

Absorption Spectroscopy

Theory of Electronic, Atomic, and Molecular Spectra

Atomic Spectra

- **Atoms** consist of electrons arranged in discrete energy levels (orbitals).
- When an electron transitions from one energy level to another, it absorbs or emits energy in the form of electromagnetic radiation.
- The energy difference between levels determines the wavelength of the spectral line:

$$\Delta E = h\nu = hc/\lambda$$

- Atomic spectra are composed of sharp lines called line spectra (e.g., hydrogen Balmer series).
- Each element produces a unique atomic spectrum, used for qualitative analysis in flame photometry or AAS.

Molecular Spectra

- **Molecules have energy levels due to:**
 - Electronic transitions
 - Vibrational transitions
 - Rotational transitions
- These energy levels are quantized, and transitions between them occur upon absorption/emission of specific photon energies.
- **Electronic transitions** involve movement of electrons to higher orbitals (e.g., $\pi \rightarrow \pi^*$, $n \rightarrow \pi^*$).
- **Vibrational transitions** involve changes in bond stretching or bending.
- **Rotational transitions** involve changes in rotational energy (microwave region).
- **Molecular spectra** are **broad**er than atomic spectra due to overlapping vibrational and rotational energy levels within each electronic level.

Electronic Spectra

- Observed in the UV-visible region (200–800 nm).
- Caused by electronic transitions within molecules.
- Particularly important for molecules with conjugated double bonds and chromophores.
- Consist of broad bands due to coupling with vibrational levels.

Fundamental Laws of Photometry

Photometry is the measurement of light intensity as it passes through a medium.

A. Lambert's Law

- Lambert's Law states that the **absorbance (A)** of a substance is **directly proportional to the path length (l)** of the absorbing medium.

$$A \propto l$$

- **Mathematical Expression**

$$A = \log_{10} \left(\frac{I_0}{I} \right) = \epsilon l$$

- **Where:**

- I_0 = Incident light intensity
- I = Transmitted light intensity
- l = Path length (cm)

B. Beer's Law

- Beer's Law states that the **absorbance (A)** is **directly proportional to the concentration (c)** of the absorbing species.

$$A \propto c$$

Beer-Lambert Law (Combined Law)

- By combining **Beer's Law** and **Lambert's Law**, the **Beer-Lambert Law** is obtained. It relates absorbance to both concentration and path length.

Mathematical Expression

$$A = \epsilon cl$$

- **Where:**

- A = Absorbance (no units)
- ϵ = Molar absorptivity or extinction coefficient ($\text{L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$)
- c = Concentration of absorbing species ($\text{mol} \cdot \text{L}^{-1}$)
- l = Path length of the sample cell (cm)

Derivation of Beer-Lambert Law

Basic Principles

- Each molecule has a probability of absorbing light proportional to its concentration.
- Absorbance is cumulative over the path length.

Steps of Derivation

1. Transmission and Absorbance

- As light passes through an absorbing medium, its intensity decreases exponentially:

$$I = I_0 e^{-\alpha l}$$

- where α is the absorption coefficient.

2. Logarithmic Form

- Taking the natural logarithm:

$$\ln \left(\frac{I_0}{I} \right) = \alpha l$$

3. Introducing Molar Absorptivity

$$\alpha = \epsilon c$$

4. Final Equation

- Substituting α in the equation:

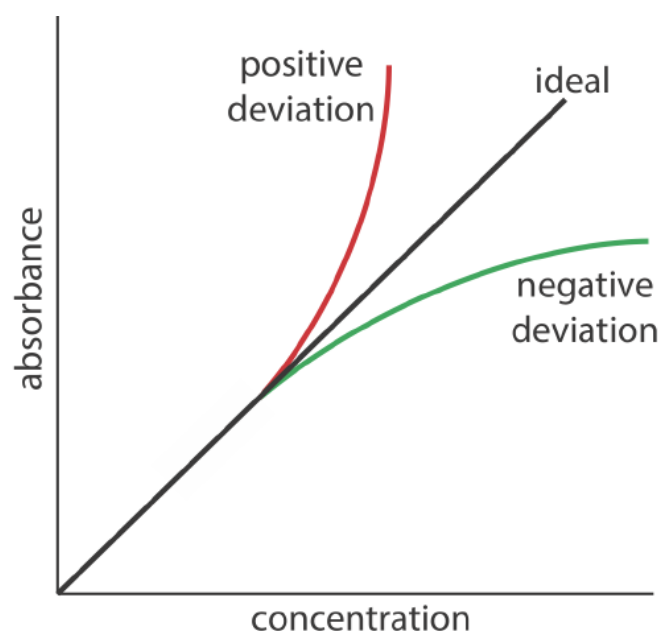
$$A = \epsilon c l$$

Assumptions of Beer–Lambert Law

- The system is homogeneous.
- Absorbing species do not interact with each other.
- The incident light is monochromatic and collimated.

Graphical Representation

- When absorbance (A) is plotted on the **y-axis** against concentration (c) on the **x-axis**, a **straight line passing through the origin** is obtained for an ideal system following Beer–Lambert law.
- The **slope of the line** is equal to ϵl .



Significance

- The combined **Beer-Lambert Law** is the foundation of **UV-visible spectrophotometry** and is used to:
 - Measure concentrations of analytes.

- Monitor reaction progress.
- Calculate rate/equilibrium constants.

Applications

1. Quantitative Analysis:

- Determine unknown concentrations by measuring absorbance.

2. Pharmaceutical Assays:

- Determine purity, content, and dosage of drugs.

3. Kinetics and Equilibrium:

- Monitor changes in absorbance over time or at equilibrium.

4. Environmental Monitoring:

- Detect pollutants in water or air samples.

5. Clinical Diagnostics:

- Measure levels of biomolecules in blood or urine.

Limitations

- Accurate only within a limited concentration range (typically 0.01–1.0 M).
- Sample must be homogeneous and clear (no turbidity or suspended particles).
- Requires monochromatic radiation.
- Path length and concentration must be uniform throughout the sample.

Deviations from Beer-Lambert Law

Situations where Beer-Lambert Law may not hold include:

1. Chemical Deviations:

- **Association/Dissociation:** At high concentrations, molecules may associate or dissociate, altering the absorbing species.
- **Reactions with Solvent:** Chemical interactions between solute and solvent can change the nature of the absorber.

2. Instrumental Deviations:

- **Stray Light:** Light outside the selected bandwidth can cause overestimated absorbance.
- **Polychromatic Radiation:** Non-monochromatic light may lead to errors, especially for samples with sharp absorption peaks.

3. Physical Deviations:

- **High Concentrations:** Changes in refractive index can affect absorption at very high solute concentrations.
- **Temperature/Solvent Effects:** Variations in solvent or temperature can alter peak shape and position.
- **Light Scattering:** Particles or droplets scatter light, distorting absorbance readings.

Application of Beer's Law to Single and Multiple Component Analysis

Single Component Analysis

- Direct application of Beer's law to determine concentration of one analyte.
- **Procedure:**
 1. Prepare standard solutions of known concentration.
 2. Measure absorbance.
 3. Plot a **calibration curve** (Absorbance vs. Concentration).
 4. Use the curve to find unknown concentration.

Multiple Component Analysis (Simultaneous Estimation)

- Used when two or more species absorb at overlapping wavelengths.
- Based on solving **simultaneous linear equations:**

$$A_1 = \epsilon_{1x}C_x + \epsilon_{1y}C_y$$

$$A_2 = \epsilon_{2x}C_x + \epsilon_{2y}C_y$$

- **Where:**
 - A_1, A_2 = absorbances at two selected wavelengths
 - C_x, C_y = concentrations of components x and y
 - ϵ = molar absorptivity values at respective wavelengths
 - Requires knowledge of **molar absorptivities** and **instrumental precision**.

Measurement of Equilibrium Constant and Rate Constant by Spectroscopy

Equilibrium Constant (K) by Spectroscopy

- Spectroscopy can be used to measure the position of equilibrium by analyzing the absorbance of species involved in a reversible chemical reaction.



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