Name of the experiment: Principle and instrumentation of UV-visible spectrophotometer.

Principle:

Spectrophotometry is a technique that uses the absorbance of light by an analyte (the substance to be analyzed) at a certain wavelength to determine the analyte concentration. UV/VIS (ultra violet/visible) spectrophotometry uses light in UV and visible part of the electromagnetic spectrum. Light of this wavelength is able to effect the excitation of electrons in the atomic or molecular ground state to higher energy levels, giving rise to an absorbance at wavelengths specific to each molecule.

When a beam of radiation (light) passes through a substance or a solution, some of the light may be absorbed and the remainder transmitted through the sample. The ratio of the intensity of the light entering the sample (I_o) to that exiting the sample (I) at a particular wavelength is defined as the transmittance (T). The absorbance (A) of a sample is the negative logarithm of the transmittance.

$$A = -\log(T)$$

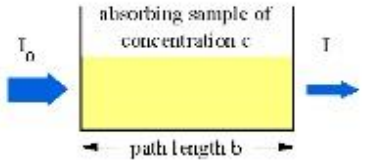


Figure: Absorption of light by a sample

Beer-Lambert Law

The absorbance of a sample at a given wavelength is proportional to the absorptivity of the substance (a constant at each wavelength), the path length (the distance the light travels through the sample) and the concentration of the absorbing substance. In these cases the Beer-Lambert Law holds:

$A = a * b * c$ where a = the absorptivity of the substance
b = path length
c = concentration of the substance

When working in concentration units of molarity, the Beer-Lambert law is written as:

$$A = \varepsilon * b * c$$

Where ε is the wavelength-dependent *molar absorptivity coefficient* with units of $M^{-1}\ cm^{-1}$.

As, Ɛ and b, both are constant, then it can be written that, $A \propto c$

Prerequisite for Beer Lambert law

- The absorbing medium must be homogeneous in the interaction volume
- The absorbing medium must not scatter the radiation – no turbidity;
- The incident radiation must consist of parallel rays, each traversing the same length in the absorbing medium;
- The incident radiation should preferably be monochromatic

Instrumentation:

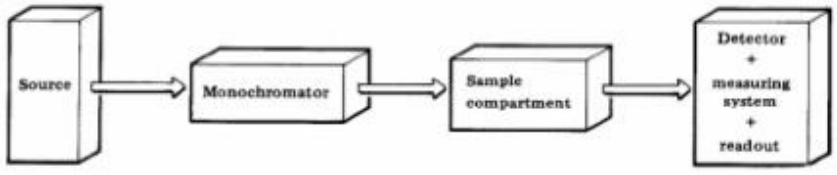
The minimum requirements of an instrument to study absorption spectra (a spectrophotometer) are shown below

1. A source of radiation of appropriate wavelengths.

2. A means of isolating light of a single wavelength and getting it to the sample compartment - monochromator and optical geometry.

3. A means of introducing the test sample into the light beam - sample handling.

4. A means of detecting and measuring the light intensity.



Light source:

The light source is usually a tungsten lamp for the visible region of the spectrum, and either a hydrogen or deuterium lamp for ultraviolet wavelengths.

Monochromator

The function of a monochromator is to produce a beam of monochromatic (single wavelength) radiation that can be selected from a wide range of wavelengths. Two basic methods of wavelength selection may be noted, filters and a dispersing system (e.g. a prism or diffraction grating).

Cuvettes

Cuvettes are optically transparent cells that hold the material(s) under study and are used to introduce samples into the light path. Glass and plastic absorb strongly below 310 nm and are not useful for measuring absorbance below that wavelength. Quartz or silica cells are used when measuring absorption of ultraviolet wavelengths by a solution since they are transparent to wavelengths greater than 180 nm.

Detectors

The most commonly encountered detectors are the photomultiplier, the silicon diode and the diode array.