CHEMISTRY 5 (INSTRUMENTAL)

AGRICULTURAL BIOTECHNOLOGY, LEVEL 2

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CONTENTS

WHAT IS A SPECTROPHOTOMETER?

- **A spectrophotometer is an instrument that measures the concentration of solutes in solution by measuring the amount of light absorbed by the sample in a cuvette at any selected wavelength.**
- **A spectrophotometer is a process where we measured absorption and transmittance of monochromatic light in terms of ratio or a function of the ratio, of the radiant power of the two beams as a functional of spectral wavelength. These two beams may be separated in time, space or both. It operates on Beer's law**

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SPECTROPHOTOMETER

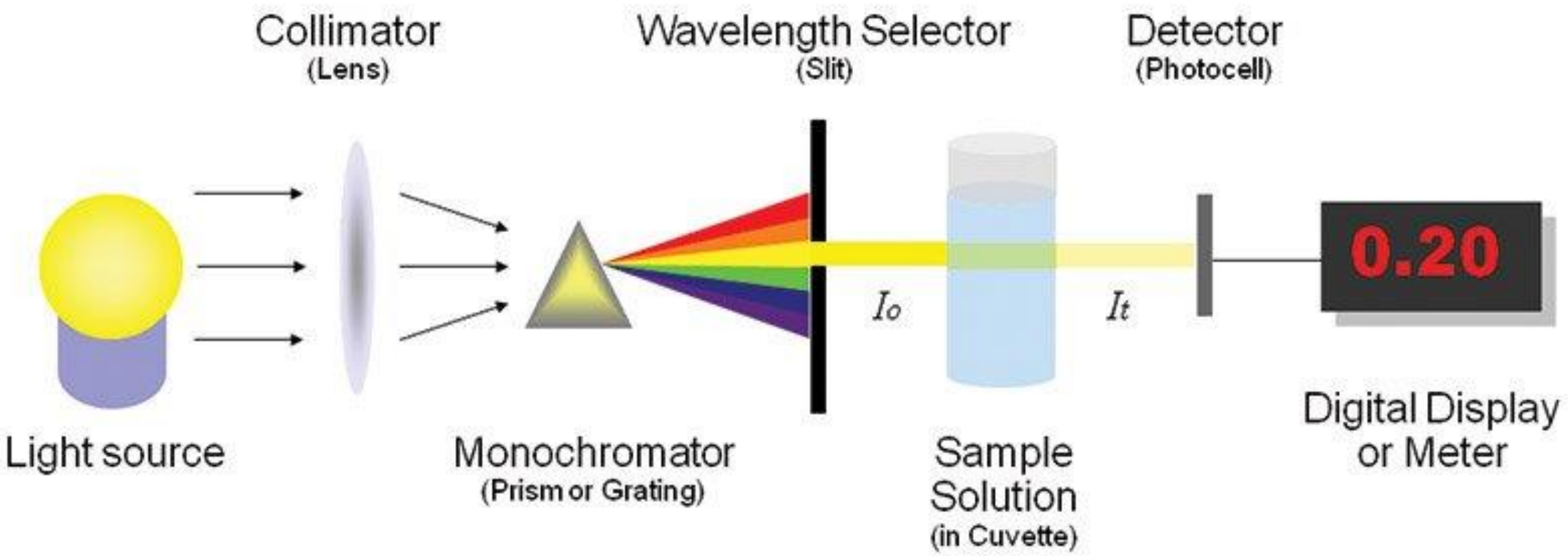
SPECTROPHOTOMETER:

- PRINCIPLE,
- INSTRUMENTATION,
- APPLICATIONS

- **Scientist Arnold O. Beckman and his colleagues at the National Technologies Laboratory (NTL) invented the Beckman DU spectrophotometer in 1940.**

Spectrophotometer

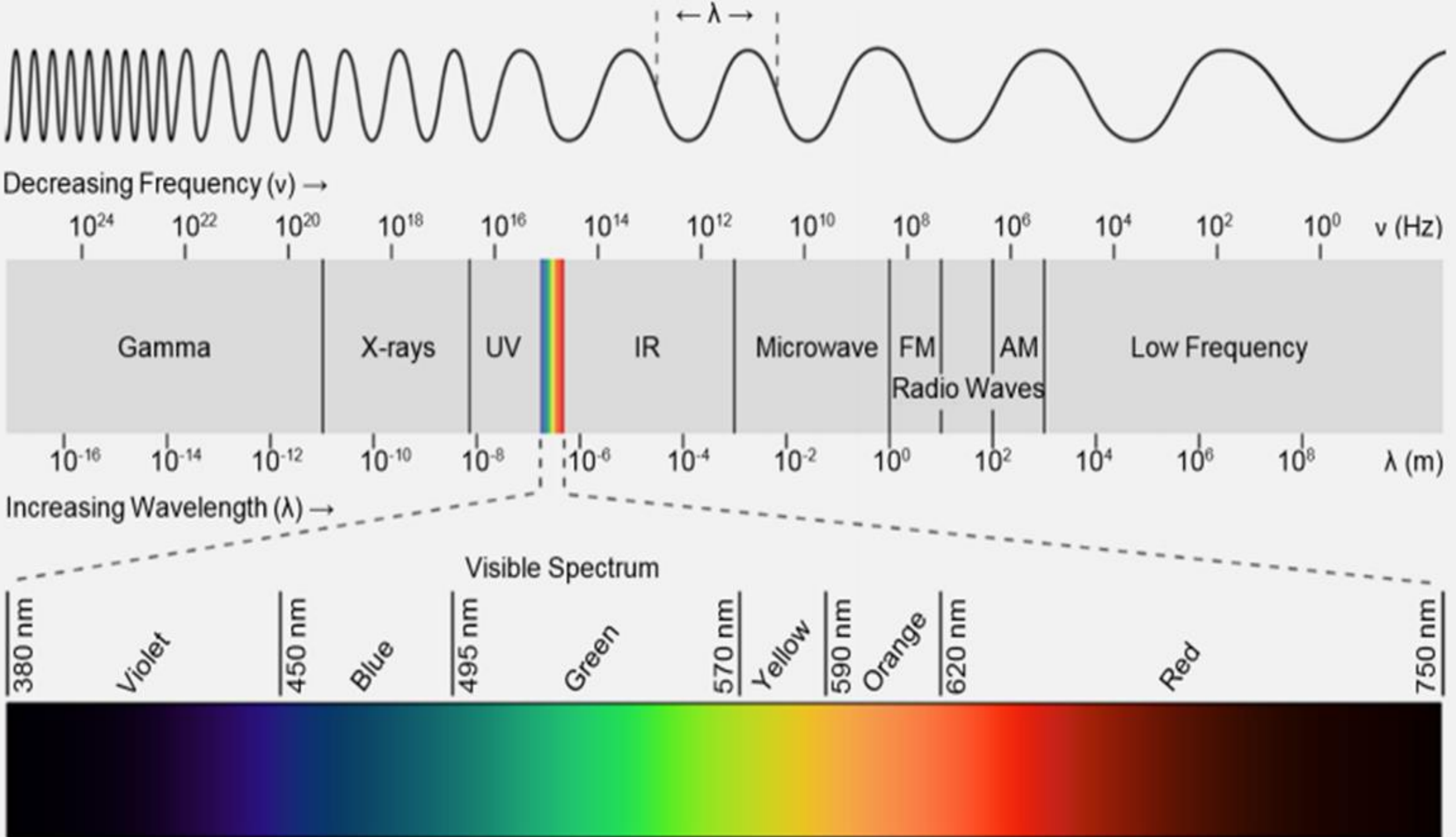
Principle, Instrumentation, Applications



PRINCIPLE

- The spectrophotometer technique is to measure light intensity as a function of **wavelength**. It does this by diffracting the light beam into a spectrum of wavelengths, detecting the intensities with a charge-coupled device, and displaying the results as a graph on the detector and then on the display device.

1. In the spectrophotometer, **a prism (or) grating** is used to split the incident beam into different wavelengths.
2. By suitable mechanisms, waves of specific wavelengths can be manipulated to fall on the test solution. The range of the wavelengths of the incident light can be as low as 1 to 2nm.
3. The spectrophotometer is useful for measuring the absorption spectrum of a compound, that is, the absorption of light by a solution at each wavelength.

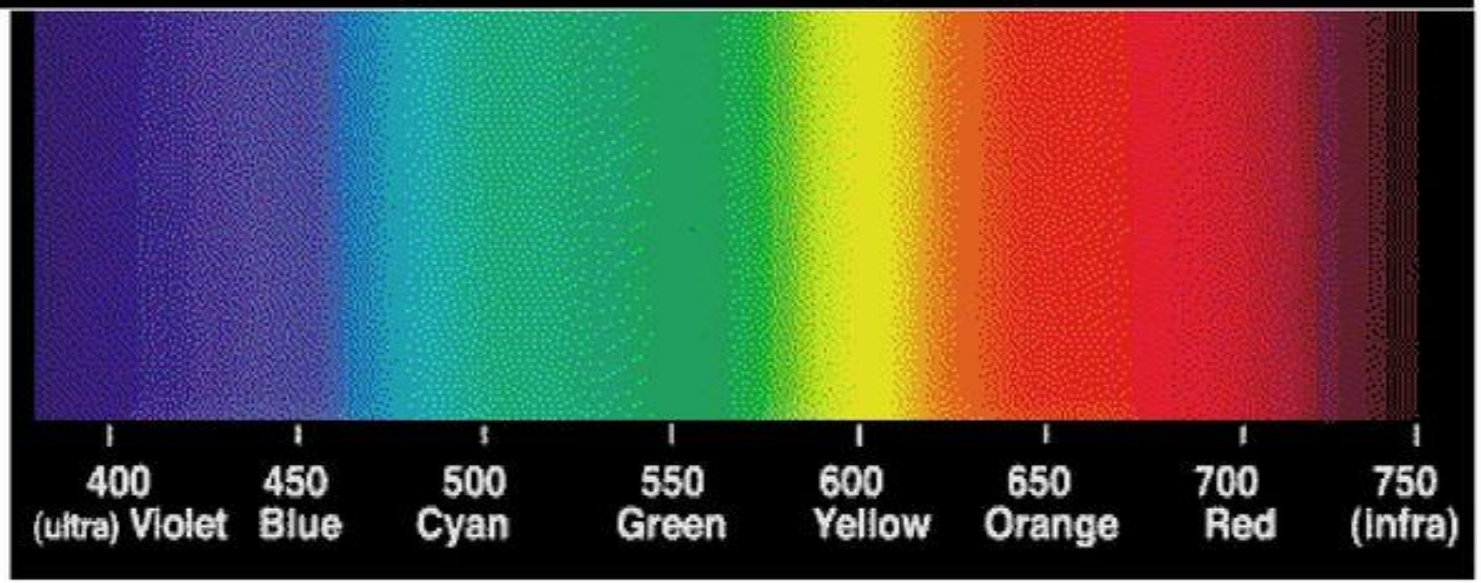

← λ →
Decreasing Frequency (ν) →
10^24 10^22 10^20 10^18 10^16 10^14 10^12 10^10 10^8 10^6 10^4 10^2 10^0 ν (Hz)
Gamma
X-rays
UV
IR
Microwave
FM
AM
Radio Waves
Low Frequency
10^-16 10^-14 10^-12 10^-10 10^-8 10^-6 10^-4 10^-2 10^0 10^2 10^4 10^6 10^8 λ (m)
Increasing Wavelength (λ) →
Visible Spectrum
380 nm
Violet
450 nm
Blue
495 nm
Green
570 nm
Yellow
590 nm
Orange
620 nm
Red
750 nm

Different Wavelengths Lead To:

<380 nm (UV)

~380-750 nm (Visible light)

>750 nm (IR)



Visible light is only a very small portion of the electromagnetic spectrum.

INSTRUMENTATION

The essential components of spectrophotometer instrumentation include:

1. **A cheap radiant energy source (Light):**
   - Materials that can be excited to high energy states by a high voltage electric discharge (or) by electrical heating serve as excellent radiant energy sources.
2. **A monochromator (Filter)**, to break the polychromatic radiation into component wavelength (or) bands of wavelengths.
   - **Prisms:**

     A prism disperses polychromatic light from the source into its constituent wavelengths by virtue of its ability to reflect different wavelengths to a different extent

   Two types of Prisms are usually employed in commercial instruments. Namely, **600 cornu quartz prism** and **300 Littrow Prism**.

   - **Grating:**

   Gratings are often used in the monochromators of spectrophotometers operating ultraviolet, visible and infrared regions.

UV Spectrophotometer

1. Hydrogen Deuterium Gas Lamp
2. Mercury Lamp

Visible Spectrophotometer

1. Tungsten Lamp

INSTRUMENTATION

The essential components of spectrophotometer instrumentation include:

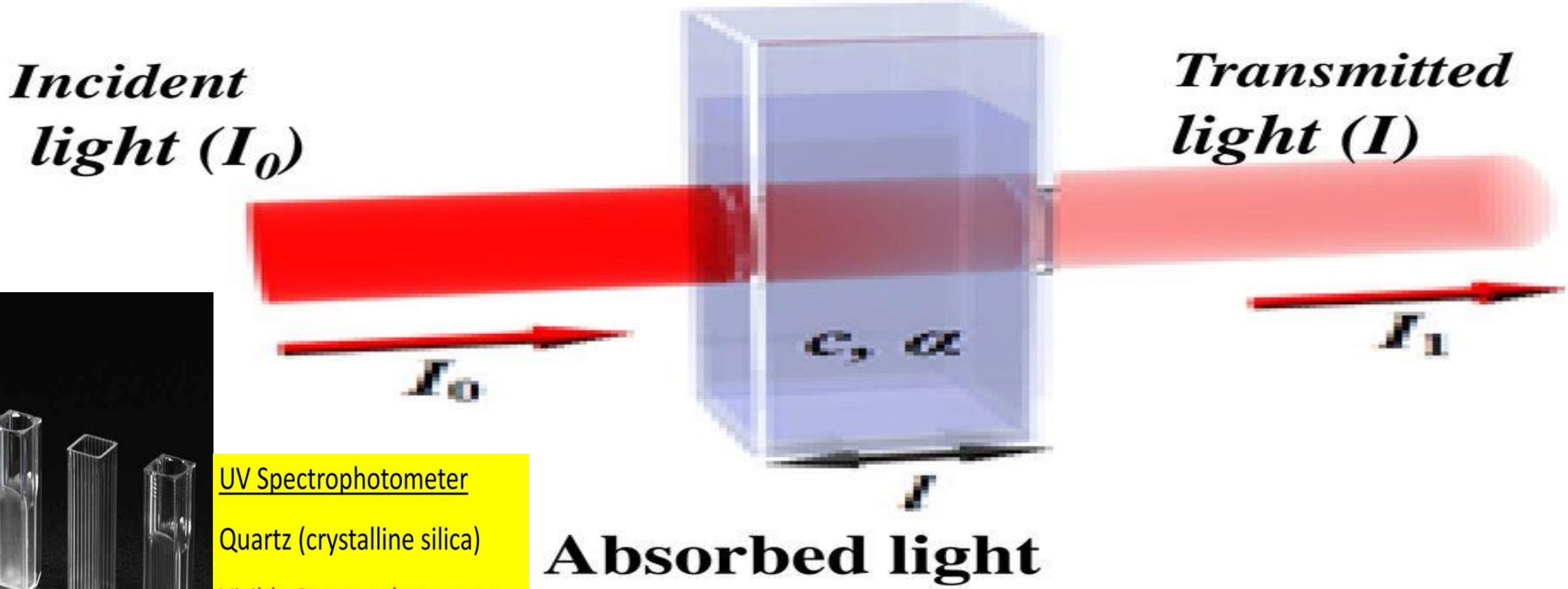
3. Transport vessels (cuvettes), to hold the sample

- Samples to be studied in the ultraviolet (or) visible region are usually glasses (or) solutions and are put in cells known as “CUVETTES”.
- Cuvettes meant for the visible region are made up of either ordinary glass (or) sometimes Quartz.

4. A Photosensitive detector and an associated readout system

- Most detectors depend on the photoelectric effect. The current is then proportional to the light intensity and therefore a measure of it.
- Radiation detectors generate electronic signals which are proportional to the transmitter light.
- These signals need to be translated into a form that is easy to interpret.
- This is accomplished by using **amplifiers**, **Ammeters**, **Potentiometers** and **Potentiometric recorders**.

Definitions & Symbols



UV Spectrophotometer

Quartz (crystalline silica)

Visible Spectrophotometer

Glass-plastic

APPLICATIONS

The essential components of spectrophotometer instrumentation include:

Some of the major applications of spectrophotometers include the following:

(Qualitative and Quantitative Applications)

- Detection of concentration of substances
- Detection of impurities
- Structure elucidation (Clarity) of organic compounds
- Monitoring dissolved oxygen content in freshwater and marine ecosystems
- Characterization of proteins
- Detection of functional groups
- Respiratory gas analysis in hospitals
- Molecular weight determination of compounds
- The visible and UV spectrophotometer may be used to identify classes of compounds in both the pure state and in biological preparations.
- Check concentration and purity of DNA/RNA present in the solution.

REFERENCES

- Link
- Link
- Link

THANK YOU