Pharmaceutical Analysis

Spectrophotometric Analysis
High Performance Liquid Chromatography
Gas Liquid Chromatography
Radioimmunoassay
Enzyme Multiplied Immunoassay
Fluoroimmunoassay

Assay Steps

- Separation (Dosage form - Biological Sample)
  - Centrifugation
  - Extraction
  - Chromatography
  - Immunoassay
- Quantitation
  - Spectrophotometric (absorbance, fluorescent)
  - Radioactivity

Separation Step

- Centrifugation
  - Excipients
  - Macromolecules
- Extraction
  - pH-pKa partition
  - Lipid/Aqueous solubility
- Chromatography
  - Adsorption, partitioning, molecular size, ion-exchange
- Immunoassay

Quantitation Step

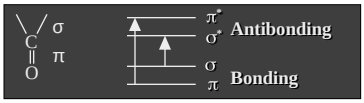
- Absorbance
- Fluorescence
- Radioactivity
- Electrochemical
- Other

Spectrophotometric Analysis

- Theory
  - Beer-Lambert's Law
- Equipment
  - Single Beam
  - Double Beam
- Application
  - Quality control
  - HPLC

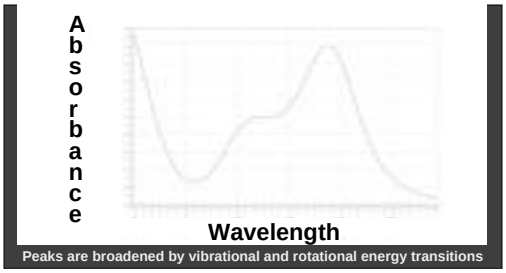
Absorption Spectroscopy

- Absorption
  - Absorption by Molecules in Solution
  - Electronic, Vibrational, and Rotational Energy

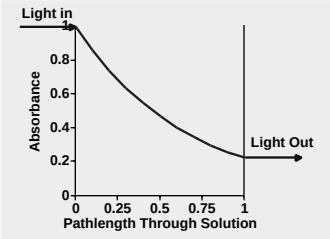


Electronic Transitions

Energy Absorption



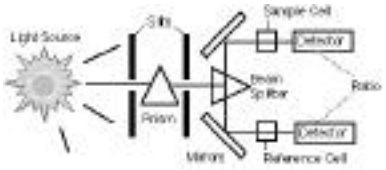
Absorption Through a Solution



Beer-Lambert's Law

- Absorbance proportional to path length and concentration
- $A = a \cdot b \cdot c$
- where A = absorbance and a = absorptivity (ε), b = pathlength, and c = concentration
- If b is 1 cm and c is in g/100 ml the absorptivity is given as $A^{1\%}_{1\ cm}$ at wavelength x. Absorptivity may also be called the extinction coefficient or absorption coefficient

Double Beam Instrument

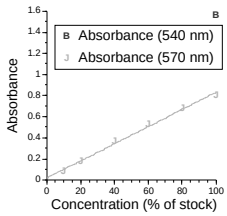


Redrawn from: Bauer, H.H., Christian, G.D., and O' Reilly, J.E. 1978 Instrumental Analysis, Figure 7.14, page 187

Single Beam Instrument

Application

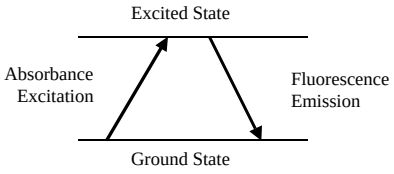
- Standard Curve



Fluorescence Spectroscopy

- Molecule absorbs energy and immediately emits energy at a higher wavelength (lower energy)
- Excitation and emission wavelengths specific to the compound
- Emission measured at 90° to the excitation light path
- Emission proportional to drug concentration

Fluorescence Transitions



Fluorescence Instrument