Spectrophotometer

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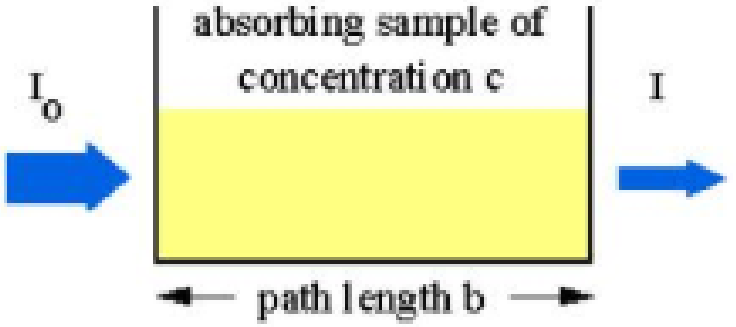
Introduction

- **Spectrophotometer:** is an instrument that measures the amount of light absorbed by a sample at selected wavelength.
- • A spectrophotometer measures light absorption at different wavelengths.
- • **Purpose:** measure the **concentration** of solutes in solution by measuring the amount of the light that is absorbed by the solution (sample), study reactions, compare optical properties.

Principle of Operation

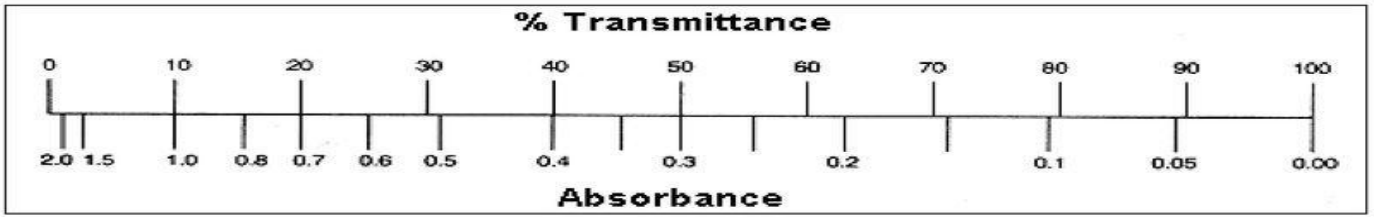
- Based on Beer–Lambert Law:
- $A = \varepsilon \times c \times b$

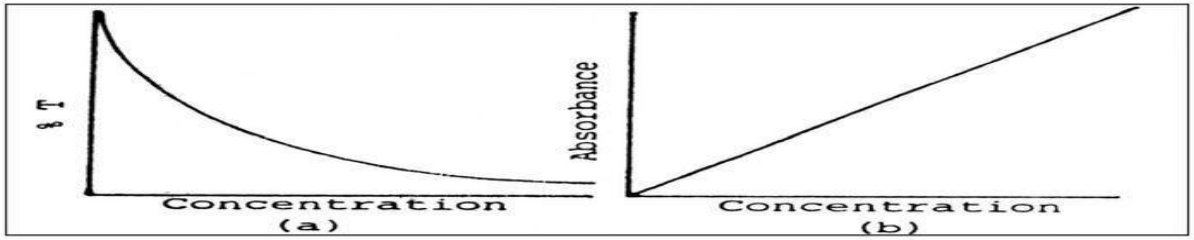


- Where:
- - A: Absorbance
- - ε: Molar absorptivity
- - c: Concentration
- - b: Path length

- By using an equation (Beer-Lambert law), it converts the transmittance or absorbance data to concentration. when transmittance 100% mean the absorbance is zero

Transmittance, absorbance, and concentration





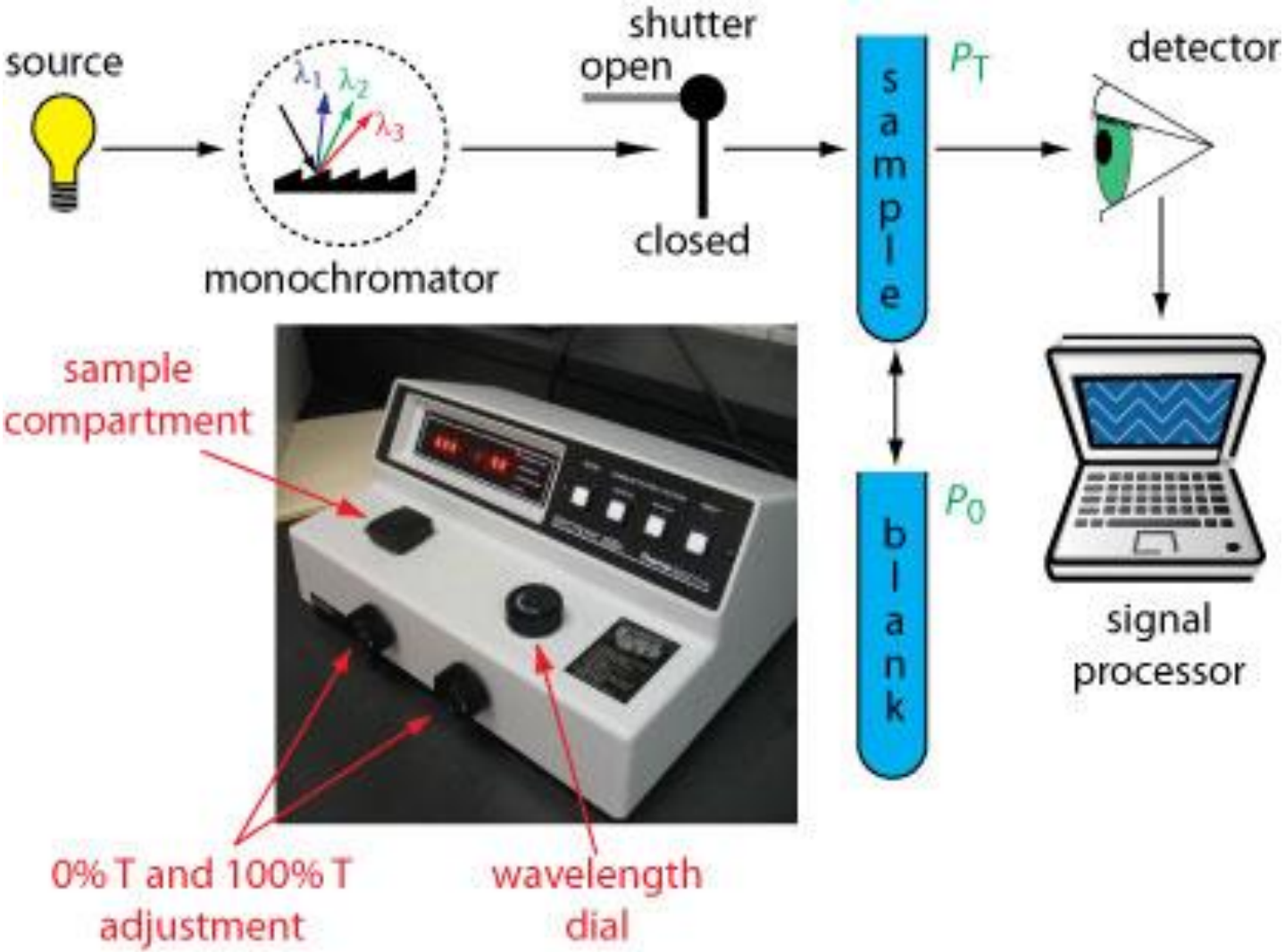
- **Why Spectrophotometers report absorbance, not transmittance?**

➢ Absorbance is linearly proportional to concentration, simplifying calculations.

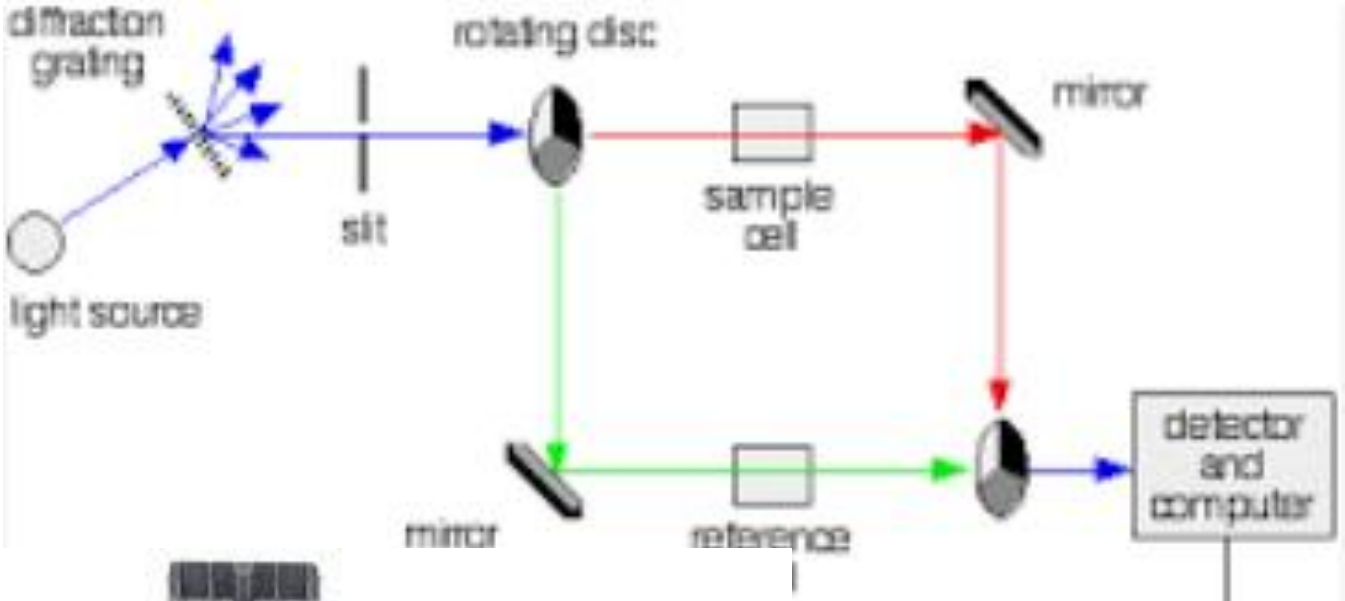
➢ Transmittance varies non-linearly, which makes it harder to directly relate to concentration.

Types of Spectrophotometers

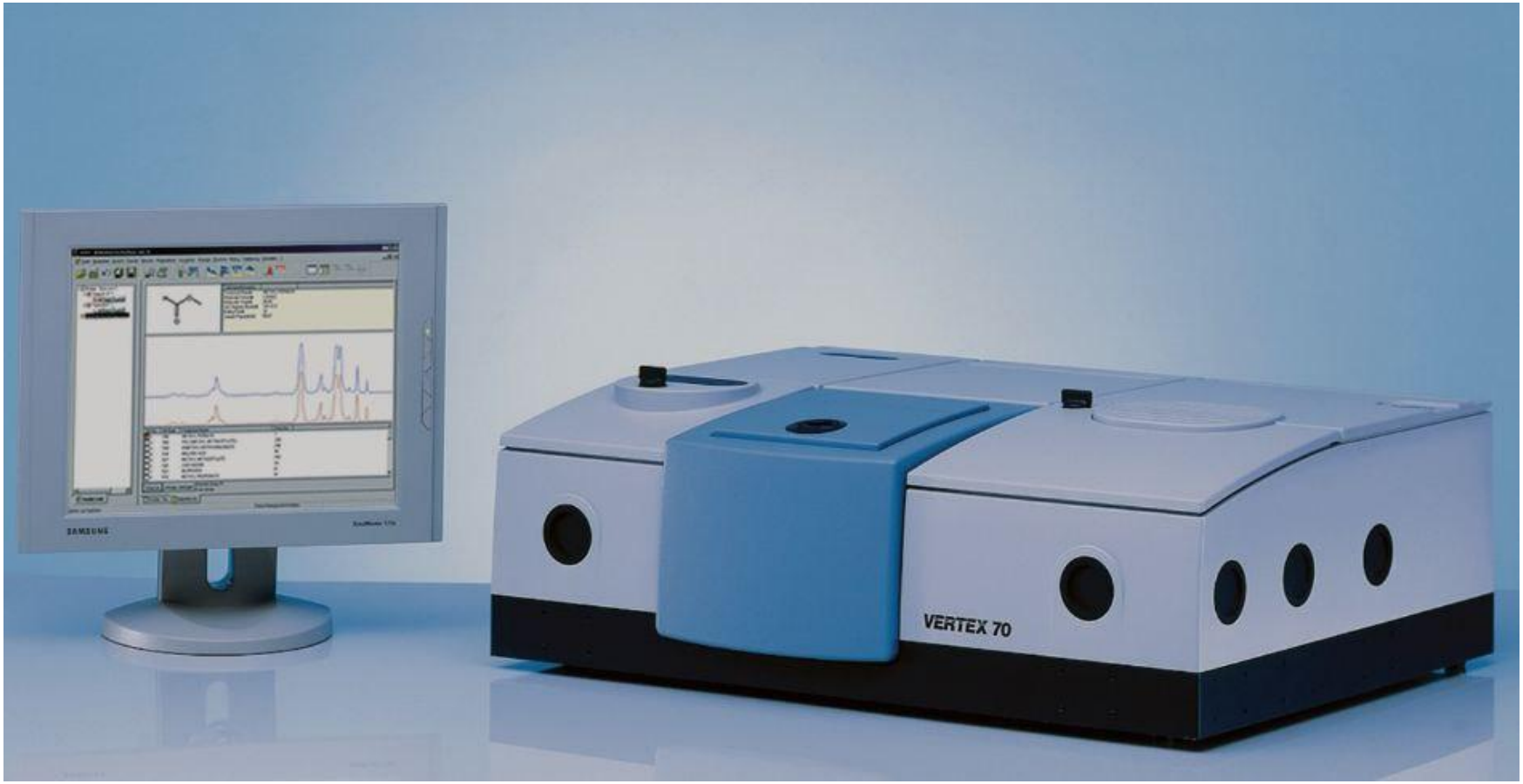
- **Single Beam Spectrophotometer:** Measures the intensity of light passing through a sample.
- **Double Beam Spectrophotometer:** Splits the light into two beams, one passing through the sample and the other through a reference.
- **UV-Vis Spectrophotometer:** Measures light absorption in the ultraviolet (200-400nm) and visible ranges (400-800 nm).
- **Infrared Spectrophotometer:** Measures infrared light absorption (wavenumbers: 400 cm −1 - 4000cm −1, wavelength=2,500 – 25,000 nm) , useful for identifying chemical bonds and functional groups .
- **Atomic Absorption Spectrophotometer (AAS):** Determines concentrations of metals by measuring light absorption (190nm – 900nm) by free atoms in the gaseous state.



Single Beam Spectrophotometer



Double Beam Spectrophotometer

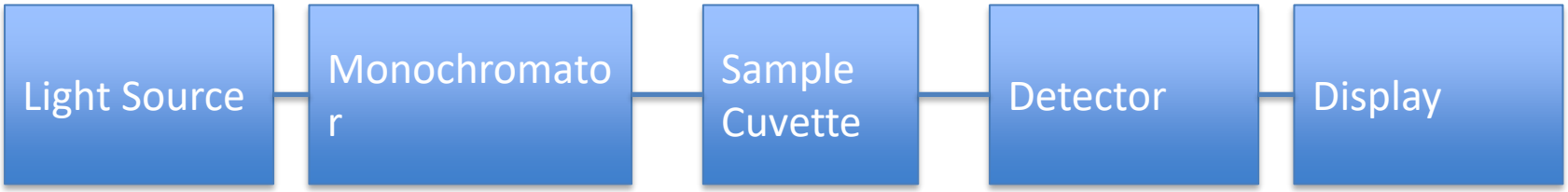


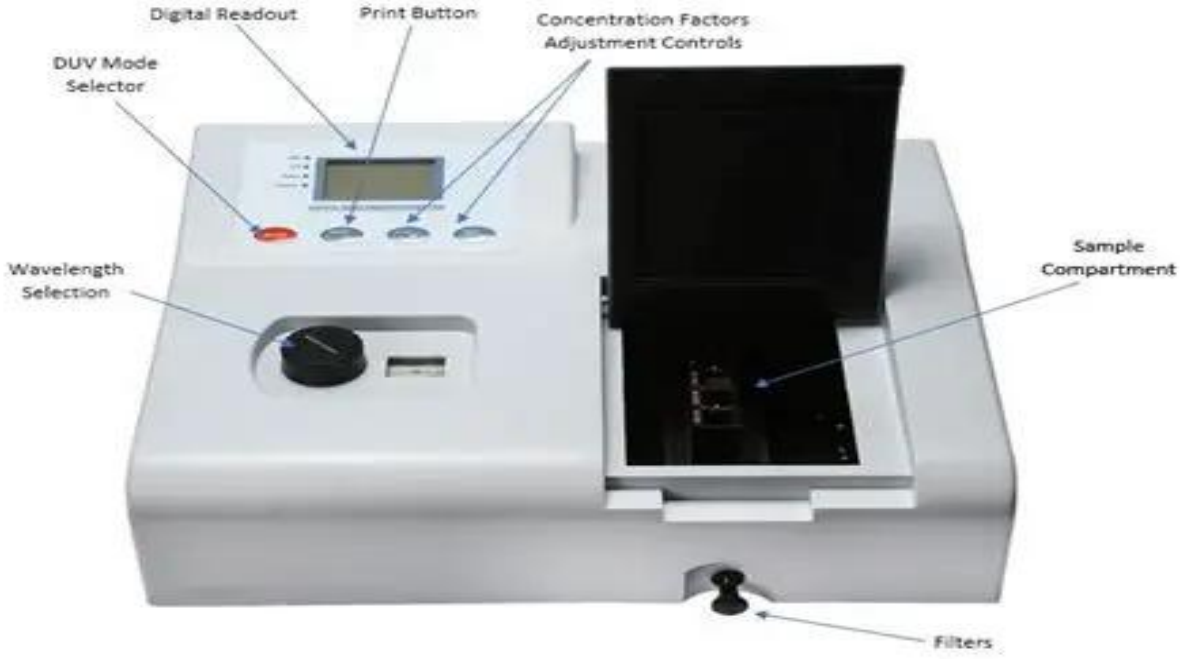
Infrared Spectrophotometer

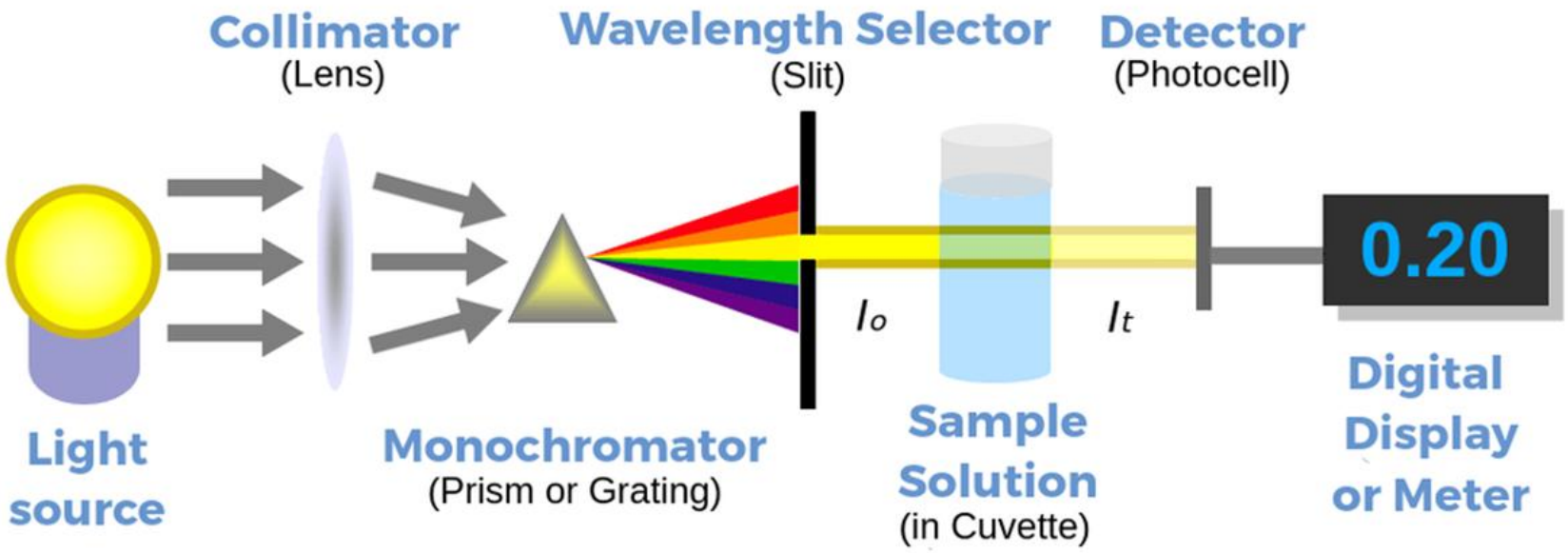
Atomic Absorption Spectrophotometer (AAS):

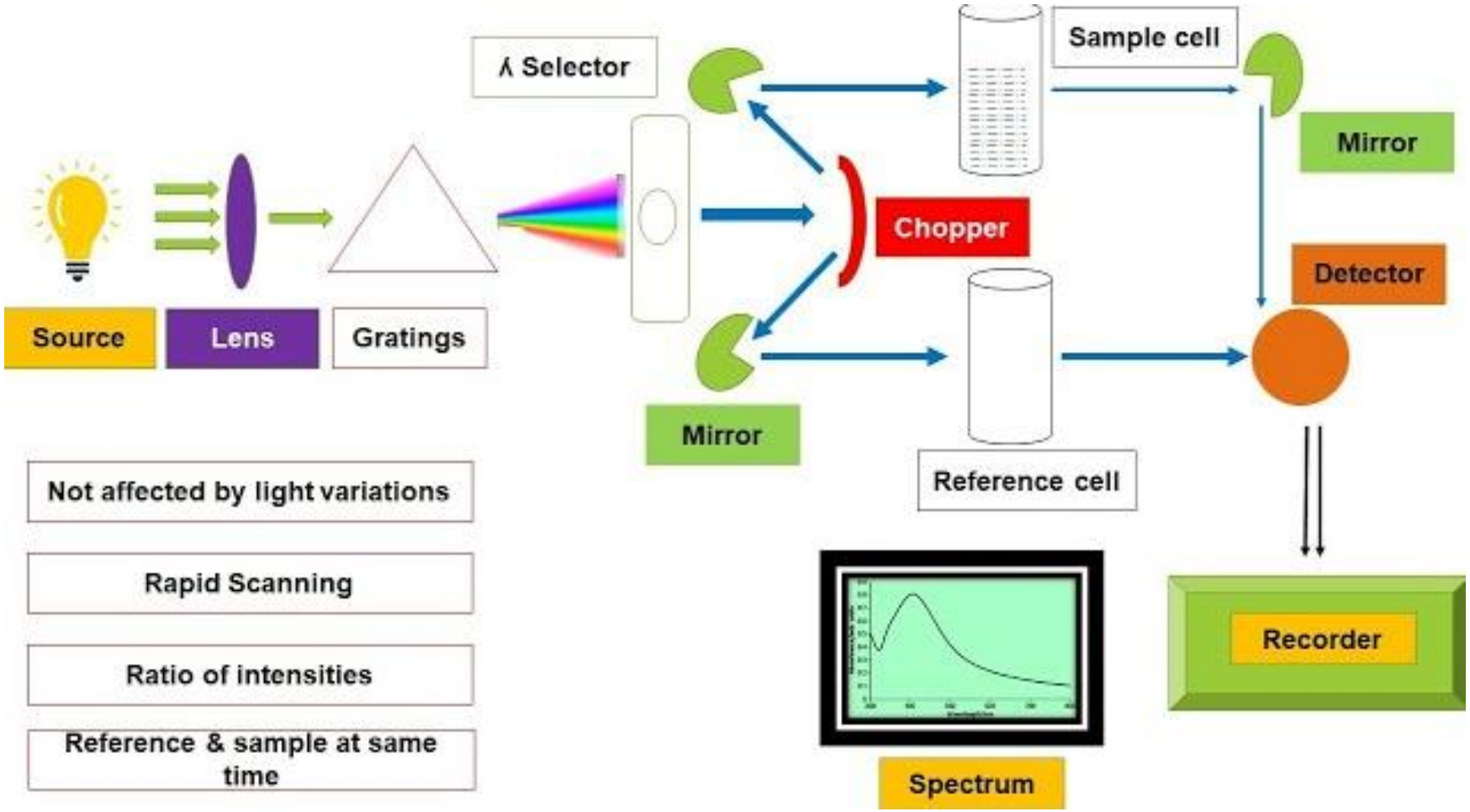
Main Components

- 1- Light source (Tungsten/Deuterium lamp).
- 2- Monochromator (Prism/Grating).
- 3- Sample holder (Cuvette).
- 4- Detector.
- 5- Display/Readout.




Digital Readout
Print Button
Concentration Factors
Adjustment Controls
DUV Mode
Selector
Wavelength
Selection
Sample
Compartment
Filters

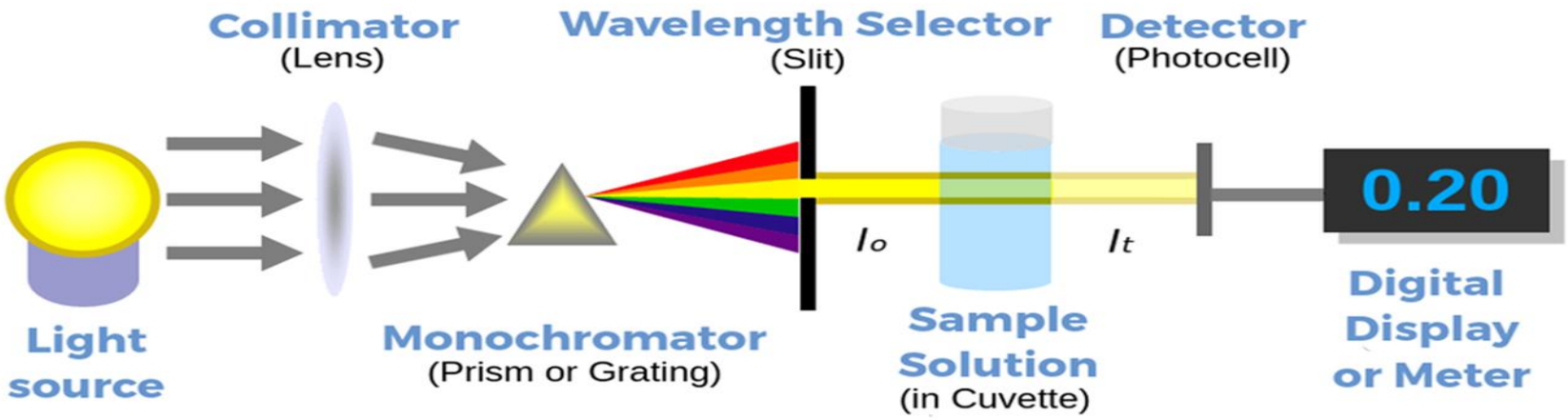

Collimator
(Lens)
Wavelength Selector
(Slit)
Detector
(Photocell)
0.20
I_o
I_t
Light
source
Monochromator
(Prism or Grating)
Sample
Solution
(in Cuvette)
Digital
Display
or Meter



- Not affected by light variations
- Rapid Scanning
- Ratio of intensities
- Reference & sample at same time

Steps of Measurement

- 1- Turn on and warm up the device.
- 2- Select appropriate wavelength.
- 3- Calibrate using Blank.
- 4- measure the absorbance of the standard
- 5- Insert sample.
- 6- Read absorbance and calculate concentration.



Types of Solutions used in Spectrophotometer measurements

- **1. Blank:** A blank contains all the test reagents but no analyte (no substance being measured).
- used to “zero” the spectrophotometer To remove background absorbance from reagents, cuvette, or solvent
- **2. Standard (Calibrator):**is a solution that contains a known concentration of the substance being measured.
- Used to calibrate the instrument, to compare with the patient sample and to calculate the concentration in unknown samples
- **3. Measured (Test) Sample**: This is the patient’s actual specimen (serum, plasma, urine, etc.) that contains an unknown concentration of the analyte.
- Used to determine the patient’s result by comparing it to the standard.

Applications

- • Medical analysis (Glucose, Urea, Creatinine).
- • Pharmaceutical industry.
- • Study of chemical reactions.
- • Monitoring bacterial growth.
- • Environmental analysis.

Advantages and Limitations

- Advantages:
- ✓ High accuracy
- ✓ Fast measurement
- ✓ Small sample volume

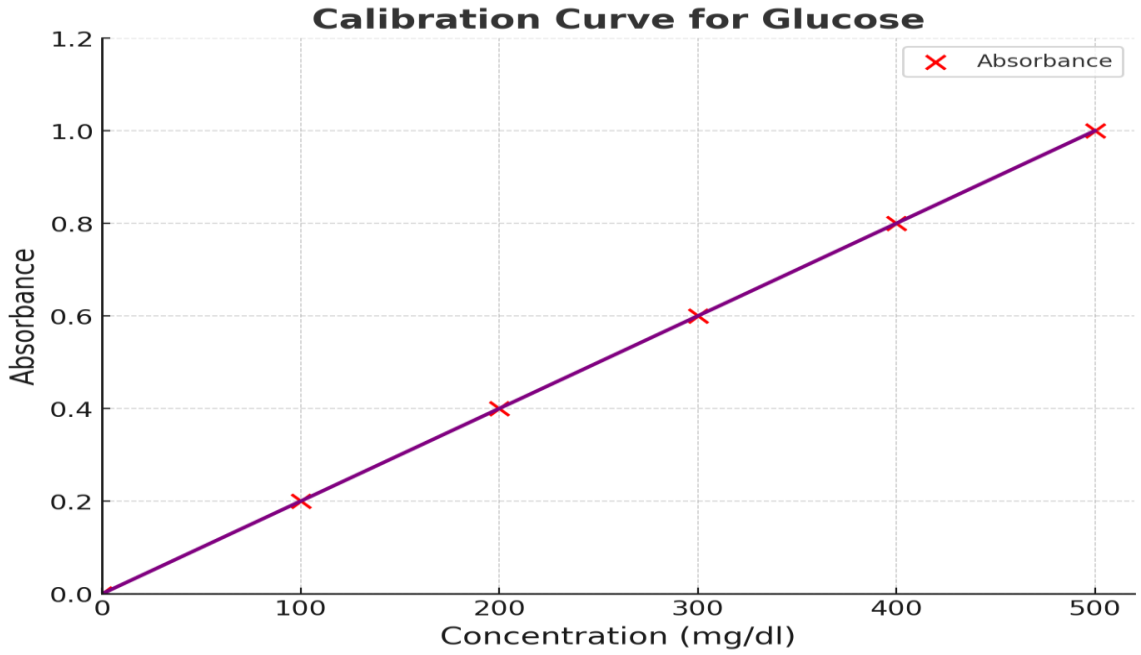
- Limitations:
- ✗ Requires calibration
- ✗ Sensitive to light
- ✗ Expensive

Practical Precautions

- Clean cuvettes carefully.
- Avoid touching cuvette walls.
- Ensure correct liquid level.
- Always use Blank before sample.
- Always check for **hemolysis, lipemia, or icterus** before interpreting spectrophotometric results. Some labs may **flag these samples as rejected samples** and suggest careful sample pretreatment (centrifugation, dilution, or blank correction) to minimize interference.

Example: Glucose Measurement

- • Glucose reacts with Glucose Oxidase enzyme.
- • Product absorbs at 505 nm.
- • Concentration calculated using calibration curve.



Good luck