D.N.R.COLLEGE (AUTONOMOUS): BHIMAVARM

DEPARTMENT OF PG CHEMISTRY

INSTRUMETNAL METHODS OF ANALYSIS –I

III SEMESTER

Presented By

B. Madhavi

DNR COLLEGE BHIMAVARAM

Unit-I

UV-VISIBLE SPECTROSCOPY

Spectroscopy is the branch of Science that deals with the study of interaction of electromagnetic radiation with matter.

- Spectrometer is an instrument design to measure the spectrum of a compound.
- Instrument used to measure the absorbance in UV (200- 400nm) or Visible (400-800nm) region is called UV-Visible Spectrophotometer.

A Spectrophotometer records the degree of absorption by a sample at different wavelengths and the resulting plot of absorbance (A) versus wavelength (2) is known as a Spectrum.

Laws of absrption

1. Beer's law

2. Lambert's law

3. Beer-lambert's law

Beer's Law: When a beam of monochromatic light is passed through a homogenous absorbing medium, the rate of decrease of intensity of radiation with increase in the concentration (c) of absorbing species is directly proportional to the intensity (I) of the incident light (radiation). $-dI/dc = k I$ $-dI/I = k\,d\,c$

On integration of above equation

$-\ln I=kc+b$

(b= integration constant)------(1)

When conc. = 0, then there is no absorbance. Here I = Io Therefor substituting in equation (1) –

$\ln I = k\times 0+b$

-In I = b

Substituting the value of b in equation (1) - In I = k c - ln Io In Io - In I = kc In Io/ln I = kc (Since log A - log B = log A/B) Io / I = e kc (removing natural logarithm) I/Io = e-kc (inverse both sides) I = I e-kc.............(2)

Lambert's law: When a beam of monochromatic light is passed through a homogenous absorbing medium, the rate of decrease of intensity of radiation with thickness of absorbing medium is directly proportional to the intensity of the incident light (radiation). $dI/dt = kI$ I= intensity of incident light of wavelength λ & t= thickness of medium

Since,

I = Io e-kt----.(3)

Now combine the eq.(2) and eq.(3), we get;

I = Io e-kct

Converting natural logarithm to base 10

I = Io 10-kct

Inverse on both sides

Io / I = 10 kct Taking log on both sides log Io / I = kct .(4) Here, transmittance (T) = I/I, and Absorbance (A) = log 1/T Hence, A = log Io / I .(5)

Using eq.(4) and eq.(5), A = kct Instead of k we can use ε, the above equation will be as follow:

A = ect

This is mathematical equation for Beer's- Lambert's Law.

A=ect

Where A = Absorbance;

ε = Molecular extinction coefficient;

c = Concentration of sample;

t = Path length (normally 10mm or 1cm)

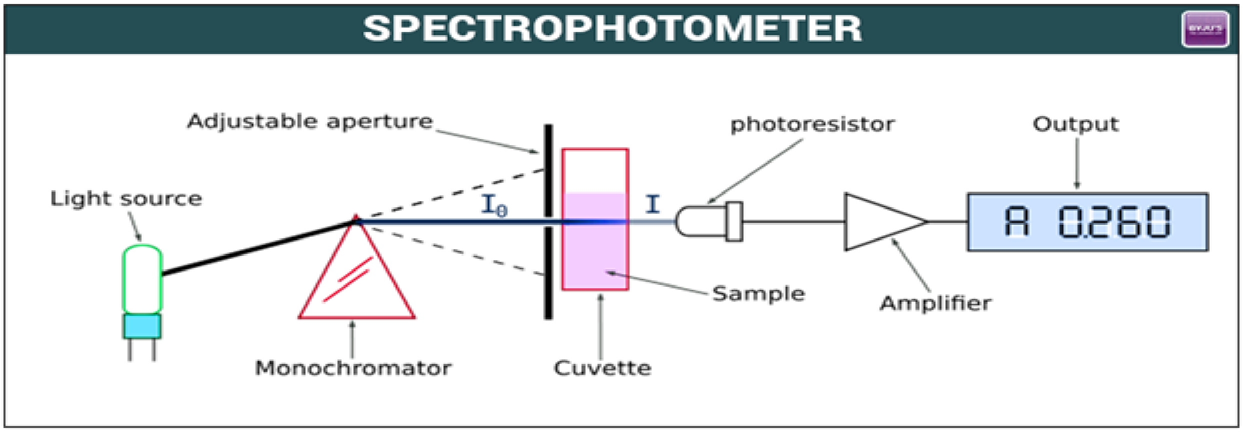
ε can be expressed as follows:

ε = E1%X Molecular weight /10

SINGLE-BEAM SPECTROPHOTOMETER

- A beam of radiation pass through a single cell, the reference cell is used to set the absorbance scale at zero for the wavelength to be studied.
- It is then replaced by sample cell to determine the absorbance of the sample at that wavelength.
- This was earliest design and is still use in both teaching and industrial labs.

It requires a stabilized voltage supply to avoid errors resulting from changes in the beam intensity



LIGHT SOURCE

Requirements:

- It should be stable.
- It should provide continuous radiation.
- It must be of the sufficient intensity for the transmitted energy to be detected at the end of the optical path.

1. HYDROGEN DISCHARGE LAMPS

- In these lamps a pair of electrodes is enclosed in a glass tube filled with hydrogen gas under relatively high pressure
- It is a continuous source.
- Covers a range 160-375nm.
- Stable, robust and widely used.

2. DEUTERIUM LAMPS

- Similar to hydrogen discharge lamp.
- Deuterium is filled in place of hydrogen.
- The intensity of radiation emitted is 3 to 5 times the intensity of a hydrogen lamp.
- More expensive than hydrogen lamp.
- Used when high intensity is required.

WAVELENGTH SELECTORS

- Converts polychromatic light to monochromatic light.
- Filters and Monochromators are used for this purposes.

FILTERS

❖ It is frequently necessary to filter or remove wide bands of radiation from a signal.

- Filters isolate a wider band than the monochromators.

* 2 types

1. ABSORPTION FILTERS

These filters have a bandwidth that ranges from 30-250µm.

The absorption filters consists of coloured glass or a dye suspended in gelatin and sandwitched between the two glass plates.

The coloured glass filter has the advantage of greater thermal stability.

Each instrument is provided with a set of 12 filters to cover the range from 390-700µm.

A narrow spectral band can be obtained by coupling cut off filters with other filters but this combination decreases the intensity of light

2. INTERFERENCE FILTERS

- Based on interference phenomenon at desired wavelength thus permitting rejection of unwanted radiation by selective reflection and producing narrow band.
- It consists of a dielectric layer(eg:CaF2) between 2 parallel silver films which is sandwitched by glassplate.
- It has a bandpass of 100-150Å and a peak transmittance of 40-60%.

MONOCHROMATORS

- It is used to disperse the heterochromic radiation into its component wavelength and to permit the isolation of desired portion of the spectrum.
- It consists of an entrance slit, an exit slit and a dispersing device either a prism or grating
- Materials of construction should be selected with care to suit the range in which it has to work.
- For eg:

Quartz for ultraviolet.

Normal glass for visual range.

Alkali halides for IR region.

- Gratings are cheaper than prism.

1. PRISM

- Made up of glass, quartz or fused silica.
- Quartz or fused silica is the choice of material of UV spectrum.
- When white light is passed through the glass prism, dispersion of polychromatic light in rainbow occurs.Now by the rotation of the prism different wavelengths of the specrum can be made to pass through in exit slit on the sample.

The effective wavelength depends on the dispersive power of prism material and the optical angle of the prism.

2. GRATING MONOCHROMATOR

- Gratings are of 2 types.

1. Diffraction grating

2. Transmission grating

DIFFRACTION GRATING

More refined dispersion of light is obtained by means of diffraction gratings.

These consists of large no. of parallel lines (grooves) about 15000-30000/inch is ruled highly polished surface of aluminum

To make the surface reflective, a deposit of aluminium is made on the surface. In order to minimize to greater amounts of scattered radiation and appearance of unwanted radiation of other spectral orders, the gratings are blazed to concentrate the radiation into a single order.

TRANSMISSION GRATING

- It is similar to diffraction grating but refracton takes place instead of reflection.

- Refraction produces reinforcement. This occurs when radiation transmitted through grating reinforces with the partially refracted radiation.

SAMPLE HOLDER/CUVETTES

- The cells or cuvettes are used for handling liquid samples.

- The cell may either be rectangular or cylindrical in nature.

- For study in UV region the cells are prepared from quartz or fused silica whereas colour corrected fused glass is used for visible region.

Cleaning is carried out washing with distilled water or with dilute alcohol, acetone.

- The surfcaces of absorption cells must be kept scrupulously clean.No fingerprints or blotches should be present on cells.

DETECTORS

- Device which converts light energy into electrical signals, that are displayed on readout devices.

- The transmitted radiation falls on the detector which determines the intensity of radiation absorbed by sample.

- The followed types of detectors are employed in instrumentation of absorption spectrophotometer.

Barrier layer cell/Photovoltaic cell

2. Phototubes/Photoemissive tube

3. Photomultiplier tube

Requirements of ideal detectors:

❖ It should give quantitative response.

* It should have high sensitivity and low noise level.

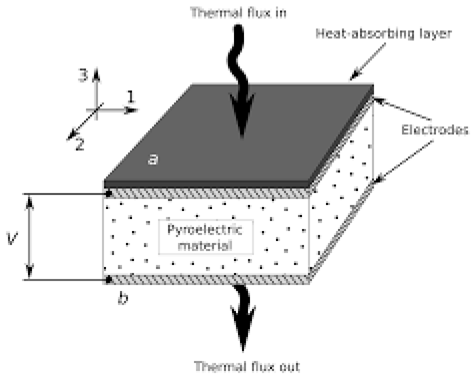
It should have a short response time.

* It should provide signal or response quantitative to 36

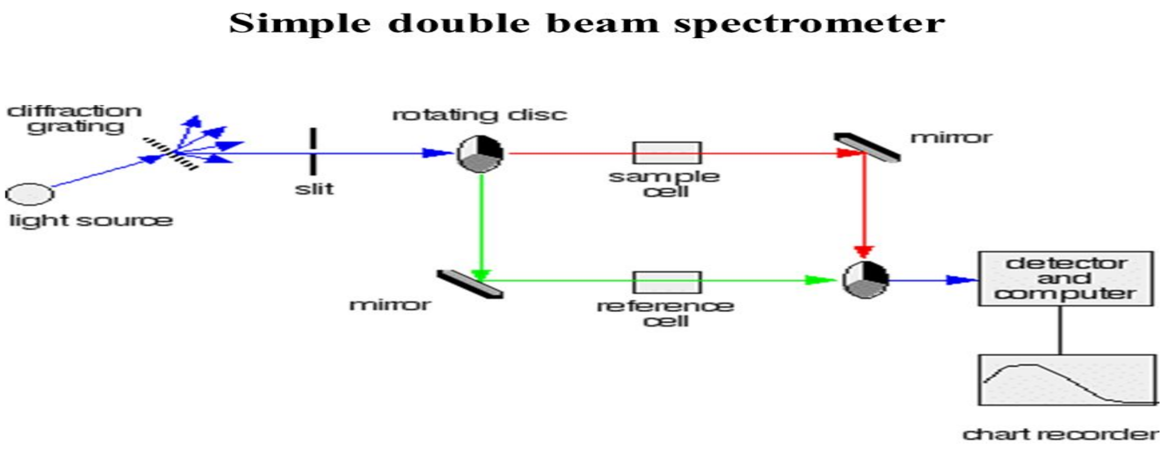
wide spectrum of radiation received

BARRIER LAYER CELL/ PHOTOVOLTAIC CELL

- The detector has a thin film metallic layer coated with silver or gold and act as another electrode.
- It also has a metal base plate which act as another electrode.
- These 2 layers are separated by a semiconductor layer of selenium. This creates a potential difference between two electrodes & causes the flow of current.
- When it is connected to galvanometer, a flow of current observed which is proportional to the intensity and wavelength of light falling on it.
- When light radiation falls on selenium layer, electrones become mobile and are taken up by transparent metal layer.



DOUBLE-BEAM SPECTROPHOTOMETER



Two beams are formed in space by a V-shaped mirror called beam splitter.

One beam passes through the reference solution to a photo detector, and the 2nd simultaneously traverses the sample to a 2nd matched detector.

The 2 outputs are amplified, and their ratio or logerithm of their ratio is determined electonically or by a computer and displayed by the readout device.

* The beams are separated in time by a rotating sector mirror that directs the entire beam from the monochromator first through the reference cell and then through the sample cell.

The pulses of radiation are recombined by another sector mirror, which transmits one pulse to other to the transducer.

* The motor-driven sector mirror is made up of pie shape segments, half of which are mirrored and half of which are transparent.

The mirrored sections are held in place by blackened metal frames that periodically interrupt the beam and prevent its reaching the transducer.

The double-beam-in-time approach is generally preferred because of the difficulty in matching the 2 detectors needed for the double-beam-in-space design.

TUNGSTEN LAMP

- Similar in its functioning to an electric bulb.
- It provides a supply of radiation in the wavelength range of 320-2500nm.
- Continuous source of light.
- When tungston filament is heated to incandescence by an electric current, the light is produced.
- The glass bulb enclosing the filament contains a low pressure of inert gas, usually argon.
- Small amounts of halogen like iodine is added to improve the intensity (Tungston-Iodine lamp)

XENON DISCHARGE LAMP

- Xenon gas is stored in lamps at 10-30 atm pressure.
- It contain 2 tungston electrodes that are separated by a distance of about 8mm.
- When current passes through xenon cause thermal excitaton.
- It produces greater UV radiation than the hydrogen lamp.
- Continuous source.

MERCURY ARC LAMP

- In this mercury vapour is stored under high pressure and the excitation of mercury atoms is done by electric discharge.

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Photomultiplier tube:

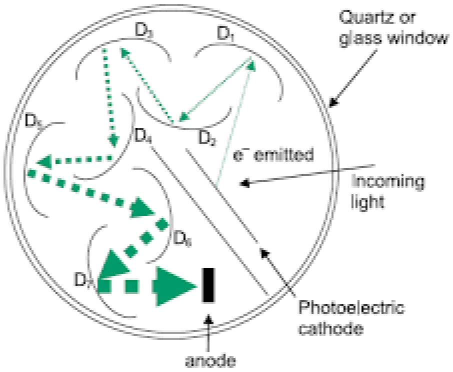
✓ The principle employed in this detector is that, multiplication of photoelectrons by secondary emission of electrons.

In a vacuum tube, a primary photo-cathode is fixed which receives radiation from the sample.

Some eight to ten dynodes are fixed each with increasing potential of 75-100V higher than preceding one.

✓ Near the last dynode is fixed an anode or electron collector electrode.

✓ Photo-multiplier is extremely sensitive to light and is best suited where weaker or low radiation is received.



Read-out device

✓ The signals from the detector after amplification are finally received by the recoding system or read-out device.

The recording is done by recorder pen**.**

Diode Array Detector

UV/UV-VIS detectors are most frequently used to measure components showing an absorption spectrum in the ultraviolet or visible region.

A DAD detects the absorption in UV to VIS region. While a UV-VIS detector has only one sample-side light-receiving section, a DAD has multiple photodiode arrays to obtain information over a wide range of wavelengths a one time, which is a merit of the DAD.

Π spectra are measured at intervals of 1 second or less during separation by HPLC with continuous eluate delivery.

■ If the measurement is performed at a fixed wavelength, components are identified from only their retention time.

■ In such a case, the DAD can be used to identify components by a comparison of the spectrum.

■ Light from the lamp is shown onto the diffraction grating, and dispersed according to wavelength.

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Spectrophotometric Titrations

• If at least one species in a complexation titration absorbs

electromagnetic radiation, we can identify the end point by monitoring

the titrand's absorbance at a carefully selected wavelength.

For example, we can identify the end point for a titration of Cu2+ with EDTA, in the presence of NH3 by monitoring the titrand's absorbance at a wavelength of 745 nm, where the Cu(NH3)42+ complex absorbs strongly.

At the beginning of the titration the absorbance is at a maximum. As we add EDTA the concentration of Cu(NH3)42+, and thus the absorbance, decreases as EDTA displaces NH3.

After the equivalence point the absorbance remains essentially unchanged

Acorr = AX (VEDTA + VCu)/VCu

where VEDTA and VCVcuu are, respectively, the volumes of EDTA and Cu.

Correcting the absorbance for the titrand's dilution ensures that the spectrophotometric titration curve consists of linear segments that we can extrapolate to find the end point. Other common spectrophotometric titration curves are shown in panels b-f.

The resulting spectrophotometric titration is shown below in panel (a). Note that the titration curve's y-axis is not the actual absorbance, A, but a corrected absorbance, Acorr

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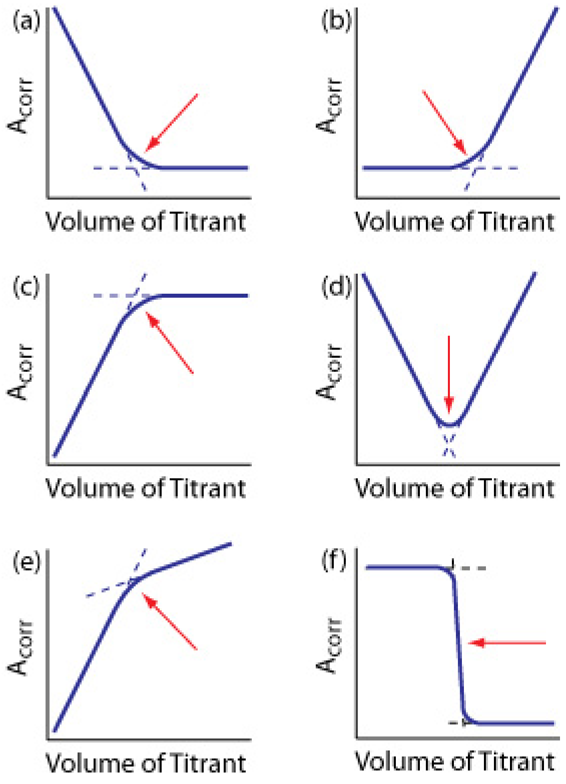


SpectroFlourimetry:

FLUORESCENCE

► When a beam of light is incident on certain substances, they emit visible light or radiations. This is known as fluorescence.

► Fluorescence starts immediately after the absorption of light and stops as soon as the incident light is cut off.

► The substances showing this phenomenon are known as fluorescent substances.

FLUORIMETRY

► Flourimetry is an analytical method for the measurement of fluorescence, based upon the emission of absorbed radiation by the molecules.

► The instrument used is called as spectroflourimeter

Factors affecting fluorescence

1.Nature of molecules

Only the molecules absorbs uv/visible radiation can show the fluorescence. Greater the absorbency of molecule more intense its fluorescence. Unsaturated molecules with π bond and good resonance stability can exhibit fluorescence.

Eg: Alkenes with conjugate double bond

Saturated molecules with sigma bond do not exhibit fluorescence.

Eg: Aliphatic unsaturated cyclic organic compounds.

2. Nature of Substituent

Electron donating groups like amino, hydroxyl groups enhance fluorescence intensity.

While electron withdrawing groups like halogens, nitro, carboxylic groups will reduce fluorescence intensity.

3. Effect of concentration

Beers law state that in a solution of an absorbing substances the absorbance is directly proportional to the concentration.

Fluorescent intensity is directly proportional to concentration of the substance but this is true in low concentrations, in high concentration it doesn't obey linearity and hence fluorescence intensity decreases.

4. Adsorption

The extreme sensitivity of the methods requires very dilute solutions.

Adsorption of fluorescent substance on the container will may therefore present a serious problems.

Stock solution must be kept diluted as required.

5.Photo decomposition

Excitation of a weakly fluorescing or dilute solution with intense light sources will cause photochemical decomposition of analyte.

To minimize decomposition :-

Use of longest feasible wavelength for excitation

Remove dissolved oxygen.

Protected the unstable solutions from ambient light by storing them in dark bottles.

6.Oxygen

Oxygen decrease the fluorescence intensity in two ways:

It oxidizes fluorescent substance to non fluorescent substance

It quenches fluorescence because of the paramagnetic properties of molecular energy.

7. PH

In the molecules containing acidic and basic functional group the changes in the pH of the medium change the degree of ionization of the functional group.

8.Conjugation

A molecule must have unsaturation i.e. 7 electrons so that UV/visible radiation can be absorbed. If there is no absorption of radiation, there will not be fluorescence

9. Effect of temperature

Increase in temperature leads to increase in collisions of molecules therefore deviation, which results in decrease in fluorescence intensity. On the other hand, decrease in temperature leads to decrease in collisions of molecules, which results in increased fluorescence intensity.

10.Viscosity

Increase in viscosity leads to decreased collision of molecules, which leads to enhancement of fluorescence intensity. Decrease in viscosity causes increased collision of molecules, which results in decreased fluorescence

11.Quantum yield of fluorescence

Quantum yield of fluorescence = number of photons emitted/number of photon absorbed Since the absorbed energy is lost by pathways, the quantum efficiency is less than 1.

12.Intensity of incident light

Intensity of light incident on the sample produces a proportional in fluorescence.

The intensity of light depends upon Light emitted from the lamp, Excitation monochromators, Excitation slit width

13.Pathlength

Effective path length depends on both excitation and emission slit width.

Use of micro cuvette does not reduce fluorescence.

Use of microcell may reduce fluorescence.

Quenching

► Quenching refers to any process that reduces the fluorescence intensity of a given substance.

► This may occur due to various factors like pH, concentration, temperature, viscosity, presence of oxygen, heavy metals or, specific chemical substances etc

Types of Quenching

- Self Quenching or Concentration Quenching
- Chemical Quenching
- Static Quenching
- Collisional Quenching

Self Quenching or Concentration Quenching

► Concentration quenching is a kind of self quenching.

► It occurs when the concentration of the fluorescing molecule increases in a sample solution. The fluorescence intensity is reduced in highly concentrated solution (>50 µg/ml).

Chemical quenching

► Chemical quenching is due to various factors like change in pH, presence of oxygen, halides and electron withdrawing groups, heavy metals etc.

► Change in pH: Aniline at pH (5-13) gives fluorescence when excited at 290 nm. But pH <5 or, pH >13 it does not show any fluorescence.

► Oxygen: Oxygen leads to the oxidation of fluorescent substance to non fluorescent substance and thus, causes quenching.

► Halides and electron withdrawing groups Halides like chloride ions, iodide ions and electron withdrawing groups like -NO2, COOH, CHO groups lead to quenching.

► Heavy metals: presence of heavy metals also lead to quenching because of collision and complex formation.

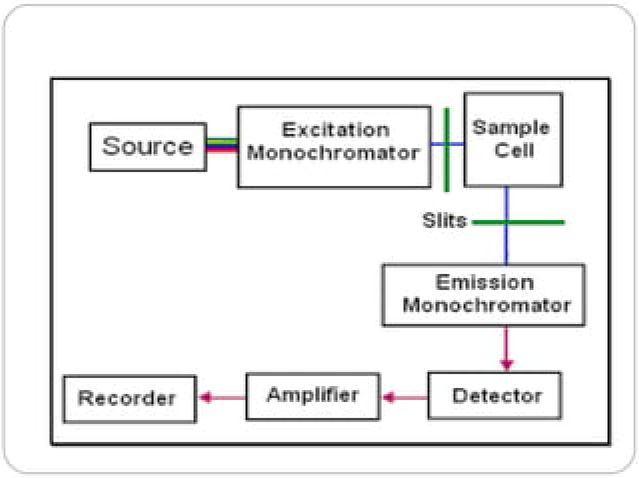
Instrumentation of Fluorescence Spectrophotometer

1. Source of light

2. Filters & Monochromators

3. Sample cells

4. Detectors



1. Source of light- the source of light should emit a radiation over a continuous region and be of adequate intensity and stability.

a. Mercury vapour lamp - Mercury vapour at high pressure(8 atmospheres) gives intense lines on a continuous background above 350nm.lines are seen at 365,398,436,546,579,690, and 734nm.Low pressure mercury vapour gives an additional line at 250nm.

b. Xenon arc lamp It gives a more intense radiation when compared to mercury vapour lamp. It is used as the source of light in Fluorescence Spectrophotometers.

c.Tungesten lamp- If excitation has to be doe in visible region, this can be used .It does not offer uv radiaton, more over the intensity of this lamp is too low.

2. Filters and Monochromators

In Flourimetry two things are important i.e. excitation wavelength and emission wavelength. As these two wavelengths are different in most cases, a filter or monochromatic is used for the purpose.

In an expensive fluorimeter, primary and secondary filter are present. Primary absorbs visible radiation and transmits uv radiation.

Secondary absorbs uv and transmits visible radiation.

In spectrofluorometer, excitation monochromators and emission monochromator re present which have gratings.

Excitation monochromator- provides a suitable radiation for excitation of

molecule which is absorbed by molecule.

Emission monochromator- It provides only the radiation emitted by the fluorescent molecule.

3. Sample cells

Sample cells are used go hold sample solutions. They are cylindrical or polyhedral.

The cells are made up of color corrected fused glass and pathlength is normally 10mm or 1cm. It need not be made up of quartz, science we are measuring only the emitted radiation and not the absorbed radiation. Hence even if there is absorption of radiation by glass, it will not affect the results.

4. Detectors

The emitted radiation is mostly visible radiation and sometimes uv radiation To measure the intensity of such radiation, Photovolatic cell, Phototubes, or Photomultiplier tubes can be used. But science, we use low concentration of substances and the intensity of emitted radiation is weak, only Photomultiplier tubes are best and accurate. In inexpensive instruments like Photoflourimeters, Photo tubes can be used

Applications

1. Determination of inorganic substances -Examples (Aluminum ion, Zinc ion, tin ion).

2. Determination of organic substances Aromatic polycyclic hydrocarbons, indoles, naphthols, proteins, plant pigments, steroids etc. can be determined at low concentrations by Fluorimetry.

3. Pharmaceutical applications

Compounds which are fluorescent e.g. p-amino Salicylate, Aminacrine, Morphine, Quinine etc. Desipramine,

Compounds converted to fluorescent products by chemical reaction e.g. Chlordiazepoxide, Chloroquine, and Methyldopa etc.

Separate the mixture of medicinal substances

Various options available are

A wavelength at which only one drug is excited is chosen.

Using chemical reaction

Using separation methods and analyzing the compounds.

4. Miscellaneous applications

Fluorescent indicators: The intensity and colour of many fluorescent substances depends on pH range and hence are used in acid-base titrations e.

Eg. Eosin, Fluoresce in, Quinine sulphate.

UNIT-II

INFRA RED SPECTROSCOPY

INTRODUCTION :

IR-Spectroscopy provides a wealth of Informations on transition between vibrational and rotational enerfy levels in molecules and on symmetry purity and structure of organic compounds.

Regions of IR spectrum:

The IR radiation refers broadly to that part of the electromagnetic spectrum which lies between the visible and microwave regions. IR region has been sub divided as follows.

1.Photographic region: this ranges from visible to 0.8μ(1μ=10^{-3}mmor10-^{4}cm-^{1})

2.Near IR region (overtone region):It ranges from 0.8to2.5μ(12500-4000cm-^{1})

3.Mid IR region (vibration-rotation region):its range from 2.5 to 15μ(4000-667 cm-^{1})

4.Far IR region (Rotation region) this ranges from 15 to 200μ (667-50 cm-^{1})

Principles of IR spectroscopy:

IR spectroscopy is concerned with study of absorption of IR radiation by which results in vibration and rotational transitions.

IR spectra mainly used in structure elucidation of determination in groups this is quantitative aspect.

Absorbance increases with conc. In according with beers law this is quantitative aspect.

Normal modes of molecular vibrations:

1.streching vibrations:-

A stretching vibration is a rhythmical movement along the bond axis such that the inter atomic distance is increasing or decreasing the symmetrical stretching vibration is inactive in the infrared since it produces no change in the dipole moment of the molecules.

Eg: O=C=O, In stretching vibrations, bond angle change only if it is required to do so by the centre of gravity resisting displacement.

These are of two types. They are

a)Symmetrical stretching:-

when the stretching and compression occur in a symmetrical fashion,.

Eg: In case of Methylene group H-C-H the 2 atoms H move away from the central carbon atom without change in the bond angle.

b)Asymmetric stretching:-

when one bond is compressing while the other is stretching.

2.Bending or Deforming vibrations:-

A bending vibration may consists of a change in bond angles between bonds with a common atom or the movement of a group of atoms with respect to the remainder of the molecule without movement of the atoms in the group.

These are of four types.

a)Scissoring :-

The 2 atoms joined to a central atom move toward and away from each other with the deformation of the valence angle(In plan)

b)Rocking :-

The structural unit swings back.

c)Wagging:-

The structural unit swings back and forth out of the plane of the molecule (out of plane)

d)Twisting:-

The structural units rotate about the bond which joins to the rest of the molecules.

Factors influencing Vibrational frequencies :-

1.Vbrational coupling :-

These takes place between two bonds vibrating with similar frequency provided that the bonds are reasonable close in the molecules.

The coupling vibrations may both be fundamentals or a fundamental vibration may couple with the overtone of some other vibration. The latter coupling is called as Fermi resonance.

Eg: For C-H stretching fundamental band is at 2,800 cm^{-1}

For bending (fundamental) vibration band is at 1389 cm^{-1}

For bending (over tone) vibration band is at 2778 cm^{-1}

2.Electronic effects:

The changes in the absorption frequencies for a particular group take place when the substituent's in the neighborhood of that particular group are changed the frequency shifts are due to electronic effects which include.

1.inductive effects:

The introduction of an electro negative atom or group causes –I effect which results in the bond order to increase. The force constant increases and hence the wave number of absorption rises.

a)Acetone (CH_3COCH_3) 1715 cm^{-1}

b)chloroacetone(CH_3COCH_2Cl) 1725cm^{-1}

c)Di chloroacetone($CH_3COCHCl_2$)

d)Tetra chloroacetone(cl_2CH-CO-$CHCl_2$) 1750,1778 cm^{-1}

The introduction of alkyl group causes + I effect which results in weakening of the bond hence force constant is lowered and wave number of absorption decreases.

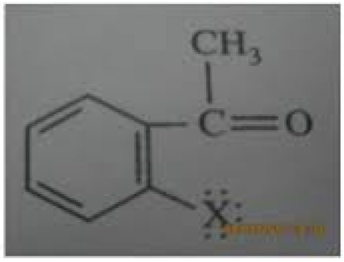
Formaldehyde and acetaldehyde exhibit wave number at 1750 and 1745 cm^{-1}

2.Mesomeric effect:-

It causes lengthening or weakening of a bond leading to the lowering of absorption frequency. In most of the cases, mesomeric effect work along the inductive effect. In few cases, inductive effect dominates over mesoromeric effect.

3. Field effect:

In Ortho substituted compounds the lone pair of electrons on two atoms influence each other through space interactions and change the vibrational frequencies of both the groups. This effect is known as field effect.



Ortho halo acetophenone

The non –bonding electrons presents on oxygen atom and halogen atom causes electro static repulsion this causes a change in the state of hybridization of c=O group and also makes it to go out of the plane of the double bond thus the conjugation is diminished and absorption occurs at a higher wave number for such Ortho substituted compounds cis absorbs field effect at a higher frequency compared to trans.

Bond angles:

In ketones the highest C=O frequencies arise in the strained cyclobutanones.This can be explain interms of bond angular strain. The C-CO-C bond angle is reduced below the normal 120o leading to increased S character in the C=O bond. Hence C=O bond shortened the therefore strengthened and so $\vartheta c = 0$ increases. If the bond angle is pushed outwards above 120^o, the opposite effect operates.

Hydrogen bonding:

IR spectroscopy is the best spectroscopy to distinguish the inter and intra molecular hydrogen bonding in a molecule.

Intra molecular hydrogen bonding is independent of conc. and dilution

Inter molecular hydrogen bonding is depend upon the conc. and dilution.

Inter molecular hydrogen bonding $\propto$ 1/conc&dilution

6.Force constant:

Force constant depends upon the strength of the bond.

$$\vartheta = 1/2\pi \times \sqrt{k}/\mu$$

If the strength of bond increases and force constant increases then frequency off molecule increases.

7.steoric effect/strain:

If strain increases in a molecule the vibrational frequency increases of ‘S’ character in a molecule.

Eg:

Cyclo propane>cyclo butane>cyclo pentane>cyclo hexane

Instrumentation of IR Spectroscopy:

IR instrumentation is divided into two classes

1.Dispersive and 2. Non dispersive

The dispersive instruments use a prism or grating and are similar to UV-Visible dispersive spectrometers except that, in the IR region different sources and detectors must be used.

Non dispersive spectrometers may use interference filters, tunable laser sources or an interferometer in FTIR spectrometers.

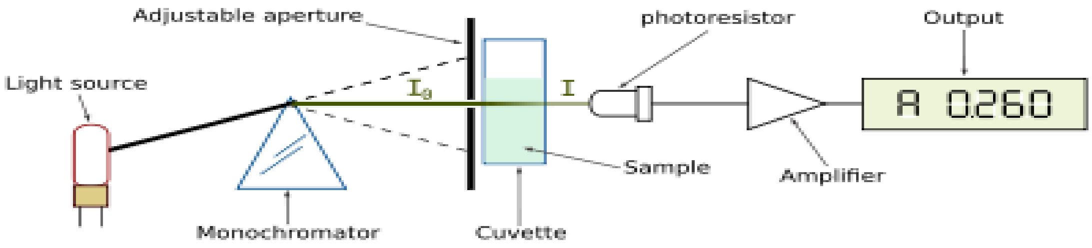
In IR region front surfaced mirrors are used since glass and quartz used in lenses are opaque to IR radiation

Radiation from a source is chopped or pulsed at a low frequency and is passed alternately through the sample and the reference cells placed before the monochromator. This minimizes the effect of stray radiation the temp & relative humidity in the room should be controlled since Nacl & KBr used in cells are hydroscopic

Components of dispersive spectrometers

	Near IR	Mid IR	Far IR
wavelength	12500 cm^{-1}	4000 cm^{-1}	6150 cm^{-1}
Source	Tungsten filament lamp	Nernst glower,coil of nichrome wire	High pressure Hg-arc lamp
Optical system	One or two quartz prisms or prism- grating double mono chromator	Two or four plans diffraction gratings with four prism monochromator	Double beam grating instruments for use to 700µm, interferometric spectrometers for use to 1000 µm
Detector	Photoconductive cells	Thermopile, thermistor, pyro-electric or semi conductor	Golay, pyro-electric detector

Single beam infrared spectrometer:



Source:

The radiation source must emit IR radiation which must be

1. Intense enough for detection
2. Steady
3. Extend over the desired wavelengths

a)coil of Nichrome wire:

a closely wound nichrome wire raised to incandescence by resistive heating a black oxide film forms on the coil giving acceptable emissivity temperature up to 1100oc are reached the nichrome coil requires no water cooling. It is simple and rugged the source is less intense.

b)Nernst glower:

it consists of hollow rod which is about 2mm in diameter and 20mm in length the glower is constructed from a fused mixture of oxides of Zr, yttium and thorium nearnst glower is non conducting at room temp hence the operating temp is15000c Nernst glowers are fragile radiation intensity is twice that of nichrome and globar sources.

The disadvantage of Nernst glower is that it emits IR radiation over wide wavelength range, one main disadvantage is its frequent mechanical failure.

c)Globar sources:

it is a rod of sintered silicon carbide about 5mm diameter and 50mm lengths It is self starting and as an operating temp near 1300oc Globar is less intensity compared to Nernst glower it is made up of metal oxides.

Monochromators:

In order to select desired frequencies from the radiation source we have to use monochromators this selection has been achieved by mans of monochromators.

a)Prism monochromator:

NaCl is the most common prism salt. This covers the range from 4000 to 650 cm^{-1}

Prism of lithium chloride fluoride or calcium fluoride give more resolution. The double pass monochromator produce more resolution than the single pass monochromator.

b)Grating monochromator:

Higher dispersion can be achieved by gratings. The gratings offer linear dispersion in ruled gratings a series of microscopically close parallel triangular grooves is engraved on to a reflective surface. Light reflected from the grating is diffracted interference arises a certain angles and so specific wavelengths appear with constructed interference at specific angles of reflection.

Halographic gratings have high wavelength purity. Gratings follows this equation

$$n\lambda = d_{(\text{sine}i \pm \text{sine}\theta)}$$

Where' n' is the order,

'd' is distance between grooves

'I' is the angle of incident of beam,

'θ'is angle of dispersion

Sampling techniques:

Infrared spectroscopy has been used for the characterization of solid liquid or gas samples the common point to the sampling of different phases is that the material containing the sample must be transparent to IR radiation.

Sampling of solids:

In this technique the finely ground solid sample is mixed with Nujol to make a thick paste this is then made to spread between IR transmitting windows this is then mounted in a path of IR beam and the spectrum is run.

Some commonly used mulling reagents are Nujol, Hexachlorobutadiene, chloro fluoro carbon oil etc, with high molecular weights.

b)sampling of liquids:

the simplest technique consists of samplings a liquid in a thin film squeezed between two Nacl plate for sample that contain water plates may be constructed with CaF2. A drop of the liquid sample is placed on the top of the Nacl plate and another Nacl plate is placed on it. The pair of Nacl plates enclosing the liquid film is then placed in the path of the sample beam.

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the gaseous sample is introduced into a gas cell the walls of the cell are made of Nacl. The cells are larger usually they are about 10cm long but they may be up to 1m long multiple reflections cab be used to make the effective path length.

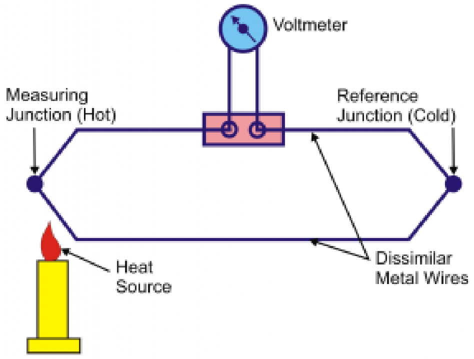
The method is not commonly used because of its lack of sensitivity, moisture must be avoided.

Detectors :

a)Thermo couple:It is based upon the fact than an electrical current will flow when two dissimilar metal wires are connected together at both ends and a temperature differential exists between the two ends. The end exposed to the IR radiation is called the hot junction. The other connection the cold junction is thermally insulated and carefully screened from stray light the electricity which flows is directly proportional to the energy differential between the two connections.

A thermocouple is made by welding two wires of different semi conductors materials of high thermo electric efficiency if two welded joins are kept at different temperatures a small electrical potential is developed between the joints. A thermo couple is closed in an evacuated steel casing with a KBr or CsI window to avoid losses of energy.

The temperature difference between the two junction generated potential difference which depends on how much IR radiation falls on the hot junction.



b)Thermistors:

A thermistor is made of fused mixture of metal oxides as the temperature of the mixture increases its electrical resistance decreases. Its response time is slow. In thermistor sintered oxides of Mn, Co,Ni (which have high temp. coefficient of resistance) are used.

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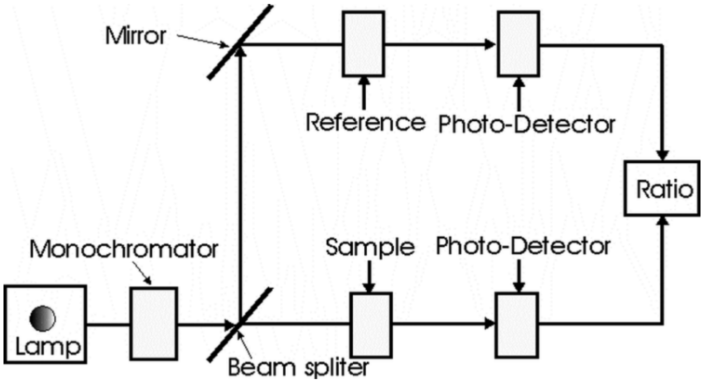
Double beam spectrometer:



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Chopper: chopper which rotates at definite speed reflects the sample and the sample and the

Reference beams to a monochromator grating as the grating rotates slowly it sends individual frequencies to detector which converts infrared energy into electrical energy it is then amplified with the help of the amplifier and recorded as spectrum by recorder.

Calibration can be carried out by using the polystyrene.

Sampling techniques:

Infrared spectroscopy has been used for the characterization of solid lilquid or gas samples the common point to the sampling of different phases is that the material containg the sample must be transparent to IR radiation.

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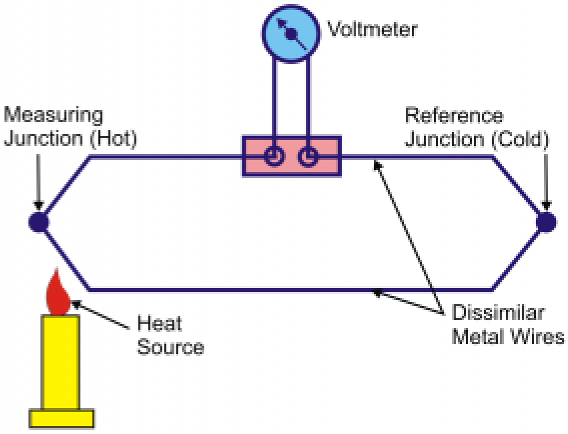

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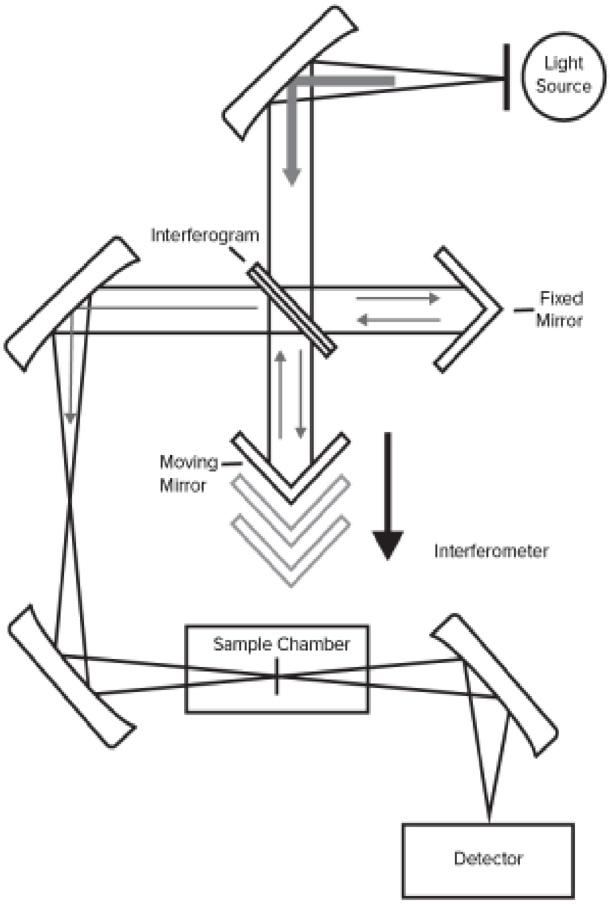
Non-dispersive IR Spectrometer (FT-IR)

*Dispersive IR spectrometers suffers from several disadvantages in sensitivity, speed and wavelength accuracy.

*Fourier transform infrared spectroscopy centers on Michelsointerferometerso that the method can be called as interferometric infrared spectroscopy.



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It is a rod of sintered silicon carbide about 5mm diameter and 50mm lengths It is self starting and as an operating temp near 1300oc Globar is less intensity compared to Nernst glower it is made up of metal oxides.

Interferometer:

This consists of a moving mirror, a fixed mirror and a beam splitter beam splitter is made up of KBr or CsI coated with germanium. Radiation from the infrared source is divided at the beam splitter, half the beam passes to a fixed mirror and half the beam passes to a fixed mirror and half is reflected to the moving mirror after reflection, the two beams recombine at the beam splitter, half the beam passes to a fixed mirror and half is reflected to the moving mirror after reflection, the two beams recombine at the beam splitter and for any particular wavelength. Constructively or destructively interfere depending on the difference in optical paths between the two arms of the interferometer with a constant mirror velocity the intensity of the emerging radiation at any particular wave length. Constructively or destructively interfere depending on the difference in optical paths between the two arms of the interferometer with a constant mirror velocity the intensity of the emerging radiation at any particular wavelength modulates in a regular sinusoidal manner In the case of a broad band source the emerging beam is a complex mixture of modulation frequencies that after passing through the sample compartment is focused on to the detector (G).Both the sampling rate and mirror velocity are controlled by a reference signal incident upon a detector(E) which is produced by modulation of the beam from the He-Ne laser the resulting signal from (G) is stored in memory and the transform is carried out of a computer using cooley –tukey transform algorithm.

Sampling techniques:

Sampling of gases:

The gaseous sample is introduced in to a gas cell the walls of the cell are made of NaCl the cells are larger usually they are about 10cm long but they may be up to 1M long multiple reflections can be used to make the effective path length.

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Comparision of Fourier –Transform and dispersive IR:

Dispersive IR	FT-IR

1.There are many moving parts, resulting mechanical splidppage.	1.only the mirror moves during on experiment.
2.Monochromators and slits are used.	2.monochromator,slits are not used. Interferometer is used.
3.Calibrating against reference spectra is required to measure frequency.	3.use of laser provides high frequency.
4.Stray light with in the instrument causes spurious readings	4.stray light does not affect the detector since all signals are modulated.
5.Only radiation of a narrow frequency range falls on the detector of any one time.	5.All frequencies fall on the detector simultaneously .
6.Scanning speed is slow.	6.scanning speed is high.
7.The sample is subject to thermal effect from the focused beam.	7.the sample is not subject to thermal effects.

Qualitative and Quantitative analysis:

Infrared spectroscopy is used to identify the compound because the spectra of any two compounds are not identical but IR spectroscopy fails to distinguish between enantiomers. The comparison of unknown spectrum with library.

Characteristic absorption of some group

Spectra is done under similar condition (solvent concentration, sampling, scab speed etc.,

1.Alkanes		
Group	Types of vibration	Wavelength cm^{-1}
C-H	C-H stre	2460-2850
C-H	C-H def	1485-1340
C-C	C-C str	1300-800
2.Alkenes		
Alkene	-C=C-H st	3100-3000
Non conjugated	C=C str	1650-1600
Conjugated diene	C=C str	~1600,~1650
3.Alkyne		
Alkyne	C≡C-H str	~3300
Mono substituted	C≡C str	2140-2100
4.aromatic hydrocarbons		
Aromatic	Ar-H str	3060-3000
Disubst(meta)	C-H def	710-690,770-730
Disubst(ortho)	C-C def	770-735
Disubstituted (para)	C-H def	840-800
5.Alcohols and phenols:		
Free O-H	O-H str	3700-3500
Inter molecular H-bonds is	O-H str	3400-3200
Intramolecular H-bond	O-H str	3570-3450
phenol	C-O str	1200,1410-1300
6.Ethers:		
C-O-C	C-O str	1150-1070

7.Aldehydes & ketones		
Saturated aldehydes	C=O str	1740-1720
Aryl aldehyde	C=O str	1715-1690
Saturated ketones	C=O str	1725-1700
Aryl ketones	C=O str	1670-1660
8.Esters		
Saturated esters	C=O str	1750-1735
Aryl esters	C=O str	1730-1715
9.carboxylic acids		
Saturared aliphatic acids	C=O str	1725-1700
Aryl carboxylic acids	C=O str	1700-1680
Carboxylic acids	O=H str	3000-2500
Acyl chlorides	C=O str	1795
10.Amines		
Primary	N=H	3500-3300
secondary	N-N	
11.Nitriles		
Nitrile	-C≡N	2210-2260

Quatitative analysis:

It is based on the Beer’s Lamberts law for this purpose it it essential to plot a calibration cureve from known standard solution when determination concentration of unknowns. The spectrometer is set at the wavelength of maximum absorbance of the material to be determined.

Analysis of petroleum hydrocarbons, oils and grease contents (by environmental protection agency)

These methods provide semi quantitative determination of petroleum hydrocarbons, oil and greases by comparision of the IR absorption of the sample extract with standards.

They all contain carbon –hydrogen bonds thus giving rise to C-H stretching absorption in the range 3100-2700 cm^{-1} fluorocarbon -113 (1,1,2-trichloro-1,2,2, tri fluro ethane) is on eof theclorofluoro carbons called freons. It contains no C-H bbonds and and thus does not absorb IR radiation in 3100-2700 cm^{-1}

The quantity of hydro carbons, oils ga grease in freo extracts can be estimated b measuring the intensity of C-H abborption band at in 2900cm^{-1}

The sample us acidified to a low pH and extracted with fluro carbon -113. Depending on the sample conc cells of path length from 10-100 mm can be used the contributions from the reference spectrum of Freon from the sample spectrum.

In the standard EPA method peak height at a single frequency in 3150-2700 cm^{-1} is also used for quantification aromatic hudrocarbons have relatively lower absorption intensity in this C-H stre. Region thus giving lower response factors compared to oil and grease standards.

Using FTIR instrument oil and grease at low ppm levels can be readily determined

Non destructive IR method for the analysis of carbon monoxide(CO)

The radiation source must emit IR radiation which must be

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2. Steady
3. Extend over the desired wavelengths

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In FTIR spectrometer the sample cell is filled with ‘CO’ gas

Sampling of gases:

The gaseous sample is introduced in to a gas cell the walls of the cell are made of NaCl the cells are larger usually they are about 10cm long but they may be up to 1M long multiple reflections can be used to make the effective path length.

The method is not commonly used because of its lack of sensitivity. Moisture must be avoided.

Detectors:

Golay detector:

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Raman spectroscopy:

Raman spectroscopy deals with the scattering of light and not with its absorption. If uses visible or UV radiation rather than Infrared radiation.

Raman discovered that when a beam of high intense monochromatic light was allowed to pass through a substance in the solids, liquid or gaseous state the scattered light contains some additional frequencies over and above that of the incident frequency this is known as Raman effect.

The lines having wavelength greater than that of the incident wavelength are called stokes's lines and those having shorter wavelength are called Anti-stokes lines.

Raman shift $\Delta v = v_i - v_s$

$v_i =$ frequency of incident radiation

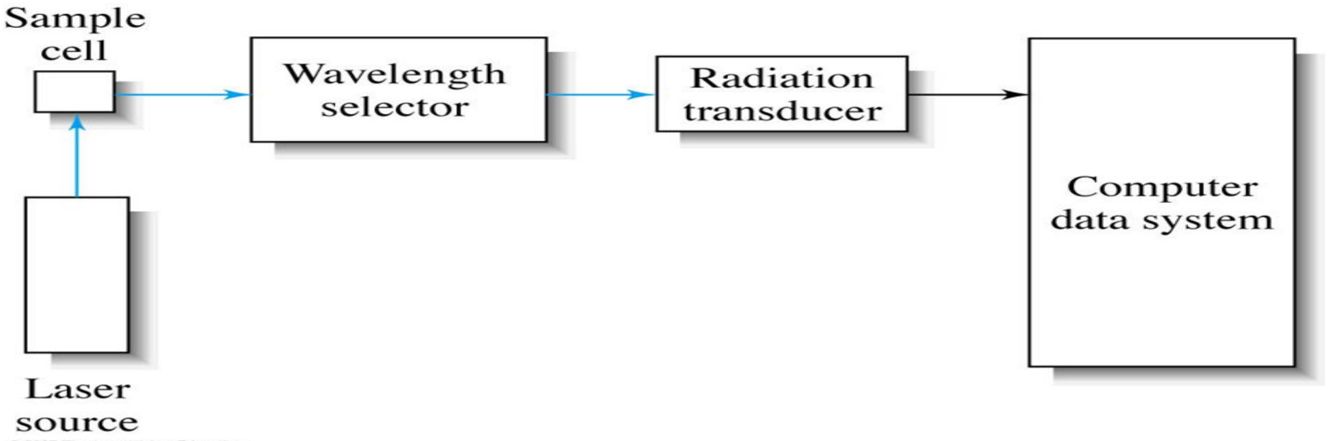
$v_s =$ frequency of scattered radiation

homo nuclear diatomic molecules such as H_2,N_2,O_2 etc do not show IR spectra since they do not posses a permanent dipole moment. They show Raman spectra since there vibration is accompanied by a change in polarisability of the molecules.

Characteristic properties of Raman lines:

1. The intensity of stokes lines is always grater than the corresponding anti- stokes lines.
2. The Raman frequencies are generally identical with IR vibrational frequencies are generally identical with IR vibrational frequencies.
3. Raman lines are symmetrically displaced about the parent lines.

Instrumentation of Raman spectroscopy:



Raman Spectrophotometer

Source of light:

Mercury arc is the most useful source (at 4358Ao) now a days higher lamp is used. It consists of 4 low pressure Hg discharge tubs completely enclosed in a hallow jacket.

He-Ne laser source: the tube is filled with a 7:1 mixture of He and Ne gas for optimum and put of 6328Ao laser line.

Sample holder: Depends upon the intensity of source and nature and availability of sample gases require bigger sample holders than those of liquids.

Solids are dissolved before subjecting to Raman spectroscopy water is used for the study of inorganic compounds.

Filters: Light from He-Ne laser beam is allowed to enter the sample compartment horizontally. Then Raman scattering from the sample cell is focused on monochromator entrance slit commonly used filters are quartz, nickel oxide and glass.

Detector: Photomultiplier tube are used as detector

Photomultiplier tubes:-

The photo cathode surface is similar in composition to the surface of the photo tubes and it emits the electrons when exposed to radiation. The tube also contains additional electrodes called dynodes. The dynode one is maintain at potential 90V more +ve than the cathode and electrons are accelerated towards as a consequence upto striking the dynode each photoelectron causes emission of several additional electrons. These intern or accelerated towards dynode2.which is 90V more +ve than dynode1 by the time this process has been repeated up to 9 times 10^6 to 10^7 electrons have been formed for each incident photon these are finally collected at anode and the resulting current is then electronically amplified and measured.

Applications:

1.Structure of CO_2:

This is a linear molecules one should expect two fundamental lines (v_2& v_3) in IR and v_1 in Raman

v_1=1340cm^{-1} ; v_2=667cm^{-1} ; v_3=2349cm^{-1}

2.Structure of N_2O:

It is assumed that N_2O is symmetrical like C_2O i.e N-O-N one should expect a similar type of behavior as in C_2O. If unsymmetrical i.e. N-N-O v_1 should become active in IR as in Raman structure of N_2O is N-N-O.

3.Structure of CO:

It gives a Raman line at 2155 cm^{-1} which is closely similar to-C≡N of 2200cm^{-1} this shows that like cyanides, CO is having -C≡O.

4.Water:

The triatomic molecules having a linear bent structure should exhibit 3 Raman lines. In the Raman spectrum of H_2O two frequencies have been recorded at 1665 and3600cm^{-1} . In addition other bonds are also observed

Differences between Raman and IR spectra:

Raman spectra	Infrared spectra
1.It is due to the scattering of light by the vibrating of light by the vibrating molecules.	1.It is the result of absorption of light by vibrating molecules.
2.Polarizability of the molecule will decide whether raman spectra will be observed or not.	2.The presence of permanent dipole moment in molecule will decide.
3.It can be recorded only in one expose.	3.It requires at least two separate runs with different prism.
4.Water can be used as a solvent.	4.Water can't be used.
5.the method is very accurate but is not very sensitive	5.The method is accurate and very sensitive.
6.Optical system are made of glass or quartz	6.Optical systems are made up of CaF_2 ,NaBr etc
7.Sometimes photo chemical reactions takes place.	7.Photochemical reactions do not takes place.

UNIT-III

NMR SPECTROSCOPY:

The basic principles are usually covered in the physical chemistry course, and now every undergraduate physical chemistry textbook has a chapter on it. In addition, numerous monographs have been written on NMR, but these are usually addressed at the advanced undergraduate and graduate levels (Ernst, Bodenhausen, & Wokaun, 1987). It is also wellknown that, conceptually, NMR is a very difficult subject to teach and learn; this is because this subject is grounded on physical concepts of electricity and magnetism and classical mechanics and is further extended with quantum mechanical treatments. It is reasonable, then, that a course in NMR poses considerable learning obstacles for the student. The purpose of this article is twofold; on the one hand to propose a teaching sequence for undergraduate student in various disciplines that, in our opinion, is conceptually sound; on the other hand, to emphasize and describe selected (both traditional and modern)

applications of NMR to chemical structure determination, (in solution and in the solid state), food analysis, clinical investigations, and NMR microscopy and imaging.

Resonance condition in NMR spectroscopy:

In the absence of an external magnetic field the direction of the spin of the nuclei will be randomly oriented (see figure below left). However, when a sample of these nuclei is place in an external magnetic field, the nuclear spins will adopt specific orientations much as a compass needle responses to the Earth’s magnetic field and aligns with it. Two possible orientations are possible, with the external field (*i.e.* parallel to and in the same direction as the external field) or against the field (*i.e.* antiparallel to the external field). See figure below right.

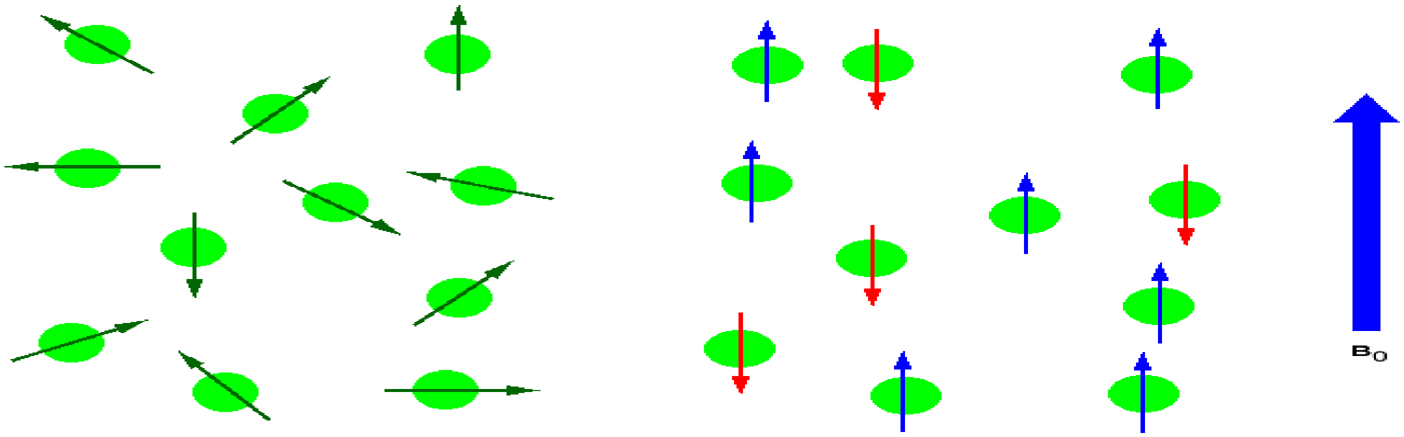
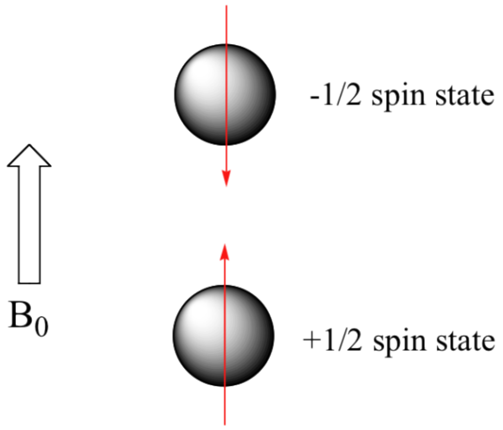


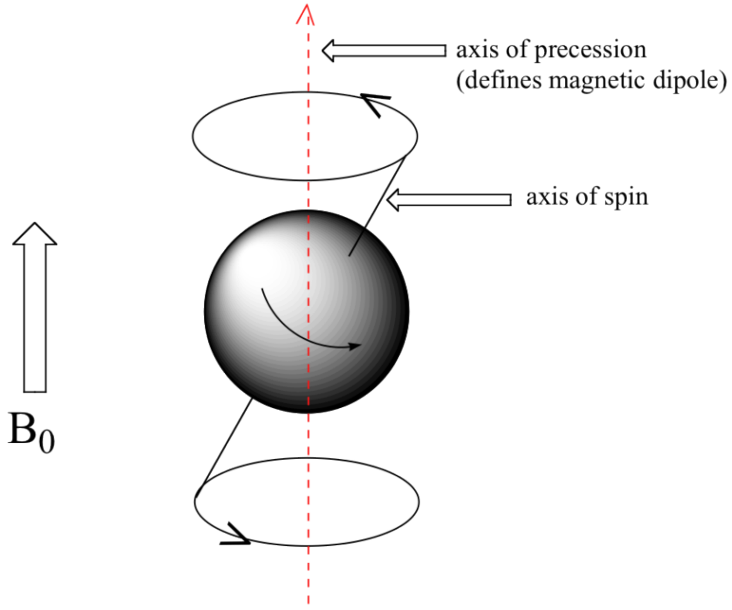
Figure 1: (Left) Random nuclear spin without an external magnetic field. (Right)Ordered nuclear spin in an external magnetic field

When the same sample is placed within the field of a very strong magnet in an NMR instrument (this field is referred to by NMR spectroscopists as the **applied field**, abbreviated $\mathbf{B_0}$) each hydrogen will assume one of two possible **spin states**. In what is referred to as the +½ spin state, the hydrogen's magnetic moment is aligned *with* the direction of B_0, while in the -½ spin state it is aligned *opposed to* the direction of B_0.



Because the +½ spin state is slightly lower in energy, in a large population of organic molecules slightly more than half of the hydrogen atoms will occupy this state, while slightly less than half will occupy the –½ state. *The difference in energy between the two spin states increases with increasing strength of B_0.*This last statement is in italics because it is one of the key ideas in NMR spectroscopy, as we shall soon see.

At this point, we need to look a little more closely at how a proton spins in an applied magnetic field. You may recall playing with spinning tops as a child. When a top slows down a little and the spin axis is no longer completely vertical, it begins to exhibit **precessional motion**, as the spin axis rotates slowly around the vertical. In the same way, hydrogen atoms spinning in an applied magnetic field also exhibit precessional motion about a vertical axis. It is this axis (which is either parallel or antiparallel to B_0) that defines the proton’s magnetic moment. In the figure below, the proton is in the +1/2 spin state.

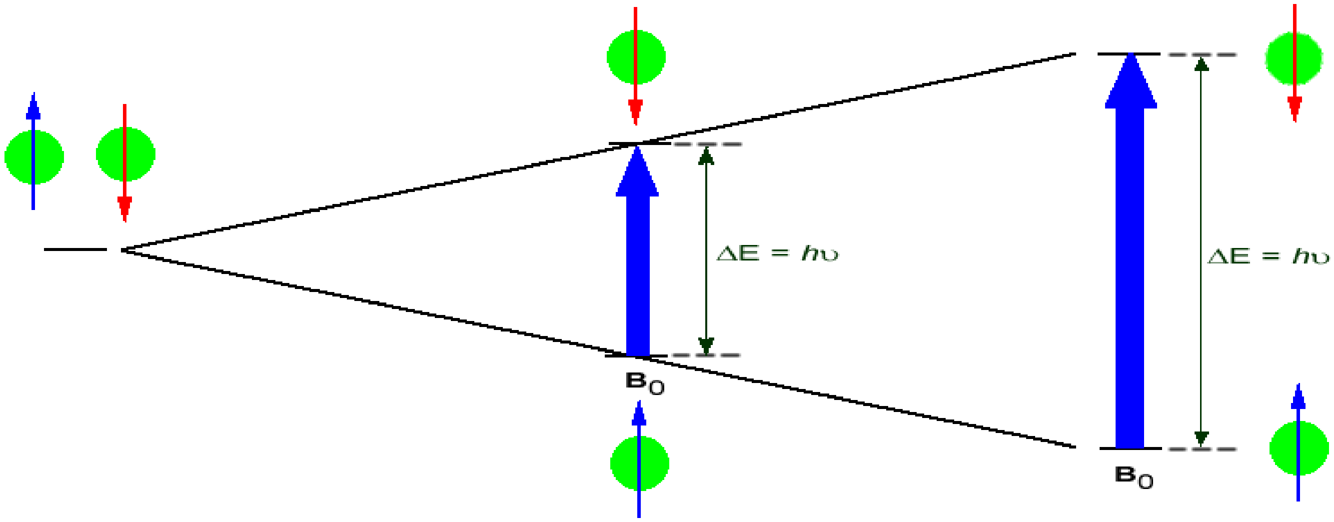


The **frequency of precession** (also called the **Larmour frequency,** abbreviated ω_L) is simply the number of times per second that the proton precesses in a complete circle. A proton`s precessional frequency increases with the strength of B_0.

If a proton that is precessing in an applied magnetic field is exposed to electromagnetic radiation of a frequency ν that matches its precessional frequency ω_L, we have a condition called **resonance**. *In the resonance condition, a proton in the lower-energy +½ spin state (aligned with B_0) will transition (flip) to the higher* *energy –½ spin state (opposed to B_0). In doing so, it will absorb radiation at this resonance frequency* $\boldsymbol{\nu} = \boldsymbol{\omega}_L$. This frequency, as you might have already guessed, corresponds to the energy difference between the proton's two spin states. With the strong magnetic fields generated by the superconducting magnets used in modern NMR instruments, the resonance frequency for protons falls within the radio-wave range, anywhere from 100 MHz to 800 MHz depending on the strength of the magnet.

If the ordered nuclei are now subjected to EM radiation of the proper frequency the nuclei aligned with the field will absorb energy and "spin-flip" to align themselves against the field, a higher energy state. When this spin-flip occurs the nuclei are said to be in "resonance" with the field, hence the name for the technique, **N**uclear **M**agentic **R**esonance or NMR.

The amount of energy, and hence the exact frequency of EM radiation required for resonance to occur is dependent on both the strength of the magnetic field applied and the type of the nuclei being studied. As the strength of the magnetic field increases the energy difference between the two spin states increases and a higher frequency (more energy) EM radiation needs to be applied to achieve a spin-flip (see image below).

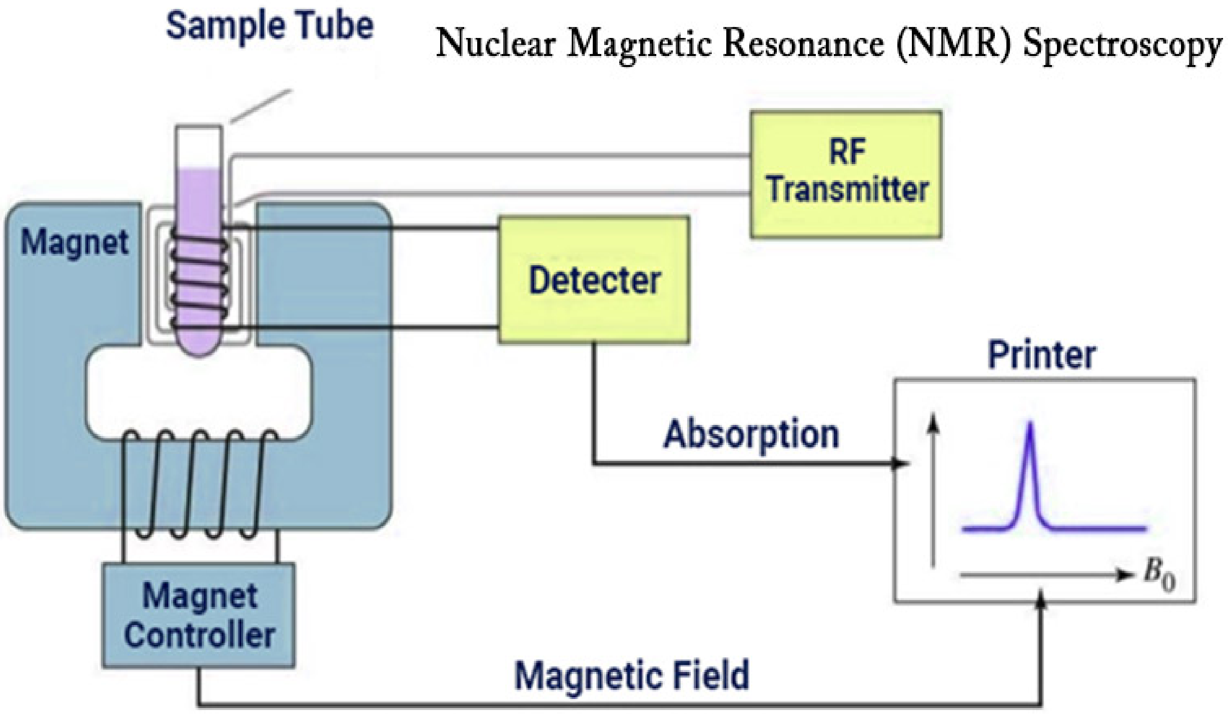


Superconducting magnets can be used to produce very strong magnetic field, on the order of 21 tesla (T). Lower field strengths can also be used, in the range of 4 - 7 T. At these levels the energy required to bring the nuclei into resonance is in the MHz range and corresponds to radio wavelength energies, *i.e.* at a field strength of 4.7 T 200 MHz bring ^{1}H nuclei into resonance and 50 MHz bring ^{13}C into resonance. This is considerably less energy then is required for IR spectroscopy, ~10^{-4} kJ/mol versus ~5 - ~50 kJ/mol.

^{1}H and ^{13}C are not unique in their ability to undergo NMR. All nuclei with an odd number of protons (^{1}H, ^{2}H, ^{14}N, ^{19}F, ^{31}P ...) or nuclei with an odd number of neutrons (*i.e.* ^{13}C) show the magnetic properties required for NMR. Only nuclei with even number of both protons and neutrons (^{12}C and ^{16}O) do not have the required magnetic properties.

Nuclear Magnetic Resonance (NMR) Spectroscopy

- Nuclear magnetic resonance spectroscopy, most commonly known as NMR spectroscopy or magnetic resonance spectroscopy (MRS), is a spectroscopic technique to observe local magnetic fields around atomic nuclei.
- It is a spectroscopy technique which is based on the absorption of electromagnetic radiation in the radio frequency region 4 to 900 MHz by nuclei of the atoms.
- Over the past fifty years, NMR has become the preeminent technique for determining the structure of organic compounds.
- Of all the spectroscopic methods, it is the only one for which a complete analysis and interpretation of the entire spectrum is normally expected.



Principle of Nuclear Magnetic Resonance (NMR) Spectroscopy

1. The principle behind NMR is that many nuclei have spin and all nuclei are electrically charged. If an external magnetic field is applied, an energy transfer is possible between the base energy to a higher energy level (generally a single energy gap).
2. The energy transfer takes place at a wavelength that corresponds to radio frequencies and when the spin returns to its base level, energy is emitted at the same frequency.
3. The signal that matches this transfer is measured in many ways and processed in order to yield an NMR spectrum for the nucleus concerned.

Working of Nuclear Magnetic Resonance (NMR) Spectroscopy

- The sample is placed in a magnetic field and the NMR signal is produced by excitation of the nuclei sample with radio waves into nuclear magnetic resonance, which is detected with sensitive radio receivers.
- The intramolecular magnetic field around an atom in a molecule changes the resonance frequency, thus giving access to details of the electronic structure of a molecule and its individual functional groups.
- As the fields are unique or highly characteristic to individual compounds, NMR spectroscopy is the definitive method to identify monomolecular organic compounds.

- Besides identification, NMR spectroscopy provides detailed information about the structure, dynamics, reaction state, and chemical environment of molecules.
- The most common types of NMR are proton and carbon-13 NMR spectroscopy, but it is applicable to any kind of sample that contains nuclei possessing spin.

Instrumentation of Nuclear Magnetic Resonance (NMR) Spectroscopy

1. **Sample holder**

Glass tube with 8.5 cm long, 0.3 cm in diameter.

2. **Permanent magnet**

It provides homogeneous magnetic field at 60-100 MHZ

3. **Magnetic coils**

These coils induce magnetic field when current flows through them

4. **Sweep generator**

To produce the equal amount of magnetic field pass through the sample

5. **Radio frequency transmitter**

A radio transmitter coil transmitter that produces a short powerful pulse of radio waves

6. **Radio frequency receiver**

A radio receiver coil that detects radio frequencies emitted as nuclei relax to a lower energy level

7. **Read out systems**

A computer that analyses and record the data.

Applications of Nuclear Magnetic Resonance (NMR) Spectroscopy

Spectroscopy is the study of the interaction of electromagnetic radiation with matter. NMR spectroscopy is the use of the NMR phenomenon to study physical, chemical and biological properties of matter.

- It is an analytical chemistry technique used in quality control.
- It is used in research for determining the content and purity of a sample as well as its molecular structure. For example, NMR can quantitatively analyze mixtures containing known compounds.
- NMR spectroscopy is routinely used by chemists to study chemical structure using simple one-dimensional techniques. Two-dimensional techniques are used to determine the structure of more complicated molecules.
- These techniques are replacing x-ray crystallography for the determination of protein structure.
- Time domain NMR spectroscopy techniques are used to probe molecular dynamics in solution.
- Solid state NMR spectroscopy is used to determine the molecular structure of solids.
- Other scientists have developed NMR methods-of measuring diffusion coefficients.

Chemical Shift

It is clear that NMR spectrum cannot be obtained on isolated nuclei. It therefore is necessary to use a suitable standard which can define the degree of shielding or deshielding of nuclei in different chemical environments. Tetramethylsilane (CH3)4Si is an ideal reference for reporting chemical shifts due to different groups.

Chemical shift is a dimensionless quantity but its magnitude is extremely small in comparison to the applied magnetic field or frequency. Therefore the observed value is multiplied by 106 and reported in parts per million (ppm). Conventionally the chemical shift scale ranges from 0 to 12 ppm. TMS is conventionally assigned 0 ppm and the values increase to the left along the x-axis.

Influencing factors on chemical shifts

Electronegativity

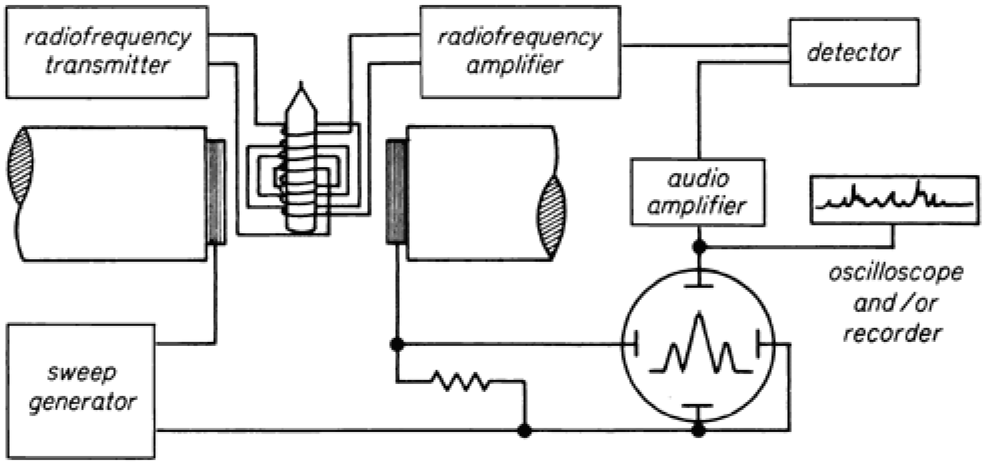
Electronegative atoms present in molecules tend to draw the electron density towards themselves and deshield the nucleus. An increase in electronegativity of the surrounding groups will result in decrease of the electron density and lead to an increase in chemical shift value due to the shielding of the nucleus.

Anisotropy

Anisotropy refers to the property of the molecule where a part of the molecule opposes the applied field and the other part reinforces the applied field. Chemical shifts are dependent on the orientation of neighbouring bonds in particular the π bonds. Examples of nucleus showing chemical shifts due to π bonds are aromatics, alkenes and alkynes. Such anisotropic shifts are useful in characterizing the presence of aromatics or other conjugated structures in molecules.

Hydrogen bonding :Hydrogen bonding results from the presence of electronegative atoms in neighbourhood of protons .The resulting deshielding leads to higher values of chemical shifts. This confirms the presence of hydrogen bonding in the molecules.Chemical shifts of NMR active protons and other nuclei serve to provide a wealth of structural information on molecules.

FT-NMR SPECTROSCOPY:



- The central component of the instrument is highly stable magnet in which the sample is placed.
- The sample is surrounded by receiver cell.
- Radio-frequency radiation is produced by a crystal controlled frequency synthesizer having an out put frequency of ν_c
- The signal passes data is amplified and creates an intense and reproducible pulse of radio- frequency current in the transmitter coil.
- The resulting RF pulse impinges on the sample contained inside the coil. The signal is amplified and transmitted to a phase sensitive detector
- The detector produces the difference between the nuclear signal ν_n and crustal oscillator output $\nu_{c.}$
- The signalis dizitized and collected in the memory of the computer for analysis by a fourier transform program and other data analysis software

- The heart of both CW and FT-NMR instruments is the magnet. The sensitivity and resolution of both types of spectrometers. it depends upon the strength and quality of the magnets.
- Both resolution and sensitivity increases with increases in field strength. In addition the field must be highly homogeneous and reproducible
- There are three types of magnets are employed in NMR studies.
  1. Permanent magnets
  2. Conventional magnets
  3. Super conducting magnets
- Permanent magnets with field strength 0.7,1.4,2.1 T have been used in the CW instruments permanents magnets are highly temperature sensitive and require extensive thermo station and shielding. But these are not ideal magnets for FT-NMR studies.
- Super conducting magnets are used in modern high- resolution instruments. These magnets attain fields as large as 21T corresponding to a proton frequency of 900MHz.
- Most super conducting magnet systems must be filled with liquid nitrogen every 10 days and with liquid helium every 80 to 130 days
- The advantage of super conducting solenoids in addition to their high field strength are their high stability, low operating cost, simplicity and small size compared with electromagnet.
- In order to reduce the effect of field fluctuation a frequency lock system is employed in commercial NMR instruments.
- Shimming: shim coils are pairs of wire loops through which carefully controlled currents are passed, producing small magnetic fields that compensate in homogeneities in the primary magnets field strength. In order in older instruments potentiometers are used to adjust the current in various pairs of the coils.
- Sample spinning: the homogeneity in the field can also be occurred hue to spinning the sample along longitudinal axis.
- Spinning is accomplished by means of small plastic turbine that slips over the sample tube a stream of air drives the turbine at a rate of 20 to 50 Rps.
- Sample probe: a key component is NMR spectrometer is sample probe. It holds the sample in a fixed position in the magnetic field, contains an air turbine to spin the sample in addition the probe ordinarily contains two other transmitter coils one for locking and other for decoupling experiments.
- The usual NMR sample cell consists 5mm ordinary glass tube containing 0.4mL of sample.
- Receiver cell/transmitter: NMR instruments consist of separate coils mounted at right angles to one another for producing excitation pulse and detecting the generated NMR signal. Single cell or single coil probe is used instead of crossed coil detectors.
- Pulse generator: R.F generators and frequency synthesizers produce a signal of essentially a single frequency. To generate FT spectra however, the sample must be irradiated with a range of frequencies sufficiently great to excite nuclei with different resonance frequencies.
- A narrow pulse generated by rapidly switching a radio frequency oscillator from off to on and then on to off will consists of band of frequencies. It it capable of exciting all nuclei of interest whose resonance will occur in the vicinity of frequency of the oscillator
- A pulse generator consists of frequency synthesizer, a gate to switch the pulse on and off at appropriate times and power amplifier to amplify the pulse at about 50 to 100 W

- Receiver system: voltage generated by the current in the detector coil is in nanovolt to microvolt range and thus it is amplified to range of about 0 to 10V. Before the signal can be further processed and digitized this amplified signal is recorded.

Magnitude of "I":

Nuclei carry a charge. So they will posse spin angular momentum. The momentum of the spin angular momentum is quantized

i.e. only those nuclei which have a finite value of spin quantum no (I>0) will process along the axis of rotation. It is known that the spin atomic no of the nuclei.

Mass No.	Atomic No	Spin quantum No,(I)
Odd	Odd or Even	½,3/2,5/2,…..
Even	Even	0
Even	Odd	1,2,3, ……

Therefore the no. of orientation taken up by a spinning nucleus is given by 2I+1

Spin tickling: it is low intensity irradiation and it is carried out by weakly irradiating line ofspecturm (of a multiplet). This technique

Spin –spin coupling:

The interaction between the spins of the neighboring nuclei in a molecule may cause the splitting of the lines in the NMR spectrum. This is known as spin-spin coupling which occurs through bonds (not space) by means of a slight umpiring of the bonding electrons.

It should be kept in mind that the area under a broad NMR signal and the total area under the several split signals remains the same.

We know that the number of signals in a NMR spectrum is equal to the number of types of protons present in the given there being one signal each for every set of equivalent protons. This found to be so in some cases. But the actual NMR spectra of most of the compounds are much more complicated than expected on the basis of above statement. For example, consider 1,1,2- tribromoethane which is having only two types of protons, and, therefore, its NMR spectrum may be expected to have only two absorption peaks. But, in actual practice, its NMR spectrum shows as many as five peaks as shown in below

^{1}H-NMR of 1,1,2-tribromoethane

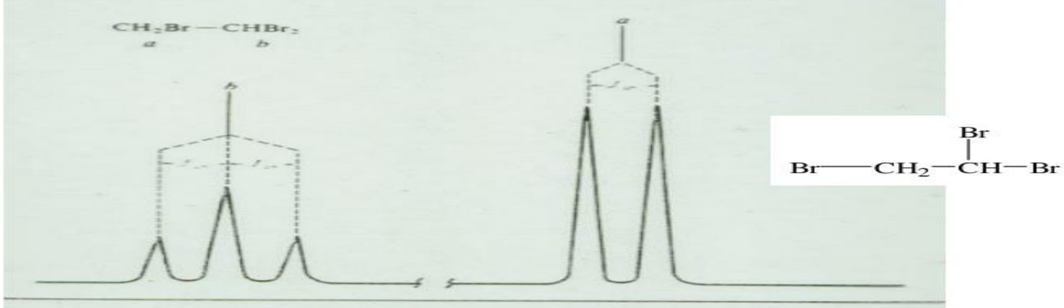


Figure 16.11 Spin–spin splitting. Signal *a* is split into a doublet by coupling with one proton: signal *b* is split into a triplet by two protons. Spacings in both sets are the same (J_{ab}).

Interpretation of NMR spectra:

NMR spectrum of a substance gives very valuable information about its molecular structure this information is gathered as follows:

1. The number of signals in the pmr spectrum tells us how many kinds of protons in different chemical environments are present in the structure under examination.
2. The positions of signals tell us about the electronic environment of each kind of proton.
3. The intensities of different signals tell us about the relative number of protons of different kinds.
4. The splitting of singles tells us about the environments of the absorbing proton with respect to the environments of the neighboring protons.

Spin de- coupling :

Multiplicity of signals arises because neighboring protons have more than one spin orientation. If we irradiate the neighboring proton with correct frequency, we can stimulate rapid transition between two spin states, so that the life time of a nucleolus in any one spin state is too short to resolve the coupling with observed nuclei.

To perform this operation, in addition to the basic NMR instrument, a second tunable radio frequency source to irradiate neighboring proton since we are making simultaneous use of two radio frequency sources, the technique is called double resonance. Since the nuclear spins during the process are less coupled than before, we also call it spin decoupling.

For the method to be successful, the chemical shift positions for the coupling multiplets should be no closer

~1ppm.

Types of spin decoupling:

a) Homo nuclear decoupling
b) Hetero nuclear decoupling
c) Spin tickling

Homo nuclear decoupling:

This term is used to describe a selective decoupling experiment where the type of nucleus decoupled is the same as that being observed (E.g.: decoupling one proton while observing other protons)

Hetero nuclear decoupling:

When the observed and the decoupled nuclei are different the decoupling is known as hetero nuclear decoupling is the decoupling of ^1H while observing ^{13}C

i)Broad band decoupling:

In this, decoupling completely nuclei of one type while observing the spectrum of one (e.g. decoupling all 1H nuclei observing 13C spectrum) broad band decoupling is usually achieved by square wave modulation of the decoupling Radio frequency radiation, effectively resulting in a speed of frequencies irradiated simultaneously.

ii) Off resonance decoupling (partial decoupling):

partial decoupling of one type of nuclei while observing the spectrum of another type of nuclei by applying an RF field not exactly equal to the larmour frequency of that nucleus, is known as off resonance decoupling. If we use fully decoupling technique we may loss valuable information. But in case of off resonance, the multplet collapses partially, then we can say about the spin active nuclei about observed nuclei.

Spin tickling :

Spin tickling is a modified spin decoupling in which a weak decoupler signal (having power one tenth of that for full decoupling) is applied to double irradiate one line in a multplet instead of irradiating the whole multiplet instead of irradiating the whole multplet with a powerful decoupler. This has the effect of perturbing the energy levels of the spin system and thus brigs about slight but definite and thus brigs about slight but definite changes in the appearance of the signals coupled to the signal being coupled.

In this experiment a weak RF field is employed and all transitions having an energy level in common with the peak being irradiated will undergo a change .

For example 1→2 transition is having and energy level C_{11} is common with the transitions 1→3and 2→4, but not the 3→4 transition. Thus the latter peak is not split by spin tickling the 1→2 peak, but the other spectral lines will be split. Experimentally, it is found that the tickling splits the lines that have an energy level in common with the line being saturated.

Shift reagents:

The addition of small amounts of certain lanthanide metal ions to particular compounds induce large changes in the chemical shifts without line broadening thus resulting in the simplification of complex second order spectra to first order.

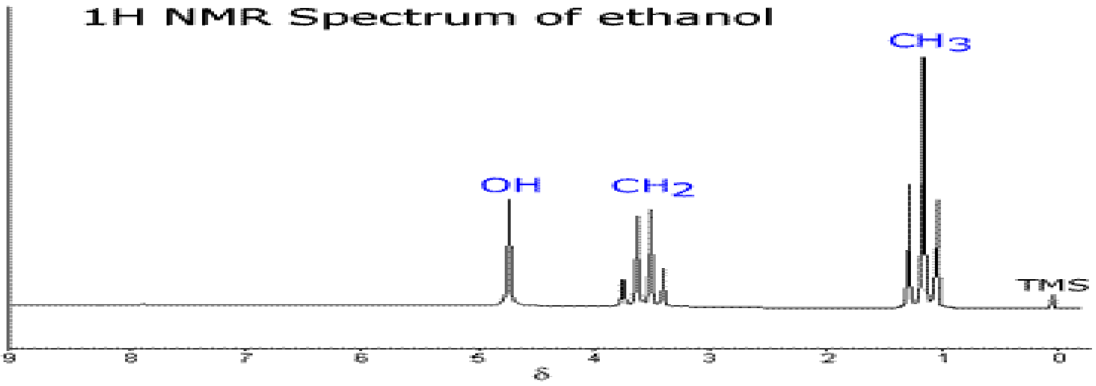
These are mild Lewis acids which attach themselves to basic sites such as amino hydroxyl and keto groups. The large shifts produced by these reagents are due to co- ordination of molecules under study to the paramagnetic lanthanide ion. The magnetic field experienced by the bound substrate changes under the influence of the magnetic field induced by the unpaired electrons of the lanthanide ion. The shift is generally down field with europium and up field with praseodymium, ytterbium, erbium and holmium compounds tend to give greater shift, but in these last two cases some line broadening occurs and complicates the analysis of multiplets. The line broadening is associated with the paramagnetic ions ability to accelerate the relaxation process.

Mechanism:

The mechanism of the shift is twofold unpaired electron spin in the paramagnetic ion is partially transferred through the interning bonds true contact shift) or space (pseudo contact shift) and creates secondary fields around protons of the organic substrate. Lanthanide ion is used in the form of following complexes for its solubility in the common NMR solvents.

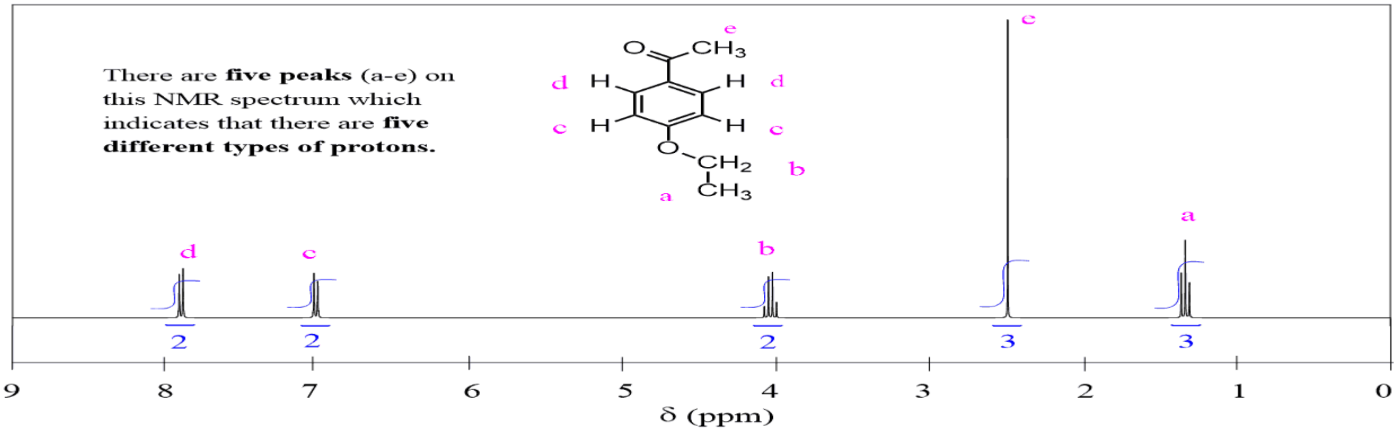
Applications of NMR spectroscopy:

All alcohols, such as **ethanol**, are very, very slightly acidic. The hydrogen on the -OH group transfers to one of the lone pairs on the oxygen of the water molecule. ... Deuterium atoms don't produce peaks in the same region of an **NMR spectrum** as ordinary hydrogen atoms, and so the peak disappears.



The number of NMR signals represents the number of different types of protons in a molecule.

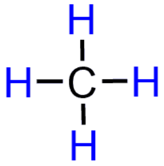
This is the example we used in the introduction to NMR spectroscopy:


There are **five peaks** (a-e) on this NMR spectrum which indicates that there are **five different types of protons.**

The spectrum has five signals which indicates five types of different protons.

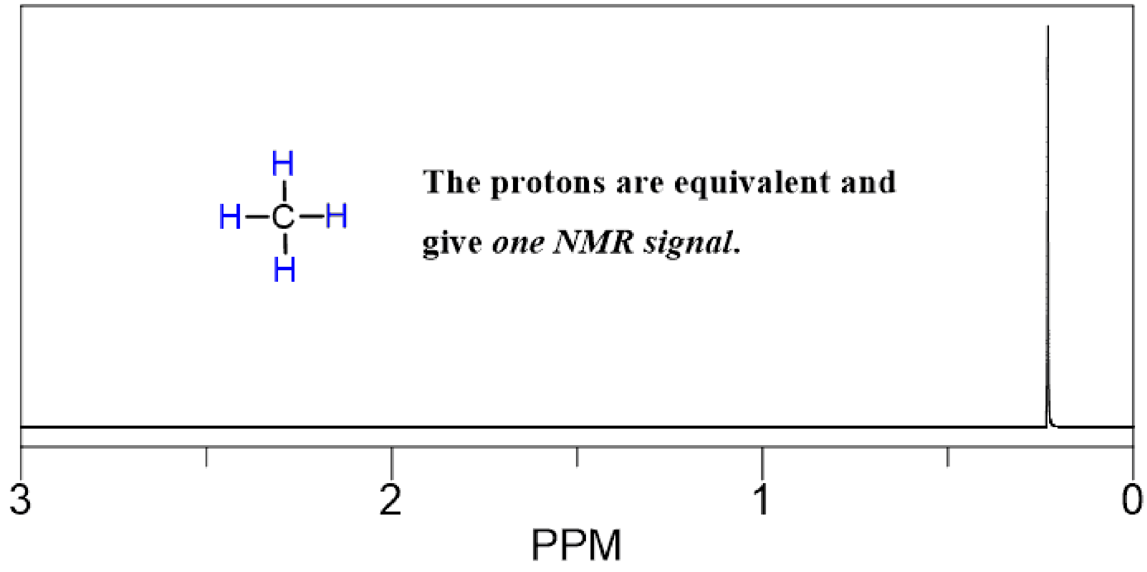
So, what does a type of proton mean?
Protons are grouped in types based on their environments.

For example, let’s stat with the simplest hydrocarbon; how many signals would you expect to see on the NMR spectrum of methane?



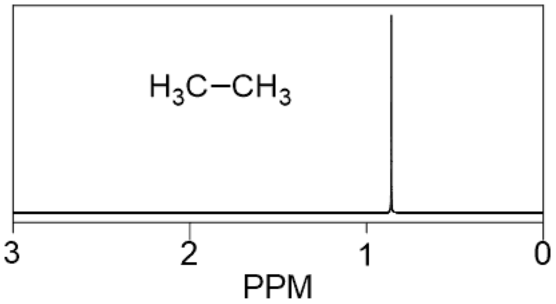
$\mathrm{H{-}\overset{H}{\underset{H}{C}}{-}H}$

Even though it has four protons, they are all connected to the same atoms and have the same neighbors on all sides – in other words, they are equivalent because they are in the same environment.
Remember, equivalent protons give one NMR signal:



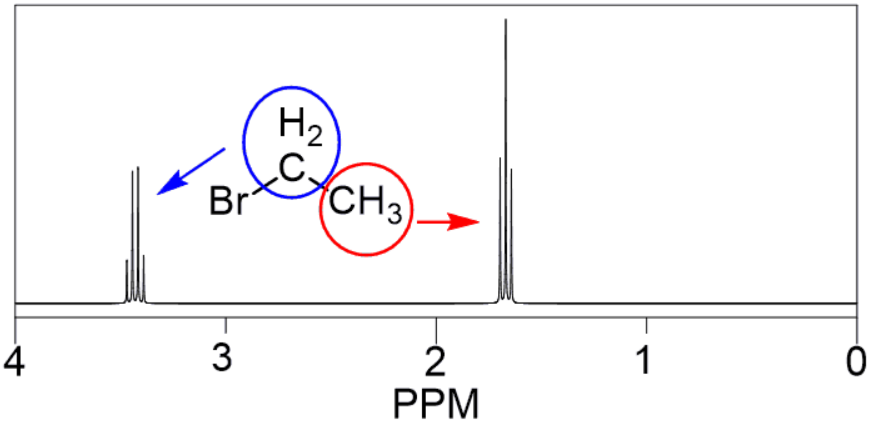
There are four protons. However, each proton has the same connectivity and they are all in the ***same environment.***

It is the same with ethane; six protons – all equivalent, therefore one NMR signal:



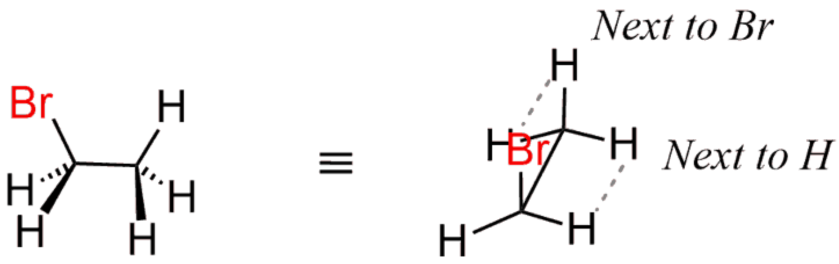
If we place another atom on any of the CH_3 groups of ethane, it makes the protons of the CH_2 and CH_3 groups different.

For example, bromoethane gives two NMR signals because the protons of the CH_2 groups, being closer to the bromine, are different from those in the CH_3 group:



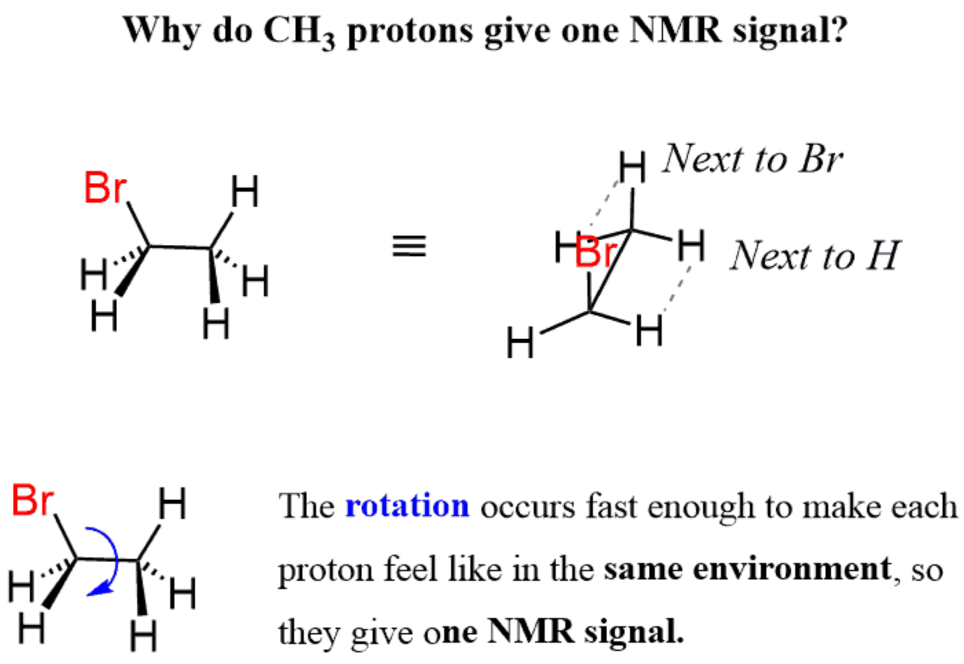
*There are **two signals**: one - from the CH_3 protons, and the other one from the protons of CH_2 group.*

There is a good question I get asked quite often;
Why are the protons of the same CH_3 group give one signal?
The carbon is tetrahedral and at any given time these protons are in a different environment since every conformation puts them next to a different atom:



So, shouldn’t they give different NMR signals?

You need to keep in mind that molecules do not freeze in one conformation unless the free rotation about a single bond is restricted by a steric or intramolecular bonding factors. The CH_3 group is very small and the rotation occurs fast enough to make each proton feel like in the same environment, so they give one NMR signal:

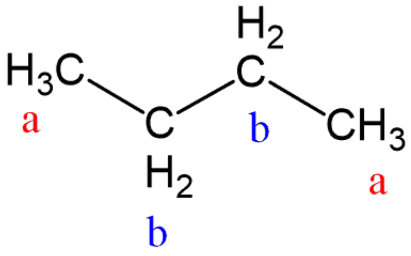


Why do CH_3 protons give one NMR signal?

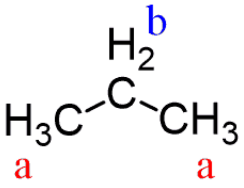
The **rotation** occurs fast enough to make each proton feel like in the **same environment**, so they give **one NMR signal.**

Let’s also look at the next two alkanes, propane and butane before trying to find some patterns for determining the number of NMR signals a little easier.

Propane and butane give two signals. One because the protons of the CH_2 group are different from those in the CH_3 group, and the other, because despite having four carbon atoms, the molecule is a combination of two identical CH_2 and CH_3 groups:



2 NMR signals



2 NMR signals

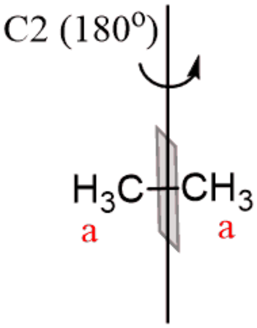
Protons a are different from protons b

Each type gives one NMR signal

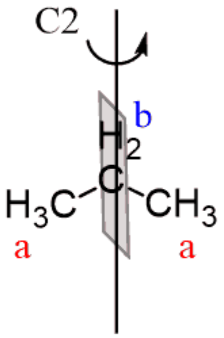
From these examples, you might have noticed that the number of NMR signals is somehow related to the symmetry of the molecule.
And yes, that is a good observation. Essentially, if two protons (or two group of protons) are exchangeable either by a symmetry axis or a plane of symmetry, they are equivalent and give one signal:

Symmetry element - equivalent protons, ne signal.

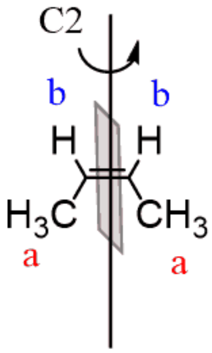
No symmetry element - not equivalent protons, different signals.



6 protons but
only 1 type
1 signal

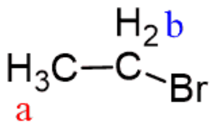


8 protons but
2 types
2 signals



8 protons but
2 types
2 signals

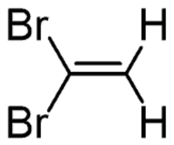
No symmetry



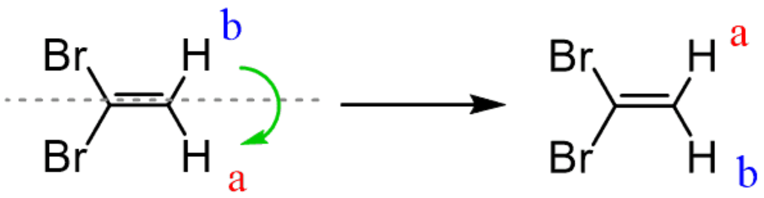
5 protons but
2 types
2 signals

Determining Equivalent Protons an Alkenes

How many signals does the NMR spectrum of the following alkene have?

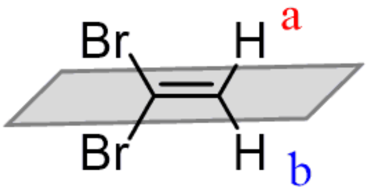


The two protons here are equivalent and will give one NMR signal. You can see this by flipping the molecule 180° which produces the same molecule:



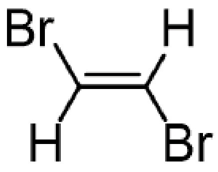
Same compound, therefore protons a and b are equivalent - **one signal**.

You can also visualize the symmetry plane reflecting protons a and b:

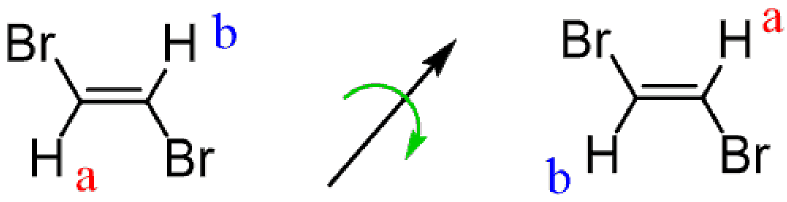


The following molecule does not have a plane of symmetry. However, protons a and b are exchangeable through a C2 (180°) symmetry axis and therefore, one NMR signal is expected too:

Equivalent protons are exchangeable through a symmetry axis



The molecule doesn't have a plane of symmetry. However, protons a and b are exchangeable through a symmetry axis.

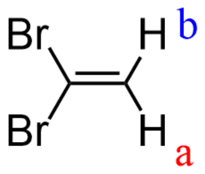


Protons a and b are equivalent - they give one NMR signal.

In both molecules, the protons were cis to a bromine and overall in the same environment.

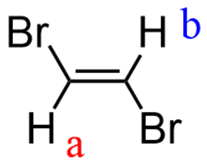
There is a trait for equivalent protons in alkenes which shows that the two protons must be cis to the same group:

Equivalent protons in alkenes must be cis to the same group



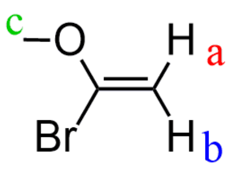
Ha and Hb are **both**
cis (trans) to the Br
They are **equivalent.**

One NMR signal



Ha and Hb are **both**
cis (trans) to the Br
They are **equivalent.**

One NMR signal



Ha is **cis to methoxy**,
while Hb is **cis to the Br**
They are NOT **equivalent.**

Two NMR signals (a, b)
and one signal from CH_3

In the last molecule, proton a is cis to the methoxy group while proton b is cis to the bromine which puts them in different environment and therefore, two NMR signals will be observed.
The non-equivalence of these two protons is also proved by the absence of symmetry elements between them.

The equivalent and non-equivalent protons are classified more rigorously as homotopic, enantiotopic, diastereotopic and constitutionally heterotopic. We will talk about these definitions and the methods for determining them in the next post and before doing that, here are some

Electron Spin Resonance (ESR)- Principle, Instrumentation, Applications

- Electron Spin Resonance (ESR) also known as Electron Magnetic Resonance (EMR) or Electron Paramagnetic Resonance (EPR) is a branch of absorption spectroscopy in which radiations having frequency in the microwave region (0.04 – 25 cm) is absorbed by paramagnetic substances to induce transitions between magnetic energy levels of electrons with unpaired spins.
- ESR is based on the fact that atoms, ions, molecules or molecular fragments which have an odd number of electrons exhibit characteristic magnetic properties. An electron has a spin and due to spin there is magnetic moment.
- Since its discovery in 1944 by E.K. Zavoisky, EPR spectroscopy has been exploited as a very sensitive and informative technique for the investigation of different kinds of paramagnetic species in solid or liquid states.

Principle of Electron Spin Resonance (ESR)

The phenomenon of electron spin resonance (ESR) is based on the fact that an electron is a charged particle. It spins around its axis and this causes it to act like a tiny bar magnet. When a molecule or compound with an unpaired electron is placed in a strong magnetic field The spin of the unpaired electron can align in two different ways creating two spin states ms = ± ½.

The alignment can either be along the direction (parellel) to the magnetic field which corresponds to the lower energy state ms = – ½ Opposite (antiparallel) to the direction of the applied magnetic field ms = + ½

The two alignments have different energies and this difference in energy lifts the degeneracy of the electron spin states. The energy difference is given by:

$\Delta E = E+ - E- = hv = gmßB$

Where,

h = Planck’s constant (6.626 x 10-34 J s-1)

v = the frequency of radiation

ß = Bohr magneton (9.274 x 10-24 J T-1) B = strength of the magnetic field in Tesla

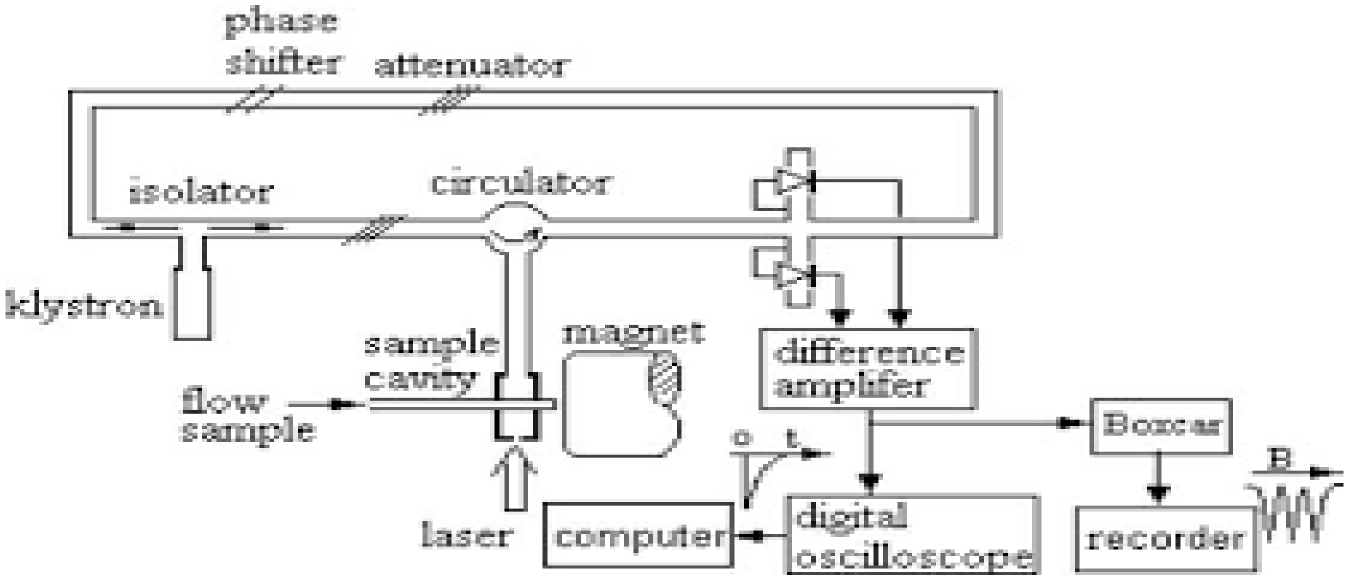
g = the g-factor which is a unit less measurement of the intrinsic magnetic moment of the electron, and its value for a free electron is 2.0023.

An unpaired electron can move between the two energy levels by either absorbing or emitting a photon of energy {\displaystyle h\nu } hv such that the resonance condition, hv = Δ E, is obeyed. This leads to the fundamental equation of EPR spectroscopy.

Working of Electron Spin Resonance (ESR)

- Although the equation permits a large combination of frequency and magnetic field values, the great majority of EPR measurements are made with microwaves in the 9000–10000 MHz (9–10 GHz) region.
- EPR spectra can be generated mostly by keeping the photon frequency fixed while varying the magnetic field incident on a sample.
- A collection of paramagnetic centers, such as free radicals, is exposed to microwaves at a fixed frequency.
- By increasing an external magnetic field, the gap between the and energy states is widened until it matches the energy of the microwaves.
- At this point the unpaired electrons can move between their two spin states. Since there typically are more electrons in the lower state, due to the Maxwell–Boltzmann distribution, there is a net absorption of energy.
- It is this absorption that is monitored and converted into a spectrum.

Instrumentation of Electron Spin Resonance (ESR)



KLYSTRONS

- Klystron tube acts as the source of radiation.
- It is stabilized against temperature fluctuation by immersion in an oil bath or by forced air cooling.
- The frequency of the monochromatic radiation is determined by the voltage applied to klystron.
- It is kept a fixed frequency by an automatic control circuit and provides a power output of about 300 milli watts.

WAVE GUIDE OR WAVEMETER

- The wave meter is put in between the oscillator and attenuator.
- To know the frequency of microwaves produced by klystron oscillator.
- The wave meter is usually calibrated in frequency unit (megahertz) instead of wavelength.
- Wave guide is a hollow, rectangular brass tube. It is used to convey the wave radiation to the sample and crystal.

ATTENUATORS

- The power propagated down the wave guide may be continuously decreased by inserting a piece of resistive material into the wave guide. This piece is called variable attenuator.
- It is used in varying the power of the sample from the full power of klystron to one attenuated by a force 100 or more.

ISOLATORS

- It’s device which minimizes vibrations in the frequency of microwaves produced by klystron oscillator.
- Isolators are used to prevent the reflection of microwave power back into the radiation source.
- It is a strip of ferrite material which allows micro waves in one direction only.
- It also stabilizes the frequency of the klystron.

SAMPLE CAVITIES

- The heart of the ESR spectrometer is the resonant cavity containing the sample.
- Rectangular TE120 cavity and cylindrical TE011 cavity have widely been used.
- In most of the ESR spectrometers, dual sample cavities are generally used This is done for simultaneous observation of a sample and a reference material.
- Since magnetic field interacts with the sample to cause spin resonance the sample is placed where the intensity of magnetic field is greatest.

COUPLERS AND MATCHING SCREWS

- The various components of the micro wave assembly to be coupled together by making use of irises or slots of various sizes.

CRYSTAL DETECTORS

- Silicon crystal detectors, which converts the radiation in D.C has widely been used as a detector of microwave radiation.

MAGNET SYSTEM

- The resonant cavity is placed between the poles pieces of an electromagnet.
- The field should be stable and uniform over the sample volume.
- The stability of field is achieved by energizing the magnet with a highly regulated power supply.
- The ESR spectrum is recorded by slowly varying the magnetic field through the resonance condense by sweeping the current supplied to the magnet by the power supply.

MODULATION COIL

- The modulation of the signal at a frequency consistent with good signal noise ratio in the crystal detector is accomplished by a small alternating variation of the magnetic field.
- The variation is produced by supplying an A.C. signal to modulation coil oriented with respect the sample in the same direction as the magnetic field.
- If the modulation is of low frequency (400 cycles/sec or less), the coils can be mounted outside the cavity and even on the magnet pole pieces.
- For higher modulation frequencies, modulation coils must be mounted inside the resonant cavity or cavities constructed of a non-metallic material e.g., Quartz with a tin silvered plating.

DISPLAY DEVICES

- In order to observe the signal a system is connected different devices can be used.

Applications of Electron Spin Resonance (ESR)

- ESR spectrometry is one of the main methods to study transition metal containing metalloproteins.
- To determine the rate of catalysis
- To know about the active site geometry
- To study denaturation and protein folding
- In studies relating to enzyme-ligand interaction
- In Biological Systems
- Study of Free Radicals
- Spin Labels
- Study of Inorganic Compounds
- Reaction Velocities & Mechanisms
- Study of naturally occurring substances such as minerals with transition elements, minerals with defects (e.g; quartz), Hemoglobin (Fe), Petroleum, Coal, Rubber etc.
- Conducting Electrons
  - This splitting occurs due to hyperfine coupling (the EPR analogy to NMR's J coupling) and further splits the fine structure (occurring from spin-orbit interaction and relativistic effects) of the spectra of atoms with unpaired electrons. Although hyperfine splitting applies to multiple spectroscopy techniques such as NMR, this splitting is essential and most relevant in the utilization of electron paramagnetic resonance (EPR) spectroscopy.
  - Introduction
  - Hyperfine Splitting is utilized in EPR spectroscopy to provide information about a molecule, most often radicals. The number and identity of nuclei can be determined, as well as the distance of a nucleus from the unpaired electron in the molecule. Hyperfine coupling is caused by the interaction between the magnetic moments arising from the spins of both the nucleus and electrons in atoms. As shown in Figure 11, in a single electron system the electron with its own magnetic moment moves within the magnetic dipole field of the nucleus.
  -

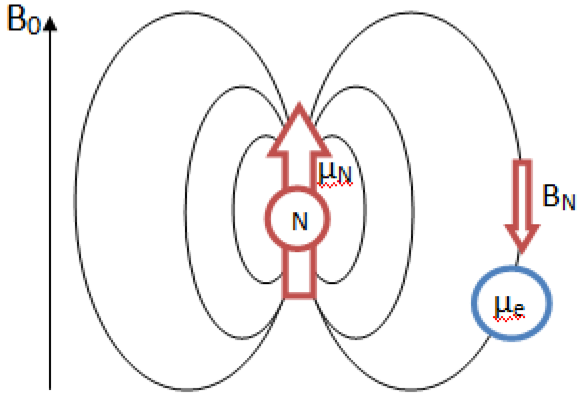


Figure 11. B is magnetic field, μ is dipole moment, 'N' refers to the nucleus, 'e' refers to the electron

This spin interaction in turn causes splitting of the fine structure of spectral lines into smaller components called hyperfine structure. Hyperfine structure is approximately 1000 times smaller than fine structure. Figure 22 shows a comparison of fine structure with hyperfine structure splitting for hydrogen, though this is not to scale.

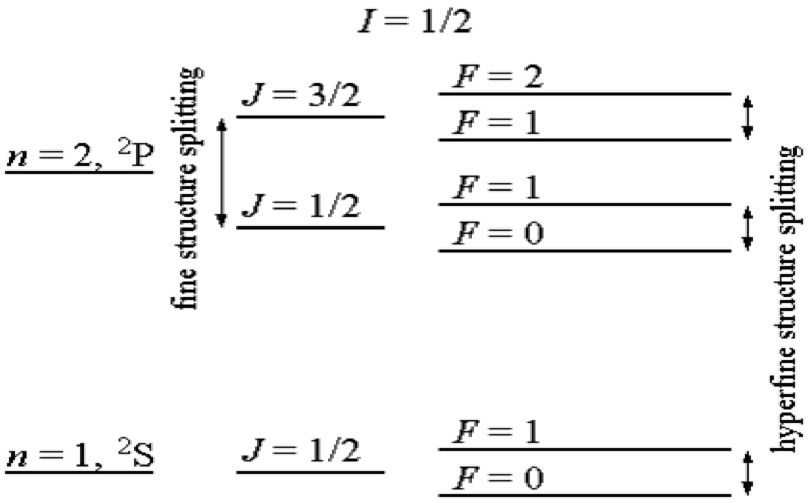


Figure 22. Splitting diagram of hydrogen

The total angular momentum of the atom is represented by F with regards to hyperfine structure. This is found simply through the relation F=J+I where I is the ground state quantum number and J refers to the energy levels of the system.

Results of Nuclear-Electron Interactions

These hyperfine interactions between dipoles are especially relevant in EPR. The spectra of EPR are derived from a change in the spin state of an electron. Without the additional energy levels arising from the interaction of the nuclear and electron magnetic moments, only one line would be observed for single electron spin systems. This process is known as hyperfine splitting (hyperfine coupling) and may be thought of as a Zeeman effect occurring due to the magnetic dipole moment of the nucleus inducing a magnetic field.

The coupling patterns due to hyperfine splitting are identical to that of NMR. The number of peaks resulting from hyperfine splitting of radicals may be predicted by the following equations where M_i is the number of equivalent nuclei:

- # of peaks=MiI+1# of peaks=MiI+1 for atoms having one equivalent nuclei
- # of peaks=(2M1I1+1)(2M2I2+1)....# of peaks=(2M1I1+1)(2M2I2+1).... for atoms with multiple equivalent nuclei

For example, in the case of a methyl radical 4 lines would be observed in the EPR spectra. A methyl radical has 3 equivalent protons interacting with the unpaired electron, each with I=1/2 as their nuclear state yielding 4 peaks.

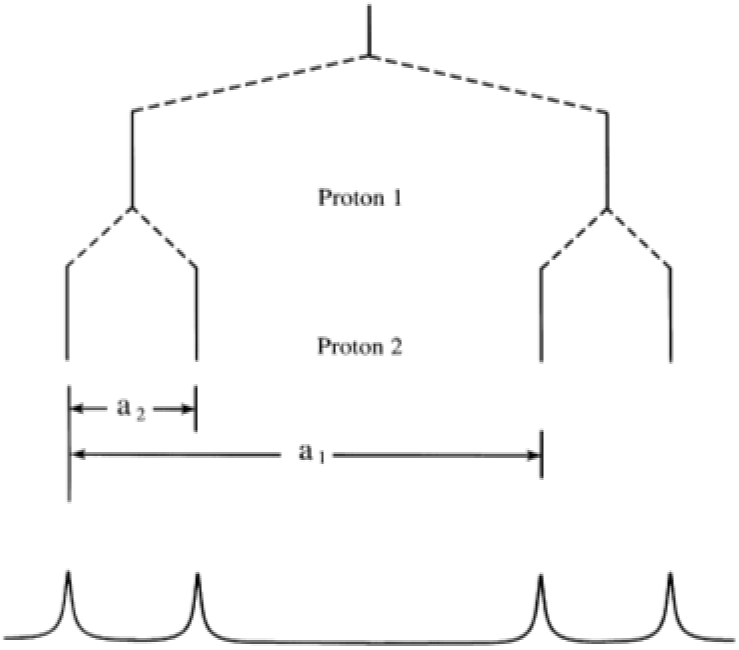


Figure 33. Approximate peaks resulting from hyperfine splitting between two unequivalent protons

The relative intensities of certain radicals can also be predicted. When I = 1/2 as in the case for ^{1}H, ^{19}F, and ^{31}P, then the intensity of the lines produced follow Pascal's triangle. Using the methyl radical example, the 4 peaks would have relative intensities of 1:3:3:1. The following figures[2] show the different splitting that results from interaction between equivalent versus nonequivalent protons.

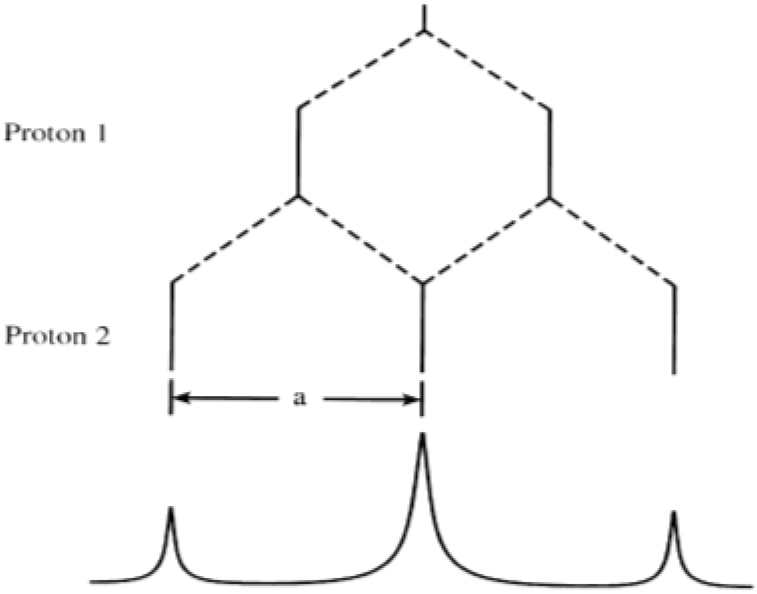


Figure 44. Approximate peaks resulting from hyperfine splitting between two equivalent protons

It is important to note that the spacing between peaks is 'a', the hyperfine coupling constant. This constant is equivalent for both protons in the equivalent system but unequal for the unequivalent protons

UNIT-IV

MASS SPECTROMETRY

Principle: In a mass spectrometer the sample to be analyzed is first bombarded with an electron beam to produce ionic fragments of the original molecule.

Mass spectrum: these ions are then sorted out by accelerating them through electric and magnetic first according to their mass/ charge ratio (m/z)

A record of the numbers of different kinds of ions is called the mass spectrum.

The uniqueness of the molecular fragmentation is the basis for the identification of different molecule in a complex mixture as no two molecules will be fragmented and ionized exactly in the same manner.

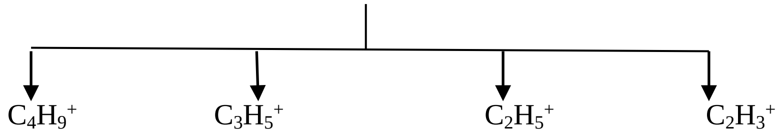
The substance whether solid/liquid/gas must be made volatile and the volatile sample must be bombarded with high energetic beam about 5-100ev.

For example consider Neo-pentane

$$C_5H_{12}+e^-\rightarrow(C_5H_{12})^++2e^-$$

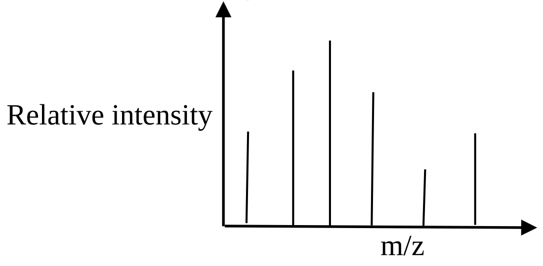
m/z =72

Molecular ion (or) parent ion fragmentation



The peak having highest intensity is base peak.

The mass spectrum of Neo-pentane may be



molecular peak

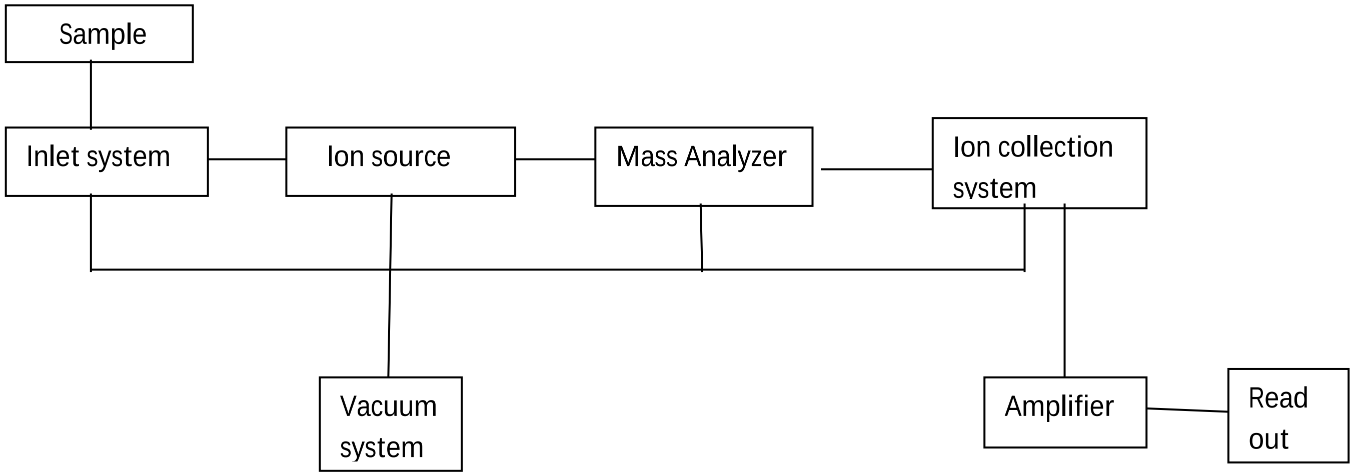
The peaks before the molecular peaks are the fragments of parent.

The species formed after bombarding with energetic electron beam will be in excited state and they lose the energy be the neighboring bonds.

To obtain parent peak the sample can be bombarded with low energetic electron. If we want fragments of parent we have to bombard with high energetic electron.

Instrumentation of mass spectrometer:

Block diagram:



Usually all mass spectrometers perform the three basic tasks.

1. Creating gaseous ion fragments from the sample.
2. Sorting these ions according to mass
3. Measuring the relative abundance of ion fragment of each atom

In general the ions are sorted into discrete mass /energy based on three properties that can be determined with relative ease energy momentum and velocity. A measurement of any two of these allows the mass/charge ration to be determined. In conventional methods, we use to measure energy and momentum i.e. accelerating the ions in an electrics field to given energy and dispersing them in a magnetic field according to the momentum.

The other methods are

1. Energy and velocity are used in time of flight mass spectrometers.

2. Momentum and velocity are used in FT-MS

1. Sample inlet system:

The purpose of the sample inlet system is to introduce a very small amount of sample into the mass spectrometer with a minimal loss of vacuum.

Most modern mass spectrometer is equipped with several types of inlets to accommodate various kinds of sample. These include.

1. Batch inlets
2. Direct probe inlets
3. Chromatographic inlets
4. Capillary electrophoresis inlets

1. Batch inlets:

- This is classical and simplest inlet system.
- Here the sample is volatized externally and them allowed to leak into the evacuated ionization region
- This is applicable to gaseous and liquid samples having boiling points up to about 500^{0}C

- The inlet system is often lined with glass to avoid loss of vacuum

2. Direct probe inlet:

- Solids and non-volatile liquids can be introduced into the ionization region by means of a sample holder or a probe, which is inserted through a vacuum lock.
- Probes are used when the quantity of sample is limited much sample is waste if batch inlet system us used.

3. Chromatographic and capillary electrophoresis inlet system:

- Mass spectrometers are often coupled with gas or high performance liquid chromatographic systems or capillary electrophoresis columns to permit the separation and determination of the components of complex mixtures.
- The eluent column is linked to the sample inlet system

2. Ion sources:

The starting point for a mass spectrometric analysis is the formation of gaseous analyte ions and the utility of the method is dictated by ionization sources. The appearance of mass spectra depends on the method used for ionization the ionization methods fall into two major categories.

a) Gas –phase sources:

In gas-phase sources, the sample is first vaporized and then ionized.

These are restricted to thermally stable compounds that have boiling points less than about 500^{0}C.

They were classified as

	Name of the source	Ionizing agent
Gas phase	Electron impact (EI) Chemical ionization(CI) Field ionization (FI)	Energetic electrons Reagent gaseous ions High potential electrode

b) Electron impact source:

- The sample is heated to a high temperature enough to produce a molecular vapor, which is then ionized by bombarding the resulting molecules with a beam of energetic electrons.
- Electrons are emitted from a heated tungsten filament and are accelerated by a potential of approximately 70V
- The electrons and molecules fare intersected at right angles at the centre of the source where collision and ionization occur.
- The primary product is a positive ion which is formed by loss of electron by electron repulsion.

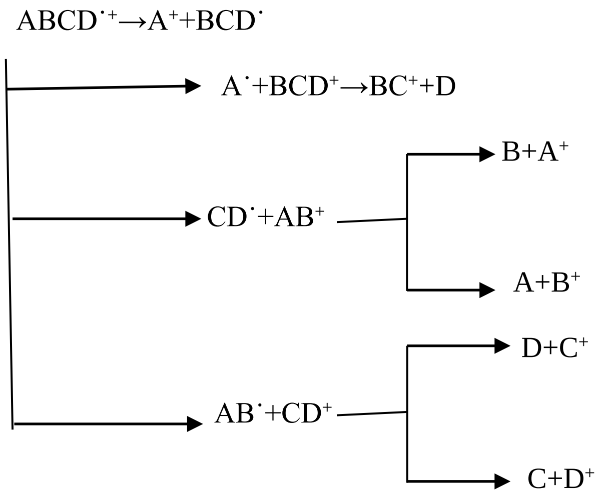
$M+e^- \rightarrow M^{\cdot+}+2e^-$

M is analyte molecule and $M^{\cdot+}$ is molecular ion.

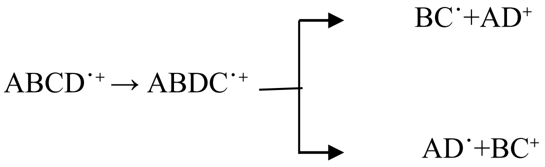
Typical reaction in electron –impact source:

Molecular ion formation $ABCD + e^{-} \rightarrow ABCD^{\cdot +} + 2e^{-}$

Fragmentation



Rearrangement followed by fragmentation



Collision followed by fragmentation

$$ABCD^{\cdot +} + ABCD \rightarrow (ABCD)^{2\cdot +} \rightarrow BCD^{\cdot} + ABCDA^{+}$$

Electron impact sources are only applicable to analytes having molecular weights smaller than about 10^{3} daltons.

If electron impact sources used the possibility of getting molecular peak is very low.

c) Chemical ionization sources:

In chemical ionization gaseous atoms of the analyte are ionized by collisions with ions produced by electron bombardment of an excess of reagent gas.

This is the second most common procedure for producing ions in the spectrometry. The gaseous reagent gas is introduced into the ionization region in an amount such that concentration ratio of reagent to sample is 10^{8} to10^{4} and reacts with the electron beam the most common reagent used is methane which reacts with high energy electrons to give several ions such as CH_4^{+}, $CH3^{+}$, and CH_2^{+} these ions react rapidly with the additional methane molecule as follows.

$$CH4^{\cdot +} + CH_4 \rightarrow CH_5^{+} + CH_3^{\cdot}$$

$$CH_3^{+} + CH_4 \rightarrow C_2H_5 + H_2$$

The collisions between the sample molecules MH and CH_5^+ or $C2H5^+$ are highly reactive and involve proton transfer or hydride transfer.

$$CH5^+ + MH \rightarrow MH_2^+ + CH_4 \text{ (Proton transfer)}$$

$$C_2H_5 + MH \rightarrow MH_2^+ + C_2H_4 \text{ (Proton transfer)}$$

$$C_2H_5 + MH \rightarrow M + C_2H_6 \text{ (Hydride transfer)}$$

It is important to note that proton transfer reaction give the($M+1)^+$ion where as the hydride transfer produce an ion whose mass is $(M-1)^+$ with some compounds an($M+2)^+$ peak is also produced from transfer of $C_2H_5{}^+$ ion to the analyte.

A variety of reagents including propane isobutene and ammonia are used for chemical ionization. Each produces a somewhat different spectrum with a given analyte.

Field ionization sources:

In field ionization sources ions are formed under the influence of large electric file (108 V/cm) which are produced by applying high voltages (10-20KV) to specially formed emitters having fine foretips having diameter less than 1µm

Usually the tungsten wire on which microscopic carbon dendrites have been grown by pyrolysis of Benzo Nitrile in high electric field.

The ionization is that its sensitivity is at least an order of magnitude less than that of electron impact sources.

Desorption sources:

These are applicable for non volatile ad thermally unstable samples.

The sample in a solid or liquid state is converted directly into gaseous ions

Desorption sources do not require vitalization of analyte molecules and are applicable in analytes having, molecular weights as large as 10^5 dalton.

In desorption methods energy in various forms is introduced in to the solid or liquid sample to cause direct formation of gaseous ions.

The spectrum is much simplified and contains only the molecular ion

Many desorption methods available are

Name	Ionizing agent
Field desorption (FD)	High potential electrode
Electro spray ionization(ESI)	High electric field
Plasma desorption (PD)	Fission fragment
Fast atom bombardment(FAB)E	Energetic atoms
Secondary ion mass spectrometry	Energetic beam

Thermo spray ionization (TSI)	High temperature

Ion sources are also classified as hard sources and soft sources.

Hard sources:

They impart sufficient energy to analyte molecules, so that they are in a highly excited energy state on relaxation, producing fragment ion that have mass to charge ratio less than molecular ion

The peaks in hard source spectrum provide information about the kinds of functional groups and their structural information about analytes.

Soft sources:

Soft sources cause little fragmentation consequently the resulting mass spectrum has molecular ion peak and only few ions.

Soft sources are useful because they supply accurate information about the molecular weight of the analyte molecules.

Mass analysis:

The function of mass analyzer is to separate the ions produced in the ion sources according to their different mass to charge ratio.

Different types of mass analyzers are

1. Magnetic sector analyzer
2. Quadruple mass analyzer
3. Ion trap analyzer
4. Time of flight mass analyzer

Ion –collection systems:

Resolved ion beams, after passage through a mass analyzer, sequentially strike a detector several types of detectors are available. The electron multiplier is most commonly used.

Faraday cup collector:

The Faraday cup collector is a simple and effective means of monitoring ion current in focal plane of the mass spectrometer.

It consists of a cup with suitable suppressor electrodes and guard electrodes.

Currents as low as 10^{-15}A may be detected.

Electron multiplier:

For ion currents less than 10^{-15}A an electron multiplier is necessary. The ion beam strikes the conversion dynode, which is a metal plate which is coated with a metal oxide that has high secondary electron emission properties.

The dynode converts ions to electrons. Either positive or negative, ions are accelerated by the constant high voltage of dynode; they are converted into electrons and positively ions when they strike the plate. The currents created are further multiplied by electron multiplier having 15 to 18 individual dynodes.

Vacuum system:

For the operation of a mass spectrometer the ion source, the mass analyzer and the detector must be kept under high vacuums conditions of 10^{-6} to 10^{-7} torr.

Most systems use a combination of oil diffusion pumps to maintain a high vacuum and backing rotatory pumps to reduce the initial pressure to approximately 0.001 torr.

Data handling:

On modern mass spectrometers the data are digitized and collected on magnetic tape or stored in the memory of a computer.

Resolving power of a mass analyzer: The function of the mass analyser is to separate an ion of mass M from of mass $M+\Delta M$. the ability of mass analyzer of measure m/z satisfactorily as quantified in the form of a parameter known as resolving power which is defined as the ratio of the ratio of the mass to be measured to the difference in the masses to be identified, I.e.,

Resolving power=$M/\Delta M$

Where M and ΔM are the m/z values of the adjacent peaks in the mass spectrum.

For example the resolving power of a mass spectrometer necessary to resolve ions of m/z 5000 is given by the following equation.

Resolving power = $M/\Delta M=5000/5001-5000=5000$

The resolving power of a mass spectrometer is based on the height of the valley between two peaks of equal intensity. The peaks at m/z equal to $M+\Delta M$ are said to be resolved when the valley between them is less than an arbitrarily Chosen percentage (commonly 10 %) of the height of the peaks. The resolving power of the mass spectrometer would then be expressed as 1 part in $M/\Delta M$ on a 10% valley definition.

The resolving power of a mass spectrometer is effective by the following factors.

1. The radius of the ion path
2. The width of accelerator and collector slits.
3. The uniformity of the kinetic energy of ions with the same m/z ration upon their entering the analyzer unit.

Determination of molecular weight

Low resolution mass spectrometer measures m/z values to the nearest whole number mass unit. However high resolution mass spectrometers measures m/z value to three or four decimal places and thus provides an extremely accurate method for determining the molecular weights.

The determination of accurate molecular weight is possible because the actual mass of the atomic particles (nuclides) are not integers. For example a molecule whose molecular weight is 32 could be O_2, N_2H_4 or CH_3OH. However, these molecules have the following precise masses.

O_2 = 2X15.9949=31.9898

N_2H_4=2X14.0031+4X1.00783=32.0375

CH_3OH=12.000+4X1.00783+15.9949 =32.0262

Thus a height resolution mass spectrometer, capable of measuring mass with an accuracy of 1 part in 40000 or better, can easily distinguish among these three molecules and consequently, provides information regarding the molecular formula of these compounds.

Qualitative analysis:

The identification of an unknown compound is possible by mass spectrometry. This is possible by calibration with a known compound like mercury vapor m/z =198.2 or perfluoro kerosin (PFR) with peaks CF_3(64),C_3F_3(93),C_4F_3(105) and C_3F_5(131)

- The mass spectrometer is used in determination of molecular weight formula. Identification of a compound from the fragmentation pattern.
- In the identification of a compound the most important information is the molecular weight.
- In the determination of molecular weight, avalotile compound is essential molecular peak is identified ignoring the peaks due to impurities.
- The molecular weight calculated may not be same as calculated from the atomic weights
- If the parent compound contains curtains certain elements with high isotopic abundance. Therefore, the mass of the heaviest ion exclusive of isotopic contributions, gives the nominal molecular weight with low resolutions mass the spectrometers and the exact molecular weight with high resolution instruments.

Quantitative analysis:

- Mass spectrometry is useful for quantitative analysis of mixtures containing closely related compounds.
- It is useful for the analysis of both inorganic and organic mixtures of low volatility.
- Since fragmentation patterns of components of a mixture are additive a mixture can be analyzed if a spectrum of a compound run under similar conditions is available vary from other at least in one peak.
- The basic principle of is that each component must show at least one peak differing substantially from another hence, by this technique high molecular weight polymeric compounds have been analyzed by pyrolysis.
- It is used in trace analysis of inorganic materials such as alloys and minerals, which permits the determination of ppb levels by using the spark source

Applications of Mass spectrometry:

i. Elucidation of the structure of organic and biological molecules.
ii. Determination of the molecular weight of peptides, proteins and oligonucleotides.
iii. Identification of components in then layer and paper chromatograms
iv. Determination of amino acid sequences in sample of polypeptides and proteins. Identification of drugs of abuse and metabolites of drugs of abuse in blood, urine and saliva.
v. Detection and identification of species separated by chromatography and capillary electrophoresis.
vi. Monitoring gases in patients breathe during surgery.
vii. Testing for the presence of drugs in blood in through bed racing horses and in Olympic athletes.
viii. Dating archaeological specimens
ix. Analysis of aerosol particles
x. Determination of pesticide residues in food
xi. Monitoring volatile organic species in water supplies.

X-Ray Fluorescence spectroscopy

Principle:

Irradiation of the sample with a beam of X-rays from an X-ray tube or a radioactive source under these circumtanceous the elements in the sample are excited by the adsorption of the primary bam and they emit their own characteristic X – Rays. This procedure is thus properly called on X-Ray fluorescence or emission method. X-Ray fluorescence (XRF) one of the most widely used of all analytical methods qualitative analysis of elements having atomic number greater than that of oxygen. It is often employed for “semi quantitative or quantitative elemental analysis”.

Matrix effect:

It is important to realize that the X-Rays produced in the fluorescence process are generated not only from atoms of the surface of a sample but also fro, atoms well below the atom surface. Thus a part of both the incident radiation and the resulting force travels a significant thickness of a sample with in which adsorption and scattering can occur. The extent to which either beam is attenuated the mass adsorption coefficient of the media. Which in turn is determined by the coefficient of all of the elements in the sample therefore, while the net intensity of a line reaching the detector in a X-Ray fluorescence measurement depends on the concentration of the element produced the line. It is also affected by the concentration and mass adsorption coefficient of the matrix effect.

The Mass adsorption coefficient:

Beers law is as applicable to the adsorption of X-Ray radiation as to other types of electromagnetic X-Ray radiation. Thus we make to write an

$\log(P_0/P)=\mu x$

or

$\ln(P_o/P)=\mu x$

where x is the sample thickness in centimeter,

P and PO are the powers of the transmitted and incident beams

μ is called the linear adsorption coefficient.

These are characteristic of the element as well as the no, of elements in the path of the beam.

A more convenient form of Beers law is

$\ln(P_o/P)=\mu x \rho x$

Where ρx is density of the sample

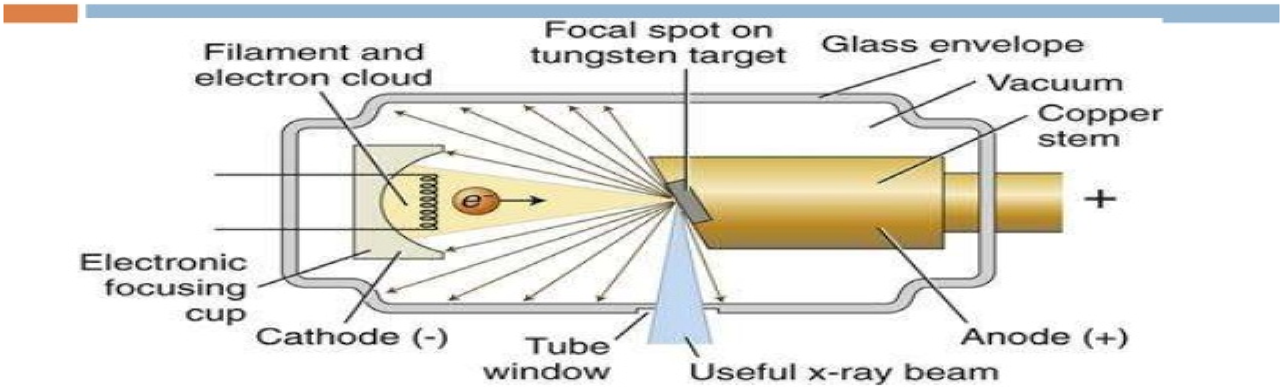
μM is mass adsorption coefficient

Mass adsorption coefficient as additive mass of the elements present in the sample.

$\mu M = wA\mu A+wB\mu B+wC\mu C$…..

where, µM is the mass adsorption coefficient of a sample containing the weight of fractions wA,wB and wC are elements of A, B, and C. the terms µA,µB,µC are respective mass adsorption coefficient for each of the elements.

X-Ray tube:



The most common source of X-rays for analytical work is the X-ray tube. An X-ray tube source is a hilly evacuated tube in which is mounted a tungsten filament cathode and metal massive anode. Thus the anode generally consists a heavy block of copper with a metal target plated on or imbedded of the copper. Target material includes such metals as Tungsten Chromium, Copper, Molybdenum Silver, Iron, Rhodium and Scandium.

Separate circuits are used to heat the filament and to accelerate the electrons to the target. Then the heater circuit provides controlling the intensity of the emitted X-rays .while the accelerating potential determines their energy or wavelength. In which water cooling of the anodes of X-ray tube was required.

Radio- isotopes :

A variety of radioactive substance have been used as source of x-ray fluorescence and absorption method.

Many of the best radioactive sources provide simple line spectra others produce a continue spectra Because of the shape of X-Ray absorption curves.

Eg. Iodine-125

X-Ray Monochromator:

The Monochromator consists of a pair of beam collimators which serve the same purpose as the slits in an optical instrument and dispersive element. The collimators for X-Ray Monochromators ordinarily consist of a series of closely space metal plates or tubes that absorb all but the parallel beams of radiation.

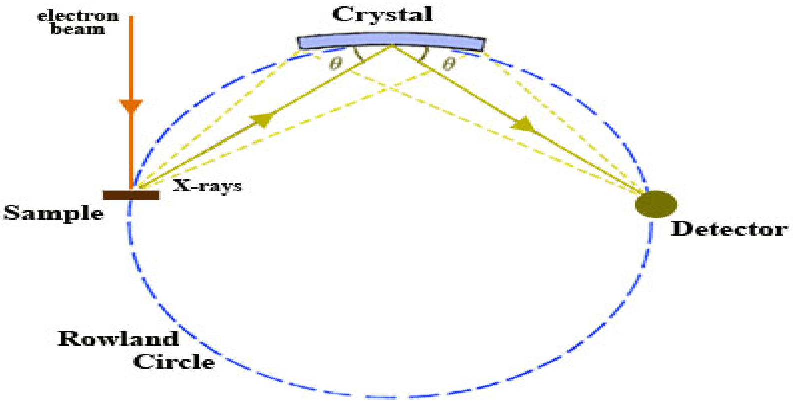
X-Ray fluorescence method instrument sun divided into three basic types.

1. Wave length dispersive instrument
2. Energy dispersive instrument
3. Non-dispersive instrument

Wavelength dispersive instrument:

This wavelength dispersive instrument further divided into two types.

I. Single channel or sequential instrument
II. Multi channel or simultaneous instrument



Single channel instrument:

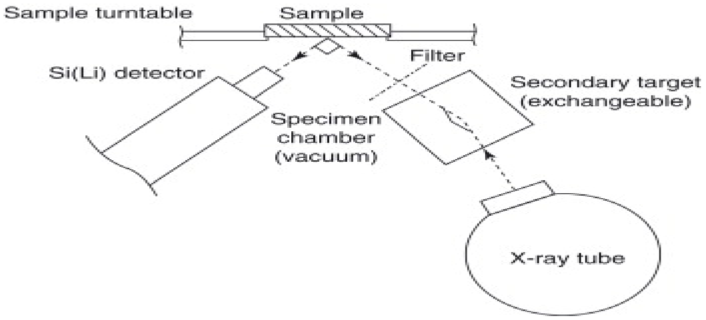
In which X- Ray tube and sample are arranged in the circular insert at the top of the figure. Single channel instruments are may be manual or automatic. The former or entirely satisfactory for the quantitative determination of few elements in

which crystal and detectors are set at the proper angles (θ and 2θ) and counting is continued until sufficient counts have accumulated for precise result most modern single channel spectrometers are provided with two X-Ray sources. Typically one has a chromium target for longer wavelength and other tungsten target for shorter wavelength.

Multi channel instruments:

Multi channel instruments are large and expensive it permits the simultaneous detection or determination of as well as 24 elements. Here individual channel consists of an appropriate crystal and detector are arranged radially around an X-Ray source and sample holder in which the crystals for all or most of the channels are fixed at a n appropriate angle for a given analyte. In some instruments one or more of the crystals can be moved to permit a spectral multi channel instruments are widely used for the determination of several components in materials of industry such as steel, other alloys. Cement ores and petroleum products. Both multi channel and single channel instruments are equipped and to form of metals, powder form evaporated few liquids or solutions.

Energy dispersive instruments:

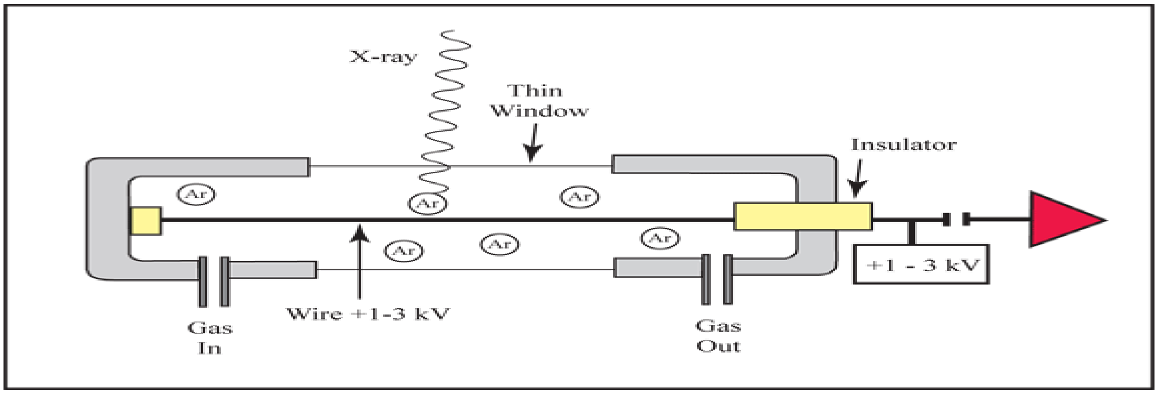


An energy dispersive spectrometer consists of a polychromatic source which may be either an X-Ray tube or a radioactive material. A sample holder, a semi conductor detector and the varies electronic components require for energy discrimination.

An obvious advantage of energy dispersive system is the simplicity and lack of moving parts in the excitation and detection components of the spectrometer, further more the absence of collimator and crystal diffractor result in a 100 fold or more increase in energy reach the detector. These features permit the use of weaker sources. Such as radioactive material or low power X-ray tubes.

The principle disadvantage of energy dispersive system when compared with crystal spectrometer is there lower resolutions at wavelength longer about 1A0 and the other hand the shorter wavelength energy dispersive system exhibit superior resolution.

Gas filled detector:



When X- radiation passes through an inert gas such as Argon, Xenon or Krypton. Interaction occurs that produce a large no of positive gaseous ions and electron (ion pairs) for each X-Ray quantum.

Radiation enter the channel through a transparent window Aluminum Mylar, Beryllium, Nylon

Each photon of X- radiation may interact with an atom of Argon, causing it to loss one of its outer electrons; this photo electron has a large kinetic energy which is equal to the difference between the X-ray photon energy and the binding energy of the electron in the Argon atom. Under the influence of an applied potential the mobile electrons migrate towards the central wire anode while slower moving electrons are attracted towards cylindrical metal anode.

The accelerating force and the ion pairs are low and the rate at which the positive and negative species separated is insufficient to present partial recombination. As a consequence the no. of electrons reaching the anodes than the number production initially by the incoming radiation.

The no. of electrons increases rapidly with applied potential. This increase is rejecting of secondary ion pair production. Then amplification of the ion current results.

Advantages of XRF:

1. The spectra are relatively simple and method is non destructive.
2. It is used for the analysis of paintings archeological specimens, jewellery, coil and other valuable objects without harm to the sample.
3. It is not sensitive but accurate.

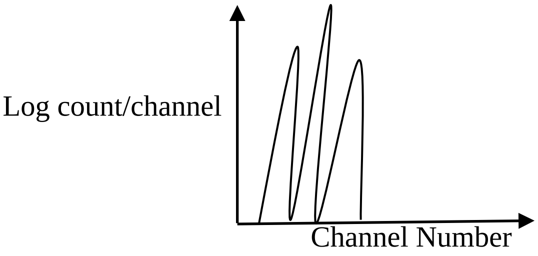
Disadvantages of XRF:

1. X-Ray fluorescence method for the lighter elements is inconvenient.
2. It is high cost of instrument**.**

Applications of XRF:

1.In Agriculture:

In agriculture technique is used for determination of trace elements in plants and foods, the detection of insecticides of fruit and leaved, continuous determination of phosphorous fertilizer.



Tube anode: Rh

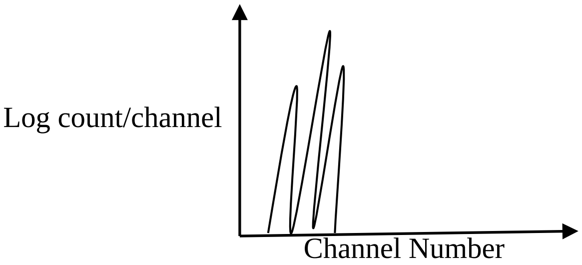
Anode voltage: 10Kv

Anode current: 50 amps

Life time: 200 S

2.In Medicine:

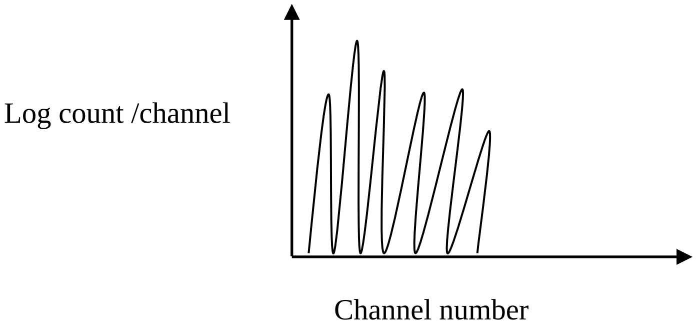
In medicine for the direct determination of sulphur protein in blood, the determination of chloride in blood serum, the determination of Sr in blood serum and bone tissue and the element analysis of tissue, bone, fluids.



Peak 1.S,2.Cl,3.Sr

3.In mining and metallurgy:

It is used for the analysis of ores determination of Chromium in stainless steel, Manganese in plain steels, Tungsten in high speed steels, determination of Zinc, Copper, Manganese and Tin in Copper metallurgy.



Peak 1.Fe,2.Mn,3.Sn,4.Zn,5.Cu 6.Cr

4. In Rubber industry:

The determination of vulcanizing element Sulphur can be done by X-Ray fluorescence.

In space technology the analysis of new allows ceramics can be carried out by X-Ray fluorescence.