SPECTROSCOPY

Spectroscopy is the branch of science dealing with the study of interaction of electromagnetic radiation with matter. The most important consequence of such interaction is that energy is absorbed or emitted by matter in discrete amounts called quanta. The absorption or emission processes are known throughout the electromagnetic spectrum ranging from the gamma region (nuclear resonance absorption or the Mössbauer effect) to the radio region (nuclear magnetic resonance). The ways in which the measurements of radiation frequency (emitted or absorbed) are experimentally made and the energy levels from these are deduced comprise the practice of spectroscopy.

ELECTROMAGNETIC RADIATION

Light or electromagnetic radiation is a form of energy that is transmitted through space at a constant velocity of $3 \times 10^8 \text{ m/s}$. These radiations are said to have dual nature exhibiting both wave and particle characteristics. The dual character is indeed useful for understanding the interactions of radiations with matter.

Wave Theory of Electromagnetic Radiation

According to this theory, the electromagnetic radiations travel in the form of waves. This wave motion consists of oscillating electric and magnetic fields directed perpendicular to each other and
perpendicular to the direction of propagation of the wave as shown in the Figure 11.1.

\[ \lambda = \text{Wavelength} \]
\[ E = \text{Amplitude of electric field} \]
\[ M = \text{Amplitude of magnetic field} \]

**Figure 11.1** The electric and magnetic components of electromagnetic radiation

In spectroscopic studies, the effects associated with the electrical component of the electromagnetic wave are important. The propagation of vibrations in the electrical field only is shown in the Figure 11.2.

**Figure 11.2** Propagation of vibration in the electric field of electromagnetic radiation

The points X, Y, P, and Q on the wave represent the maximum disturbances in the electric field. The distance from the mean position is known as the amplitude of the wave. The distance from the crest X to crest Y (or from valley P to valley Q) is the wavelength \( \lambda \). The number of complete wavelength units passing through a given point per second is called frequency \( v \). These two quantities are related to each other, and given

\[ v = \frac{c}{\lambda} \]  

(1)
where \( c \) is the velocity of the electromagnetic wave. Since \( c \) is constant \((3 \times 10^8 \text{ ms}^{-1} \text{ in vacuum})\) for all types of electromagnetic radiations, the above relation may be expressed as

\[
\nu \propto \frac{1}{\lambda}
\]

(2)

Reciprocal of wavelength, i.e., \( \frac{1}{\lambda} \) is called wave number, \( \bar{\nu} \).

Hence equation 1 may be written as,

\[
\nu = c\bar{\nu}
\]

(3)

The wavelength is expressed in terms of centimetre (cm), metre (m), micron (\( \mu \)) or micrometre (\( \mu \)m) or angstrom (Å) units. The other commonly used unit is nanometer (nm) where \( 1 \text{ nm} = 10^{-9} \).

Frequency is measured as cycles per second (cps) called Hertz (Hz) or kilocycles per second (kHz) or megacycles per second (MHz).

\[
1 \text{ kHz} = 10^3 \text{ Hz}
\]
\[
1 \text{ MHz} = 10^6 \text{ Hz or cps}
\]

The wave number is the number of waves per unit distance and is expressed in the units of \( \text{cm}^{-1} \) called \textbf{Kaysers} (K). Sometimes kilokaysers (kK) is also used.

\[
1 \text{kK} = 1000 \text{ K} = 1000 \text{ cm}^{-1}
\]

**Quantum Theory of Electromagnetic Radiation**

The quantum theory describes electromagnetic radiation as one consisting of a stream of energy packets called photons or quanta, which travel in the direction of propagation of the beam with the velocity of light. Orbital electrons accept only those radiations which have the exact amount of energy to push it into the permitted next higher energy level. In other words, it can absorb radiations of only a particular wavelength, which provides exact requirement of energy. Hence, the absorption spectrum is always specific for a substance. During the emission or absorption of light by chemical substances, the energy changes take place directly, always as integral multiples of small units of energy, i.e., photon. The energy
$E$ of photon is proportional to the frequency of radiation and is given by the equation,

$$E = h\nu$$  \hspace{1cm} (4)

where $h$ is the Planck's constant ($6.625 \times 10^{-34}$ erg/second; $6.625 \times 10^{-34}$ joule/second). The energy of photon is called quantum of energy.

By substituting $\frac{c}{\lambda}$ for $\nu$ as expressed in equation 1, equation 4 can be alternatively expressed as

$$E = h\nu = \frac{hc}{\lambda}$$

By substituting $c\bar{\nu}$ for $\nu$ as expressed in equation 3, equation 4 can be alternatively expressed as.

$$E = hc\bar{\nu}$$

**Electromagnetic Spectrum**

The electromagnetic spectrum for most spectroscopic purposes is considered to be consisting of the region of radiant energy ranging from wavelengths of 10 metres to $1 \times 10^{-12}$ centimetres. When a molecule absorbs electromagnetic radiation, it can undergo various types of excitation. This excitation may be electronic excitation, rotational excitation, excitation leading to a change in nuclear spin, excitation resulting in bond deformation and so on. Vacuum UV, UV, visible and near infrared ranges of spectrum are produced due to transitions which occur at the valence electron level. Far infrared range of spectrum is produced due to molecular vibration and rotation. If the energy available approaches the ionization potential of the molecule, an electron may be ejected and ionization may occur. Since each mode of excitation requires a specific quantity of energy, different absorptions appear in different regions of the electromagnetic spectrum. The various regions of electromagnetic spectrum are shown in the Table 11.1.
### Table 11.1  Different regions of the electromagnetic spectrum

<table>
<thead>
<tr>
<th>Type of radiation</th>
<th>Wavelength</th>
<th>Wave number</th>
<th>Type of molecular spectrum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radio frequency</td>
<td>&gt; 100 mm</td>
<td>&lt; 3 x 10⁸ Hz</td>
<td>NMR (spin orientations)</td>
</tr>
<tr>
<td>Microwave</td>
<td>1 to 100 mm</td>
<td>10⁻² to 0.1 cm⁻¹</td>
<td>Rotational</td>
</tr>
<tr>
<td>Far-IR</td>
<td>50 μm to 1 mm</td>
<td>200 to 10 cm⁻¹</td>
<td>Vibrational fundamentals or rotational</td>
</tr>
<tr>
<td>Mid-IR</td>
<td>2.5 to 50 μm</td>
<td>4000 to 667 cm⁻¹</td>
<td>Vibrational fundamentals</td>
</tr>
<tr>
<td>Near-IR</td>
<td>780 nm to 2.5 μm</td>
<td>(13 to 4) x 10⁹ cm⁻¹</td>
<td>Vibrational (overtones)</td>
</tr>
<tr>
<td>Visible</td>
<td>380 to 780 nm</td>
<td>(2.6 to 1.3) x 10⁹ cm⁻¹</td>
<td>}</td>
</tr>
<tr>
<td>Near UV</td>
<td>200 to 380 nm</td>
<td>(5 to 2.6) x 10⁹ cm⁻¹</td>
<td>Electronic (valence orbitals)</td>
</tr>
<tr>
<td>Vacuum UV</td>
<td>10 to 200 nm</td>
<td>10⁸ to 5 x 10⁹ cm⁻¹</td>
<td>}</td>
</tr>
<tr>
<td>X-rays</td>
<td>10 pm to 10 nm</td>
<td>10⁷ to 10⁸ cm⁻¹</td>
<td>Electronic (core orbitals)</td>
</tr>
<tr>
<td>Gamma rays</td>
<td>10⁻¹⁶ cm</td>
<td>10¹⁸ cm⁻¹</td>
<td>}</td>
</tr>
<tr>
<td>Cosmic rays</td>
<td>10⁻¹² cm</td>
<td>10¹⁰ cm⁻¹</td>
<td>Mossbauer (Nuclear transitions) (excited states of nuclei)</td>
</tr>
</tbody>
</table>

The major characteristics of various spectrum regions are outlined as follows:

1. **γ-ray region**  The γ-rays are short waves emitted by atomic nuclei involving energy changes of 10⁹ to 10¹¹ Joules/gram atom.
2. **X-ray region**  X-rays emitted or absorbed by movement of electrons close to the nuclei of relatively heavy atoms, involve energy changes of the order of 10000 kilo Joules.
3. **Visible and ultraviolet region**  This region further consists of vacuum ultraviolet, ultraviolet and visible regions. The distinction
between vacuum ultraviolet and ultraviolet is made because air and quartz start absorbing below 180 nm. Radiations, which we call light, form the visible region of the electromagnetic spectrum.

4. **Infrared region** This has been further divided into near infrared, mid infrared and far infrared regions. All the three subregions of the infrared part of the electromagnetic spectrum are associated with the changes in the vibration of molecules and distinction between them is due to difference in instrumentation.

5. **Microwave region** This region corresponds to changes in the rotation of molecules.

6. **Radio frequency region** The energy change involved in this region arises due to the reversal of a spin of nucleus or electron.

When a molecule absorbs radiation, its energy increases in proportion to the energy of the photon. The lowest state of energy of an atom or molecule is called the ground state. By absorbing one quantum of energy, \( h\nu \), the molecule is raised to the next higher level and is said to be in the excited state. Absorption of more energy in integral multiples of \( h\nu \), will result in further excitation to higher energy levels.

(Study of absorbed radiations from a continuous source that are utilized in raising the internal energy of a molecule constitutes absorption spectroscopy.) After the absorption of energy, \( \text{the excited species returns to the ground state by emitting this energy as radiations. The study of this emitted radiation constitutes emission spectroscopy} \)

The internal energy of a molecule may be regarded as the sum of the translational, rotational, vibrational and electronic energies. The kinetic energy component due to free motion of molecules through space is called translational energy, which is not of concern in molecular spectroscopy. Rotational energy is associated with the rotational motion of a molecule as a whole. The energy component associated with the vibration of the constituent atoms in the molecule is called vibrational energy. Electronic energy is associated with the motion of electrons.
TYPES OF SPECTROSCOPY

Absorption of photons by the molecules may change its internal energy (electronic, vibrational or rotational energy) or may cause transitions between different spin orientations of nuclei in the magnetic field. It is thus possible to affect a change in a particular type of molecular energy using appropriate frequency wavelength or wave number of the incident radiation. However, the spectra may result from transitions in which more than one type of molecular energy changes. For example, electronic spectra arise from transitions between electronic energy levels accompanied by changes in both vibrational and rotational states. Although almost all parts of the electromagnetic spectra are used for studying matter, the following types of spectroscopy are in common use:

1. γ-ray spectroscopy
2. Mössbauer spectroscopy
3. X-ray spectroscopy
4. Colorimetry
5. Ultraviolet and Visible spectroscopy (UV-VIS)
6. Electronic micromotility meter
7. Flame photometry
8. Nephelometry and turbidimetry
9. Fluorimeter and phosphorimeter
10. ORD and CD spectroscopy
11. Infrared spectroscopy (IR)
12. Raman spectroscopy
13. Electron Spin Resonance spectroscopy (ESR)
14. Nuclear Magnetic Resonance spectroscopy (NMR)
15. Atomic spectroscopy
16. Mass spectrometry (MS)

γ-RAY SPECTROSCOPY

γ-rays are of nuclear origin, but they are also part of electromagnetic spectrum. Due to their considerable penetrating power, their main
the help of the Oxford crystallographer, Dorothy Hodgkin, Franklin described the helical backbone and correct crystallographic space group for DNA. Her work was instrumental in Watson's and Crick's correct modelling of DNA, for which they received the Nobel Prize.

X-Ray Crystallography

It is a technique in crystallography, in which the pattern produced by the diffraction of X-rays through the closely spaced lattice of atoms in a crystal is recorded and then analysed to reveal the nature of that lattice. This generally leads to an understanding of the material and molecular structure of a substance. The spacings in the crystal lattice can be determined. The electrons that surround the atoms, rather than the atomic nuclei themselves, are the entities which physically interact with the incoming X-ray photons. This technique is widely used in chemistry and biochemistry to determine the structures of immense variety of molecules, including inorganic compounds, DNA and proteins. X-ray diffraction is commonly carried out using single crystals of a material, but if these are not available, microcrystalline powdered sample may also be used, although this requires different equipment.

COLORIMETRY

Colorimetry is a form of photometry, which deals with measurement of light absorption by coloured substances in solutions. The instrument which measures the intensity of the colour is known as colorimeter.

Principle

The colorimeter is one of the most widely used instruments in biological research. It is based on the principle that when a beam of incident light passes through a coloured solution, the coloured substances in the solution absorb a part of light and hence, the intensity of the transmitted light is always less than that of the incident light. As the number of light-absorbing molecules increases,