

UCB008 - APPLIED CHEMISTRY



Molecular Spectroscopy Series Lecture - VIII

UV-Visible Spectroscopy – Instrumentation and Analysis

by

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Learning Outcomes

At the end of this session participants should be able to:

- distinguish various functional components of a UV-visible spectrophotometer
- differentiate between single-beam and double-beam UV-visible spectrophotometer

UV-Vis Spectrophotometer

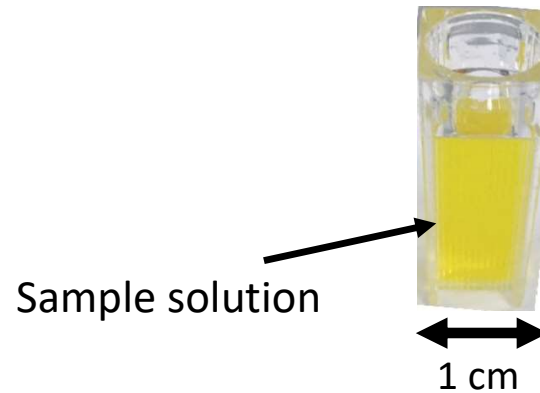
- Light source
 - Deuterium lamp – UV radiation
 - Tungsten filament lamp – Visible radiation
- Sample containers
 - Cuvettes
 - Plastic
 - Glass
 - Quartz



Sample Handling

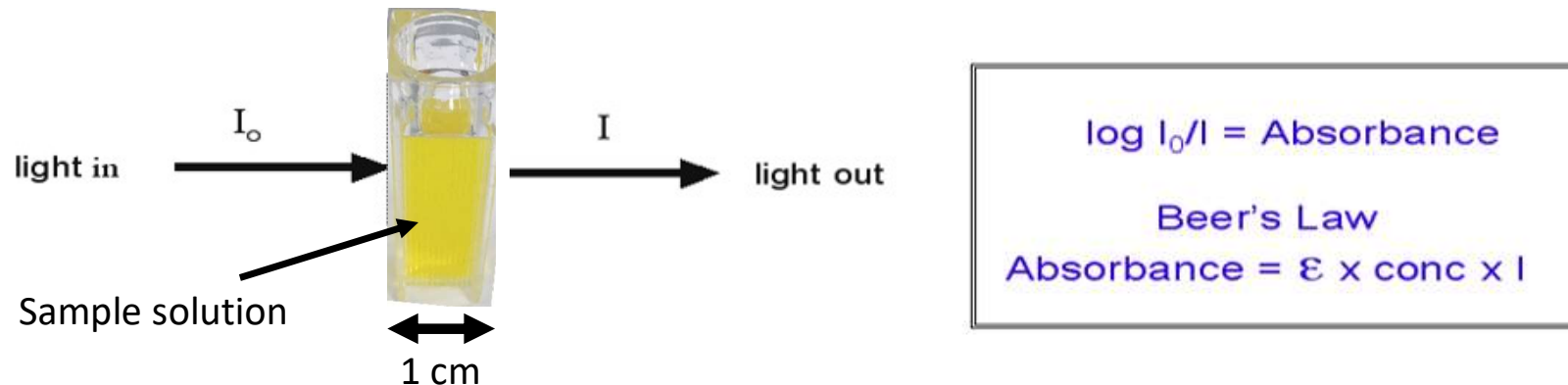
Solvent - Should not absorb in the region under investigation

Solvent
1 mg sample \longrightarrow 100ml



Solvents – water, ethanol, n-hexane, cyclo-hexane, benzene, methyl alcohol, diethyl ether etc.

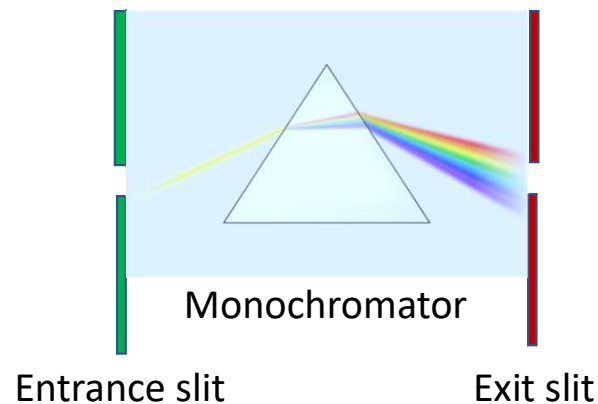
Sample Handling



- Cuvettes are used for keeping the sample solution in the instrument.
- Thickness of sample solution in cuvette – 1cm
- **Cuvette material should not absorb in the region under investigation.**
 - Glass/plastic – used only for visible region as they absorb UV radiation
 - Quartz – used for UV-visible region

Monochromator

- Monochromator is used to isolate the required wavelength from polychromatic radiations
- **Entrance slit:** Sends a uni-directional beam to prism
- **Prism:** Disperses the radiations into constituent wavelengths
- **Exit slit:** Sends monochromatic beam to sample solution



Detector

- Detector detects the radiation received and converts them into electrical signal.

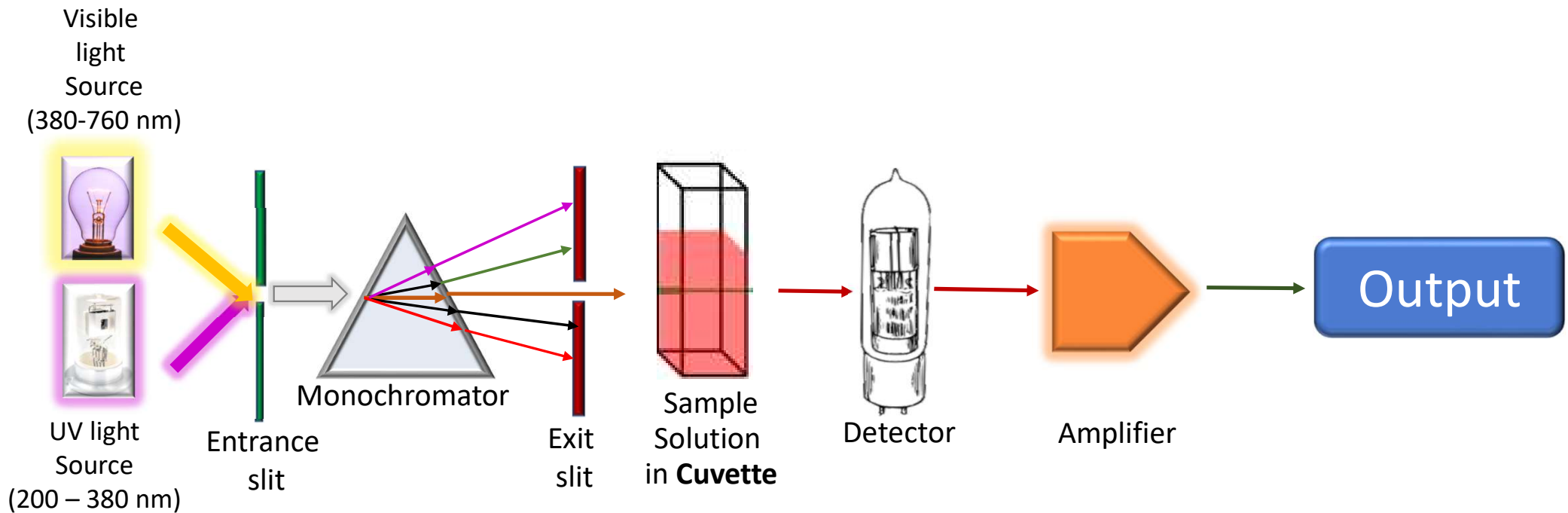
Amplifier

- The signal received from the detector is amplified by the amplifier and sent to read-out device.

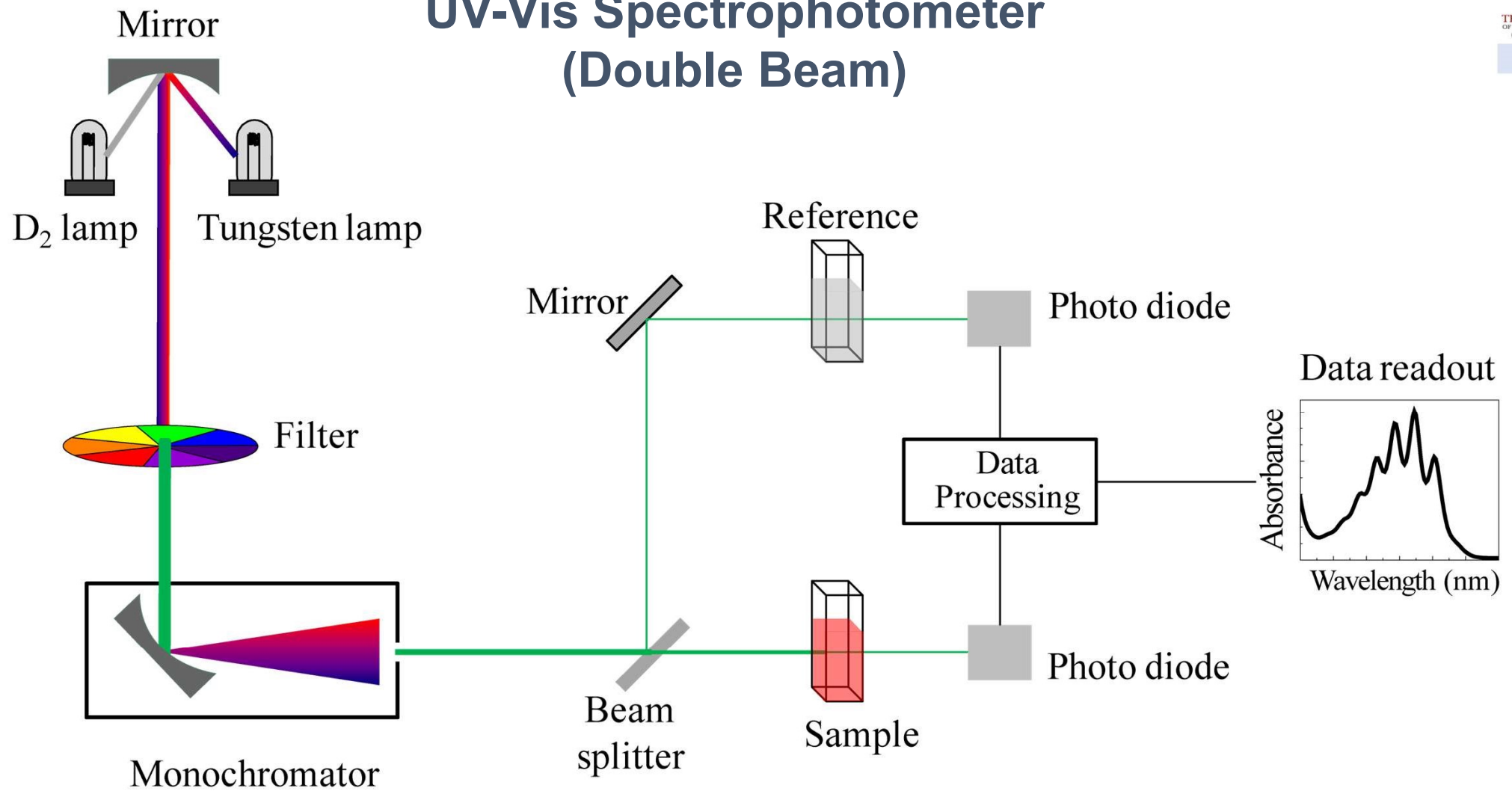
Output

- The read-out device gives the absorbance value at different wavelengths which can be plotted as an absorbance vs wavelength graph.

UV-Vis Spectrophotometer (Single Beam)

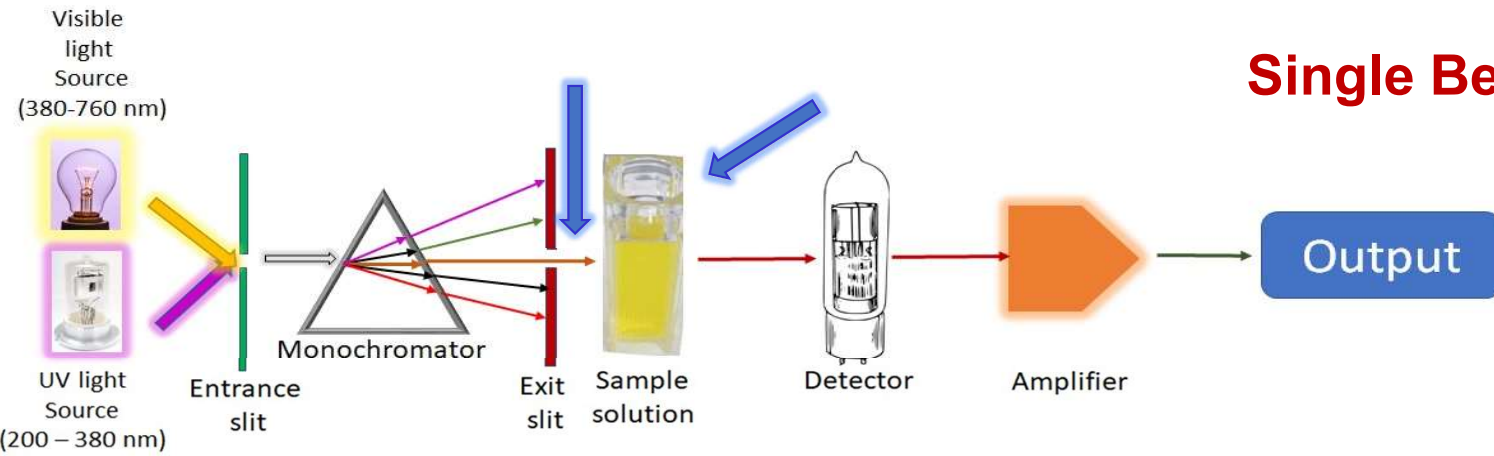


UV-Vis Spectrophotometer (Double Beam)

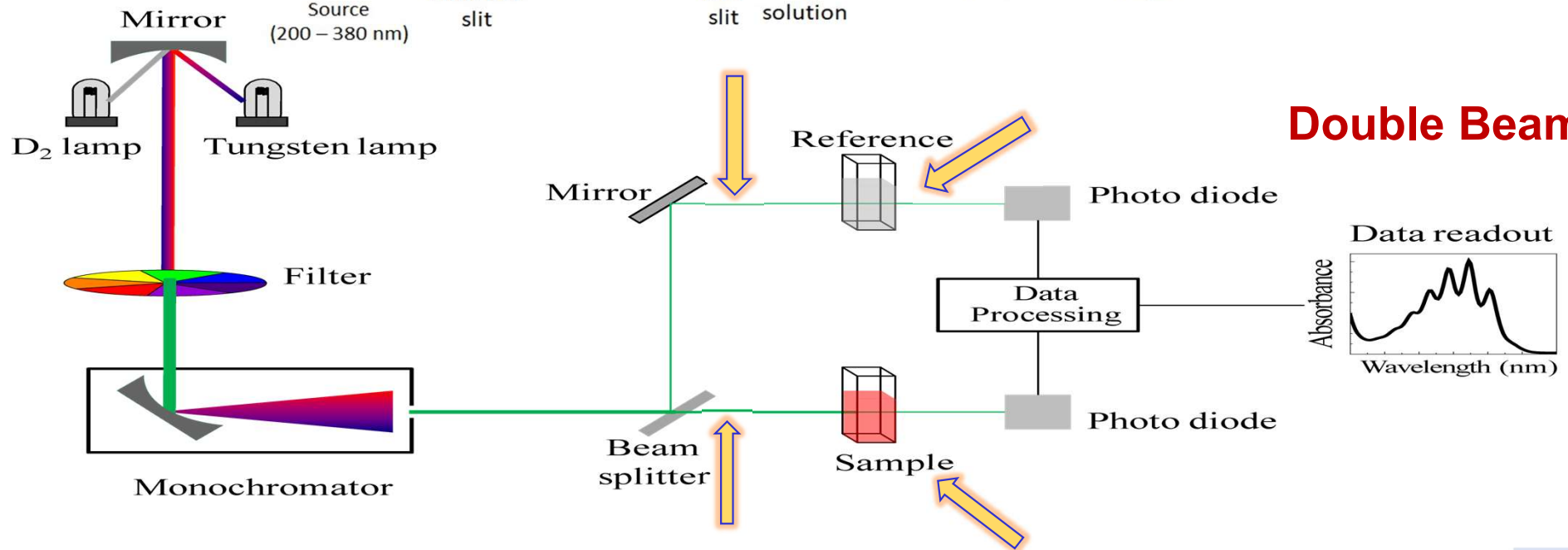


UV-Vis Spectrophotometer

Single Beam

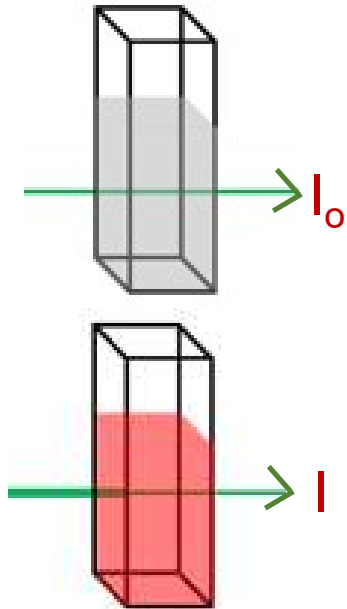


Double Beam

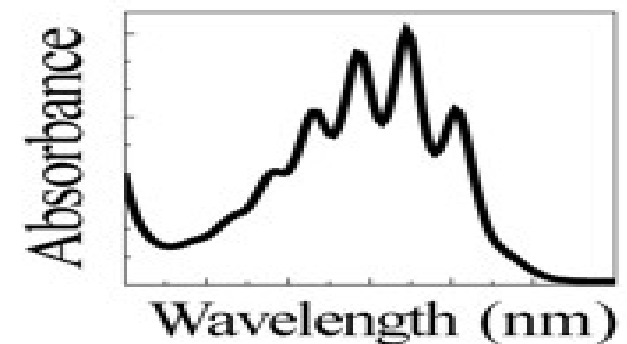


Single Beam

- Solvent is taken as **blank solution** for which absorbance is measured and absorbance value is set to **Zero** and transmitted intensity is considered as I_o .
- Absorbance of sample solution is measured, and if sample absorbs radiations, then transmitted intensity I is less than I_o .
- Instrument gives output graph which is plot of wavelength of entire region vs absorbance of radiation at each wavelength of the region which is termed as **Spectrum**.

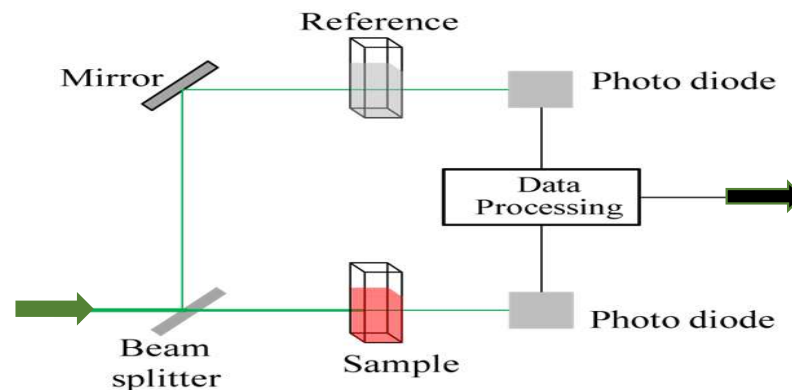


$$\text{Absorbance} = \log I_o / I$$

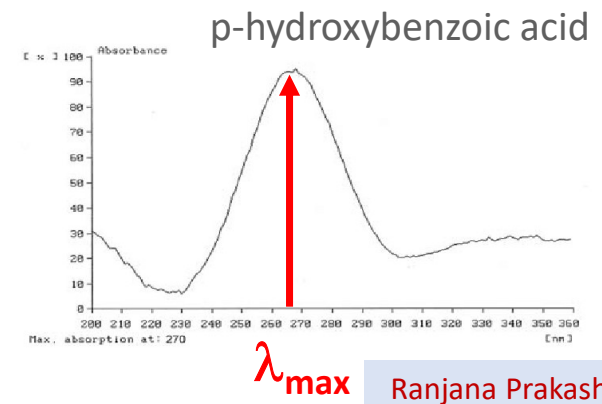


• Double Beam

- In Double beam spectrophotometer, each absorbance measurement of solution of sample is accompanied by simultaneous measurement of the pure solvent
- Instrument is capable in comparing the intensities of the two beams at each wavelength of the region



- In UV-visible spectrophotometers, The ratio between reference (blank) beam and sample beam intensities (I_0 / I), i.e., *ratio recording* is carried out.
- Absorbance = $\log I_0 / I$
- $A = \epsilon cx$ – Beer's Law, where concentration (c) and thickness of sample solution (x) are constant for a given sample



UV-visible spectrophotometers in our laboratories..



...at School of Chemistry and Biochemistry



...at School of Energy and Environment

In the next session.....

- Qualitative and quantitative analysis
- Applications of UV-visible spectroscopy