ROHINI
COLLEGE OF ENGINEERING AND TECHNOLOGY
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DEPARTMENT OF BIOMEDICAL ENGINEERING

BM3491 Biomedical Instrumentation

UNIT- V BIOCHEMICAL MEASUREMENTS

5.7 Spectrophotometer

A spectrophotometer is an instrument that measures the amount of photons (the intensity of light) absorbed after it passes through sample solution. It is commonly used in chemistry, physics, biology, and industrial applications to quantify the concentration of substances in a solution by measuring the amount of light absorbed by the solution.

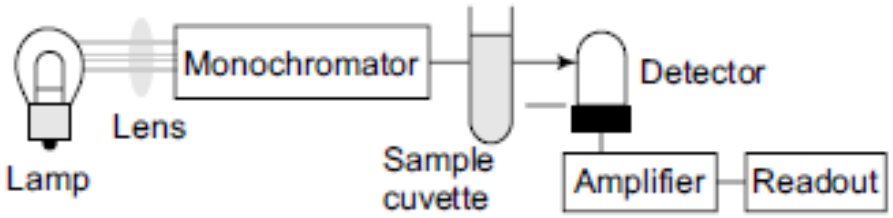


Fig. 5.7.1 *Various components of a spectrophotometer type instrument*

Fig. 5.7.1 shows the various components used in a spectrophotometer.

Basic Components of a Spectrophotometer

1. **Light Source**:
   - ❒ Provides the initial light that will pass through the sample.
   - ❒ Common light sources include tungsten lamps for visible light and deuterium lamps for ultraviolet light.

2. **Monochromator**: ***(Filtering arrangement)***

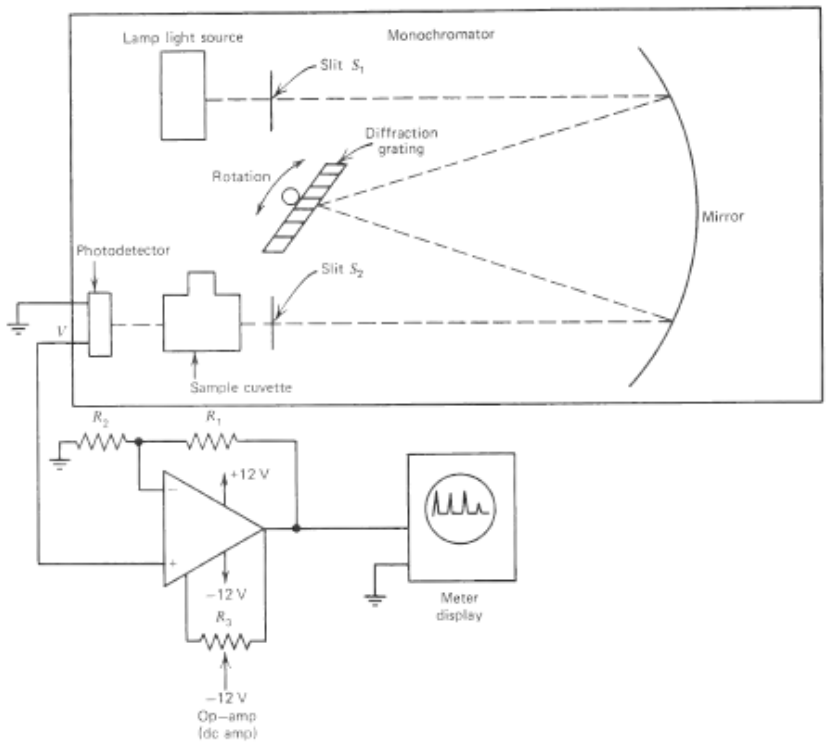
- ❒ Separates the light into its component wavelengths (i.e., it selects a specific wavelength of light).
- ❒ Can be a prism or diffraction grating.

3. **Optical System**:
   - ❒ An Optical system produces a parallel beam of filtered light for passage through an absorption cell / sample holder (cuvette).
   - ❒ This may include lens, mirrors, slits, diaphragms etc.

4. **Sample Holder**:
   - ❒ Contains the sample being analyzed.
   - ❒ Often a cuvette, which is a small, transparent container.

5. **Detector**:
   - ❒ Measures the intensity of light passing through the sample (unabsorbed radiant energy)
   - ❒ Common detectors include human eye, a barrier-layer cell, phototube, photo-multiplier tube or photodiodes.

6. **Digital Display or Output Device**:
   - ❒ Shows the results of the measurement.
   - ❒ Can be a digital readout or a connected computer.



***5.7.2** Spectrophotometer—simplified schematic*

5.7.1 Radiation Sources:

- The function of the radiation source is to provide a sufficient intensity source of light which is suitable for making a measurement.
- The most common and convenient source of light is the ***tungsten lamp***.
- This lamp consists of a ***tungsten filament*** enclosed in a glass envelope.
- It is cheap, intense and reliable.
- A major portion of the energy emitted by a tungsten lamp is in the visible region and only about 15 to 20% is in the infrared region.
- When using a tungsten lamp, it is desirable to use a ***heat absorbing filter*** between the lamp and the sample holder to absorb most of the infrared radiation.
- For work in the ultraviolet region, a ***hydrogen or deuterium*** discharge lamp is used.
- For work in the infrared region, a tungsten lamp may be used. However, due to high absorption of the glass envelope and the presence of unwanted emission in the visible range, tungsten lamps are not preferred. In such cases, nernst filaments or other sources of similar type are preferred.

- Modern instruments use a ***tungsten-halogen*** light source, which has a higher intensity output than the normal tungsten lamp.

5.7.2 Optical Filters:

- Ideal filters should be monochromatic, i.e. they must isolate radiation of only one wavelength. A filter must meet the following two requirements:
  - ✓ high transmittance at the desired wavelength and
  - ✓ low transmittance at other wavelengths

- **Absorption Filters**:
  a) The absorption type optical filter usually consists of colour media: colour glasses, coloured films (gelatin, etc.), and solutions of the coloured substances.
  b) This type of filter has a wide spectral bandwidth. Their efficiency of transmission is very poor and is of the order of 5 to 25%.

- **Interference Filters**:
  1. These filters usually consist of two semi-transparent layers of silver, deposited on glass by evaporation in vacuum and separated by a layer of dielectric (ZnS or MgF2).
  2. Interference filters allow a much narrower band of wavelengths to pass and are similar to monochromators in selectivity.
  3. The transmittance of these filters varies between 15 to 60per cent with a spectral bandwidth of 10 to 15 nm.
  4. For efficient transmission, multilayer transmission filters are often used.
  5. They are characterized by a bandpass width of 8 nm or less and a peak transmittance of 60-95%.

5.7.3 Monochromators:

1. Monochromators are optical systems, which provide better isolation of spectral energy than the optical filters, and are therefore preferred where it is required to isolate narrow bands of radiant energy.

2. Monochromators usually incorporate a small glass of quartz prism or a diffraction grating system as the dispersing media.
3. The radiation from a light source is passed either directly or by means of a lens or mirror into the narrow slit of the monochromator and allowed to fall on the dispersing medium, where it gets isolated

❒ **Prism Monochromators**:

i. Prism may be made of glass or quartz. The glass prisms are suitable for radiations essentially in the visible range whereas the quartz prism can cover the ultraviolet spectrum also.

ii. Prism spectrometers are usually expensive, because of exacting requirements and difficulty in getting quartz of suitable dimensions.

❒ **Diffraction Gratings**:

i. Monochromators may also make use of diffraction gratings as a dispersing medium.

ii. A diffraction grating consists of a series of parallel grooves ruled on a highly polished reflecting surface.

iii. When the grating is put into a parallel radiation beam, so that one surface of the grating is illuminated, this surface acts as a very narrow mirror.

iv. The reflected radiation from this grooved mirror overlaps the radiation from neighbouring grooves.

❒ **Holographic Gratings**:

i. Precision spectrophotometers use holographic or interference gratings, which have superior performance in reducing stray light as compared to diffraction gratings.

ii. Holographic gratings are made by first coating a glass substrate with a layer of photo-resist, which is then exposed to interference fringes generated by the intersection of two collimated beams of laser light.

5.7.4 Detectors:

❒ The radiation is fall on a photosensitive element, in which the light energy is converted into electrical energy.

- The electric current produced by this element can be measured with a sensitive galvanometer directly or after suitable amplification.
- Any type of photosensitive detector may be used for the detection and measurement of radiant energy, provided it has a linear response in the spectral band of interest and has a sensitivity good enough for the particular application.
- There are two types of photo-electric cells; photo-voltaic cells and photo-emissive cells.
- Modern spectrophotometers use semiconductor array detectors which allow rapid recording of absorption spectra.
- These spectrometers use photodiode arrays (PDAs) or charge-coupled devices (CCDs) as the detector.

5.7.5 Sample Holders:

- Liquids may be contained in a cell or cuvette made of transparent material such as silica, glass or perspex.
- The faces of these cells through which the radiation passes are highly polished to keep reflection and scatter losses to a minimum.
- Solid samples are generally unsuitable for direct spectrophotometry.
- It is usual to dissolve the solid in a transparent liquid.
- Gases may be contained in cells which are sealed or stoppered to make them air-tight

Working Principle :

1. **Emission of Light**:
    - The light source emits a broad spectrum of light.
2. **Selection of Wavelength**:
    - The monochromator isolates a specific wavelength from the emitted light.
    - This selected wavelength is directed towards the sample.
3. **Light Passage Through Sample**:
    - The light passes through the sample held in the cuvette.

- The sample absorbs some of the light, and the remaining light passes through.

4. **Detection of Transmitted Light**:
   - The detector measures the intensity of the transmitted light (It).
5. **Calculation of Absorbance**:
   - The instrument calculates absorbance (A) using the formula:
   - $$A = -\log\left(\frac{I_t}{I_0}\right)$$

     Where I_t is the intensity of transmitted light and
     I_0 is the intensity of the incident light.
6. **Display of Results**:
   - The absorbance value is displayed on the digital screen or sent to a connected computer for analysis.

Applications

1. **Quantitative Analysis**:
   - Determines the concentration of substances in a solution using Beer-Lambert Law:

     $$A = \epsilon c l$$

     where A is absorbance,
     ϵ is the molar absorptivity,
     c is the concentration, and
     l is the path length of the cuvette.
2. **Kinetic Studies**:
   - Monitors reaction rates by measuring changes in absorbance over time.
3. **Nucleic Acid and Protein Analysis**:
   - Measures concentrations and purity of DNA, RNA, and proteins by detecting specific absorbance wavelengths (e.g., 260 nm for nucleic acids, 280 nm for proteins).
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4. **Colorimetric Assays**:
    - Used in assays where a color change indicates the presence or concentration of a substance.

Calibration and Maintenance :

- **Calibration**: Regular calibration with standard solutions is necessary to ensure accurate measurements.
- **Maintenance**: Includes cleaning the light source, monochromator, and sample holder to prevent contamination and maintain precision.

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