

Spectrophotometry

Spectrophotometry – Quick Reference Notes

1. What Is Spectrophotometry?

- **Definition:** An analytical technique that measures how much a chemical substance absorbs light by quantifying the intensity of light before and after it passes through a sample.
- **Key Principle:** According to Beer-Lambert law, absorbance (A) is proportional to the concentration (c) of the absorbing species, the path length (l) of the cuvette, and the molar absorptivity (ϵ).

$$[A = \epsilon \cdot l \cdot c]$$

2. Core Components of a Spectrophotometer

Component	Function
Light Source	Provides continuous radiation (e.g., deuterium lamp for UV, tungsten-halogen for visible).
Monochromator / Filter	Selects a narrow wavelength band (λ) from the source.
Sample Holder (Cuvette)	Holds the solution; standard path length = 1 cm (square-wave quartz or glass).
Detector	Converts transmitted light into an electrical signal (photodiode, PMT, CCD).
Readout/Display	Shows absorbance (A) or transmittance ($T = I/I_0$). Often linked to a computer for data storage.

3. Fundamental Concepts

3.1. Absorbance vs. Transmittance

- **Transmittance (T)** = I / I_0 (ratio of transmitted to incident light intensity).
- **Absorbance (A)** = $-\log_{10}(T)$.

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- Relationship: $A = -\log_{10}(I/I_0)$.

3.2. Beer-Lambert Law

- Valid for dilute solutions (generally $<0.1\text{ M}$) and when the absorbing species does not interact with the solvent or other solutes.
- Linearity range:** Plot of A vs. c should be straight; deviations occur at high absorbance (>1.0) due to stray light or chemical effects.

3.3. Molar Absorptivity (ϵ)

- Units: $\text{L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$.
- Indicates how strongly a species absorbs at a given wavelength.
- Large ϵ = strong absorber = low concentration needed for measurable absorbance.

4. Typical Procedures

Step	Action	Tips
1. Calibration / Blank	Run a blank (solvent only) to set 0% absorbance.	Use the same cuvette type as for samples.
2. Wavelength Selection	Choose λ_{max} (maximum absorbance) from a spectrum scan.	Maximize sensitivity and minimize interference.
3. Sample Preparation	Dilute sample so that expected A is between 0.1–0.8.	Avoid bubbles; use matched cuvettes for reference and sample.
4. Measurement	Record absorbance at λ_{max} .	Perform duplicate/triplicate readings for precision.
5. Quantitation	Use calibration curve (A vs. known c) or directly apply Beer-Lambert law.	Verify linearity; include standards that bracket sample concentration.

5. Applications

Field	Example Uses
Biochemistry	Protein concentration (Bradford, BCA assays), nucleic acid quantification (260 nm).

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Field

Example Uses

Environmental
Chemistry

Determination of nitrate, phosphate, heavy?metal complexes.