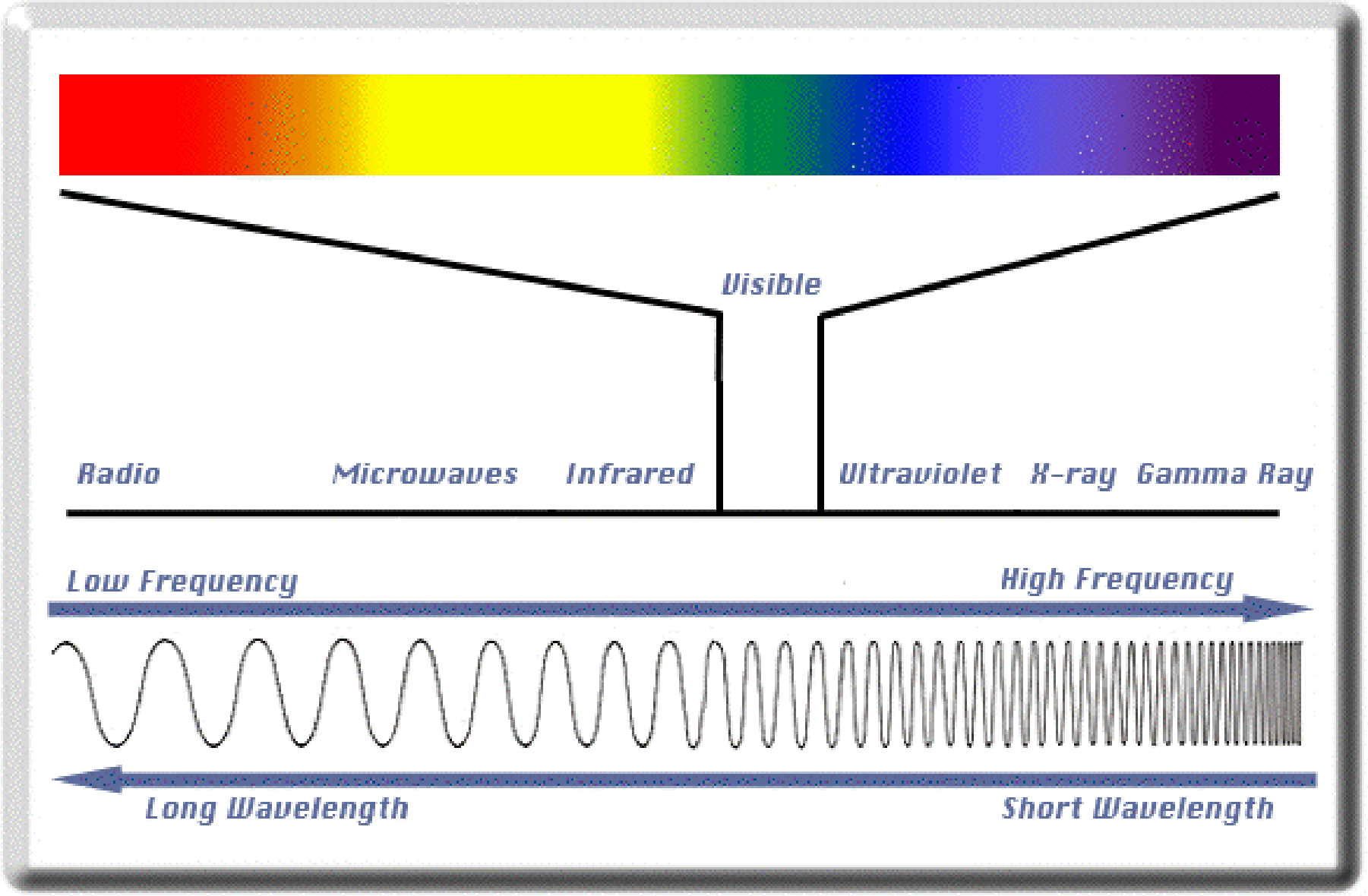
Electromagnetic(EM) Radiation:

EM radiation is the transmission of energy in the form of a wave. Ex: light.

Wavelength(λ) measured in nanometers(nm).



EM Radiation:

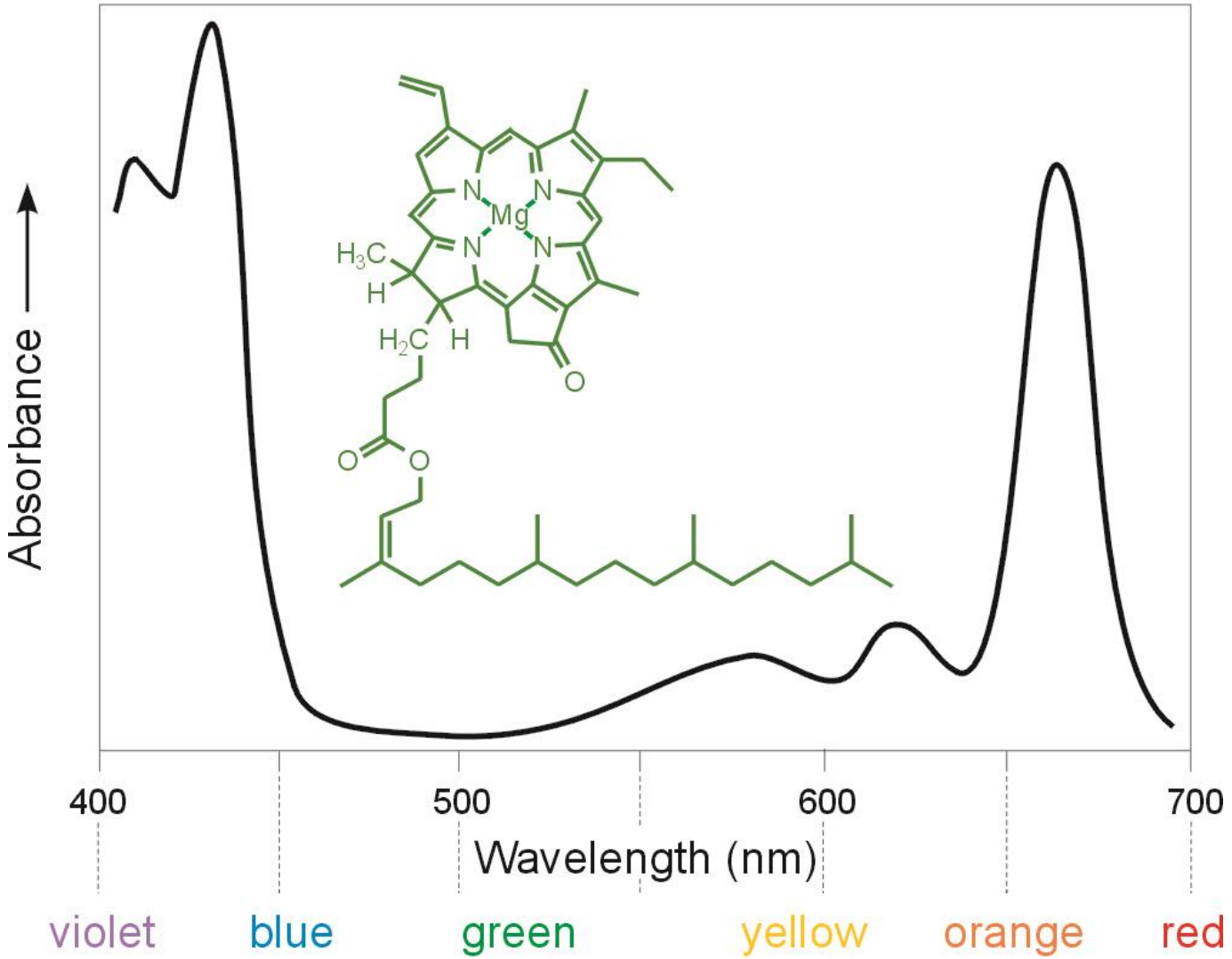


UV-Visible:

Refers to EM radiation in the Ultaviolet – visible region.

UV: 200 – 400 nm
visible: 400 – 700 nm

UV-Visible Absorption Spectrum of Chlorophyll:

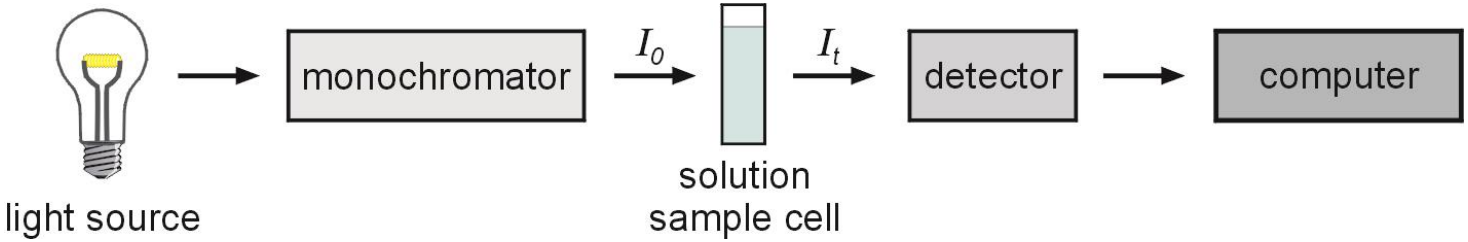


Spectrophotometry:

Involves exposing a sample to UV-visible EM radiation and measuring the amount of light Absorbed by the sample.

$$\%T = 100 \times \frac{I_t}{I_o} \qquad A = -\log \%T$$

Spectrophotometer:



Beer's Law:

The amount of light Absorbed depends on

1. **the sample**
2. **path length**
3. **number of absorbing moleucles**

$$A = \varepsilon lc$$

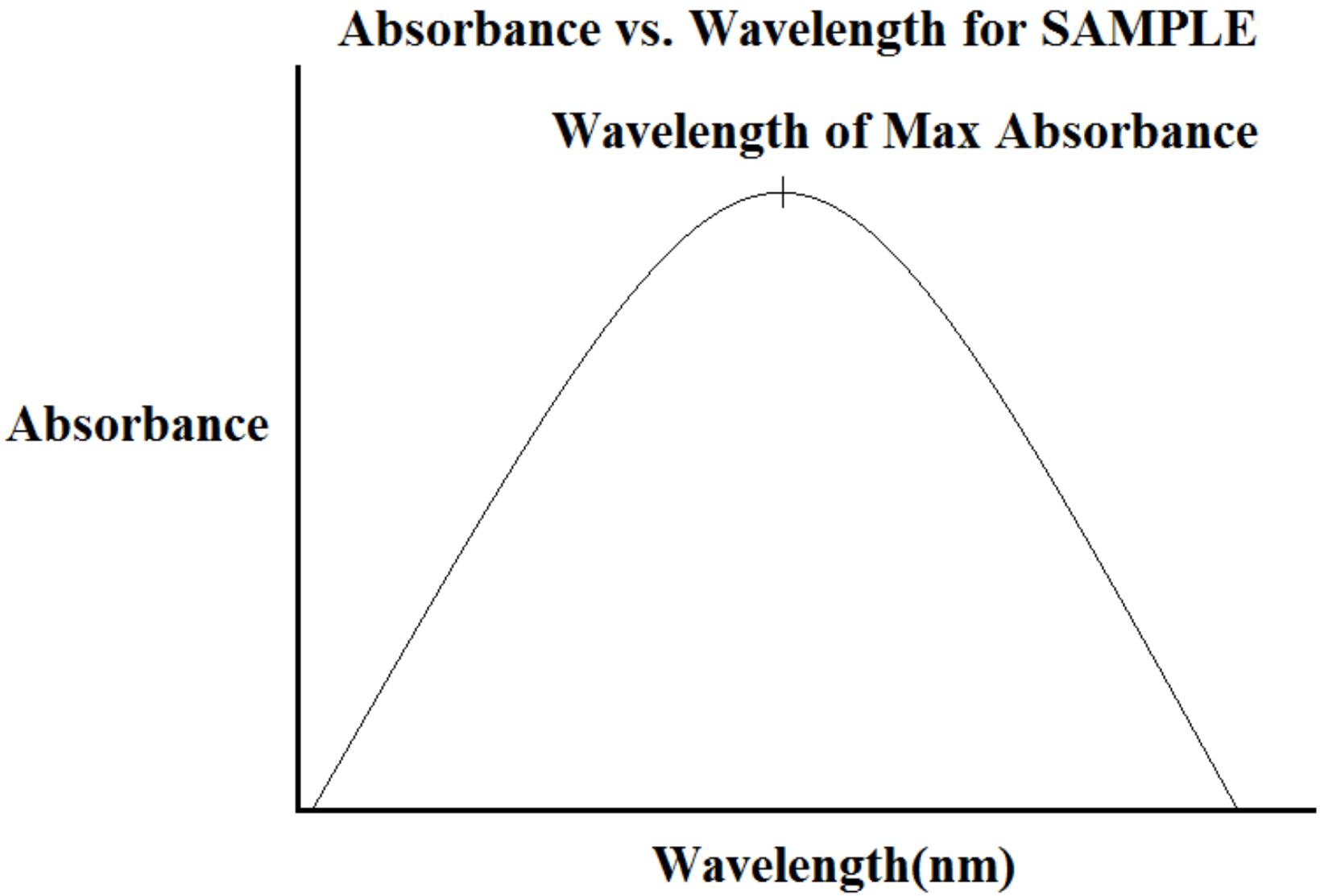
A = Absorbance

ε = Molar Absorptivity

l = path length(1 cm)

c = sample concentration

Part I: Construct an Absorption Spectrum



Part II: Construct a Calibration Curve

Calibration curve is a plot of Absorbance(Y-axis) vs. Concentration(X-axis).

Calibration curve determined by measuring the absorbance of a series of standard solutions of KNOWN concentration.

Preparations of Standards:

Calibration standards prepared by dilution of a stock solution.

$$C_1 \times V_1 = C_2 \times V_2$$

Consider Solution#1:

$$0.250 \text{ mg/mL} \times 3.00 \text{ mL} = C_2 \times 6.00 \text{ mL}$$

$$C_2 = 0.130 \text{ mg/mL}$$

Calibration Curve: