Spectrophotometer
Lecture 3

Spectrophotometer

- It is an instrument that is of great significance for measurement of absorbance experimentally.
- It has a light source that produces light of a preselected wavelength, directed through the solution and amount of light that is absorbed is measured through detectors and read on the recorder.
- It works on the ***Beer-Lambert Law***

Components of Spectrophotometer

The spectrophotometer consists of following components.

- Light source
- Collimator (Lens)
- Monochromator (filters, slits, and mirrors)
- Wavelength selector (slit)
- Sample chamber (Cuvette)
- Detector
- Meter or recorder

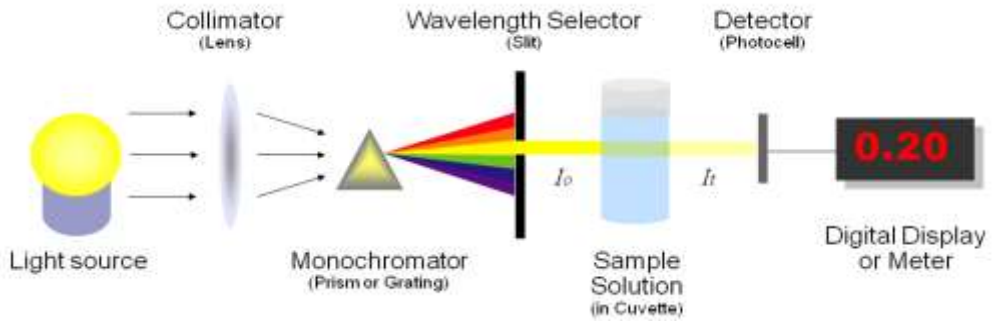

Fig. Instrumentation of spectrophotometer

Light Source

- The light sources used in the spectrophotometer produce light in the UV and visible region.
- A high-pressure ***hydrogen or deuterium lamp*** is used for production of ***UV light*** in the 200 to 340 nm range.
- However, ***tungsten-halogen lamp*** is used for production of ***visible light*** in the range of 340 to 800 nm.

Monochromator

- The lamps produce light of various wavelengths called polychromatic light, however, for accurate estimations monochromatic light (single wavelength) is needed.
- Therefore, the spectrophotometer possesses an optical system in the form of prism or diffraction grating that reduces polychromatic light to monochromatic light.
- The presence of accessary elements like series of slits, lenses, filters, and mirrors concentrates the light, increases the spectral purity, and focuses it toward the sample.

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Sample Chamber

- The monochromatic light is incident on the sample which is filled in a cuvette made of glass, or quartz.
- ***Glass cuvettes are used in the visible region***
- ***Quartz cuvettes are used in the UV-range.***

Detector

- When light is incident on the sample, some of the fraction is absorbed and the remaining is transmitted depending on the nature of the compounds under study.
- The intensity of the light that passes through the sample is measured by a light-sensitive detector, usually a photomultiplier tube (PMT).
- This detector first detects the light and then amplifies it through a cascade of electrons accelerated by dynodes.
- This is followed by conversion of this light into electrical signal.
- The electrical signal is passed to the recorder or meter.

Printers and Recorders

- There are some less expensive instruments that yield a direct readout of absorbance and/or transmittance for monochromatic light and the signal is manifested in an analog or digital form.

- Nevertheless, for the measurements that depict a scan between absorbance and wavelength, more sophisticated system is needed for displaying the spectrum.

- Moreover, modern instruments are fitted with computers that are programmed to enable various functions, such as:
  - subtraction of solvent spectrum
  - spectral overlay
  - storage
  - difference spectra
  - derivative spectra
  - calculation of concentrations and rate constants.