Chemistry For Biologist

Presented By-
Swati G. Swami
Assist. Professor
R. S. M. L.

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- Electromagnetic radiation
- Classification of Methods
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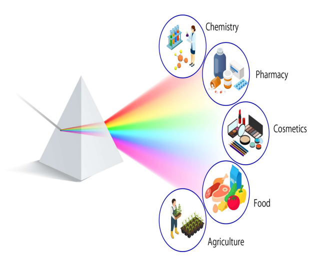
Spectroscopy

- It is a branch of science that deals with interaction of matter with light or electromagnetic radiation.

OR

- It is a branch of science in which electromagnetic radiation of particular wavelength or range of wavelength is used for qualitative and quantitative analysis of matter.
- Matter – anything which occupies space i universe and having mass is called matter.



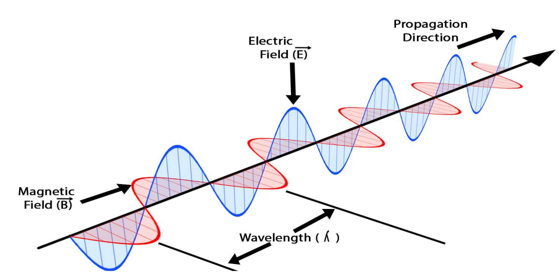
Spectrometry:

- It is the measurement of these responses and an instrument which performs such measurements is a spectrometer or spectrograph, although these terms are more limited in use to the original field of optics from which the concept sprang.
- Spectroscopy is often used in physical and analytical chemistry for the identification of substances through the spectrum emitted from or absorbed by them.
- Spectroscopy is also heavily used in astronomy and remote sensing. Most large telescopes have spectrometers, which are used either to measure the chemical composition and physical properties of astronomical objects or to measure their velocities from the Doppler Shift of their spectral lines.

Electromagnetic radiation

- EM is a form of energy that is all around us
- EM is a form of energy and has both electrical and magnetic characteristics
- Electricity and magnetism were once thought to be separate forces
- James clerk maxwell developed a unified theory of electromagnetism
- The study of electromagnetism deals with how electrically charged particles interact with each other and with magnetic fields
- The electric and magnetic fields in n electromagnetic wave oscillate along directions perpendicular to the propagation direction of the wave

Electromagnetic Wave



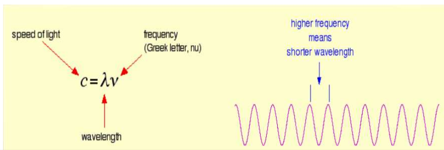
Various terms...

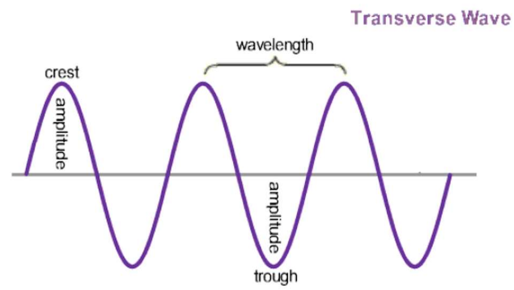
- Frequency :- number of waves produced each second (measured in Hz).
- Wavelength (λ) :- the distance between two successive waves (measured in m).
- Amplitude :- is the maximum distance a wave extends beyond its middle position.
- Relation between frequency and wavelength – velocity (speed) of propagation

$$C = \upsilon \lambda$$

Where,

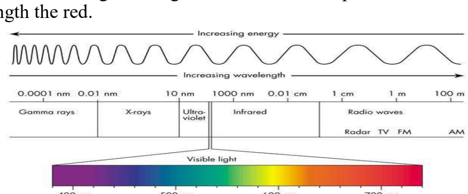
C = 2.99792 x 108 m/s

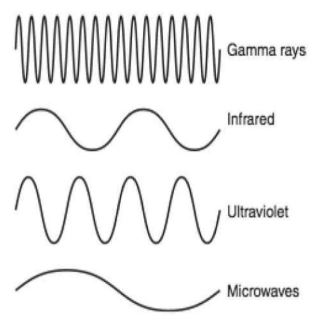




Electromagnetic spectrum

- Electromagnetic spectrum ranges from very short wavelength (gamma rays) to very long wavelengths (radio waves)
- The visible region of the spectrum extends approximately over the wavelength range 400-700nm
- The shorter wavelengths being the blue end of the spectrum and the longer wavelength the red.





Which kind of wave has the greatest frequency?

Classification of Methods

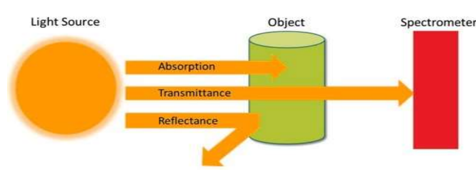
- The type of spectroscopy depends on the physical quantity measured.
- Normally, the quantity that is measured is an intensity, either of energy absorbed or produced.
- Most spectroscopic methods are differentiated as either atomic or molecular based on whether or not they apply to atoms or molecules.
- Along with that distinction, they can be classified on the nature of their interaction:

1) Absorption spectroscopy
2) Emission spectroscopy
3) Scattering spectroscopy

1) Absorption spectroscopy:

It uses the range of the electromagnetic spectra in which a substance absorbs.

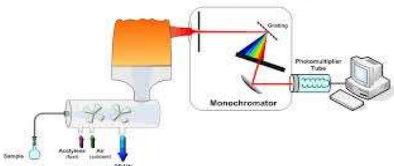
This includes atomic absorption spectroscopy and various molecular techniques, such as infra-red spectroscopy in that region and Nuclear Magnetic resonance spectroscopy in the radio region.



2) Emission spectroscopy:

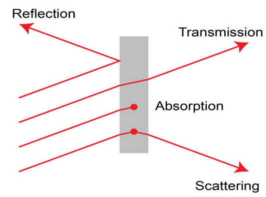
- It uses the range of electromagnetic spectra in which a substance radiates (emits).
- The substance first must absorb energy. This energy can be from a variety of sources, which determines the name of the subsequent emission, like luminescence. Molecular luminescence techniques include spectroflourimetry.

Atomic Emission Spectroscopy



3) Scattering spectroscopy:

- It measures the amount of light that a substance scatters at certain wavelengths, incident angles, and polarization angles.
- The scattering process is much faster than the absorption/emission process. One of the most useful applications of light scattering spectroscopy is Raman Spectroscopy.

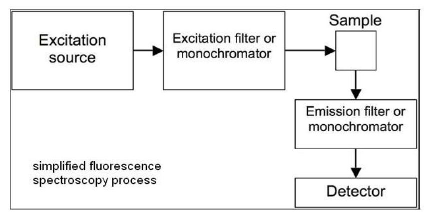


Common types

- Fluorescence spectroscopy
- X-ray spectroscopy and crystallography
- Flame spectroscopy 1- Atomic emission spectroscopy 2- Atomic absorption spectroscopy 3- Atomic fluorescence spectroscopy
- Plasma emission spectroscopy
- Spark or arc emission spectroscopy
- UV/VIS spectroscopy
- IR spectroscopy
- Raman spectroscopy
- NMR spectroscopy
- Photo thermal spectroscopy
- Thermal infra-red spectroscopy
- Mass Spectroscopy

1- **Fluorescence spectroscopy**:

Fluorescence spectroscopy uses higher energy photons to excite a sample, which will then emit lower energy photons. This technique has become popular for its biochemical and medical applications.



2- **X-ray spectroscopy:**

X-rays of sufficient frequencies interact with material and excite the atoms contained. Due to this excitation Auger Effect is produced and some excitation radiations are absorbed or evolved if vice versa occurs.

X-ray absorption and emission spectroscopy is used in chemistry and material sciences to determine elemental composition and chemical bonding.

Very good and versatile technique but a little complex. It needs some scattering light detectors along with X-ray source. Overall X-ray diffraction technique is one that is used most widely for bond length and angle measurements.

3- **Flame Spectroscopy**:

Liquid solution samples are aspirated into a burner or nebulizer/burner combination, desolvated, atomized, and sometimes excited to a higher energy electronic state. The use of a flame during analysis requires fuel and oxidant, typically in the form of gases. Common fuel gases used are acetylene (ethyne) or hydrogen. Common oxidant gases used are oxygen, air, or nitrous oxide. These methods are often capable of analyzing metallic element in the PPM, billion, or possibly lower concentration ranges. Light detectors are needed to detect light with the analysis information coming from the flame.

- Atomic absorption Spectroscopy
- Atomic emission spectroscopy
- Atomic fluorescence spectroscopy

4- Spark or arc (emission) spectroscopy :

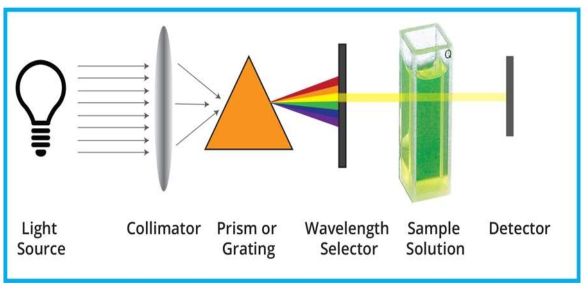
It is used for the analysis of metallic elements in solid samples.

For non-conductive materials, a sample is ground with graphite powder to make it conductive.

In traditional arc spectroscopy methods, Since the conditions producing the arc emission typically are not controlled quantitatively, the analysis for the elements is qualitative. Nowadays, the spark sources with controlled discharges under an argon atmosphere allow that this method can be considered eminently quantitative, and its use is widely expanded worldwide through production control laboratories of foundries and steel mills.

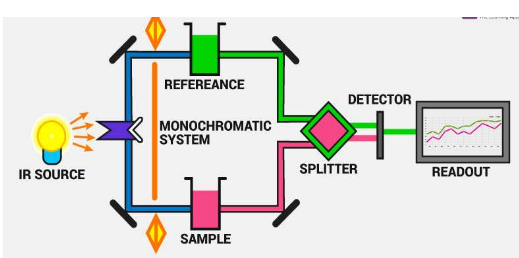
5- **UV/VIS spectroscopy:**

- It basically involves the spectroscopy of photons and spectrophotometery.
- It uses light in the visible and adjacent near ultraviolet (UV) and near infrared (NIR) ranges.
- UV/Vis spectroscopy is routinely used in the quantitative determination of solutions of transition metal ions and highly conjugated organic compounds.
- For the quantitative measurements, Beer-Lambert law is followed.
- The Beer-Lambert Law is useful for characterizing many compounds but does not hold as a universal relationship for the concentration and absorption of all substances. A 2nd order polynomial relationship between absorption and concentration is sometimes encountered for very large, complex molecules such as organic dyes.



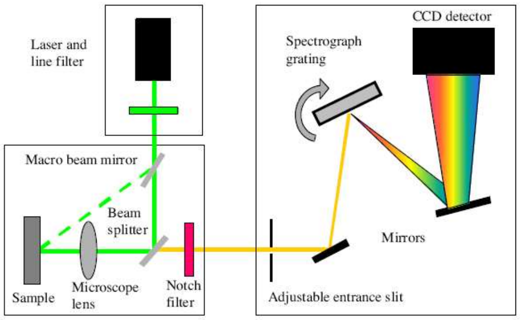
6- Infra-red Spectroscopy:

- (IR spectroscopy) is the subset of spectroscopy that deals with the infrared region of the electromagnetic spectrum. It covers a range of techniques, the most common being a form of absorption spectroscopy. As with all spectroscopic techniques, it can be used to identify compounds or investigate sample composition.
- Infrared spectroscopy offers the possibility to measure different types of inter atomic bond vibrations at different frequencies. Especially in organic chemistry the analysis of IR absorption spectra shows what type of bonds are present in the sample.
- Infrared spectroscopy exploits the fact that molecules have specific frequencies at which they rotate or vibrate corresponding to discrete energy levels.



7- Raman Spectroscopy:

- It relies on inelastic scattering, or Raman scattering of monochromatic light, usually from a laser in the visible, near infrared, or near ultraviolet range. The laser light interacts with phonons or other excitations in the system, resulting in the energy of the laser photons being shifted up or down. The shift in energy gives information about the phonon modes in the system.
- Spontaneous Raman scattering is typically very weak, and as a result the main difficulty of Raman spectroscopy is separating the weak inelastically scattered light from the intense Rayleigh scattered laser light. Raman spectrometers typically use holographic diffraction gratings and multiple dispersion stages to achieve a high degree of laser rejection.



8- Nuclear magnetic resonance:

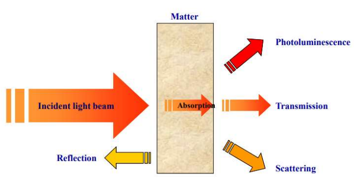
Nuclear magnetic resonance spectroscopy analyzes the magnetic properties of certain atomic nuclei to determine different electronic local environments of hydrogen, carbon, or other atoms in an organic compound or other compound. This is used to help determine the structure of the compound.

9- Photo thermal spectroscopy:

It is a group of high sensitivity spectroscopy techniques used to measure optical absorption and thermal characteristics of a sample. The basis of photo-thermal spectroscopy is the change in thermal state of the sample resulting from the absorption of radiation. Light absorbed and not lost by emission results in heating. The heat raises temperature thereby influencing the sample thermodynamic properties. Measurement of the temperature, pressure, and/or density changes that occur due to optical absorption are ultimately the basis for the photo-thermal spectroscopic measurements.

Principles of Spectroscopy

- If matter is exposed to electromagnetic radiation, e.g. infrared light, the radiation can be absorbed, transmitted, reflected, scattered or undergo photoluminescence.
- Photoluminescence is a term used to designate a number of effects, including fluorescence, phosphorescence, and Raman scattering.



Application of Spectroscopy

1. Spectroscopy is the important detector system in advanced methods like HPLC, HPTLC etc.
2. It is also important and a main detector system in multi sample analyzer instruments like Elisa plate reader, micro-plate reader, auto-analyzers etc.
3. It is also a part of continuous culture broths like in fermentation tanks to keep the concentration of microbes or any chemical substance at a constant and helps regulate the rate of addition or deletion into the tank.
4. It is useful to determine bio molecules like corticosteroids, testosterone, aldosterone etc.
5. It is also useful in analysis of phyto-chemicals like glycosides, tannins, alkaloids etc.
6. It is also useful in determination of inorganic substance like Fe, Mg, Ca, Cu and other salts and their derivatives. Further chemicals like potassium permanganate, Ferrous sulphate etc.