MUTHAYAMMAL ENGINEERING COLLEGE

(An Autonomous Institution)

(Approved by AICTE, New Delhi, Accredited by NAAC & Affiliated to Anna University)

Rasipuram - 637 408, Namakkal Dist., Tamil Nadu

LECTURE HANDOUTS

BIOTECH | **III/VI**

Course Name with Code : **BIOLOGICAL SPECTROSCOPY 16BTE15**

Course Faculty : **Dr. G. Pratap Kumar**

Unit : **I** **Date of Lecture:**

Topic of Lecture: Optical Rotatory Dispersion - Introduction

Introduction :

- ORD is the production of colors that results from passing white light through an optically active substance (quartz) that causes the amount of optical rotation to vary with the wavelength.
- Shorter wavelengths are rotated more than longer wavelengths per unit of distance. This dependence of specific rotation on wavelength is called as optical rotatory dispersion.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on knowing the concept of ORD.
- Prerequisite knowledge on understanding the polarization of light on sample.

Detailed content of the Lecture:

- Optical rotatory dispersion (ORD) is the variation in the optical rotation of a substance with a change in the wavelength of light
- ORD can be used to find the absolute configuration of metal complexes.
- Example, when plane-polarized white light from an overhead projector is passed through a cylinder of sucrose solution, a spiral rainbow is observed perpendicular to the cylinder.

Video Content / Details of website for further learning (if any):

Important Books/Journals for further learning including the page nos.:

Banwell, Colin N. and E.M. McCash, "Fundamentals of Molecular Spectroscopy" Tata McGraw-Hill, 2007 (Pg. No. 3-5).

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LECTURE HANDOUTS

BIOTECH | **III/VI**

Course Name with Code : **BIOLOGICAL SPECTROSCOPY 16BTE15**

Course Faculty : **Dr. G. Pratap Kumar**

Unit : **I** **Date of Lecture:**

Topic of Lecture: Polarized light

Introduction :

- Polarized light is illustrated that a non-polarized beam of light incident on two linear polarizers.
- Electric field vectors are depicted in the incident light beam as sinusoidal waves vibrating in all directions.
- In reality, the incident light electric field vectors are vibrating perpendicular to the direction of propagation with an equal distribution in all planes before encountering the first polarizer.
- Polarized light can be produced from the common physical processes that deviate light beams, including absorption, refraction, reflection, diffraction and the process known as birefringence.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on understanding the basic information of polarized light.
- Prerequisite knowledge on knowing the term 'polarization' and it's types.
- Prerequisite knowledge on learning the importance of light waves with respect to Instrumental methods of analysis.

Detailed content of the Lecture:

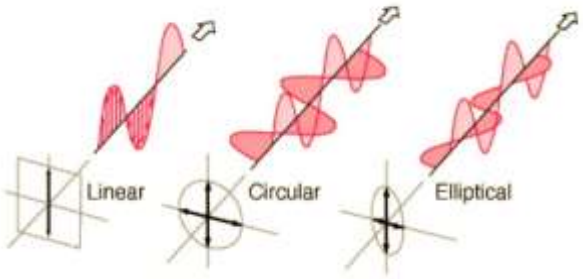
- A light wave is an electromagnetic wave that travels through the vacuum of outer space.
- A light wave that is vibrating in more than one plane is referred to as unpolarized light.
- Polarized light waves are light waves in which the vibrations occur in a single plane. Thus, the process of transforming unpolarized light into polarized light is known as 'polarization'.

Types of polarized light:

a) **Liner or plane polarized light** - Vibrating in a single plane perpendicular to the direction of propagation is called 'plane polarised light'.

b) **Circular polarized light** - When vibration of light are along a circle lying in a plane perpendicular to the direction of propagation the light is called 'circular polarized light'.

c) Elliptical polarized light - When vibration are along a ellipse lying in a plane perpendicular to the direction of propagation the light is called 'elliptically polarized light'.



Types of polarized light

Video Content / Details of website for further learning (if any):

Important Books/Journals for further learning including the page nos.:

Banwell, Colin N. and E.M. McCash, "Fundamentals of Molecular Spectroscopy" Tata McGraw-Hill, 2007 (Pg. No. 5-11).

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LECTURE HANDOUTS

BIOTECH

III/VI

Course Name with Code : **BIOLOGICAL SPECTROSCOPY 16BTE15**

Course Faculty : **Dr. G. Pratap Kumar**

Unit : **I** **Date of Lecture:**

Topic of Lecture: Instrumentation of polarimeter

Introduction :

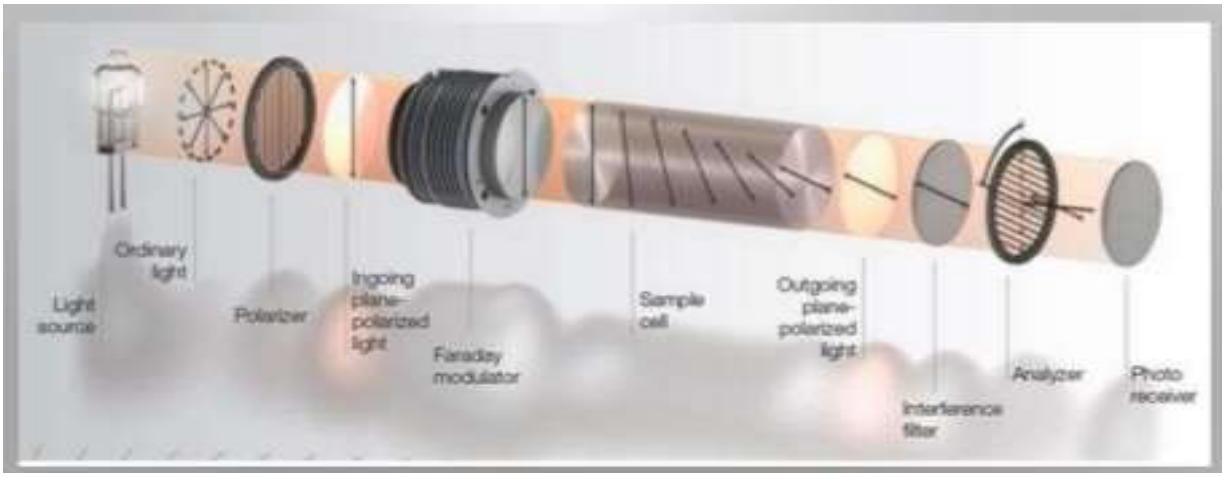
- Polarimetry is an instrument analytical method using rotation of polarized light by some substances as a measure of their concentration in a solution.
- When it's adapted for measuring quality of sugar the name saccharimeter is used. In both instruments it is the rotation of polarized light by a substance in a solution which is measured.
- Usually, it is only one instrument which has two interchangeable scales, one labelled in angular degrees °, the other in units °Z, named International Sugar Scale (I. S. S).

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge for understanding the working and principle behind polarimetry.
- Prerequisite knowledge for learning the basics of polarimeter.

Detailed content of the Lecture:

INSTRUMENTATION:



WORKING:

- Normal monochromatic light contains light that possesses oscillations of the electrical field in all possible planes perpendicular to the direction of propagation.
- When light is passed through a polarizer (i.e., Nicol prism, Polaroid film) only light oscillating in one plane will leave the polarizer ("picket fence model").
- This linear polarized light can be described as a superposition of two counter-rotating components, which propagate with different velocities in an optical active medium. If one

component interacts stronger than the other with a chiral molecule, it will slow down and therefore arrive later at the observer.

- The result is that the plane of the light appears to be rotated because the two vectors are not canceling each other anymore due to the phase shift.

Video Content / Details of website for further learning (if any):
https://www.chem.ucla.edu/~bacher/General/30BL/tips/Polarimetry.html

Important Books/Journals for further learning including the page nos.:
Banwell, Colin N. and E.M. McCash, "Fundamentals of Molecular Spectroscopy" Tata McGraw-Hill, 2007 (Pg. No. 15-25).

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LECTURE HANDOUTS

BIOTECH | **III/VI**

Course Name with Code : **BIOLOGICAL SPECTROSCOPY 16BTE15**

Course Faculty : **Dr. G. Pratap Kumar**

Unit : **I** **Date of Lecture:**

Topic of Lecture: Optical rotation

Introduction :

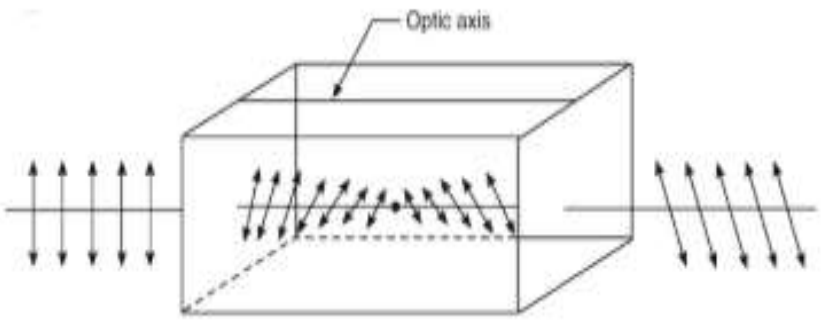
- Optical rotation, also known as polarization rotation or circular birefringence, is the rotation of the orientation of the plane of polarization about the optical axis of linearly polarized light as it travels through certain materials.
- Optical activity occurs only in chiral materials, those lacking microscopic mirror symmetry. Unlike other sources of birefringence which alter a beam's state of polarization, optical activity can be observed in fluids.
- This can include gases or solutions of chiral molecules such as sugars, molecules with helical secondary structure such as some proteins, and also chiral liquid crystals.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on understanding the concepts of optical activity and optical rotation.
- Prerequisite knowledge on learning the importance of optical rotation.

Detailed content of the Lecture:

- The angle through which the plane of polarization is rotated when polarized light passes through a layer of liquid.
- The ability to rotate the plane of polarization of plane-polarized light by a certain substance is called optical activity.
- Quartz and cinnabar are examples of optically active crystals while aqueous solutions of sugar, tartaric acid are optically active solutions.



Optical rotation

Optically active substances are classified into two types.

a. Dextrorotatory substances – Substances that rotate the plane of polarization of the light towards the right are known as right-handed.

b. Laevorotatory substances – Substances which rotate the plane of polarization of the light toward the left are known as left-handed.
Video Content / Details of website for further learning (if any):
Important Books/Journals for further learning including the page nos.: Banwell, Colin N. and E.M. McCash, "Fundamentals of Molecular Spectroscopy" Tata McGraw-Hill, 2007 (Pg. No. 25-27).

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LECTURE HANDOUTS

BIOTECH

III/VI

Course Name with Code : **BIOLOGICAL SPECTROSCOPY 16BTE15**

Course Faculty : **Dr. G. Pratap Kumar**

Unit : **I** **Date of Lecture:**

Topic of Lecture: Instrumentation of ORD

Introduction :

- ORD is the variation in the optical rotation of a substance with a change in the wavelength of light.
- Short wavelengths are rotated more than longer wavelengths, per unit of distance. Because the wavelength of light determines its color with distance through the tube is observed. This dependence of specific rotation on wavelength is called optical rotatory dispersion.
- The speed of the circularly polarized components are retarded by an optically active substance to different extent resulting in the rotation of plane of polarization.

Prerequisite knowledge for Complete understanding and learning of Topic:

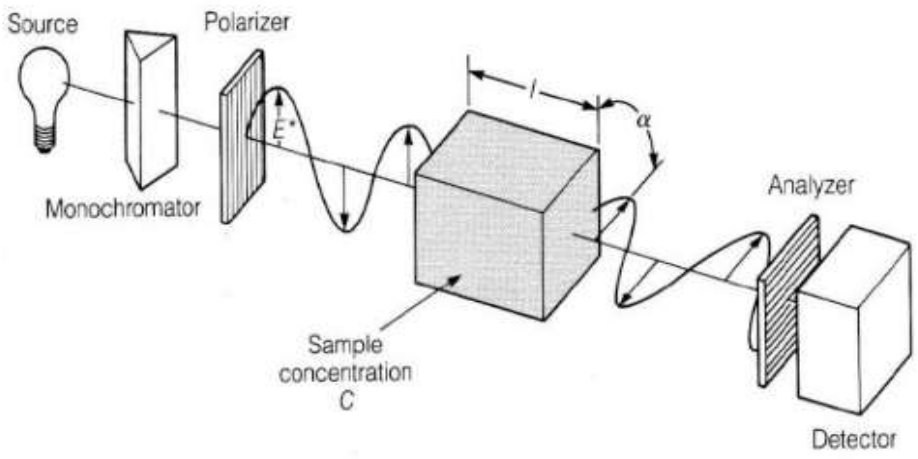
- Prerequisite knowledge on understanding the working of ORD.
- Prerequisite knowledge on how the concentration of substance is measured when polarized light passes.

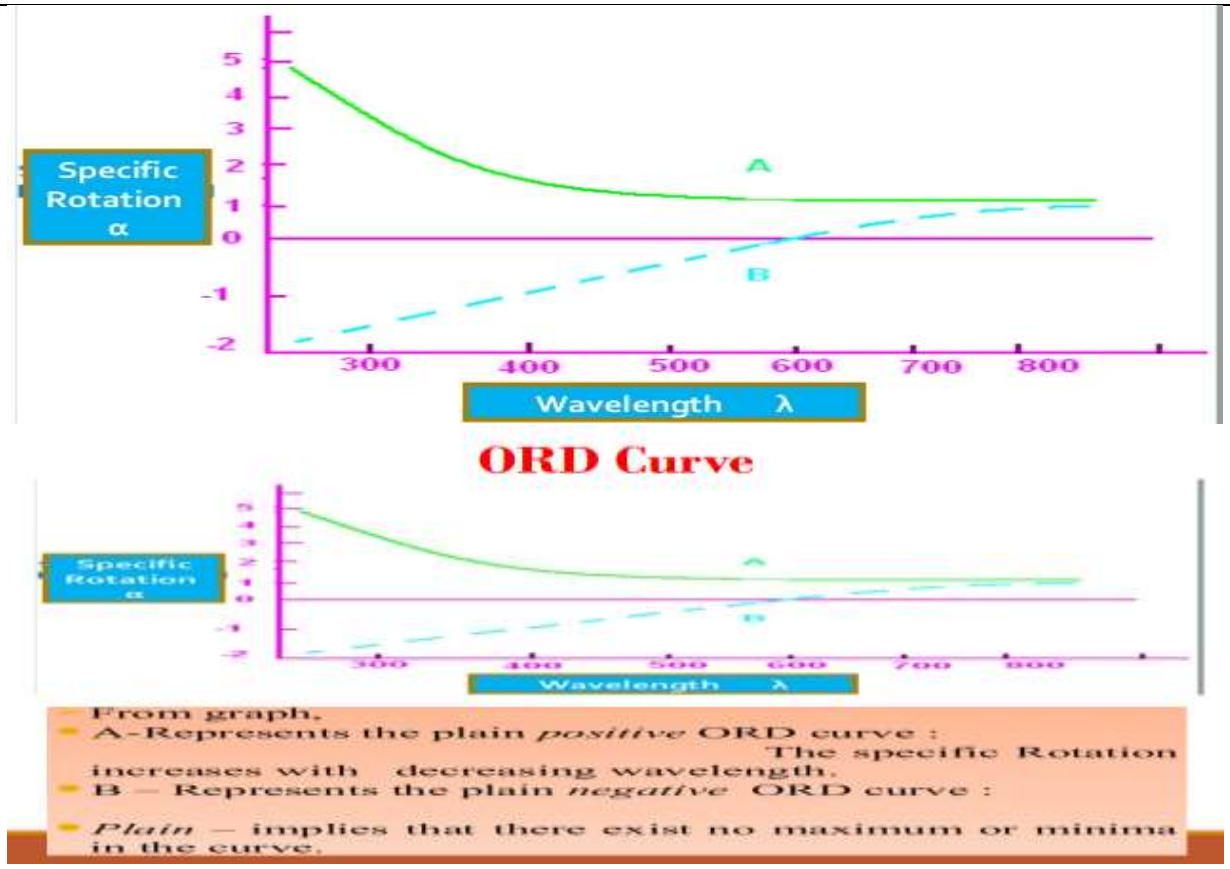
Detailed content of the Lecture:

PRINCIPLE:

- When the white light passes through a polarizer the extent of rotation of light depends on its wavelength.
- This dependence of specific rotation on wavelength is called ORD.

INSTRUMENTATION:





Video Content / Details of website for further learning (if any):

Important Books/Journals for further learning including the page nos.:

Banwell, Colin N. and E.M. McCash, "Fundamentals of Molecular Spectroscopy" Tata McGraw-Hill, 2007 (Pg. No. 30-33).

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BIOTECH | **III/VI**

Course Name with Code : **BIOLOGICAL SPECTROSCOPY 16BTE15**

Course Faculty : **Dr. G. Pratap Kumar**

Unit : **I** **Date of Lecture:**

Topic of Lecture: Circular dichroism spectroscopy (CD)

Introduction :

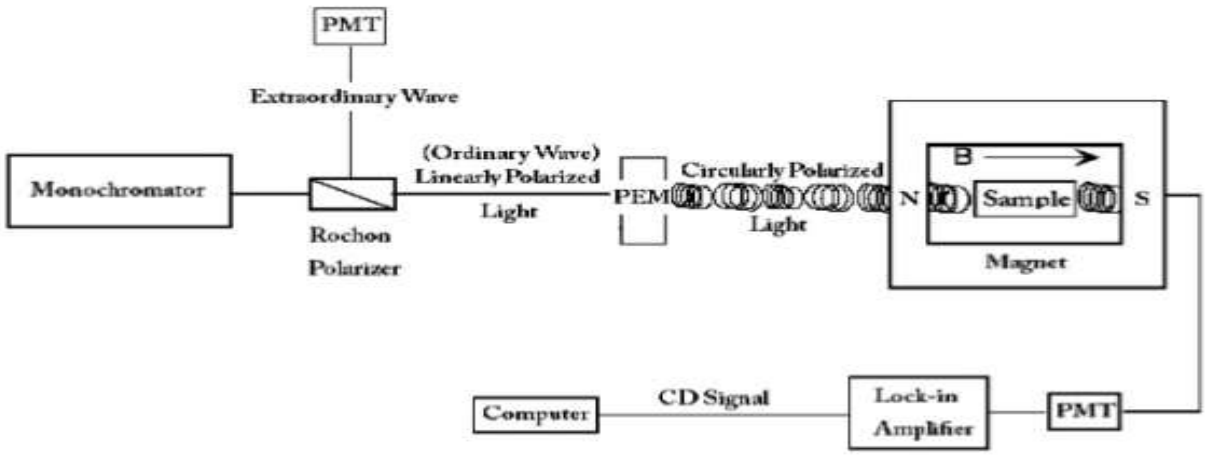
- It is used for study of biomolecules, their structure and interaction with metals and other molecules.
- Involves measurement of how an optically active compound absorbs circular polarized light both left and right handed.
- The instrument needs to be able to measure accurately in the far UV at wavelengths down to 190 to 170 nm. In addition, the difference in left and right handed absorbance A(l)-A(r) is very small, therefore should be sensitive. The CD is a function of wavelength.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on the working and components of CD spectroscopy.
- Prerequisite knowledge on the measurement of compounds for studying the interaction.

Detailed content of the Lecture:

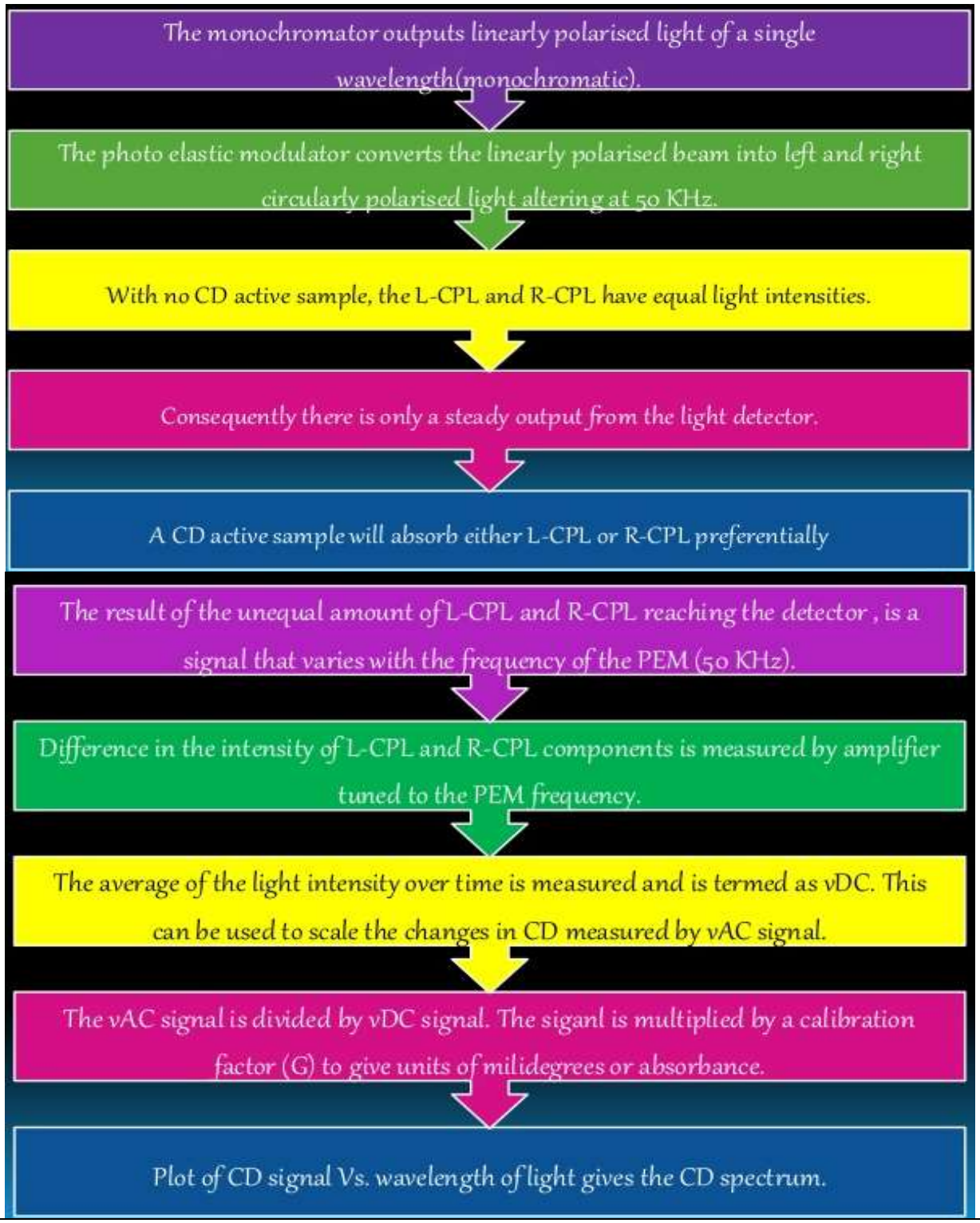
INSTRUMENTATION:



COMPONENTS:

- Lamp – Xe/Hg
- Monochromator
- Polarizaer
- Photo-elastic modulator
- Sample compartment
- Detector

WORKING:



Video Content / Details of website for further learning (if any):

Important Books/Journals for further learning including the page nos.:

Banwell, Colin N. and E.M. McCash, "Fundamentals of Molecular Spectroscopy" Tata McGraw-Hill, 2007 (Pg. No. 37-45).

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LECTURE HANDOUTS

BIOTECH | **III/VI**

Course Name with Code : **BIOLOGICAL SPECTROSCOPY 16BTE15**

Course Faculty : **Dr. G. Pratap Kumar**

Unit : **I** **Date of Lecture:**

Topic of Lecture: Circular dichroism of nucleic acids

Introduction :

- Estimation of nucleic acid conformation using Circular dichroism spectrophotometer for application purposes.
- Determination of the thermodynamics o folding and unfolding of nucleic acids.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on role of CD in analyzing nucleic acids (DNA).
- Prerequisite knowledge on applications of CD with interaction of nucleic DNA.

Detailed content of the Lecture:

Application of CD to Nucleic acids:

The major application of CD to the study of nucleic acids is to determine the degree of base stacking. The CD of a dimer is very dependent on the interaction of the monomers. For example: poly C has the following spectral properties:

Solvent	Ellipticity	A_{260}
Water	35,000	1.0
Ethylene glycol	7,000	1.3

In this case both the CD and the hyper-chromicity show that polyC is a helix in water and that this helix is due to base stacking.

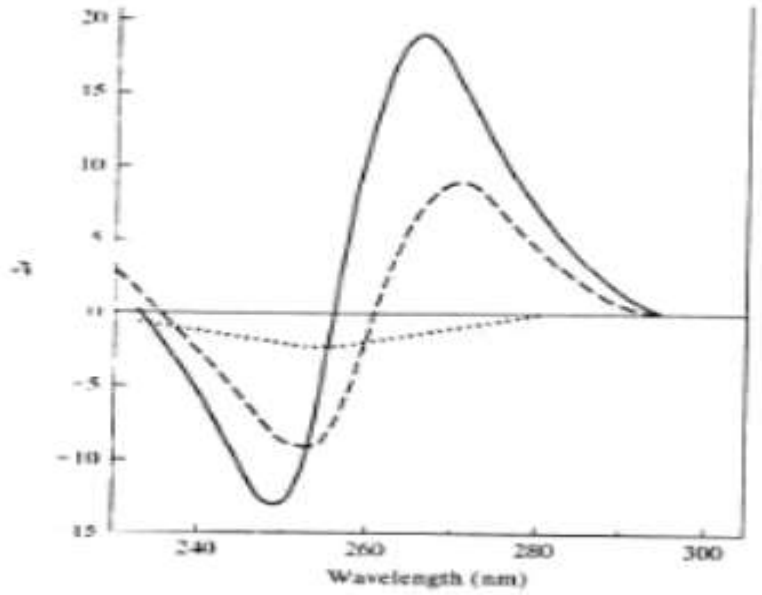

Figure 10.9 The circular dichroism of single stranded oligo(rA) in aqueous solution at pH 7: polymer (———); dimer (– – – – –); monomer (· · ·). [Adapted from data in K. E. van Holde, J. Brahms, and A. M. Michelson (1965) *J. Mol. Biol.* **12**, 726–739.]

Video Content / Details of website for further learning (if any):

Important Books/Journals for further learning including the page nos.:

Banwell, Colin N. and E.M. McCash, "Fundamentals of Molecular Spectroscopy" Tata McGraw-Hill, 2007 (Pg. No. 50-55).

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LECTURE HANDOUTS

BIOTECH

III/VI

Course Name with Code : **BIOLOGICAL SPECTROSCOPY 16BTE15**

Course Faculty : **Dr. G. Pratap Kumar**

Unit : **I** **Date of Lecture:**

Topic of Lecture: Circular dichroism of proteins

Introduction :

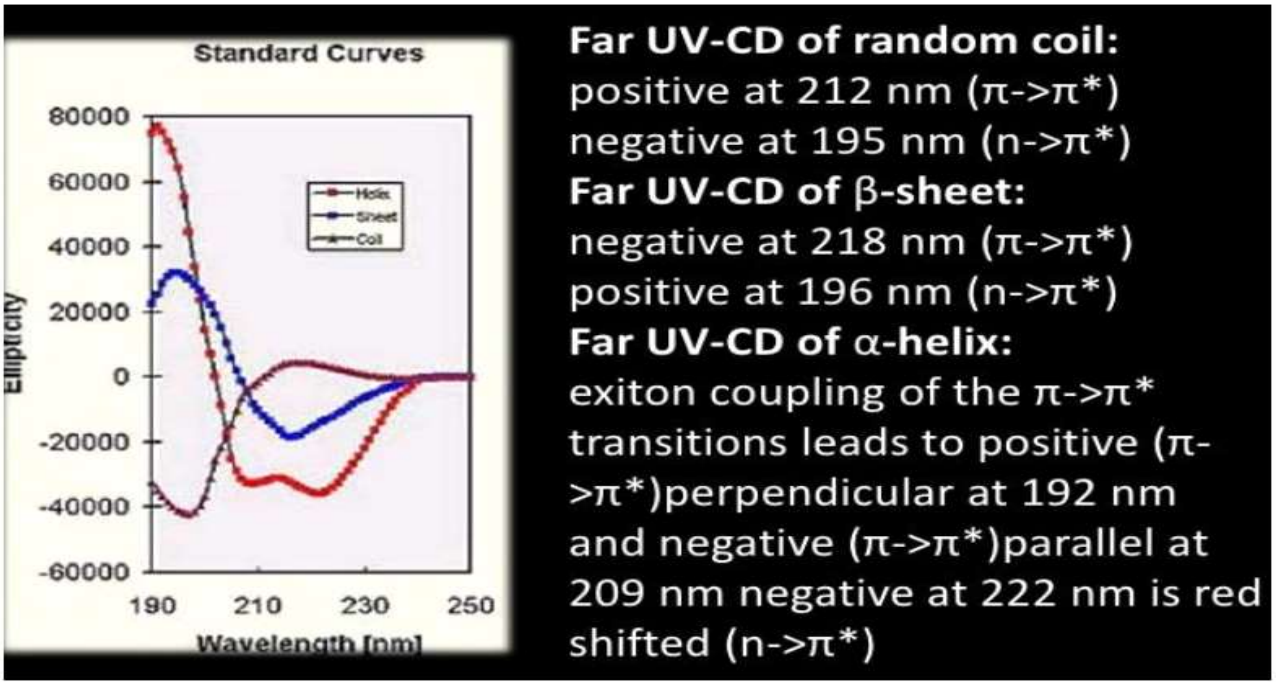
- Determination of conformational changes due to the interaction of asymmetric molecules such as; protein-protein interactions, protein-DNA interactions, Protein-Ligand interactions, DNA-ligand interactions.
- CD bands in the near UV region (260-350 nm) are observed in a folded protein where aromatic side chains are immobilized in an asymmetric environment.
- The CD of aromatic residues is very small in the absence of ordered structure (For ex: short peptides).

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on role of CD on protein analysis.
- Prerequisite knowledge on applications of CD to view the protein sample interaction.

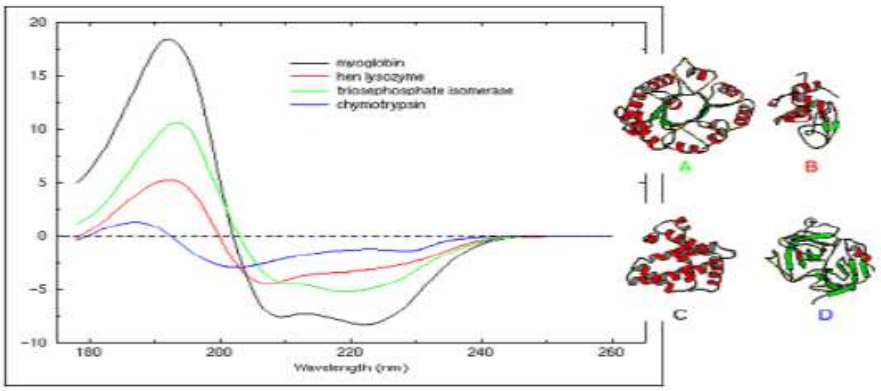
Detailed content of the Lecture:

It has been shown that CD spectra between 260 and approximately 180 nm can be analyzed for the different secondary structural types: alpha helix, parallel and anti-parallel beta sheets, turns, and other.



• A number of excellent review articles are available describing the technique and its application. Modern secondary structure determination by CD are reported to achieve accuracies of 0.97 for helices, 0.75 for beta sheet, 0.50 for turns, and 0.89 for other structure types. For proteins we will be mainly concerned with absorption in the ultraviolet region of the spectrum from the peptide bonds (symmetric chromophores) and amino acid sidechains in proteins.

Proteins with different compositions of 2° structure give different CD spectra



• Protein chromophores can be divided into three classes: the peptide bond, the amino acid sidechains, and any prosthetic groups.

Video Content / Details of website for further learning (if any):

Important Books/Journals for further learning including the page nos.:

Banwell, Colin N. and E.M. McCash, "Fundamentals of Molecular Spectroscopy" Tata McGraw-Hill, 2007 (Pg. No. 55-65).

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Course Faculty : **Dr. G. Pratap Kumar**

Unit : **I** **Date of Lecture:**

Topic of Lecture: Applications of CD

Introduction :

- Circular dichroism has applications in variety of modern research fields ranging from biochemistry to inorganic chemistry.
- Far UV and Near-UV CD spectrum allow the correct folding and conformations to be verified which can help to determine structural modifications during formulation, processing, release, administration and monitor protein sample impurities.
- A powerful application of CD is to compare two macromolecules or the same molecule under different conditions and determine if they have a similar structure.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on the advantages of CD.
- Prerequisite knowledge on different areas of applications of CD.

Detailed content of the Lecture:

- CD is a particularly powerful tool to follow dynamic changes in protein structure which may result due to the effect of changing temperature, pH, ligands or denaturants etc.
- CD can be used to follow the kinetics of refolding of the secondary structure of a protein using changes in denaturant concentration.
- It can also be used to follow the unfolding of proteins by thermal denaturation.
- Determination of secondary structure of proteins that cannot be crystallised. Investigation of the effect of e.g. drug binding on protein secondary structure.
- Secondary structure and super-secondary structure of membrane proteins. Study of ligand-induced conformational changes, carbohydrate conformation.

Video Content / Details of website for further learning (if any):

Important Books/Journals for further learning including the page nos.:

Banwell, Colin N. and E.M. McCash, "Fundamentals of Molecular Spectroscopy" Tata McGraw-Hill, 2007 (Pg. No. 66-67).

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LECTURE HANDOUTS

BIOTECH | **III/VI**

Course Name with Code : **BIOLOGICAL SPECTROSCOPY 16BTE15**

Course Faculty : **Dr. G. Pratap Kumar**

Unit : **II** **Date of Lecture:**

Topic of Lecture: Chemical shift

Introduction :

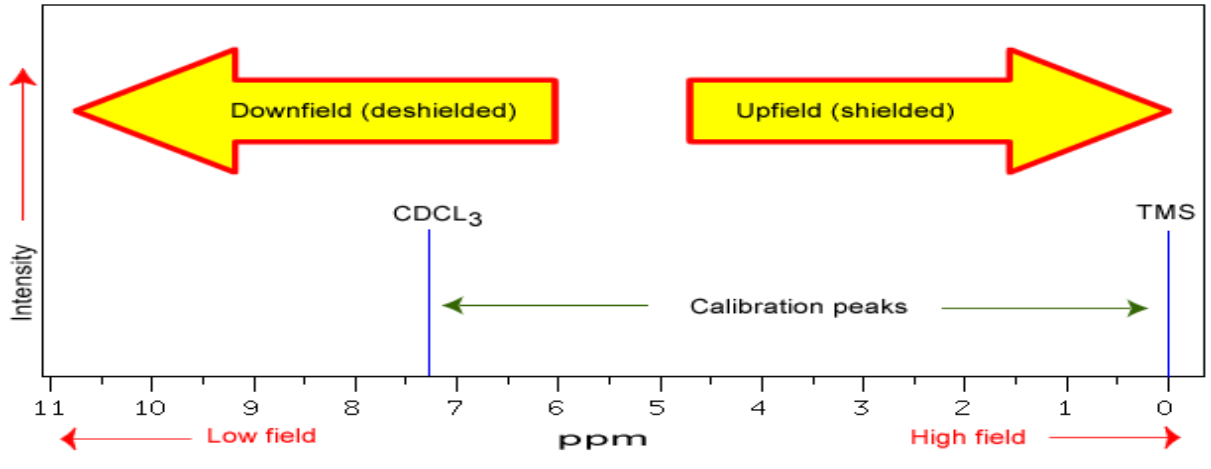
- In NMR spectroscopy, the chemical shift is the resonant frequency of a nucleus relative to a standard in a magnetic field.
- The variations of nuclear magnetic resonance frequencies of the same kind of nucleus due to variations in the electron distribution is called chemical shift.
- It's used to describe signals in other forms of spectroscopy such as photoemission spectroscopy. Some atomic nuclei possess a magnetic moment (nuclear spin) which gives rise to different energy levels and resonance frequencies in a magnetic field.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on electromagnetic radiations.
- Prerequisite knowledge on describing signals in other forms of spectroscopy.
- Prerequisite knowledge on calculations in NMR using chemical shift.

Detailed content of the Lecture:

- The NMR spectra is displayed as a plot of the applied radio frequency versus the absorption.
- The applied frequency increases from left to right, thus the left side of the plot is the low field, downfield or deshielded side and the right side of the plot is the high field, upfield or shielded side.
- The size of the chemical shift is given with respect to a reference frequency or reference sample, usually a molecule with a barely distorted electron distribution.



- The position on the plot at which the nuclei absorbs is called the chemical shift.
- The two most common standards are TMS (tetramethylsilane, (Si(CH3)4) which has been assigned a chemical shift of zero, and CDCl3 (deuterochloroform) which has a chemical shift of 7.26 for 1H NMR and 77 for 13C NMR.
- The scale is commonly expressed as parts per million (ppm) which is independent of the spectrometer frequency. The scale is the **delta (δ) scale**.

$$\delta = \frac{\text{frequency of signal} - \text{frequency of standard}}{\text{spectrometer frequency}} \times 10^6$$

- The range at which most NMR absorptions occur is quite narrow. Almost all 1H absorptions occur downfield within 10 ppm of TMS. For 13C NMR almost all absorptions occurs within 220 ppm downfield of the C atom in TMS.

Video Content / Details of website for further learning (if any):

Important Books/Journals for further learning including the page nos.:

Banwell, Colin N. and E.M. McCash, "Fundamentals of Molecular Spectroscopy" Tata McGraw-Hill, 2007 (Pg. No. 75-79).

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BIOTECH | **III/VI**

Course Name with Code : **BIOLOGICAL SPECTROSCOPY 16BTE15**

Course Faculty : **Dr. G. Pratap Kumar**

Unit : **II** **Date of Lecture:**

Topic of Lecture: Spin-spin coupling

Introduction :

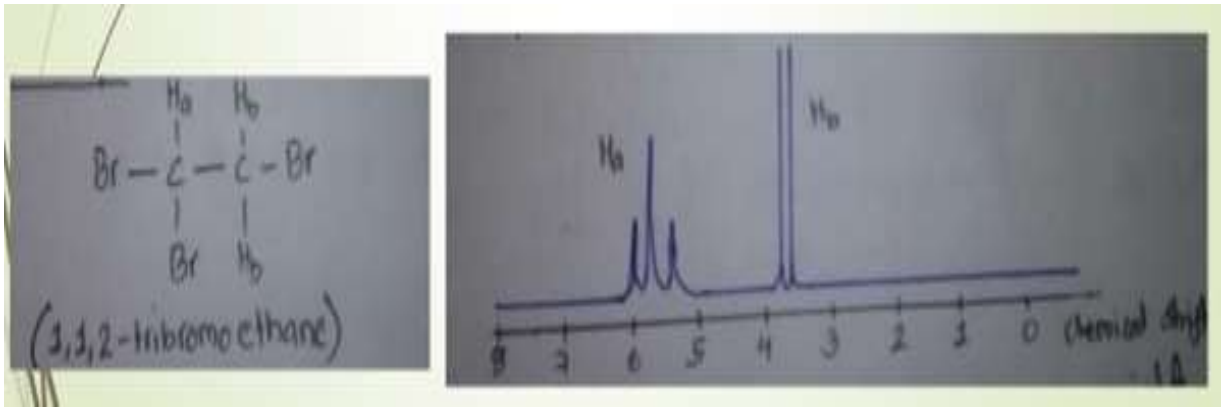
- The interaction between the spin magnetic moments of the different sets of H atoms in the molecule is known as spin-spin coupling.
- It's imperative that a minimum of 2 sets of protons are present in adjacent positions.
- The magnetic spins of these resonating nuclei interact with each other and affect each other's precession frequencies.
- The effective magnetic field (B_{eff}) experienced by neighboring protons as a result of magnetic spins thereby affect the chemical shift values.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on analysis of NMR spectrum through spin-spin coupling.
- Prerequisite knowledge on understanding the importance of magnetic spin in NMR spectroscopy.

Detailed content of the Lecture:

- The magnetic interaction between the spins of neighbouring, non-equivalent NMR-active nuclei may cause splitting of NMR spectrum which is known as spin-spin coupling.
- The splitting pattern is related to the number of equivalent H-atom at the nearby nuclei.
- Example: 1,1,2-tribromoethane.



- By examining the structure of 1,1,2-tribromoethane, we might expect only two single peaks that correspond to two different types of hydrogen. However, what we see is slightly different. Instead of two singlet peaks, each peak consists of multiple lines.

- The signals for Hb consists of a doublet that for Ha consists of a triplet. The splitting of the peaks into multiple peaks is called spin-spin coupling which is the direct interaction between the neighboring hydrogen nuclei.
- The chemical shift of Ha is affected both by it's own density and also by neighboring hydrogen nuclei.
- Each one of nuclei can spin either one of two ways: spin up (+1/2) or spin down (-1/2). Since there are two Hb nuclei, there are 4 possible spin combination around Ha atom. (↑↑), (↑↓), (↓↑) or (↓↓).
- Net magnetic field of Ha hydrogen can be modified by each one of the different combination. Two identical combination sums up to give higher intensity.

Video Content / Details of website for further learning (if any):

Important Books/Journals for further learning including the page nos.:

Banwell, Colin N. and E.M. McCash, "Fundamentals of Molecular Spectroscopy" Tata McGraw-Hill, 2007 (Pg. No. 83-85).

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III/VI

Course Name with Code : **BIOLOGICAL SPECTROSCOPY 16BTE15**

Course Faculty : **Dr. G. Pratap Kumar**

Unit : **II** **Date of Lecture:**

Topic of Lecture: Relaxation mechanisms

Introduction :

- Spin rotation (SR) relaxation mechanism arises from an interaction between the nuclear spin and a coupling to the overall molecular rotational angular momentum.
- The fluctuations produce transitions between the nuclear spin states in a similar manner to the magnetic dipole-dipole interaction.
- There are two mechanisms involved: spin-lattice and spin-spin.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on NMR spectroscopy for molecule analysis of sample.
- Prerequisite knowledge on different types of mechanisms of relations in NMR.

Detailed content of the Lecture:

- Relaxation describes how a spin returns to equilibrium.
- The distribution of spins follows the Boltzmann Distribution:

$$\frac{n_i}{n} = \frac{g_i e^{-E_i / k_B T}}{Z(t)}$$

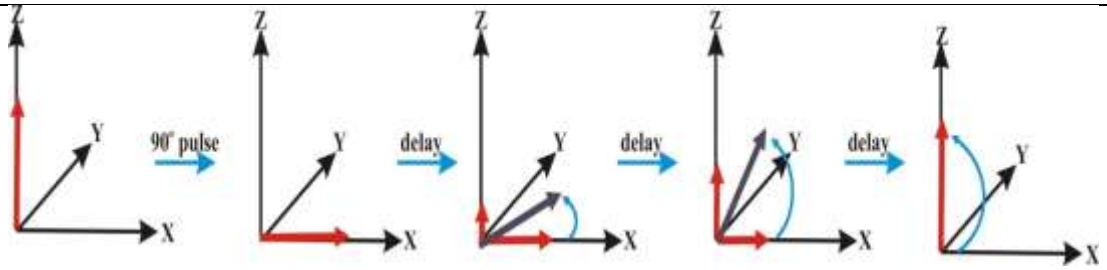
- There is no transverse magnetization.
- There is no phase coherence.

Two types of relaxation:

a. **Longitudinal relaxation:** along the axis of the external magnetic field (spin-lattice)
b. **Transverse relaxation:** perpendicular to the external magnetic field (spin-spin)

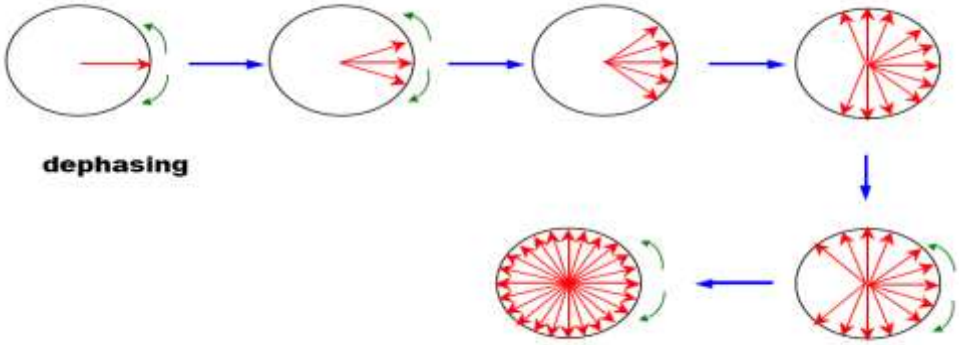
a. Longitudinal relaxation:

- Relaxation process occurs along z-axis
- transfer of energy to the lattice or solvent material
- coupling of nuclei magnetic field with magnetic fields created by the ensemble of vibrational and rotational motion of the lattice or solvent.
- results in a minimal temperature increase in sample
- Relaxation time (T_1) → exponential decay



b. Transverse relaxation:

- exchange of energy between excited nucleus and low energy state nucleus
- randomization of spins or magnetic moment in x,y-plane
- related to NMR peak line-width
- relaxation time (T_2)



Video Content / Details of website for further learning (if any):

Important Books/Journals for further learning including the page nos.:

Banwell, Colin N. and E.M. McCash, "Fundamentals of Molecular Spectroscopy" Tata McGraw-Hill, 2007 (Pg. No. 90-92).

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LECTURE HANDOUTS

BIOTECH | **III/VI**

Course Name with Code : **BIOLOGICAL SPECTROSCOPY 16BTE15**

Course Faculty : **Dr. G. Pratap Kumar**

Unit : **II** **Date of Lecture:**

Topic of Lecture: Nuclear overhauser effect (NOE)

Introduction :

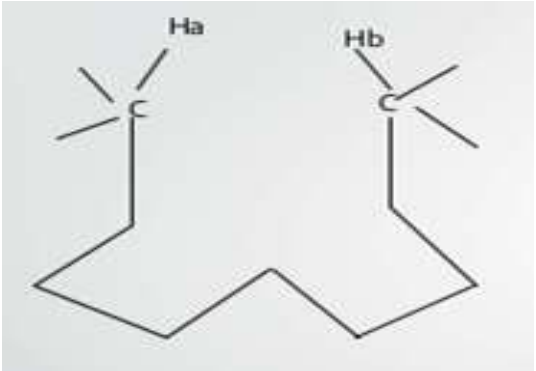
- NOE is the resonance line intensity changes caused by dipolar cross relaxation from neighboring spins with perturbed energy level populations.
- To understand the nature of the NOE, we have to look at a two-spin system I^1 and I^2.
- Since NOE does not involves coherences, but merely polarization, i.e. population differences between the a and b states, we can use the energy level diagram here.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on understanding the importance of NOE.
- Prerequisite knowledge on knowing the mechanism of NOE in NMR.

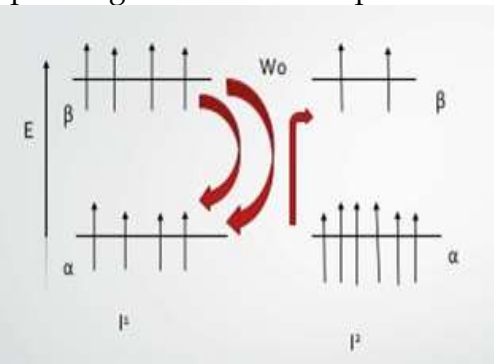
Detailed content of the Lecture:

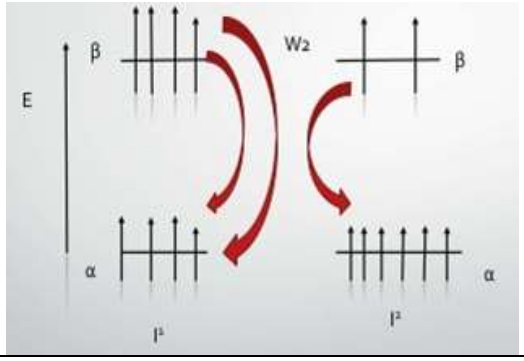
- The nuclear overhauser effect is of great value in studying the molecular geometry of the compounds. It tells whether the two protons are in close proximity within the molecules or not.
- An important consequence of this effect is that the line intensities observed in the normal spectrum may not be the same as in the decoupled spectrum.
- Consider a molecule in which two protons are close enough to allow through space interactions of the fluctuating magnetic vector for this effect, the number of intervening bonds between the two concerned protons have no significance.
- Consider a hypothetical molecule in which two protons are in close proximity. In such a compound if we double irradiate Hb then this proton gets stimulated and the stimulation is transferred through space to the relaxation mechanism of Ha.
- Thus due to the increase in the spin lattice relaxation of Ha, its signal will appear more intense by 15-50%. Thus we say that if the intensity of absorption of Ha signal is increased by double irradiating Hb then the protons Ha and Hb must be in close proximity in a molecule.



- The possible transitions for this two-spin system can be classified into three groups:

a. W1 transitions involving a spin flip of only one of the two spins (either I1 or I2), corresponding to relaxation of the spin.
b. a W0 transition involving a simultaneous spin flip $\alpha \rightarrow \beta$ for one spin and $\beta \rightarrow \alpha$ for the other one (i.e., in summa a zero-quantum transition).
c. a W2 transition involving a simultaneous spin flip of both spins in the same direction, corresponding to a net double-quantum transition.





Video Content / Details of website for further learning (if any):

Important Books/Journals for further learning including the page nos.:

Banwell, Colin N. and E.M. McCash, "Fundamentals of Molecular Spectroscopy" Tata McGraw-Hill, 2007 (Pg. No. 91-95).

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LECTURE HANDOUTS

BIOTECH

III/VI

Course Name with Code : **BIOLOGICAL SPECTROSCOPY 16BTE15**

Course Faculty : **Dr. G. Pratap Kumar**

Unit : **II** **Date of Lecture:**

Topic of Lecture: ESR - Instrumentation

Introduction :

- ESR is a method for observing the behavior of the electrons within a suitable molecule and for analyzing various phenomena by identifying the electron environment.
- ESR measurements afford information about the existence of unpaired electrons as well as quantities, type, nature, environment and behavior.
- ESR instruments provide the only means of selectively measuring free radicals non-destructively and in any sample phase (gas, liquid or solid).

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on understanding the working and components of ESR.
- Prerequisite knowledge on learning the principle and importance behind ESR.

Detailed content of the Lecture:

- It is a branch of absorption spectroscopy in which radiation having frequency in microwave region.
- Electron spin resonance (ESR) is also known as Electron Paramagnetic Resonance (EPR). This is a technique for detecting paramagnetism.
- The technique may be used for detecting transitional metal ion and their complexes, free radicals and their excited states.
- ESR Phenomenon is shown by:

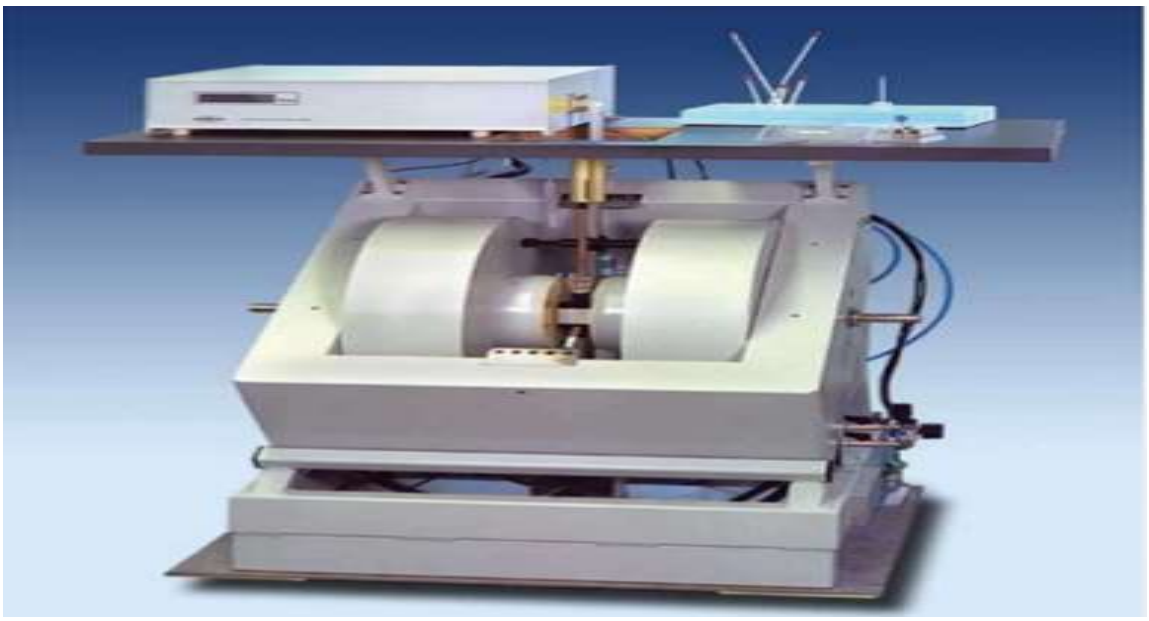
a) Atoms having odd number of electrons.
b) Ions having partly filled inner electron shells
c) Free radicals having unpaired electrons

- The unpaired electrons are excited to a high energy state under the magnetic field by the absorption of microwave radiations. The excited electron changes its direction of spin and relaxes in to the ground state by emitting its energy.
- The transition between two different energy levels takes place by absorbing a quantum of radiation of frequency in the microwave region. Microwave absorption is measured as a function of the magnetic field by ESR Spectroscopy.
- In ESR the energy levels are produced by the interaction of magnetic moment of an unpaired electron in a molecule with an applied magnetic field. The ESR spectrum results in due to the transitions between these energy levels by absorbing radiations of microwave frequency.

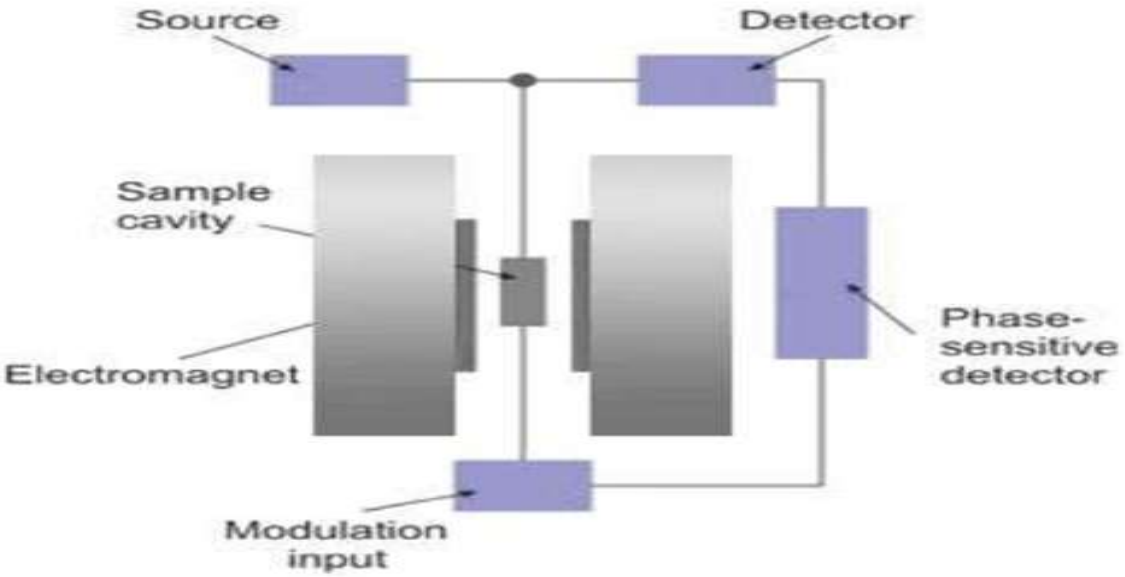
INSTRUMENTATION:

- ❑ Source
- ❑ Sample Cavity

- Magnet System
- Crystal Detector
- Auto amplifier and Phase sensitive Detector
- Oscilloscope



- **Klystron Source**. It is a vacuum tube which can produce microwave oscillations centered on a small range of frequency. The frequency of the monochromatic radiation is determined by the voltage applied to Klystron.
- **Isolator:** It is a device which minimizes vibrations in the frequency of microwaves produced by Klystron oscillator. Isolator is a strip of ferrite material.
- **Wave meter:** It is fixed in between the isolator and attenuator to know the frequency of microwaves produced by Klystron oscillator.
- **Attenuator:** Attenuator is used to adjust the level of the microwave power incident upon the sample.



- **Sample Cavity:** This resonant cavity which contains the sample is called the heart of ESR.
- **Magnet System:**

• The sample cavity is placed between the pole pieces of an electromagnet.
• This provides a homogenous magnetic field and can be varied from zero to 500 gauss.

- **Crystal Detectors:**

• The most commonly used detector is a silicon crystal which acts as a microwave rectifier.
• This converts microwave power into a direct current input.

- **Oscilloscope:**
  - The signal from phase sensitive detector and sweep unit is recorded by the oscilloscope.

Video Content / Details of website for further learning (if any):

Important Books/Journals for further learning including the page nos.:

Banwell, Colin N. and E.M. McCash, "Fundamentals of Molecular Spectroscopy" Tata McGraw-Hill, 2007 (Pg. No. 95-99).

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LECTURE HANDOUTS

BIOTECH | **III/VI**

Course Name with Code : **BIOLOGICAL SPECTROSCOPY 16BTE15**

Course Faculty : **Dr. G. Pratap Kumar**

Unit : **II** **Date of Lecture:**

Topic of Lecture: ESR multi-dimensional NMR spectroscopy

Introduction :

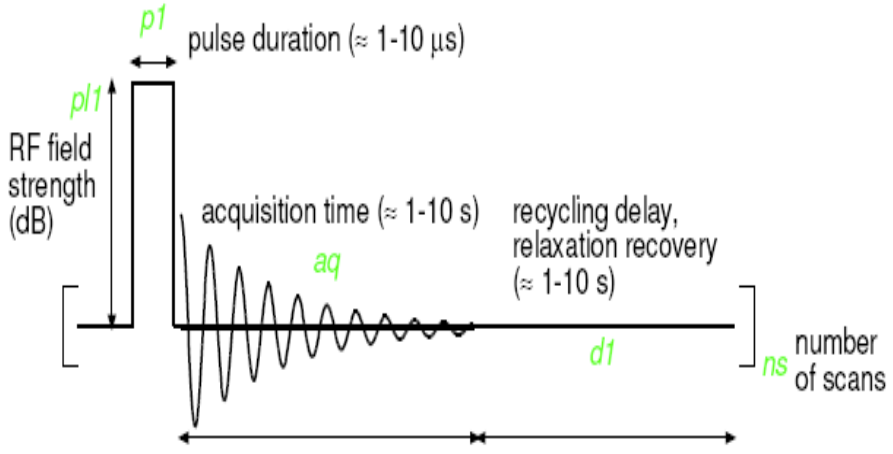
- NMR-spectroscopy observes the resonance interaction of atomic nuclei with electromagnetic waves.
- The major advantages of MD NMR are improved resolution and magnetization transfer.
- Improved resolution mean signals are spread over a surface (2D) or in a 3D space (3D, 4D), whereas magnetization transfer are signals result from the interaction between nuclei.
- Transfer of magnetization takes place between like nuclei. Both axis exhibit the chemical shift of the same type of nucleus.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on learning the concepts of multi-dimensional NMR.
- Prerequisite knowledge on understanding the importance of NMR experiments

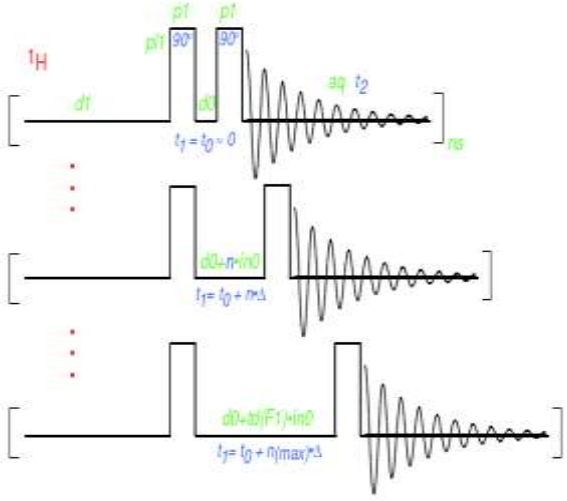
Detailed content of the Lecture:

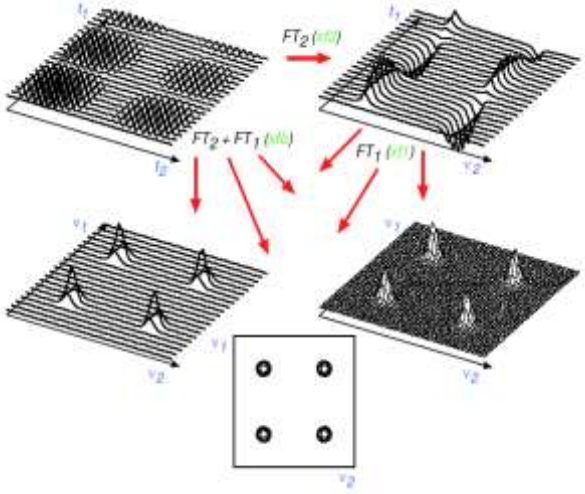
- More complex NMR experiments will use multiple "time-dimensions" to obtain data and simplify the analysis.
- In a 1D NMR experiment the FID acquisition time is the time domain (t_1)
  - Multidimensional NMR experiments may also observe multiple nuclei (^{13}C,^{15}N) in addition to ^{1}H.
  - But usually detect ^{1}H.



- *2D COSY* (Correlated SpectroscopY): Correlate J-coupled NMR resonances → Identify the spins that are coupled to each other.

- A series of FIDs are collected where the delay between 90° pulses (t1) is incremented. t_2 is the normal acquisition time.
- During the t_1 time period, peak intensities are modulated at a frequency corresponding to the chemical shift of its coupled partner.





Video Content / Details of website for further learning (if any):
Basic principles of multidimensional NMR spectroscopy – Peter Schmieder AG Solution NMR, 2009.

Important Books/Journals for further learning including the page nos.:
Banwell, Colin N. and E.M. McCash, "Fundamentals of Molecular Spectroscopy" Tata McGraw-Hill, 2007 (Pg. No. 100-103).

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LECTURE HANDOUTS

BIOTECH | **III/VI**

Course Name with Code : **BIOLOGICAL SPECTROSCOPY 16BTE15**

Course Faculty : **Dr. G. Pratap Kumar**

Unit : **II** **Date of Lecture:**

Topic of Lecture: Determination of macromolecular structure by NMR

Introduction :

- The use of NMR data to determine macromolecular structures relies on the existence (to a first approximation) of two types of interactions between pairs of nuclei that are manifested in NMR spectra.
- The first of these interactions is the dipolar interaction, particularly between protons.
- ^1H, ^1H NOEs are the most important source of structural information in NMR beacause they provide an indirect measure of the distances between the chemically abundant hydrogen nuclei; pairs of protons that are closer in space give rise to larger NOEs.
- For even a modest-sized protein of 100 residues, one would expect to measure several thousand distances from NOE data.
- The second interaction is manifested between pairs of nuclei that are close in the covalent structure of the molecule (separated by less than three of four covalent bonds).

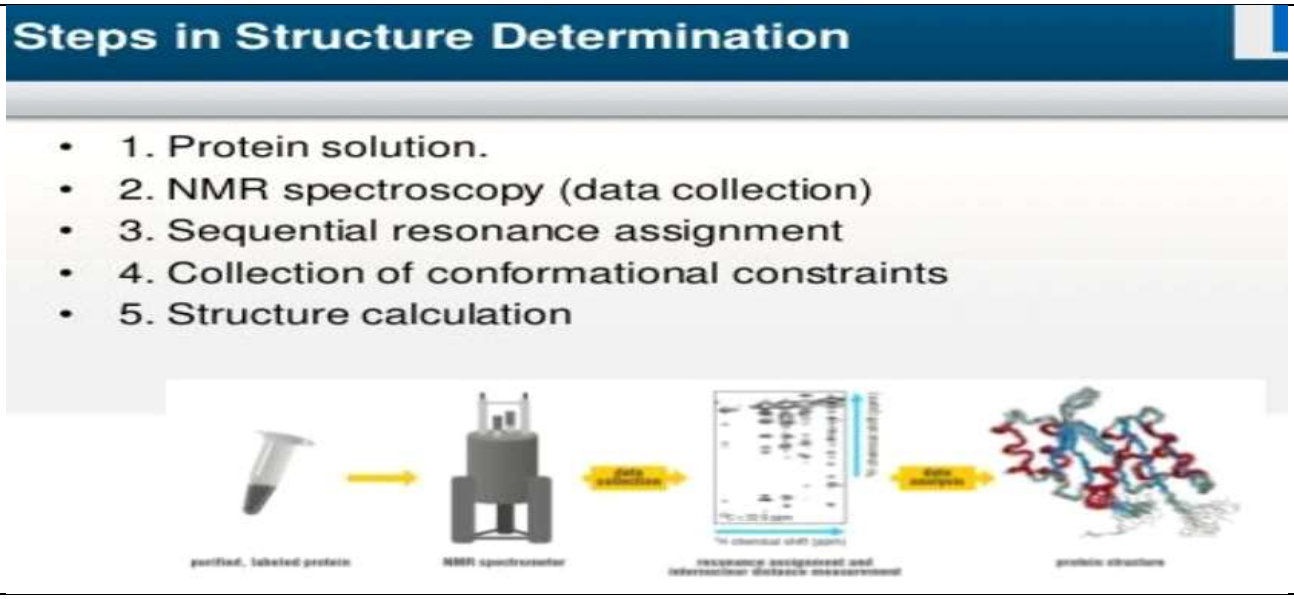
Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on understanding the importance of protein structure determination through NMR.
- Prerequisite knowledge on knowing the NMR spectra for protein folding and other structure determination parameters.

Detailed content of the Lecture:

Structure Determination

- Various functions of biological system depend upon the structure and function of proteins.
- Determination of structure and functions of proteins assist in scrutinizing the dynamics of proteins.
- To understand the functions of proteins at a molecular level, it is often necessary to determine their three-dimensional structure.



Video Content / Details of website for further learning (if any):

Important Books/Journals for further learning including the page nos.:

Banwell, Colin N. and E.M. McCash, "Fundamentals of Molecular Spectroscopy" Tata McGraw-Hill, 2007 (Pg. No. 105-112).

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LECTURE HANDOUTS

BIOTECH | **III/VI**

Course Name with Code : **BIOLOGICAL SPECTROSCOPY 16BTE15**

Course Faculty : **Dr. G. Pratap Kumar**

Unit : **II** **Date of Lecture:**

Topic of Lecture: Magnetic Resonance Imaging

Introduction :

- MRI (Magnetic Resonance Imaging) is a radiology technique.
- This MRI uses magnetism, radio waves, and a computer to produce images of body structures.
- It is based on the principle of NMR. The first MRI exam was performed on a human being in 1997.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on understanding the basic working of MRI instrument.
- Prerequisite knowledge on learning the principle behind MRI.

Detailed content of the Lecture:

Introduction

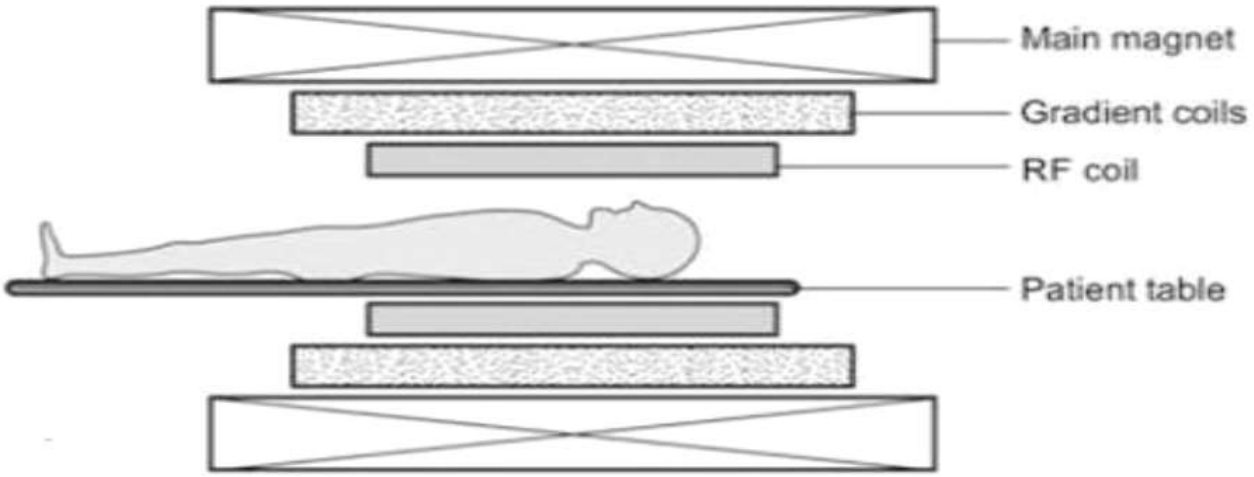
- MRI is a type of scan that uses strong magnetic fields and radio waves to produce detailed images of inside of the body.
- An MRI scanner is a large tube that contains powerful magnets.You lie inside the tube during scan.
- MRI perhaps the best application of superconductivity which directly affected the humanity across the globe.

PRINCIPLE:

- MRI makes use of the magnetic properties of certain atomic nuclei.
- Hydrogen nucleus (single proton) present in water molecules, and therefore in all body tissues.
- The hydrogen nuclei partially aligned by a strong magnetic field in the scanner.

COMPONENTS:

- Scanner
- Computers
- Recording hardware



Schematic diagram of MRI scanner

Video Content / Details of website for further learning (if any):

Important Books/Journals for further learning including the page nos.:

Banwell, Colin N. and E.M. McCash, "Fundamentals of Molecular Spectroscopy" Tata McGraw-Hill, 2007 (Pg. No. 115-117).

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BIOTECH **III/VI**

Course Name with Code : **BIOLOGICAL SPECTROSCOPY 16BTE15**

Course Faculty : **Dr. G. Pratap Kumar**

Unit : **II** **Date of Lecture:**

Topic of Lecture: Applications of MRI

Introduction :

- MRIs are a relatively new technology to hit the medical world and have completely revolutionized medical imaging and the diagnosing process as we know.
- In vivo images can be taken of the human body, meaning that internal images can be seen without making any incisions.
- Completely non-intrusive procedures are used which makes MRI's very effective but somewhat expensive for doctors to use.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on understanding various applications of MRI.
- Prerequisite knowledge on how the procedure for MRI's are taken into consideration for analysis.

Detailed content of the Lecture:

MRI is used for a huge range of clinical applications:

- **Clinical neurology**
  a. Segmentation and classification
  b. Measuring volumes of brain structures
  c. Multiple sclerosis, neurodegeneracy and stroke
- **Cardiology**
  a. Either need to image fast or deal with heart motion
- **Cancer**
  a. Breast, colorectal, liver, prostrate
- **Soft tissue damage**
  a. Cartilage and ligament tear

MRI is also used a great deal in basic science to study brain function and cancer growth.

Video Content / Details of website for further learning (if any):

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LECTURE HANDOUTS

BIOTECH | **III/VI**

Course Name with Code : **BIOLOGICAL SPECTROSCOPY 16BTE15**

Course Faculty : **Dr. G. Pratap Kumar**

Unit : **III** **Date of Lecture:**

Topic of Lecture: Ion sources in MS

Introduction :

- Ion source in MS is used for producing gaseous ions from the substance being studied.
- Once in the source, sample molecules are subjected to ionization. Ions formed in the source (molecular and fragment ions) acquire some kinetic energy and leave the source.
- Ionization methods are selective for analysis of sample is extremely important.

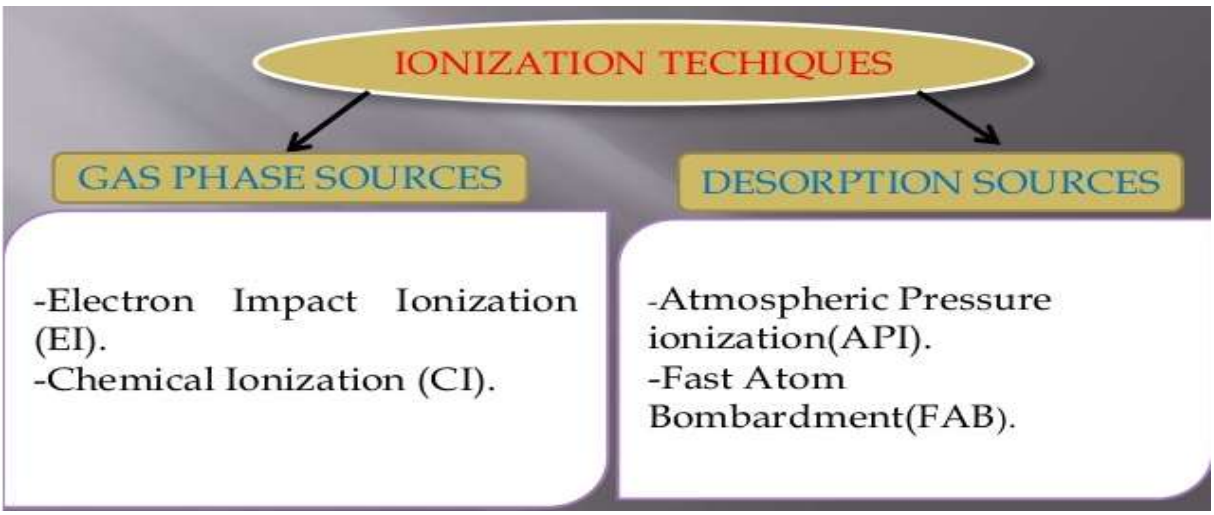
Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on learning the principles of ion sources present in MS
- Prerequisite knowledge on understanding the mechanism behind ion sources in MS.

Detailed content of the Lecture:

Ion sources:

Several methods are there for converting the sample into the gaseous ionic phase these are as under:

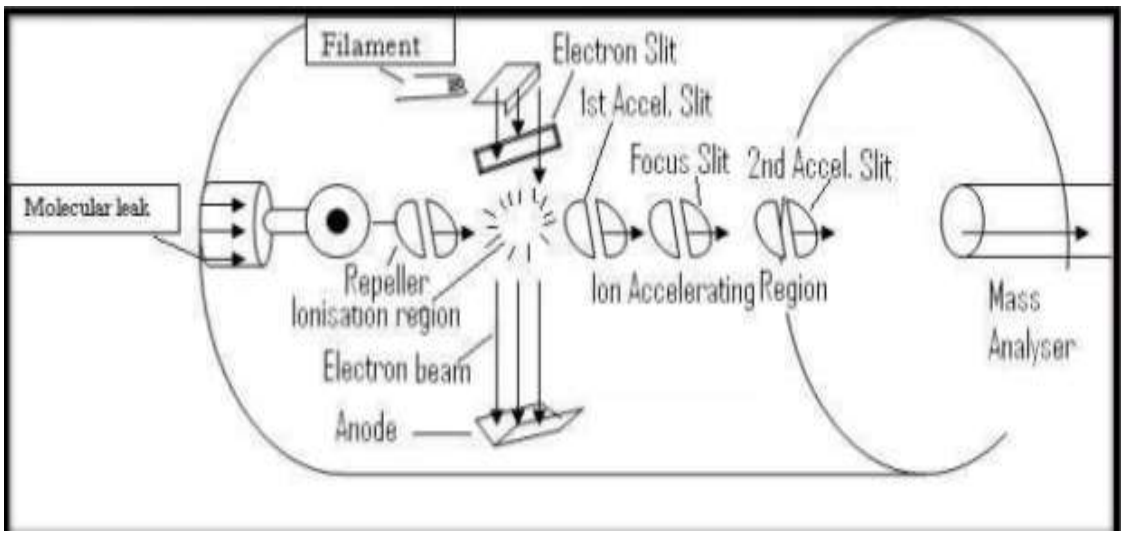


EI:

- It is the type of hard ionization technique due the high energy of Electron Impact. Ions are accelerated at the voltage of ~104 V.
- Ionization method as name includes the impact of beam of high energetic electron to a gaseous phase or the volatile organic sample.
- Due to the electron impact the sample is broken into positive or negative ions.
- The energetic electron beam is emitted by a electrically heated tungsten or rhenium which are then accelerated by the potential difference of 70eV.

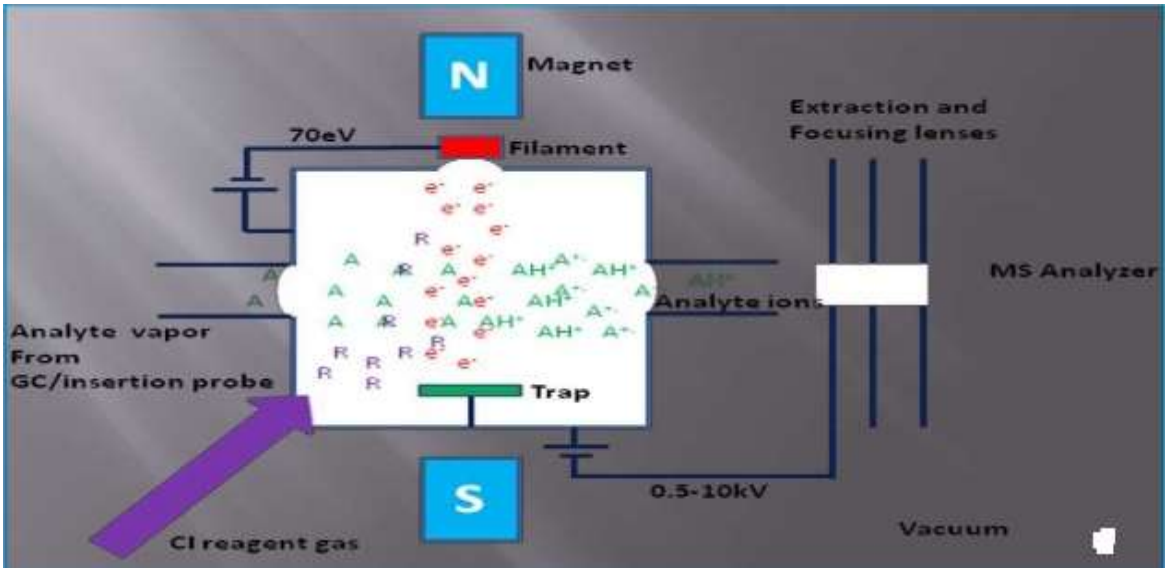
- Collision between ions and molecules may also result in ion with higher m/z values than the molecular ion. Where M+ is a radical cation which gives molecular weight

$$M(g) + e^- \rightarrow M^+. (g) + 2e^-$$



CI:

- EI is not appropriate for certain compounds due to the excessive fragmentation. Chemical ionization includes the ionization of reagent gas in high volume approx 1000 times more.
- Typically used reagent gas is methane, ammonia, isobutane.
- Firstly at high pressure the reagent gas is ionized and subsequently this ionized gas molecule collide with sample as gaseous phase and bring about fragmentation.
- It is a soft ionization technique. Generally have less fragmentation and molecular ion is abundant.

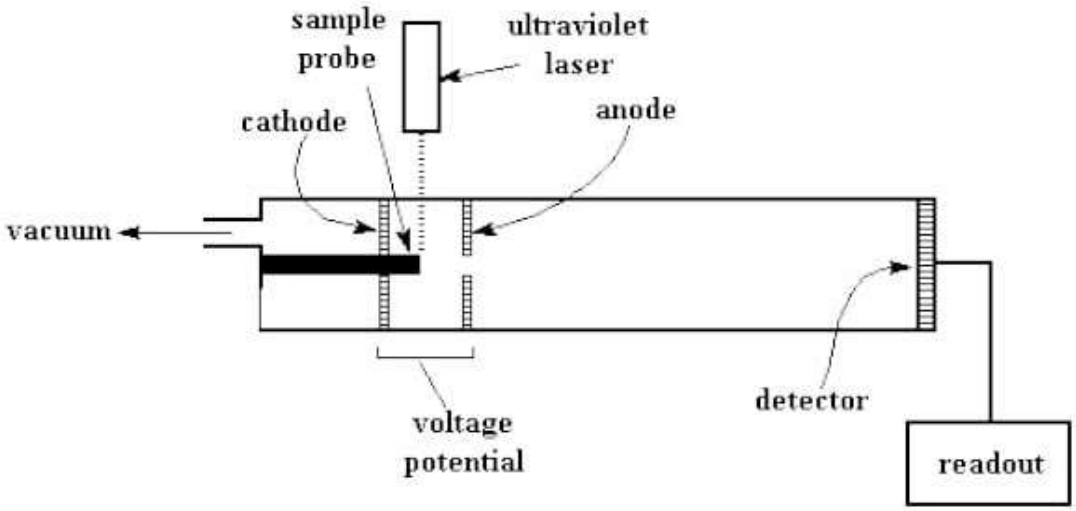


API:

- It operates at the atmospheric pressure. It is used for a mixture of high molecular weight non-volatile compound.
- It is of various types which are:
  a) Matrix Assisted Laser Desorption Ionization (MALDI)
  b) Electrospray Ionization (ESI)
  c) Atomic Pressure Chemical Ionization (APCI)
  d) Atomic Pressure Photon Ionization (APPI)

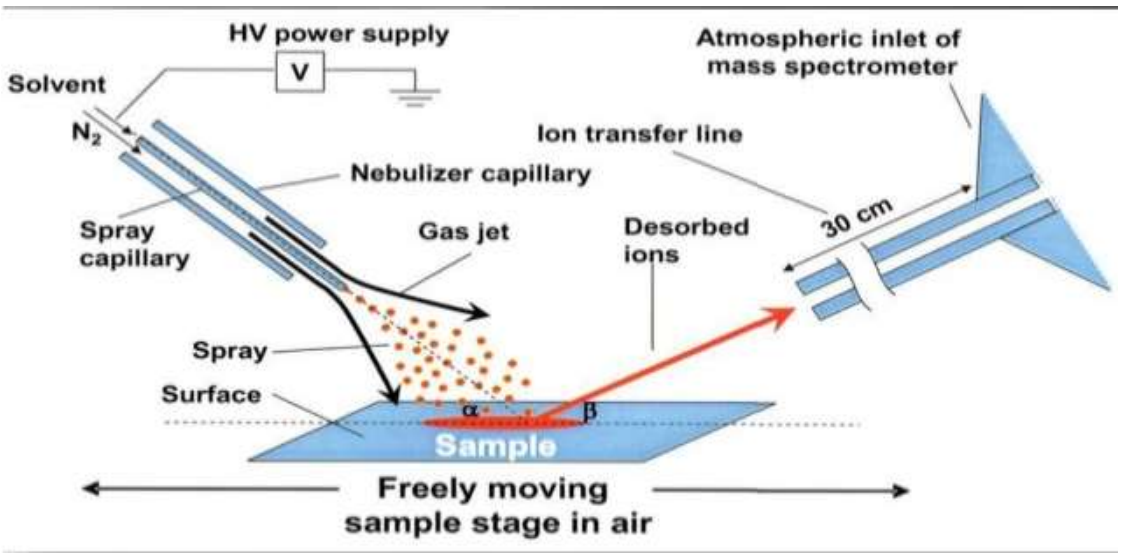
a) MALDI:

- Matrix Assisted Laser Desorption Ionization technique that in contrast to vacuum MALDI operates at normal atmospheric environment.
- In this method, ionization is carried out by bombarding a laser beam on the sample dissolved in a matrix solution.
- Matrix is used in MALDI to:
    1. Absorb the laser energy.
    2. Prevent analyte agglomeration.
    3. Protect analyte from being destroyed by direct laser beam.



b) <u>ESI:</u>

- It operates at atmospheric pressure. A sample solution is sprayed from a small pore into electric field in the presence of flow of warm nitrogen to assist desolvation.
- The droplets thus formed evaporates in the region of vacuum maintained at high pressure to form ions. The increased pressure causes the charge to increase in the ion thus formed.
- Generally used for molecule such as peptides, proteins, organometallic and polymers but cannot be used for buffer of phosphates as the trace level of this can interfere with ESI process.



c) <u>APPI:</u>

- A mixture of the analyte and the solvent i.e. a liquid solution is first vaporized with the help of nebulizing gas N2.

- The mixture enters the ionization chamber at atmospheric pressure. The mixture is then exposed to the UV source of krypton lamp.
- The photon emitted from this lamp has a specific energy level i.e. 10eV.
- It is high enough to ionize sample excluding the unwanted species. Hence analyte molecule is analyzed or measured.
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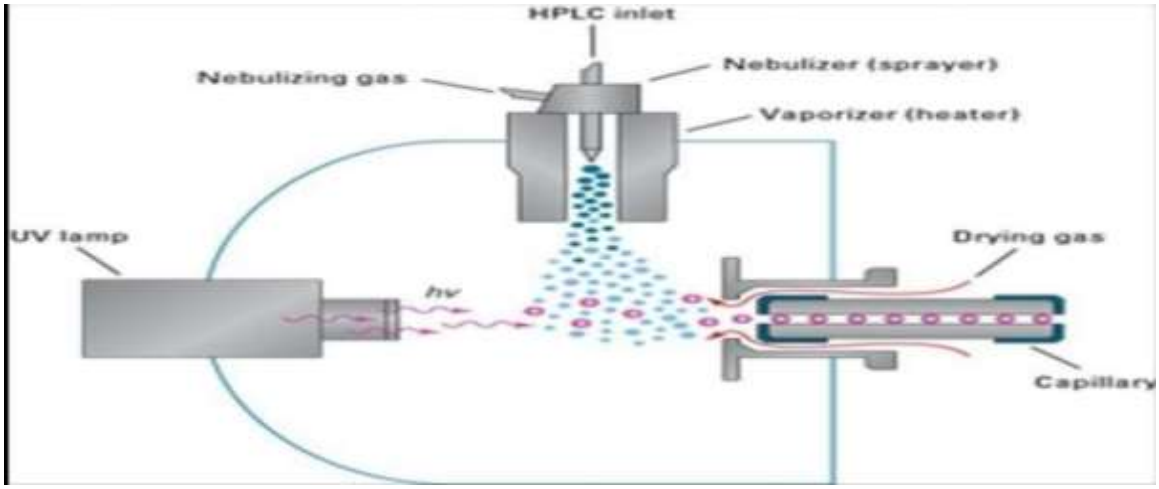
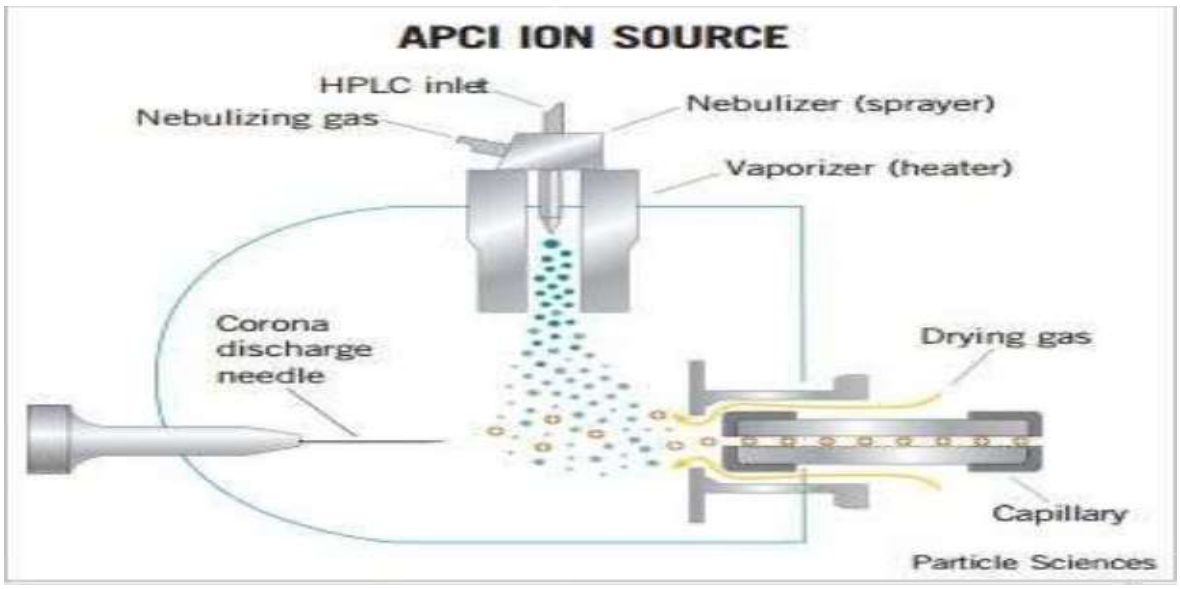


d) APCI:

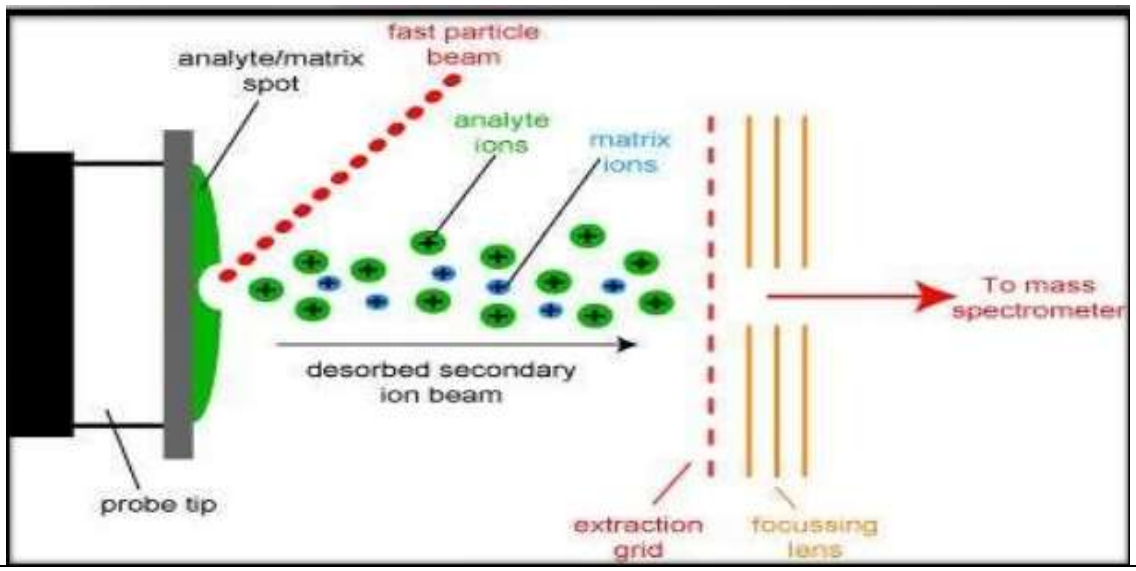
- The corona discharge produces primary ions in this technique.
- The nebulized sample via high speed nitrogen gas is displaced to a quartz tubing called as desolvation chamber.
- In desolvation chamber these droplets are converted to mixture of compound which are subsequently carried to a corona discharge electrode.
- Due to these molecule are thus ionized in two ways or modes : Positive mode: proton transfer or charge exchange occurs . Negative mode: proton abstraction or electron capture or adduct formation is their.
- It produces singly charged species. Generally employed for large biomolecules and polymers. It is a high mass pulsed technique hence it is generally combined with TIME OF FLIGHT.



FAB:

- For polar molecules such as peptides with molecular weight up to 10000 can be analyzed by soft ionization technique called as Fast Atom Bombardment.

- Thermally unstable molecule it works well as it works at room temperature. The beam for bombardment is generally consist of Xenon or Argon gas atom of high energy, the beam is produced by ionizing xenon atom by the electrons.
- The sample is dissolved in glycerol and fine layer is formed over metal probe which is then ionized by fast beam of xenon or argon striking the sample.
- Generally it causes less fragmentation and molecular ion is obtained. Hence sample mass is analyzed in this way



Video Content / Details of website for further learning (if any):

Important Books/Journals for further learning including the page nos.:

Banwell, Colin N. and E.M. McCash, "Fundamentals of Molecular Spectroscopy" Tata McGraw-Hill, 2007 (Pg. No. 125-145).

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Rasipuram - 637 408, Namakkal Dist., Tamil Nadu

LECTURE HANDOUTS

BIOTECH | **III/VI**

Course Name with Code : **BIOLOGICAL SPECTROSCOPY 16BTE15**

Course Faculty : **Dr. G. Pratap Kumar**

Unit : **III** **Date of Lecture:**

Topic of Lecture: Sample introduction in MS

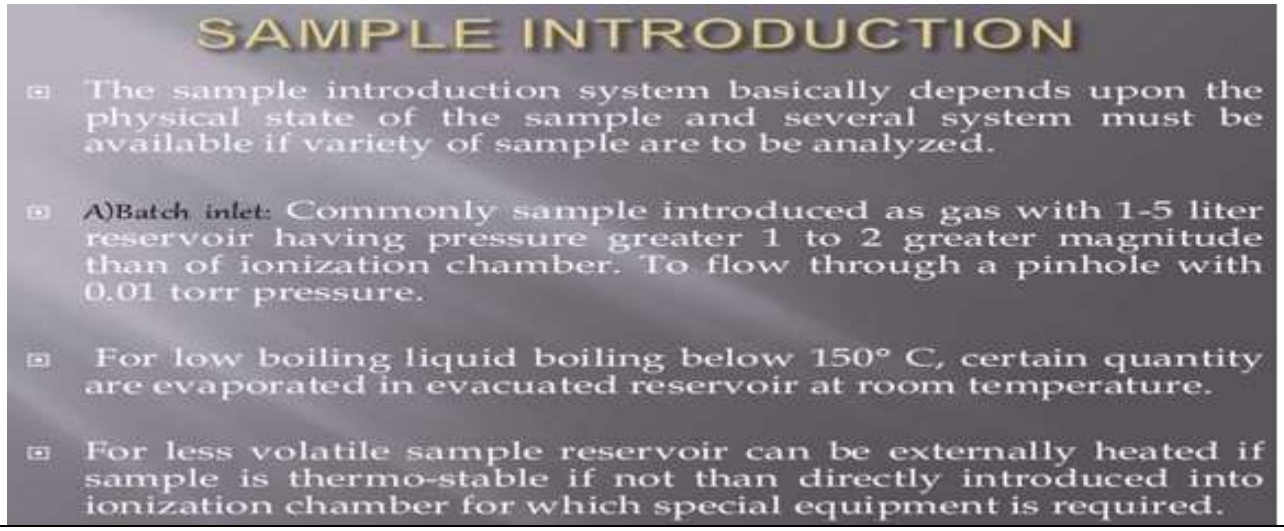
Introduction :

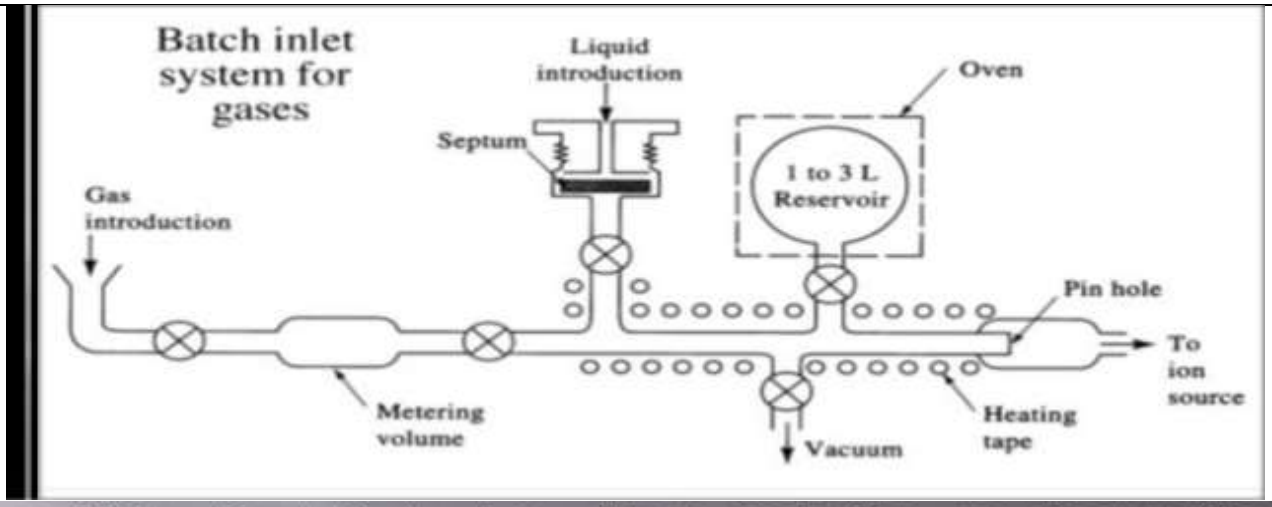
- The selection of a sample inlet depends upon the sample and the sample matrix. Most ionization techniques are designed for gas phase molecules so the inlet must transfer the analyte into the source as a gas phase molecule.
- Gases and samples with high vapour pressure are introduced directly into the source region. Liquids and solids are usually heated to increase the vapor pressure for analysis.
- If the analytes are thermally labile (it decomposes at high temperatures) or if it does not have a sufficient vapor pressure, the sample must be directly ionized from the condensed phase.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on how sample is injected into the instrument.
- Prerequisite knowledge on knowing the MS analysis of compound.

Detailed content of the Lecture:





- B)The Direct Probe Inlet: Non volatile or thermally unstable introduced directly into the ion chamber by sample probe via vacuum lock.
- Consist of small capillary tube or cup containing µg of sample.
- Heater is their to volatilize the sample at low pressure.
- The probe is used to study carbohydrates, steroids and low molecular weight polymers.

Video Content / Details of website for further learning (if any):

Important Books/Journals for further learning including the page nos.:

Banwell, Colin N. and E.M. McCash, "Fundamentals of Molecular Spectroscopy" Tata McGraw-Hill, 2007 (Pg. No. 155-165).

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LECTURE HANDOUTS

BIOTECH

III/VI

Course Name with Code : **BIOLOGICAL SPECTROSCOPY 16BTE15**

Course Faculty : **Dr. G. Pratap Kumar**

Unit : **III** **Date of Lecture:**

Topic of Lecture: Mass analyzers in MS

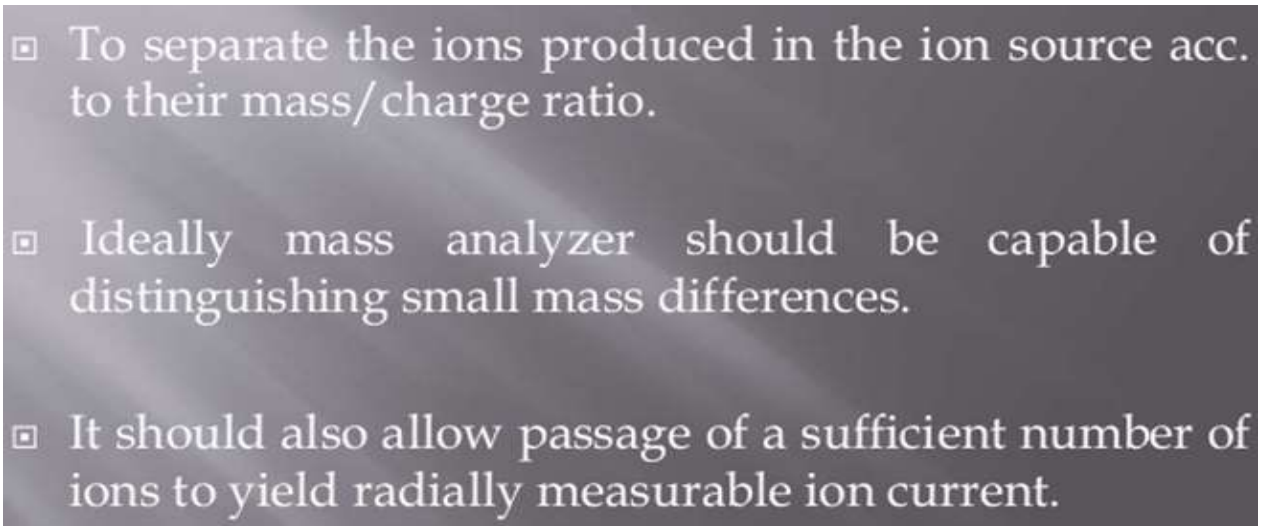
Introduction :

- After ions are formed in the source region they are accelerated into the mass analyzer by an electric field. The mass analyzer separates these ions according to their m/z value.
- The selection of mass analyzer depends upon the resolution, mass range, scan rate and detection limits required for an application.
- Each analyzer has very different operating characteristics and the selection of an instrument involves important tradeoffs. Mostly analyzers are typically described as either continuous or pulsed.

Prerequisite knowledge for Complete understanding and learning of Topic:

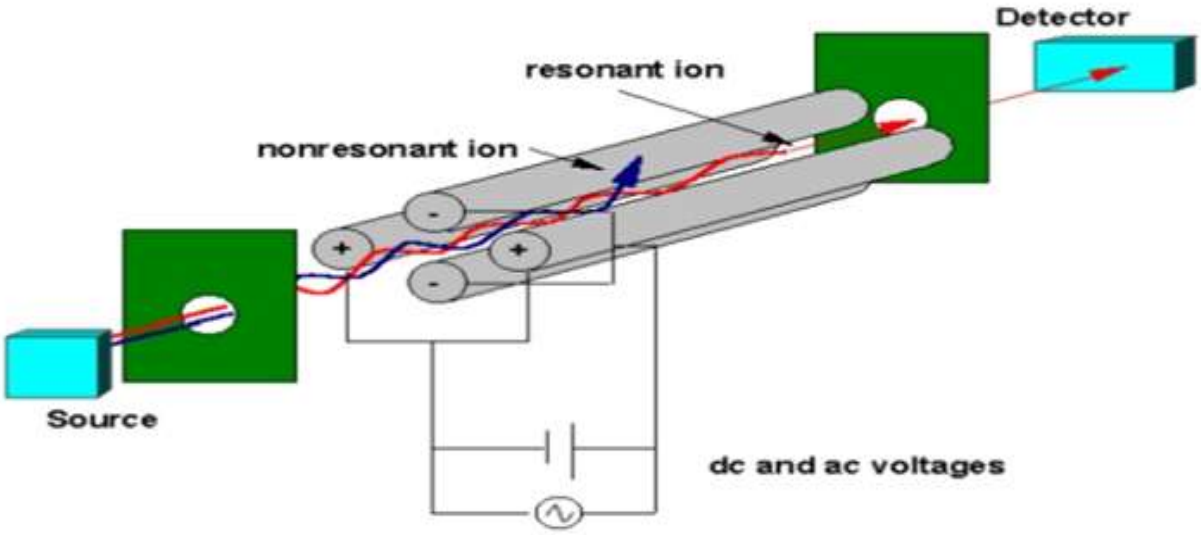
- Prerequisite knowledge on knowing the types of analyzers used in MS.
- Prerequisite knowledge on understanding the concepts and importance of analyzers in MS.

Detailed content of the Lecture:



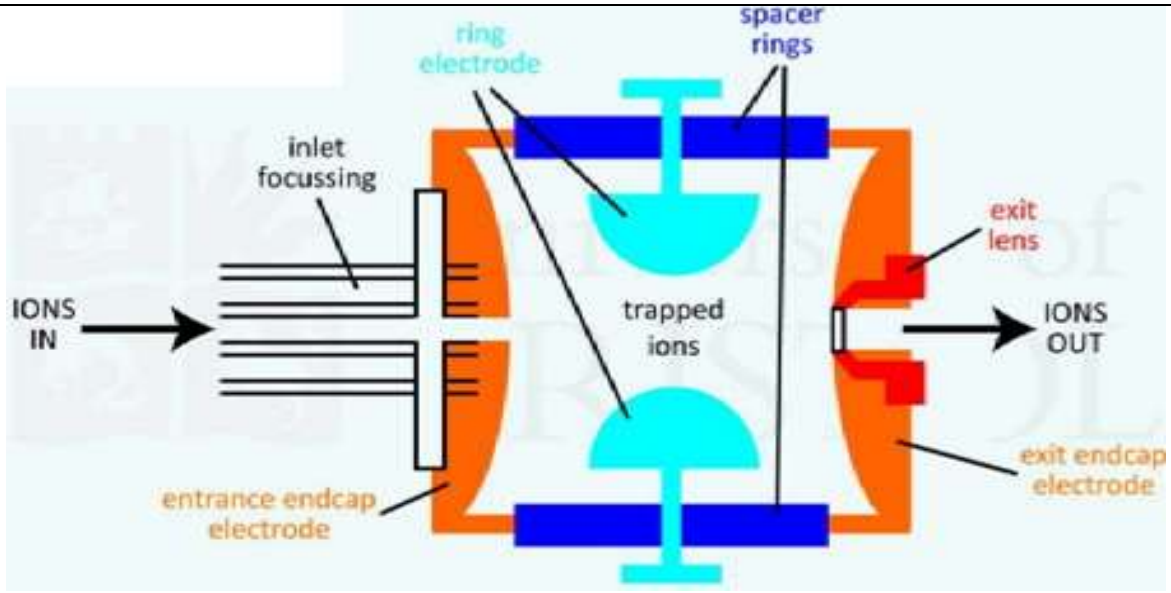
QUADRUPOLE ANALYZER

- Consists of 4 parallel metal rods, or electrodes.
- The ions are accelerated by a potential of 5-15 V and injected into the area between the 4 rods .
- Opposite electrodes have potentials of the same sign .
- One set of opposite electrodes has applied potential of $[U+V\cos(\omega t)]$.
- Other set has potential of $-[U+V\cos(\omega t)]$.
- U= DC voltage, V=AC voltage, ω= angular velocity of alternating voltage



ION TRAP ANALYZER

- The ion trap is a variation of the Quadrupole mass filter, and consequently is sometimes refer to as a Quadrupole Ion Trap.
- The trap contains ions in a 3-dimensional volume rather than along the center axis.
- Helium gas is added to the trap causing the ions to migrate toward the center.
- After trapping, the ions are detected by placing them in unstable orbits, causing them to leave the trap.



Video Content / Details of website for further learning (if any):

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Banwell, Colin N. and E.M. McCash, "Fundamentals of Molecular Spectroscopy" Tata McGraw-Hill, 2007 (Pg. No. 168-175).

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LECTURE HANDOUTS

BIOTECH

III/VI

Course Name with Code : **BIOLOGICAL SPECTROSCOPY 16BTE15**

Course Faculty : **Dr. G. Pratap Kumar**

Unit : **III** **Date of Lecture:**

Topic of Lecture: Ion detectors

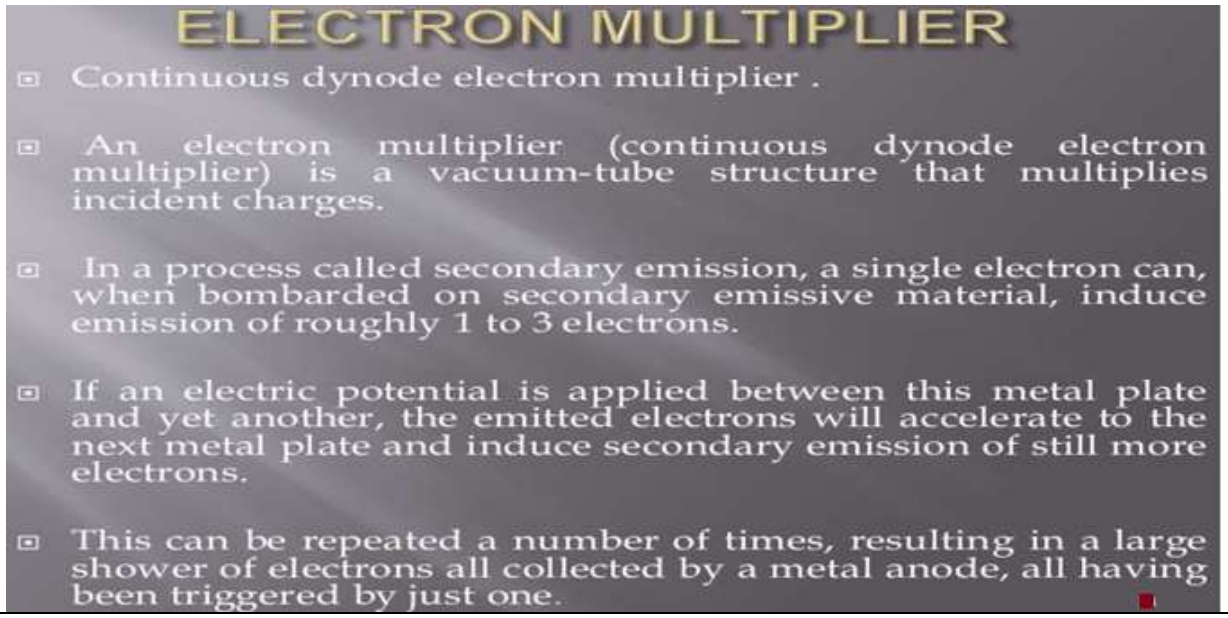
Introduction :

- Detection of ions is based up on their charge or momentum. For large signals a faraday cup is used to collect ions and measure the current.
- Older instruments used photographic plates to measure the ion abundance at each mass to charge ratio.
- Most detectors currently used amplify the ion signal using a collector similar to a photomultiplier tube. These amplifying detectors include various types like channeltrons, etc.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on types of detectors in MS used for analysis of samples.
- Prerequisite knowledge on understanding the working of detectors in MS.

Detailed content of the Lecture:



Video Content / Details of website for further learning (if any):

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Banwell, Colin N. and E.M. McCash, "Fundamentals of Molecular Spectroscopy" Tata McGraw-Hill, 2007 (Pg. No. 180-185).

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III/VI

Course Name with Code : **BIOLOGICAL SPECTROSCOPY 16BTE15**

Course Faculty : **Dr. G. Pratap Kumar**

Unit : **III** **Date of Lecture:**

Topic of Lecture: Biomolecular Mass spectrometry

Introduction :

- Mass spectrometry enables the characterization of molecules that are present in cells and allows thereby the identification and characterization of proteins and other biomolecules that work together and are involved in cellular processes and in disease.
- MS is an indispensable field for analyzing biomolecules like analysis of glycans, analysis of lipids, analysis of proteins and peptides, analysis of oligonucleotides.
- The characterization of oligosaccharides is more difficult than that of proteins and oligonucleotides because of the isomeric nature of the subunit and its ability to form branched structures.
- MS has become a vital tool in proteomic research which give information on the identity of a protein, the amount of the protein present, etc.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on understanding the analysis of biomolecules using MS.
- Prerequisite knowledge on knowing the characterizing parameters of biomolecules using MS.

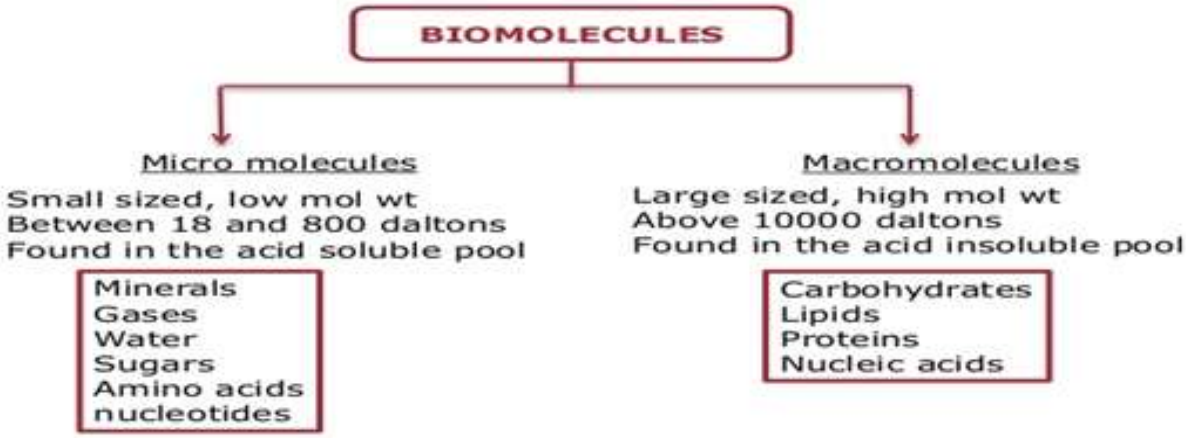
Detailed content of the Lecture:

Chemicals or molecules present in the living organisms are known as **Biomolecules**

The sum total of different types of biomolecules, compounds and ions present in a cell is called as **cellular pool**

Biomolecules are compounds of **carbon**.
Hence the chemistry of living organisms is organized around carbon
Carbon is the most versatile and the most predominant element of life.

ELEMENT	Non living (Earth crust)	Living Matter
Hydrogen	0.14	0.5
Carbon	0.03	18.5
Oxygen	46.6	65.0
Nitrogen	Very less	3.3
Sulphur	0.03	0.3
Sodium	2.8	0.2
Calcium	3.6	1.5
Magnesium	2.1	0.1
Silicon	27.7	Very less



The major complex biomolecules of cells

Biomolecule	Building block	Major functions
Protein	Amino acid	Basic structure and function of cell
DNA	Deoxyribonucleotide	Hereditary information
RNA	Ribonucleotide	Protein synthesis
Polysaccharide	Monosaccharide	Storage form of energy
Lipids	Fatty acids & glycerol	Storage form of energy to meet long term demands

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Banwell, Colin N. and E.M. McCash, "Fundamentals of Molecular Spectroscopy" Tata McGraw-Hill, 2007 (Pg. No. 187-190).

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BIOTECH | **III/VI**

Course Name with Code : **BIOLOGICAL SPECTROSCOPY 16BTE15**

Course Faculty : **Dr. G. Pratap Kumar**

Unit : **III** **Date of Lecture:**

Topic of Lecture: Protein analysis

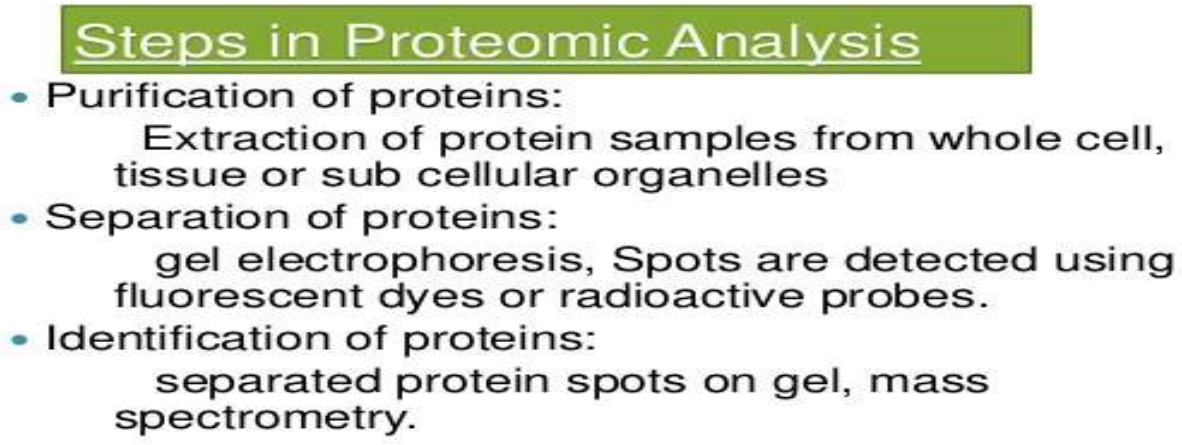
Introduction :

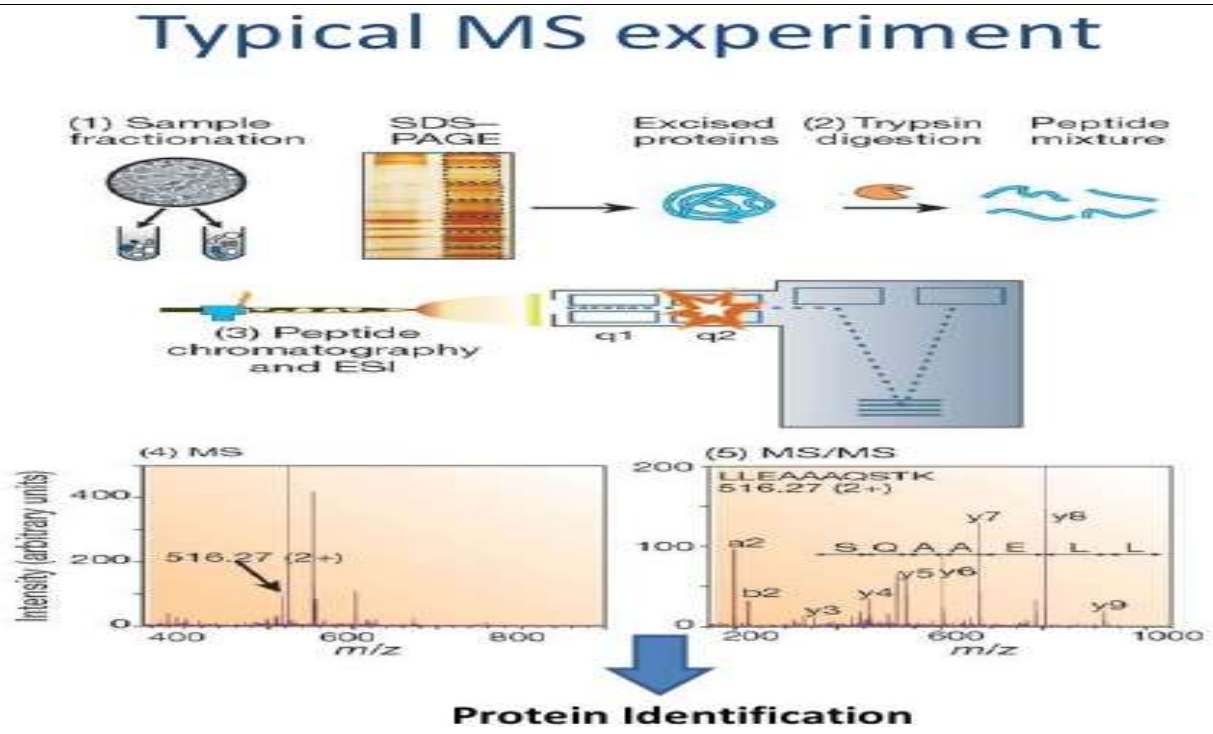
- MS analysis of proteins measures the m/z ratio of ions to identify and quantify molecules in simple and complex mixtures.
- The approach involves enzymatic and/or chemical degradation of the protein to a collection of peptides which are then fractionated by HPLC.
- MS of proteins require that proteins in solution or solid state be turned into an ionized form in the gas phase before they are injected and accelerated in an electric or magnetic field for analysis.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on understanding the analysis of protein sample using MS.
- Prerequisite knowledge on knowing the importance of protein Sample in the field of proteomics.

Detailed content of the Lecture:





Video Content / Details of website for further learning (if any):

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Banwell, Colin N. and E.M. McCash, "Fundamentals of Molecular Spectroscopy" Tata McGraw-Hill, 2007 (Pg. No. 190-192).

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LECTURE HANDOUTS

BIOTECH **III/VI**

Course Name with Code : **BIOLOGICAL SPECTROSCOPY 16BTE15**

Course Faculty : **Dr. G. Pratap Kumar**

Unit : **III** **Date of Lecture:**

Topic of Lecture: Peptide analysis

Introduction :

- In MS, the peptide masses are determined and through MS/MS we can confirm their sequence.
- Any peptide sequences detected are then matched against a protein database to confirm which protein they derive from and thus which proteins were originally present in the sample.
- As peptide mass fingerprinting has a sample throughput similar to AA analysis, this combined identification approach is suitable for rapid protein identification.

Prerequisite knowledge for Complete understanding and learning of Topic:

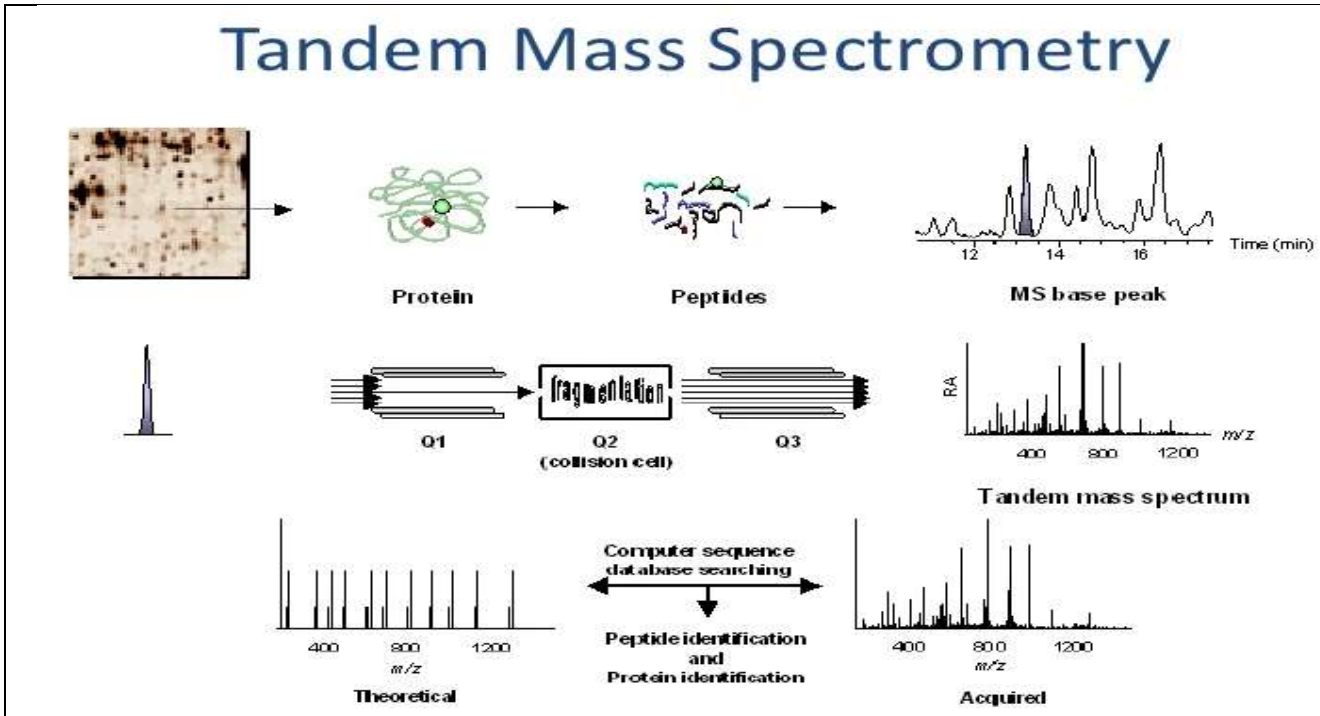
- Prerequisite knowledge on digestion of peptides samples in MS analysis.
- Prerequisite knowledge on how the structural components of cells are analyzed using MS.

Detailed content of the Lecture:

- It is basically a technique that is used for identification of the protein in which the protein of interest is splitted into smaller peptide then the mass of these peptides is measured by MS such as MALDI-TOF or ESI-TOF.
- Peptide – Specific protein fragment usually generated with Trypsin
  Mass – The size of the peptide
  Fingerprint – Uniqueness
- The identification of protein is one of the hardest task among proteomics but MS is the excellent method for identification of protein allowing to measure with high precision the m/z ratio of charged molecules such as peptides.

PROCEDURE:

1. The protein of interest from a sample are separated on 2D PAGE.
2. Protein of interest is digested by Trypsin (or any other site specific cleavage).
3. Ionization of peptides in a MALDI/ESI MS.
4. m/z values detected and plotted as mass spectra.
5. PMF database search to identify the protein.



Video Content / Details of website for further learning (if any):

Important Books/Journals for further learning including the page nos.:

Banwell, Colin N. and E.M. McCash, "Fundamentals of Molecular Spectroscopy" Tata McGraw-Hill, 2007 (Pg. No. 195-198).

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LECTURE HANDOUTS

BIOTECH

III/VI

Course Name with Code : **BIOLOGICAL SPECTROSCOPY 16BTE15**

Course Faculty : **Dr. G. Pratap Kumar**

Unit : **III** **Date of Lecture:**

Topic of Lecture: Carbohydrates and small molecules

Introduction :

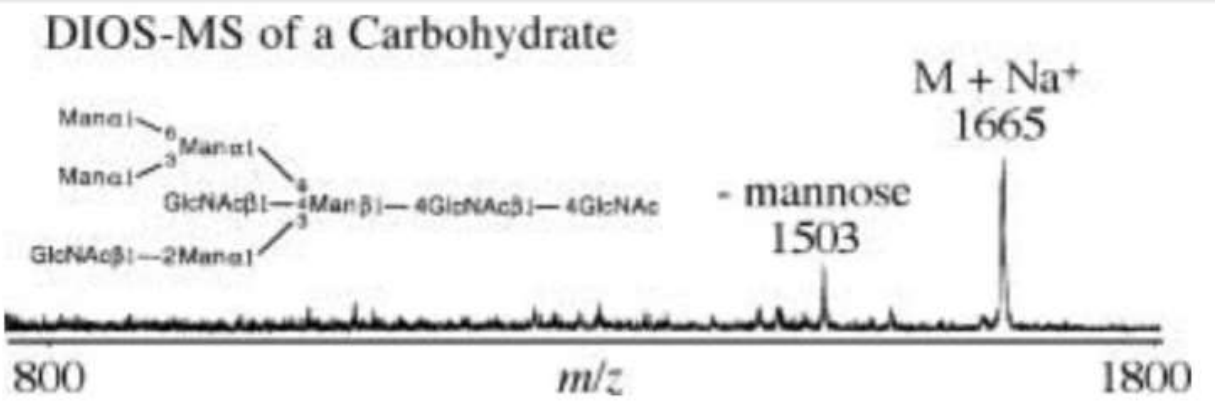
- Carbohydrates is an organic compound technically they are polyhydroxy aldehydes and ketones. They are linked to proteins and lipids that play important roles in cell interactions.
- The analysis of carbohydrates by MS provides information on molecular mass, constituent monosaccharides, sequence of the monosaccharides, linkage type, stereochemistry, etc.
- Glucose is the most important carbohydrate; the major metabolic fuel of mammals and a universal fuel of the fetus.
- It's the precursor for synthesis of all other carbohydrates in the body.

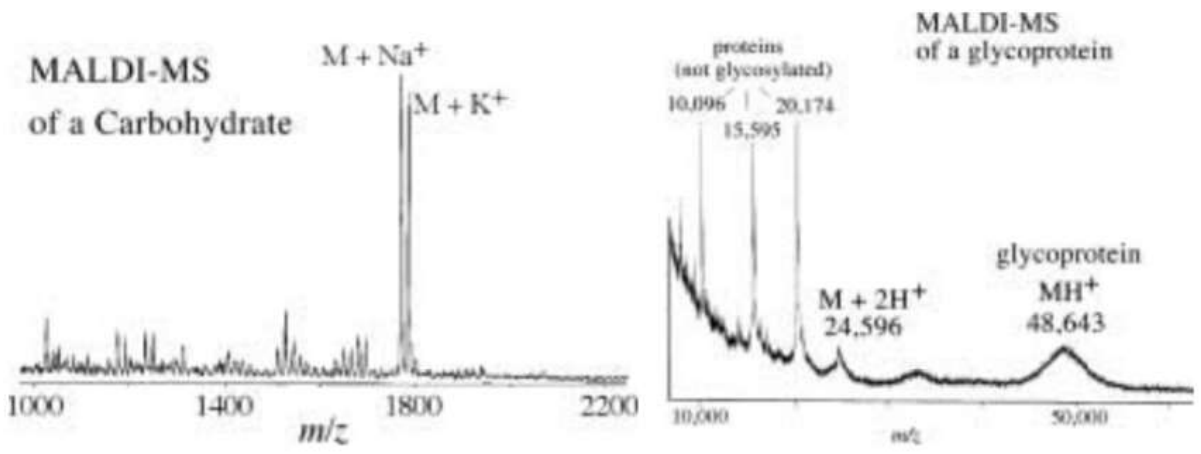
Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on knowing the classification and analysis of carbohydrates present in the sample.
- Prerequisite knowledge on understanding the main presence of carbohydrate when done using preliminary tests for the sample in laboratories.

Detailed content of the Lecture:

- Carbohydrates can be ionized in both positive and negative ion mode to give MH^+, M + alkali metal $[M-H]^-$.
- MALDI-MS analysis of carbohydrates typically yields the best spectra when using 2,5-dihydrobenzoic acid as a matrix.
-





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Banwell, Colin N. and E.M. McCash, "Fundamentals of Molecular Spectroscopy" Tata McGraw-Hill, 2007 (Pg. No. 198-200).

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LECTURE HANDOUTS

BIOTECH

III/VI

Course Name with Code : **BIOLOGICAL SPECTROSCOPY 16BTE15**

Course Faculty : **Dr. G. Pratap Kumar**

Unit : **III** **Date of Lecture:**

Topic of Lecture: Specific applications in MS

Introduction :

- MS is applicable across diverse fields including forensic toxicology, metabolomics, proteomics, pharma/biopharma and clinical research.
- Specific applications of MS include drug testing and discovery, food contamination detection, pesticide residue analysis, isotope ratio determination, protein identification and carbon dating.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on some of the applications of MS in various fields.
- Prerequisite knowledge on understanding and knowing the techniques applied through MS in PMF or mapping, protein folding, etc.

Detailed content of the Lecture:

- **Applications of MS in proteomics:**

Characterization of proteins and protein complexes sequencing of peptides and identification of posttranslational modifications.

- **Applications of MS in metabolomics:**

Cancer screening and diagnosis, global metabolic finger printing analysis, biomarker discovery and profiling, biofuels generation and use, lipidomics studies and metabolic disorder profiling.

- **Applications of MS in pharmaceutical analysis:**

Drug discovery and absorption, distribution, metabolism and elimination (ADME) studies, pharmacokinetics and pharmacodynamics, metabolite screening and preclinical development.

- **Applications of MS in forensic analysis:**

Analysis of trace evidence, arson investigation, confirmation of drug abuse and identification of explosive residues.

Video Content / Details of website for further learning (if any):

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Banwell, Colin N. and E.M. McCash, "Fundamentals of Molecular Spectroscopy" Tata McGraw-Hill, 2007 (Pg. No. 200-201).

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LECTURE HANDOUTS

BIOTECH

III/VI

Course Name with Code : **BIOLOGICAL SPECTROSCOPY 16BTE15**

Course Faculty : **Dr. G. Pratap Kumar**

Unit : **IV** **Date of Lecture:**

Topic of Lecture: Scattering by X-rays

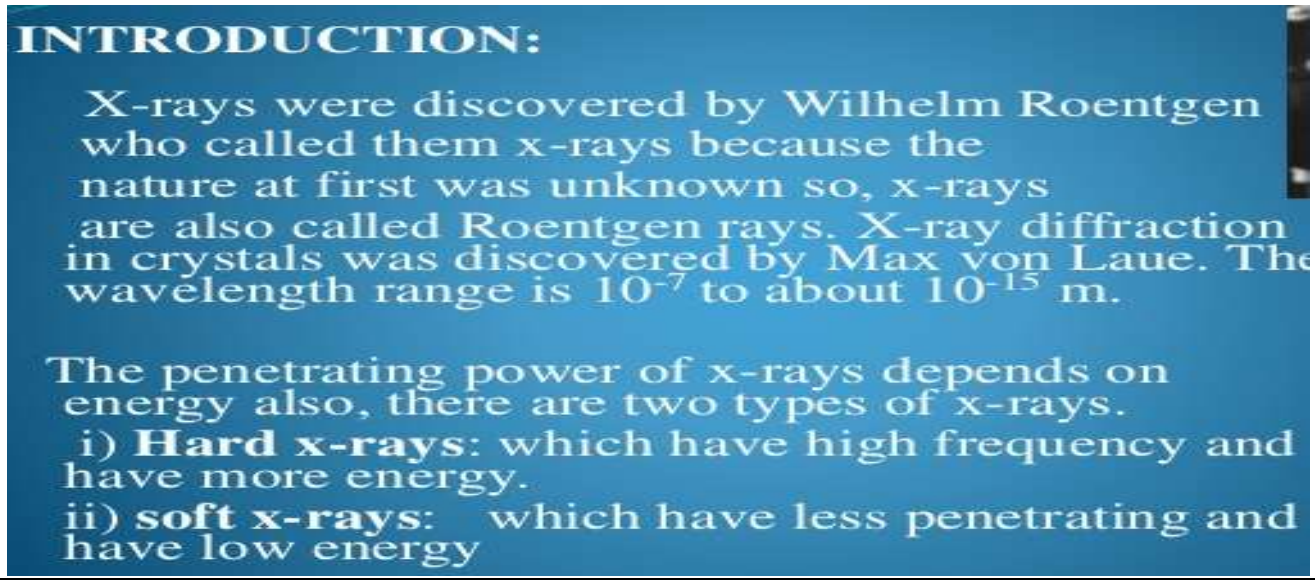
Introduction :

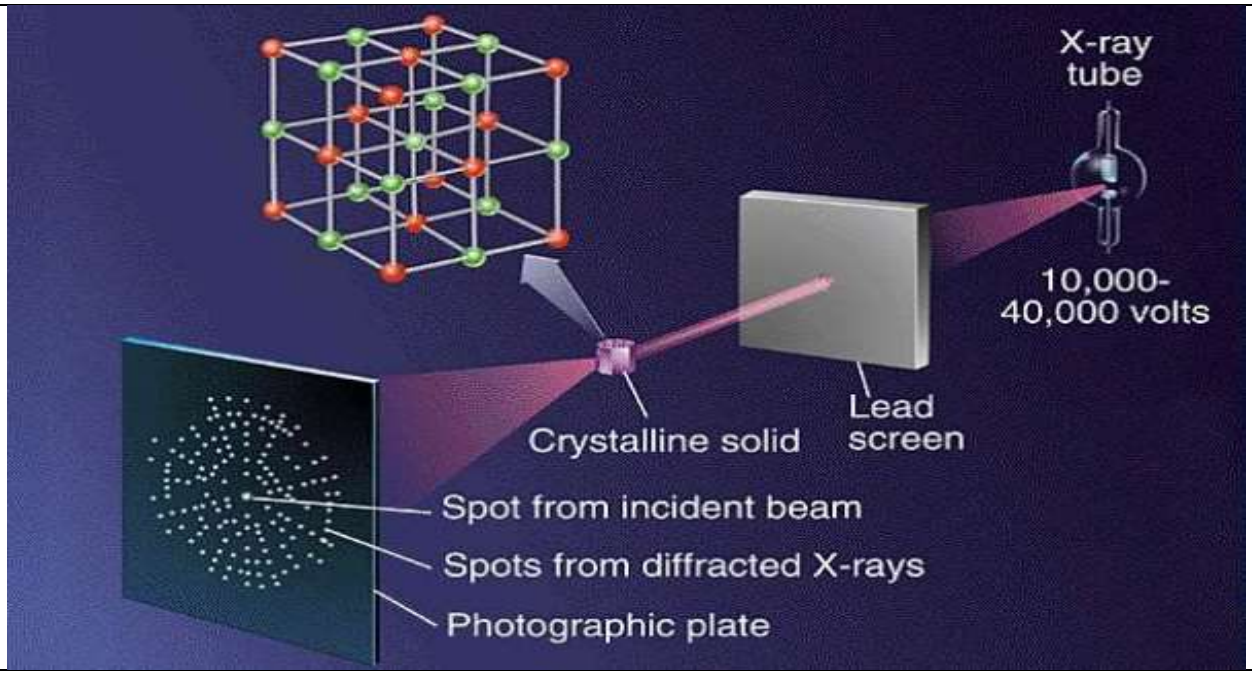
- X-rays are scattered at the electrons of the atomic shell. When a sample is illuminated by x-rays these incident x-rays can be deflected and scattered by the sample producing complex patterns.
- Analysis of these patterns, their intensities as well as the angel of scatter, changes in polarization, wavelength and energy can reveal structural, elemental and atomic information about the sample and are known as x-ray scattering techniques.
- X-ray scattering can be applied to a wide range of different sample types, from simple repeating crystals to novel materials and complex biological molecules.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on some of the applications of MS in various fields.
- Prerequisite knowledge on understanding and knowing the techniques applied through MS in PMF or mapping, protein folding, etc.

Detailed content of the Lecture:





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Banwell, Colin N. and E.M. McCash, "Fundamentals of Molecular Spectroscopy" Tata McGraw-Hill, 2007 (Pg. No. 203-208).

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BIOTECH

III/VI

Course Name with Code : **BIOLOGICAL SPECTROSCOPY 16BTE15**

Course Faculty : **Dr. G. Pratap Kumar**

Unit : **IV** **Date of Lecture:**

Topic of Lecture: Diffraction by a crystal

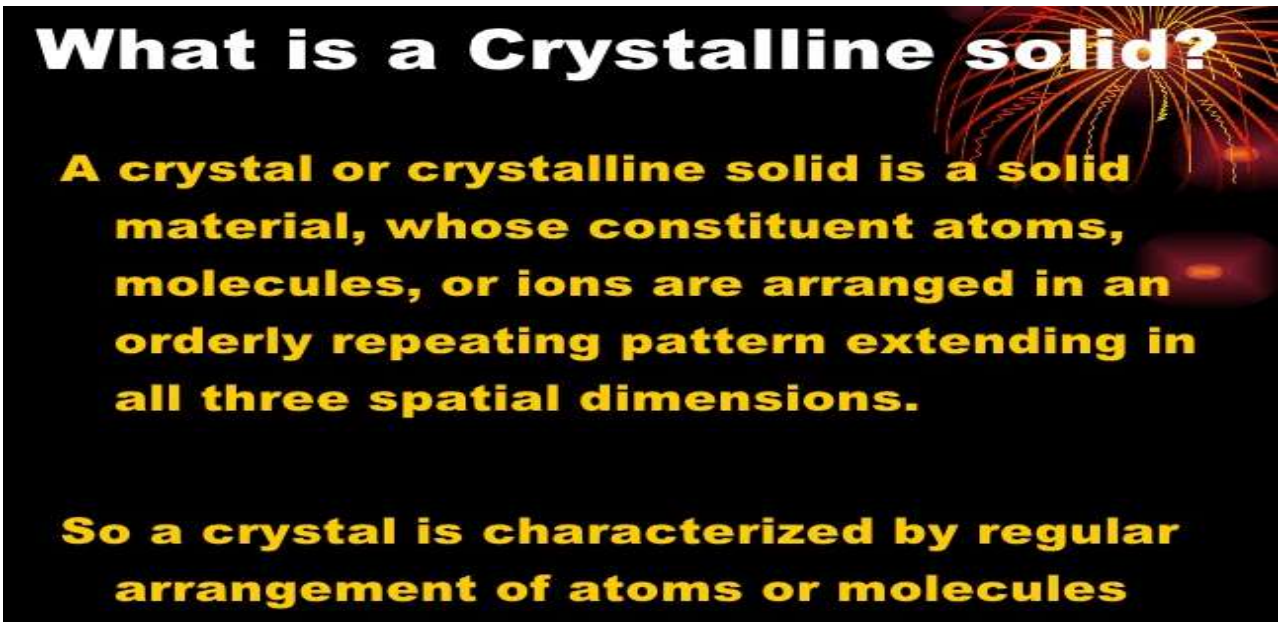
Introduction :

- In crystal diffraction, everything moves like a wave and exchanges energy and momentum like a particle.
- When waves move through a crystal they diffract. Light, sound, neutrons, atoms and electrons are all diffracted by crystals.
- The shape and the dimensions of the unit cell can be deduced from the position of the Bragg reflections; the content of the unit cell, on the other hand must be determined from the intensities of the reflections.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on some of the applications of MS in various fields.
- Prerequisite knowledge on understanding and knowing the techniques applied through MS in PMF or mapping, protein folding, etc.

Detailed content of the Lecture:



- To get the diffraction pattern from all parts of crystal, the primary beam must strike the crystal from many different directions. This is achieved by rotating the crystal in the beam during the experiment.

- The diffracted spots are recorded either on a film or by an electronic detector feed the signals directly in a digitized form into a computer. Several thousand diffraction spots are collected.

- All diffraction methods are based on generation of X-rays in an X-ray tube. These X-rays are directed at the sample, and the diffracted rays are collected.

Video Content / Details of website for further learning (if any):

Important Books/Journals for further learning including the page nos.:

Banwell, Colin N. and E.M. McCash, "Fundamentals of Molecular Spectroscopy" Tata McGraw-Hill, 2007 (Pg. No. 211-215).

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LECTURE HANDOUTS

BIOTECH

III/VI

Course Name with Code : **BIOLOGICAL SPECTROSCOPY 16BTE15**

Course Faculty : **Dr. G. Pratap Kumar**

Unit : **IV** **Date of Lecture:**

Topic of Lecture: Measuring diffraction pattern

Introduction :

- Diffraction pattern from single crystals produced by divergent radiation and consisting of continuous sets of lines have been observed with x-rays, electrons and other radiations under a great variety of experimental conditions.
- It means bending of waves around the corners of an obstacle or through an aperture into the region of geometrical shadow of the aperture.
- These patterns of interference rely on the size of the diffracting object and the size of the wave.

Prerequisite knowledge for Complete understanding and learning of Topic:

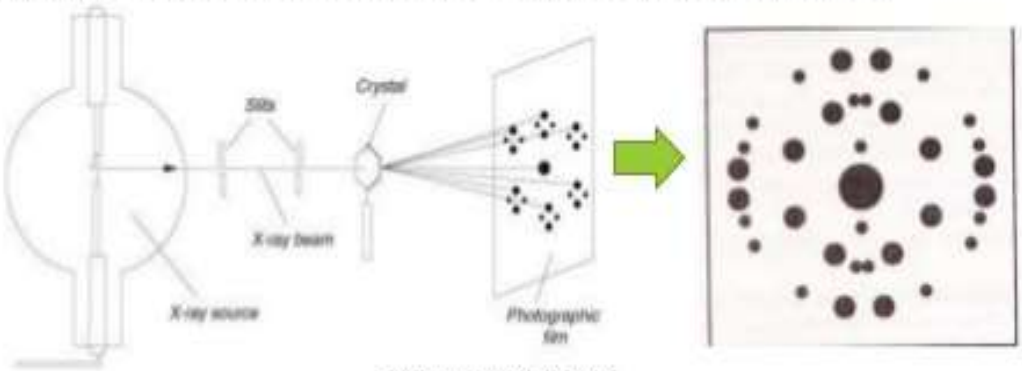
- Prerequisite knowledge on some of the applications of MS in various fields.
- Prerequisite knowledge on understanding and knowing the techniques applied through MS in PMF or mapping, protein folding, etc.

Detailed content of the Lecture:

There are several XRD methods which are generally used for investigating the internal structures and crystal structures of various solid compounds.

The Laue method

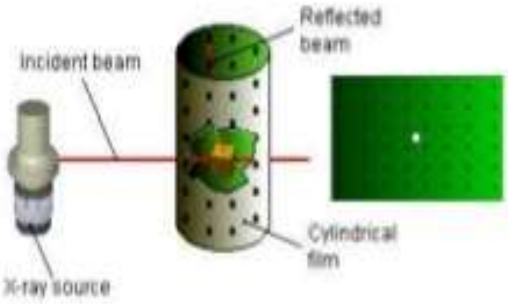
Laue in his very first experiments used white radiation of all possible wavelengths and allowed this radiation to fall on a stationary crystal. The crystal diffracted the X-ray beam and produced a very beautiful pattern of spots which conformed exactly with the internal symmetry of the crystal. Let us analyze the experiment with the aid of the Bragg equation. The crystal was fixed in position relative to the X-ray beam, thus not only was the value for *d* fixed, but the value of was also fixed.



X-Ray Laue Method

Rotating Crystal Method

- ← Single crystal mounted with one axis normal to a monochromatic x-ray beam
- ← Cylindrical film placed around the sample
- ← As sample rotates, some sets of planes momentarily satisfy Bragg condition
- ← When film is laid flat, a series of horizontal lines appears
- ← Because crystal rotates about a single axis, possible Bragg angles are limited - not every plane is able to produce a diffracted spot
- ← Sometimes used to determine unknown crystal structures

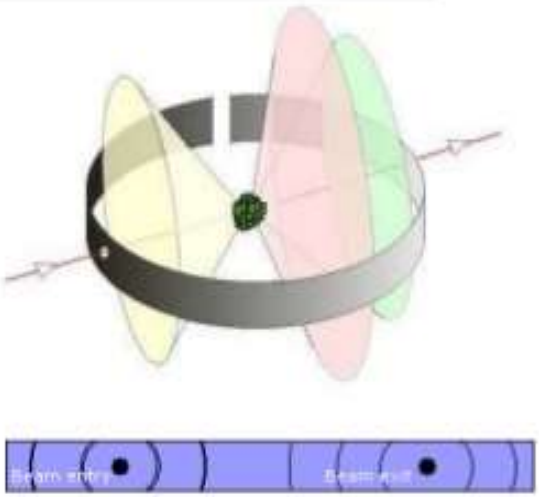


POWDER CRYSTAL METHOD:

- If the angle of incidence is θ then the angle of reflection will be 2θ.
- If the radius is r the circumference 2πr corresponds to a scattering angle of 360°.

$$\theta = 360 * 1/\pi r$$

- From the above equation the value of θ can be calculated and substituted in Bragg's equation to get the value of d.



POWDER CRYSTAL DIFFRACTION

Video Content / Details of website for further learning (if any):

Important Books/Journals for further learning including the page nos.:

Banwell, Colin N. and E.M. McCash, "Fundamentals of Molecular Spectroscopy" Tata McGraw-Hill, 2007 (Pg. No. 217-225).

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Rasipuram - 637 408, Namakkal Dist., Tamil Nadu

LECTURE HANDOUTS

BIOTECH | **III/VI**

Course Name with Code : **BIOLOGICAL SPECTROSCOPY 16BTE15**

Course Faculty : **Dr. G. Pratap Kumar**

Unit : **IV** **Date of Lecture:**

Topic of Lecture: Bragg's reflection

Introduction :

- Bragg's law, a special case of Laue diffraction gives the angles for coherent scattering of waves from a crystal lattice.
- It encompasses the superposition of wave fronts scattered by lattice planes leading to a strict relation between wavelength and scattering angle, with respect to the crystal lattice.
- Bragg's diffraction occurs when radiation of wavelength comparable to atomic spacings, is scattered in a specular fashion by atoms of a crystalline system and undergoes constructive interference.
- The phenomena of Bragg diffraction by a crystal lattice shares similar characteristics with that of thin film interference, which has an identical condition in the limit where the refractive indices of the surrounding medium and the interfering medium.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on some of the applications of MS in various fields.
- Prerequisite knowledge on understanding and knowing the techniques applied through MS in PMF or mapping, protein folding, etc.

Detailed content of the Lecture:

BRAGG'S LAW:

- After few months, In 1913, English physicists **Sir William Henry Bragg** and his son **Sir William Lawrence Bragg** developed a relationship to explain why the cleavage faces of crystals appear to reflect X-ray beams at certain angles of incidence (theta, θ).

- The variable *d* is the distance between atomic layers in a crystal, and the variable lambda λ is the wavelength of the incident X-ray beam; n is an integer.

- Although Bragg's law was used to explain the interference pattern of X-rays scattered by crystals, diffraction has been developed to study the structure of all states of matter with any beam.

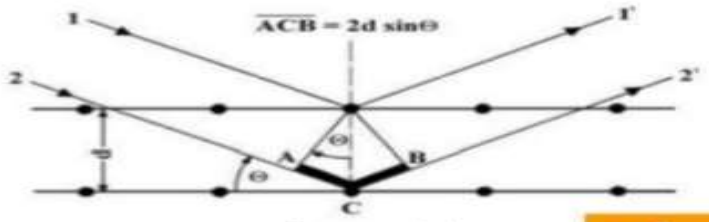
- Bragg carried out a series of experiments, the result of which he published the Bragg equation –

$$n\lambda = 2\,d\sin\theta$$

where, assume n = 1 for the first order reflection
λ = wavelength
θ = X-ray incidence angle
d = distance between atomic layer

BASIC PRINCIPLE

Bragg's law of diffraction:



$\overline{AC} = d\sin\theta$

$\overline{ACB} = 2d\sin\theta$

$n\lambda = \overline{ACB}$

Bragg's Law: $n\lambda = 2d\sin\theta$

When bragg's law is satisfied, reflected beams are in phase and interfere constructively to produce diffraction patterns

Video Content / Details of website for further learning (if any):

Important Books/Journals for further learning including the page nos.:

Banwell, Colin N. and E.M. McCash, "Fundamentals of Molecular Spectroscopy" Tata McGraw-Hill, 2007 (Pg. No. 227-230).

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LECTURE HANDOUTS

BIOTECH **III/VI**

Course Name with Code : **BIOLOGICAL SPECTROSCOPY 16BTE15**

Course Faculty : **Dr. G. Pratap Kumar**

Unit : **IV** **Date of Lecture:**

Topic of Lecture: Unit cell

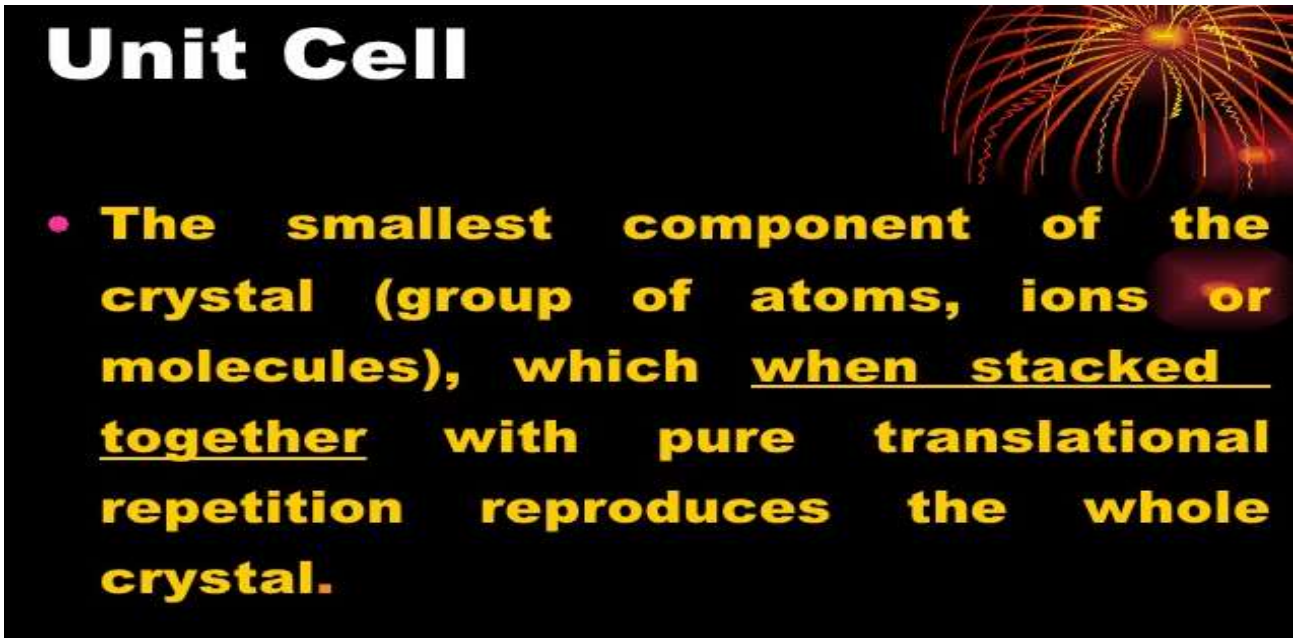
Introduction :

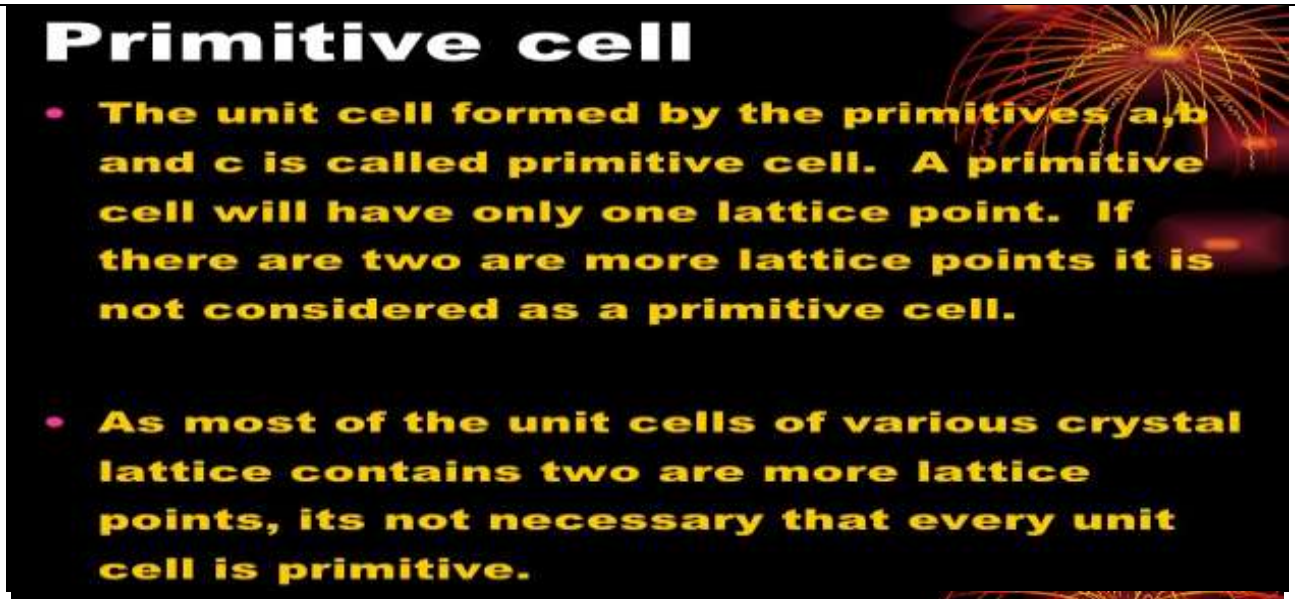
- A unit cell is the smallest repeating portion of a crystal lattice. Unit cells occur in many different varieties.
- A crystal can be thought of as the same unit cell repeated over and over in three dimensions.
- Each sphere represents an atom or an ion.

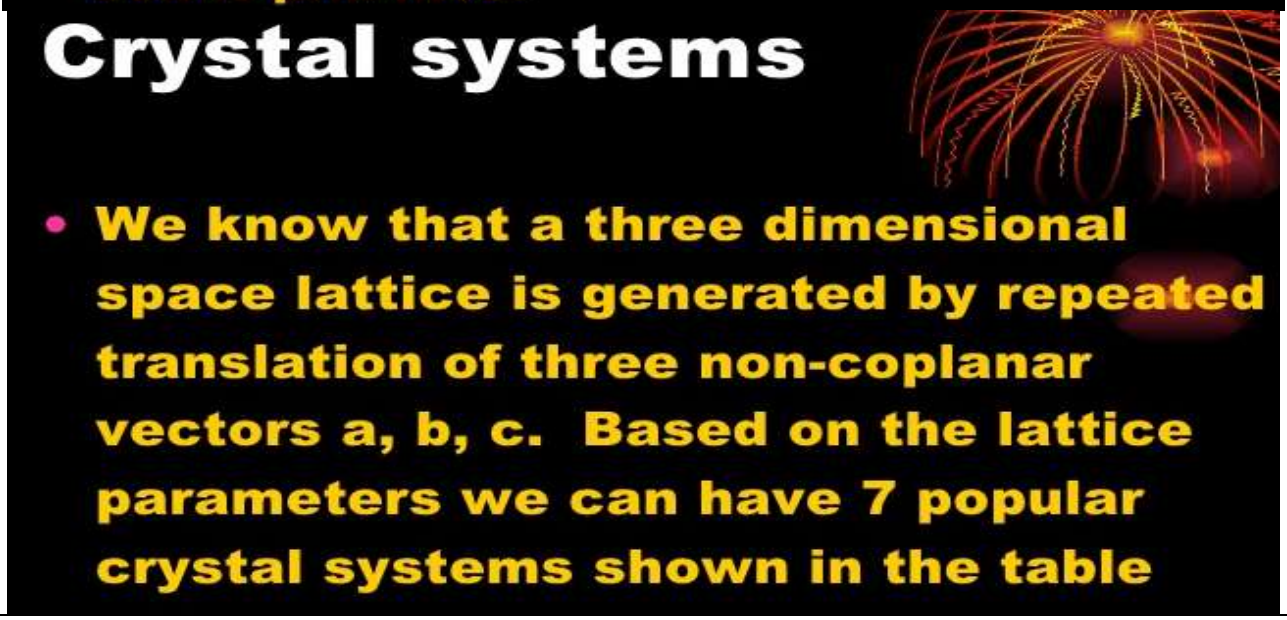
Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on some of the applications of MS in various fields.
- Prerequisite knowledge on understanding and knowing the techniques applied through MS in PMF or mapping, protein folding, etc.

Detailed content of the Lecture:







Video Content / Details of website for further learning (if any):

Important Books/Journals for further learning including the page nos.:

Banwell, Colin N. and E.M. McCash, "Fundamentals of Molecular Spectroscopy" Tata McGraw-Hill, 2007 (Pg. No. 231-235).

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LECTURE HANDOUTS

BIOTECH | **III/VI**

Course Name with Code : **BIOLOGICAL SPECTROSCOPY 16BTE15**

Course Faculty : **Dr. G. Pratap Kumar**

Unit : **IV** **Date of Lecture:**

Topic of Lecture: Phase problem- Methods

Introduction :

- Phase problem is the problem of loss of information concerning the phase that can occur when making a physical measurement.
- When waves are diffracted from a crystal they give rise to diffraction spots. Each diffraction spot corresponds to a point in the reciprocal lattice and represents a wave with an amplitude and represents a wave with an amplitude and a relative phase.
- The phase problem must be solved in x-ray crystallography, neutron crystallography and electron crystallography. Not all of the methods of phase retrieval work with every wavelength used in crystallography.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on some of the applications of MS in various fields.
- Prerequisite knowledge on understanding and knowing the techniques applied through MS in PMF or mapping, protein folding, etc.

Detailed content of the Lecture:

- ❑ From x-ray diffraction, we have obtained two parameters.

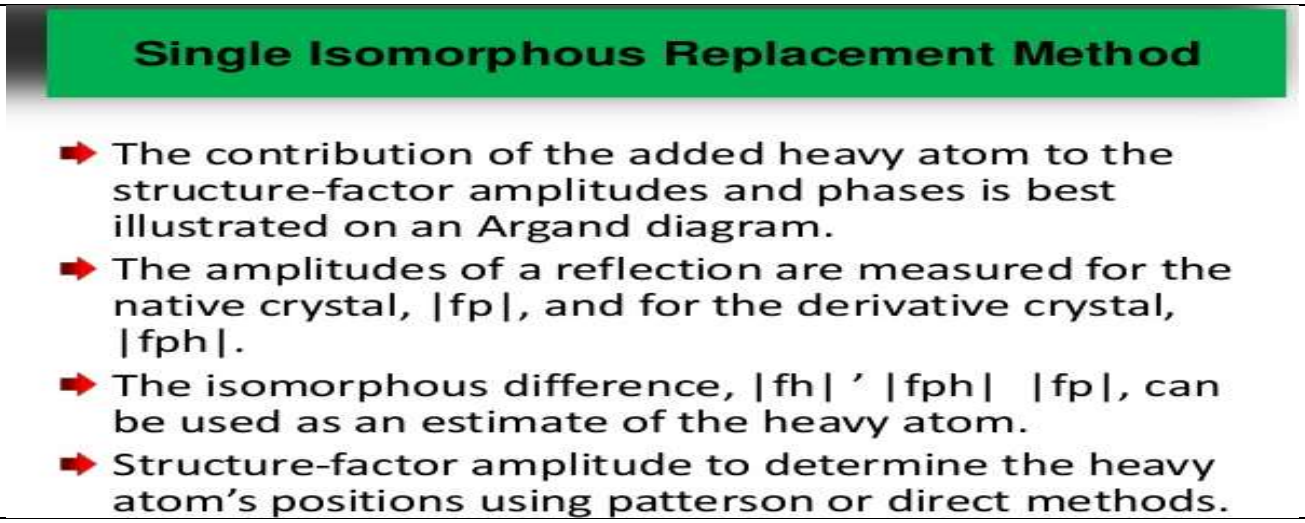
A. Amplitudes

B. Phases

- ❑ In almost most of the cases amplitudes are retrieved but retrieving of phases is a bit difficult issue.
- ❑ In small molecule crystallography basic assumptions on atomicity and amplitudes can give rise to phase extraction. But, it is not possible in macromolecular crystallography.

Methods to solve phase problem:

- **Molecular Isomorphous Replacement Method**
  - A. Single Isomorphous Replacement Method
- **Anomalous Scattering Method**
  - A. Single wavelength anomalous diffraction method (SAD)
  - B. Multiple wavelength anomalous diffraction method (MAD)



Video Content / Details of website for further learning (if any):

Important Books/Journals for further learning including the page nos.:

Banwell, Colin N. and E.M. McCash, "Fundamentals of Molecular Spectroscopy" Tata McGraw-Hill, 2007 (Pg. No. 235-240).

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LECTURE HANDOUTS

BIOTECH

III/VI

Course Name with Code : **BIOLOGICAL SPECTROSCOPY 16BTE15**

Course Faculty : **Dr. G. Pratap Kumar**

Unit : **IV** **Date of Lecture:**

Topic of Lecture: Anomalous diffraction

Introduction :

- Anomalous scattering which contains atoms called anomalous scatterers. By changing the wavelength of the X-rays, you can change the degree to which the anomalous scatterers perturb the diffraction pattern.
- Scattering information of an atom whose absorption frequency is close to the wavelength of the source beam produces phase information
- Resolved anomalous scattering requires intensity measurements at one wavelength.
- Multi-wavelength anomalous dispersion, requires intensity measurements at several wavelengths.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on some of the applications of MS in various fields.
- Prerequisite knowledge on understanding and knowing the techniques applied through MS in PMF or mapping, protein folding, etc.

Detailed content of the Lecture:

Anomalous Dispersion Methods

- All elements display an anomalous dispersion (AD) effect in X-ray diffraction .
- For elements such as e.g. C,N,O, etc., AD effects are negligible.
- For heavier elements, especially when the X-ray wavelength approaches an atomic absorption edge of the element, these AD effects can be very large.
- The scattering power of an atom exhibiting AD effects is:

$$f_{AD} = f_n + \Delta f' + i\Delta f''$$

f_n is the normal scattering power of the atom in absence of AD effects
$\Delta f'$ arises from the AD effect and is a real factor (+/- signed) added to f_n
$\Delta f''$ is an imaginary term which also arises from the AD effect
$\Delta f''$ is always positive and 90 ahead of $(f_n + \Delta f')$ in phase angle

The values of $\Delta f'$ and $\Delta f''$ are **highly dependent on the wave-length** of the X-radiation.

In the absence AD effects, $I_{hkl} = I_{-h-k-l}$ (Firedel's Law).

With AD effects, $I_{hkl} \neq I_{-h-k-l}$ (Friedel's Law breaks down).

SINGLE WAVELENGTH ANOMALOUS DIFFRACTION

- SAD can simply utilize the intrinsic anomalous scatterers present in the macromolecule, such as the S atoms of cysteine and methionine or bound ions.
- The challenge is in maximizing and measuring the very small signal, since the Bijvoet ratio can be as low as 1% when the typical merging R factor is several times this value.
- The trick lies in making multiple measurements of reflections at an appropriate wavelength in order to achieve a high multiplicity that will give statistically accurate measurements of the anomalous difference.
- The data should also be as complete as possible

Multiple Wavelength Anomalous Diffraction method

- Isomorphous replacement has several problems:
- Nonisomorphism between crystals (unit-cell changes, reorientation of the protein.
- Conformational changes, changes in salt and solvent ions.
- Problems in locating all the heavy atoms.
- Problems in refining heavy-atom positions, occupancies.
- Thermal parameters and errors in intensity measurements.
- Data are collected from a single crystal at several wavelengths, typically three, in order to maximize the absorption and dispersive effects.
- Wavelengths are chosen at the absorption (f'') peak (λ1), at the point of inflection on the absorption curve (λ 2), where the dispersive term f ' has its minimum, and at a remote wavelength (λ 3 and/or λ 4) to maximize the dispersive difference to λ 2.

Video Content / Details of website for further learning (if any):

Important Books/Journals for further learning including the page nos.:

Banwell, Colin N. and E.M. McCash, "Fundamentals of Molecular Spectroscopy" Tata McGraw-Hill, 2007 (Pg. No. 293-295).

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LECTURE HANDOUTS

BIOTECH

III/VI

Course Name with Code : **BIOLOGICAL SPECTROSCOPY 16BTE15**

Course Faculty : **Dr. G. Pratap Kumar**

Unit : **IV** **Date of Lecture:**

Topic of Lecture: Determination of crystal structure

Introduction :

- To solve a crystal structure means to determine the precise spatial arrangements of all of the atoms in a chemical compound in the crystalline state.
- Crystal structures are determined by scattering experiments using a portion of the crystal as the target.

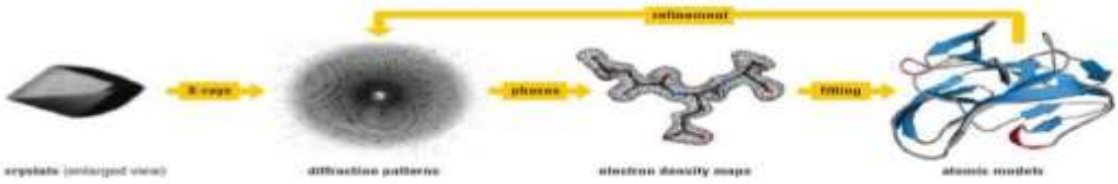
Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on some of the applications of MS in various fields.
- Prerequisite knowledge on understanding and knowing the techniques applied through MS in PMF or mapping, protein folding, etc.

Detailed content of the Lecture:

Steps in Structure Determination

1. Protein purification.
2. Protein crystallization.
3. Data collection.
4. Structure Solution (Phasing)
5. Structure determination (Model building and refinement)



Video Content / Details of website for further learning (if any):

Important Books/Journals for further learning including the page nos.:

Banwell, Colin N. and E.M. McCash, "Fundamentals of Molecular Spectroscopy" Tata McGraw-Hill, 2007 (Pg. No. 288-289).

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LECTURE HANDOUTS

BIOTECH | **III/VI**

Course Name with Code : **BIOLOGICAL SPECTROSCOPY 16BTE15**

Course Faculty : **Dr. G. Pratap Kumar**

Unit : **IV** **Date of Lecture:**

Topic of Lecture: Electron and Neutron diffraction

Introduction :

- Electron diffraction refers to the wave nature of electrons. However, from a technical or practical point of view, it may be regarded as a technique used to study matter by firing electrons at a sample and observing the resulting interference pattern.
- Electron diffraction is most frequently used in solid state physics and chemistry to study the crystal structure of solids.
- In neutron diffraction, the application of neutron scattering to the determination of the atomic and magnetic structure of a material. A sample to be examined is placed in a beam of thermal or cold neutrons to obtain a diffraction pattern that provides information of the structure of the material.
- The technique requires a source of neutrons. Neutrons are usually produced in a nuclear reactor or spallation source.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on some of the applications on MS in various fields.
- Prerequisite knowledge on understanding and knowing the techniques applied through MS in PMF or mapping, protein folding, etc.

Detailed content of the Lecture:

How X-ray Differs From Electron Waves?

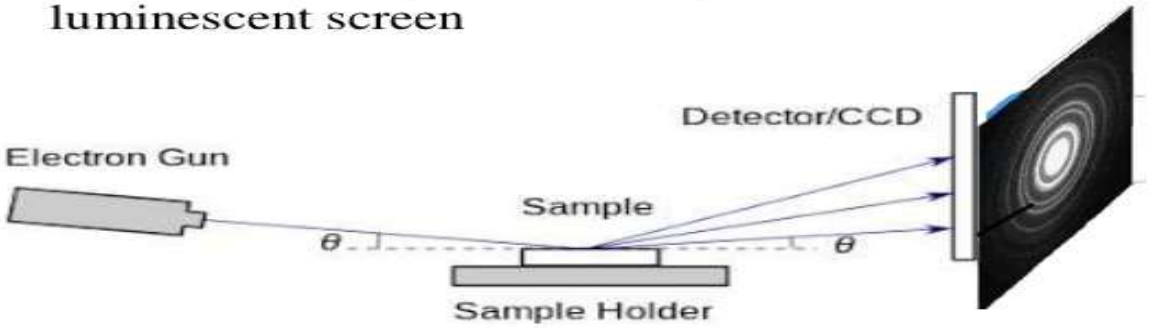
- As Louis De-broglie predicted that wave properties should also be associated with moving electrons and hence the wavelength associated with the electrons are given by

$$\lambda = \frac{h}{mv}$$

- v depends on potential difference(p.d)
- For 10 to 10,000 volts λ varies between 3.89 to 0.12Å hence such electrons act as X-Ray towards crystal. 10,000 to 40,000 volts applied to get high speed electrons to be used in diffraction

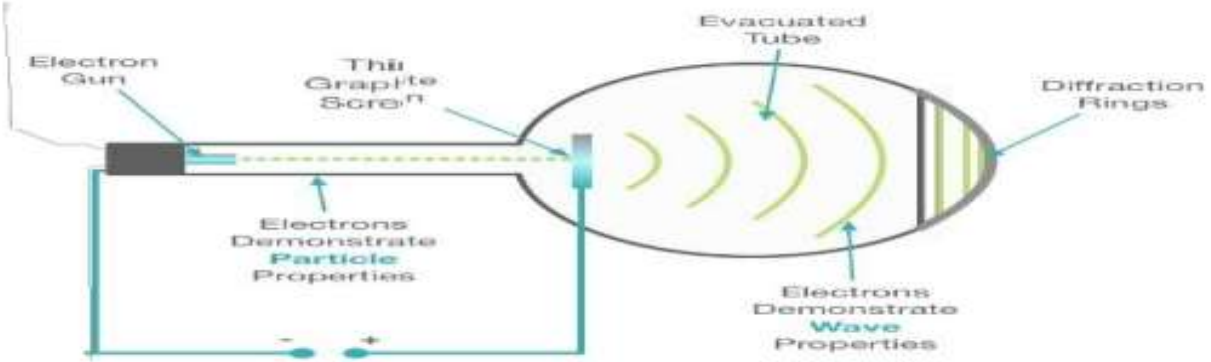
INSTRUMENTATION

- It consists mainly of electron gun target and luminescent screen



WORKING

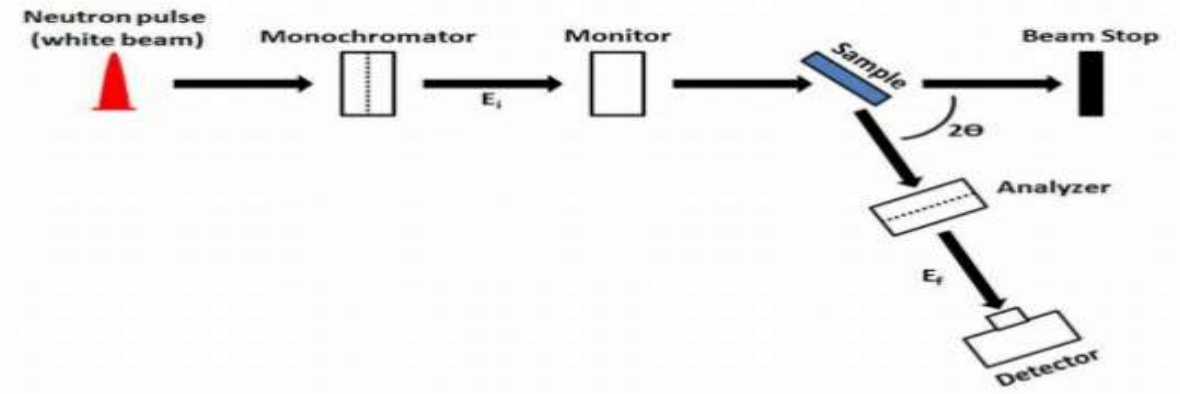
- ➢ It involves generation of electrons by a hot filament, made accelerated by applying p.d of about 40,000 volts, passage of electrons passing through the sample
- ➢ As a result it get diffracted and the effect is seen on the fluorescent screen in the form of concentric rings



NEUTRON DIFFRACTION

- Neutron diffraction is the application of neutron scattering to the determination of the atomic structure of the material
- When a beam of neutrons emanating from a reactor is slowed down and selected properly by their speed, their wavelength lies near one angstrom (0.1 nanometer), the typical separation between atoms in a solid material. Such a beam can then be used to perform a diffraction experiment.

It consists mainly of neutron source, monochromator and detector.



WORKING

- The technique requires a source of neutrons. Neutrons are usually produced in a nuclear reactor or spallation source.
- At a research reactor, other components are needed, including a crystal monochromators, as well as filters to select the desired neutron wavelength.
- Sample requirement: Single crystal work is also possible, but the crystals must be much larger than those that are used in single-crystal X-ray crystallography.

Video Content / Details of website for further learning (if any):

Important Books/Journals for further learning including the page nos.:

Banwell, Colin N. and E.M. McCash, "Fundamentals of Molecular Spectroscopy" Tata McGraw-Hill, 2007 (Pg. No. 280-290).

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LECTURE HANDOUTS

BIOTECH | **III/VI**

Course Name with Code : **BIOLOGICAL SPECTROSCOPY 16BTE15**

Course Faculty : **Dr. G. Pratap Kumar**

Unit : **V** **Date of Lecture:**

Topic of Lecture: Electron microscopy

Introduction :

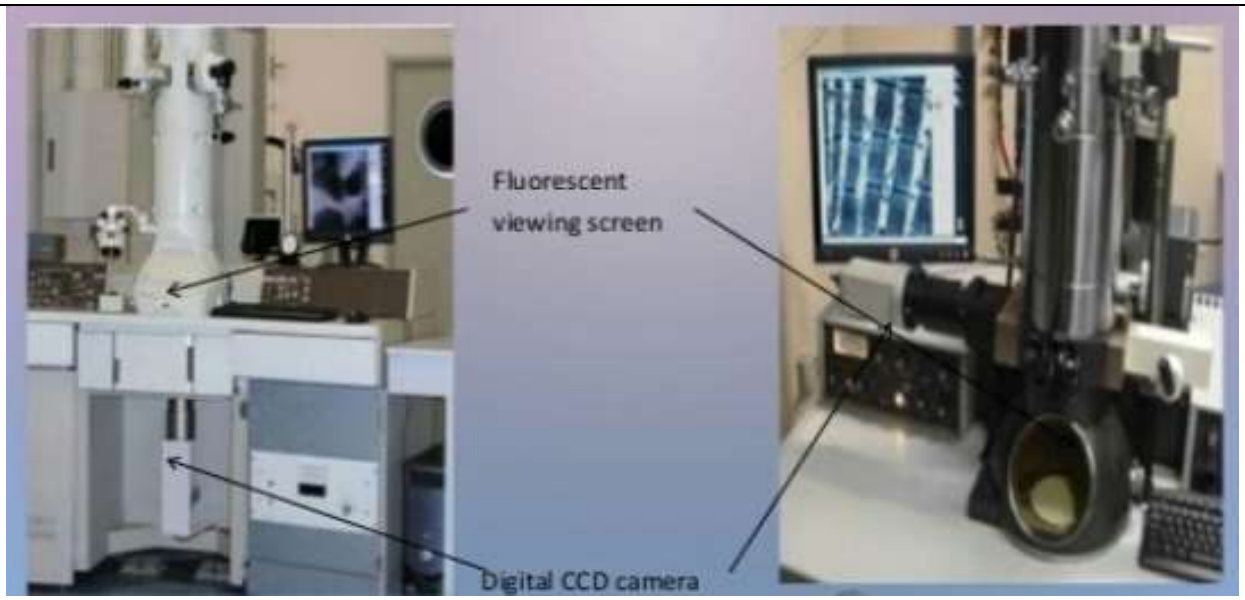
- Electron microscopy uses an electron beam to create an image of a sample. The EM operates under vacuum which means the samples are placed in a vacuum system during analysis.
- It's a special type of microscope having a high resolution of images, able to magnify objects in nm, which are formed by controlled use of electrons in vacuum captured on a phosphorescent screen.
- The image formed results from a scattering of electrons by atoms in the specimen.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on knowing the principles of microscopy.
- Prerequisite knowledge on understanding the techniques applied through microscopes in sample, specimens, etc.

Detailed content of the Lecture:

- Electron microscopes use signals arising from the interaction of an electron beam with the sample to obtain information about structure, morphology and composition.
- The electron gun generates electrons. Two sets of condenser lenses focus the electron beam on the specimen and then into a thin tight beam.
- To move electrons down the column, an accelerating voltage is applied between the tungsten filament and anode.
- The specimen to be examined is made extremely thin, at least 200 times thinner than those used in the optical microscope. Ultra-thin sections of 20-100 nm are cut which is already placed on the specimen holder.
- The electronic beam passes through the specimen and electrons are scattered depending upon the thickness or refractive index of different parts of the specimen.
- The denser regions in the specimen scatter more electrons and therefore appear darker in the image since fewer electrons strike that area of the screen. In contrast, transparent regions are brighter.
- The electron beam coming out of the specimen passes to the objective lens, which has high power and forms the intermediate magnified image.
- The ocular lenses then produce the final further magnified image.



There are two types of electron microscopes, with different operating styles: TEM and SEM

Video Content / Details of website for further learning (if any):

Important Books/Journals for further learning including the page nos.:

Banwell, Colin N. and E.M. McCash, "Fundamentals of Molecular Spectroscopy" Tata McGraw-Hill, 2007 (Pg. No. 293-295).

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LECTURE HANDOUTS

BIOTECH | **III/VI**

Course Name with Code : **BIOLOGICAL SPECTROSCOPY 16BTE15**

Course Faculty : **Dr. G. Pratap Kumar**

Unit : **V** **Date of Lecture:**

Topic of Lecture: Transmission Electron Microscopy

Introduction :

- TEM is a microscopy technique in which a beam of electrons is transmitted through a specimen to form an image. The specimen is most often an ultrathin section less than 100 nm thick or a suspension on a grid.
- An image is formed from the interaction of the electrons with the sample as the beam is transmitted through the specimen. The image is then magnified and focused onto an imaging device, such as a fluorescent screen, a layer of photographic film attached to a charge-coupled device.
- TEM are capable of imaging at a significantly higher resolution than light microscopes, owing to the smaller de Broglie wavelength of electrons.

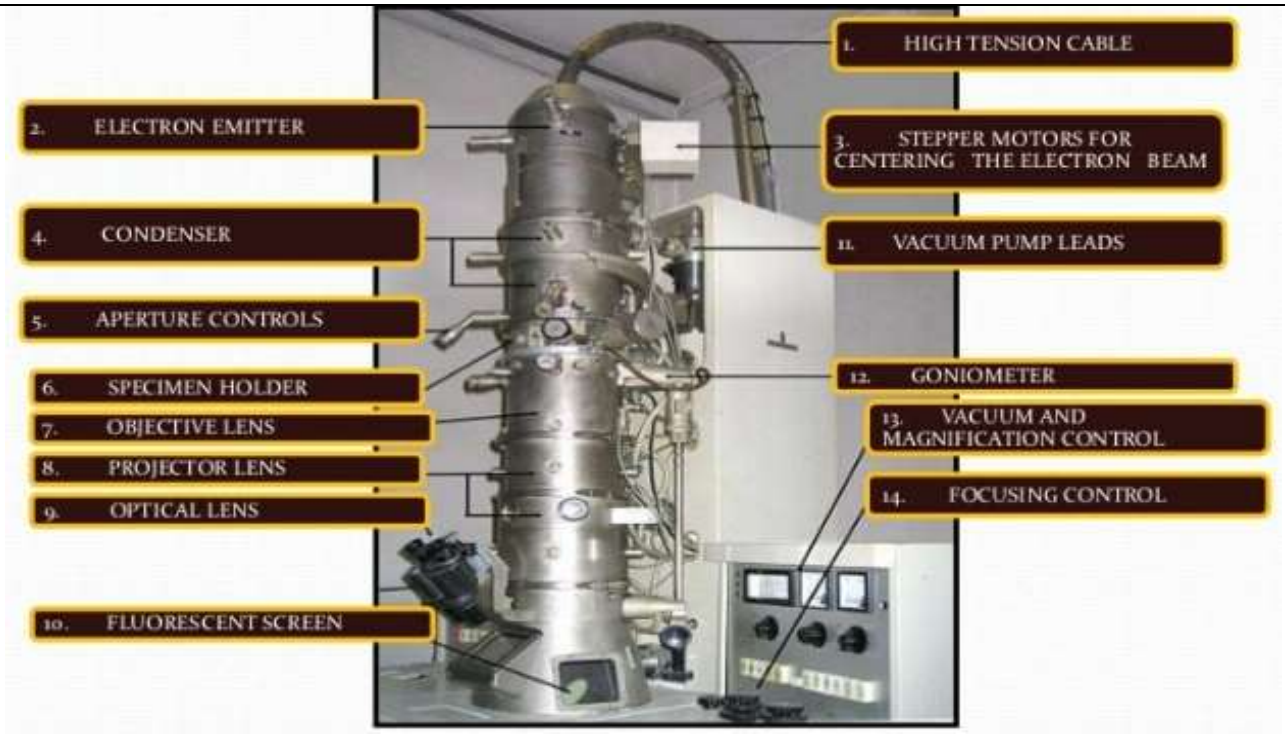
Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on applications of TEM.
- Prerequisite knowledge on understanding and knowing the principles of TEM in sample analysis.

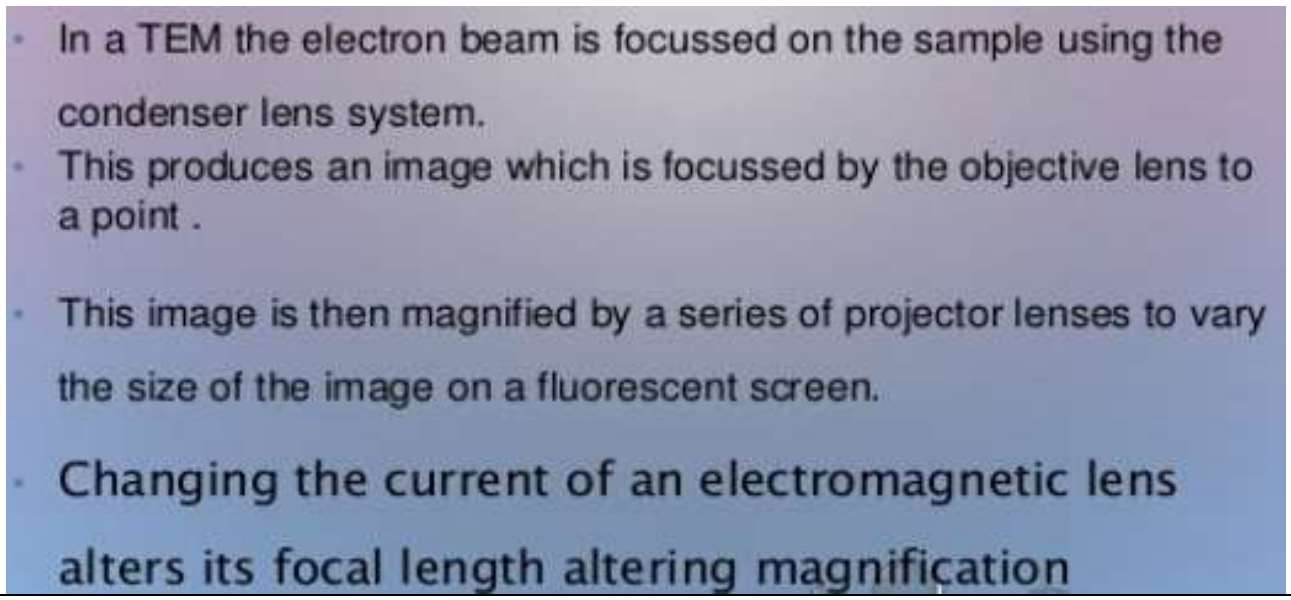
Detailed content of the Lecture:

- TEM is a microscopy technique where a beam of electrons is transmitted through an ultra-thin specimen. An image is formed from the interaction of the electrons transmitted through the specimen; the image is magnified and focused onto an imaging device, such as fluorescent screen, on a layer of photographic film or to be detected by a sensor such as a CCD camera.

INSTRUMENTATION:



WORKING:



Video Content / Details of website for further learning (if any):

Important Books/Journals for further learning including the page nos.:

Banwell, Colin N. and E.M. McCash, "Fundamentals of Molecular Spectroscopy" Tata McGraw-Hill, 2007 (Pg. No. 295-298).

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BIOTECH | **III/VI**

Course Name with Code : **BIOLOGICAL SPECTROSCOPY 16BTE15**

Course Faculty : **Dr. G. Pratap Kumar**

Unit : **V** **Date of Lecture:**

Topic of Lecture: Scanning Electron Microscopy

Introduction :

- Scanning Electron Microscope is a type of electron microscope that produces images of a sample by scanning the surface with a focused beam of electrons.
- The electrons interact with atoms in the sample producing various signals that contain information about the surface topography and composition of the sample.
- In the most common SEM mode, secondary electrons emitted by atoms excited by the electron beam are detected using a secondary electron detector.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on some of the applications of SEM in various fields.
- Prerequisite knowledge on understanding and knowing the techniques on SEM in analysis of sample and specimen.

Detailed content of the Lecture:

- SEM is a type of electron microscope that images a sample by scanning it with a high-energy beam of electrons in a raster scan pattern. The electrons interact with the atoms that make up the sample producing signals that contain information about the sample's surface topography, composition and other properties.

INSTRUMENTATION:

WORKING:

- The virtual source at the top represents the electron gun, producing a stream of monochromatic electrons.
- The stream is condensed by the first condenser lens. This lens is used to both form the beam and limit the amount of current in the beam. It works in conjunction with the condenser aperture to eliminate the high-angle electrons from the beam.
- The beam is then constricted by the condenser aperture, eliminating some high-angle electrons.
- The second condenser lens forms the electrons into a thin, tight, coherent beam and is usually controlled by the fine probe current knob.
- A user selectable objective aperture further eliminates high-angle electrons from the beam.
- A set of coils then scan or sweep the beam in a grid fashion, dwelling on points for a period of time determined by the scan speed.
- The final lens, the objective focusses the scanning beam onto the part of the specimen desired. When the beam strikes the sample interactions occur inside the sample and are detected with various instruments.
- Before the beam moves to its next dwell point these instruments count the number of electron interactions and display a pixel on a CRT whose intensity is determined by this number.
- This process is repeated until the grid scan is finished and then repeated, the entire pattern can be scanned 30 times/sec.

Video Content / Details of website for further learning (if any):

Important Books/Journals for further learning including the page nos.:

Banwell, Colin N. and E.M. McCash, "Fundamentals of Molecular Spectroscopy" Tata McGraw-Hill, 2007 (Pg. No. 298-300).

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LECTURE HANDOUTS

BIOTECH

III/VI

Course Name with Code : **BIOLOGICAL SPECTROSCOPY 16BTE15**

Course Faculty : **Dr. G. Pratap Kumar**

Unit : **V** **Date of Lecture:**

Topic of Lecture: Scanning Tunneling Microscopy

Introduction :

- Scanning Tunneling Microscopy (STM) is a type of microscope used for imaging surfaces at the atomic level. It is widely used in both industrial and fundamental research to obtain atomic-scale images of metal surfaces.
- The electron cloud associated with metal atoms at a surface extends a very small distance above the surface. When a very sharp tip-in practice a needle which has been treated so that a single atom projects from its end is brought sufficiently close to such surface, there is a strong interaction between the electron cloud on the surface and that of the tip atom and an electric tunneling current flows when a small voltage is applied.
- At a separation of a few atomic diameters, the tunneling current rapidly increases as the distance between the tip and the surface decreases. This rapid change of tunneling current with distance results in atomic resolution if the tip is scanned over the surface to produce an image.

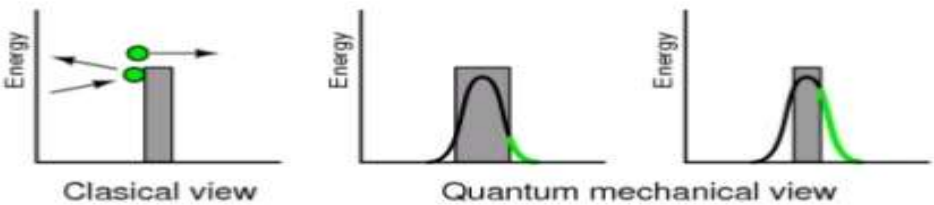
Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on some of the applications of STM in various fields.
- Prerequisite knowledge on understanding and knowing the techniques and principle of STM in biomolecules.

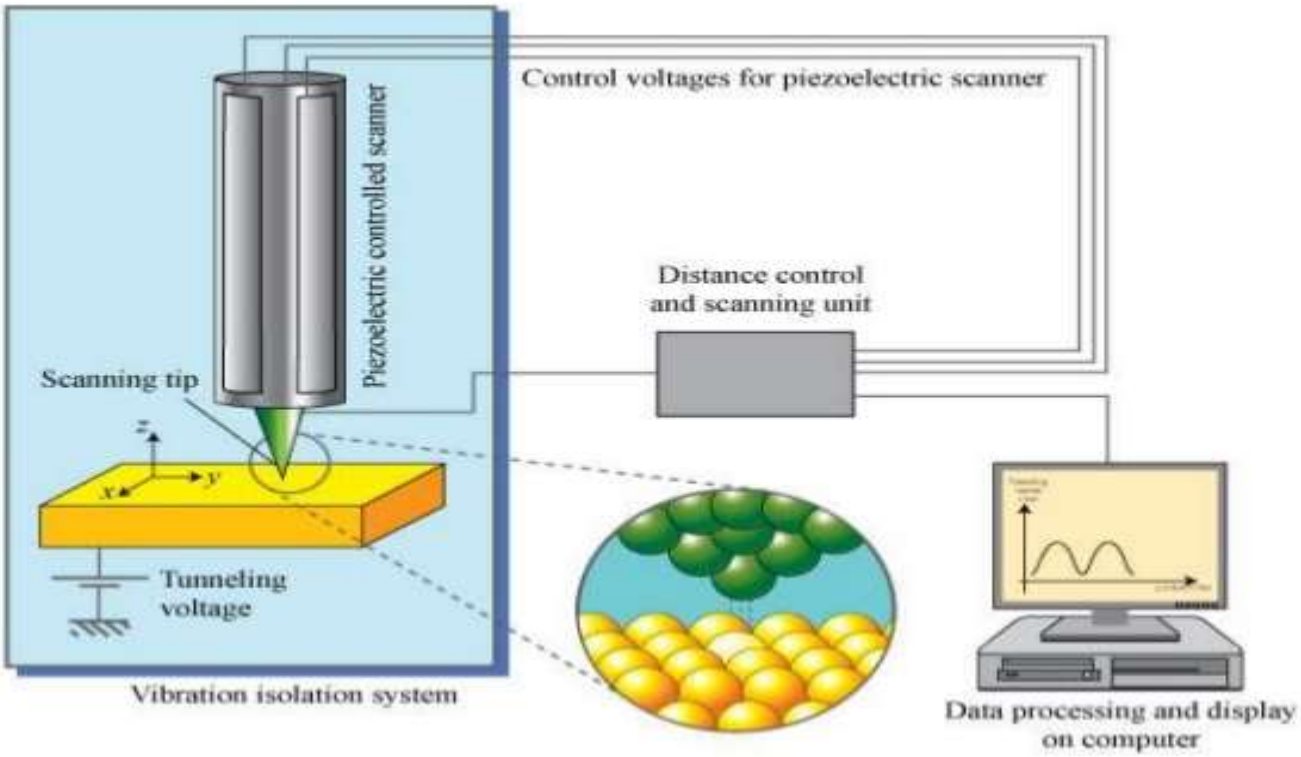
Detailed content of the Lecture:

PRINCIPLE:

- The principle of scanning tunneling microscopy is in quantum mechanics which is different from classical mechanics.
- While classical mechanics deals up to macroscopic level, quantum mechanics deals with microscopic level.
- Quantum mechanics explains the wave and particle like behavior of tiny particles like photons and electrons.
- The quantum mechanics phenomenon which explains tunneling effect is the working principal of scanning tunneling microscopy.



INSTRUMENTATION:



WORKING:

- A small voltage is applied between the tip and the sample surface. This applied voltage is typically a few millivolt to a few volt which depends upon the material of the sample.

- When the tip is brought close enough (5 to 10Å) to the sample, the tunneling phenomenon occurs which results in a net current in the range of 10pA to 10 nA .

- Tunneling is purely a quantum mechanical phenomenon and it is well known that according to classical mechanics, if there is no contact between the tip and surface, no current can flow.

- The tunneling current varies exponentially with respect to the separation between the tip and the surface (d) of the sample.

$$IH = V \exp(-2Kd)$$

Where, K is the wave vector associated with the particles in the tunnel barrier, in this case, the vacuum between the tip and the sample,

Video Content / Details of website for further learning (if any):

Important Books/Journals for further learning including the page nos.:

Banwell, Colin N. and E.M. McCash, "Fundamentals of Molecular Spectroscopy" Tata McGraw-Hill, 2007 (Pg. No. 312-315).

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LECTURE HANDOUTS

BIOTECH

III/VI

Course Name with Code : **BIOLOGICAL SPECTROSCOPY 16BTE15**

Course Faculty : **Dr. G. Pratap Kumar**

Unit : **V** **Date of Lecture:**

Topic of Lecture: Atomic Force Microscopy

Introduction :

- Atomic Force Microscopy (AFM) or Scanning Force Microscopy (SFM) is a very high resolution type of scanning probe microscopy (SPM) with demonstrated resolution on the order of fractions of a nanometer more than 1000 times better than the optical diffraction limit.
- It has the advantage of imaging almost any type of surface, including polymers, ceramics, composites, glass and biological samples.
- The AFM relies on the forces between the tip and sample, these forces impact AFM imaging. The force is not measured directly but calculated by measuring the deflection of the lever, knowing the stiffness of the cantilever.

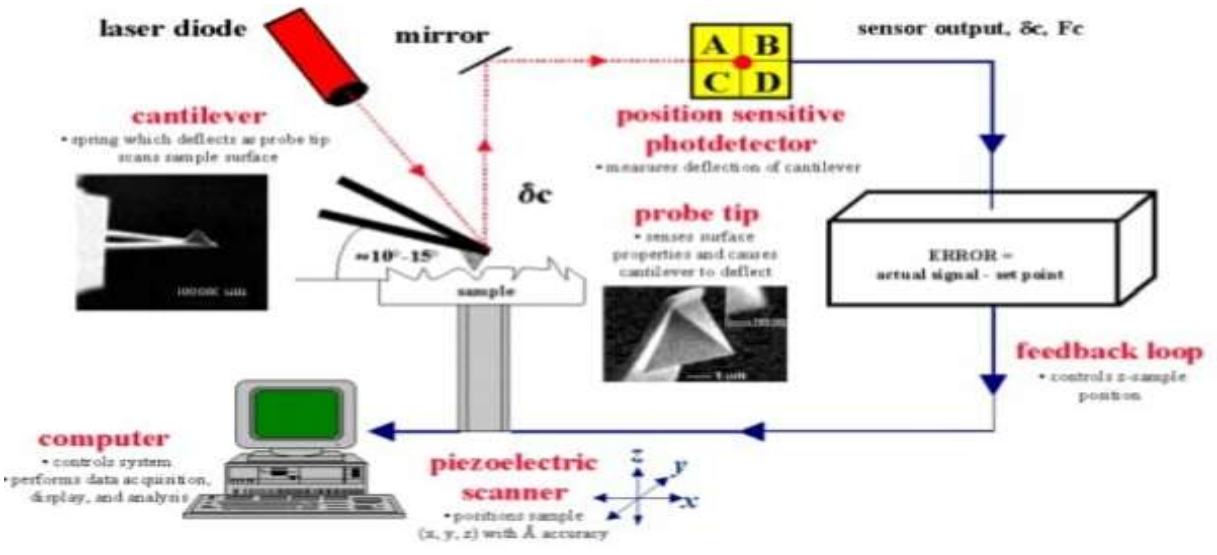
Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on some of the applications of AFM in various biological fields.
- Prerequisite knowledge on understanding and knowing the applications of AFM in analysis of samples.

Detailed content of the Lecture:

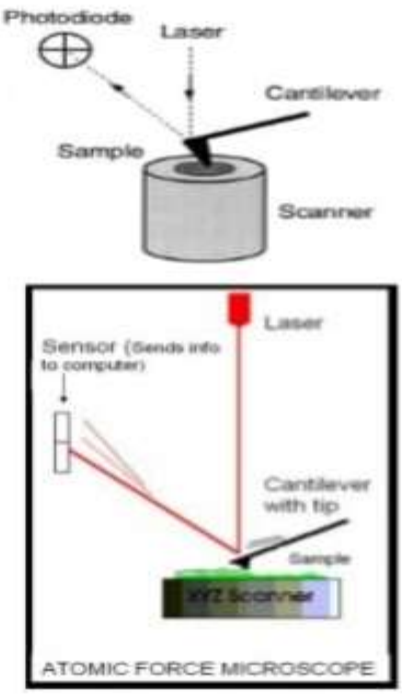
- AFM was developed when people tried to extend STM technique to investigate the electrically non-conductive materials like proteins. It is also a types of scanning probe microscopy with demonstrated resolution on the order of fractions of a nm, more than 1000 times better than the optical diffraction limit.

INSTRUMENTATION:



WORKING:

- The AFM brings a probe in close proximity to the surface
- The force is detected by the deflection of a spring, usually a cantilever (diving board)
- Forces between the probe tip and the sample are sensed to control the distance between the the tip and the sample.
- The cantilever is designed with a very low spring constant (easy to bend) so it is very sensitive to force.
- The laser is focused to reflect off the cantilever and onto the sensor
- The position of the beam in the sensor measures the deflection of the cantilever and in turn the force between the tip and the sample.



Video Content / Details of website for further learning (if any):

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BIOTECH

III/VI

Course Name with Code : **BIOLOGICAL SPECTROSCOPY 16BTE15**

Course Faculty : **Dr. G. Pratap Kumar**

Unit : **V** **Date of Lecture:**

Topic of Lecture: Combinatorial chemistry

Introduction :

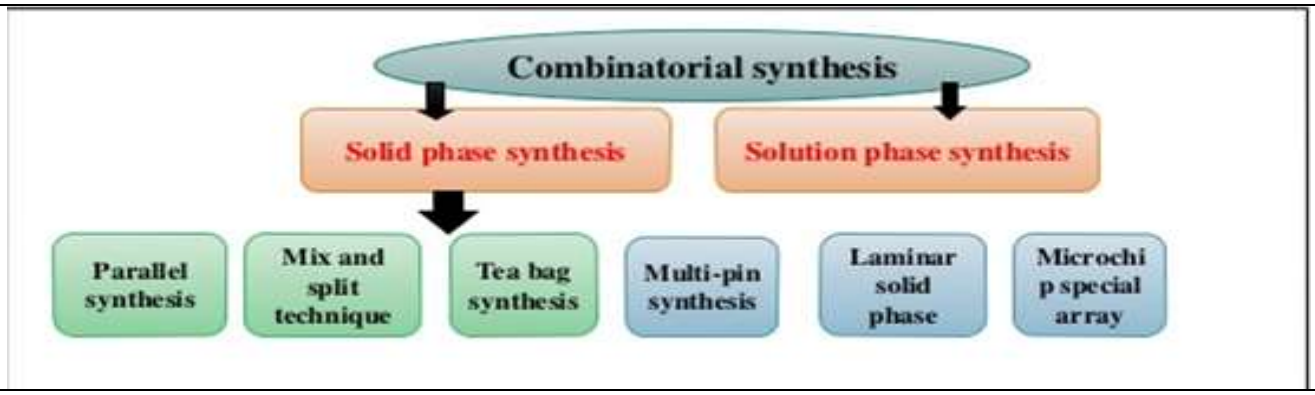
- Combinatorial chemistry is a new method developed by academic and research to decrease to the time and cost of producing effective marketable competitive new drugs.
- Scientists use combinatorial chemistry to create large number molecules that can be detected efficiently.
- The range of combinatorial techniques is highly diverse, and these products could be made individually in a parallel or in mixtures, using either solution or solid phase techniques.
- The development of new processes for the generation of collection of structurally related compounds (libraries) with the introduction of combinatorial approaches has revitalized random screening as a paradigm for drug discovery and has raised enormous excitement about the possibility of finding new and valuable drugs in short times and at reasonable costs.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on some of the applications of drug discovery.
- Prerequisite knowledge on understanding and knowing the phases involved in combinatorial chemistry.

Detailed content of the Lecture:

- Is a technique by which large numbers of different but structurally similarly molecules are produced rapidly and submitted for pharmacological assay.
- The techniques uses the same reaction condition with the same reaction vessels to produce a large of range of analogues.
- Is to prepare very large number of compound then identify more component from these compound
- This technique by which distinct molecule which is structurally large may synthesized in a short time and submit by pharmacological study



Video Content / Details of website for further learning (if any):

Important Books/Journals for further learning including the page nos.:

Banwell, Colin N. and E.M. McCash, "Fundamentals of Molecular Spectroscopy" Tata McGraw-Hill, 2007 (Pg. No. 220-230).

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LECTURE HANDOUTS

BIOTECH | **III/VI**

Course Name with Code : **BIOLOGICAL SPECTROSCOPY 16BTE15**

Course Faculty : **Dr. G. Pratap Kumar**

Unit : **V** **Date of Lecture:**

Topic of Lecture: High Throughput Screening

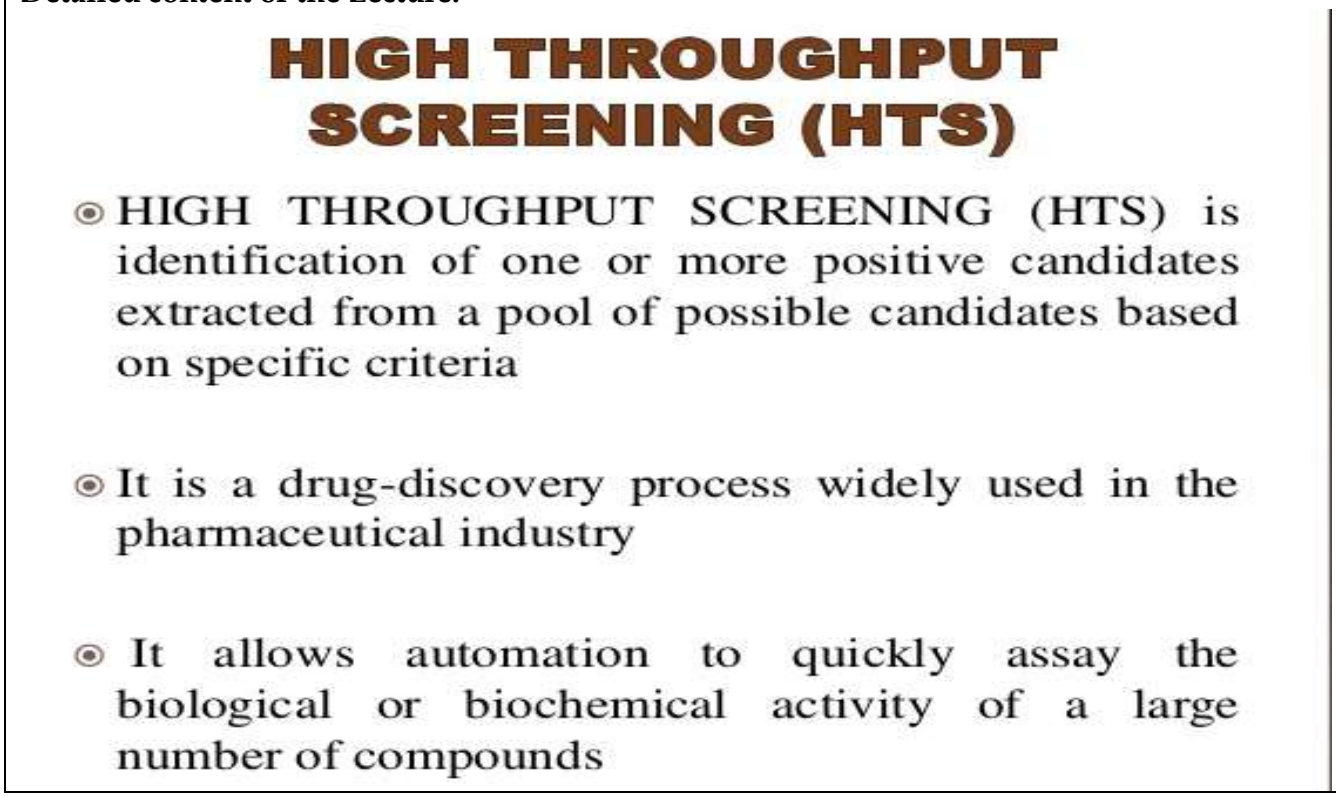
Introduction :

- High Throughput Screening (HTS) is the use of automated equipment to rapidly test thousands to millions of samples or biological activity at the model organism, cellular pathway or molecular level.
- There are multiple steps in any HTS experiment, which can take weeks to complete. However, these steps can be generalized into 3 categories: sample preparation, sample handling and readouts and data acquisition.
- The search for compounds with activity against a promising new drug target – such as an enzyme involved in a disease-critical pathway – will often begin by screening libraries containing several thousands of compounds, with the help of HTS technologies.

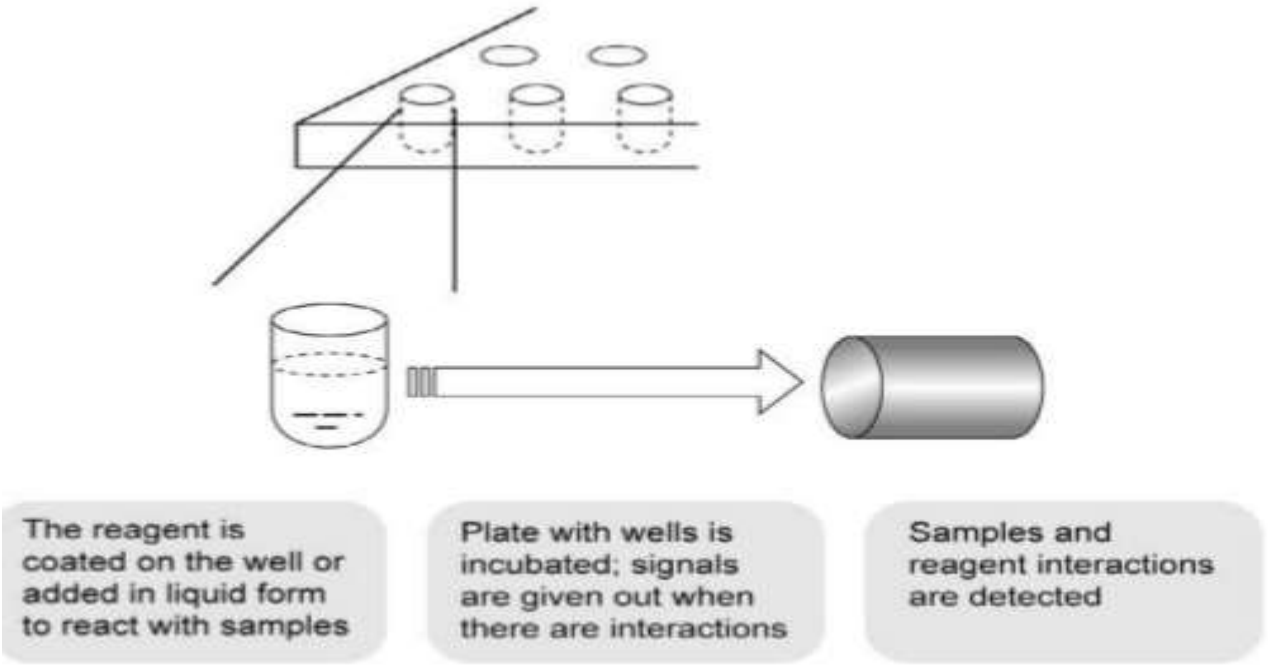
Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on some of the concepts of HTS in various fields.
- Prerequisite knowledge on understanding and knowing the techniques applied through HTS in protein and compounds.

Detailed content of the Lecture:



- The heart of the HTS system is a plate, or tray, which consists of tiny wells where assay reagents and samples are deposited, and their reactions monitored
- The configuration of the plate has changed from 96 wells (in a matrix of 8 rows by 12 columns) to 384, and now to a high - density 1536 - well format, which enables large - scale screening
- Assay reagents may be coated onto the plates or deposited in liquid form together with test samples into the wells
- Both samples and assay reagents may be incubated, and those that interact show signals, which can be detected



Video Content / Details of website for further learning (if any):

Important Books/Journals for further learning including the page nos.:

Banwell, Colin N. and E.M. McCash, "Fundamentals of Molecular Spectroscopy" Tata McGraw-Hill, 2007 (Pg. No. 249).

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BIOTECH | **III/VI**

Course Name with Code : **BIOLOGICAL SPECTROSCOPY 16BTE15**

Course Faculty : **Dr. G. Pratap Kumar**

Unit : **V** **Date of Lecture:**

Topic of Lecture: High Throughput screening methods

Introduction :

- The most common targets used in HTS campaigns are enzymes – catalytic proteins that promote chemical reactions within the cell. This is not only due to enzymes being well-established drug targets for many diseases, but also because their catalytic reactions make them particularly amendable to drug discovery research using HTS.
- Generally, these molecules are identified during HTS campaigns and the most commonly used analysis methods are fluorescence, chemiluminescence and Surface Plasmon Resonance (SPR).
- A number of assays can be used to measure enzymatic activity, but fluorescence-based methods, including fluorescence anisotropy and Forster Resonance Energy Transfer (FRET) are the most common due to their sensitivity, ease and adaptability to HTS formats.
- A novel MS-based HTS method, High-Affinity Spectrometry Screening (HAMS), which uniquely evades the detection of false positive hits.

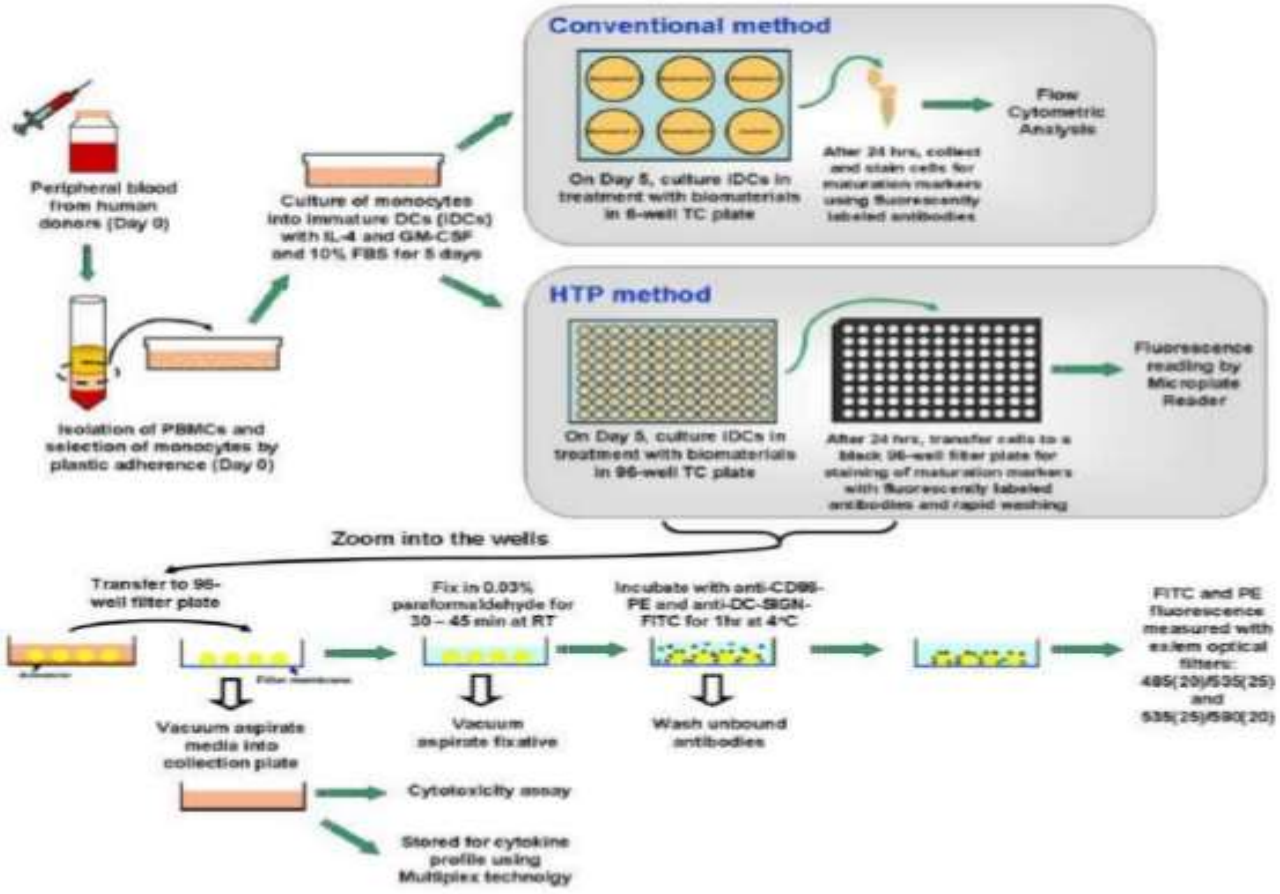
Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on the importance in the early stages of drug discovery.
- Prerequisite knowledge on understanding and knowing the techniques used in HTS in the discovery of newer drugs.

Detailed content of the Lecture:

- A CELL-BASED ASSAY IS: one where the fundamental unit of expression is the cell, either cell populations or single cells

- FOUR KEY ELEMENTS OF CELL BASED ASSAY:
  - A cellular component e.g. a cell line or a primary cell population
  - A target (substrate) molecule that records the cellular response
  - An instrument to conduct and monitor the assay An informatics component to manage and analyse data from the assay



Video Content / Details of website for further learning (if any):

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Banwell, Colin N. and E.M. McCash, "Fundamentals of Molecular Spectroscopy" Tata McGraw-Hill, 2007 (Pg. No. 249-250).

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LECTURE HANDOUTS

BIOTECH

III/VI

Course Name with Code : **BIOLOGICAL SPECTROSCOPY 16BTE15**

Course Faculty : **Dr. G. Pratap Kumar**

Unit : **V** **Date of Lecture:**

Topic of Lecture: Applications of microscopy and screening methods

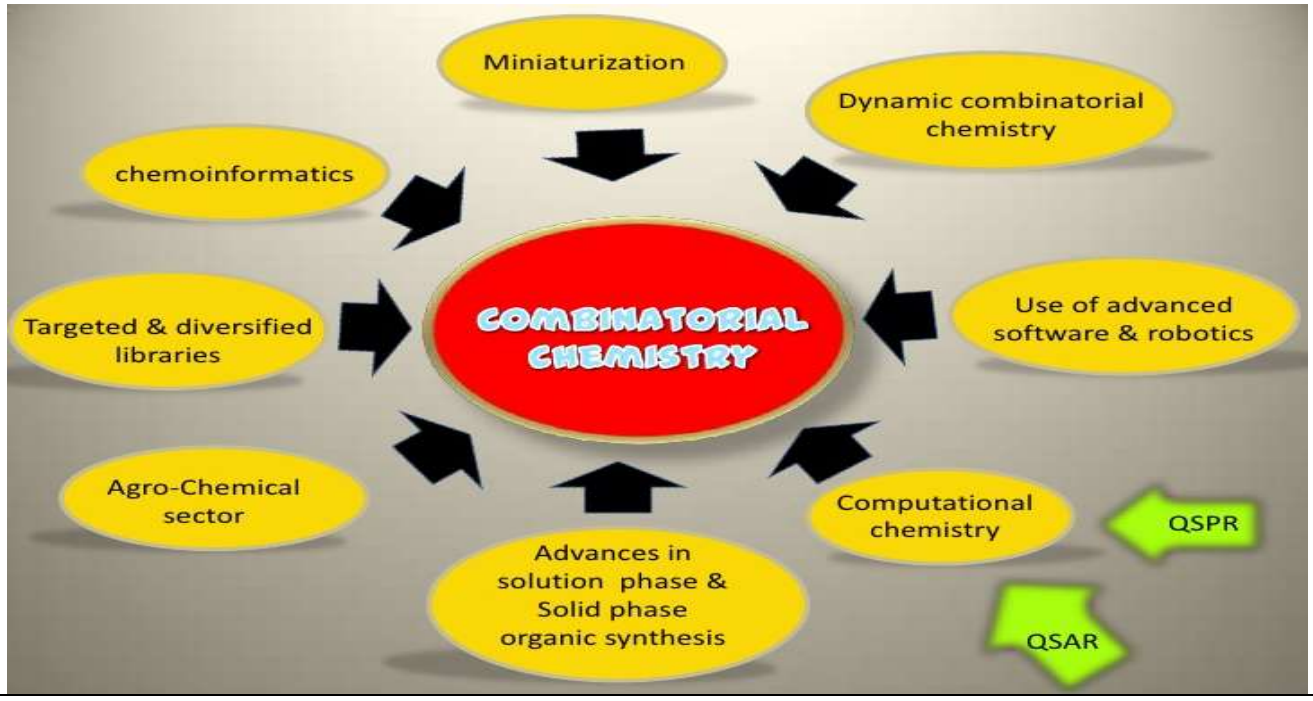
Introduction :

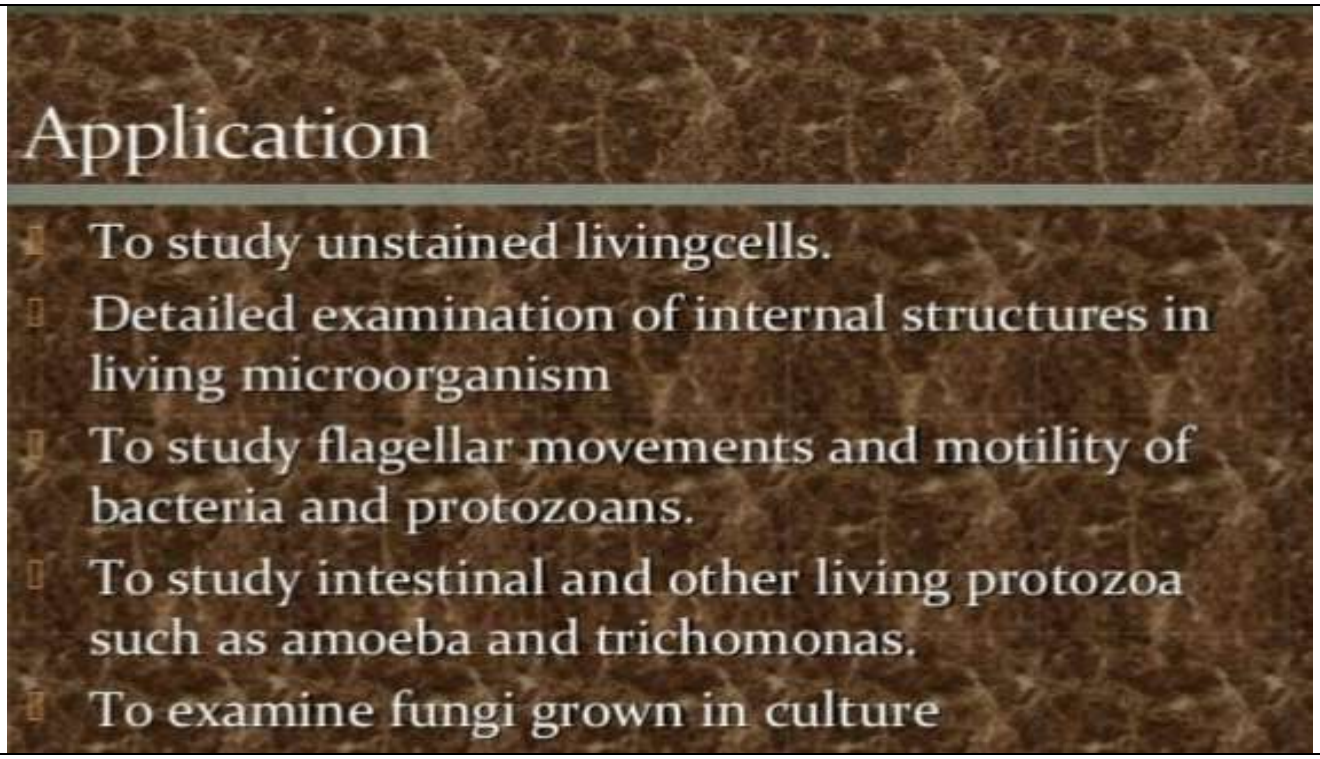
- In fact, microscopes are even used directly in medicine to analyze biological samples from patients.
- The main application of microscopes is scientific research in biology to study cells with optical/light microscopes, develop nanotechnology like carbon nanotubes with electron and scanning probe, and pathology to understand how diseases work.
- The application of high-throughput screening has particularly been of paramount significance in the drug discovery process. This automated process enables very large numbers of chemical or biological compounds to be investigated for their therapeutic potential.
- High throughput screening methods are extensively used in the pharmaceutical industry, leveraging robotics and automation to quickly test the biological or biochemical activity of a large number of molecules, usually drugs.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on some of the applications of microscopes in various fields.
- Prerequisite knowledge on understanding and knowing the principle behind microscopes and screening methods.

Detailed content of the Lecture:





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