

Principles of Spectrophotometry

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A concise, organized guide for quick reference and deeper study

1. What Is Spectrophotometry?

Aspect	Description
Definition	Quantitative measurement of the amount of light absorbed (or transmitted) by a sample as a function of wavelength.
Core Idea	Molecules absorb light at specific wavelengths; the intensity of absorption is proportional to the concentration of the absorbing species.
Typical Output	Absorbance (A) vs. wavelength spectra; single-wavelength absorbance values for quantitative analysis.

2. Fundamental Concepts

Concept	Equation / Principle	Key Points
Beer-Lambert Law	$A = \epsilon \cdot b \cdot c$	A = absorbance (unitless) ϵ = molar absorptivity ($L \cdot mol^{-1} \cdot cm^{-1}$) – intrinsic to the analyte at a given λ b = path length (cm) – usually the cuvette width c = concentration ($mol \cdot L^{-1}$)
Absorbance vs. Transmittance	$A = -\log_{10}(T)$, $T = \frac{I}{I_0}$	I_0 : incident light intensity; I : transmitted intensity.
Molar Absorptivity (ϵ)	Determined experimentally; high ϵ = strong absorber, low detection limits. Region where $A \propto c$ (typically $0.1 - 1.0 AU$).	
Linear Range	Outside this range, deviations occur due to stray light, detector saturation, or chemical effects.	

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Selectivity	Depends on wavelength choice; select a λ where the analyte has a strong absorbance and interferences have minimal absorbance.	

3. Instrument Components & Their Functions

Component	Function	Typical Variations
Light Source	Emits continuous or discrete radiation covering the UV–Vis region (λ 190–1100 nm).	Deuterium lamp (UV), Tungsten halogen lamp (visible–NIR), LEDs, lasers.
Monochromator / Filter	Isolates a narrow band of wavelengths ($\Delta\lambda$ 1–5 nm).	Diffraction grating, prism, interference filters.
Sample Holder (Cuvette)	Holds the liquid/solid sample; defines path length b .	Standard 1 cm quartz cuvettes (UV–Vis), glass (visible only), plastic (visible only).
Detector	Converts transmitted light into an electrical signal.	Photomultiplier tube (PMT), photodiode, charge-coupled device (CCD).
Readout / Data System	Calculates absorbance, displays spectra, stores data.	Analog meters, digital displays, PC-based software.
Reference Beam (optional)	Compensates for source intensity fluctuations.	Dual-beam spectrophotometers have sample and reference channels.

4. Measurement Procedure (Step-by-Step)

- Instrument Warm-up** – Allow the lamp to stabilize (usually 15–30 min).
- Zero/Blank Calibration** – Fill reference cuvette with solvent or blank matrix; set absorbance to zero at the chosen wavelength.
- Wavelength Selection** – Choose λ_{max} (peak absorbance) of