

Unit IV: Instrumental Methods and Applications

Introduction to spectroscopy—types of energy present in molecules, types of spectra, UV-Vis spectroscopy – principle, types of electronic transitions, chromophore, auxochrome, Bathochromic shift, Hypsochromic shift, Instrumentation of UV-Vis spectrophotometer, applications; Infrared spectroscopy – principle, types of vibrational modes, group frequencies, Instrumentation of IR spectrophotometer, applications. Principle and applications of physicochemical methods (SEM, TEM, X-ray diffraction).

Spectroscopy is the study of interaction of matter and electromagnetic radiation.

Electromagnetic radiation:- A kind of radiation including visible light, radio waves, gamma rays, and X-rays, in which electric and magnetic fields vary simultaneously. This electromagnetic radiation has a dual nature. It has the properties of both particles and waves. The particles are called as photons and each photon possesses certain amount of energy called as a quantum. The energy (ΔE) of a photon is directly proportional to its frequency (γ).

$$\Delta E \propto \gamma = h \gamma = \frac{hc}{\lambda}$$

E = energy of one photon, h = plank's constant - its value is 6.625×10^{-34} erg sec or 6.625×10^{-34} joule sec, γ = frequency, λ = wavelength, C = speed of light (3×10^{10} cm/sec)

The energy of an electromagnetic radiation is directly proportional to frequency (γ) and inversely proportional to wavelength (λ).

Wave number ($\tilde{\gamma}$) is the number of waves in one cm. It is expressed in units of cm^{-1} and it is reciprocal of the wavelength.

$$\text{Wave number } (\tilde{\gamma}) = \frac{1}{\lambda}$$

$$E = hc\tilde{\gamma}$$

Hence the energy of an electromagnetic radiation is directly proportional to its wave number. _____

TYPES OF ENERGY PRESENT IN MOLECULES

If E is the energy of a molecule, it can be expressed as: $E = (E_{trans}) + (E_{rot}) + (E_{vib}) + (E_{ele})$

- i. **Translational Energy (E_{trans})** is concerned with the overall movement of the molecules along the three axes. It is significant only in gases and to a lesser extent for liquids.
- ii. **Rotational energy (E_{rot})** involves the spinning of molecules about the axes passing through their centre of gravity.
- iii. **Vibrational energy (E_{vib})** is associated with vibrations within a molecule such as the stretching or the bending of bonds.
- iv. **Electronic energy (E_{ele})** involves promotion of electrons to higher levels on absorption of energy

Except translational energy levels of a molecule the other three forms of energy are quantized. The magnitude of the various forms of these energies and the difference in energy levels vary considerably. _____

Difference between the energy levels for:

- Electronic energy (E_{ele}) change = About $10^1 - 10^4$ kJ / mol
- Vibrational energy (E_{vib}) change = About 1 – 100 kJ / mol
- Rotational energy (E_{rot}) change = About 0.01 – 1 kJ / mol

TYPES OF SPECTRA:

The two types of spectra can be seen in spectroscopy.

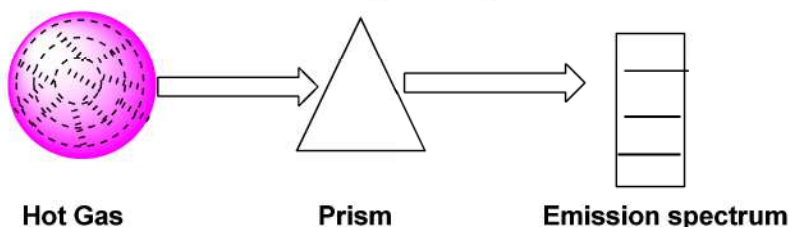
i) Emission spectra.

ii) Absorption spectra.

(i) Emission spectra:

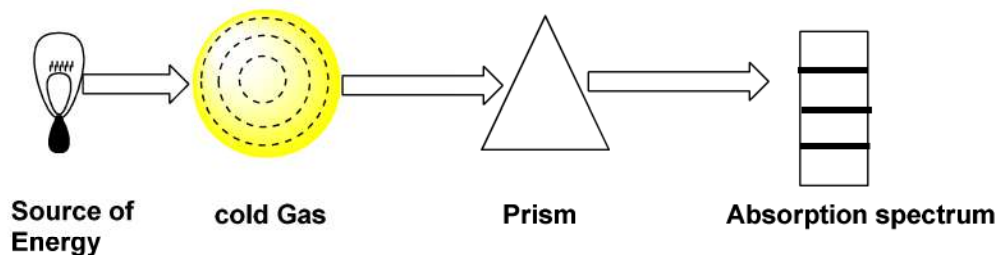
- Molecules give emission spectra when subjected to intense heat or electric discharge.
- In this situation the molecules get excited by obtaining the necessary energy and these excited states are unstable and as a result, the molecules return to their lower energy state, by emitting radiation in the form of photon and the corresponding frequency is recorded as the emission spectrum.
- If the transition is from upper energy level (E_2) to lower energy level (E_1), the frequency (ν) of the emission spectrum is given by:

$$\nu = \frac{E_2 - E_1}{h} = \frac{\Delta E}{h}$$



(ii) Absorption Spectra:

- When a substance is irradiated with electromagnetic radiation, the molecules may be transferred from the ground state to the excited state, by absorbing the incident photons.
- This process is known as absorption and the resultant spectrum is known as absorption spectrum.



- Energy absorption occurs only when the energy difference between the ground state and higher energy level is exactly matched by the energy of the incident electromagnetic radiation. The molecular spectra are more complicated than atomic spectra, because they cover wider regions of the electromagnetic spectrum and their interpretation is often more difficult.
- The energy absorbed (ΔE) by a molecule may bring about changes in one or more of its energy levels such as rotational, vibrational and electronic.
- Absorption spectra helps to elucidate the structure of molecules.
- The absorption spectra further divided into three different types on the basis of the radiation absorbed.
 - a) Microwave absorption spectra
 - b) Infrared absorption spectra
 - c) Ultraviolet and Visible absorption spectra

	Microwave	Infrared	Ultraviolet and Visible
Radiation absorbed	0.01 – 1 kJ / mol	1 – 100 kJ / mol	100 – 10 ⁴ kJ / mol
Effect on the molecule	Brings change in <u>rotational energy levels</u> of the molecule.	Brings change in the <u>vibrational and rotational energy levels</u> of the molecule.	Brings change in <u>electronic energy levels</u> within the molecule. <small>Vibrational and rotational transitions are also brought about, but their resolution is not possible in solids and liquid states.</small>
Spectra Information obtained helps	To calculate force constant, bond length, bond angle etc.	<ul style="list-style-type: none"> • For detection of functional groups present in the compounds • For quantitative analysis. • <u>To calculate</u> force constant, bond length bond angle etc. 	For qualitative and quantitative analysis.

Ultraviolet (UV) Spectroscopy

UV spectroscopy is an important tool in analytical chemistry. The other name of UV (Ultra-Violet) spectroscopy is Electronic spectroscopy as it involves the promotion of the electrons from the ground state to the higher energy or excited state and this is the fundamental principle here. UV Spectroscopy primarily used to measure the multiple bond or aromatic conjugation with in molecules.

Electronic Transitions

According to theory of electronic spectroscopy, when the molecule absorbs UV or visible light, its electrons promoted to higher energy levels. i.e. From bonding to anti-bonding molecular orbitals.

When excitation occurs, an electron from one of the filled σ , π or n molecular orbitals get excited to vacant σ^* or π^* anti-bonding molecular orbitals (shown in below fig). The σ electrons requires a high energy for excitation to σ^* .

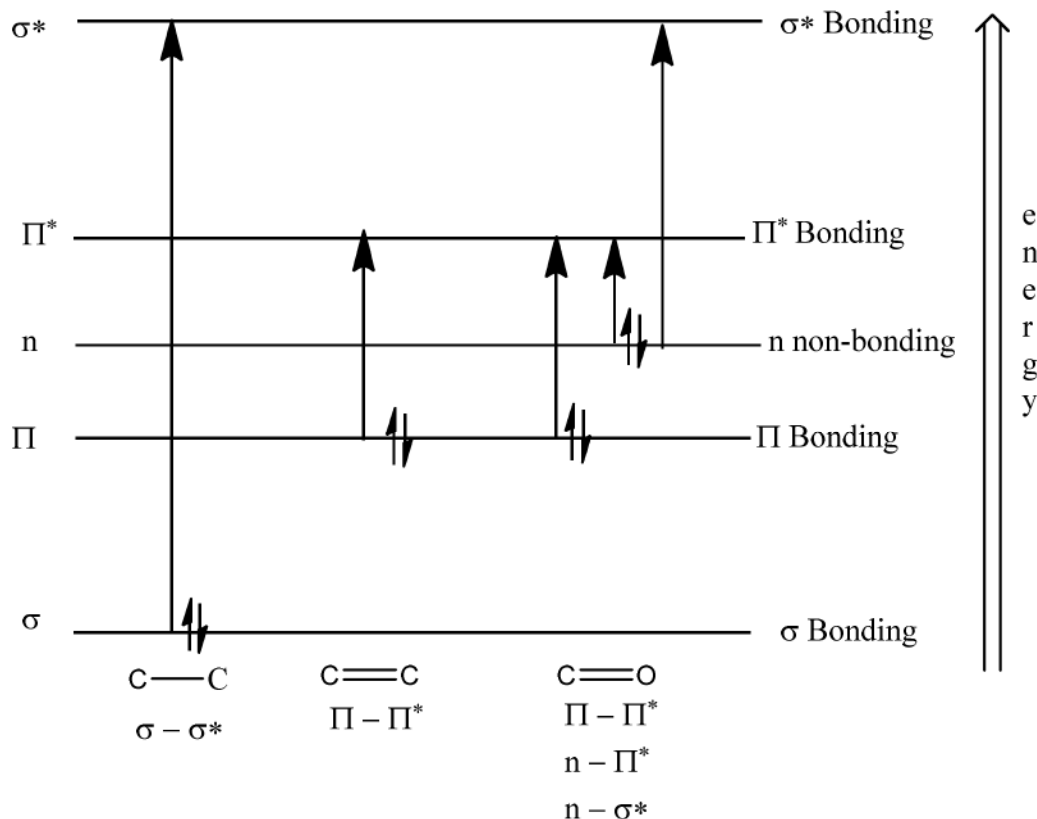


Fig: Relative energies of orbitals and possible transitions between them

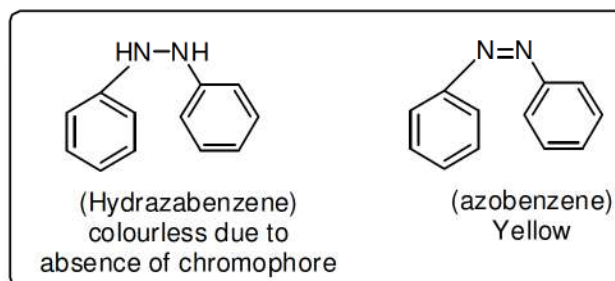
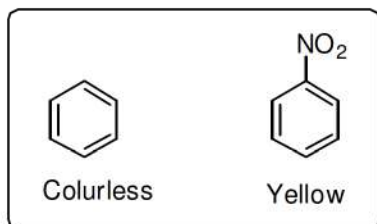
Electronic energy levels $\sigma \rightarrow \sigma^* > n \rightarrow \sigma^* > \pi \rightarrow \pi^* > n \rightarrow \pi^*$

 $\xrightarrow{\hspace{10em}}$ Energy decreases

TRANSITION PROBABILITY:

i) Chromophores:

- i) An unsaturated group responsible for electronic absorption and whose presence gives colour to compound is called chromophore. Exact colour depends upon the wavelength of light absorbed by a compound.
- ii) In another way the part of the molecule having n or π electrons is responsible for absorption and these fragments are known as chromophores. Eg. $\text{C}=\text{C}$, $\text{C}=\text{O}$, $-\text{N}=\text{N}-$, $-\text{C}\equiv\text{N}$, NO_2
- iii) Compounds with chromophore unit are called *chromogen*.
- iv) The Organic compound which exhibits $\pi \rightarrow \pi^*$, $n \rightarrow \sigma^*$ and $n \rightarrow \pi^*$ transitions acts as chromophore.
 - $\pi \rightarrow \pi^*$ transitions occur in ethylene, acetylenes
 - Both $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions occur in $>\text{C}=\text{O}$, $-\text{NO}_2$, $-\text{NO}$

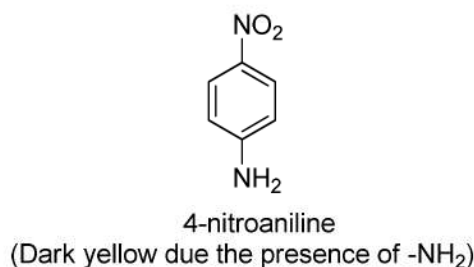
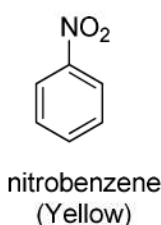


List of some common chromophores:

Chromophore	Example	Type of transition	λ_{max}
	CH_3OH	$n \rightarrow \pi^*$	183
	CH_3Cl	$n \rightarrow \sigma^*$	173
$>\text{C}=\text{C}<$	$\text{H}_2\text{C}=\text{CH}_2$	$\pi \rightarrow \pi^*$	171
$-\text{C}\equiv\text{C}-$	$\text{H}-\text{C}\equiv\text{C}-\text{H}$	$\pi \rightarrow \pi^*$	173

ii. Auxochrome

- An auxochrome can be defined as any group which does not itself acts as a chromophore but whose presence brings (enhancement of colour) about a shift of the absorption band towards the longer wavelength of the spectrum.
- A saturated group with non-bonding electrons attached to chromophore altering both wavelength as well as intensity of absorption.
- Examples: $-\text{OH}$, $-\text{OR}$, $-\text{NH}_2$, $-\text{NHR}$, $-\text{SH}$, $-\text{X}$ etc. having unshared pair of electrons and show absorption.



- The effect of the auxochrome is due to its ability to extend the conjugation of a chromophore by the sharing of non-bonding electrons.

Difference between chromophore & auxochrome

Chromophores are responsible for giving dyes their color by absorbing certain wavelengths of visible light and transmitting or reflecting others.

Auxochromes modify the ability of a chromophore to absorb light. They often result in the deepening and intensifying of the color of compounds.

ABSORPTION SHIFTS:

Bathochromic shift, Hypsochromic shift, are the most important types of shifts observed in the UV spectroscopy. It is due the effect of substituents on a chromophore.

- i. Bathochromic shift (red shift) – a shift to longer λ ; lower energy
- ii. Hypsochromic shift (blue shift) – shift to shorter λ ; higher energy

A) Bathochromic shift-

Shift of absorption maximum towards the longer wavelength due to the presence of an auxochrome **or** change in solvents is called as Bathochromic shift. In general the Bathochromic shift is produced when two or more chromophores are present in conjugation in a molecule.

- Eg.** i) Ethylene shows absorption max at 170 nm, whereas 1,3-butadiene shows max absorption at 217 nm.
- ii) Absorption max of acetone is 280 nm in hexane and 260 nm in water. _____.

B) Hypsochromic shift-

Shift of absorption maximum towards the shorter wavelength is called as Hypsochromic shift. Generally it is caused due to the removal of conjugation or by changing the polarity of the solvents.

Eg. Aniline shows absorption max at 280 nm. It is because by using lone pairs on nitrogen atom shows conjugation with the π bond system of aromatic ring. And anilinium ion is generated in acidic solution; as a result conjugation is removed and results the blue shift at absorption max at 200 nm.

INSTRUMENTATION OF UV-VIS SPECTROPHOTOMETER

Principle of UV spectroscopy

UV spectroscopy obeys the Beer-Lambert law, which states that: when a beam of monochromatic light is passed through a solution of an absorbing substance, the rate of decrease of intensity of radiation with thickness of the absorbing solution is proportional to the incident radiation as well as the concentration of the solution.

The mathematical expression of Beer-Lambert law is $A = \log(I_0/I) = ECL$

I_0 = intensity of light leaving reference cell

I = intensity of light leaving sample cell

C = molar concentration of solute

L = length of sample cell (cm.)

A = absorbance

E = molar absorptivity

Molar absorptivity, is a measure of how well a chemical species absorbs a given wavelength of light.

Instrumentation and working of UV spectroscopy

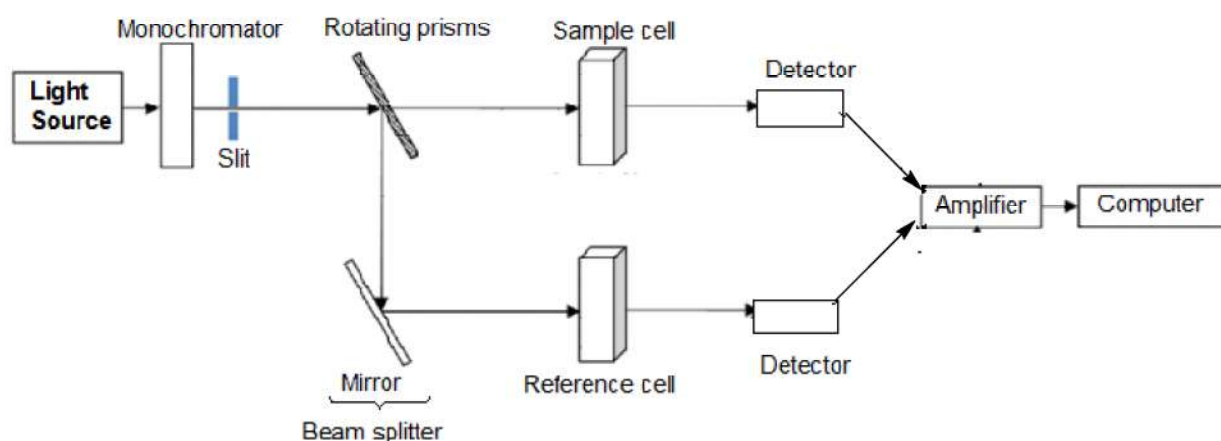
Most of the modern UV spectrometers consist of the following parts.

Light Source:

Tungsten filament lamps and Hydrogen-Deuterium lamps are most widely used and suitable light source as they cover the whole UV region. The electrical excitation of deuterium or hydrogen at low pressure produces a continuous UV spectrum

Monochromator:

Monochromators generally composed of prisms and slits. A beam of monochromatic light is split with the help of beam splitter (rotating prisms) into two equal halves called as sample beam and reference beam.



Block diagram of uv-vis spectrophotometer

Sample and reference cells:

The sample beam is directed through a transparent cell containing a solution of the compound being analyzed and the reference beam is directed through an identical cell that contains only the solvent. These cells are made of either silica or quartz. Glass can't be used for the cells as it also absorbs light in the UV region.

Detector:

Generally two photocells serve the purpose of detector in UV spectroscopy. One of the photocells receives the beam from the sample cell and the second detector receives the beam from the reference. The intensity of the radiation from the reference cell is stronger than the beam of the sample cell. This results in the generation of alternating currents in the photocells.

Amplifier:

The alternating current generated in the photocells is transferred to the amplifier. Generally, the current generated in the photocells is of very low intensity. The amplifier is used to convert this information into a physically measurable information.

Computer / printer:

The amplifier is connected to the computer to store and print the data generated for the desired compound. The instrument gives output graph contains a plot of the wavelength of the entire region versus the absorbance (A) of the light at each wavelength. _____

APPLICATIONS OF UV-VIS SPECTROPHOTOMETER

i) Detection of Impurities:

- a) Additional peaks can be observed due to impurities in the sample.
- b) Further the absorption of the sample solution is compared with the absorption of the reference solution. The intensity of the absorption can be used for the relative calculation of the purity of the sample substance. _____

ii) Identification of an unknown compounds:

The spectrum of unknown compound is compared with the spectrum of a standard compound and if both the spectrums coincide then it confirms the identification of the unknown compound.

iii) Identification of extent of conjugation:

With the increase in double bonds the absorption shifts towards the longer wavelength.

iv) Structure elucidation of organic compounds:

Helps in the structure elucidation of organic molecules by knowing the presence of saturated or unsaturated bonds and hetero atoms. However, UV spectroscopy is not helpful in the detection of individual functional groups _____

v) Quantitative analysis:

Used for the quantitative determination of compounds that absorb UV radiation. This determination is based on Beer's law

vi) Qualitative analysis:

By comparing the absorption spectrum with the spectra of known compounds the quality of the newly synthesized compound can be studied _____

vii) Chemical kinetics:

To study the kinetics of reaction, and in determination of dissociation constants of acids and bases from the change of absorption spectra with pH. _____

viii) Determination of configurations of geometrical isomers:

Cis-isomer suffers distortion and absorbs at lower wavelength as compared to trans-isomer

ix) Detection of Functional Groups:

UV spectroscopy is used to detect the presence or absence of chromophore in the compound. Absence of a band at particular wavelength regarded as an evidence for absence of particular group _____

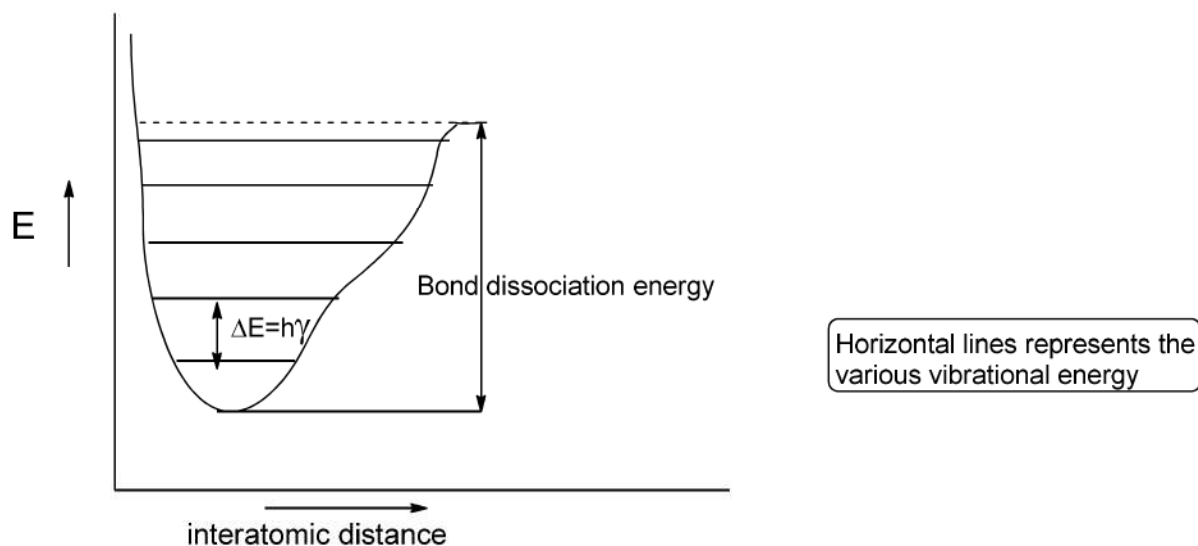
x) **In determining the structure of vitamins:**

It helps in predicting the relation between different groups this helps in determining the structure of several vitamins.

Infrared spectroscopy

Origins of Infrared spectrum (introduction)

IR spectrum is considered as vibrational –rotational spectrum. IR radiation is not enough to produce the excitations of electrons; however, it causes vibrational excitation of the covalent bonds within that molecule. These vibrations are quantized and they occur only when the compound absorbs IR energy in a particular region.



The energy possessed by a molecule at any given moment is defined as the sum of the following contributing energy terms.

$$E_{\text{Total}} = E_{\text{electronic}} + E_{\text{vibrational}} + E_{\text{Translation}}$$

Absorption of appropriate electromagnetic radiation produces different excitations in the molecules. The fundamental requirement for absorption of an IR radiation is that there must be a net change in dipole momentum during the vibration for the molecule or the functional group under study. The change in the dipole moment of the molecule gives the absorption bands in the IR. Otherwise, if no absorption i.e. the net dipole moment is zero, they are said to be IR inactive.

Theory of Molecular vibrations (types of vibrational modes or fundamental frequencies)

The vibrational and the rotational energies of the molecules are increases with the passage of infra-red light through the sample. High energetic radiations change the molecular vibrations and the low energy radiations change the molecular rotational levels.

The energy required to bring the changes in vibrational energies of a molecule depends on its

- i) Molecular weight
- ii) Bond strength
- iii) The arrangement of atoms within the molecule.

There are two types of molecular vibrations 1) stretching vibrations 2) bending vibrations.

- 1) Stretching vibrations: it is a rhythmical moment along the bond axis, such that the inter-atomic distance is increases or decreases but the atoms remains in the same bond axis.
- 2) Bending vibrations: It brings the change in bond angle between bonds and it does not occur along the axis of the bond.

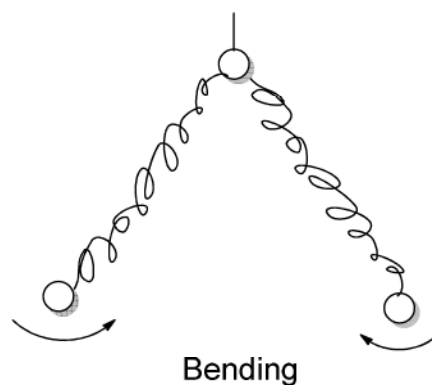
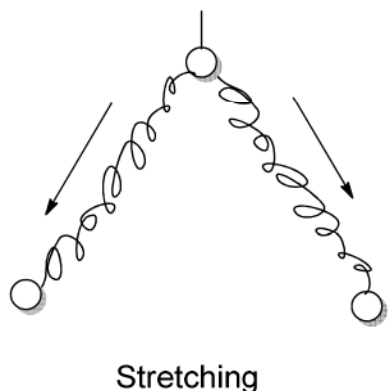
Energy required for Stretching vibrations > Bending vibrations. As the bending vibrations requires lesser energy and hence occur at higher wavelengths as compared to stretching absorption of the same band.

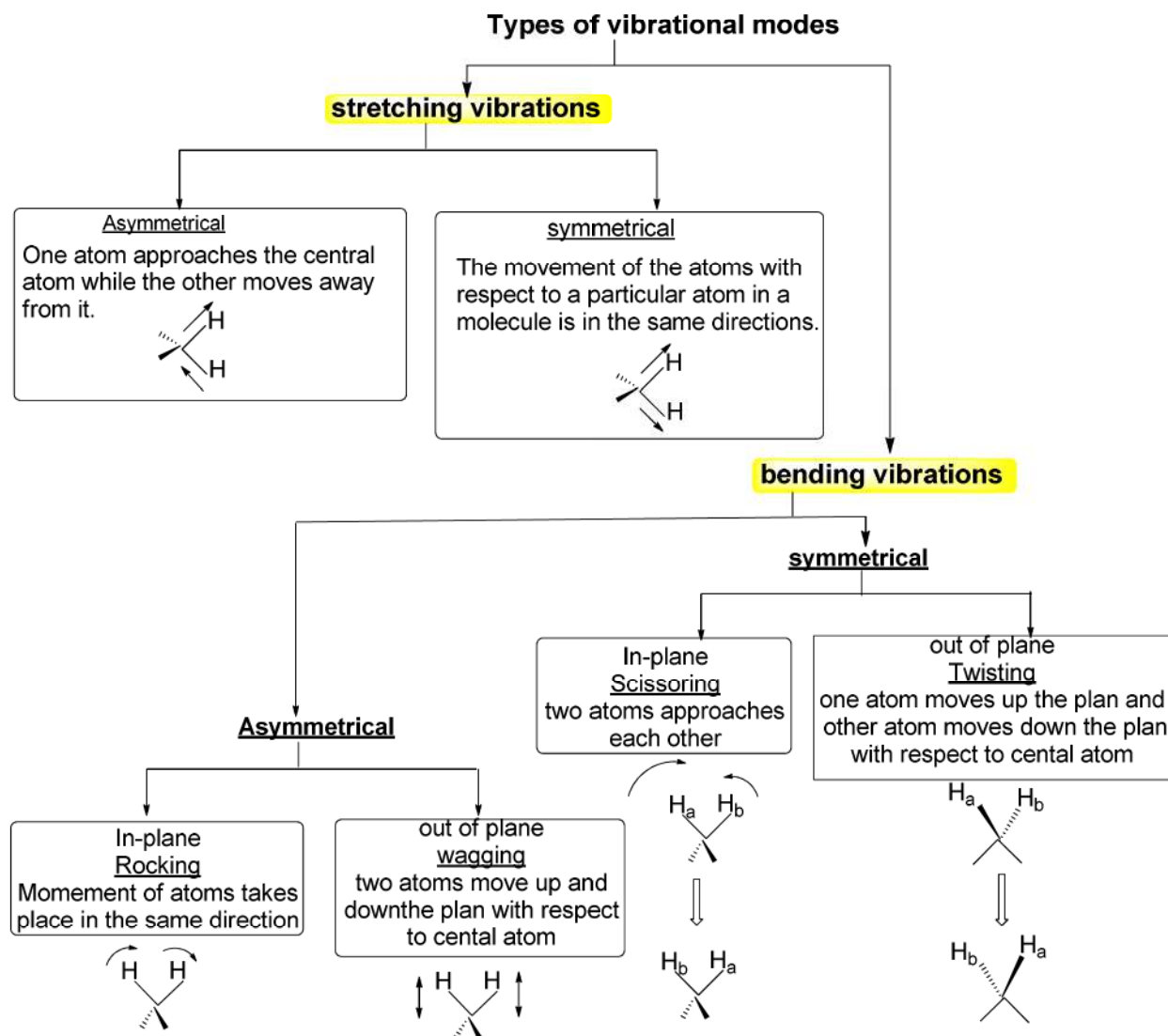
Each stretching and bending vibrations of a molecule occurs with a characteristic frequency. When a compound is exposed to an IR radiation of a frequency the molecules in it will absorb energy that exactly matches the frequency of one of its vibrations.

A diatomic molecule such as HCl and only undergo a stretching vibration.



The vibrations of a molecule containing three atoms are more complex. There are symmetric and asymmetric stretches and bends. Bending vibrations can be either in-plane or out of plane. Bending vibrations are often called as scissor, rock, twist and wag.





GROUP FREQUENCIES

IR spectrum is not a simple but shows several bands. The main reason for this type of complex nature of the IR spectrum is due to the various types of vibration in a molecule. Pentane and Decane shows 45 and 90 possible IR absorption bands respectively. However, the vibrational bands for several functional groups appear at characteristic frequencies. As a result in case of poly atomic molecules *group frequencies* are used for the recognizing the molecular structure and other information.

In the general regions (4000 to 1400 cm^{-1}) of the IR spectrum, various kinds of vibrational frequencies are observed as characteristic of particular functional group in any molecule. Such frequencies are known as 'group frequencies'. The group frequencies remains constant from molecule to molecule, because the value of force constant for a particular bond is constant.

For example: The absorptions at 3050 cm^{-1} and 1700 cm^{-1} in the IR spectrum of acetophenone ($\text{C}_6\text{H}_5\text{COCH}_3$) are typical of stretching modes of C-H (aromatic) and C = O groups respectively. Thus the IR spectra permits recognition of the types of functional groups present in organic molecule.

The characteristic IR absorptions frequencies (cm^{-1}) for different groups:

Nitro compound ($-\text{NO}_2$)	1585-1565	O-H stretching alcohol (free)	3700 - 3584
Phenols ($-\text{OH}$)	3500	O-H stretching alcohol (intermolecular bonded)	3550 - 3200
Aldehyde (aliphatic)	1740-1720	Ketone (aliphatic)	1725 - 1705
Aldehyde (aromatic)	1715-1495	Ketone (aromatic)	1715 - 1695
C - Cl	700 – 600	C – Br	600 -500

IR Instrumentation (IR Spectrometer)

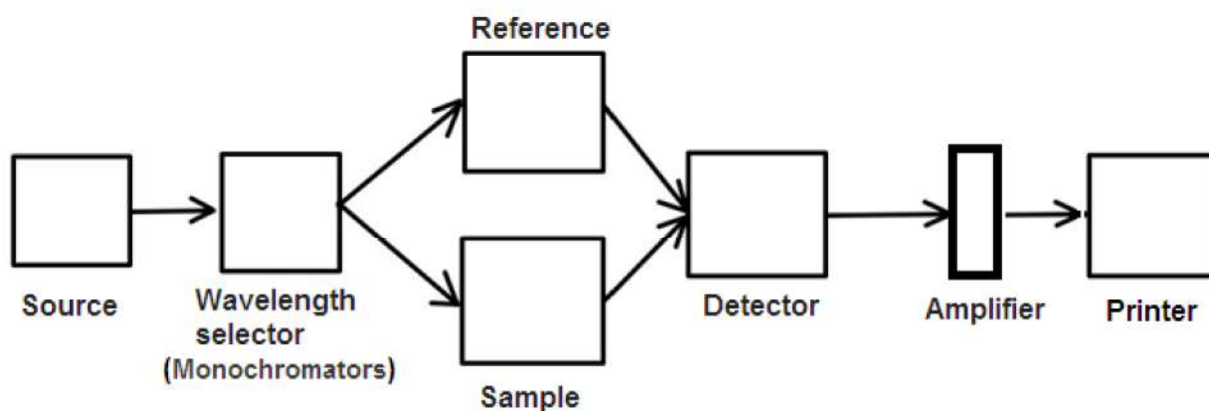
Principle of Infrared Spectroscopy:

The IR spectroscopy theory utilizes the concept that molecules tend to absorb specific frequencies of light that are characteristic of the corresponding structure of the molecules.

The instrumentation of infrared spectroscopy is illustrated below.

IR radiation source:

The Nernst Glower is electrically heated to about $2000\text{ }^\circ\text{C}$ for the production of IR radiation. Nernst Glower composed of a mixture of rare earth oxides such as zirconium oxide (ZrO_2), yttrium oxide (Y_2O_3) and thorium dioxide (ThO_2) sealed by platinum leads to the ends to permit electrical connection. Consist of cylindrical hollow rod or tube having a diameter of 1- 2 mm and length of 30 mm.



Block diagram of typical IR spectrometer

Monochromators:

A device used to select radiation of (or very close to) a single wavelength or energy. Sodium chloride is most commonly prism salt used

Sample:

IR spectroscopy has been used for the characterization of solids, liquids or gas samples. As a result a suitable sampling technique has to be selected. However, material containing sample must be transparent to the IR radiation.

Detector:

Detectors are used to measure the intensity of unabsorbed infrared radiation. Detectors like thermocouples, pyro-electric detectors and Photoconducting are used.

- i) Thermal detectors- Their responses depend upon the heating effect of radiation
- ii) Pyroelectric detectors - depends on the rate of change of the detector temperature rather than on the temperature itself.
- iii) Photoconducting detectors - Most sensitive to IR radiation.

Amplifier:

Helps to convert electrical signals into measurable physical quantities

Computer/Printer:

Computers are used to store the IR spectrum and for printing.

Working procedure of IR spectrometer:

First, a beam of IR light from the source is split into two and passed through the reference and the sample respectively. Now, both of these beams are reflected to pass through a detector. The data obtained from detector are converted into readable form with amplifier and finally the required reading is printed out.

In infrared spectrophotometer, IR radiation of successively decreasing frequency is passed through the sample of the compound and the percent (%) transmittance is measured. An IR spectrum is the graph of percent transmittance (%T) versus decreasing frequency expressed in wave numbers ($4000\text{--}600\text{ cm}^{-1}$).

APPLICATION OF IR SPECTROSCOPY

IR finds many applications in industry as well as in research because different molecules with different combination of atoms produce their unique spectra.

a) Identification of functional group and structure elucidation

The main advantage of infrared spectroscopy is the ability to confirm the presence of functional groups. It will give more accurate information about the presence of functional groups.

b) Identification of substances

If two compounds have identical IR spectra then both of them must be samples of the same substances. IR spectra of two enantiomeric compounds are identical. So IR spectroscopy fails to distinguish between enantiomers.

c) Studying the progress of the reaction

Progress of chemical reaction can be determined by examining the small portion of the reaction mixture withdrawn from time to time. The rate of disappearance of a characteristic absorption band of the starting material and/or the rate of appearance of the characteristic absorption band of the product group due to formation of product is observed.

d) Detection of impurities

IR spectrum of the test sample to be determined is compared with the standard compound. If any additional peaks are observed in the IR spectrum, then it is due to impurities present in the compound.

e) Quantitative analysis

It is a dynamic measurement used for quantitative analysis. The quantity of the substance can be determined either in pure form or as a mixture of two or more compounds. This can be done by comparing the standard peak corresponding to the drug and test sample.

f) In forensic analysis

It is also employed in forensic analysis in civil and criminal analysis

There is difference between IR and FTIR.
IR is used to identify compounds and FTIR is used for quantitative analysis.
IR is done with a monochromatic light source and FTIR is done with a polychromatic light source.
FTIR is done with a broadband light source and it is used to identify compounds.

Principle and applications of physicochemical methods

Principle and applications of SEM

Principle: A scanning electron microscope (SEM) is a type of electron microscope that produces images of a sample by scanning the surface with a focused beam of electrons.

The sample in SEM is exposed to the high-energy electron beam as a result electrons interact with atoms in the sample, producing various signals that gives information about topography, morphology, orientation of grains etc. of a material. Hence SEM is a very useful tool for the characterization of materials.

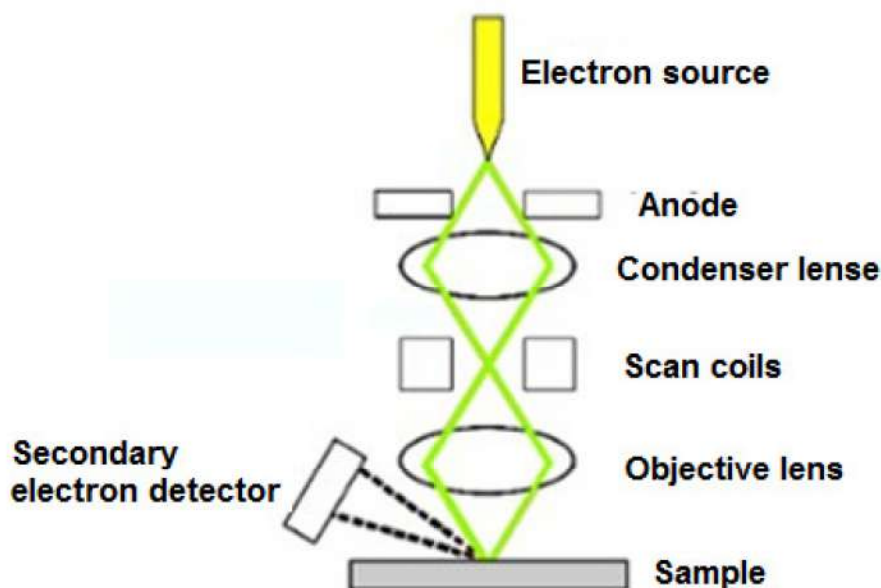
- Topography- the arrangement of atoms/ molecules on the surface of material
- Morphology- a particular form, shape, or structure appearing externally.
- crystallography means the arrangement of atoms in the materials

A schematic representation of an SEM is shown in Figure. Electrons are generated at the top of the column by the electron source, move more quickly down the column under vacuum to prevent the ionization and gases molecules.

Electromagnetic lenses are used to control the path of the electrons. Scanning coils are used to allow the beam onto the sample only.

- When a primary electrons are allowed to hit on the metal, results the secondary electron emitted from the surface of the sample. These two types of electrons used as signals in SEM imaging.

(SEM) Scanning Electron Microscope



Block diagram of SEM

What are secondary electrons?

Secondary electrons are electrons generated as ionization products. They are called 'secondary' because they are generated by other radiation (the primary radiation). The primary radiation can be in the form of ions, electrons, or photons with sufficiently high energy, i.e. exceeding the ionization potential.

APPLICATIONS OF SEM

SEMs can be used in a variety of industrial, commercial, and research applications

a) Materials Science:

Used in investigation / analysis of nano materials (like nanotubes, nanofibres), superconductors, electronics etc,

b) Semiconductor Inspection:

Reliable performance of semiconductors requires accurate topographical information. SEM offers a speedy, accurate measurement of the composition of the semiconductor.

C) Microchip Assembly:

The high resolution, three-dimensional image of SEM is invaluable to design and production microchips.

d) Forensic Investigations:

Criminal and other forensic investigations utilize SEMs to uncover evidence and gain further forensic deep analysis. Uses include:_____.

- analysis of gunshot residue
- bullet marking comparison
- handwriting and print analysis
- Examination of banknote authenticity.

e) Biological Sciences

SEM can be used on anything from insects and animal tissue to bacteria and viruses for study. Uses include:

- Measuring the effect of climate on change of species.
- identifying new bacteria and virulent strains
- vaccination testing
- in the field of genetics

f) Soil and Rock Sampling (Geological sampling)

SEM can determine weathering processes, to identify composition of rock. Valid uses include:

- Identification of tools and early human artefacts _____.
- soil quality measurement for farming and agriculture
- Dating historic ruins _____.
- Forensic evidence is soil quality, toxins etc.

g) Medical Science _____.

Broadly speaking, SEMs are used in medical science to compare blood and tissue samples in determining the cause of illness and measuring the effects of treatments on patients

Disadvantages of SEM

- SEMs are expensive
- Need training to operate and for analysis of the sample.
- Must be placed in the area free of any possible electric, magnetic or vibration interference.
- The maintenance involves keeping a steady voltage, currents to electromagnetic coils

PRINCIPLE AND APPLICATIONS OF TEM

A **Transmission Electron Microscope (TEM)** produces images via the interaction of electrons with a sample. They are also the most powerful microscopic tool available to-date for material science, capable of producing high-resolution, detailed images 1 nanometer in size.

Principle in TEM:

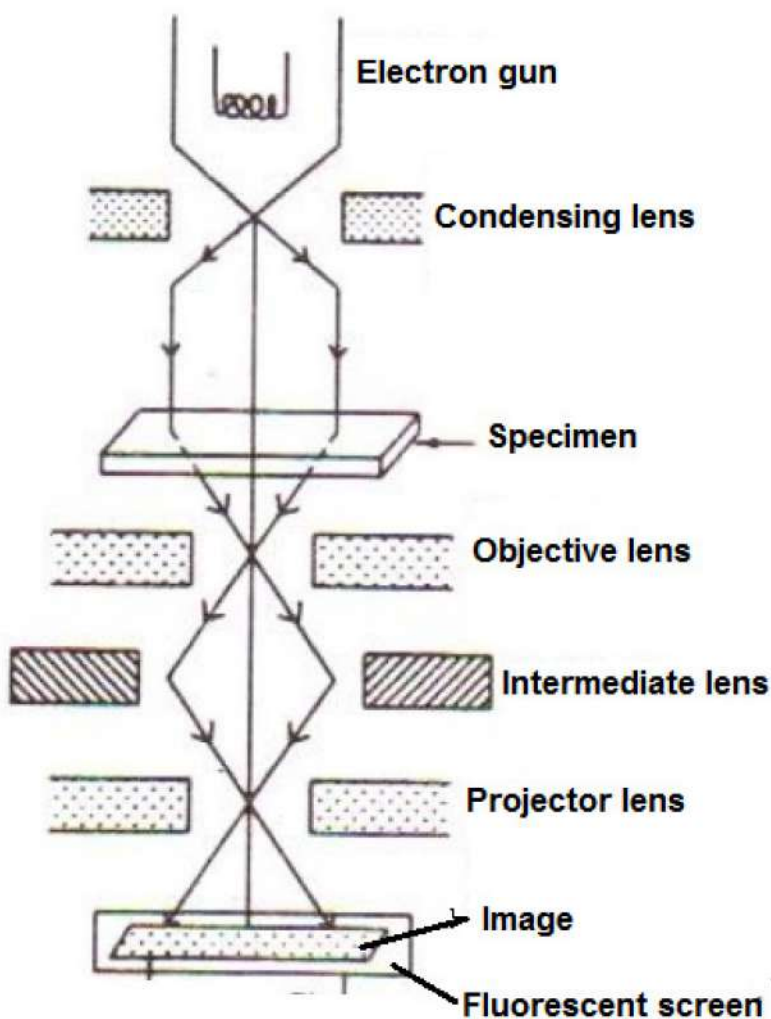
In TEM high energy beam of electrons is shone through a very thin sample and the interactions between the electrons and the atoms can be used to analyze the chemical sample.

Working:

A schematic representation of TEM is shown in Figure. A beam of high velocity electrons accelerated under vacuum (to avoid ionization/ interference of molecule in air) focused by condenser lens onto specimen and these electrons interacting with the specimen as it passes through. The emergent electron beam is focused by objective lens. Final image forms on a fluorescent screen for viewing.

To obtain a TEM analysis, samples need to have certain properties:

- i) The material need to be sliced thin enough for electrons to pass through it. _____.
- ii) Samples need to be able to withstand the vacuum chamber.



Block diagram of TEM

TEM Applications

TEMs have a wide-range of applications in a variety of scientific, education, research and industrial fields.

a) In structure analysis:

TEMs provide topographical, morphological, compositional and crystalline information.

- i) The images allow researchers to view samples on a molecular level, making it possible to analyze structure and texture.
- ii) This information is useful in the study of crystals and metals.
- iii) TEMs provide information on element and compound structure

b) Industrial applications:

- i) TEMs can be used in semiconductor analysis and production
- ii) In the manufacturing and design of silicon chips.
- iii) Technology companies use TEMs to identify flaws, fractures and damages to micro-sized objects; this data can help fix problems and / or help to make a more durable, efficient product.

c) In education and research:

- i) Very useful in understanding the materials especially nano-sized
- ii) Image morphology of chemical samples suspended on a thin film

d) In medical and pharmaceutical field:

- i) For detailed studies of thin specimens (tissue sections, molecules, etc) for understanding the tissue arrangements and component.
- ii) To study the small or whole organisms such as viruses or bacteria, and frozen solutions.
- iii) Also in contaminant identification.

e) Forensic analysis:

TEM can be very useful in forensic analysis due to its ability to analyze from a very small particles.

f) Other fields:

In gemology and metallurgy industry for determination of the internal structure from small particles.

Disadvantages of TEM

- TEMs are large and very expensive
- TEM sample preparation is a quite complex and tedious procedure
- Operation and analysis requires special training

- Samples are limited to those that are electron transparent, able to tolerate the vacuum chamber and small enough to fit in the chamber
- Images are black and white
- High maintenance cost. A Transmission Electron Microscope requires constant upkeep including maintaining voltage, currents in the electron gun, coils and cooling water.

Which is better TEM or SEM?

- This all depends on what type of analysis want to perform. For example, SEM used to get information about the surface features of sample, like roughness or contamination detection. If the crystal structure of sample can be determine by using TEM only.
- SEMs provide a 3D image of the surface of the sample whereas TEM images are 2D image of a small area of the sample, which in some cases makes the interpretation of the results more difficult for the operator. _____

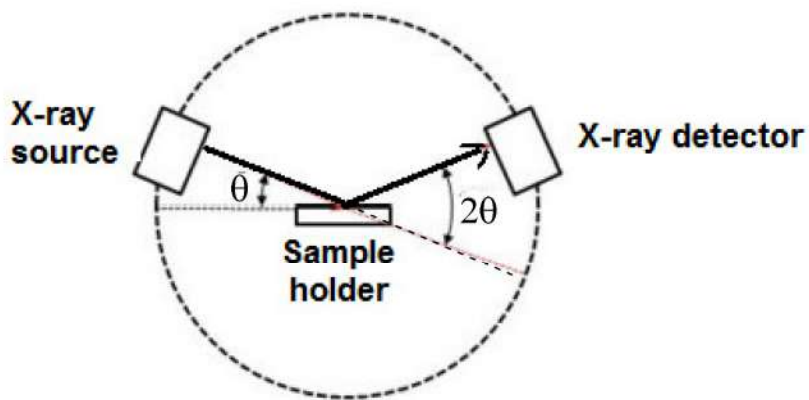
List the differences between SEM and TEM?

- SEM is based on scattered electrons while TEM is based on transmitted electrons.
- SEM focuses on the sample's surface and its composition whereas TEM provides the details about internal composition.
- The sample in TEM has to be cut thinner (generally below 150 nm) whereas SEM samples require little or no effort for sample preparation Since SEM gives only surface information the working of electron.
- The most important differences between a transmission electron microscope and a scanning electron microscope are: Rather than the broad static beam used in TEM, the SEM beam is focused to a fine point and scans line by line over the sample surface in a rectangular raster pattern.
- In TEM a broad static beam used and in SEM electron beam is focused to a fine point and scans line by line over the sample surface _____.
- SEMs usually use acceleration voltages up to 30 kV, while TEM users can set it in the range of 60 – 300kV.
- SEMs usually use acceleration voltages up to 30 kV, while TEM users can set it in the range of 60 – 300kV.

Principle and applications of X-ray diffraction

The principle behind XRD analysis:

XRD analysis is based on constructive interference of monochromatic X-rays and a crystalline sample. The interaction of the incident rays with the sample produces constructive interference when conditions satisfy Bragg's law ($n\lambda = 2d \sin \theta$)



Block diagram of XRD

As the wavelength of X-ray are similar to the distance between atoms, X-ray Diffraction (XRD) techniques uses this principle to elucidate the crystalline nature of materials. The diffracted of X-rays from atoms produce a diffraction pattern that contains information about the atomic arrangement in crystal.

X-ray diffractometers consist of three basic units: an X-ray tube (source), a sample holder and an X-ray detector. The X-rays are generated by a cathode ray tube and these X-rays are directed at the sample and the diffracted rays are collected. A key component of all diffraction is the angle between incident (θ) and diffracted rays (2θ).

Applications of XRD:

- XRD is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information of unit cell dimensions.
- For the identification of unknown crystalline materials (e.g. minerals, inorganic compounds).
- Determination of unknown solids is critical to studies in geology, environmental science, material science, engineering and biology.
- For measurement of sample purity
- XRD can be used to determine material structure and to determine the atomic arrangement
- To determine of modal amounts of minerals (quantitative analysis)
- For characterization of thin films samples. XRD studies helps to identify the chemical composition on the film and to determine the thickness, roughness and density of the film
- Forging final confirmation to the organic compound structures which are confirmed by various analytical techniques earlier.
- To know the exact grain size of the material that are difficult to determine optically.
- Measuring superlattices in multilayered epitaxial structures.
- To make textural measurements, such as the orientation of grains in a polycrystalline sample.

Note: This study material gives basic information only, Refer text books for more details