Shree H. N. Shukla Institute of science

B. Sc
Semester-6

STUDY MATERIAL

Subject Name: ANALYTICAL TECHNIQUES AND BIOINFORMATICS
Subject Code: 602

UNIT I BASIC ANALYTICAL TECHNIQUES IN BIOSCIENCES

1.1 Concept Of Good Laboratory Practices and Quality Management
1.2 Colorimetry and Spectrophotometry
1.3 Introduction to Mass spectroscopy
1.4 Introduction to IR And NMR and Their Applications
1.5 Applications of Radioisotopes in Biosciences
1.6 Atomic Spectroscopy: Principles and Applications of Atomic Absorption/Emission Spectrometer

- **Good laboratory practice** or good laboratory practices are accepted methods to carry out activities or operations in a laboratory. The authorities and laboratory organizations say that these practices help ensure safety. They also have a positive influence on the quality of the result. For pharmaceutical companies, for example, GLP compliance is extremely important.

- Lab furniture supplier, *Labtech Supply*, says that good laboratory practices are not guidelines; they have the force of law. We also refer to good laboratory practice as **GLP**. GLP is part of the quality assurance that ensures that organizations consistently produce and control goods to a high quality standard.

- Good laboratory practice is not only concerned with production, but also quality control.

- According to the *European Commission*:

- "The principles of Good Laboratory Practice (GLP) promote the quality and validity of data generated in the testing of chemicals and prevent fraudulent practices."

- Non-clinical good laboratory practice

- Non-clinical good laboratory practice also refers to experimental research. The term refers to a quality system of management controls for laboratories where research is taking place.

- GLP aims to ensure the consistency, reliability, uniformity, and quality of chemical non-clinical safety tests. It also aims to ensure their consistency and reproducibility. Non-clinical means not related to human patient care.

- According to LabCompliance: "Good Laboratory Practice (GLP) deals with the organization, process and conditions under which laboratory studies are

planned, performed, monitored, recorded and reported. GLP practices are intended to promote the quality and validity of test data."

- Included in the term *'chemicals'* are substances and molecules that the pharmaceutical industry creates. In other words, substances we use when creating medications.

- In 1972, New Zealand was the first country to introduce good laboratory practice. The United States subsequently followed in 1978 after the Industrial BioTest Laboratories (IBT Labs) scandal.

- IBT Labs had been engaging in extensive scientific misconduct, including fraud. IBT Labs' President and several top executives were indicted.

- Today, all OECD countries follow the Principles of GLP, as do many other nations across the world. OECD stands for the **O**rganization for **E**conomic **C**o-operation and **D**evelopment.

- *Wikipedia* says that good laboratory practice:

- "Applies to non-clinical studies conducted for the assessment of the safety or efficacy of chemicals (including pharmaceuticals) to man, animals, and the environment."

- Do not confuse the term GLP with **Standards for Laboratory Safety**, which is all about wearing the right gloves, glasses, and clothing.

- Regulatory agencies globally oversee many different types of 'practices.' For example, they supervise good agricultural practices, good clinical practices, and good manufacturing practices.

GLP in the USA and EU

USA

The United States Food and Drug Administration (FDA) has GLP rules in 21CFR58. In the US, people carrying out preclinical trials on animals use these rules. The FDA is America's regulatory agency for medications, medical devices, food, cosmetics, and tobacco products.

In other words, researchers follow the rules before carrying out clinical research on humans.

If an organization does not follow the FDA rules or OECD Guidelines, it will find it hard to apply for a New Drug Application in the US. In fact, the New Drug Application in the US will probably stay on hold perpetually.

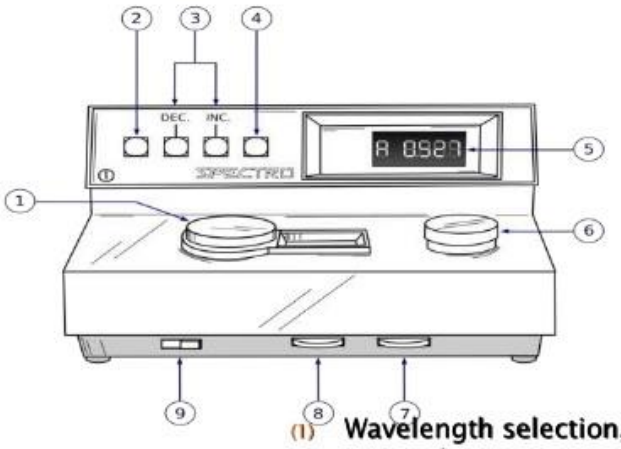
EU

Since 1987, the European Council has adopted two basic Directives as well as a Decision related to the application of the GLP principles.

The rules and regulations regarding good laboratory practice in the EU apply across the whole economic bloc. Therefore, if a laboratory complied with the rules in one country, its processes are valid in all other EU member states.

Working Principle & Applications of Colorimeters

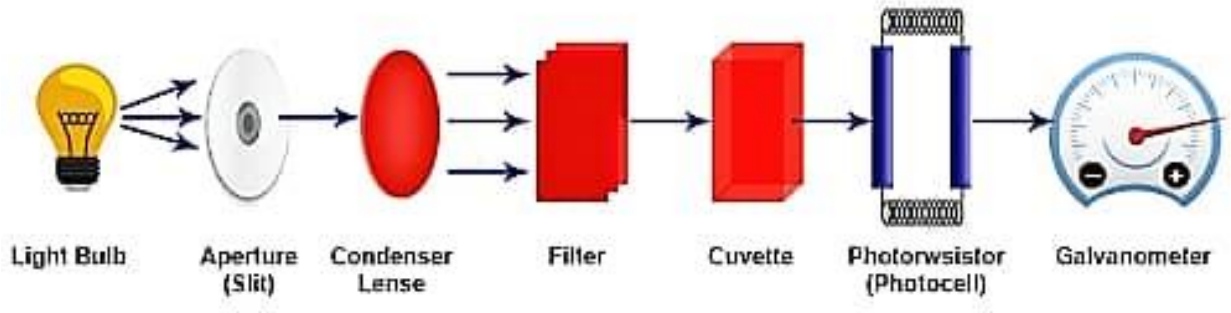
- When it comes to the appearance of products, the most important aspect of appearance is the color of the product.
- The color contributes a major portion of the appearance and hence it is essential to ensure that the color of the products is of the best quality and level. To ensure that the color of the products is of the best quality and level, a colorimeter is used.
- The **colorimeter** is a device that is mainly used in industries and laboratories for analyzing the color quality of the products along with color measurement.



Colorimeter

(1) Wavelength selection,
(2) Printer button
(3) Concentration factor adjustment,
(4) UV mode selector (Deuterium lamp)
(5) Readout
(6) Sample compartment
(7) Zero control (100% T),
(8) Sensitivity switch

What is Colorimeter?



The colorimeters are highly sensitive devices that can measure the concentration and intensity of a particular color that is used in a product.

There are mainly two different types of colorimeters that are used in industries that are color densitometers and color photometers.

The color densitometers measure the color density of primary colors in a color combination in a test sample. The color photometers are used for measuring the reflectance of a color as well as the transmission.

Working Principle of Colorimeter

- The working of colorimeters is mainly based on the Beer-Lambert's Law. This law states that the light absorption when passes through a medium are directly proportional to the concentration of the medium.
- When a colorimeter is used, there is a ray of light with a certain wavelength is directed towards a solution. Before reaching the solution the ray of light passes through a series of different lenses.
- These lenses are used for navigation of the colored light in the colorimeter. The colorimeter analyzes the reflected light and compares with a predetermined standard. Then a microprocessor installed in the device is used for calculation of the absorbance of the light by the solution.
- If the absorption of the solution is higher than there will be more light absorbed by the solution and if the concentration of the solution is low then more lights will be transmitted through the solution.

How Colours can be analysed using Colorimeters?

To analyze the concentration of an unknown sample, there are several specimen prepared from the test sample and then tested using an efficient colorimeter. After analysis, the transmittance and concentration of the tested specimen are plotted on a graph to obtain a graphical representation of the concentration creating a calibration curve. This curve is then compared with the curve of a known sample, and the concentration is measured.

Applications of Colorimeters

The Testronix's colorimeters can be used for measuring the carol concentration and intensity of a variety of materials such as:

- Food ingredients,
- Building materials,
- Textile products,
- Beverages,
- Chemical solutions and many others.

The **Testronix colorimeters** offer high accuracy of test finding along with a high rate of repeatability which allows the manufacturers to ensure the best color quality and appearance of their products.

1.2 spectrophotometry

Analytical techniques: it is same determine the concentration of a chemical compound e.g. Gravimetry, titrimetric, spectroscopy, chromatography etc.

Spectrometry and colorimetry are analytical methods of measuring the amount of light absorbed by a substance in solution. They are commonly used techniques for quantitatively determining substance encountered biochemistry.

Absorbance is a characteristic of a substance just like melting point, boiling point, density and solubility. But because it can be related to the amount of the substance in solution, absorbance can be used to quantitatively determine the amount of substance in solution.

Spectroscopic techniques employ light to interact with matter and thus prob certain features of a sample. Because these techniques use optical materials to disperse and focus. The radiation, they often are identified as optical spectroscopies.

Terminologies:

Light is an electromagnetic radiation composed of photons of electric and magnetic field moving in a wave that oscillates along the path of motion. Oscillation of two fields is perpendicular to each other and to the direction of propagation forming transverse waves.

Electromagnetic radiation is a form of energy whose behaviour is described by the properties of both waves and particles. Some properties of electromagnetic radiation, such as its refraction when it passes from one medium to another are explained best by describing light as a wave.

Other properties such as absorption and emission, are better described by treating light as a particle. An electromagnetic wave is characterized by several fundamental properties including its velocity, amplitude, frequency, wave lengths.

The wave length of light is defined as the distance between two consecutive peaks and is measured in submultiples of meters mostly nanometer, which can be presented by

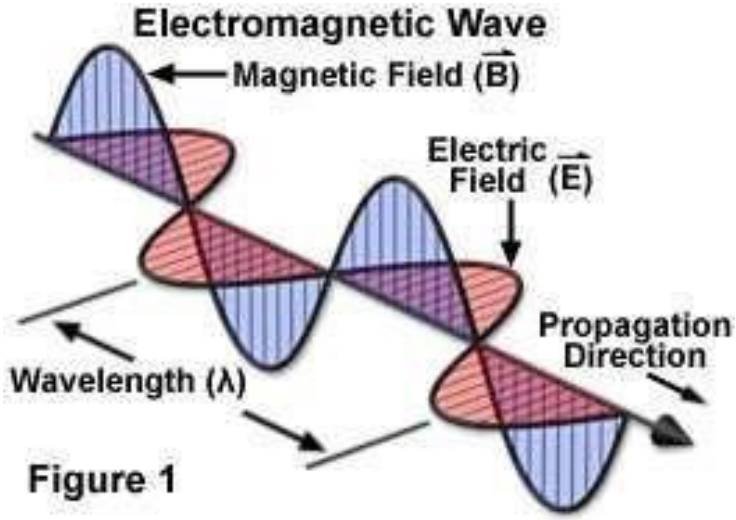


Figure 1

$\lambda = c/v$.....(1)

Where,

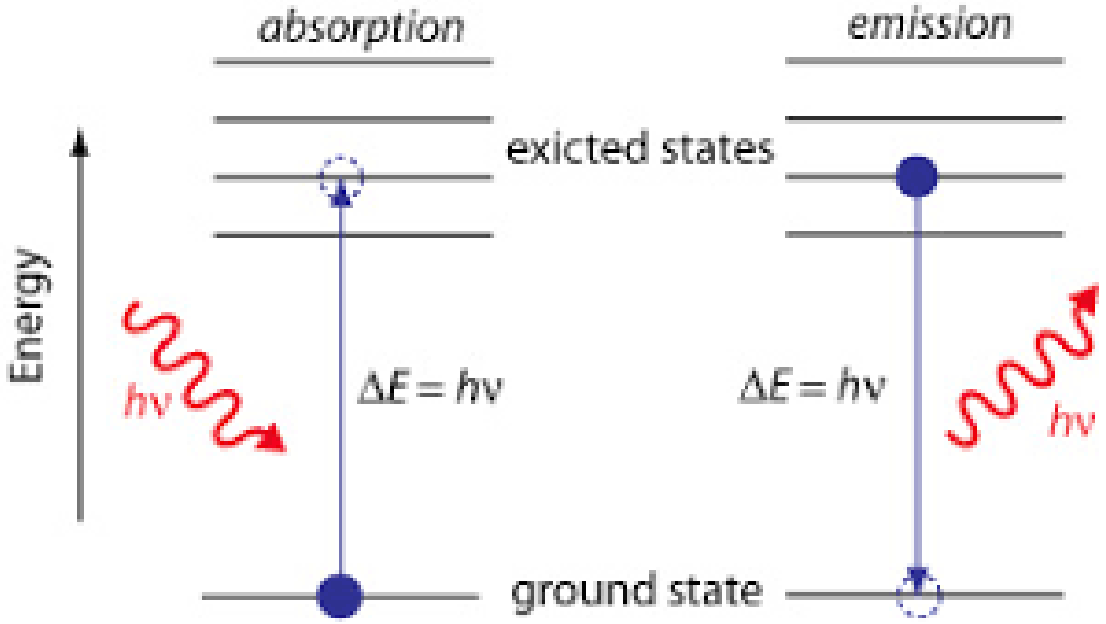
- lambda (λ) -wave length of light, c-speed of light (2.999× 108m/sec) and v-factory. Frequency(U): Is the number of oscillations made by A wave within one second or number of waves passing through a fixed point on the time axis per second, which is measured by one per second (1Hz).
- Now relation of frequency with wavelength this
- $v=c/\lambda$.....(2)
- (Which can be derived from equation 01) i.e. wavelength and frequency share inverse relationship Maximum length of a vector is called amplitude.

- Photons of different wave lengths have different energies that are given by the following equation E= hc / λ =hu (see equation2) Where, h-plank's constant=6.63×10-34Jsec. Thus, shorter the wavelength, greater would be the energy of the electromagnetic radiation.

Region	Wavelength
Gamma-rays	0.01NM-0.1NM
X-rays	0.1NM-10NM
Ultraviolet (UV)rays	200NM-400NM
Visible rays	400NM-700NM
Infrared (IR)rays	700NM-500micrometer
Microwaves (Radar)	1.0cm-10cm
Radio waves	1.0m-5.0m

- Electromagnetic radiation can be divided into various regions according to the wave length The range of all type of electromagnetic radiation with different wavelengths is called electromagnetic spectrum. Electrons principally reside in the lowest level (ground state). In order for electronsto be promoted to a higher level (excited state) energy must be put in to a system.
- If this energy E=hv is derived from electromagnetic radiation, this givesrise to an absorption spectrum and an electron is transferred from electronic ground state to excited state. The molecules once arrived at an excited electronic, state do not continue forever because then energy originally gained from photon absorption is lost by collision with other. Eventually, the molecules returned to the ground state with liberation of energy.
- When sample absorb selector magnetic radiation the number of Photons passing through a sample decrease.
- The measurement of this decrease in photons, is called absorbance. Absorption occurs only when the photons energy hv matches the difference in energy, ΔBetween two energy levels.

- When an atom or molecule in an excited state returns to a lower energy state, the excess energy often is released as a photon, a process we call emission.

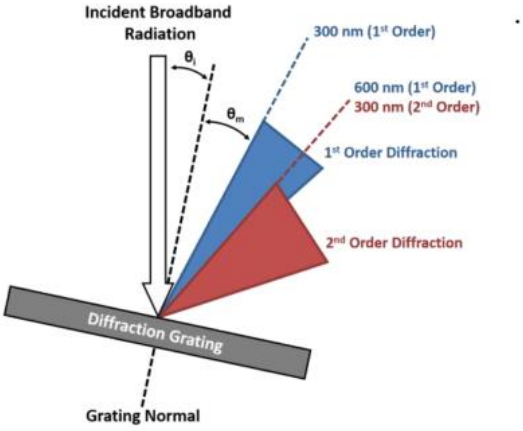


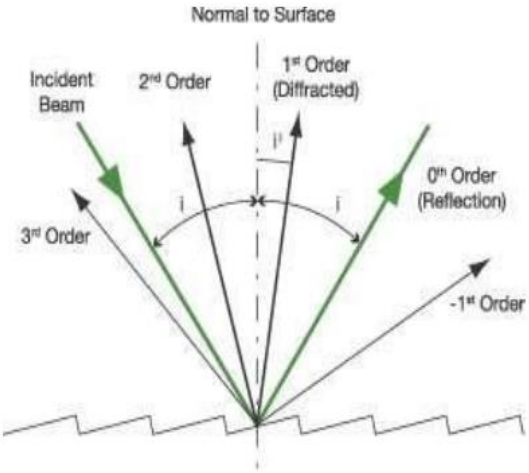
- A graph of wavelength v/s absorbance is called absorption spectra.
- Number of complete cycle occurring per centimetres is called wavenumber (wavenumber=1/ λ)
- A beam of radiation from an electric bulb consisting of several wavelength this called polychromatic light while a beam in which all the rays have same wavelength his called monochromatic light

Reflection: Throwing back of light by a surface without absorbing it. Law of reflection states that the angle at which the wave is incident on the surface equals the angle at which it is reflected, Mirrors exhibit reflection.

Refraction: It is the bending of a wave when it enters a medium where its speed is different

Diffraction: spreading out of waves past small opening





Quantitative aspects of light absorption:

Laws of absorption:

Two laws govern the absorption of monochromatic light by any absorbing material:

1.Boucher-Lambert'sLaw:

The amount of light absorbed by a substance in

A solution is proportional to the thickness of the absorbing material i.e. The path length and is independent of the intensity of the incident light. It is expressed by

I/Io=e-kb.....(3)

Where I=intensity of transmitted light, Io=Intensity of incident light, b= path length, k=linear

absorption coefficient of absorbing material.

To remove the power term in equation3itcanbeconverted in to logarithmic form. Thus,

In I o /I=-kb

Removing negative sign, it can be written as

InI o /I=kb

Changing to common logarithms we get,

2.303log 10 I o /I=kb.....(4)

2. Beer's Law:

The amount of light absorbed by a substance in a solution is proportional to the number of as absorbing molecules i.e. the concentration of the absorbing solutions. These Is mathematically expressed in the form of an equation similar to the one above

2.303log 10 I 0 /I= k'c.....(5)

Where k'=absorptivity constant and C=the concentration of the absorbing material

Beer-Lambert's Law:

We can combine equation 4&5 of Bouger-LambertLaw and the beers law Here, k and k 'merge to become a single Constanta. The combined equation is written.

Log 10 I 0 /I= ab C.....(6)

This equation has been alternately referred to as the Beer Lambert's Law, the Bouger-Beer law or more simply, Beer's law.

This combined law states that the amount of light absorbed is proportional to the concentration of the absorbing material and to the thickness of the material (pathlength).

Comparison of percent transmittance and absorbance scales:

Transmittance: ratio of intensity of transmitted light to the intensity of incidents light I/I 0 .

Absorbance: logarithm to the base10ofthereciprocalof transmittancelog 10 I 0 /Iorlog 10 1/T

The quantity I 0 /I is known as absorbance or optical density (O.D.)

A=log10I0/I.....(1)

Transmittance, T=I/I0.....(2)

%Transmittance, %T=100T

Now from eqn..1, we can write

A=log101/T

A=log10100/%T

A=2-log10%T

Limitation of beer-Lambert's Law:

1. These laws are applicable for monochromatic light and not applicable for polychromatic to light.

2.These laws are applicable only at lower solute concentration but not applicable at very high solute concentration. At lower concentrations, with increased in concentration there is proportionate increase in absorbance while at very high solute concentration the increase in the absorbance is not

proportionate to the concentration

Instrumentation:

The main type of instruments used for measuring absorbance or are colorimeter and Spectro photometer The instrument that are used to study that absorption or emission of electromagnetic radiation as a function of wavelength are called spectrophotometer while colorimeter is the instrument which applies wavelength only in visible range. The basic components of these instruments include

1.Stable and a cheap light source.

2.Monochromator or colored filter (wavelengths selector) means for isolating a narrow range of

wavelengths.

3.A sample holder (cuvette) to hold the sample.

4.Photo detector for measuring the signal.

5.Single process or are recorder that displays the signal in a form convenient for the analyst .

Radiation (light source):

Materials which can be exited to high energy states high voltage electric discharge serve as excellent radiant energy source. As the electrons of these material return to their ground state, they emit radiation of characteristic

energies corresponding to delta E (differences between energy level of excited state and ground state.

Visible radiation Light source The function of the light source is to provide a sufficient of light the light source typically yields a high output of polychromatic light over a wide range of the spectrum.

1) Tungsten lamp:

Tungsten Halogen Lamp, it is the most common light source used in spectrophotometer. This lamp consists of a tungsten filament enclosed in a glass envelope, with a wavelength range of about 330 to 900 nm, are used for the visible region. It has long life about 1200h

Xenon flash lamps:

1)Their range between (190nm - 1000 nm)

2) Emit both UV and visible wavelengths

3) Long life

4) Do not heat up the instrument

5) Reduce warm up time

U.V radiation Hydrogen / Deuterium Lamps:

- For the ultraviolet region, hydrogen or deuterium lamps are frequently used. their range is approximately 200 to 450 nm. Deuterium lamps are generally more stable and has long life about 500h.
- This lamp generates continuous or discontinuous spectral. Wave length selector All the sources discussed so far emit radiation over wide range of wave lengths.
- However, the laws of absorption apply only to the monochromatic radiation. Thus, absorption of narrow band width will tend to show greater support to beers law. moreover, narrow bandwidth radiation will allow the resolution of absorption bands which are quite close to each other. Therefore, a narrow band width is made possible by using wavelength selectors.

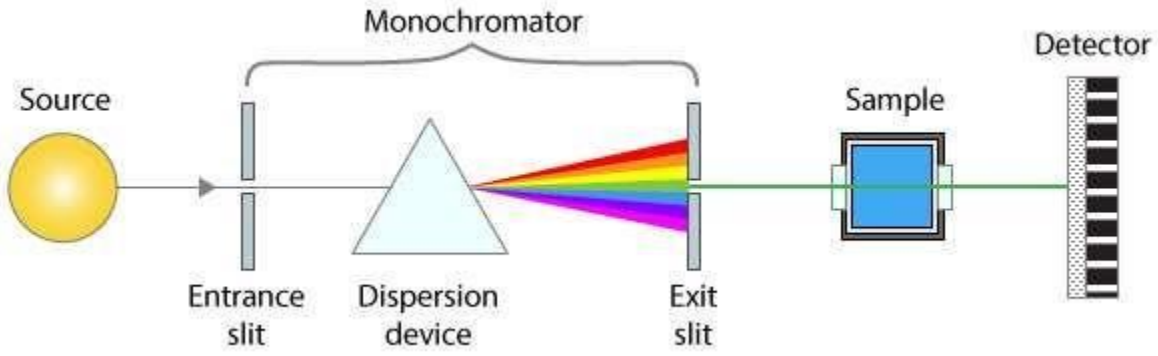
Wave length selectors are of two type

A) Filters B) Monochromators.

- A light filter is a device that allows light of the required wavelength to pass but absorbs light of other wave lengths. filters resolve polychromatic light into a relatively wide bandwidth of about40 nm and are used as wavelength selectors only in colorimeters.
- Filter is used to select the colors of light which the solute absorbs the most. filter used generally are of complementary colored i.e. a blue colored solution absorbs the red color maximally therefore blue colored solution a red colored filter is used. for absorbance measurement of a red colored solution blue colored filter is used because red colored solution absorbs maximally the blue colored light.

Monochromators:

The monochromators are preferred wavelength selectors in spectrophotometers because of the several advantages they offer compared to the filter. As the name suggests a monochromator resolves polychromatic light into its individual wavelengths and isolates these wavelengths into very narrow bends. This increase. this sensitivity of detection by closely obeying the beers law secondly with the help of monochromators it is possible to resolve the two compounds in a which are having absorption maxima quite close to other both the compounds in same solution can be estimated quantitatively



without separation. An entrance silt which admits polychromatic light from the source. A collimating device such as lens or mirror which collimates the polychromatic light on the dispersion devices.

A wavelength resolving device or a dispersion device like prism grating which breaks the radiation in to component wavelength A focusing lens or mirror for focusing rays with specific wavelength

An exit slit which allow monochromatic beam to escape Entire assembly is mounted in a tight box and all the component in a monochromator assembly must not absorb in the range of wavelength which are to be studied.

The construction of typical monochromator having prism and grating as a dispensing component is shown in figure alcove radiation from the source enters the monochromator through an entrance silt. The radiation is collected by a collimating mirror which reflects a parallel beam of radiation to a diffraction grating. The diffraction grating is an optically reflecting surface with a large number of parallel grooves. The diffraction grating disperses the radiation and second mirror focus the radiation on to a planar surface containing an exit silt.

In some monochromators a prism is used in place of the diffraction grating radiation exits the monochromator and passes to the detector as shown in figure

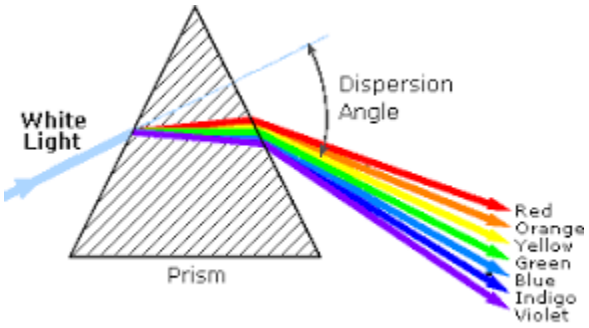
a monochromator converts a polychromatic source of radiation at the entrance silt to a monochromatic source of finite effective band width at the exit silt.

The choice of which wave length exits the monochromator is determined by rotating the diffraction grating a narrower exit silt provides a smaller effective band width and better. Resolution but allows a smaller through put of radiation.

Monochromators are classified as either fixed wavelength or scanning in a fixed wavelength monochromator we select the grating normally a fixed wave length monochromators used for a quantitative analysis where measurement are made at one two wave length.

Prism

A prism splits the incoming light in to its components by refraction occurs because radiation of different wavelength travels along different of dispersion by prism depend upon 1 apical angle of prism which is usually 60 deg and 2 the material of which it is made up of for visible range simple glass prism areused and for UV range Silla fused silica or quartz prism are used.

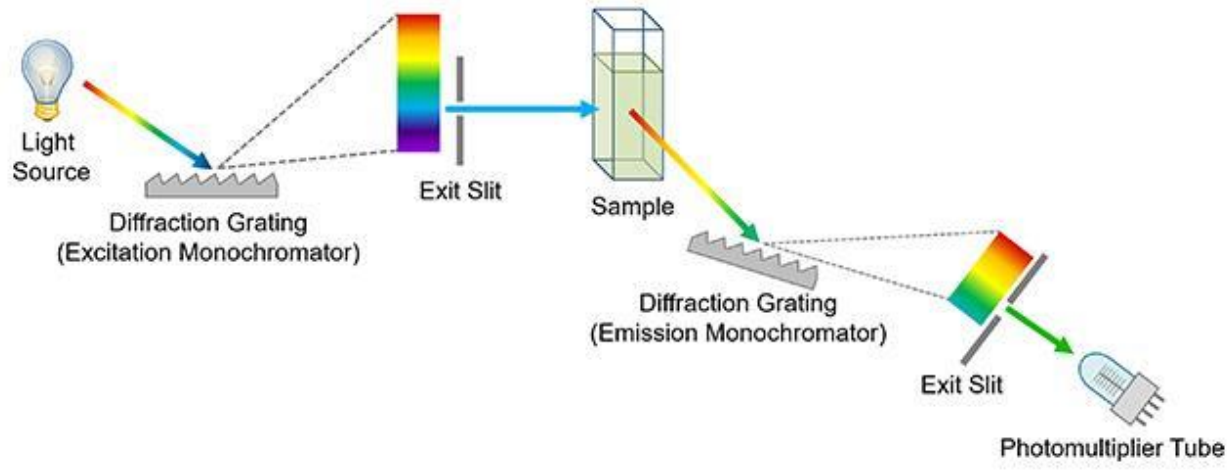


Grating:

At a grating the splitting of wavelength is achieved by diffraction it possesses highly alumized surface having large number or parallel grooves which are equally spaced also known as lines.

Grating may have 60-2000 liter/nm on the region of spectrum in which its intended to operate it the resolution achieved by grating is much higher than the one available by prisms now days instrument almost exclusively contain grating as monochromator as they can be reproducibly made in high quality by photo reproduction when monochromators consist of prism and grating both and prism is placed before grating which is known as

which is then allowed to be diffracted by the grating this arrangement allows the resolution of single wave length



Sample containers(holders):

The solutions are dispended in containers known as cuvettes. Cuvettes meant for visible region are made up of either ordinary glass or sometimes quartz.

Glass absorbs in U.V. region, quartz or fused silica are used in this region. Standard path length his usually1cm.

However, cuvetteofpathlengthof1mmto10cmare Available. Surface of cuvette must be kept clean; fingerprint and traces of previous samples causes interference in the optical path and might cause error in quantitative measurements.

Cuvettes hold be rinsed with water every time. If, however dirtisnot removed sulfonic detergents or nitric acid may be used to clean it.

Two sides of cuvettes through which light passes are polished to be optically flat and the other 2sidesarerough glass and the cuvette may be handled by this side.

Detection devices:

most detectors depend on the photoelectric effect where incident light liberates electrons from a metal or other material surface. Some sort of external circuitry collects their electrons and measuring their number as current as current. the current is than proportional to the light intensity.

Important requirement for a detector includes.

1. High sensitivity to allow the detection of low levels of radiant energy

2. Short response time

3. Long term stability

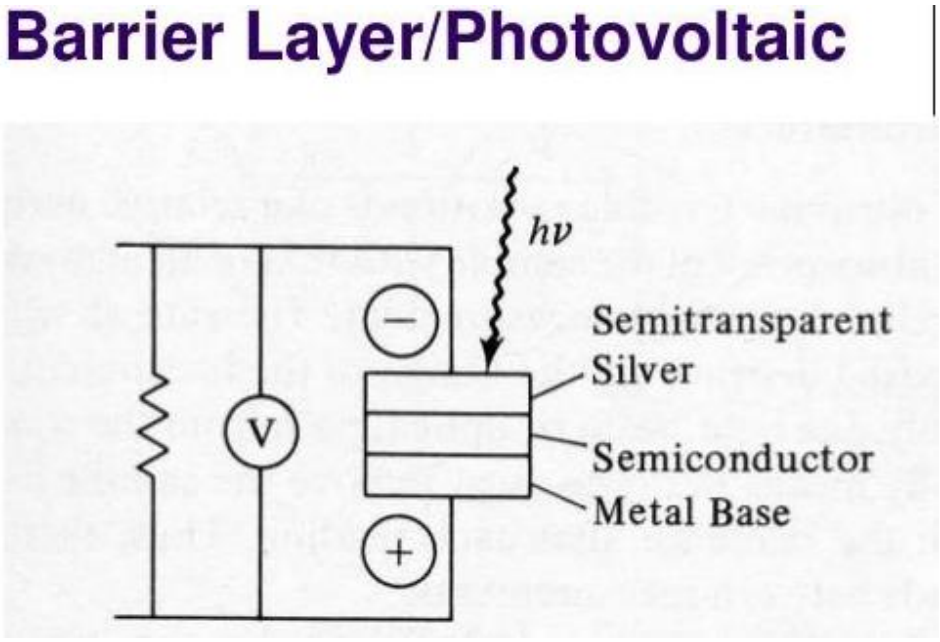
4. Electronic signal which is easily amplified for typical readout apparatus

UV – visible radiation detectors mostly uses photocells phototubes, photomultiplier tubes

photodiodes

1 photo voltaic / photocells / barrier layer cells

It employs a semiconductor material like cadmium sulphide silicon selenium etc. selenium-based photocells are most common. A typical photocell consists of a thin coating of selenium above which a silver thin film is present having steel base. The arrangement ensure that electron pass easily from selenium to silver but not in a reverse direction. due to the inability of electrons to move away from the silver film the silver act as the collecting electrode for electron. Current flowing between 2 electrodes is than measured by a micro ammeter, photocell have long life inexpensive and reliable it is widely used in colorimeters but their use in spectrophotometer is becoming limited.



2 phototubes or photo emissive tube

The components of a phototube include;

i) An evacuated glass envelope (with a quartz window)

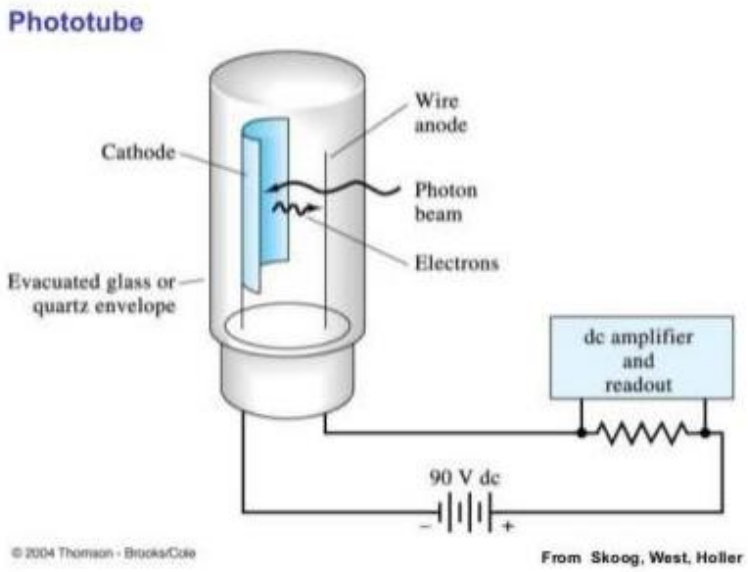
ii) A semi-cylindrical cathode whose inner surface is coated with alkali or alkaline earth oxide and

iii) A centrally located metal wire anode

The energy of the photon is transferred to the loosely bound electrons of the cathode surface. the electrons become excited and finally leave the surface and travel toward the anode causing current in the circuit.

If the electron collection is 100% efficient the phototube current should be proportional to the light intensity.

Photo tube currents are quite small and require amplification. this is usually accomplished by placing a high resistance (R in the figure) in the phototube circuit.



3. photo multiplier tube

These detectors are designed to amplify the initial photoelectric effect and are suitable for use at very low light intensities a photomultiplier consists of

1) An evacuated glass tube into which is sealed the cathode and anode

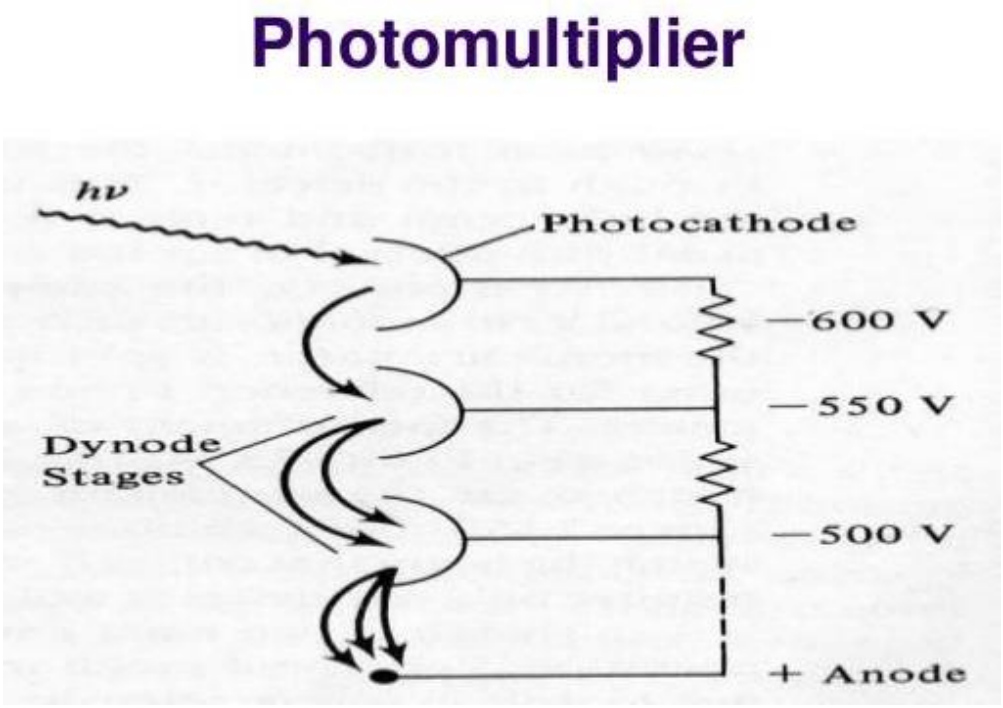
2) Additional intervening electrodes known as dynodes

The arrangement is shown in fig. The external circuitry is arranged so that voltage (100 volts) Exists between anode and the cathode As the radiation strikes

lie photocathode electron are liberated the applied potential difference accelerates the electron first dynode each successive dynode is at a higher electric potential and thus acts. As an amplification stage for the original photon. the applied voltage causes sufficient electron acceleration to knock out other electrons from each dynode surface.

The liberated electrons are dragged on to the next dynode where electrons are released and this process goes on as cascade till the last dynode By the time the electrons arrive at the collecting anodes the initial photoelectric current is amplified by a factor of approximately 10

In particle photomultiplier tube are used only for low light intensities at higher intensities power photomultiplier exhibit great instability in splits of this tendency to be unstable photomultiplier are the detectors of choice ill modern spectrophotometers.



3) Photodiode

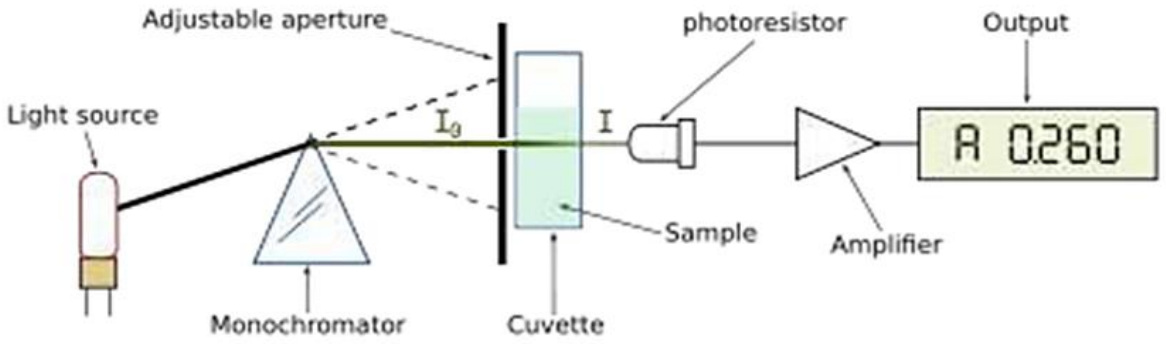
It is a semiconductor device that converts light into current the current generated when photons are absorbed in the photodiode and is measured a photodiode aery is a 2-D matrix composed of hundreds of thin semiconductor spaced very closely together light from the instrument is dispersed by either a grating or a prism on to photodiode array each position of diode on the array is

calibrated to correspond to a specific wavelength each diode is scanned and the resultant electronic change is calculated to be proportional to absorption entire spectrum is essentially recorded within mill second.

Single beam spectrophotometer:

This type is cheaper because there are less parts and the system is less complicated low cost, high Sensitivity, because the optical system is simple.

The disadvantage is that an appreciable amount of Time elapses between taking the reference and Making the sample measurement

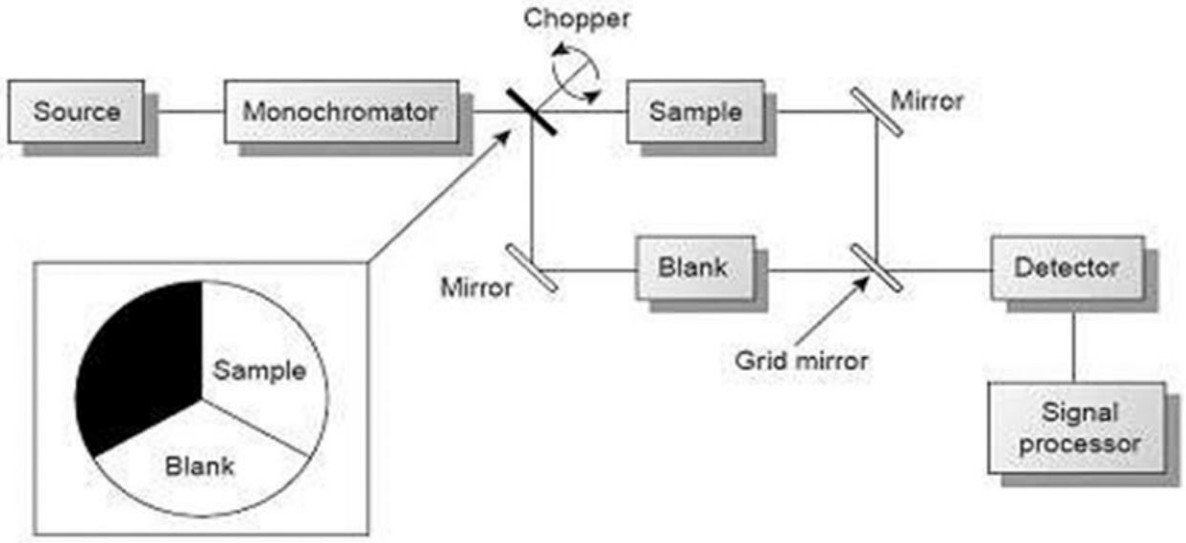


Double beam spectrophotometer:

The double beam instrument design aims to eliminate drift by measuring blank and sample virtually simultaneously.

A chopper alternately transmits and reflects the light beam so that it travels down the blank and the sample optical paths to a single detector.

high stability because reference and sample are measured virtually at the same moment in time.



Advantage:

it is not naceeasry to continually replaced the blank with the sample or to zero adjust at each wavelength as in the single beam units. The ratio of the power sample and reference beams is constantly obtained and used. Any error due to variation in the intensity of the source and fluctuation in the detector is minimized.

Disadvantage

higher cost, lower sensitivity Split beam spectrophotometer: The split beam spectrophotometer is similar to the double beam spectrophotometer but it uses a beam splitter instead of a chopper.

Thus, blank and sample measurements can be made at the same moment in time. Spectra are measured in the same way as with a double beam spectrophotometer. The advantage of this design is good stability,

Application of colorimeter and spectrophotometers: UV visible Spectro photometry has diverse applications in quantitative and qualitative an alysis in biochemistry. Only a few important applications are summarized below.

Qualitative analysis: visible and ultraviolet spectra may be used to identify classes of compounds in both pure state and in biological preparation. This is based on the fact that absorption spectra are specific for a class of compounds.

Quantitative analysis: Some compounds have specific max and have higher extinction coefficient can be estimated quantitatively by absorbance

measurement. Many substances do not possess significant extinction coefficient in the visible region will react quantitatively with some other reagents to give a color product. This property is used to estimate such substances.

Colorimetry: is widely used in biochemistry to assays wide range of biologically important substance like; inorganic phosphorus, calcium, amino acid, proteins, carbohydrates, nucleic acid, lipids, cholesterol, sterols and steroids hormones, vitamins, drugs etc.

Enzyme assays and kinetic studies: The quantitative assay of enzymes activities carried out when substrate or product is colored or absorbs light in UV range because the rate of appearance or disappearance of light absorbing products or substrate can be followed spectrophotometrically.

E.g. Lactose+NAD + ____> pyruvate+NADH+H + NADHstronglyabsorbsat340nmwhileNADdoesnot.

Molecular weight determination: The molecular weight of amines, picrates, Sulphur and aldehyde and ketone compounds can be determined by using the absorbance data with accuracy.

Control of purity: Impurities in a compound can be detected easily by spectrometric studies E.g. carbon disulfide impurity in the carbon tetra chloride can be detected easily by measuringabsorbanceat318nmwhereonlythecarbon disulfide absorbs.

Testing or Purity of compounds: It can be based on their specific Extinction coefficient. Study of

Cis-Trans isomerism: Trans isomers is usually more elongated than its cis counterpart and has higher absorption maximum. Absorption spectroscopy can be thus utilized to study the cis trans isomerism.

Turbidometry and nephiometry: Measurement of the intensity of the transmitted light will give an idea about number of particles in the suspension. Using this turbidimetric technique one can get approximate number of particles in suspension. This technique is routinely used to measure the number of bacteria in a given suspension. The wavelengthusedforthispurposeis600nm.

1. Mass Spectrometry

Mass spectrometry is a powerful analytical technique used to quantify known materials, to identify unknown compounds within a sample, and to elucidate the structure and chemical properties of different molecules. The complete process involves the conversion of the sample into gaseous ions, with or without fragmentation, which are then

characterized by their mass to charge ratios (m/z) and relative abundances.

This technique basically studies the effect of ionizing energy on molecules. It depends upon chemical reactions in the gas phase in which sample molecules are consumed during the formation of ionic and neutral species.

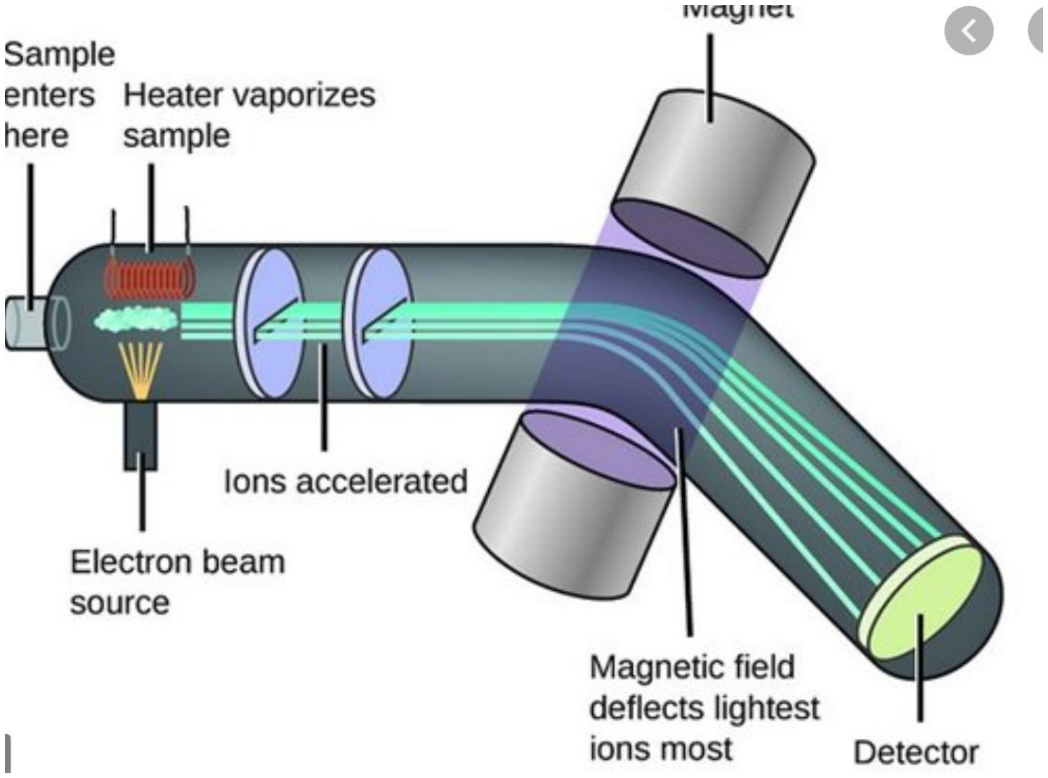
Basic Principle

A mass spectrometer generates multiple ions from the sample under investigation, it then separates them according to their specific mass-to-charge ratio (m/z), and then records the relative abundance of each ion type.

The first step in the mass spectrometric analysis of compounds is the production of gas phase ions of the compound, basically by electron ionization. This molecular ion undergoes fragmentation. Each primary product ion derived from the molecular ion, in turn, undergoes fragmentation, and so on.

The ions are separated in the mass spectrometer according to their mass-to-charge ratio, and are detected in proportion to their abundance.

A mass spectrum of the molecule is thus produced. It displays the result in the form of a plot of ion abundance versus mass-to-charge ratio. Ions provide information concerning the nature and the structure of their precursor molecule. In the spectrum of a pure compound, the molecular ion, if present, appears at the highest value of m/z (followed by ions containing heavier isotopes) and gives the molecular mass of the compound.



Components

The instrument consists of three major components:

1. **ion Source:** For producing gaseous ions from the substance being studied.
2. **Analyzer:** For resolving the ions into their characteristics mass components according to their mass-to-charge ratio.
3. **Detector System:** For detecting the ions and recording the relative abundance of each of the resolved ionic species.

In addition, a sample introduction system is necessary to admit the samples to be studied to the ion source while maintaining the high vacuum requirements (~10-6 to 10-8 mm of mercury) of the technique; and a computer is required to control the instrument, acquire and manipulate data, and compare spectra to reference libraries.

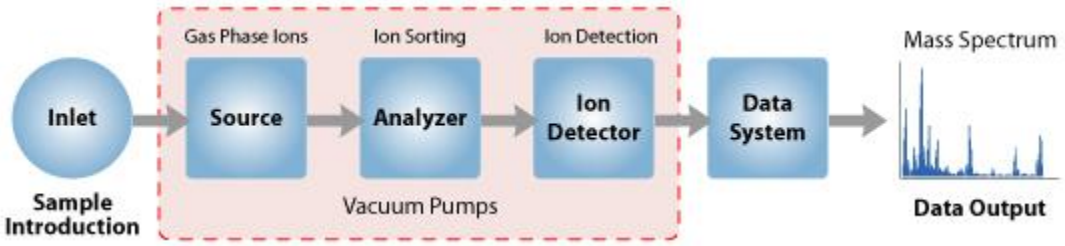


Figure: Components of a Mass Spectrometer

With all the above components, a mass spectrometer should always perform the following processes:

1. Produce ions from the sample in the ionization source.
2. Separate these ions according to their mass-to-charge ratio in the mass analyzer.
3. Eventually, fragment the selected ions and analyze the fragments in a second analyzer.
4. Detect the ions emerging from the last analyzer and measure their abundance with the detector that converts the ions into electrical signals.
5. Process the signals from the detector that are transmitted to the computer and control the instrument using feedback.

Analysis of Biomolecules using Mass Spectrometry

Mass spectrometry is fast becoming an indispensable field for analyzing biomolecules. Till the1970s, the only analytical techniques which provided similar information were electrophoretic, chromatographic or ultracentrifugation methods. The results were not absolute as they were based on characteristics other than the molecular weight. Thus the only possibility of knowing the exact molecular weight of a macromolecule remained its calculation based on its chemical structure.

The development of desorption ionization methods based on the emission of pre-existing ions such as plasma desorption (PD), fast atom

bombardment (FAB) or laser desorption (LD), allowed the application of mass spectrometry for analyzing complex biomolecules.

Analysis of Glycans

- Oligosaccharides are molecules formed by the association of several monosaccharides
  linked through glycosidic bonds. The determination of the complete structure of oligosaccharides is more complex than that of proteins or oligonucleotides. It involves the determination of additional components as a consequence of the isomeric nature of monosaccharides and their capacity to form linear or branched oligosaccharides. Knowing the structure of an oligosaccharide requires not only the determination of its monosaccharide sequence and its branching pattern, but also the isomer position and the anomeric configuration of each of its glycosidic bonds.
- Advances in glycobiology involves a comprehensive study of structure, bio-synthesis, and biology of sugars and saccharides. Mass spectrometry (MS) is emerging as an enabling technology in the field of glycomics and glycobiology.

Analysis of Proteins and Peptides

- Proteins and peptides are linear polymers made up of combinations of the 20 amino acids linked by peptide bonds. Proteins undergo several post translational modifications, extending the range of their function via such modifications.

- The term Proteomics refers to the analysis of complete protein content in a living system, including co- and post-translationally modified proteins and alternatively spliced variants. Mass Spectrometry has now become a crucial technique for almost all proteomics experiments. It allows precise determination of the molecular mass of peptides as well as their sequences. This information can very well be used for protein identification, de novo sequencing, and identification of post-translational modifications.

2.INFRARED SPECTROSCOPY

Introduction:

IR radiation so much higher wave length has compared to uv and visible region i.e. considerably have low energy.

It refers to that region of E.M. spectrum which lies b/w visible and micro wave region Range of IR radiation

Divided into 4 section

1.Photo graphic region: range–visibleto1.2um

2.Very near IR (over to region): Range–1.2to2.5to um

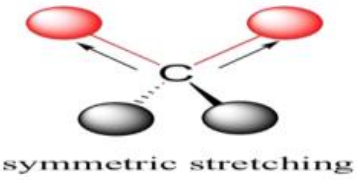
3.Near IR region (vibration region): Range–2.5to25 um

4.Far IR region (rotation region): Range–25to300– 400um.

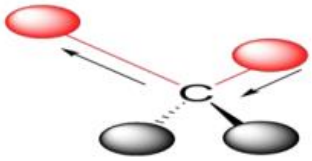
IRspectroscopyusuallymeanstherangefrom2.5to25um.

It gives the important information about vibration of molecules and hence about the structure of

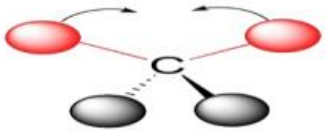
molecule.



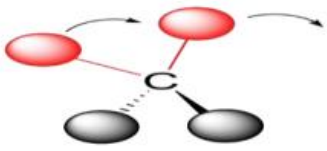
symmetric stretching



asymmetric stretching



scissoring



rocking

Principle:

Due to low energy, IR is not associated with electronic transitions, rather it is associated with the vibrational transitions of molecule.

Thesevibrationsareof2type

1.Stretchingvibration

2.Bendingordefomation

Stretching vibration: Bound distance (bond length) b/w the atoms in molecule fluctuate to about+/- 0.5A: Atoms remain in same bond axis.

1.Bending or deformation: involve changes in a position of the atom with respect to original bond axis i.e. variations bond angle may be about+/-0.5deg.

IR spectra: The technique is based upon the simple fact that chemical substance shows selective

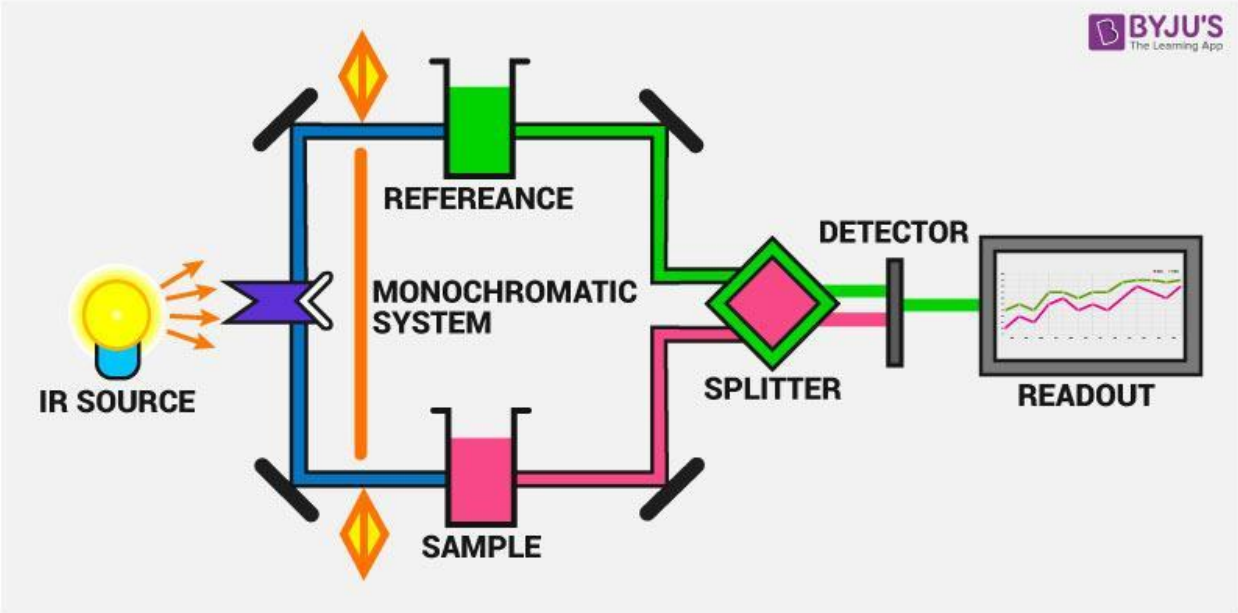
absorption in IR region. After absorption of IR radiation, molecule of chemical substance vibrates at many rates of vibration, giving rise to absorption bands, called IR absorption spectrum.

Various bands will be present in IR spectrum which will correspond to characteristic functional groups and bonds present in chemical substance.

Thus, IR spectrum of chemical substance is a finger print for it identifications.

A molecule can absorb IR of app wave length, which is accompanied by promotion of molecule to an excited vibrational state.

Infrared spectra differ from uv-visible spectra, here wave number is used rather than wavelength presented by% transmittances wavenumber.



INSTRUMENTATION:

Main components

1.IRradiationsource

2.Monochromators

3.Samplecells

4.Detectors

1.IRradiationsource

Radiation source must emit IR radiation which must b

1.Intenerenoughfordetection

2.steady

3.extendeverdesiredwavelength

Popular IR source

1.In can descent lamp Is an electric light with a wire filament heated to high temperature? By passing an electric current through it, until it glows Used in near infrared instrument

Disadvantage: it falls in far IR

2.Nernstglower

Consist of hollow rod of 2mm in diameter and 30mm in length

Glower is composed of rare earth oxides such as zirconium oxide (z r o 2), Ytterbium oxide(y 2 o 3) and erbium oxide (E r2 o 3) Nernst glower is non-conducting at room temperature and must be heated to bring it to conductor state. Generallyheatedat1000to1800degC

Advantage:

1.Emits IR radiation over wide wave length range

2.Intensity of radiate one main steady and constant over long period of tune \

Disadvantages

1.Frequent mechanical failure

2.Its energy is also concentrated invisible region of spectrum

3.Globarsource

It is rod of silicon carbide which is about 50mm in length and 4mm in diameter.

When it Is heated to a temperature b/w1300to100deg. It strongly emits radiation in IR region.

Disadvantages

it is less intense source than Nernst glower

4.Mercuryare

Special high-pressure mercury lamps are used for infrared region

Beckman devised quartz mercury lamp in a unique manner.

For shorter wave length the heated quartz envelope emits the radiation For longer wave length the mercury provides radiation through the quartz

2.Monochromators

The radiation source emits radiation of various frequencies thus it becomes necessary to select desired frequencies from radiation source and reject radiation of other frequencies

2 type monochromators

1.Prism

2.Grating Prism

Glass and quartz absorb IR. Hence are unsatisfactory in IR region. Must be contracted of material such as metal halide salts.

Nacl is probably the most common salt.

These salts are subject to mechanical and thermal instability and water solubility, protection against damaged must be continuously exercised.

Grating

If prism is replaced by a grating higher dispersion can be achieved.

It is made with material like aluminum, which is not attacked by moisture, while metal salt prisms are subject to catch moisture

3.Sample cell

IR spectroscopy is used for characterization of solid, liquid and gas samples, hence samples of different phase have to be handled.

These samples have to be had to be treated differently Common point to the sampling of different phases is the material containing the samples must not absorb IR

Hence only certain metal halide salt (Nacl, Kbr, ThBr)

4.Detectors

Some detectors used in IR region are bolometers, thermo couple, thermistors, photoconductivity cells, semiconductor detectors golay cell, pyroelectric detectors, Fourier trans form systems working.

A beam of infrared light produced, passed through monochromators (not shown), and then split in to two separate beams.

One is passed through the samples, the other passed through a reference.

The beams are both reflected back towards a detector, however first they pass through a splitter, the two beams enters the detector.

The two signals are then compared and a printout is obtained.

Applications

Identification of compound

Macro molecules possess a large no of atoms and show numerous, specific vibration, thus providing a detailed IR spectrum, which helps in molecular identification, another approach is identification of functional groups by studying their specific absorption bands.

As saying the rate of reactors:

This is done by studying the loss and appearance and shift of bonds due to functional groups involved in enzymatic reaction.

Ex. If the substrate processes a -OH groups &the product does not the rate of reaction can measure by measuring the rate of disappearance of the O-H stretching vibration.

Study of conformation of molecules Conformation of proteins &other molecule have been studied using the certain techniques of IR.

Study of interaction between macro molecules.

Hydrogen bond can be studied using IR spectroscopy because of the fact that the strength of bond of functional groups involved in H bonding get alter.

EX. H bond to starching, thereby reducing their starching vibration.

Some specific applications of IR spectroscopy are as follows.

1)determination of steroids, hormone & pharmaceutical chemical.

2)study of vitamins, enzyme, lipid, protein, etc.

3)study of sub micro amount of asbestoses styrene but adios rubber extract on high ways, co 2 in air.

4)study of geological materials &soil in land and terrestrial material

5)characterization natural products, polymers, detergents, fats and resins.

6)Drug, organic compound and polymer manufacturing.

7)Determination of nutritive values in foods industry.

1.3 Atomic-spectroscopy

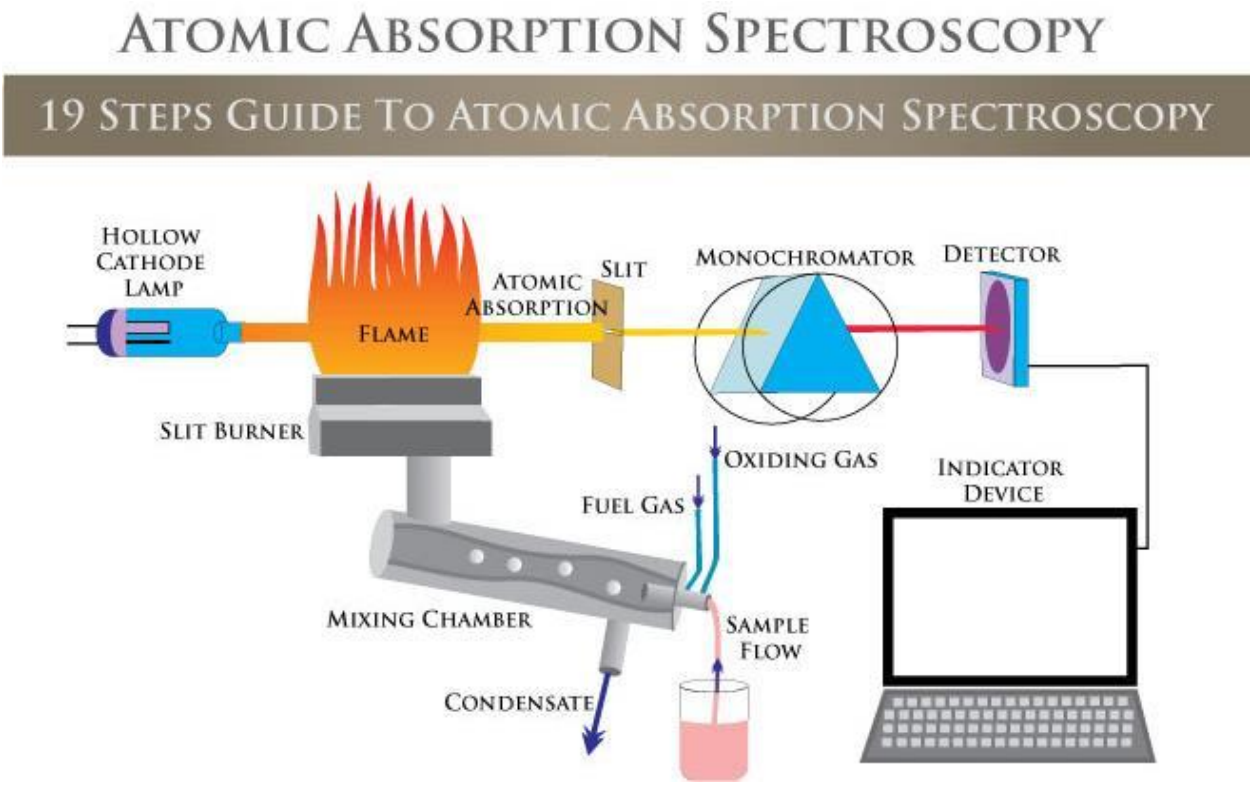
The science of atomic spectroscopy has yielded3techniques

1) atomic absorption 2) atomic emission 3) atomic fluorescence

Then energy absorbed to move an electron to a more energetic level and the energy emitted as the electron moves to a lessen energetic level is in a form of photon.

The wave length of the emitted radiant energy is directly related to the electronic transitions which has occurred. since every element has a unique electronic structure, the wave length of light emitted is a unique property of each individual element electronic transitions can occur by absorbing the energy, each transition resulting in the emission flight.

The process of excitation and decay to the ground level is involved in both the field so atomic spectroscopy. either the energy absorbed in the excitation process, on the energy emitted in the decay process is measured and used for analytical purposes.



Atomic absorption:

Technique was introduced by walsh, alkernadeandmilatzin1956.

It is an absorption method where radiation is absorbed by atom in the vapour state. It involves study of absorption of radiation [usually in uv visible region] by atom in gaseous state.

thus, the sample is1stconvertedintoatomicvapourandtheabsorptionofatomicvapouris measured at a selected wavelength.

it involves spraying a solution of the sample in to the flame, hence it is also known as absorption flame photometry.

The measured absorbance is proportional to the concentration and analyses is made by comparing it with reference sample of known concentration.

The principle of atomic absorption is similar basically to those considered of UV and visible radiation by solution, but the equipment and sample handling techniques are quite different.

To dissociatethechemicalcompoundsintofreeatomsthermalenergyinaformofflameisprovidedhere the sample is vaporized and imposed in the beam of radiation from source, can be done by spraying the sample in to a suitable flame.

It is normally arranged so the radiation is focused by a lens to a flame in to which solution to be measured is sprayed. A second lens then re-Image it into the entrance slit of the monochromator.

Thus, if a light just impinges on a ground state atom, the atom absorbs the light as it enters an excited state in a process called atomic absorption. It measures the amount of light at the resonant wave length which is absorbed as it passes through cloud of atoms. As the number of atoms in the light pass increases, the amount of light absorbed increases.

By measuring the amount of light absorbed, a quantitative determination of the amount of analyte present can be made

The ease and speed at which precise and accurate determination can be made, have made atomic absorption one of the most popular methods for determination of metals.

Atomicspectroscopyisverysensitiveformostelements.concentrationattheppmlevel mayberoutinely determined using flame atomization.

Light source:

Hollow cathode lamps are the most common radiation source in AAS. It contains a tungsten anode and hollow cylindrical cathode. These are sealed in a glass tube filled with an inert gas. Each element has its own unique lamps which must be used for that analysis.

Nebulizer:

It sucks up liquid sample at controlled a tend create fines pray for introduction in to flame.

Atomizer

elements to be analyzed needs to be in atomic state. Atomization is separation of particles in to individual molecules and breaking molecules in to atoms. This is done by exposing the analyte to high temperature resin a flame or graphite furnace.

Monochromator:

This is a very important par to fan AAS spectrometer. It is used to separate out different lines. A monochromatic is used to select the specific wave length of light which is absorbed by the sample, and to exclude other wavelengths.

The selection of the specific light allows the determination of the selected element in the presence of others.

Detector:

The light selected by the monochromator is directed on to a detector, whose function is to convert the light signal in to an electrical signal proportional to the light intensity.

The processing of electrical signal is fulfilled by a signal amplifier. The signal could be displayed for Read out, or further fed into a data station for print out by the requested format.

Applications:

1.Clinicalanalysis;

Analyse metals in biological fluids such as blood and urine. Used for analysis of elements in various biological samples. Used for routine estimation no f sodium, potassium, calcium, magnesium, manganese etc. in biological samples.

2.Environmental analysis;

Used form on it or in go environment e.g. Find out the various elements in rivers, sea water, drinking water, air etc. It can be used for sensitive estimation of alkali, alkaline earth and are earth elements.

3.Lubricatingoilsandgreases; Ba, Ca, Mg, Zn, Li, Na

4.Water and effluents; many elements e.g. Ca, Mg, Fe, Si, Al, Ba

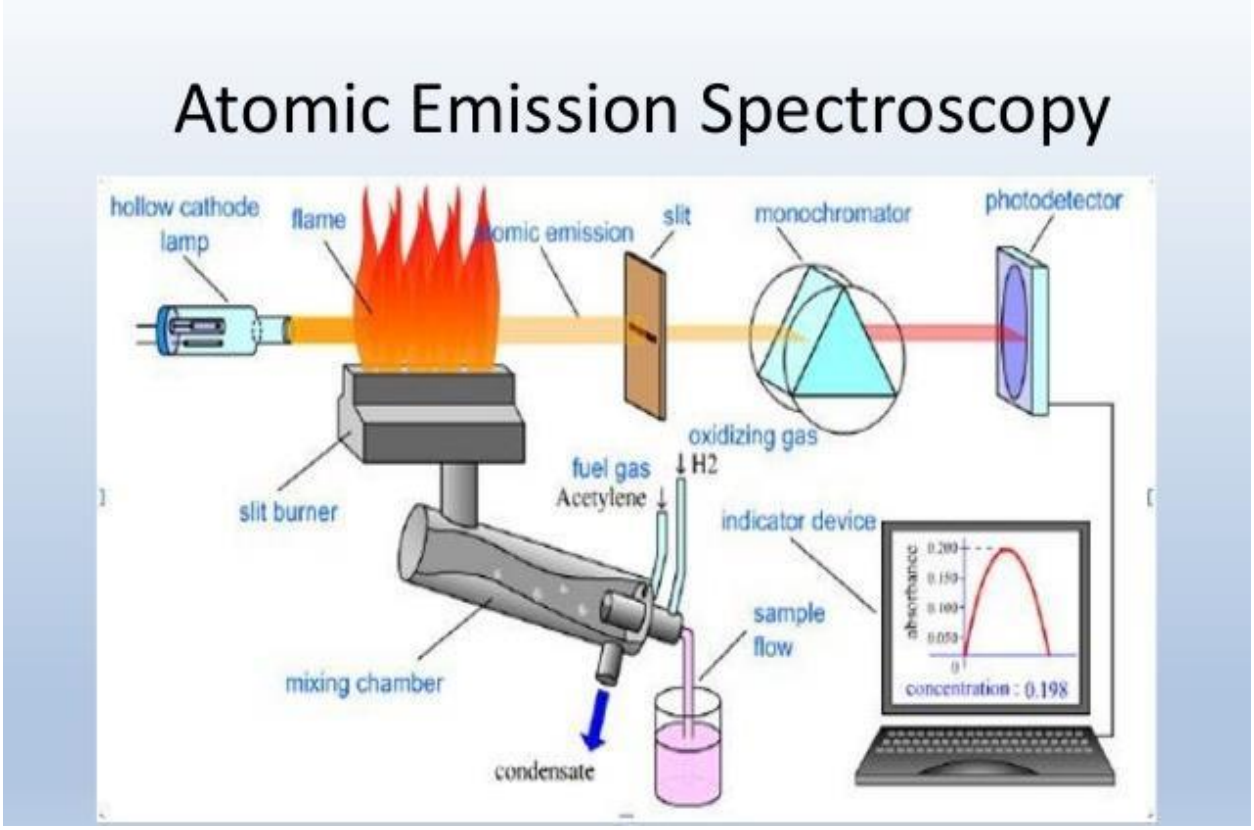
5.Food; Wide range of elements

6.Medicines; range of elements

Atomic emission spectroscopy:

Atomic emission spectroscopy (AES)is a method of chemical analysis that uses the intensity of

light emitted from a flame to determine the quantity of an element in sample.



The wavelength of the atomic spectral line gives the identity of the element while the intensity of the emitted light is proportional to the number of atoms of the element.

Sample of a material (analyte) is brought in to the flame as either gas, sprayed solution, or directly inserted into the flame by use of a small loop of wire, usually platinum.

The heat from the flame evaporates the solvent and breaks chemical bonds to create free atoms.

The thermal energy so excites the atoms into excited electronic states that subsequently emit light when they return to the ground electronic state.

Flame temperature is a very important factor in the analysis it is regulated by adjusting the flow rates and the ratio of fuel oxidant in the flame atomizer.

Because the number of atoms in a particular excited state increase with rise in temperature, a high temperatureatomreservoirisdesirableforatomicemissionspectrometry.

Each element emits light at a characteristic wavelength, which is dispersed by a grating or prism and detected in the spectrometer.

Instrumentation:

The major difference from AAS is the absence fan external radiation source, because here the flamer itself acts as the excitation source for the sample to be analysed. The system consists of flame regulating excitation source with a gas regulating system, optics to focus the emitted light a monochromator, and detector &finally an amplifier readout system.

Various gas mixtures producing differing flame temperatures are used, some combination is listed below.

The emitted radiation is focused by a collimating on to a monochromator which select the specific and analytical spectral lines for small other radiation emitted from the flame. This radiation of appropriately selected wave length strikes a photo detector the magnitude of the electrical

signal developed is readout.

Applications of AES:

Can determine proportional quantity of a particular element in a given sample. *Used to examine different substance such as foods and drinks, oil and soil samples.

Analysis of many elements in tissues & other biological samples e.g. Ca, Mg, Mn.

It has been used to detect about 40elements in plants &oils, thus helps in diagnosis of deficiency problems in plants.

1.4 Nuclear Resonance and NMR spectra:

When a nucleus is placed in a system where it absorbs energy it become excited, it than loses energy to return to unexcited state. This nucleus which alternately becomes excited and unexcited is said to be in a state of resonance.

In order to determine resonance frequency, the energy absorbed, by nuclei is measured as the magnetic field is varied. Energy absorbed in this process produces a signal at the detector and this is simple is amplified and recorded as a band in spectrum.

Thus, a plot between absorption signal at the detector to the strength of magnetic field Ho [freq]. The effective field strength this different for different nuclei as one

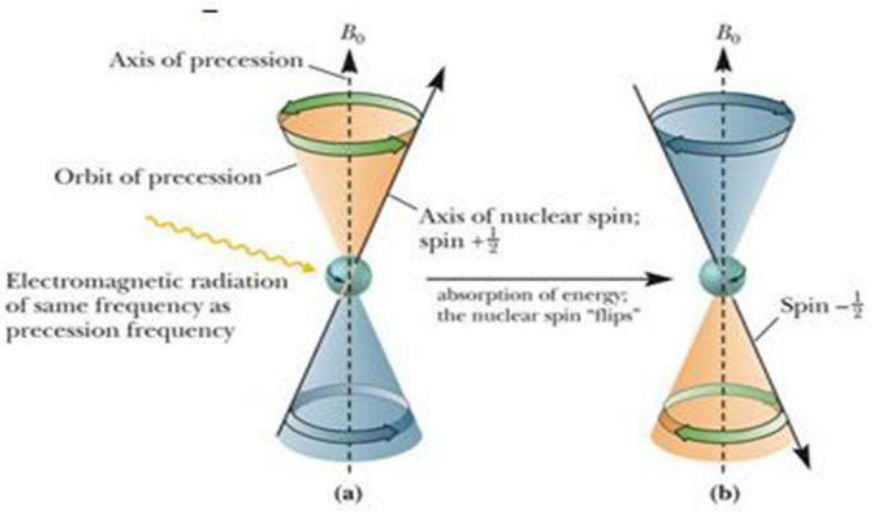
nucleus will haves lightly different environment for many other nucleus [Even magnitude varies with the nucleus].

Hence, all nuclei do not absorb at the same applied field but the absorption depends upon the magnetic field thus at a given radio waves, different nuclei will requires lightly different magnetic field.

Hence, to measure the absorbance of the sample a monochromatic radio wave is used, varying the magnetic field intensity.

Nuclear Magnetic Resonance

– (a) Precession and (b) after absorption of electromagnetic radiation.



Instrumentation:

1.Sampleholder:

Should be chemically inert, durable, cheap, not absorb radio waves. Generally, glass tubes are employed which 8.5cm long and approx. 0.3cm in diameter.

2.Permanentmagnet:

It should give homogeneous magnetic field i.e. Strength hand direction of magnetic field must not change from point to point. The magnet must be capable of producing strong magnetic field of least

10,000 gauss.

3.Magnetic coil and sweep generator:

It is not easy to the magnetic field of large, stable magnet; however, it can be done super imposing a small variable magnetic field on the main field.

It can be done by using coils, which induce a magnetic field that can be varied by varying the current following through them.

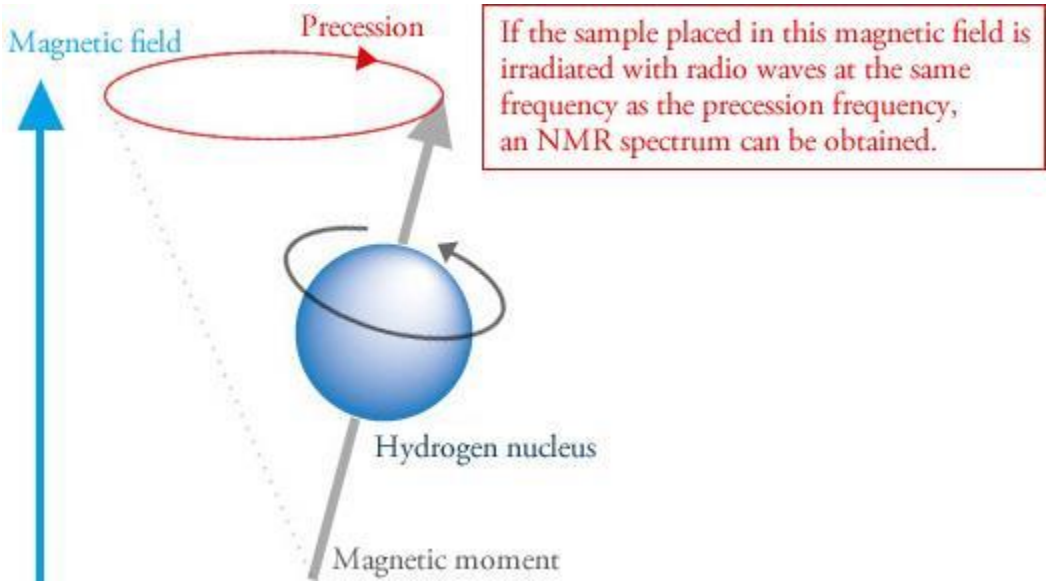
The small magnetic field produced is in the same direction as the main field. Hence the sample is exposed to both fields, which appear as one field to the molecule.

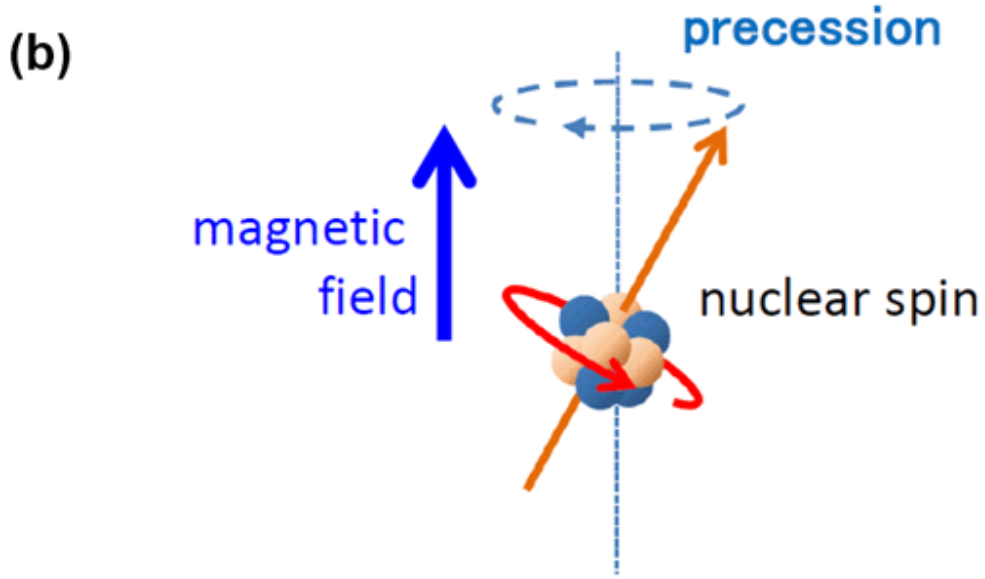
In commercial equipment it is to keep the RF radiation constant in attaining the resonance. It is similar to scanning the spectrum by varying the wavelength in IR or U.V. spectroscopy. [Sweeping the field]

Radiofrequency transmitter: produce powerful pulse of radio waves.

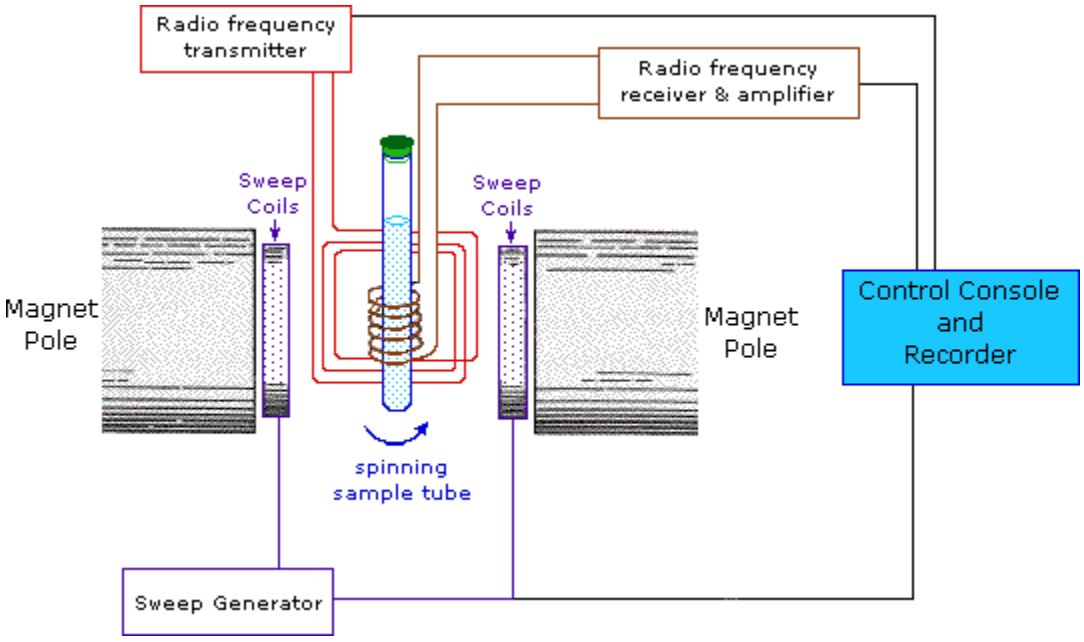
Radiofrequency receiver: detects radiofrequency emitted as nuclei relax to lower energy level.

Read out system: Analyse and record the data.





(b) Precession of a nuclear spin. The axis of nuclear spin shows precessional motion when a magnetic field is applied along the tilt direction. The precessional motion of nuclear spin has a proper frequency specified by the kind of atomic nucleus, so that the frequency measurement through nuclear magnetic resonance (NMR) can identify the materials.



Working of instrument:

Sample placed in glass tube specially designed for NMR. Dissolve the sample in app solvent like

chloroform, acetone, benzene, place the sample between two magnetic poles. Radio waves are

incident to the sample. Detector detects the radio-frequency signals when resonance occur.

Application

1.StructuralDiagnosis:

Proper interpretation of the spectra should tell us about the structure of an organic molecules

It is mainly applied to study the structure of small organic molecules and globular proteins like

antibiotics, neuro toxin, various cytochrome, membrane binding protein etc...

However, for protein study it is combined with x-ray study for better information.

2.Study of dynamic characteristics of protein structures:

Internal motions like opening of secondary structure or any chemical exchange can affect the

property of NMR resonance.

3.ComplexFormation:

Using NMR, it is possible to detect very small conformational changes like binding of ligand to an enzyme, a drug to DNA, antigen to anti body etc...

4.Biological Structure and Component:

Helpful in studying lipid head groups of the biological membranes and their interaction with

integral protein of the membrane.

5.Quantitative studies:

Over the year NMR has been used to determine the concentration of metabolites.

6.Intact Organ Study:

An intact biological specimen such as heart, kindly and skeleton muscles by NMR is one of the

most availability ground in G area. This can be done by 3 presonance of small molecules such as

ATP, ADP, Pi, and creation phosphate.

Limitations:

Minimum sample size is required is 0.1ml having minimum concentration of about 0.1%. If two

type of atom resonate at same resonance frequencies hence over lapping spectra is difficult to

interpret. While characterizing organic molecule, no information of molecular weight is given.

In most cases, only liquids can be studied by NMR, although polymers when preheated with various solvents, become fluids which can be treated as liquids.

1.5 Application of Radioactive Substances

Introduction

Atomic nuclei are composed of two major particles, protons and neutrons.

Protons are positively charged with a mass approximately 1850 times greater than that of an electron. The number of protons present in the nucleus is known as the atomic number (*z*), and it determines what the element is, for example six protons is carbon.

Neutrons are uncharged particles with a mass approximately equal to that of a proton.

The sum of protons and neutrons in a given nucleus is atomic mass

$$A = Z + N$$

Since the number of neutrons in a nucleus is not related to the atomic number, it does not affect the chemical properties of the atom. Atoms of a given element may not necessarily contain the same number of neutrons. Atoms

of a given element with different mass numbers (i.e. different numbers of neutrons) are called isotopes.

The ratio of neutrons to protons will determine whether an isotope of an element is stable enough to exist in nature.

Stable isotopes for elements with low atomic numbers tend to have an equal number of neutrons and protons, whereas stability for elements of higher atomic numbers requires more neutrons.

Unstable isotopes are called radioisotopes. They become stable isotopes by the process of radioactive decay: radiation are emitted

They can also be defined as atoms that contain an unstable combination of neutrons and protons.

Thus, radioactivity is the spontaneous transformation of an unstable atom and Often results in the emission of radiation. This process is referred to as a transformation, decay or disintegrations of an atom.

Application

Mutagen: -

Radioisotopes can cause mutations particularly in microorganism. Mutants are desirable in industries for development of new strains which produce higher yield of products.

Sterilization of food and equipments: -

Food irradiation is a method of treating food in order to make it safer to eat and have a longer

shelf life very strong. Y – emitters are widely used in the food industry for sterilizes of prepacked food.

The energy from Y-rays passing through the food is enough to destroy many disease-causing bacteria as well as that cause food to spoil, but is not strong enough to change quality, flavour or texture of the food.

Irradiation of food have additional benefits since the energy passing through the food can disrupt cellular processes, it also halt the cellular processes that lead to sprouting & ripening of foods.

Fruits and vegetables are irradiated to slow down the ripening process. In this way delicate fruits won't reach their peak ripeness before they arrive at the supermarket.

Irradiation doesn't make the food radioactive during this process energy passes through food much like a ray of light passes through a window since food never actually touches radioactive source, the food cannot become radioactive.

It is also used for sterilization of plastic disposable equipment such as Petri dishes syringes.

Traces: -

A radioactive tracer, or radioactive label, is a chemical compound in which one or more atoms

have been replaced by a radioisotope so by virtue of its radioactive decay it can be used to explore the mechanism of chemical reactions by tracing the path that the radioisotope follows from reactants to product.

Radioisotopes of hydrogen, carbon, phosphorus, sulphur, and iodine have been used extensively to trace the path of biochemical reactions.

Insect Irradiation: -

Male insects are raised in laboratory then irradiated with a dose which makes them sterile.

They are often released into environment in large nos, a normal female mating with one of these male produce infertile eggs.

So by this way harmful insects can be reduced such bio control have potential benefit to reduce

use of harmful pesticides to control insects.

Molecular biology techniques:

Radioisotopes are used in DNA-RNA sequencing synthesis of DNA & RNA probes r-DNA tech. etc,

Labelling of Nucleic acid will be helpful for detection. Advantages of radio labelling are as follow.

A. Easier to handle

B. Sensitivity is more

C. Detection procedure is simple

3 1H, 32 15P, 35 16S, 14 6C etc used for labelling different component of nucleic acid.

Membrane transfer studies: -

It is also used in the study of movement of ions and compounds across the membranes.

It involves addition of radioactive ions & compounds to one side of the membrane and measurement of their appearance on other side.

Study of ion exchange by kidneys, transport of nutrients in intestine can be done with radioisotopes.

Immune assays: -

Useful in analysis of hormones, growth factors, bacterial antigens & various biological molecules.

In RIA either antigen or antibody is radio labelled. Radiolabelling must not interfere in the binding of antigen & antibody has to be compared with unlabelled ones.

Pharmacological studies: -

Radioisotopes are widely used in development of new drugs radioisotopes are used in study of site of drug accumulation end product of drug metabolism etc.

Therapy and Diagnosis: -

There are nearly one hundred radioisotopes whose radiation is used in diagnosis, therapy, or investigations in nuclear medicine.

It is used for brain bone, liver, spleen, kidney, lung and thyroid imaging.

1311, is used to diagnose and treat thyroid disorders.

A very effective role for radioisotopes such as 11C, 13N, 15O, or 18F in a process known as

Positron Emission Tomography (PET).

Positron emission tomography (PET) is a imaging technique that produces a three-dimensional

image of functional processes in the body.

The system detects [camera] rays emitted indirectly by a positron-emitting radionuclide

(tracer), which is introduced into the body on a biologically active molecule.

PET's most important clinical role is in oncology, with fluorine-18 as the tracer, since it has

proven to be the most accurate method of detecting most cancers. It is also well used in cardiac and brain imaging.

Radio isotopes have been particularly useful in treating cancers.

Rapidly dividing cells are particularly sensitive to damage by radiation. For this reason, cancerous growths can be controlled or eliminated by irradiating the area containing the growth.

Radiation therapy uses high-energy radiation to kill cancer cells. The radiation may be delivered by a machine outside the body (external-beam radiation therapy), or it may come from radioactive material placed in the body near cancer cells (internal radiation therapy).

Internal radionuclide therapy is by administering or planting a small radiation source, usually

a gamma or beta emitter, in the target area. This is becoming the main means of treatment.

Iodine-131 is commonly used to treat thyroid cancer. Iridium-192 implants are used especially in the head and breast.

They are produced in wire from and are introduced into a body to the target area. After administering the correct dose, the implant wire is removed. This internal radiation procedure gives less overall radiation to the body, is more localised to the target tumor.

Radiation therapy kills cancer cells by damaging their DNA. Radiation therapy can either damage DNA directly or create free radicals within the cells that can in turn damage the DNA.

Cancer cells whose DNA is damaged beyond repair stop dividing or die. When the damaged cells die, they are broken down and eliminated by the body's natural processes. Radiation therapy can also damage normal cells, leading to side effects.

Ecological studies: -

Radio isotopes are also useful for ecologist Migratory pattern. Like behaviour pattern of many animals can be monitored using radio tracers.

Pesticide levels: -

To measure pesticide levels, a pesticide can be tagged with a radioisotope such as chlorine-36, and this is applied to a field of test plants.

Over a period of time, radioactivity measurements are made. Estimates can then be made about how much accumulates in the soil, how much is taken up by the plant and how much is carried