**Beer - Lambert Law**

- **Fundamental Law of Spectrophotometry**

  \[ T = \frac{P}{P_0} = \frac{S}{B} \]

  \[ A = -\log T \]

  \[ A = -\log \frac{P}{P_0} = \varepsilon \cdot bc \]

  - Assumes:
    - monochromatic radiation
    - system not saturated in light
    - absorbers behave independently and are distributed homogeneously
  - The product corresponds to the number of absorbers per cm² area as beam passes through cell.
  - \( A = abc \) vs \( A = \varepsilon bc \)

- **Typical analytical application: Calibration curve**

  \[ \text{Absorbance} \]

  \[ \text{Concentration} \]

  - Also works for mixtures: For a given \( \lambda \),

    \[ A_{\text{net}} = A_1 + A_2 + A_3 + \ldots \]

    \[ A_{\text{net}} = \varepsilon_1 \cdot bc_1 + \varepsilon_2 \cdot bc_2 + \varepsilon_3 \cdot bc_3 + \ldots \]
Limitations/Deviations Affecting Linearity

A. Real Deviations: due to derivation of BL
   - Law only works at low concentrations (~mM)
   - At higher concentration, $\eta$ of solution changes, causing $\varepsilon$ to change.

B. Instrumental Deviations:
   1. Deviations due to polychromatic radiation
      - due to bandpass of measurement
      - narrow features + wide bandpass $\rightarrow$ changing $\varepsilon$
      - [Link](http://www.chem.uoa.gr/applets/AppletBeerLaw/Appl_Beer2.html)

![Diagram of polychromatic radiation and Beer's law](image)

2. Deviations due to stray light
   - Increased light reaching detector
   - Contributes most when $P << P_0$
   - Causes negative deviation at high concentration (High Abs.)
   - Decreasing bandpass lowers stray light
   - increased linearity

![Diagram of apparent deviation from Beer's law](image)
Limitations/Deviations Affecting Linearity

C. Chemical Deviation: Shifting Equilibria
   - As position of equilibrium changes, concentration of absorber changes
   - Equilibrium affected by:
     - Compensate by using isosbestic point: point where molar absorptivity for components of equilibria are identical.

Optimizing UV-Vis Analysis

- Using UV-Vis for quantitative analysis:
  1. Temperature: changing temp cause shifting equilibria
  2. Solvents: Transparency, Solubility, Purity
  3. Photoeffects: luminescence
  4. Appropriate wavelength:
     choose $\lambda_{\text{max}}$ for best sensitivity and linearity
  5. Appropriate sample cells: minimize scatter, etc.
Example Applications of UV-VIS

• Determination of equilibrium constants

• Determination of reaction kinetics

• Quantitative Analysis - Calibration curves...

• Detectors for separations (HPLC)

Example Applications of UV-VIS

• Photometric Titrations
  – Monitor absorbance of analyte (product, titrant) during titration
  – Beer’s law applies!
    • away from eq. pt., observe linear regions
    • magnitude of absorbance depends on concentration
    • slope of linear portion is determined by Beer’s law
    • Intersection of linear portions = eq. pt.

  – Need to account for effect of dilution on the absorbance

  – Endpoint is determined by data taken away from it
    • points near the endpoint aren’t as critical
    • don’t need sharp transition