Program Name : **B. Pharm**

Course Name : **Instrumental Methods of Analysis**

Course Code : **BP 701 T**

Semester : **VIIth**

Session : **2020-2021**

Unit : **1 (Part-2)**

Topic : **UV- Visible Spectroscopy**

Sub-topic :

- Derivation
- Deviations
- Instrumentation

Derivation

Mathematically law can be describe as

$$\frac{dI}{dx} \propto I \ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where

dI is little bet decrease is intensity of light when passing through the small distance dx and I is the intensity of monochromatic light just before entering in the absorbent medium.

Eq. (1) may be written as –

$$\frac{dI}{dx} = \propto aI \ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where

$\frac{dI}{dx}$ is the rate of decrease of intensity with thickness of cell dx, a is absorption coefficient

Integration of equation (2) after rearrangement gives

$$-ln\, I = ax + C \quad \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

Where

C is constant of integration. At $x = 0$, $I = I_0$

So C = $-ln\, I_0$. Introduction of this in eq. (3) results-

$$ln\frac{I}{I_0} = -ax \ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

Eq. (4) can be written as-

$$I = I_0 e^{-ax} \ldots\ldots\ldots\ldots\ldots (5)$$

Eq. (5) can be written as-

$$\log\frac{I}{I_0} = \frac{-a}{2.303}x \ \ldots\ldots\ldots\ldots\ldots\ldots\ldots (6)$$

or

$$\log\frac{I}{I_0} = -\mathrm{a}'x \ \ldots\ldots\ldots\ldots\ldots\ldots\ldots (7)$$

Where

a' $(=\frac{a}{2.303})$ is extension coefficient and $ln\frac{I}{I_0}$ absorbance of medium, absorbance is represented by A

Lambert's law is extended by Beer who showed that absorbance is not only depends upon intensity of light but also on the concentration of the solution.

$$-\frac{\mathrm{dI}}{\mathrm{d}x} \propto \mathrm{C} \ \ldots\ldots\ldots\ldots\ldots\ldots\ldots . (8)$$

Combination of both laws resulting

$$-\frac{\mathrm{dI}}{\mathrm{d}x} \propto \mathrm{I} \times \mathrm{C} \ \ldots\ldots\ldots\ldots\ldots\ldots\ldots . (8)$$

or

$$-\frac{\mathrm{dI}}{\mathrm{d}x} = \mathrm{bI} \times \mathrm{C} \ \ldots\ldots\ldots\ldots\ldots\ldots\ldots . (9)$$

When the concentration "C" is expressed in *mol*/L, b is molar absorption coefficient

In the case of Lambert's law eq. (9) may be transformed into-

$$\log\frac{I}{I_0} = \frac{-b}{2.303} \times \mathrm{c} \times x \ \ldots\ldots\ldots\ldots\ldots\ldots\ldots (10)$$

or

$$\log\frac{I}{I_0} = \varepsilon \times \mathrm{c} \times x \ \ldots\ldots\ldots\ldots\ldots\ldots\ldots (11)$$

or

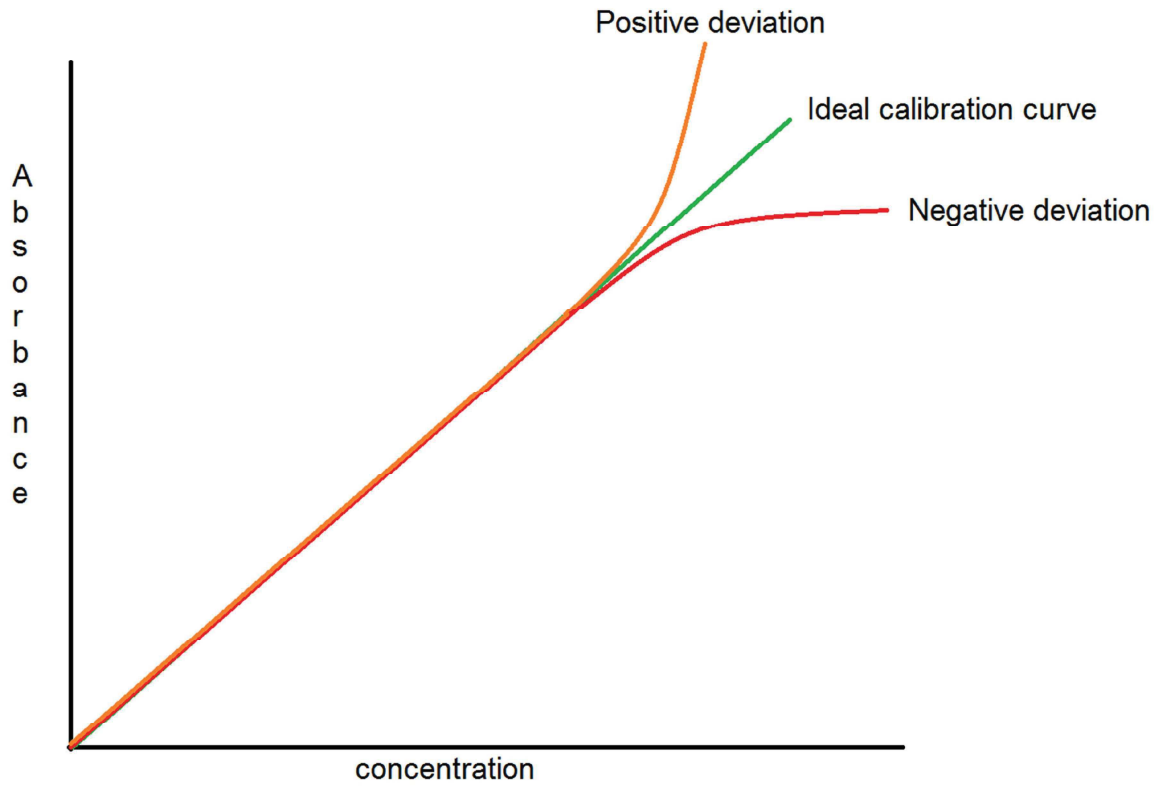
$$\log\frac{I}{I_0} = \varepsilon \times c \times l \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (12)$$

Where

$\varepsilon \left(= \frac{-b}{2.303}\right)$ is molar extinction coefficient which is expressed in *mol/L/cm* and it is depends upon the nature of absorbing analyte and wavelength of incident light

Equation 11or 12 is generally known as Beer-Lambert's law

Deviations



There are two types of deviation- (1) Positive Deviation- when a little bet change in analyte concentration results in a greater change in absorbance. (2) Negative deviation- when a grater change in analyte concentration results in small change in absorbance.

Deviation from Beer Law

A non-linear curve results deviation from beer law. A system is said to obey Beer's law when a platted graph gives a straight line (concentration vs absorbance).

The Beer's law deviations can be classified into three categories

Real Deviations- These are fundamental deviations due to the limitations of the law itself.

a) Beer's law requires dilute solutions only so it is a limiting law.
b) At high concentration (exceeding 0.01M) solute molecules can cause different charge distribution on their adjacent species in the solution.
c) High concentration experienced shift in spectra.
d) In case of large ions or molecules experience deviations even at very low concentrations

Chemical Deviations–

- Deviations occur when a specific chemical species of the sample is being analyzed.
- Solvent undergoes association, dissociation and interaction results different product. For example, phenol red experiences a resonance transformation when change in pH occurs, the acidic form (yellow) to the basic form (red). The molecule bonds retransformed as the electron distribution occur due to the pH of the solvent in which it is dissolved. So Acid and Base forms of phenol red give chemical deviations of Beer law in UV- Vis. Spectroscopy

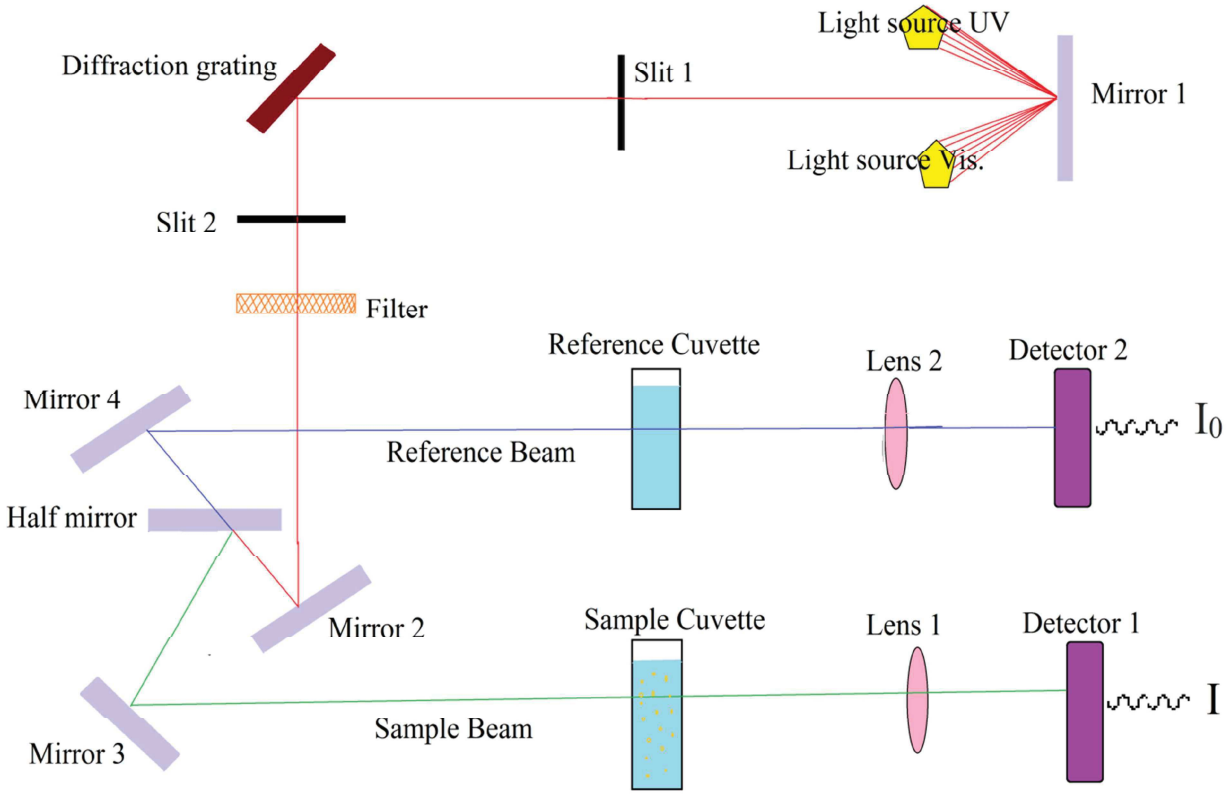
Instrument Deviations

- Deviation occur due to improper handling of instrument
- Polychromatic radiation- Beer law is strictly followed a monochromatic source of radiation.
- Stray radiation- Scattered radiation is the radiation from the instrument that is outside the nominal wavelength band selected due to reflection and scattering by the surfaces of lenses, mirrors, gratings, filters and windows.
- The wavelength of the stray radiation is different from the wavelength band selected.
- The radiation exiting from a monochromator is often affected with minute quantities of scattered or stray radiation.
- When the analyte absorb the stray radiation (wavelength), Beer's law deviation formed.
- Mismatched cell- when the reference and standard cells are having different path-lengths, Beer's law deviation formed.
- Improper slit- when the width of the slit is not proper, undesirable radiation fall on the detector cause deviations.

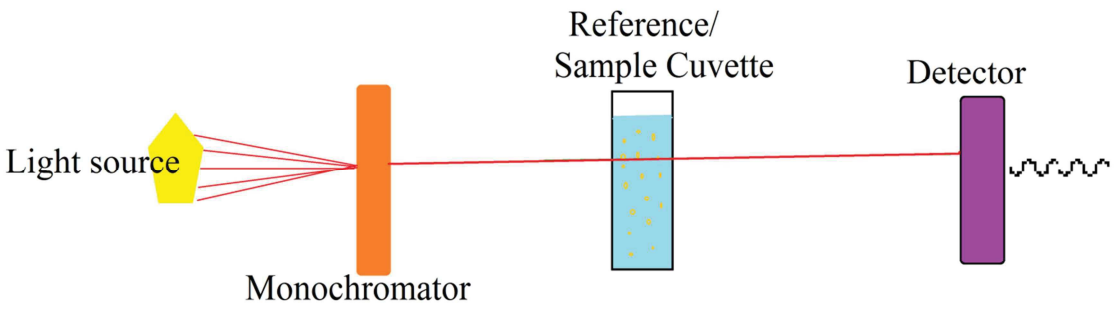
Instrumentation

Ultra Violet or Visible Spectroscopy

➢ Double Beam UV Spectrophotometer(Schematic Diagram)



➢ Single Beam UV Spectrophotometer (Schematic Diagram)



Light Source

UV or visible radiation source

Deuterium and Hydrogen lamps

A pair of electrodes is enclosed in a glass tube containing hydrogen or deuterium gas. When current is passed in electrodes electron discharge is occurring which exited the gas molecule which results in the emission of radiation (UV & Visible).

Wavelength: 160-800 nm

Quartz window must be employed

D2 lamp

Hydrogen lamp

Xenon arc Lamp

It consists of two tungsten electrodes form an arc at a specific distance and xenon gas is stored (under pressure) in quartz or fused silica tube. It emits radiation with a higher intensity (500 nm) than a hydrogen discharge lamp.

Wavelength: 750-1000 nm.

Xenon arc Lamp

Tungsten Halogen Lamp

It is also known as a halogen lamp. It is an incandescent light source. It is consists of a filament made up of tungsten enclosed in a quartz vessel containing an inert gas and a small quantity of Iodine or bromine (Halogen).

Its 85% emitted light lies in IR and near IR region, 15 % in the visible region, and less than 1% in the UV region.

Tungsten Halogen Lamps

Mercury Vapor Lamp

These lamps are ideal light sources that provide high-intensity light in the deep UV to visible regions.

It consists of 2 alloys (tungsten) electrodes which are placed together in a medium containing mercury vapor and 25-50 torr of pure argon gas. These electrodes are enclosed in an elliptically shaped in a silica glass tube.

It provides clear white light, high intensity with 24000 hrs of life.

Mercury Vapor Lamp

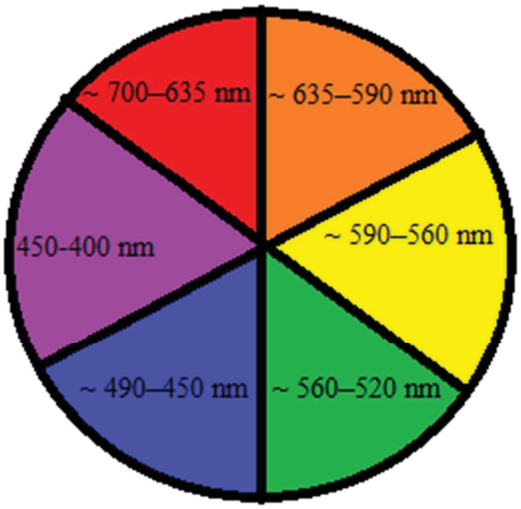
Wavelength Selector

Filter and Monochromator

Filter

Filter is a device used to get selected wavelength. It allows the light pass through it but absorbed the light of different wavelength may partially and fully. A specific filter is used to obtain the desired wavelength for special analysis like absorption filters and interference filters.

Absorption Filters: These filters are made up of glass and gelatin solid sheets colored by the pigments of oxides of Vanadium, iron, nickel, copper, Chromium, Cobalt, etc. It works by absorbing the unwanted radiation and transmit only the required radiation. Selection of filters done as per filter color chart, for example If the color of solution is BLUE a filter having a complimentary color ORANGE is used in the analysis.



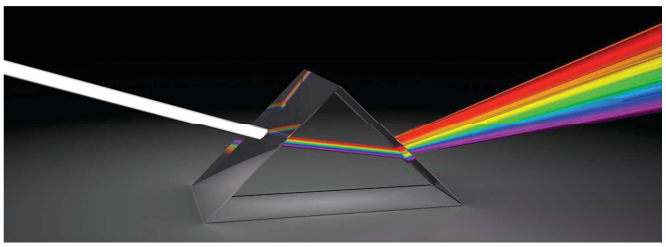
Similarly, we can select the required filter in colorimeter, based upon the color of the solution. Absorption Filters are Simple in construction, Cheaper, and Selection of the filter is easy, while these are less accurate and having more (±20-30nm) bandwidth.

Interference Filter

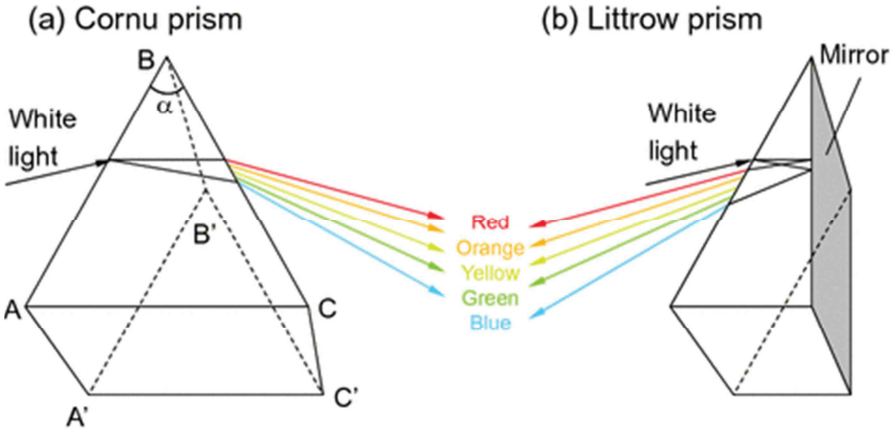
These filters give selective wavelength by declining the unwanted wavelength. These filters are made up of glass plates, which are silvered internally and separated by thin film of CaF2, SiO, and MgF2. These filters have 40-60% peak transmittance and 10-15nm band pass. These are Provide narrower band pass and greater transmittance than absorption filter.

Prism

It is made from glass, Quartz or fused silica. It convert a white light to the rainbow color light.



The effective wavelength depends on the dispersive power of prism material and the optical angle of the prism. There are two types of mounting (1) 'Cornu type'(refractive), which has an optical angle of 60^{o} and its adjusted such that on rotation the emerging light is allowed to fall on exit slit. While (2) "Littrow type"(reflective), which has optical angle 30^{o} and its one surface is aluminized with reflected light back to pass through prism and to emerge on the same side of the light source i.e. light doesn't pass through the prism on other side.



Gratings

These are most effective one in converting a polychromatic light to monochromatic light and achieved resolution near +/- 0.1nm

Two types of gratings are used-

(1) Diffraction grating- give more refined dispersion of light These are consist large number of grooves about 15000-30000/ inch is ruled on highly polished surface of aluminum.

For best result aluminum is spread over the surface. To overcome scattered radiation the gratings are blazed to concentrate the radiation into a single order

(2) Transmission gratings- give higher and linear dispersions than prism monochromator and used over a wide wavelength ranges. These are made up of aluminum.

Sample hander or cells or cuvettes

Cuvettes are used for the handling of samples. These are rectangular or cylindrical in shape with two rough and two smooth sides, and made up of glass, quartz or fused silica.

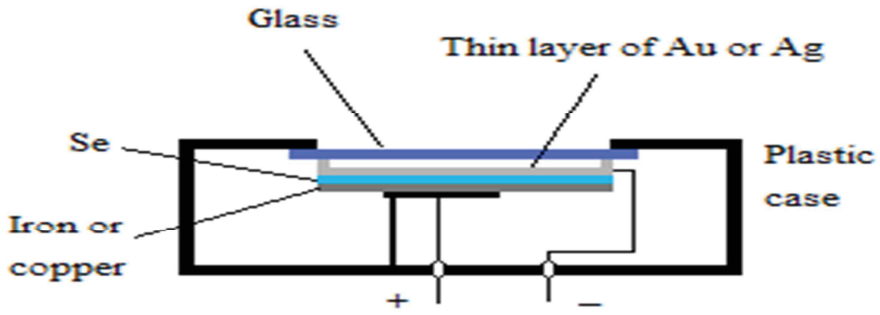
Detector

Detector is a device which transforms light energy into electrical signals that are observed on recorder. The characteristics of ideal detector is give quantitative response, high sensitivity, low noise, short response time, and response quantitative to wide spectrum of radiation received.

Some commonly used detectors are as follows

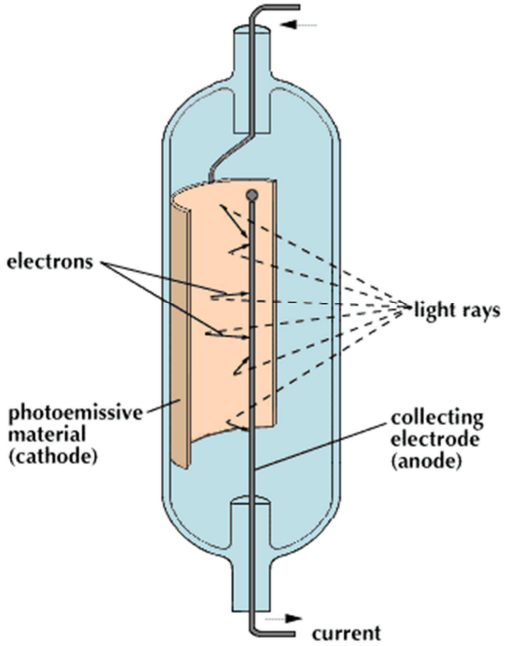
(1) Barrier layer cell/Photovoltaic cell

It is consist of a coated silver or gold thin layer of metallic film which acts as an electrode and another metal plate acts as another electrode. Both of the layers are separated by selenium layer that act as a semiconductor. When UV radiation falls on selenium layer, an electron become mobile and is taken up by transparent metal layer that results a potential difference between the electrodes & causes the flow of current. When it is connected to galvanometer, a flow of current observed which is proportional to the intensity and wavelength of light falling on it.



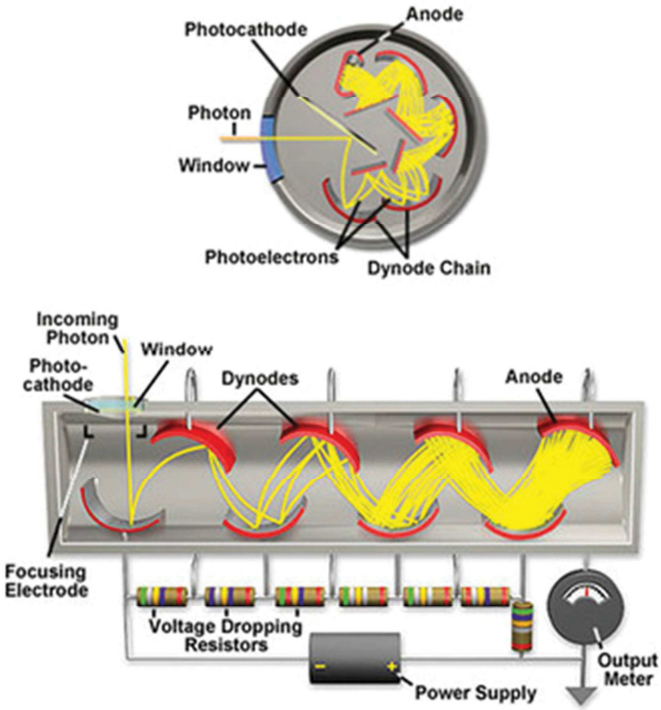
(2) Phototubes/ Photo emissive tube

It is consists of a evacuated glass tube with a photocathode and a collector anode. The surface of photocathode is coated with a layer of elements like cesium, silver oxide and its mixtures. When radiant energy falls on photosensitive cathode, electrons are emitted which are attracted to anode causing flow of current. It is more sensitive than barrier layer cell.



(3) Photomultiplier tube

Photomultiplier tube is multiply the photoelectrons by secondary emission of electrons. A primary photo-cathode is fixed in a vacuum tube which receives radiation from the sample. Some 08 to 10 dynodes are fixed each with increasing potential of 75-100V higher than preceding one. Near the last dynode an electron collector electrode is fixed. It is extremely sensitive to light and detect weaker or low radiation.



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