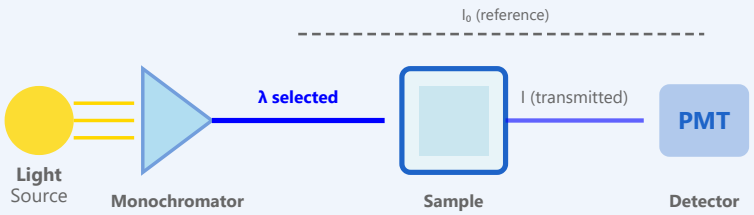
UV-Vis Spectroscopy

$$A = \varepsilon bc = -\log_{10}(I/I_0)$$

ε: molar absorptivity ($M^{-1}cm^{-1}$) | b: path length (cm) | c: concentration (M)

Spectrophotometer Design



Chromophores in Biology

Proteins: Trp, Tyr (280 nm)
DNA/RNA: 260 nm
Heme: Soret band (420 nm)

Cuvette Selection

Quartz: UV region
Glass/Plastic: Visible only
Standard: 1 cm path length

Applications

Protein quantification | DNA/RNA purity | Enzyme kinetics | Drug screening

Baseline Corrections

Buffer blank essential | Scatter correction for turbid samples | Temperature control

Linear Range

A = 0.1-1.0 optimal | Beyond A=2: non-linear | Dilute if necessary

Light Sources

Deuterium (UV) | Tungsten-halogen (Visible) | Xenon flash lamps