SPECTROPHOTOMETRY

Spectrophotometer is an instrument which measures light absorption as a function of wavelength in the UV as well as visible regions (Figure 11.9). It also follows essentially the laws of light absorption, viz. the Beer-Lambert’s law. Unlike colorimeters, in spectrophotometers the compounds can be measured at precise wavelengths. Spectrophotometry has become a powerful tool for qualitative and quantitative measurements.

A spectrophotometer consists essentially of six parts.

1. Light source
2. Condensing lens
3. Monochromator
4. Sample holder(s)
5. Detector(s) and
6. Recorder

![Figure 11.9 Spectrophotometer](image)

A spectrophotometer has two light sources, an UV light (for measuring light absorption from ~200 to ~400 nm) and white light (for measuring light absorption from ~400 to 900 nm). With the help of a shutter, only one of the lights is allowed to fall on a silvered mirror (SM). The reflected light from the mirror passes through an entrance slit and a condensing lens. The lens renders the light rays into parallel beams and the parallel beams of light...
now fall on a monochromator (grating). The monochromator disperses the light into its component wavelengths. Using the wavelength selector, the desired wavelength is selected. Now the selected beam of monochromatic light passes again through a lens to a light-tight compartment where the sample is kept in a cuvette. After passing through the sample, the transmitted light falls on a photomultiplier tube (PMT). The PMT converts the light energy into electrical energy, which is amplified, measured and recorded on the analog/digital read-out (Figure 11.10).

![Diagram](image)

**Figure 11.10** Schematic diagram of a single-beam spectrophotometer

In double beam spectrophotometers, the monochromatic light coming out from the lens is split into two halves by placing a half-silvered mirror (HSM) on its path. Now 50% of the light passes directly through the mirror and falls on the reference cuvette and 50% of the light reflected onto a second silvered mirror and then allowed to fall on the sample cuvette. At any given time, the intensities of the transmitted light from the reference and sample cuvettes are measured, amplified, the difference in intensities computed and sent to the read-out (Figure 11.11).
ULTRAVIOLET AND VISIBLE SPECTROSCOPY (UV-VIS)

Ultraviolet and visible regions of the electromagnetic spectrum and their associated techniques are most widely used in analytical and biological research.

Parts of a UV-VIS Spectrophotometer

Light sources A UV-VIS spectrophotometer has two light sources, a tungsten lamp for visible light, and a deuterium or a hydrogen lamp for UV light respectively (deuterium lamp gives wider and more intense light in UV region than a hydrogen lamp). The light from the light source is composed of a wide range of wavelengths. This light is called polychromatic or heterochromatic. The polychromatic light reflected back using a plane mirror, passes through an entrance slit and through a condensing lens and falls onto a monochromator. The monochromator disperses the light and the desired wavelength is focused on the exit slit using the wavelength selector.

Monochromators The monochromators which produce radiations of single wavelength are based either upon refraction by a prism or diffraction by a grating. Prisms are made of glass for visible region and of quartz or silica for UV region. A grating consists
of ruled lines (as many as 2000 lines per millimetre) on a transparent or reflecting base. The resolving power of a grating is directly proportional to the closeness of these lines. Gratings are superior to prisms as they yield linear resolution of the spectrum for the entire range of wavelengths. The efficiency of monochromation is enhanced by using double monochromators in which a selected part of the spectrum from the first grating is further resolved by a second grating, resulting in a bandwidth of as low as 0.1 mm.

**Cuvettes**  The optically transparent cells (cuvettes) are made up of glass, plastic, silica or quartz. Glass and plastic absorb UV light below 310 nm. Hence, they cannot be used for light measurements in UV region. Silica and quartz do not absorb UV light and hence they are used for both UV and visible light measurements. Since quartz absorbs light below 190 nm, cuvettes of lithium fluoride can be used which transmit radiation down to 110 nm. Oxygen also absorbs light at wavelengths less than 200 nm. Therefore, if spectra are required in this region the apparatus must be evacuated. The standard cuvettes are made up of quartz, have an optical path of 1 cm, and hold a volume of 1–3 ml. Microcuvettes (0.3–0.5 ml) are used for measurement of expensive chemicals.

**Photocell and photomultiplier tubes**  A photocell (Figure 11.12) is a photoelectric device, which converts light energy into electrical energy. The photocell consists of two electrodes, the photocathode and the anode, which are vacuum-sealed inside a glass envelope. Light falling on the photocathode causes electrons to be emitted into the enclosed gas. The current of these electrons (and not the light) can be amplified by a photomultiplier tube. A typical photomultiplier tube has a series of anodes, each subsequent anode being biased with a higher positive voltage than the one before it, until the last anode is the same potential as the cathode. As the current of each anode is higher than its predecessor, the overall amplification factor can be extremely high. The output of the photomultiplier is then fed to an amplifier and readout device.

![Figure 11.12 Schematic diagram of a photocell](image-url)
energy, which is then amplified, detected and recorded. In photocells, the photons strike a semicylindrical photoemissive cathode in vacuum. This causes emission of electrons, which is proportional to the intensity of radiation. When a potential difference is applied across the electrodes, the emitted electrons flow to the anode wire generating a photocurrent. This current is amplified electronically and measured.

A photomultiplier tube (Figure 11.13), has a cathode with photoemissive surface (a selenium layer) and a wire anode. In addition to the photoemissive cathode, it also contains a circular array of nine additional cathodes called dynodes.

![Figure 11.13](image)

**Figure 11.13** Cross-section of a photomultiplier tube

The electrons emitted from the photoemissive cathode strike dynode 1, which emit several additional electrons. The electrons are accelerated towards dynode 2, which again emit several electrons. The amplified electrons flow to the anode generating a much larger photoelectric current than in a photocell.
Applications

UV-VIS spectrophotometer is a more refined instrument and it gives a far better precision and resolution than a colorimeter. It has a wide range of applications in biological research.

1. It is used to estimate the concentration of both coloured as well as colourless solutions, which could absorb light.
2. Because of its higher sensitivity, it is used to estimate extremely small quantities of substances in a matter of a few minutes.
3. It usually does not degrade or modify the materials studied (unless a photochemical reaction occurs) and hence the materials can be recovered and reused.
4. It is also used to find out the absorption maxima of compounds with a wide range of wavelengths.
5. It offers selectivity in that each component in a solution or reaction mixture can be singled out and estimated.
6. It also enables one to follow details of fast reactions and fast enzyme kinetics.
7. It is also used to measure the growth of bacteria and yeasts and to determine the number of cells in a culture.
8. Small volumes (as small as 0.3 ml) can be used for estimation of precious samples.

**Electronic Micromotility Meter**

Electronic micromotility meter (EMM) is highly sensitive and gives accurate quantitative measures of the motility of bacteria, larval and adult trematodes and nematodes. This instrument can also be used to record the motility of sperms. A new application has been developed for UV bio-spectrophotometer and the instrument is renamed as electronic micromotility meter (EMM) (Figure 11.14), which can be used to monitor the motor activity of microorganisms (Veerakumari, 2003).
- Natural organic chemistry
- Biochemistry and macromolecules
- Metal complex chemistry
- Polymer chemistry
- Medical science
- Agrochemistry
- Physical chemistry
- Rapid scanning (time-resolved) experiments

**INFRARED SPECTROPHOTOMETRY**

The technique is based upon the simple fact that a chemical substance shows marked selective absorption in the infrared region. After absorption of IR radiations, the molecules of a chemical substance vibrate at many rates of vibration, giving rise to close-packed absorption bands, called an IR absorption spectrum, which may extend over a wide wavelength range. Various bands will be present in IR spectrum that will correspond to the characteristic functional groups and bonds present in a chemical substance. Thus, an IR spectrum of a substance is a *fingerprint* for its identification.

**SINGLE-BEAM INFRARED SPECTROPHOTOMETER**

A diagram of the optical system of a single-beam infrared spectrophotometer is shown in Figure 11.29.

![Schematic diagram of single-beam infrared spectrophotometer](image)

*Figure 11.29 Schematic diagram of single-beam infrared spectrophotometer*
In the single-beam system, the radiation is emitted by the source through the sample and then through a fixed prism and a rotating Littrow mirror. Both prism and Littrow mirror select the desired wavelength and then allow it to pass on to the detector. The detector measures the intensity of radiation after it passes through the sample. Knowing the original intensity of radiation, one can measure how much radiation has been absorbed. By measuring the degree of absorption of wavelengths, the absorption spectrum of the sample can be obtained.

Disadvantages

The various disadvantages of a single-beam spectrophotometer are as follows:

1. This type of instrument has a basic disadvantage in that the intensity of the emission of the radiation source varies from point to point in IR absorption spectrum; therefore, the resulting spectrum is considerably deformed. The necessary correction by the continuous variation of slit is cumbersome.

2. When the sample is analysed in solution, the bands of solvent appear in the spectrum. In this case, the spectrum of the sample is obtained by subtracting the spectrum of the solvent from the resultant spectrum, the former must be recorded under identical conditions (thickness of layer, etc.)

In order to overcome the above mentioned difficulties, a double-beam spectrophotometer is used.

DOUBLE-BEAM INFRARED SPECTROMETER

The essential features of double-beam infrared spectrometer (Figure 11.30a, b) are described briefly.

Radiation source The source of radiation in a typical infrared spectrometer is a small ceramic rod, heated electrically in the range 1100–1800°C and is made of either silicon carbide
(Glowbar) or Nernst filament (a high-resistance, brittle element composed of mixture of a sintered oxides of zirconium, thorium and cerium held together by a binding material). The radiation is divided into two beams, one of which passes through the sample while the other functions as a reference beam. The reference and the sample beams are then passed alternately into a monochromator at very short intervals by means of a rotating mirror.

**Figure 11.30a** Schematic diagram of double-beam infrared spectrophotometer

1. **Absorption cells and sample preparation.** The cells generally employed are made up of rock salt or potassium bromide (glass and quartz cells are unsuitable as these materials themselves absorb IR radiation). A compound (0.55-mg)
may be examined in solution in a suitable minimum absorbing solvent (CCl$_4$, CHCl$_3$, etc.) as a liquid film. In the case of solids which are not soluble in these solvents the compound can be examined as a mull in nujol or as a pellet obtained by pressing the sample in a hydraulic press.

2. Monochromator The pulse beam enters the monochromator through an entrance slit and is dispersed by a grating or by a Littrowmount prism. In the monochromator, the emergent beams are sorted out into individual wavelengths by means of a sodium chloride prism, which is transparent to infrared radiation throughout the range 4000–650 cm$^{-1}$. Lithium fluoride has more favourable dispersion properties than sodium chloride at high wave numbers but is not transparent below 1000 cm$^{-1}$. In order to extend the operating range below 650 cm$^{-1}$, prisms made up of potassium bromide (transparent up to 400 cm$^{-1}$) and caesium iodide (transparent up to 200 cm$^{-1}$) are also used.

In high resolution rating instruments, a filter is used to reject radiation of unwanted orders.

Detector, Amplifier and Recorder The pulsating single beam now emerging through the slit is a narrow band consisting of only a very few frequencies. After dispersion, the beams are focused alternately at each particular wavelength throughout the spectral range, by means of a mirror system on to the detector, usually a sensitive fast thermocouple. The signals from this are amplified electronically. The spectrum, which is really a measure of the difference in intensities of the reference and sample beams throughout the wavelength range, is recorded on a special graph paper mounted on a rotating drum.

Applications

Infrared spectroscopy is an ideal and rapid method for measuring certain contaminants in foodstuffs. It is also widely used in biochemical research to identify metabolic intermediates, drug metabolites, and environmental pollutants. Further, infrared
Spectroscopy is used in the study of photosynthesis and respiration in plants. Infrared spectroscopy is one of the valuable methods for characterizing both qualitatively and quantitatively the multitude of inorganic compounds encountered in research as well as in industry.

**Fourier Transform Infrared Spectroscopy**

Fourier transform infrared spectroscopy is a technique for obtaining high-quality infrared spectra by mathematical conversion of an interference pattern into a spectrum.

**Principle**

Infrared radiations consisting of all wavelengths (e.g., 5000–400 cm⁻¹) is split into two beams, which recombine after a path difference has been introduced (one beam is of fixed length and the other is of variant path length). A condition is, therefore, set up under which interference between the beams can occur. When the difference in the corresponding wavelengths is an integral multiple of the invariant beam, the interference is constructive; destructive interference occurs when the difference is an odd integer multiple of one quarter of the wavelength. The result of a complete variation of wavelengths is an oscillatory series of destructive and constructive combinations. This is called interferogram. Fourier transformation converts this interferogram from the time domain into one spectral point on the more familiar form of the frequency domain. Similar transformation at successive points throughout this variation gives rise to the complete IR spectrum.

**Instrumentation**

A simple form of FTIR spectrometer including Michelson interferometer is shown in the Figure 11.31.

It consists of two mutually perpendicular plane mirrors, one of which is fixed (M₁) while the other (M₂) can move along an axis that is perpendicular to its plane. The movable mirror is either moved at a constant velocity or is held at equally spaced points for