UV-VIS SPECTROSCOPY

Spectrophotometer

INTRODUCTION

- ***Spectroscopy*** is the branch of science which deals with interaction of electromagnetic radiation with materials. In other words it is an analytical method for qualitative and quantitative analysis by use of light.

- ***The Lambert Beer law*** in 1852 made the basis for the quantitative evaluation of absorption measurements.[2]

1.SPECTROSCOPY

- When an Electromagnetic radiation is incident on a matter, phenomena like reflection, transmission, absorption ,are occurring.[4]

- Spectroscopy is the study of interaction of electromagnetic radiation with matter based on the Bohr-Einstein frequency relationship E=hv , here h is the proportionality constant called Planck's constant (6.626 x 10-34 J s) and v is frequency.

- Measurement of radiation intensity as a function of wavelength is described by ***spectroscopy,*** as shown in figure 2.

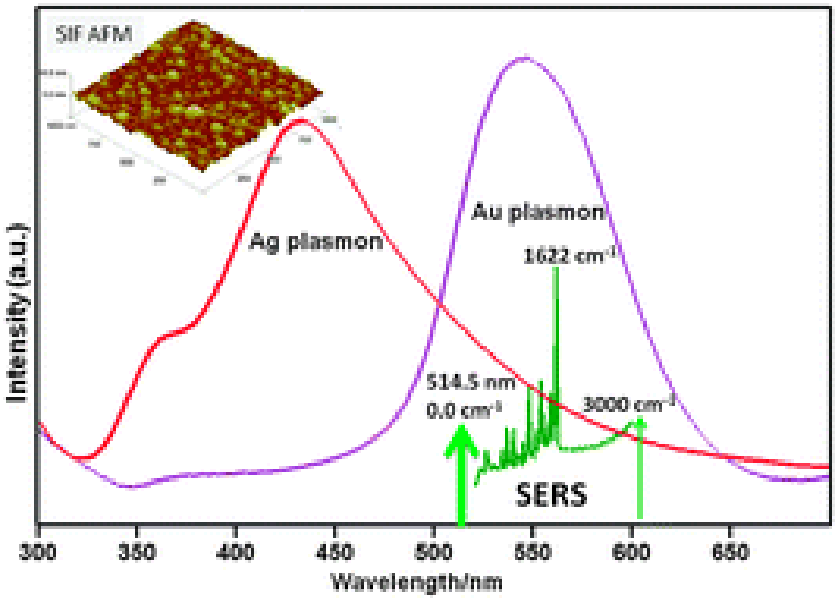


FIG.2.Spectroscopy graph.

- All forms of ***spectroscopy*** use part of the electromagnetic radiation to give us information about the materials.

1.1 SPECTRUM

- The ***spectrum*** is formed by electromagnetic waves and the wavelength is varies. See figure 3.

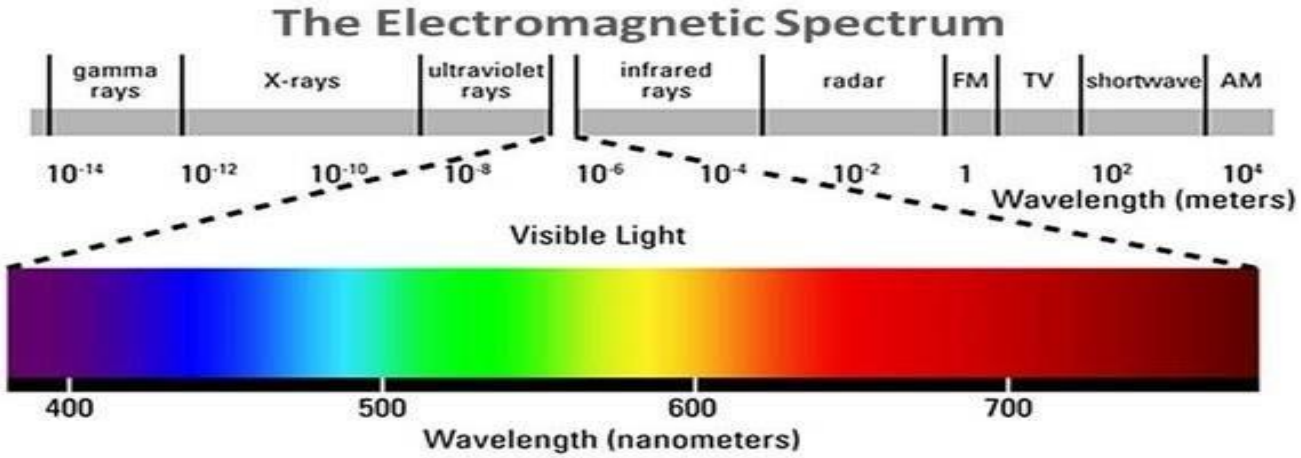


FIG.3.Electromagnatic spectrum.

- When a narrow beam of light is allowed to pass through a prism/grating, it is dispersed into seven colors from red to violet and the band is called ***Spectrum***

  See figure 4.

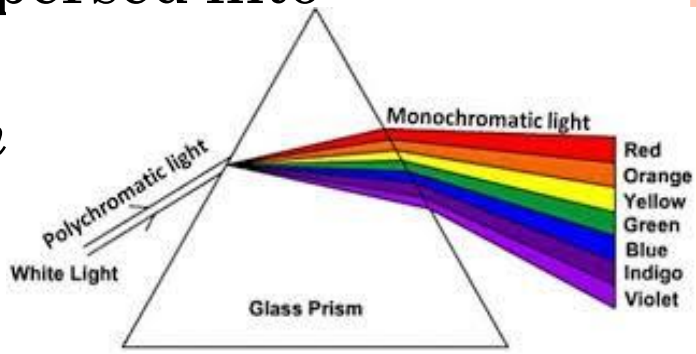


FIG.4.Glase prism dispersion

1.2 PRINCIPLE

Basic principle of *spectroscopy* is the ***Beer-Lambert's law***.[2]

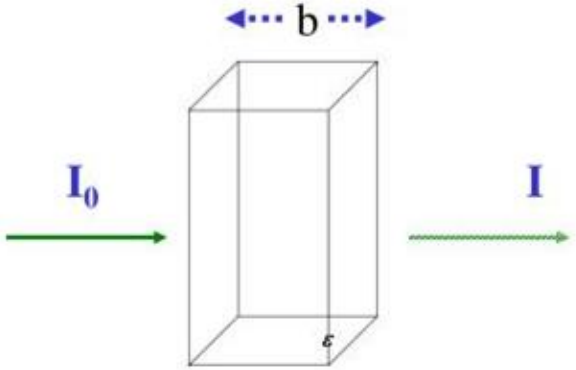
1.2.1 BEER LAW

- ***Beer's law*** stated that absorbance is proportional to the concentrations of the material sample.

1.2.2 LAMBERT LAW

- ***Lambert's law*** stated that absorbance of a material is directly proportional to its thickness (path length).

- The modern derivation of the Beer–Lambert law combines the two laws and correlates the absorbance to both the concentrations and the thickness of the material.



$$A = \varepsilon bc$$

A = Absorbance = $- \log_{10} I / I_0$

b = Optical path length (cm)

c = Solution Concentration (M)

ε = Molar Absorptivity 1/(cm.M)

(measure of how well a compound absorbs a given wavelength of light)

I_0 = Incident beam intensity

I = Transmitted beam intensity

FIG 5. Beer–Lambert law.

2. UV-VISIBLE SPECTROSCOPY

- ***Ultraviolet–visible spectrum*** can be generated when ultraviolet light and visible light(200-900nm) are absorbed by materials. The spectrum can be used to analyze the composition and the structure of the materials. For a particular wavelength in the ultraviolet–visible ranges, the absorption degree is proportional to the components of the materials. Therefore, the characteristics of the materials are quantitatively reflected by the spectrum, which changes with the wave-length.[1]

- ***Ultraviolet–visible spectrum*** consists of an absorption spectrum. An absorption spectrum gives information about the *molar absorptivity, concentration of the sample, optical bath length*. See figure6, in previous slid.

2.1 INSTRUMENTATION

- **2.1.1 SOURCE of LIGHT.**
- **2.1.2 MONOCHROMATOR.**
- **2.1.3 SMPLE SOLIOTION in CUVETTE.**
- **2.1.4 PHOTO DETECTOR.**
- **2.1.5 READOUT DEVICE.**

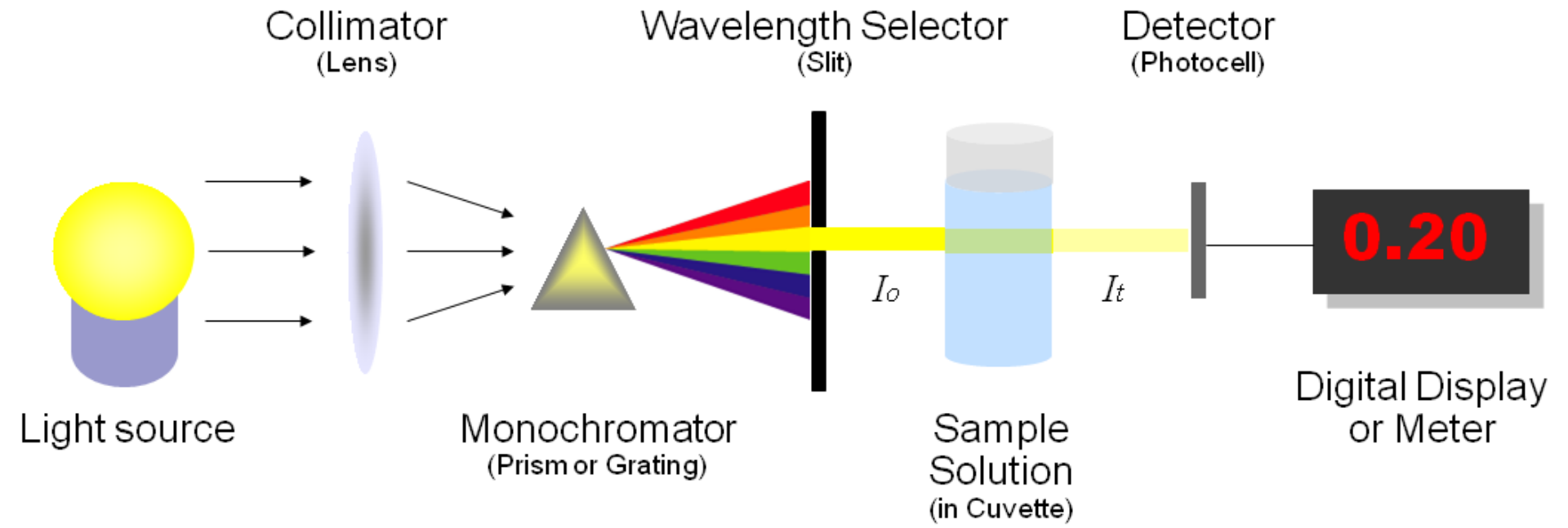


FIG 6.Components of spectrophotometer.

2.1.1 SOURCE OF LIGHT

- **Part of the UV and Visible radiation source** is **Tungsten lamp. See figure 7.**

FIG.7.Tungsten lamp

- **UV radiation source** is **Deuterium or Hydrogen lamp . See figure 8.**
- Range of wavelength 200-400 nm.

FIG.8.Deuterium lamp

2.1.2 MONOCHROMATOR

- It is a device that breaks the polychromatic radiation into component wavelengths. See figure 9.

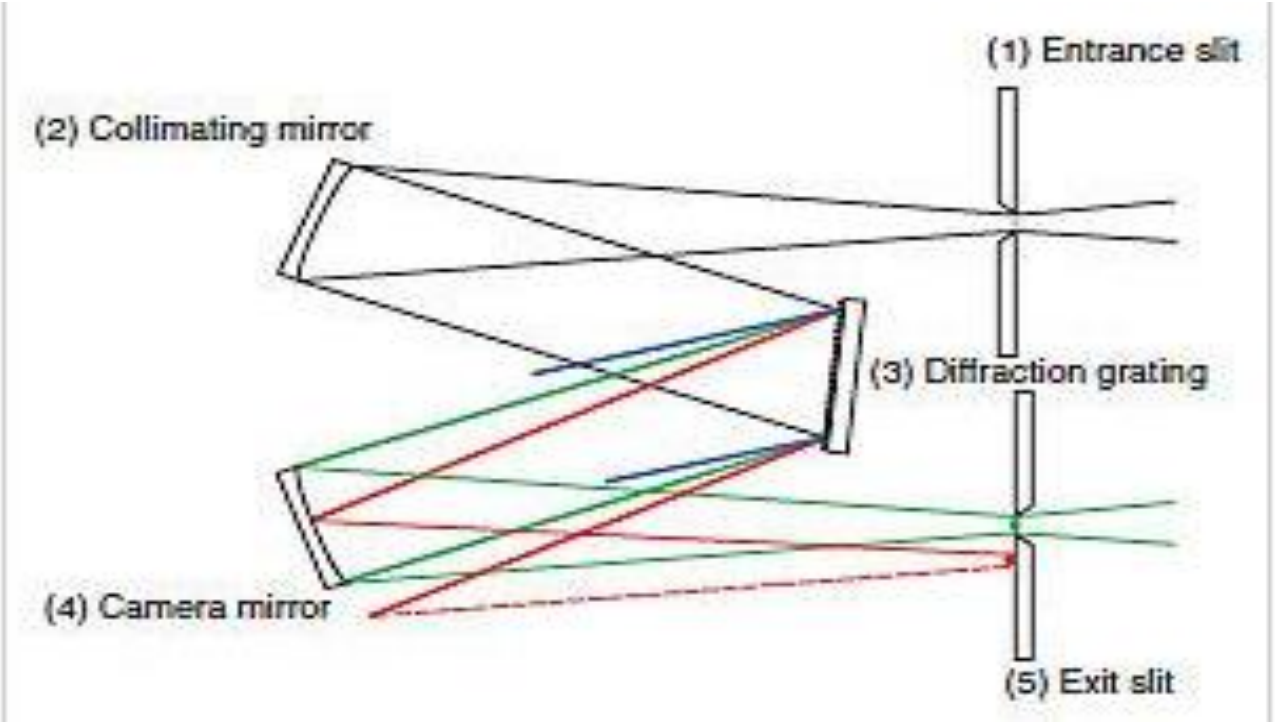


FIG.9.Monochromator components.

The monochromator unit consists of :

- **Entrance slit:** defines narrow beam of radiation from source.

- **Collimating mirror:**(polished surface) collimates the lights.

- **Diffraction grating or Prism** (make of quartz): disperses the light into specific wavelength.

- **Focusing mirror:** captures the dispersed light & sharpens the same to the sample via exit slit

- **Exit slit:** allows the corrected wavelength of light to the sample .

2.1.3 SMPLE SOLIOTION IN CUVETTE

- liquid sample is usually contained in a cell called a ***cuvette.*** See figure10.
- Fingerprints and droplets of water disrupt light rays during measurement.
- Cuvette from ***Quartz*** can be used in UV as well as in visible spectroscopy.
- Cuvette from ***Glass*** is suitable for visible but not for UV spectroscopy because it absorbs UV radiation.

FIG 10.sample solution in cuvette

2.1.4 PHOTO DETECTOR

- A photo detector is a semiconductor device which converts light energy to electrical energy. It consists of a simple P-N junction diode and is designed to work in reverse biased condition. The photons approaching the diode are absorbed by the photodiode and current is generated.[4] See figure 11.

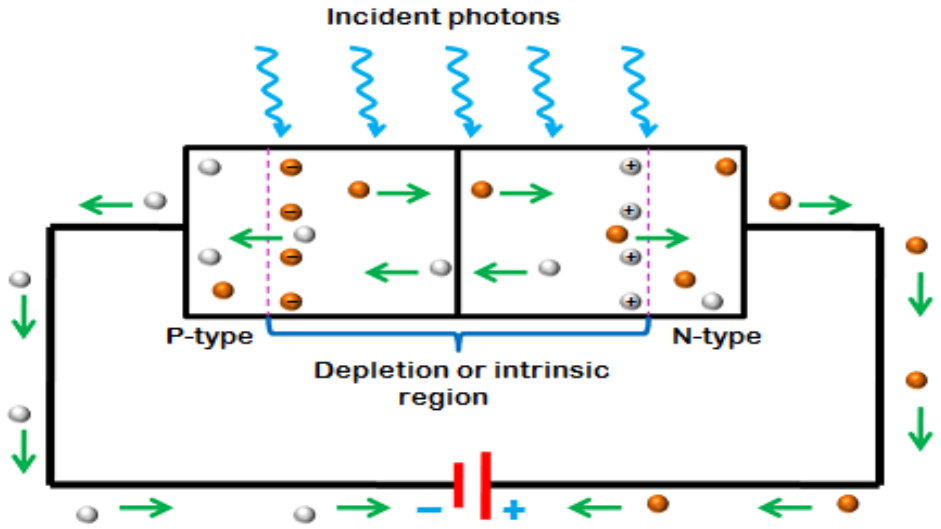


FIG 11. Photodiode

2.1.5 READOUT DEVICE.

- Digital screen to record an uv spectrograph with absorbance against the wavelength.

2.2 TYPES of SPECTROPHOTOMETER

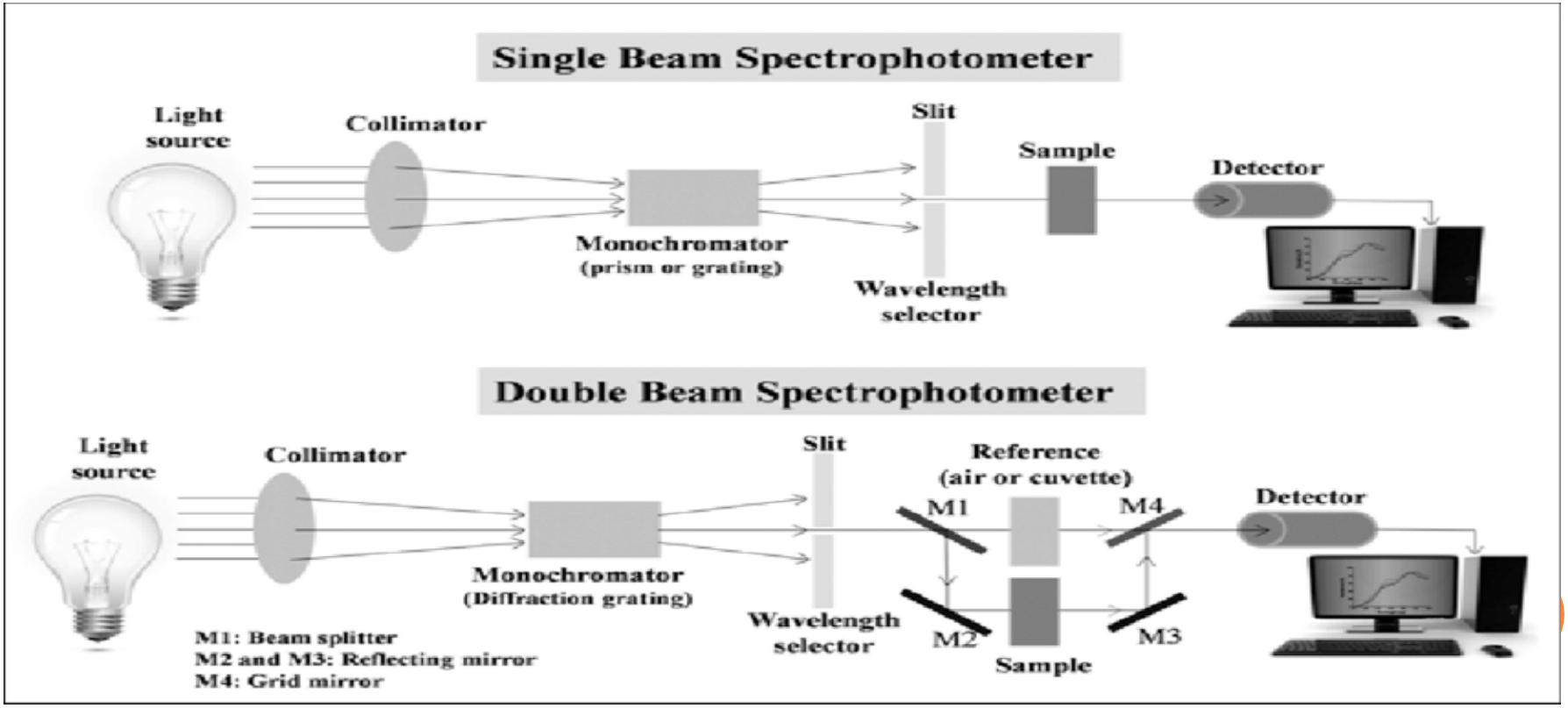


FIG 12. Types of spectrophotometer.

2.3 Applications of UV-VIS Spectroscopy.

- UV-Vis spectroscopy is used heavily in many different research areas to identify or quantify a sample.[3]
- **Chemical field**:

1. Detection of impurities.
2. Structure of organic compounds (single or double bond, presence or absence of functional group).
3. Kinetics of reaction.
4. Manufacturing drugs.

- **Biological fields**

1. quantify the amount of protein and DNA in a sample
2. quantify the amount of bacterial cells in a cell culture

- Major **advantages** of uv-vis spectroscopy are:

1. High sensitivity.
2. Require only small volume of sample.
3. Linearity over wide range of concentration.
4. Can be used with gradient elution.[4]

- Major **disadvantages** of uv-vis spectroscopy are:

1. Not linear for high concentration.
2. Does not work with compounds that do not absorb light at this wavelength region.
3. Generates significant heat and requires external cooling.[4]

- Thank you