AL- MUSTAQBAL UNIVERSITY COLLEGE
DEPARTMENT OF BIOMEDICAL ENGINEERING

Laboratory Instrumentation

BME 422

Lecture 4

- Spectrophotometer -

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Spectrophotometer

Spectrophotometer

- Spectrophotometry is the basis for many of the instruments used in clinical chemistry.

- The primary reasons for this are
  - Ease of measurement.
  - Satisfactory accuracy and precision.
  - The suitability of spectrophotometric techniques to use in automated instruments.

Spectrophotometer

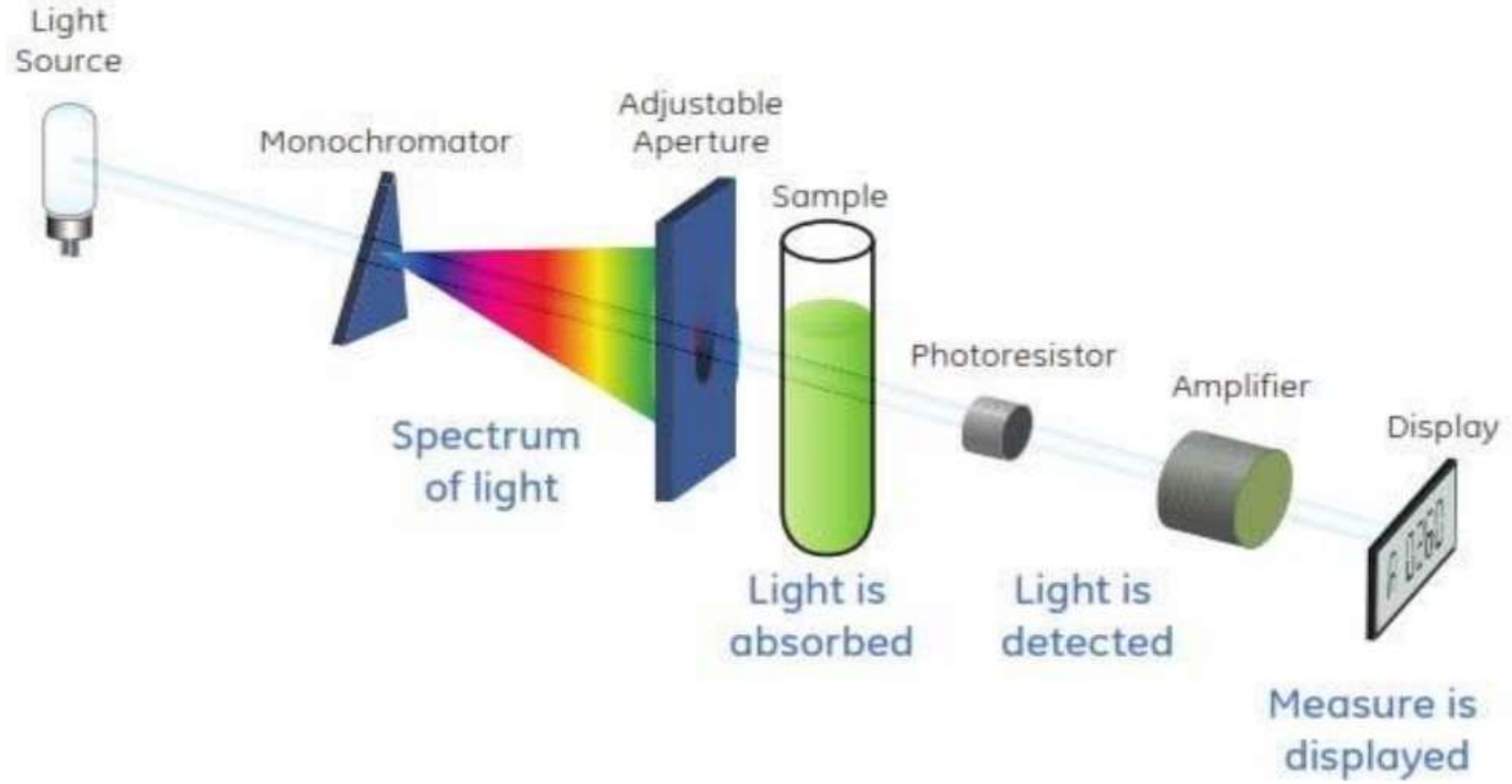
- Spectrophotometry is based on the fact that substances of clinical interest selectivity absorb or emit electromagnetic energy at different wavelengths.

- For most laboratory applications, wavelengths in the range of
  - The ultraviolet (200-400 nm).
  - The visible (400-700 nm).
  - The near infrared (700-800 nm) are used.

- The majority of the instruments operate in the visible range.

Basic components

Spectrophotometer: is an instrument that measures the amount of light absorbed by a sample.



Spectrophotometer main components

1- A source of radiant energy, which may be a tungsten lamp, a xenon-mercury arc, hydrogen discharge lamp, etc.

2- Filtering arrangement for the selection of a narrow band of radiant energy. It could be a single wavelength absorption filter, or an interference filter.

3- An optical system for producing a parallel beam of filtered light for passage through an absorption cell (cuvette). The system may include lenses, mirrors, slits, diaphragm, etc.

Spectrophotometer main components

4- A detecting system for the measurement of unabsorbed radiant energy, which could be the human eye, a barrier-layer cell, phototube or photo-multiplier tube.

5- A readout system or display, which may be an indicating meter or a numerical display.

Power Sources

- Hydrogen discharge lamps are used to provide power in the 200-to-360 nm range. While, tungsten lamps are used for the 360-to-800 nm range.

- Hydrogen lamps produce a continuous spectrum; but a problem with this power source is that they produce many of their power in the infrared range.

- The output in the ultraviolet and visible ranges can be increased by operating the lamp at voltages above the rated value, but this stratagem significantly reduces the expected life of the lamp.

Wavelength Selectors

- A variety of devices are used to select those portions of the power spectrum produced by the power source that are to be used to analyze the sample.

- These devices can be divided into two classes: filters and monochromators.

- There are two basic types of filters: glass filters and interference filters.

Glass filters

- Glass filters function by absorbing power.

- For example, a blue colored filter absorbs in the higher-wavelength visible range (red region) and transmits in the lower wavelength visible range (blue-green region).

- These filters (consisting of one or more layers of glass plates) are designed to be low-pass, high pass, or band pass (a combination of low- and high -pass) filters.

Interference filters

- Interference filters are made by spacing reflecting surfaces such that the incident light is reflected back and forth a short distance.

- The distance is selected such that light in the wavelength band of interest tends to be in phase and to be reinforced; light outside this band is out of phase and is canceled (the interference effect).

- Harmonics of the frequencies in this band are also passed and must be eliminated by glass cutoff filters.

Interference filters

- Glass filters are used in applications in which only modest accuracy is required.

- Interference filters are used in many spectrophotometers, devices that use filters as their wavelength selectors are called colorimeters or photometers.

Monochromators

- Monochromators are devices that utilize prisms and diffraction gratings.
- They provide very narrow bandwidths and have adjustable nominal wavelengths.
- The basic principle of operation of these devices is that they disperse the input beam spatially as a function of wavelength.
- A mechanical device is then used to allow wavelengths in the band of interest to pass through a slit.

Cuvette

- The cuvette holds the substance being analyzed.

- Its optical characteristics must be such that it does not significantly alter the spectral characteristics of the light as that light enters or leaves the cuvette.

- The degree of care and expense involved in cuvette design is a function of the overall accuracy required of the spectrophotometer.

Purpose

- A spectrophotometer is an analytical instrument used to make qualitative and quantitative measurement of an analyte in a solution.

- It is the most versatile, reliable, and widely used instrument in clinical chemistry to examine blood, urine, or tissues for clinical diagnosis.

Purpose

- The majority of clinical chemistry procedures have been tailored to produce an end product (color), which can be detected and measured by some type of a spectrophotometer.

- Even highly automated analyzer systems make use of a spectrophotometer as a final readout device.

Principle

- Every chemical compound absorbs, transmits, or reflects light (electromagnetic radiation) over a certain range of wavelength.

- Spectrophotometry is the method that is based on the absorption of electromagnetic radiation in the visible, ultraviolet (UV), and infrared (IR) ranges.

- This electromagnetic spectrum ranges from very short wavelengths including X-rays to very long wavelengths including microwaves and broadcast radio waves.

Principle

- The nature of all these radiations is the same and all travel with the speed of light.

- They differ from each other in terms of their frequency and wavelength and the effect they produce on interaction with matter.

- Molecules possess three types of internal energy, namely, electronic, vibrational, and rotational.

Spectrophotometer

The most common spectrophotometers are used in the UV and visible regions of the spectrum, and some of these instruments also operate into the near-IR region as well.