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 | **II/IV**

Course Name with Code : **Instrumental Methods of Analysis 19BTD08**

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

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

Topic of Lecture: General aspects – Instrumental Methods of Analysis in terms of Research

Introduction :

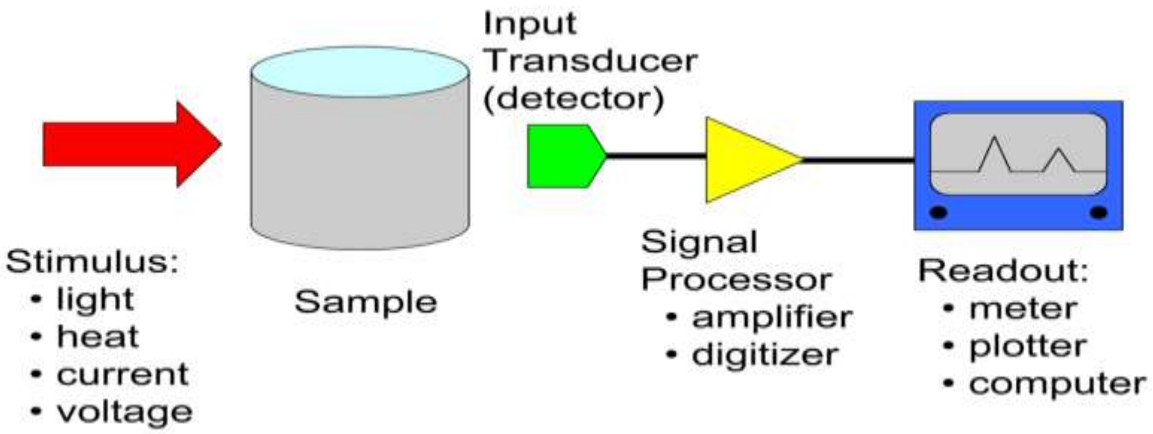
- The most instrumental techniques fit into one of three principal areas: spectroscopy, electrochemistry and chromatography.
- Although the instrument is often the most visible and exciting element of the analytical method, it is only one component of the total analysis.
- Principle types of chemical instrumentation includes, spectroscopic techniques, electrochemical techniques, chromatographic techniques, etc.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on different instruments used in research areas.
- Prerequisite knowledge on basics of chemistry and technology.

Detailed content of the Lecture:

- Analytical instrumentation is the study of the separation, identification and quantification of the chemical components of natural and artificial materials.
- Qualitative analysis gives an indication of the identity of the chemical species in the sample whereas quantitative analysis determines the amount of certain components in the substance.



<u>Importance of Instrumental methods:</u>

A modern, well-educated scientist is one who is capable of solving problems with an analytical approach and who can apply modern instrumentation to problems.

1. Fundamental principles of instrumental measurements.
2. Applications of these principles to specific types of chemical measurements.

3. Examples of modern instrumentation.
4. Use of instruments to solve real analytical problem.

Some of the basic functions of instrumentation is as follows:

- The purpose of chemical instrumentation is to obtain information from the substance being analyzed.
- Each analytical instrument may be divided into four components: a signal generator, an input transducer, an electronic signal modifier and an output transducer.
- Quantitative methods are abundant in the literature of analytical chemistry, and it is relatively simple to search or the problem under consideration.

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

https://www.youtube.com/watch?v=dAM0CVa8IkQ

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

Willard, Hobart, et al., "Instrumental Methods of Analysis" 7th Edition, CBS, 1986 (Pg. No. 1-11).

Course Faculty

Verified by HoD

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 | **II/IV**

Course Name with Code : **Instrumental Methods of Analysis 19BTD08**

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

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

Topic of Lecture: Electromagnetic Radiation and properties

Introduction :

- Electromagnetic radiation refers to the waves of the electromagnetic field, propagating through space, carrying electromagnetic radiant energy.
- It includes radio waves, microwaves, infrared, visible light, X-rays and gamma rays.
- Electromagnetic waves are emitted by electrically charged particles undergoing acceleration and these waves can subsequently interact with other charged particles exerting force on them.
- It is characterized by it's intensity ad frequency of the time variation of electric and magnetic fields.
- The basic properties and behavior of EMR has various forms including their sources, characteristics and applications.

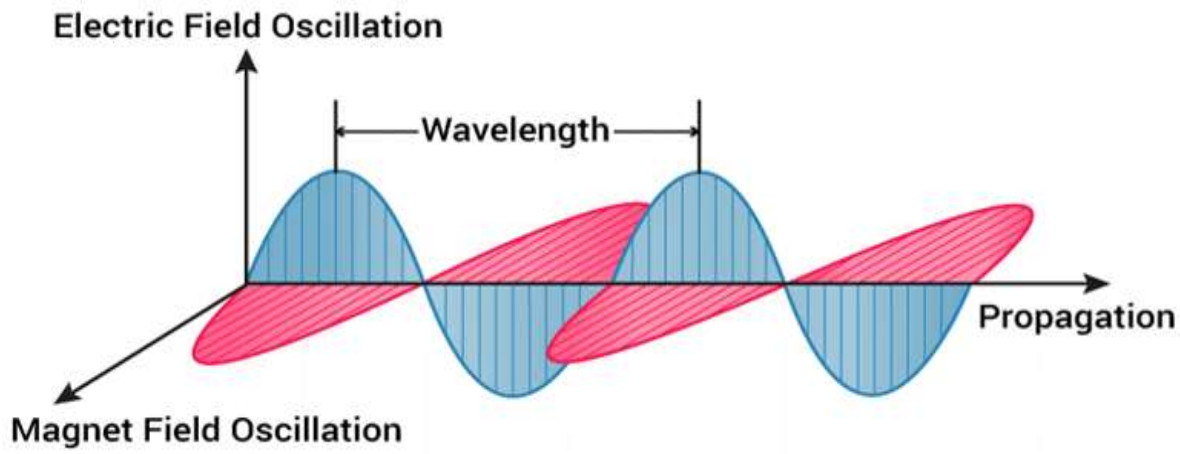
Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on wavelength, frequency and energy.
- Prerequisite knowledge on various ranges on EMR spectrum.
- Prerequisite knowledge on quantum physics in biology.

Detailed content of the Lecture:

Electromagnetic Radiation:

Electromagnetic radiation can be defined as a form of energy that is produced by the movement of electrically charged particles travelling through a matter or vacuum or by oscillating magnetic and electric disturbance.



Electromagnetic Radiation

Properties of Electromagnetic Radiation:

- When electromagnetic radiation occurs, the electron radiations are released as photons.
- These are bundles of light energy or quantized harmonic waves which travel at the speed of light.
- Then based on the wavelength of the electromagnetic spectrum, the energy is grouped into different categories.
- These magnetic and electric waves travel perpendicular to each other and have some characteristics like wavelength, amplitude, and frequency.

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

https://www.youtube.com/watch?v=Ja7hq3YYIWo

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

Willard, Hobart, et al., "Instrumental Methods of Analysis" 7th Edition, CBS, 1986 (Pg. No. 97-100).

Course Faculty

Verified by HoD

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 | **II/IV**

Course Name with Code : **Instrumental Methods of Analysis 19BTD08**

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

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

Topic of Lecture: Wave properties

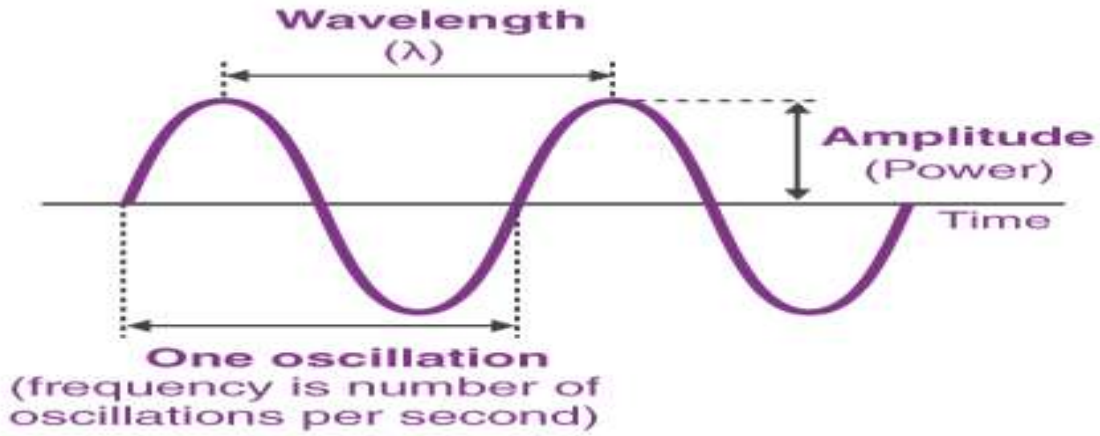
Introduction :

- A wave is a disturbance in a medium that carries energy without a net movement of particles.
- Waves transfers energy and usually involves a periodic and repetitive movement.
- The properties of a wave include frequency, amplitude, wavelength and speed.
- The velocity of a wave is the product of the wavelength and the frequency.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on wave and it's properties.
- Prerequisite knowledge on types of waves such as transverse and longitudinal.

Detailed content of the Lecture:



Waves and their Characteristics

- Electromagnetic radiation occurs when an atomic particle, like an electron, is accelerated by an electric field, causing it to accelerate.
- Electromagnetic waves and their characteristics is explained briefly in the points mentioned below.

Wavelength

- Wavelength (λ) is the distance between successive crests of a wave, especially points in an electromagnetic wave or sound wave.

- It can be simply defined as the distance of one full cycle of the oscillation. If 'λ' is the wavelength, 'c' is the speed of light and 'ν' is frequency.
- Then we can derive the relation given below.

$$c = \lambda \nu$$

The shorter the wavelength, greater the frequency and greater the frequency, the higher the energy.

Amplitude

- It is the distance from the middle of the wave to the maximum vertical displacement of the wave.
- Larger the amplitude, higher the energy and lower the amplitude, lower the energy.
- Amplitude tells us about the brightness or intensity of a wave compared to other waves.

Frequency

- The number of cycles per second is defined as Frequency.
- It is defined as Hertz (Hz) or sec-1. If 'E' is the energy, 'h' is Planck's constant which is equal to 6.62607 x 10-34 and 'ν' is the frequency we can derive the relation given below.

$$E = h\nu$$

Thus, we can see that frequency is directly proportional to energy.

Period

- Period is commonly characterised by the symbol 'T'.
- It is the total time which a wave takes to travel 1 wavelength.

Velocity

- In relation with electromagnetic radiation, the velocity is normally expressed as:

$$Velocity = \lambda\nu$$

Video Content / Details of website for further learning:
https://www.youtube.com/watch?v=ekQtbsYesCo

Important Books/Journals for further learning including the page nos.:
Willard, Hobart, et al., "Instrumental Methods of Analysis" 7th Edition, CBS, 1986 (Pg. No. 97-100).

Course Faculty

Verified by HoD

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 | **II/IV**

Course Name with Code : **Instrumental Methods of Analysis 19BTD08**

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

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

Topic of Lecture: Components of optical instruments and Sources of radiation

Introduction :

- An optical instrument is a device that processes light waves either to enhance an image for viewing or to analyze and determine their characteristic properties.
- The spectroscopic techniques use instruments that share several common basic components which includes, source of energy, isolating narrow range of wavelengths, a detector for measuring the signal and signal processor that displays the signal.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on types of optical instruments.
- Prerequisite knowledge on different sources of radiation.

Detailed content of the Lecture:

Components of Optical Instruments

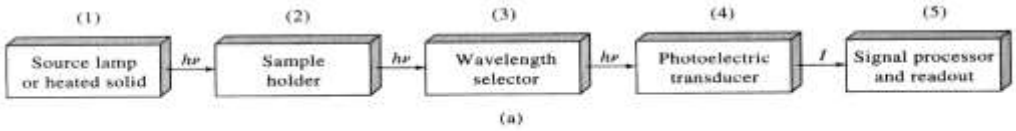
- Instruments for optical spectroscopy include instruments which detect EM radiation in the ultraviolet (UV), visible, and infrared (IR) regions of the EM spectrum.
- Although UV and IR instruments are not technically optical techniques, since they are outside the visible region of the EM spectrum, they are included in this class because their design is similar to visible spectrophotometers.

Optical spectroscopic methods are based on six phenomena:

1) absorption
2) fluorescence
3) phosphorescence
4) scattering
5) emission
6) chemiluminescence

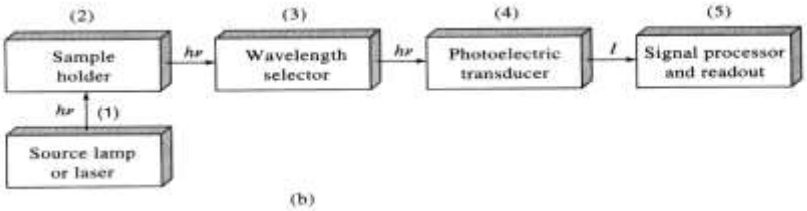
I. General Design of Optical Instruments

Absorption



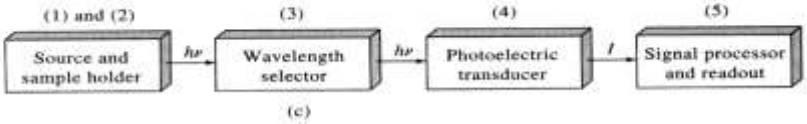
(a)

Fluorescence, Phosphorescence, and Scattering



(b)

Emission and Chemiluminescene



(c)

Five Basic Optical Instrument Components:

1) Source - A stable source of radiant energy at the desired wavelength (or □ range).
2) Sample Holder - A transparent container used to hold the sample (cells, cuvettes, etc.).
3) Wavelength Selector - A device that isolates a restricted region of the EM spectrum used for measurement (monochromators, prisms, & filters).
4) Photoelectric Transducer - (Detector) Converts the radiant energy into a useable signal (usually electricity).
5) Signal Processor & Readout – Displays the transduced signal on a readout device such as a meter, digital readout, chart recorder, computer, etc.

II. Sources of Radiation -Generate a beam of radiation that is stable and has sufficient power.

A. Continuum Sources - emit radiation over a broad wavelength range and the intensity of the radiation changes slowly as a function of wavelength.

- This type of source is commonly used in UV, visible, IR, and fluorescence instruments.
- Deuterium lamp is the most common UV source.
- Tungsten lamp is the most common visible source.
- Glowing inert solids are common sources for IR instruments.
- High pressure, gas filled (argon, xenon, mercury) lamps are used when an intense source is required (i.e. fluorescence)

B. Line Sources - Emit a limited number *lines* or bands of radiation at specific wavelengths.

- Used in atomic absorption spectroscopy, atomic and molecular fluorescence spectroscopy, and Raman spectroscopy.

- Usually provide radiation in the UV and visible region of the EM spectrum.

- Types of line sources:

1) Hollow cathode lamps
2) Electrodeless discharge lamps
3) Lasers - Light amplification by stimulated emission of radiation

Video Content / Details of website for further learning (if any): https://www.youtube.com/watch?v=UI7TsblcXak
Important Books/Journals for further learning including the page nos.: Willard, Hobart, et al., "Instrumental Methods of Analysis" 7th Edition, CBS, 1986 (Pg. No. 118-139).

Course Faculty

Verified by HoD

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 | **II/IV**

Course Name with Code : **Instrumental Methods of Analysis 19BTD08**

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

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

Topic of Lecture: Wavelength selectors, sample containers and radiation transducers

Introduction :

- In a sample, if there are two components then it absorbs different wavelengths of light.
- It's used to select a given wavelength of the light from the light source.
- A container that contains a sample is usually called 'cell' with a fixed length and volume.
- The shape of cuvette is usually in round or square and made of material that does not absorb light in the wavelength range.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on different types of filters and monochromators.
- Prerequisite knowledge on types of sample holders used for experiments.
- Prerequisite knowledge on various detectors used for sample analyses.

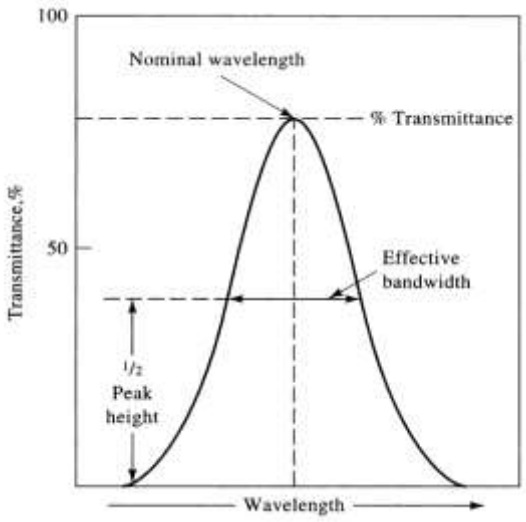
Detailed content of the Lecture:

II. Wavelength Selectors

• An ideal wavelength selector would output a single (line) wavelength or frequency of radiation. Realistically this is impossible. Wavelength selectors output a limited, narrow, continuous group of wavelengths called a *band*.

• The quality of a wavelength selector is measured by the inverse of the *effective bandwidth*.

• *Effective bandwidth* is defined as the peak width at half height of a plot of the output of a wavelength selector (% transmittance) as a function of wavelength.



- Two types of wavelength selectors:

A) filters

B) monochromators

A) Filters

- Two types of filters:

1) Interference filters

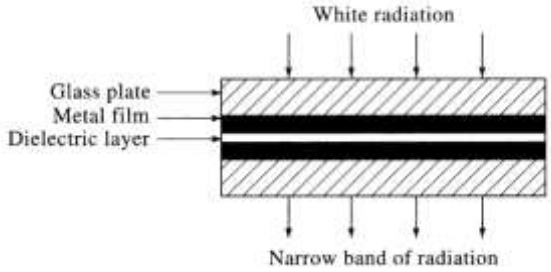
2) Absorption filters

1) Interference Filters

• Rely on constructive and destructive interference in order to select a narrow bandwidth of radiation.

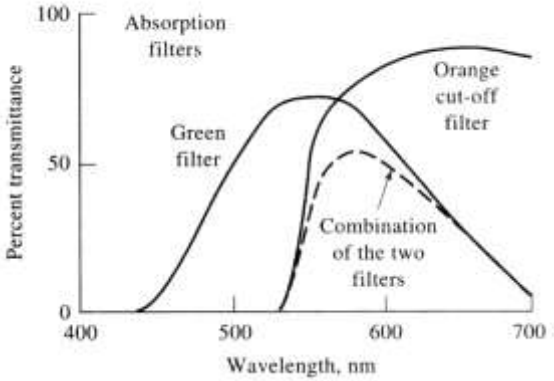
• Constructed of a transparent dielectric layer (usually CaF2 or MgF2) sandwiched between two semitransparent metallic films and two plates of glass or other transparent material.

• Useful in the UV, visible, and IR regions of the EM spectrum.



2) Absorption Filters

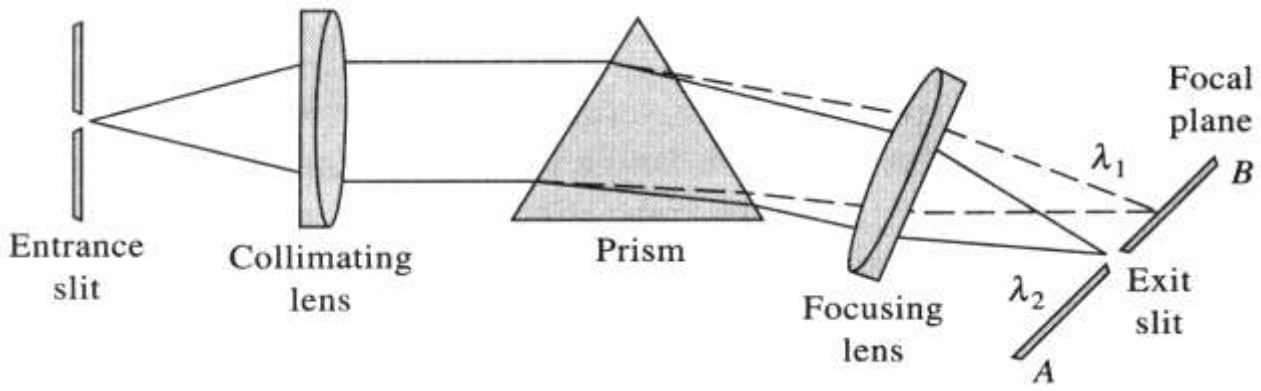
- Cut-off (band pass) filters which have 100% transmittance over a visible region and rapidly fall off to 0% transmittance.
- Commonly used in visible spectrophotometry.
- Normally constructed of colored glass or a dye in a gelatin.
- Often two absorption filters are paired to give a narrower band of transmittance.



B) Monochromators – Designed for spectral scanning.

- <u>Scanning</u> - Varying the wavelength of radiation over a considerable range.

- Used in most scanning spectrometers including UV, visible, and IR instruments.



• Prisms were the dispersion elements in older instruments but the dispersion of radiation is nonlinear as a function of wavelength (i.e. dispersion increases as wavelength shortens).

• Virtually all modern instruments incorporate grating in their monochromators because gratings are cheaper and disperse radiation linearly as a function of wavelength.

Components of a Monochromator:

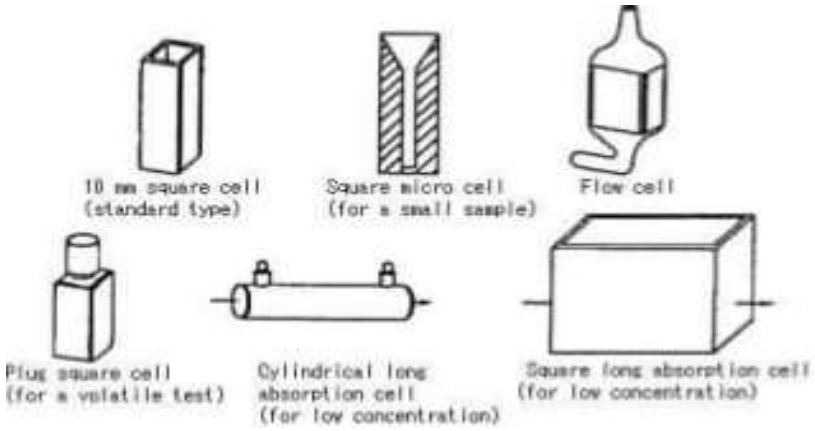
1) Entrance Slit – provides a rectangular optical image of the incoming polychromatic radiation.
2) Colliminating Lens or Mirror - provides a parallel beam of radiation that impinges upon the dispersive element.
3) Prism or Grating - (dispersive element) disperses the polychromatic radiation by the process of diffraction.
4) Focusing Lens or Mirror - Focuses the dispersed radiation on the exit slit.
5) Exit Slit - Isolate the wavelength band of interest.

III. Sample containers

- Most experiments using absorption of emissions spectroscopy interrogate samples that are gases, liquids or solutions.
- For liquids and solutions cuvettes are the most common sample containers.
- Two types are available: Glass – visible region and Quartz – UV region

The key characteristics for sample containers are:

1. The window material or cuvette material is transparent in the spectral region of the experiment.
2. The window, cell or cuvette material doesn't react with sample.
3. The path length of the cell is matched to the experiment and instrument.
4. The cell volume is matched to the sample.



IV. Radiation Transducers (Detectors)

•Early detectors in spectroscopic instruments were the human eye or photographic plates or films. Modern instruments contain devices that convert the radiation to an electrical signal.

A. Types of Radiation Transducers and Ideal Properties

1. Two general types of radiation transducers
a. Photon detectors
b. Thermal detectors

a. Photoelectric (or quantum) detectors have an active surface, which is capable of absorbing EM radiation.

• In some of these detectors the absorbed radiation causes the emission of electrons which results in a photocurrent.
• Other types of these detectors the absorption of radiation promotes electrons into the conduction band of a semicondutor thus enhancing conduction (photoconduction).

• Photoelectric detectors are commonly useful in ultraviolet, visible, and near infrared instruments.

b. Thermal detectors measure the heat induced by the impinging radiation.
• Commonly used in infrared instruments.

2. Properties of an ideal detector
a. High sensitivity
b. High signal-to-noise ratio
c. Constant response over a large range of wavelengths
d. Fast response time
e. Electrical signal (S) produced is proportional to the radiant power (P)

B. Photon Detectors
• Several types of photon detectors are available:
1. Vacuum phototubes
2. Photomultiplier tubes
3. Photovoltaic cells
4. Silicon photodiodes
5. Diode array transducers
6. Photoconductivity transducers

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

Important Books/Journals for further learning including the page nos.:
Willard, Hobart, et al., "Instrumental Methods of Analysis" 7th Edition, CBS, 1986 (Pg. No. 118-139).

Course Faculty

Verified by HoD

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 | **II/IV**

Course Name with Code : **Instrumental Methods of Analysis 19BTD08**

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

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

Topic of Lecture: Signal process and Read outs

Introduction :

- A signal is defined as the output of a transducer that is responding to the chemical system of interest.
- The signal is divided into two parts, one caused by analyte and other caused by other components of sample and instrumentation.
- The DC signals are produced that are amplified by DC amplifiers and read on analog meters, recorders, digital voltameters or display of computer systems.
- The measurement is displayed on the analog meter.
- A current is converted to voltage before display for a sample.

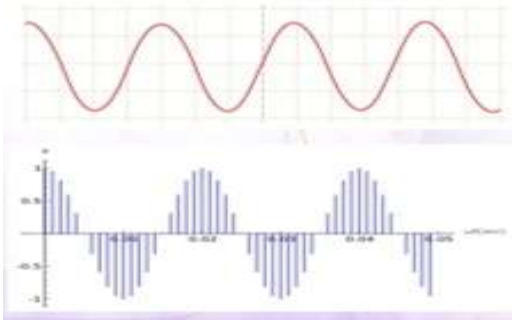
Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on difference between signal and noise.
- Prerequisite knowledge on read out devices and amplification of signals.

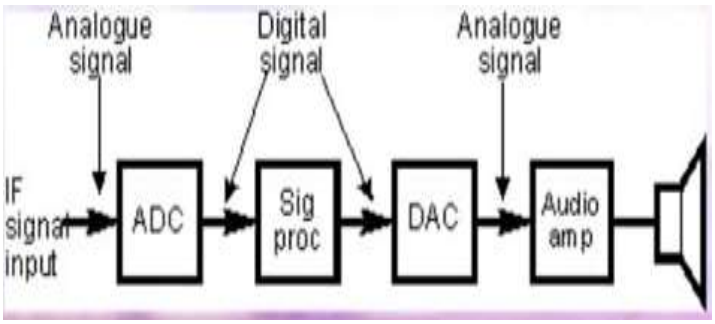
Detailed content of the Lecture:

Signal processing:

- Signal processing is the analysis, interpretation and manipulation of like sound, images, time-varying measurement values and sensor data etc.
- Types of signal processing: 1. Analog signal processing 2. Digital signal processing.



Signal processing



Digital signal processing

Read outs:

- The modified signal is converted into sample absorption for signal readout.
- A readout device such as a chart recorder, an analog meter, an oscilloscope or a computer that converts the electrical signal into a form that is usable by the analyst.

- Several types of readout devices are found in modern instruments. Some of these devices include the digital meters, the scale of potentiometer, cathode ray tubes and computers.
- The instrument is caliberated so that there are 100 units on the meter from (It = 0) to (I = Io) and these units are linear with respect to It. When an absorbing sample is substituted for the 'blank', the detector response will show between 0 and 100 units on the meter.

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

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

Willard, Hobart, et al., "Instrumental Methods of Analysis" 7th Edition, CBS, 1986 (Pg. No. 13; 148).

Course Faculty

Verified by HoD

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 | **II/IV**

Course Name with Code : **Instrumental Methods of Analysis 19BTD08**

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

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

Topic of Lecture: Signal to Noise ratio and Sources of noise

Introduction :

- It's a representative marker that is used in describing the quality of an analytical method or the performance of an instrument.
- The signal is what is been measured which finally shows the presence of your analyte.
- Noise is extraneous information that can interfere with or alter the signal.
- It's important for the analyst who uses a particular instrumental method to be aware of the sources of noise due to which noise determines both accuracy and detection limits of a measurement.
- Noise enters a measurement system from environmental sources external to the measurement system.

Prerequisite knowledge for Complete understanding and learning of Topic:

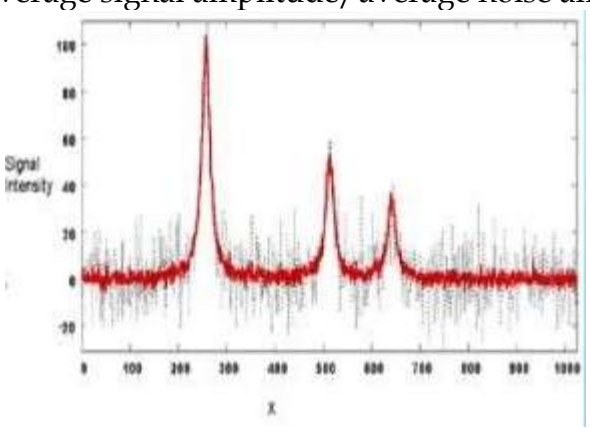
- Prerequisite knowledge on various signals and noises from the measurement system.
- Prerequisite knowledge on how noise is produced from the source to the system.

Detailed content of the Lecture:

- As concentrations decrease to trace levels or as signal sources become weak, the problem of distinguishing signals from noise becomes increasingly difficult, resulting in decreased accuracy and precision in measurements.

- The ability of an instrument system to discriminate between signals and noise is usually expressed as a signal to noise ratio (S/N), where

S/N = average signal amplitude/average noise amplitude



Signal to noise ratio

Sources of Noise:

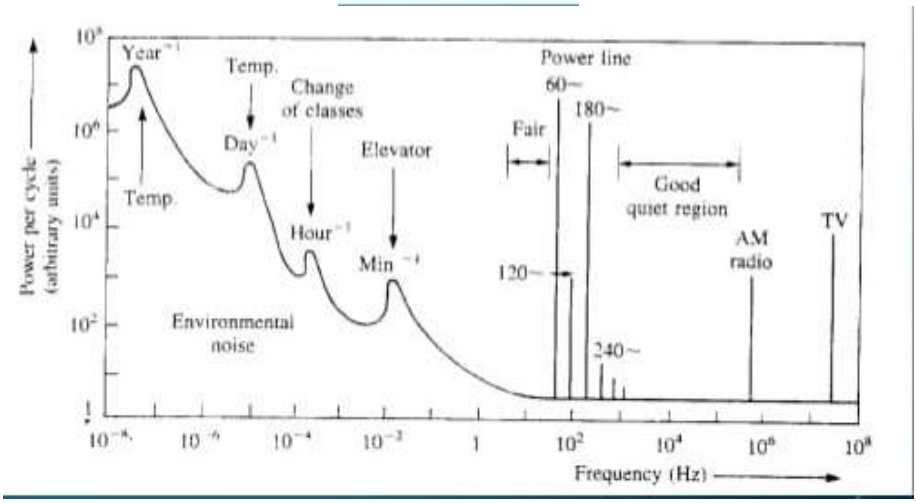
Chemical: This noise arises from uncontrollable variables in the chemistry of the system such as variation in temperature, pressure, humidity, light and chemical fumes present in the room.

Instrumental Noise: Noise that arises due to the instrumentation itself. It could come from any of the following components- source, input transducer all signal processing elements, and the output transducer. This noise has many types and can arise from several sources.

CATEGORIES OF INSTRUMENTAL NOISE:
- Thermal or Johnson
- Shot Noise
- Flicker Noise
- Environmental Noise

THERMAL NOISE:
- Noise that originates from the thermally induced motions in charge carriers is known as thermal noise. It exists even in the absence of current flow.
- Since thermal noise is independent of the absolute values of frequencies, it is also known as "white noise."
- V is the average voltage due to thermal noise, k is the Boltzmann constant, T is the absolute temperature. R is the resistance of the electronic device, and Af is the bandwidth of measurement frequencies.

$$V_{av} = \sqrt{4kTR\,\Delta f}$$



SHOT NOISE:
- Shot noise refers to the random fluctuations of the electric current in an electrical conductor, which are caused by the fact that the current is carried by discrete charges (electrons).
- The strength of this noise increases for growing magnitude of the average current flowing through the conductor. Shot noise is to be distinguished from current fluctuations in equilibrium, which happen without any applied voltage and without any average current flowing. These equilibrium current fluctuations are known as Johnson-Nyquist noise.
- The sub-Poissonian shot-noise power, S, of a metallic resistor as a function of its length, L, as predicted by theory. Indicated are the elastic mean-free path, l, the electron- electron scattering length, lee, and the electron-phonon scattering length lep.

$$iav = \sqrt{2Ie\Delta f}$$

where, iav is the shot noise, I is the intensity of the signal. e is the charge on the electron and Δf is the measurement frequency bandwidth.

FLICKER NOISE:

• Its magnitude is inversely proportional to frequency of signal.
• Can be significant at frequencies lower than 100 Hz.
• Causes long term drift in de amplifiers, meters, and galvanometers. Can be reduced significantly by using wire- wound or metallic film resistors rather than composition type.
• Flicker Noise is associated with crystal surface defects in semiconductors and is also found in vacuum tubes.
• The noise power is proportional to the bias current, and, unlike thermal and shot noise, flicker noise decreases with frequency.

$$Vav = \sqrt{KI2}/f$$

Where K is a constant depending on factors such as resistor such as resistor materials, I is the dc current and f is the frequency.

ENVIRONMENTAL NOISE:

• Environmental noise is due to a composite of noises from different sources in the environment surrounding the instrument.
• Much environmental noise occurs because each conductor in an instrument is potentially an antenna capable of picking up electromagnetic radiation and converting it to an electrical signal.
• There are numerous sources of electromagnetic radiation in the environment including ac power lines, radio and TV stations, gasoline engine ignition systems, arcing switches, brushes in electrical motors, lightening, and ionospheric disturbances.

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

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

Willard, Hobart, et al., "Instrumental Methods of Analysis" 7th Edition, CBS, 1986 (Pg. No. 13-17).

Course Faculty

Verified by HoD

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 | **II/IV**

Course Name with Code : **Instrumental Methods of Analysis 19BTD08**

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

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

Topic of Lecture: Enhancement of signal to noise – Hardware and Software techniques

Introduction :

- For some measurements only minimal efforts are required for maintaining a good signal to noise ratio because the signals are relatively strong and the requirements for precision and accuracy are low.
- The signal to noise ratio of a signal can be enhanced by either hardware or software techniques.
- The wide use of personal computers in chemical instrumentation and their inherent programming flexibility make software signal smoothing techniques more attractive.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on types of algorithms used for enhancement.
- Prerequisite knowledge on filters used such as low pass, high pass and band pass.

Detailed content of the Lecture:

Hardware techniques:

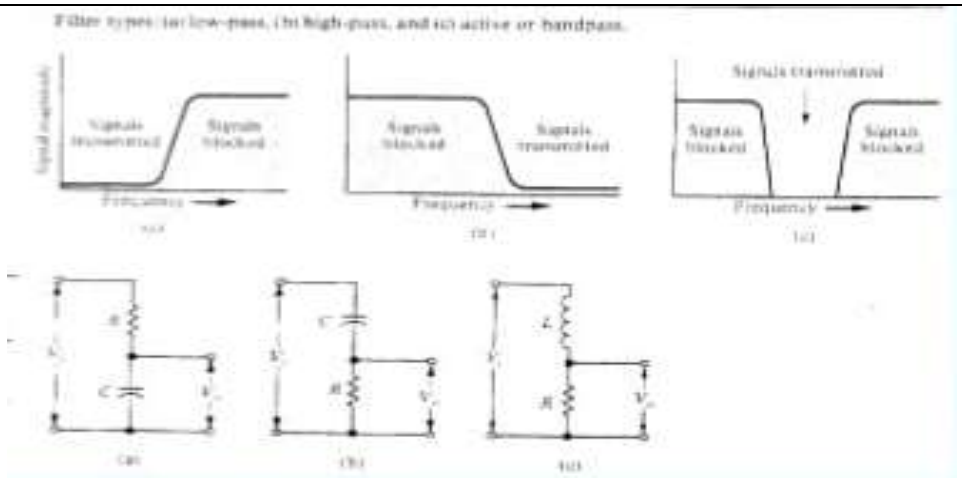
To avoid losing data, the signal from the input transducer should be sampled at a rate twice that of the highest frequency component of the signal according to the Nyquist sampling theorem.

FILTERING:

• Although amplitude and the phase relationship of input and output signals can be used to discriminate between meaningful signals and noise, frequency is the property most commonly used.

• White noise can be reduced by narrowing the range of measured frequencies, environmental noise can be eliminated by selecting the proper frequency.

• Three kinds of electronic filters are used to select the band of measured frequencies: Low Pass Filters High Pass Filters Band Pass Filters.



INTEGRATION:

- Integration of DC signals for precisely limited time periods is a powerful way to reduce white noise.
- The coherent signal adds directly with respect to the integration time, whereas the random noise adds as the square root of the integration time; therefore, the S/N ratio increases with the square root of the integration time.
- Although a simple RC filter can be used to integrate signals, an operational amplifier with a capacitor in the feedback loop usually serves as a hardware integrator.
- Analog to digital convertors such as voltage to frequency or dual slope devices have built in S/N enhancement as a result of the integration techniques used in the signal conversion circuits.

MODULATION/DEMODULATION:

- If the signal and noise can't be separated by filtering, it's often advantageous to shift the signal of interest away from the noise frequency.
- To accomplish this, the signal is first transposed onto a carrier wave that has a desirable frequency, then it is transmitted to an amplifier tuned to the frequency of the carrier signal and finally the original signal is recovered from the carrier wave. The first process is modulation and the final is demodulation.
- Modulation/demodulation techniques can be used to process a signal in a region of minimum noise and also discriminate between signal and noise on the basis of signal's unique modulation configuration relative to the random pattern of noise.

ACTIVE FILTERING:

- Even when the signal is processed in a relatively noise free environment, some noise will always be passed because of the bandwidth necessary to transmit the signal and the difficulty of obtaining and holding a match between signal frequencies and the filter band pass.
- Using a combination of signal frequency and phase relationships, it discriminates between both flicker and white noises. The functional components of a lock-in amplifier include a modulator (chopper), a multiplier and a low-pass filter.
- The data containing signal at frequency f is superimposed onto the carrier wave frequency f0 to produce a modulated signal, f0 + Δf, that is then transmitted to an electronic device known as multiplier.

BOXCAR INTEGRATORS:

- It is a relatively simple method of signal enhancement for repetitive signals. It periodically samples the same portion of a signal for a fixed period of time and then averages the samples using a low-pass RC filter.

- This triggerable, gated intergrator is a versatile measurement device. It provides S/N enhancement for the portion of signal that is sampled.
- It's best used for S/N ratio reduction in repetitive signals, although it can be used for more complex variable input waveforms.

Software techniques:

- The increased use of instruments that contain built-in microcomputers has increased the importance of software techniques for data acquisition and signal to noise enhancement.
- The minimum hardware required for software signal processing functions is analog signal conditioning circuits and an analog to digital component as well as the microcomputer chips.
- Once the data are in digital form a variety of software enhancement techniques may be used to increase the signal to noise ratio.

Digital filtering technique: Three of the most commonly used software signal enhancement techniques are boxcar averaging, ensemble averaging and weighted digital filtering.

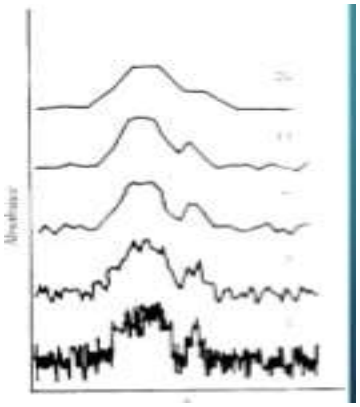
a) Boxcar averaging:

- In this method, a group of closely spaced digital data points depicting a slowly changing analog signal is replaced by a single point representing the average of the group.
- In this mode of operation, 1 boxcar of points can be acquired and averaged before the next boxcar of data arrives. Enhancement of the S/N ratio can be calculated by the following equation:

$$S/N = \sqrt{n}\left(\frac{S}{N}\right)o$$

Where (S/N)o is the signal to noise of the untreated data and n is the number of points averaged in each boxcar. The effect of increasing n on the S/N ratio and signal resolution.

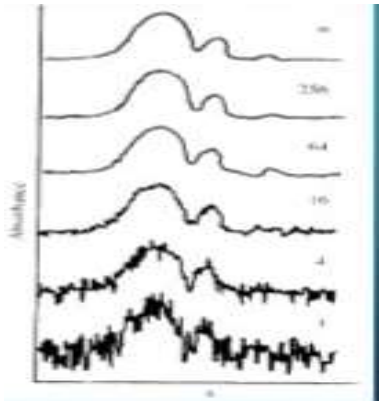
- Boxcar averaging can also be used for very rapidly changing signals when a short delay can be precisely controlled and the desired sampling interval is too fast for the available instrumentation.



b) Ensemble averaging:

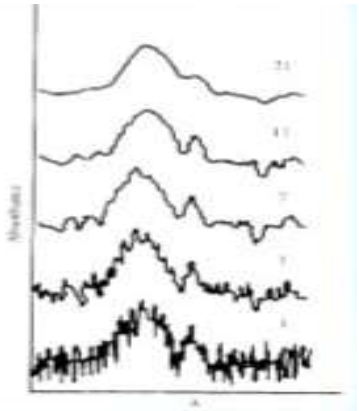
- It can be applied to signals that are changing rapidly. The results of n repeated sets of measurements of the same phenomenon are added and the sum is divided by n to obtain an average scan.
- If each set of measurements is recorded in the same way, the data contained in the measurements will sum coherently, whereas the random noise should average to a value smaller than the enhanced signal.
- To the extent that n represents a normal statistical distribution, the resulting S/N will be increased by a factor of n over that of a signal scan.

- The principle liability of this technique is the time required to obtain a significant increase in the S/N ratio – 100 scans to obtain an order of magnitude increase in the S/N ratio.



c) Weighted digital filtering:

- In digital filtering each of the data points to be averaged contributes equally to the calculation of the average.
- Assigning different weights to points as a function of their position relative to the central point can produce more realistic filtering.
- Adjustable filtering parameters include the mathematical smoothing function, the number of points and their positions relative to the central point in the moving average and the number of times the data are processed by the smoothing function.



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

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

Willard, Hobart, et al., "Instrumental Methods of Analysis" 7th Edition, CBS, 1986 (Pg. No. 18-24).

Course Faculty

Verified by HoD

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 | **II/IV**

Course Name with Code : **Instrumental Methods of Analysis 19BTD08**

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

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

Topic of Lecture: Types of optical instruments and Principle of Fourier Transform optical measurements

Introduction :

- An optical instrument (or 'optic') is a device that processes light waves either to enhance an image for viewing or to analyze and determine their characteristic properties.
- The first optical instruments were telescopes used for magnification of distant images, and microscopes used for magnifying very tiny images.
- Since the days of Galileo and Van Leeuwenhoek these instruments have been greatly improved and extended into other portions of the electromagnetic spectrum.
- The use of converging lenses makes things appear larger, and on the other hand diverging lenses always get you smaller images.
- FTIR stands for Fourier Transform InfraRed Spectrophotometer – the preferred method of infrared spectroscopy.
- A method used for measuring all of the infrared frequencies simultaneously rather than individually as with dispersive instruments.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on definition and types of optical instruments.
- Prerequisite knowledge on IR spectroscopy and Fourier transforms.

Detailed content of the Lecture:

- Optics and optical instruments are the devices that process photons to enhance images for viewing/analyzing their characteristics.
- Another class of optical instrument is used to analyze the properties of light or optical materials. They include:

1. **Interferometer:** for measuring the interference properties of light waves.
2. **Photometer:** for measuring light intensity.
3. **Polarimeter:** for measuring dispersion or rotation of polarized light.
4. **Reflectometer:** for measuring the reflectivity of a surface or object.
5. **Refractometer:** for measuring refractive index of various materials.
6. **Spectrometer:** for generating or measuring a portion of the optical spectrum for the purpose of chemical or material analysis.
7. **Autocollimator:** which is used to measure angular deflections.
8. **Vertometer:** which is used to determine refractive power of lenses such as glasses, contact lenses and magnifier lens.
9. **DNA sequencers:** can be considered optical instruments as they analyse the color and intensity of light emitted by a fluorochrome attached to a specific nucleotide of a DNA strand.

10. **Surface plasmon resonance:** based instruments use refractometry to measure and analyse biomolecular interactions.

- Optical instruments examples are:

a) Eyes
b) Lenses
c) Magnifying glass
d) Telescope
e) Microscope

Principle of Fourier Transform optical measurements:

- The mathematical operations known as Fourier transformations (FT) provide a powerful method of S/N enhancement.
- Applications of this technique in instrumental analysis usually fall into one of two categories. The first involves the use of FT to produce spectroscopic methods that are much faster than conventional frequency domain methods. Second, tansformations of conventional signals may be multiplied by appropriate conditioning functions to achieve digital filtering and other useful signal modifications.
- Two methods of data representations are the frequency-amplitude function. F(v), and the less common time-amplitude function, f(t). The functions known as a Fourier transform pair, are related by the following equations:

$$\mathrm{F}(v) = \int_{-\infty}^{\infty} f(t)e - i(2\pi)vt\ dt$$

$$f(\mathrm{t}) = \int_{-\infty}^{\infty} F(v)e - i(2\pi)vt\ 2\pi\ dv$$

- In Fourier transform spectroscopy the data are rapidly generated in the time domain [f(t)] form by either an interferometer or a pulsed magnetic resonance signal.
- The resulting data are in the form of superimposed waves and include all the frequencies of the spectral range of the instrument.

$$\mathrm{F}(vj) = \sum_{k=1}^{N} f(tk)e - i(2\pi)vjtk; j = 0,1,2\ \ldots$$

Video Content / Details of website for further learning (if any):
https://byjus.com/physics/optical-instruments/
https://www.rp-photonics.com/fourier_transform_spectroscopy.html

Important Books/Journals for further learning including the page nos.:
Willard, Hobart, et al., "Instrumental Methods of Analysis" 7th Edition, CBS, 1986 (Pg. No. 25-28).

Course Faculty

Verified by HoD

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 | **II/IV**

Course Name with Code : **Instrumental Methods of Analysis 19BTD08**

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

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

Topic of Lecture: Molecular absorption spectrometry – Introduction and Basics

Introduction :

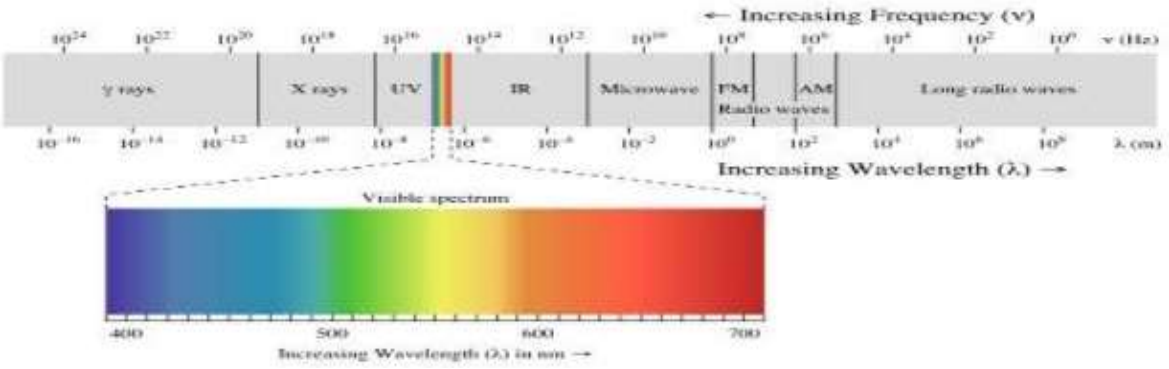
- In analytical chemistry, Atomic Absorption Spectrometry (AAS) is a technique for determining the concentration of a particular metal element in a sample.
- It is the most widely used method in analysis of elements which is based on the absorption of radiation.
- In absorption spectroscopy a beam of light is incident on a sample and most of the light passes through the sample.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on spectrophotometer and their uses.
- Prerequisite knowledge on different types of spectrometry instruments used.

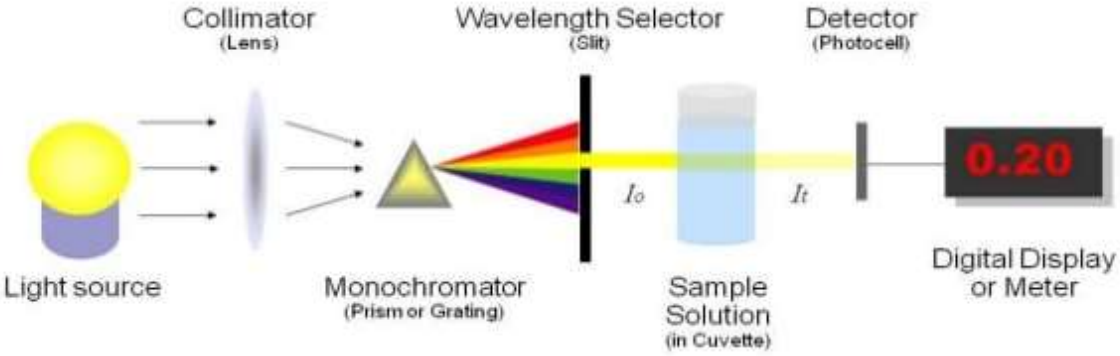
Detailed content of the Lecture:

- Absorption spectroscopy measures the absorption of radiation as a function of frequency or wavelength due to it's interaction with a sample.
- The intensity of absorption varies as a function of frequency and this variation is 'absorption spectrum'.
- Light is a spectrum of different wavelengths which the eye recognizes as 'white' but can be isolated into discrete portions and measured.
- Wavelength describes a position within a spectrum. It's the distance between 2 peaks as the light travels in a wave-like manner.
- Light also is composed of discrete energy packs called photons whose energy is inversely proportional to the wavelength.



- Certain molecules absorb light in a characteristic way: helps to identify and quantify biological molecules.

- Absorption occurs when the energy contained in a photon is absorbed by an electron resulting in a transition to an excited state.
- The absorption efficiency of an analyte is affected by: The nature of the analyte, number of available microstates, the solvent.



- The light absorption is directly related to the concentration of the compound in the sample.

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

Important Books/Journals for further learning including the page nos.:
Willard, Hobart, et al., "Instrumental Methods of Analysis" 7th Edition, CBS, 1986 (Pg. No. 243-245).

Course Faculty

Verified by HoD

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 | **II/IV**

Course Name with Code : **Instrumental Methods of Analysis 19BTD08**

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

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

Topic of Lecture: Measurement of Transmittance and Absorbance

Introduction :

- Transmittance is defined as the light passing through an object that is not reflected or absorbed.
- Absorbance and transmittance are measurements used in spectrophotometry. It measures how much radiant energy a substance absorbs at varying wavelengths of light.
- Absorbance (A), also known as optical density (OD), is the quantity of light absorbed by a solution.
- Absorption measurements based upon UV and visible light find widespread application for the quantitative determination of a large variety of species.

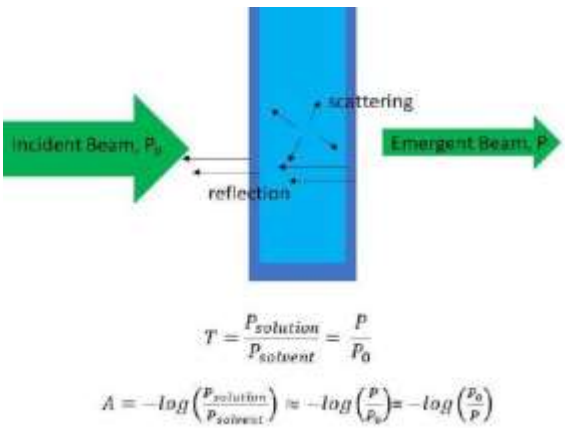
Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on scattering of light using absorbance and transmittance.
- Prerequisite knowledge on how absorption spectroscopy quantifies the sample.

Detailed content of the Lecture:

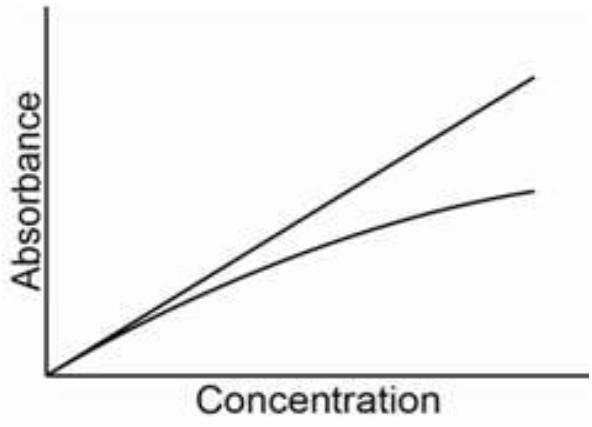
Absorbance measurement:

- The intensity of light emerging from the sample is attenuated by reflections losses at each of four interfaces where the refractive index of the media change possibly attenuated by particles scattering of light in the sample and most importantly by the absorption of the light by the sample.
- In order for the sample to absorb the light must meet 2 conditions: a) there must be a mechanism by which a component of the sample can interact with the electric or magnetic field components of the light; b) the wavelength or energy of the light must be resonant the difference in energy between two of the quantized energy levels of the absorbing component.



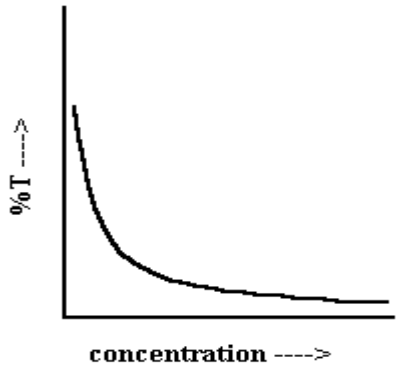
$$T = \frac{P_{solution}}{P_{solvent}} = \frac{P}{P_0}$$

$$A = -log\left(\frac{P_{solution}}{P_{solvent}}\right) \approx -log\left(\frac{P}{P_0}\right) = -log\left(\frac{P_0}{P}\right)$$



Transmittance measurement:

- The transmittance, T, is simply the fraction of light intensity passing through the sample and the absorbance A is the $-\log_{10}$ of the intensity of the light passing though the solvent relative to the intensity of light passing through the sample.
- For a non-absorbing solvent $A = -\log(P_o/P)$.
- The power of the beam transmitted by the analyte solution is usually compared with the power of the beam transmitted by an identical cell containing only solvent. An experimental transmittance and absorbance are then obtained with the equations.



Video Content / Details of website for further learning (if any):
https://chem.libretexts.org/Courses/Providence_College/CHM_331_Advanced_Analytical_Chemistry_1/08%3A_An_Introduction_to_Ultraviolet-Visible_Absorption_Spectrometry/8.01%3A_Measurement_of_Transmittance_and_Absorbance

Important Books/Journals for further learning including the page nos.:
Willard, Hobart, et al., "Instrumental Methods of Analysis" 7th Edition, CBS, 1986 (Pg. No. 161).

Course Faculty

Verified by HoD

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 | **II/IV**

Course Name with Code : **Instrumental Methods of Analysis 19BTD08**

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

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

Topic of Lecture: Beer Lambert's law Equation

Introduction :

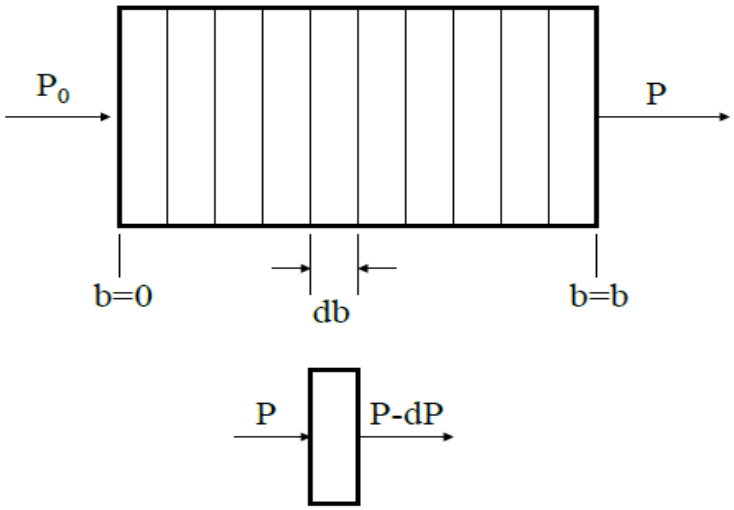
- The Beer-Lambert's law states that the quantity of light absorbed by a substance dissolved in a fully transmitting solvent is directly proportional to the concentration of the substance and the path length of light through the solution.
- The Beer-Lambert law is the linear relationship between absorbance and concentration of an absorbing species.
- It implies that both the type and the concentration of the molecules are important in the process of radiation absorption.

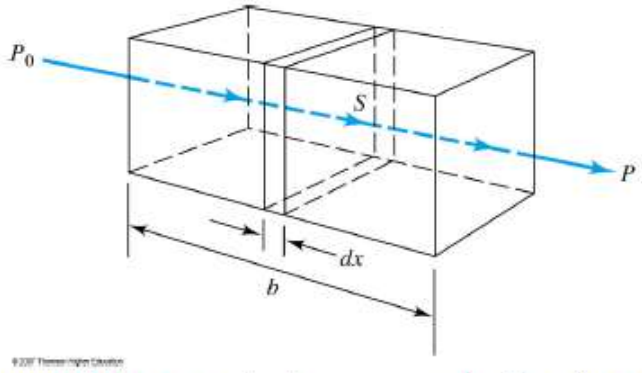
Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on EMR and in spectrophotometer instruments.
- Prerequisite knowledge on deriving absorbance and transmittance using Beer-Lambert's equation.
- Prerequisite knowledge on Engineering Physics and Biological Sciences.

Detailed content of the Lecture:

Sample with Absorbing molecule





dP ∝ P Incremental power lost ∝ power in; i.e., increase power in, increase power absorbed

dP ∝ db Longer pathlength, greater number of molecules in incremental slice and more power absorbed

Therefore, dP ∝ Pdb dP = -kPdb

k = proportionality constant (function of λ, c)

negative sign: because power is lost (i.e., absorbed)

Rearrange:

$$\frac{dP}{P} = -kdb$$

Integrate: $$\int_{P_0}^{P} \frac{1}{P} dP = -k \int_{0}^{b} db$$

$$InP - InP_0 = -kb - (-k)(0)$$

$$In\frac{P}{P_0} = -kb$$

Factor out concentration part of k: k = k"c

$$In\frac{P}{P_0} = -k"bc$$

Convert fraction (remove –sign) and change In to log:

(1/2.303)k" = ε

$$\log\frac{P_0}{P} = \frac{1}{2.303} k:"bc$$

$$A = \log\frac{P_0}{P} = \varepsilon bc$$

Applications

Beer's law applies to a medium containing more than one kind of absorbing substance. Provided there is no interaction among the various species, the total absorbance for a multicomponent system is given by

$$A_{total} = A_1 + A_2 + \ldots + A_n$$
$$= \varepsilon_1 bc_1 + \varepsilon_2 bc_2 + \ldots + \varepsilon_n bc_n$$

where, the subscripts refer to absorbing components 1, 2, …, n.

Assumption

- incident radiation is Monochromatic(all molecules absorb light of one λ)
- Absorbing molecules act independently of one another i.e, low c
- Pathlength is uniform (all rays travel the same distance in sample)
- No scattering
- Absorbing medium is optically homogeneous
- Incident beam is not large enough to cause saturation
- All rays should be parallel to each other and perpendicular to surface of medium.

Limitations

* *Real Limitations*

High concentration > 0.01 M

- the extent of solute-solvent interactions, solute-solute interactions, or hydrogen bonding can affect the analyte environment and its absorptivity.

* *Chemical Deviations*

- Analyte dissociates, associates or reacts to give molecule with different absorption characteristics (e.g., pH-dependent indicators)
  - Example 13-1

* *Instrumental Deviations*

- Polychromatic radiation
- Stray Radiation

Video Content / Details of website for further learning (if any):
https://www.youtube.com/watch?v=WP6JpnHZJIQ&t=13s

Important Books/Journals for further learning including the page nos.:
Willard, Hobart, et al., "Instrumental Methods of Analysis" 7th Edition, CBS, 1986 (Pg. No. 159-162).

Course Faculty

Verified by HoD

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 | **II/IV**

Course Name with Code : **Instrumental Methods of Analysis 19BTD08**

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

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

Topic of Lecture: Molecular absorption spectroscopy – Instrumentation and applications

Introduction :

- AAS (Atomic Absorption Spectroscopy) is a spectroanalytical procedure for the quantitative determination of chemical elements.
- It determines over 70 different elements in solution. Metals like Fe, Cu, Al, Pb, Ca, Zn, Cd and many more.
- This analytical technique established by Robert Bunsen and Robert Kirchhoff. The modern form was developed in 1950's by Sir Alan Walsh.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on principle and working of the instrument.
- Prerequisite knowledge on the instrument used and their applications.

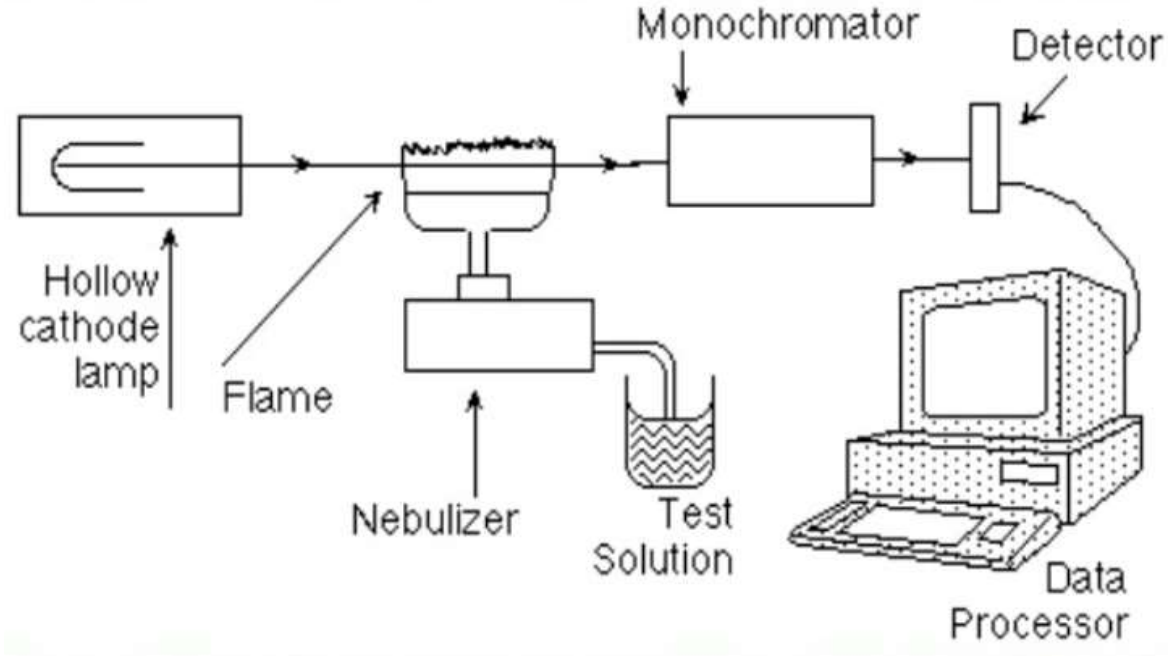
Detailed content of the Lecture:

- Absorbance spectroscopy is a molecular spectroscopy method that uses the wavelength dependent absorption characteristics of materials to identify and quantify specific substances.
- In analytical chemistry, Atomic Absorption Spectrometry (AAS) is a technique for determining the concentration of a particular metal element in a sample.
- It is the most widely used method in analysis of elements which is based on the absorption of radiation.

PRINCIPLE:

- The technique uses basically the principle that free atoms (gas) generated in an atomizer can absorb radiation at specific frequency.ν Atomic-absorption spectroscopy quantifies the absorption of ground state atoms in the gaseous state.
- The atoms absorb ultraviolet or visible light and make transitions to higher electronic energy levels. The analyte concentration is determined from the amount of absorption.
- Concentration measurements are usually determined from a working curve after calibrating the instrument with standards of known concentration.
- Atomic absorption is a very common technique for detecting metals and metalloids in environmental samples.

INSTRUMENTATION:



COMPONENTS:

1. LIGHT SOURCE:

- Hollow Cathode Lamp are the most common radiation source in AAS.
- It contains a tungsten anode and a hollow cylindrical cathode made of the element to be determined.
- These are sealed in a glass tube filled with an inert gas (neon or argon).
- Each element has its own unique lamp which must be used for that analysis.

2. NEBULIZER:

- Suck up liquid samples at controlled rate.
- Create a fine aerosol spray for introduction into flame.
- Mix the aerosol and fuel and oxidant thoroughly for introduction into flame.

3. ATOMIZER:

- Elements to be analyzed needs to be in atomic sate.
- Atomization is separation of particles into individual molecules and breaking molecules into atoms.
- This is done by exposing the analyte to high temperatures in a flame or graphite furnace.
- Types: flame atomizers and graphite tube atomizers.

4. MONOCHROMATOR:

- This is a very important part in an AA spectrometer.
- It is used to separate out all of the thousands of lines.
- A monochromator is used to select the specific wavelength of light which is absorbed by the sample, and to exclude other wavelengths.
- The selection of the specific light allows the determination of the selected element in the presence of others.

5. DETECTOR:

- The light selected by the monochromator is directed onto a detector that is typically a photomultiplier tube, whose function is to convert the light signal into an electrical signal proportional to the light intensity.
- The processing of electrical signal is fulfilled by a signal amplifier.
- The signal could be displayed for readout, or further fed into a data station for printout by the requested format.

APPLICATIONS:

- Determination of even small amounts of metals (lead, mercury, calcium, magnesium, etc) as follows:
- Environmental studies: drinking water, ocean water, soil.
- Food industry.
- Pharmaceutical industry.

Video Content / Details of website for further learning (if any):
https://www.slideshare.net/sharmasuriti/atomic-absorption-spectroscopy-15185397

Important Books/Journals for further learning including the page nos.:
Willard, Hobart, et al., "Instrumental Methods of Analysis" 7th Edition, CBS, 1986 (Pg. No. 243-245).

Course Faculty

Verified by HoD

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 | **II/IV**

Course Name with Code : **Instrumental Methods of Analysis 19BTD08**

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

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

Topic of Lecture: Theory of fluorescence – Instrumentation and applications

Introduction :

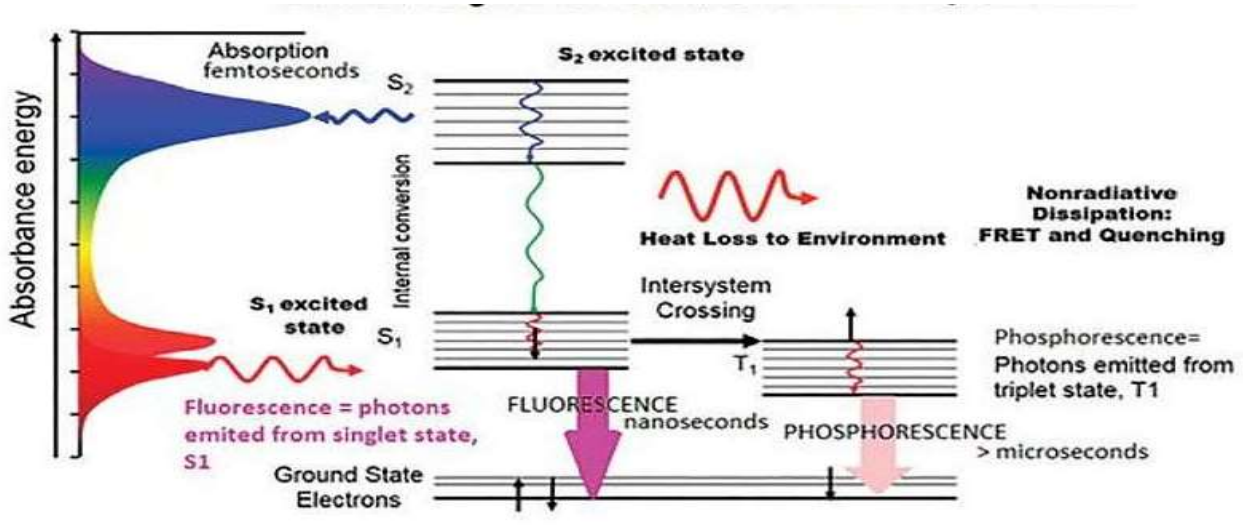
- Luminescence is defined as reemission of previously absorbed radiation.
- It is a type of luminescence caused by photons exciting a molecule raising it to an electronic excited state.
- As the excited molecule returns to ground state emits a photon of lower energy which corresponds to a longer wavelength than the absorbed photon. Fluorescence is shown as no change in electron spin.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on Fluorescence and applications of it.
- Prerequisite knowledge on working and instrumentation.
- Prerequisite knowledge on different components of fluorescence spectroscopy.

Detailed content of the Lecture:

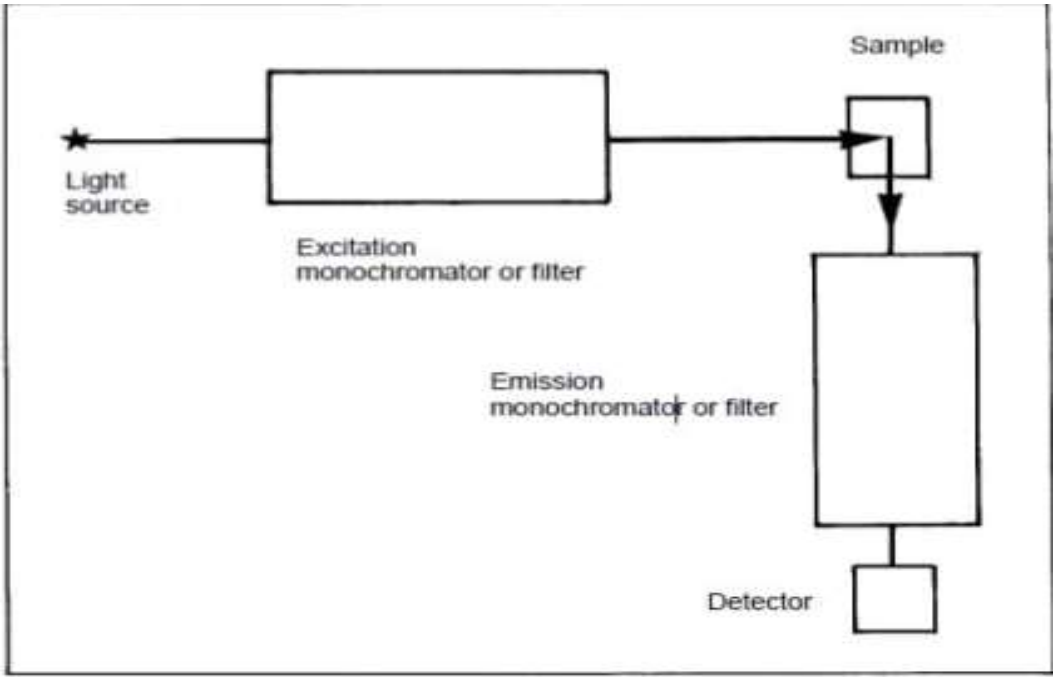
- Fluorescence spectroscopy analyzes fluorescence from a molecule based on it's fluorescent properties.
- Fluorescence is measured by the detection of this emitted radiation. The reason for the transition from higher to lower states is an earlier excitation event. This earlier event is due to input of energy by absorption of electromagnetic radiation.
- The wavelength of absorbed radiation must be at lower values (higher energy) than the emitted (fluoresced) wavelength. The difference between these two wave lengths is known as the stoke shift.



Jablonski's diagram

INSTRUMENTATION:

All fluorescence instruments contain three basic items: a source of light, a sample holder and a detector.



Working and Instrumentation diagram of Fluorescence spectroscopy

COMPONENTS:

LIGHT SOURCES:

- Commonly employed sources in fluorescence spectrometry have spectral outputs either as a continuum of energy over a wide range or as a series of discrete lines.
- An example of the first type is the tungsten-halogen lamp and of the latter, a mercury lamp.
- It is advantageous to employ a source whose output is a continuum and the most commonly employed type is the xenon arc.

SAMPLE HOLDERS:

- The majority of fluorescence assays are carried out in solution, the final measurement being made upon the sample contained in a cuvette or in a flow cell. Cuvettes may be circular, square or rectangular (the latter being uncommon), and must be constructed of a material that will transmit both the incident and emitted light.
- Square cuvettes, or cells will be found to be most precise since the parameters of path length and parallelism are easier to maintain during manufacture. However, round cuvettes are suitable for many more routine applications and have the advantage of being less expensive. The cuvette is placed normal to the incident beam.
- The resulting fluorescence is given off equally in all directions, and may be collected from either the front surface of the cell, at right angles to the incident beam, or in-line with the incident beam.

DETECTORS:

- All commercial fluorescence instruments use photomultiplier tubes as detectors and a wide variety of types are available.
- The material from which the photocathode is made determines the spectral range of the photomultiplier and generally two tubes are required to cover the complete UV visible range.

APPLICATIONS:

- A very few compounds exhibit the phenomenon of fluorescence.

- The effects of pH, solvent composition and the polarization of fluorescence may all contribute to structural elucidation.
- Some of the fluorescent dyes are sensitive to the presence of metal ions and can thus be used to track changes of these ions in *in vitro* samples as well as whole cells.

Video Content / Details of website for further learning (if any):
https://m.youtube.com/watch?v=awrN615hF8w&t=18s

Important Books/Journals for further learning including the page nos.:
Willard, Hobart, et al., "Instrumental Methods of Analysis" 7th Edition, CBS, 1986 (Pg. No. 197-212).

Course Faculty

Verified by HoD

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 | **II/IV**

Course Name with Code : **Instrumental Methods of Analysis 19BTD08**

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

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

Topic of Lecture: Theory of Phosphorescence – Instrumentation and applications

Introduction :

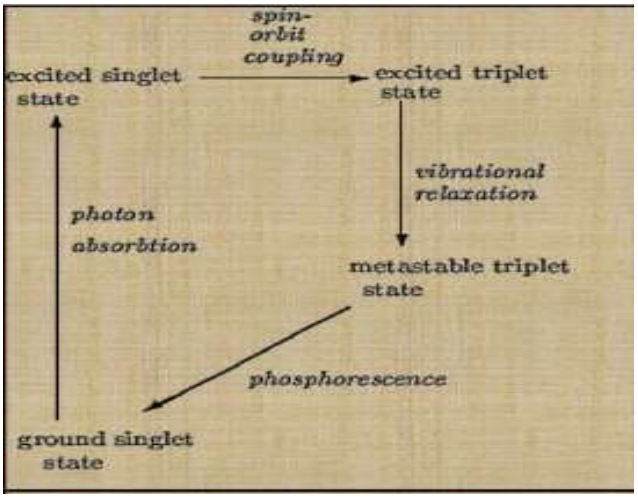
- Phosphorescence: The emission of radiation in a similar manner to fluorescence but on a longer timescale, so that emission continues after excitation ceases.
- Delayed and long lived emission of light energy in the form of a photon after an electron has been excited due to radiation.
- Phosphorescence is shown as change in electron spin.

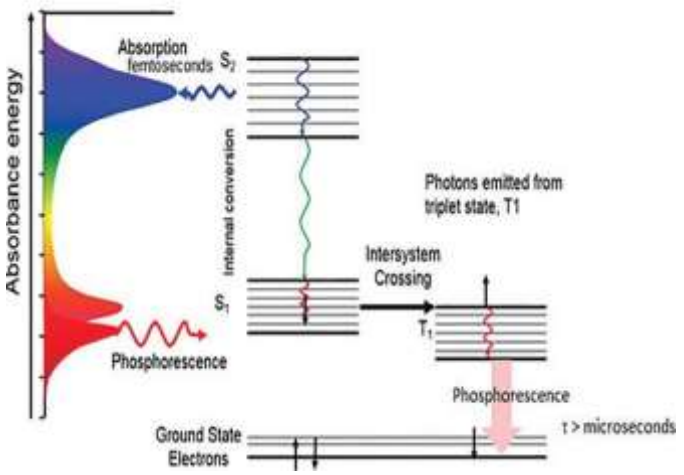
Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on phosphorescence spectroscopy instrumentation.
- Prerequisite knowledge on working and components of phosphorescence spectroscopy.
- Prerequisite knowledge on applications of phosphorescence spectroscopy.

Detailed content of the Lecture:

- While the molecule is in the excited state, it is possible for one electron to reverse its spin. The molecule is transferred to a lower – energy triplet state by a process called intersystem crossing.
- Through the processes of internal conversion and vibrational relaxation, the molecule rapidly attains the lowers vibrational level of the first excited triplet stage. From here, the molecule can return to the ground state by emission of photon. This emission is referred to as phosphorescence.
- It is much longer – lived than fluorescence. Phosphorescence measurements are made by cooling samples to liquid nitrogen temperature (-196°C) to minimize collision with other molecules.



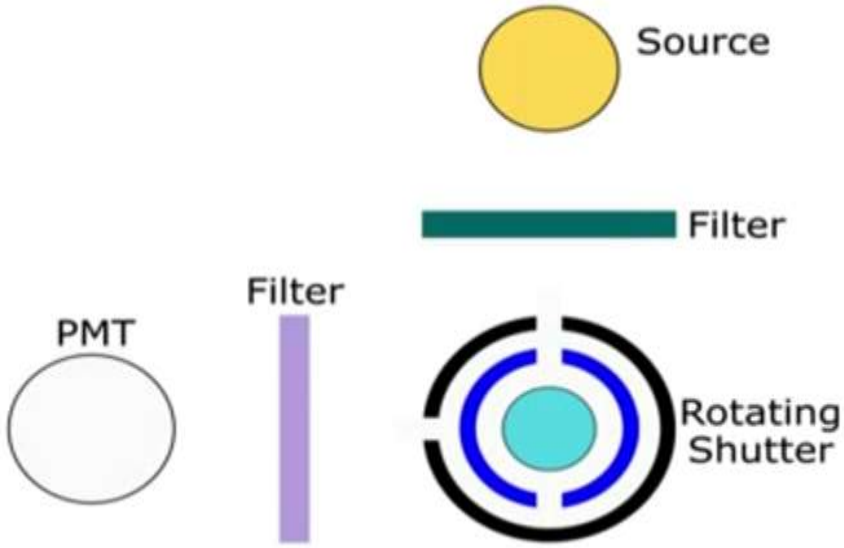


Principle of phosphorescence using Jablonski diagram

INSTRUMENTATION:

Spectrophosphorimeter is similar to a Spectrofluorimeter except that the former instrument must be fitted with

1) A sample system which is maintained at liquid nitrogen temperature.
2) A Rotating-shutter device commonly called a phosphoroscope.



EXCITATION SOURCE:

High intensity source of UV light are used;

- **Lasers –** A laser makes it possible to have narrow wavelength intervals that offer very high energy irradiation. This is useful when a large amount of energy is needed to produce the Phosphorescence in the sample.
- **Photodiodes –** Photodiodes are specialized diodes that can be configured in a manner that allows electrons to flow towards the sample so that the excess energy excites the phosphorescent particles.
- **Xenon Arcs –** Arcs of Xenon can produce the right amount of radiation for Phosphorescent materials.
- **Mercury Vapor –** Since mercury vapor can create ultraviolet radiation when electrical current is passed through it, it is good for use with materials that shows Phosphorescence under the ultraviolet radiation.

FILTERS AND MONOCHROMATORS:

Filters are of;

- Absorption
- Interfernce

Monochromators allow wavelength adjustment. Monochromators make it possible to do so with a diffraction grating.

- The primary filters that excite the sample provide the appropriate wavelength and
- The secondary filters monochromate the emitted light when sent to the detector.

PHOSPHOROSCOPE:

- A rotating disk excitation optical chopper, with three open and three larger opaque areas, is used to alternately excite the sample and allow phosphorescence to be measured.
- By measuring the phosphorescence intensity at several time intervals along the emission decay curve, a recorder trace of the decay with respect to time can be produced.
- The analytical precision and accuracy for quantitative measurements is improved by rotating the sample tube.

DETECTORS:

- A single channel (single wavelength from sample) or

- Multiple channels (multiple wavelengths detection)

APPLICATIONS:

- The majority of phosphorescence applications have been applied in the drug and pharmaceutical field and in the analysis of pesticides.
- The phosphorescence intensity of the rare earths increases tremendously when they are covalently bound to certain molecules and this feature has been used in the analysis of transferin in blood.
- Phosphorescence has been used in the detection of air and water-borne pollutants for the analysis of impurities in polycyclic aromatic hydrocarbons and in petroleum products

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

https://m.youtube.com/watch?v=YhAy-exSwZo&t=19s

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

Willard, Hobart, et al., "Instrumental Methods of Analysis" 7th Edition, CBS, 1986 (Pg. No. 217-220).

Course Faculty

Verified by HoD

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 | **II/IV**

Course Name with Code : **Instrumental Methods of Analysis 19BTD08**

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

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

Topic of Lecture: Theory of IR spectroscopy – Instrumentation and applications

Introduction :

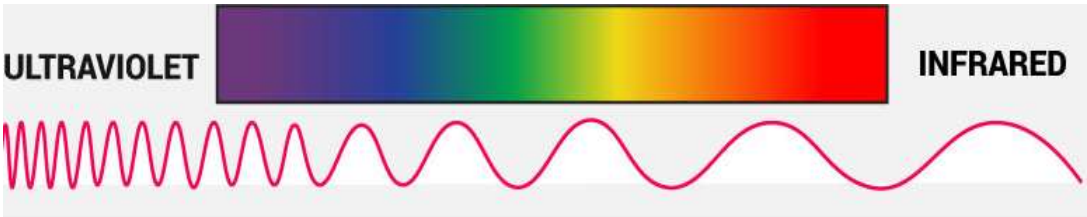
- IR (InfraRed) deals with the infrared region of the electromagnetic spectrum i.e light having a longer wavelength and a lower frequency than visible light.
- It refers to the analysis of the interaction of a molecule with IR light.
- The major use of IR spectroscopy is to determine the functional groups of molecules, relevant to both organic and inorganic chemistry.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on instruments used for absorption and emission.
- Prerequisite knowledge on different types of spectrometry instruments used.

Detailed content of the Lecture:

- An IR spectrum is essentially a graph plotted with the infrared light absorbed on the Y-axis against frequency or wavelength on the X-axis.
- IR Spectroscopy detects frequencies of infrared light that are absorbed by a molecule. Molecules tend to absorb these specific frequencies of light since they correspond to the frequency of the vibration of bonds in the molecule.
- Solid samples can be prepared by crushing the sample with a mulling agent which has an oily texture. A thin layer of this mull can now be applied on a salt plate to be measured.
- Liquid samples are generally kept between two salt plates and measured since the plates are transparent to IR light. Salt plates can be made up of sodium chloride, calcium fluoride, or even potassium bromide.

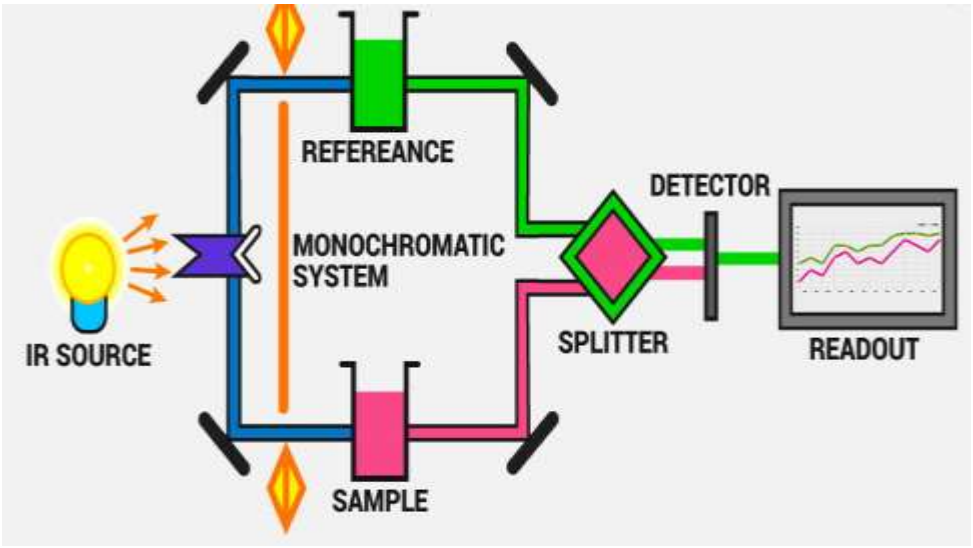


IR spectrum range

PRINCIPLE:

- The IR spectroscopy theory utilizes the concept that molecules tend to absorb specific frequencies of light that are characteristic of the corresponding structure of the molecules.
- The energies are reliant on the shape of the molecular surfaces, the associated vibronic coupling, and the mass corresponding to the atoms.
- For instance, the molecule can absorb the energy contained in the incident light and the result is a faster rotation or a more pronounced vibration.

INSTRUMENTATION:



COMPONENTS:

The main parts of IR spectrometer are as follows:

- Radiation source
- Sample cells and sampling of substances
- Monochromators
- Detectors
- Recorder

➢ **Radiation Source:** IR instruments require a source of radiant energy which emit IR radiation which must be steady, intense enough for detection and extend over the desired wavelength.

Various sources of IR radiations are as follows.

- Nernst glower
- Incandescent lamp
- Mercury arc
- Tungsten lamp
- Glober source
- Nichrome wire

➢ **Sample cells and sampling of substances:** IR spectroscopy has been used for the characterization of solid, liquid or gas samples.

- Solid – Various techniques are used for preparing solid samples such as pressed pellet technique, solid run in solution, solid films, mull technique etc.
- Liquid – Samples can be held using a liquid sample cell made of alkali halides. Aqueous solvents cannot be used as they will dissolve alkali halides. Only organic solvents like chloroform can be used.
- Gas– Sampling of gas is similar to the sampling of liquids.

➢ **Monochromators:**

- Various types of monochromators are prism, gratings and filters.
- Prisms are made of Potassium bromide, Sodium chloride or Caesium iodide.
- Filters are made up of Lithium Fluoride and Diffraction gratings are made up of alkali halides.

➢ **Detectors:**

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

➢ **Recorders:**

- Used to record the IR spectrum.

APPLICATIONS:

- Protein characterization

- Nanoscale semiconductor analysis and
- Space exploration.
- Analysis of gaseous, liquid or solid samples
- Identification of compounds
- Quantitative analysis

Video Content / Details of website for further learning (if any):
https://byjus.com/chemistry/infrared-spectrascopy/

Important Books/Journals for further learning including the page nos.:
Willard, Hobart, et al., "Instrumental Methods of Analysis" 7th Edition, CBS, 1986 (Pg. No. 287-301).

Course Faculty

Verified by HoD

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 | **II/IV**

Course Name with Code : **Instrumental Methods of Analysis 19BTD08**

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

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

Topic of Lecture: Theory of Raman spectroscopy – Instrumentation and applications

Introduction :

- A monochromatic radiation is incident upon a sample then this light will interact with the sample in some fashion.
- It is the scattering of the radiation that occurs which can tell the Raman spectroscopist something of the samples molecular structure.
- If the frequency of the scattered radiation is analysed not only is the incident radiation wavelength seen but also a small amount of radiation that is scattered at some different wavelengths.

Prerequisite knowledge for Complete understanding and learning of Topic:

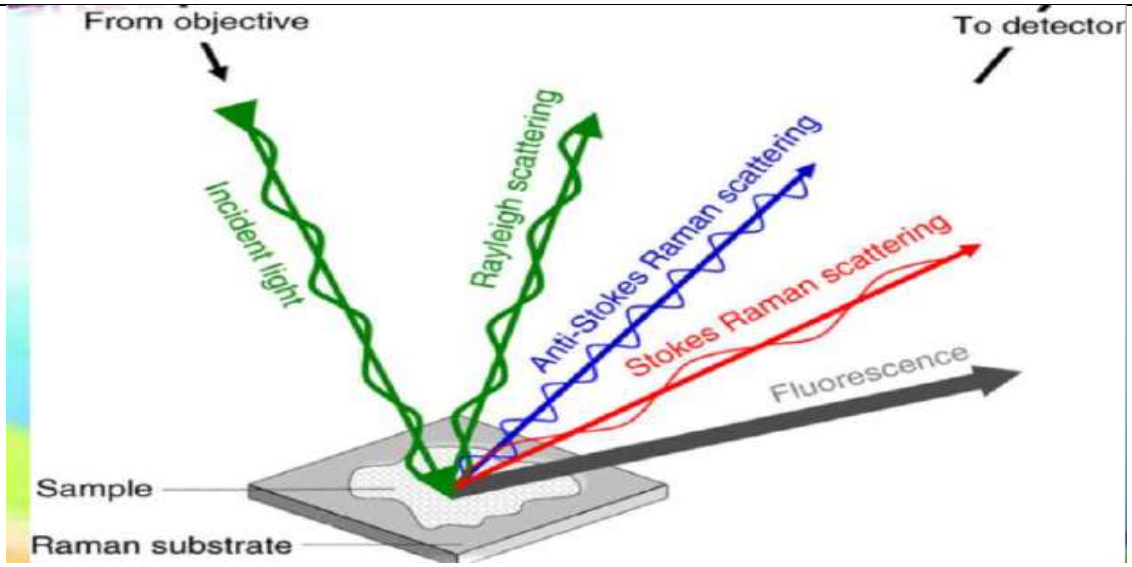
- Prerequisite knowledge on molecules systems through scattered radiation.
- Prerequisite knowledge on different rotational and vibrational transitions.
- Prerequisite knowledge on different various scattering types.

Detailed content of the Lecture:

- In molecular systems, these frequencies are principally in the ranges associated with rotational, vibrational and electronic level transitions.
- The scattered radiation occurs over all directions and may also have observable changes in it's polarization along with it's wavelength.
- The scattering process without a change of frequency is called Rayleigh scattering, a change in the frequency of light is called Raman scattering.

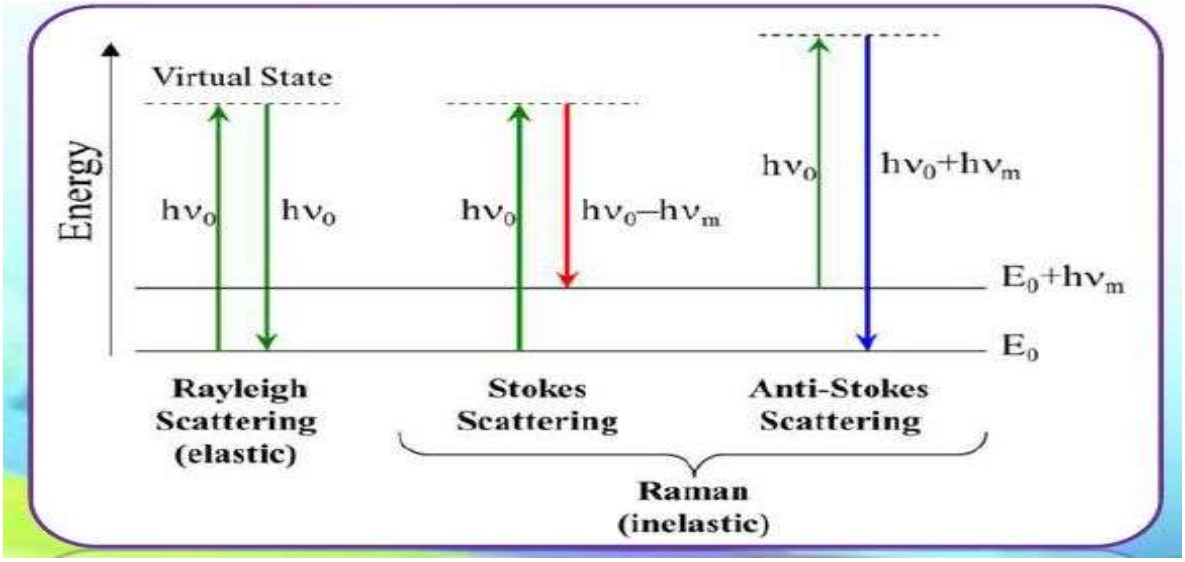
Theory:

- The inelastic scattering of a photon by molecules which are excited to higher vibrational or rotational energy levels.
- Phenomenon of inelastic light scattering.
- Scattering of light at the same frequency as incident light is called RAYLEIGH SCATTERING.
- Light scattered with different frequency is called RAMAN SCATTERING.

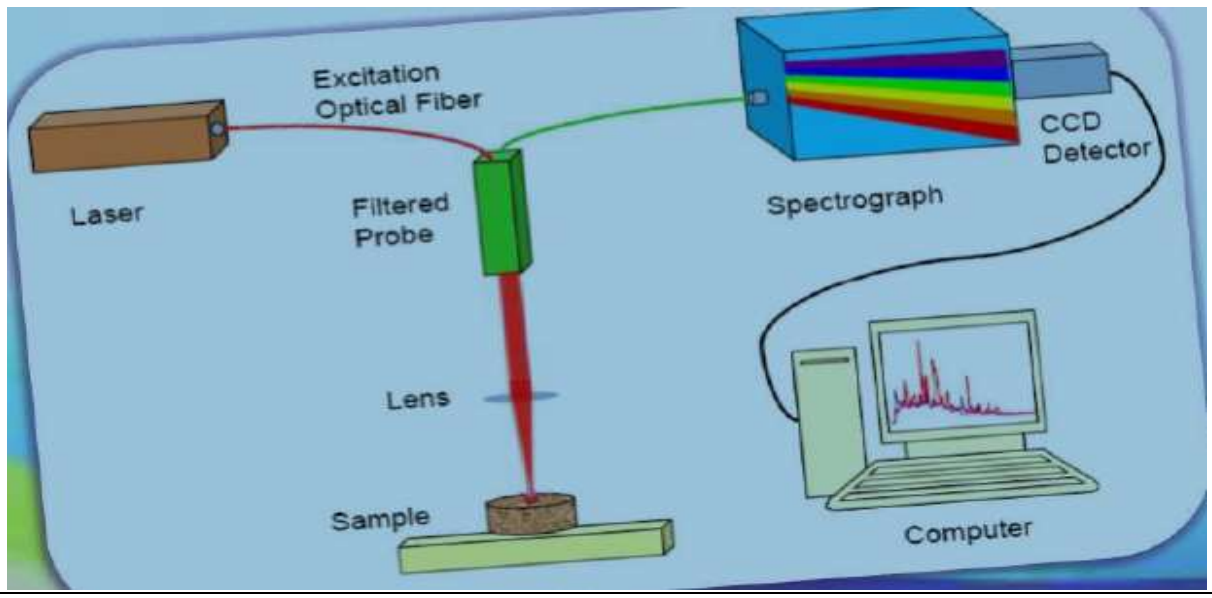


Two possible outcomes:

- The material absorbs energy and the emitted photon has a lower energy than the absorbed photon -Stokes Raman scattering.
- The material loses energy and the emitted photon has a higher energy than the absorbed photon - Anti Stokes Raman scattering.



INSTRUMENTATION:



Three main components-

1. The laser Small form factor, low power consumption, narrow linewidth, a stable power output, and a stable wavelength output.
2. The sampling interface Block the laser wavelength as much as possible so that the raman shift can be observed.
3. The spectrometer Small form factor, high resolution, low power consumption, and low noise.

APPLICATIONS:

- To determine the nature of chemical bonds and symmetry of molecules
- As a fingerprint to identify molecules
- In solid state physics to crystallographic orientation of sample
- To detect explosives for airport security
- To investigate chemical composition of historical documents
- In medicine

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

https://youtu.be/SsIYDEma_cU

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

Willard, Hobart, et al., "Instrumental Methods of Analysis" 7th Edition, CBS, 1986 (Pg. No. 323-329).

Course Faculty

Verified by HoD

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 | **II/IV**

Course Name with Code : **Instrumental Methods of Analysis 19BTD08**

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

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

Topic of Lecture: Theory of NMR

Introduction :

- NMR is Nuclear Magnetic Resonance spectroscopy is a powerful and theoretically complex analytical tool.
- It's important to remember that, with NMR we are performing experiments on the nuclei of atoms not the electrons.
- The chemical environment of specific nuclei is deduced from information obtained about the nuclei.

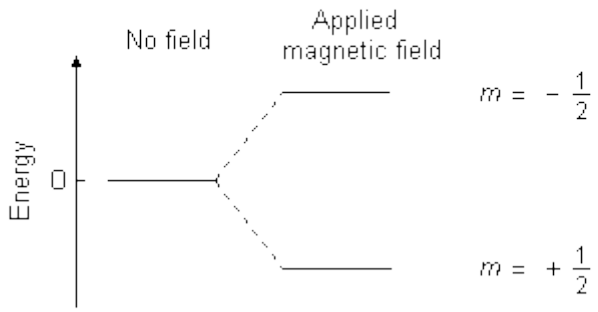
Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on atomic particles and their spin across the axis.
- Prerequisite knowledge on the energy levels for a particular nucleus with their spin.
- Prerequisite knowledge on precesssional movement of a molecule.

Detailed content of the Lecture:

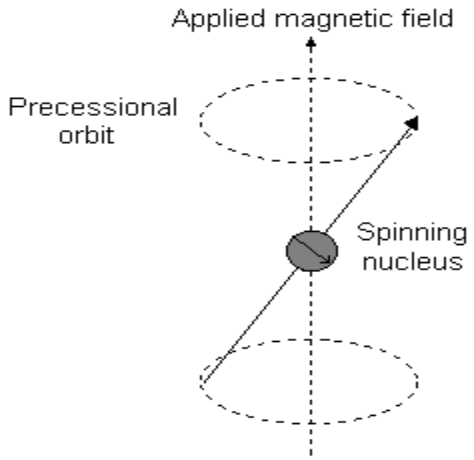
- Subatomic particles (electrons, protons and neutrons) can be imagined as spinning on their axes. In many atoms (such as ^{12}C) these spins are paired against each other, such that the nucleus of the atom has no overall spin. However, in some atoms (such as ^{1}H and ^{13}C) the nucleus does possess an overall spin. The rules for determining the net spin of a nucleus are as follows;
  1. If the number of neutrons **and** the number of protons are both even, then the nucleus has **NO** spin.
  2. If the number of neutrons **plus** the number of protons is odd, then the nucleus has a half-integer spin (i.e. 1/2, 3/2, 5/2).
  3. If the number of neutrons **and** the number of protons are both odd, then the nucleus has an integer spin (i.e. 1, 2, 3).
- The overall spin, I, is important. Quantum mechanics tells us that a nucleus of spin I will have 2I + 1 possible orientations. A nucleus with spin 1/2 will have 2 possible orientations. In the absence of an external magnetic field, these orientations are of equal energy. If a magnetic field is applied, then the energy levels split. Each level is given a magnetic quantum number, m.

Energy levels for a nucleus with spin quantum number 1/2



The absorption of radiation by a nucleus in a magnetic field

- Imagine a nucleus (of spin 1/2) in a magnetic field. This nucleus is in the lower energy level (i.e. its magnetic moment does not oppose the applied field). The nucleus is spinning on its axis. In the presence of a magnetic field, this axis of rotation will precess around the magnetic field



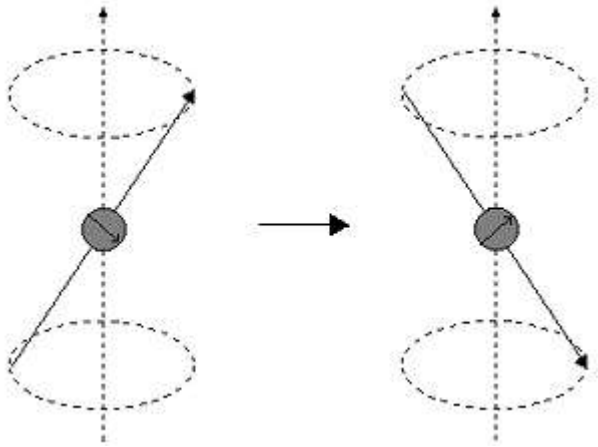
- The frequency of precession is termed the Larmor frequency, which is identical to the transition frequency.

The potential energy of the precessing nucleus is given by;

$$E = - \mu B \cos \theta$$

where θ is the angle between the direction of the applied field and the axis of nuclear rotation.

- If energy is absorbed by the nucleus, then the angle of precession, θ, will change. For a nucleus of spin 1/2, absorption of radiation "flips" the magnetic moment so that it opposes the applied field (the higher energy state).



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

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

Willard, Hobart, et al., "Instrumental Methods of Analysis" 7th Edition, CBS, 1986 (Pg. No. 203-205).

Course Faculty

Verified by HoD

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 | **II/IV**

Course Name with Code : **Instrumental Methods of Analysis 19BTD08**

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

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

Topic of Lecture: Environmental effects on NMR spectra

Introduction :

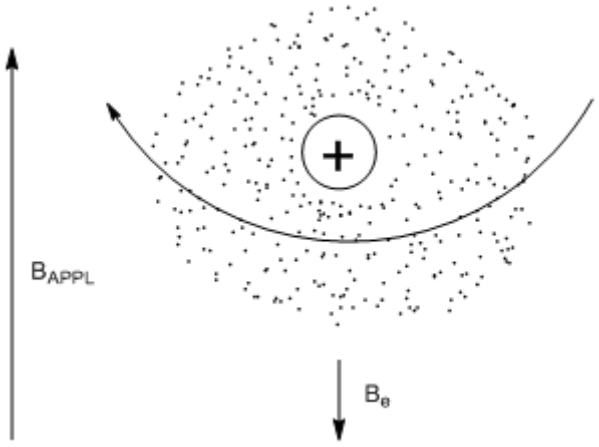
- Spinning electrons generate a magnetic field that in some way is responsible for shielding.
- The spin of two paired electrons in a molecular orbitals generate opposing magnetic fields that cancel each other out.
- NMR spectra are represented with the magnitude of absorbance on the y-axis and frequency on the x-axis.
- In NMR spectroscopy, it's also necessary to adopt a uniform zero reference.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on understanding the importance of NMR spectroscopy.
- Prerequisite knowledge on nature and effects of compounds in magnetic field in NMR.
- Prerequisite knowledge on knowing how electrons in an atoms is shielded.

Detailed content of the Lecture:

- While it might be tempting to think that spinning electrons generate a magnetic field that in some way is responsible for shielding.
- The spin of two paired electrons in a molecular orbital generate opposing magnetic fields that cancel each other out.
- For instance, a circulating charged 'cloud' of electrons does create a magnetic field. This magnetic field usually opposes B_{APPL}, hence the use of term 'shielding' to describe the effect.
- Altering the applied magnetic field ends up altering the rate at which the electrons circulate about the nucleus.
- At higher values of B_{APPL}, the electrons circulate faster with the result of a proportionally larger value of B_e.



Circulation pattern for the electron cloud around a hydrogen nucleus

Video Content / Details of website for further learning (if any): https://chem.libretexts.org/Bookshelves/Analytical_Chemistry/Map%3A_Principles_of_Instrumental_Analysis_(Skoog_et_al.)
Important Books/Journals for further learning including the page nos.: Willard, Hobart, et al., "Instrumental Methods of Analysis" 7th Edition, CBS, 1986 (Pg. No. 206).

Course Faculty

Verified by HoD

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 | **II/IV**

Course Name with Code : **Instrumental Methods of Analysis 19BTD08**

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

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

Topic of Lecture: Chemical shift (δ) in NMR

Introduction :

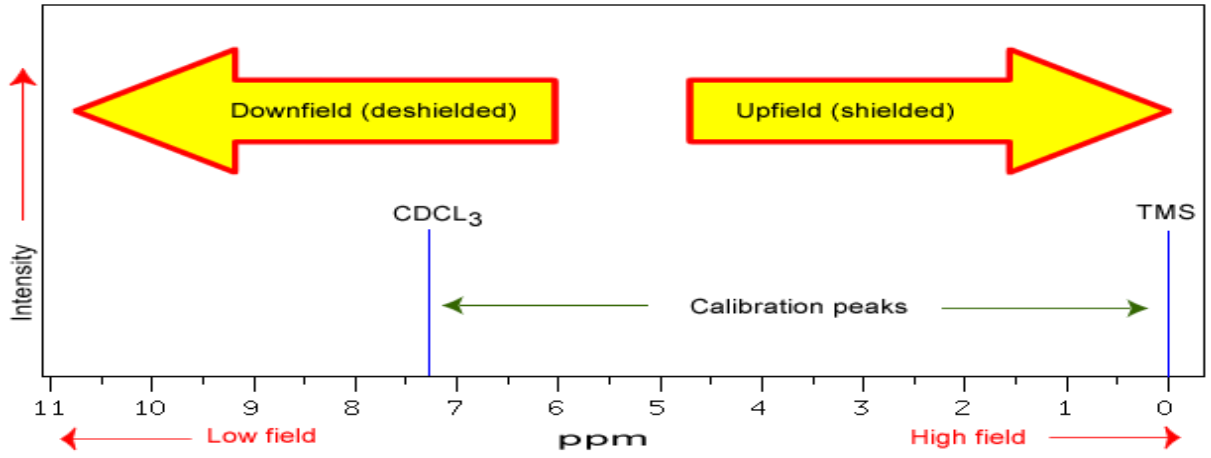
- In NMR spectroscopy, the chemical shift is the resonant frequency of a nucleus relative to a standard in a magnetic field.
- The variations of nuclear magnetic resonance frequencies of the same kind of nucleus due to variations in the electron distribution is called chemical shift.
- It's used to describe 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.:

Willard, Hobart, et al., "Instrumental Methods of Analysis" 7th Edition, CBS, 1986 (Pg. No. 387-388; 440-442).

Course Faculty

Verified by HoD

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 | **II/IV**

Course Name with Code : **Instrumental Methods of Analysis 19BTD08**

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

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

Topic of Lecture: NMR spectrometers and applications of ^{1}H and ^{13}C NMR

Introduction :

- NMR is a spectroscopic technique to observe local magnetic fields around atomic nuclei.
- The sample is placed in a magnetic field and the NMR signal is produced by excitation of the nuclei sample with radio waves into NMR which is detected with sensitive radio receivers.
- The intramolecular magnetic field around an atom in a molecule changes the resonance frequency, thus giving access to details of the electronic structure of a molecule and it's individual functional groups.
- In modern organic chemistry, NMR spectroscopy is the definitive method to identify monomolecular organic compounds.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on studying the principle and working of NMR.
- Prerequisite knowledge on compounds subjected to magnetic field for analysis.
- Prerequisite knowledge on studying of molecules by recording the interaction of R_f EMR with the nuclei of molecules placed in a strong magnetic field.

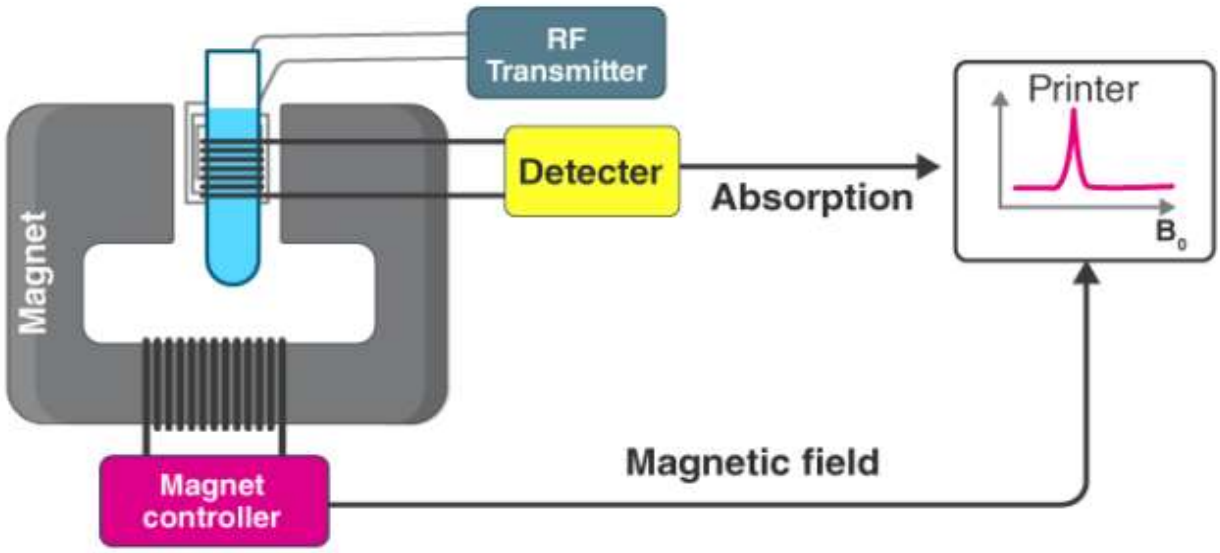
Detailed content of the Lecture:

- NMR spectroscopy is a crucial analytical tool for organic chemists. The research in the organic lab has been significantly improved with the aid of the NMR.
- It can also determine the content and purity of the sample. Proton (^{1}H) NMR is one of the most widely used NMR methods by organic chemists.
- The protons present in the molecule will behave differently depending on the surrounding chemical environment, making it possible to elucidate their structure.

PRINCIPLE:

- Many nuclei have spin, and all nuclei are electrically charged, according to the NMR principle.
- An energy transfer from the base energy to a higher energy level is achievable when an external magnetic field is supplied. All nuclei are electrically charged and many have spin.
- Transfer of energy is possible from base energy to higher energy levels when an external magnetic field is applied. The transfer of energy occurs at a wavelength that coincides with the radio frequency.
- Also, energy is emitted at the same frequency when the spin comes back to its base level.
- Therefore, by measuring the signal which matches this transfer the processing of the NMR spectrum for the concerned nucleus is yield.

INSTRUMENTATION:



NMR Spectroscopy Instrumentation

WORKING:

- Place the sample in a magnetic field. Excite the nuclei sample into nuclear magnetic resonance with the help of radio waves to produce NMR signals.
- These NMR signals are detected with sensitive radio receivers. The resonance frequency of an atom in a molecule is changed by the intramolecular magnetic field surrounding it.
- This gives details of a molecule's individual functional groups and its electronic structure. Nuclear magnetic resonance spectroscopy is a conclusive method of identifying monomolecular organic compounds.
- This method provides details of the reaction state, structure, chemical environment and dynamics of a molecule.

NMR SPECTROMETERS:

There are two types of NMR spectrometers,

a. continuous-wave (cw) and
b. pulsed or Fourier-Transform (FT-NMR)

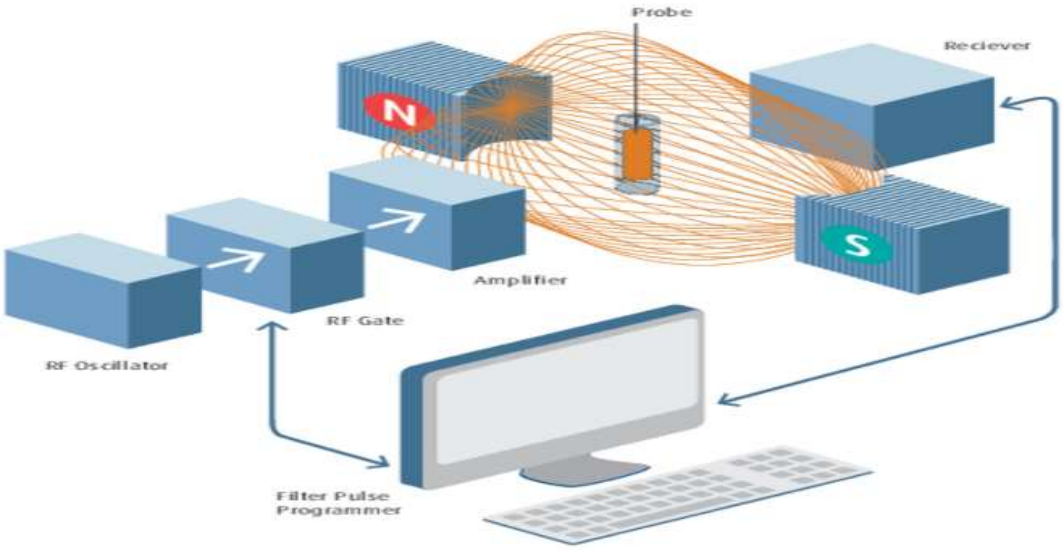
1. **A continuous-wave NMR instrument:** Consists of the following units:

a. a magnet to separate the nuclear spin energy states
b. at least two radiofrequency channels: one for field/frequency stabilization and one to furnish RF irradiation energy;
c. a sample probe containing coils for coupling the sample with the RF field;
d. a detector to process the NMR signals; a sweep generator for sweeping either the magnetic or RF field through the resonance frequencies of the sample;
e. a recorder to display the spectrum.

- ❑ The spectrum is scanned by the field-sweep method or the frequency-sweep method.
- ❑ In the field sweep method, the RF signal is held constant, then the magnetic field is swept, which varies the energy levels, to determine the magnetic field strengths that produce resonance at fixed resonance frequency.
- ❑ In the frequency-sweep method, the magnetic field is held constant, which keeps the nuclear spin energy levels constant, then the RF signal is swept to determine the frequencies at which energy is absorbed.

2. Fourier-Transform NMR spectrometers: Use a pulse of radiofrequency radiation to cause nuclei in a magnetic field to flip into the higher-energy alignment.

- The length of the RF pulse is 1-10 μs and is wide enough to simultaneously excite nuclei in all local environments.
- The interval between pulses T is typically one to several seconds. During T, a time-domain RF signal called the free induction decay (FID) signal is emitted as nuclei return to their original state.



FT-NMR spectroscopy

APPLICATIONS OF ^{1}H and ^{13}C NMR:

1. NMR spectroscopy is a Spectroscopy technique used by chemists and biochemists to investigate the properties of organic molecules, although it is applicable to any kind of sample that contains nuclei possessing spin.
2. For example, the NMR can quantitatively analyze mixtures containing known compounds. NMR can either be used to match against spectral libraries or to infer the basic structure directly for unknown compounds.
3. Once the basic structure is known, NMR can be used to determine molecular conformation in solutions as well as in studying physical properties at the molecular level such as conformational exchange, phase changes, solubility, and diffusion.

Video Content / Details of website for further learning (if any):
https://byjus.com/chemistry/nmr-spectroscopy/
https://www.youtube.com/watch?v=ywR6aLpfjI0

Important Books/Journals for further learning including the page nos.:
Willard, Hobart, et al., "Instrumental Methods of Analysis" 7th Edition, CBS, 1986 (Pg. No. 422-437).

Course Faculty

Verified by HoD

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 | **II/IV**

Course Name with Code : **Instrumental Methods of Analysis 19BTD08**

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

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

Topic of Lecture: Molecular mass spectra

Introduction :

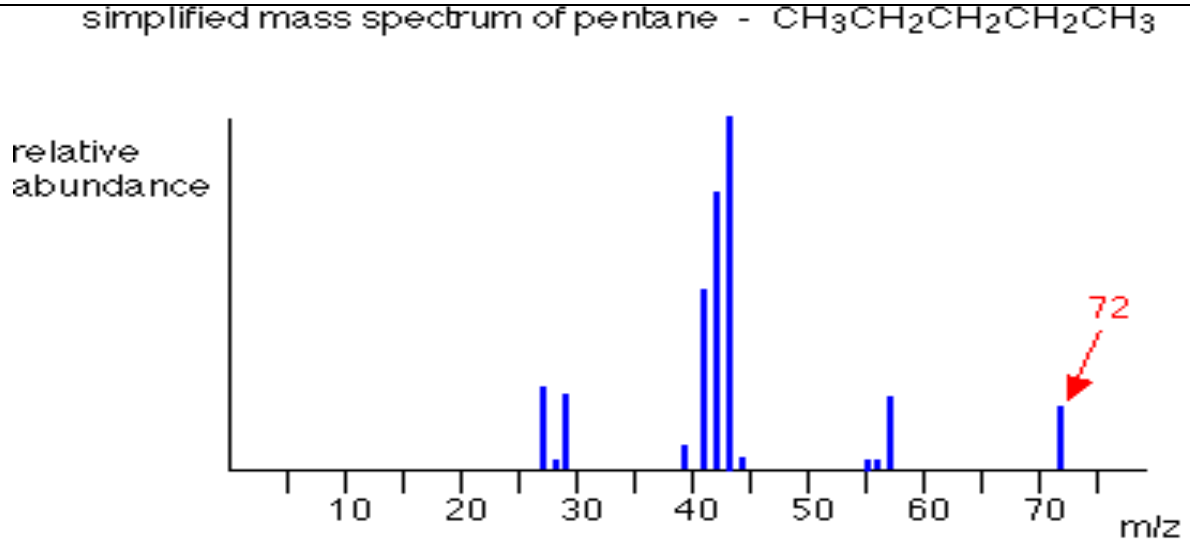
- A mass spectrum will usually be represented as a vertical bar graph in which each bar represents an ion having a specific mass-to-charge ratio (m/z) and the length of the bar indicates the relative abundance of the ion.
- The most intense ion is assigned an abundance of 100 and it is referred to as base peak.
- These spectra are used to determine the elemental or isotopic signature of a sample, the masses of particles and of molecules and to elucidate the chemical identity or structure of molecules and other chemical compounds.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on the mass spectrum of molecule analysed.
- Prerequisite knowledge on the fragmentation of ions of the molecule.
- Prerequisite knowledge on the difference between base peak and parent ion.

Detailed content of the Lecture:

- Most of the ions formed in a mass spectrometers have a single charge so the m/z value is equivalent to mass itself.
- The highest mass ion in a spectrum is normally considered to be the molecular ion and lower mass ions are fragments from the molecular ion assuming the sample is a single pure compound.
- The nature of the fragments often provides a clue to the molecular structure, but if the molecular ion has a lifetime of less than a few microseconds it will not survive long enough to be observed.
- Modern mass spectrometers easily distinguish ions differing by only a single atomic mass unit (amu), and thus provide completely accurate values for the molecular mass of a compound.
- The molecular ion in a mass spectrum is always a radical cation, but the fragment ions may either be even electron cations or odd electron radical cations depending on the neutral fragment lost.
- For Ex: mass spectra of pentane is as follows:



NMR spectra of a single pure compound

Video Content / Details of website for further learning (if any):
https://www2.chemistry.msu.edu/faculty/reusch/virttxtjml/spectrpy/massspec/masspec1.htm

Important Books/Journals for further learning including the page nos.:
Willard, Hobart, et al., "Instrumental Methods of Analysis" 7th Edition, CBS, 1986 (Pg. No. 453-454).

Course Faculty

Verified by HoD

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 | **II/IV**

Course Name with Code : **Instrumental Methods of Analysis 19BTD08**

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

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

Topic of Lecture: Ion sources in MS

Introduction :

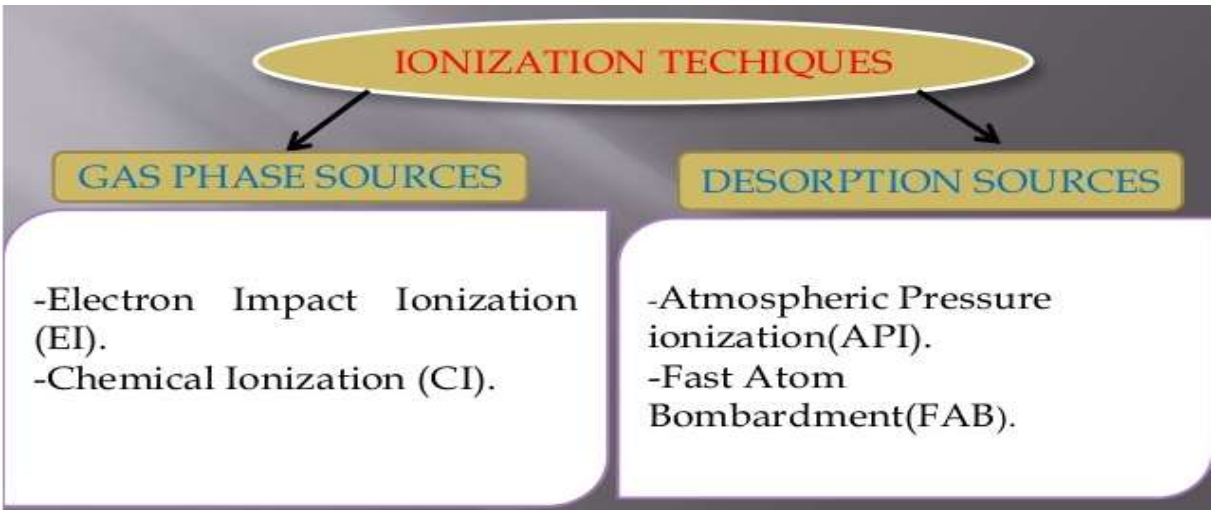
- An ion source is a device that creates atomic and molecular ions.
- Ion sources are used to form ions for MS, optical emission spectrometers, particle accelerators, ion implanters and ion engines.
- Liquids and solids are first converted into gas from the gaseous sample, ions are produced in a box like enclosure called ion source.
- Ion source is the heart of Mass spectrometry.

- **Prerequisite knowledge for Complete understanding and learning of Topic:**
- Prerequisite knowledge on different types of ion sources used in MS.
- Prerequisite knowledge on how molecules are passed through and vaporized for detection.
- Prerequisite knowledge on how sample is detected.

Detailed content of the Lecture:

Ion sources:

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

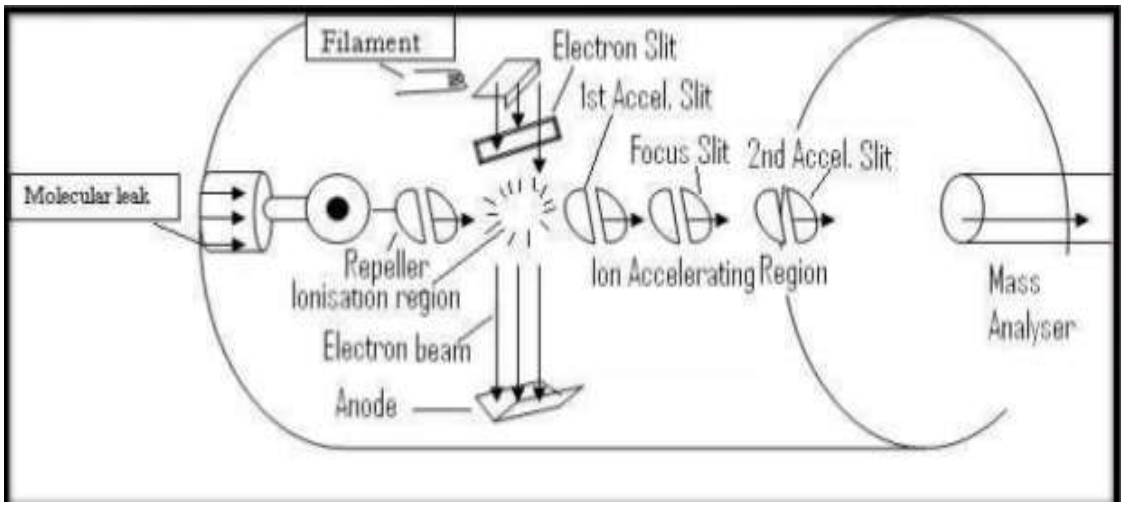


EI:

- 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.

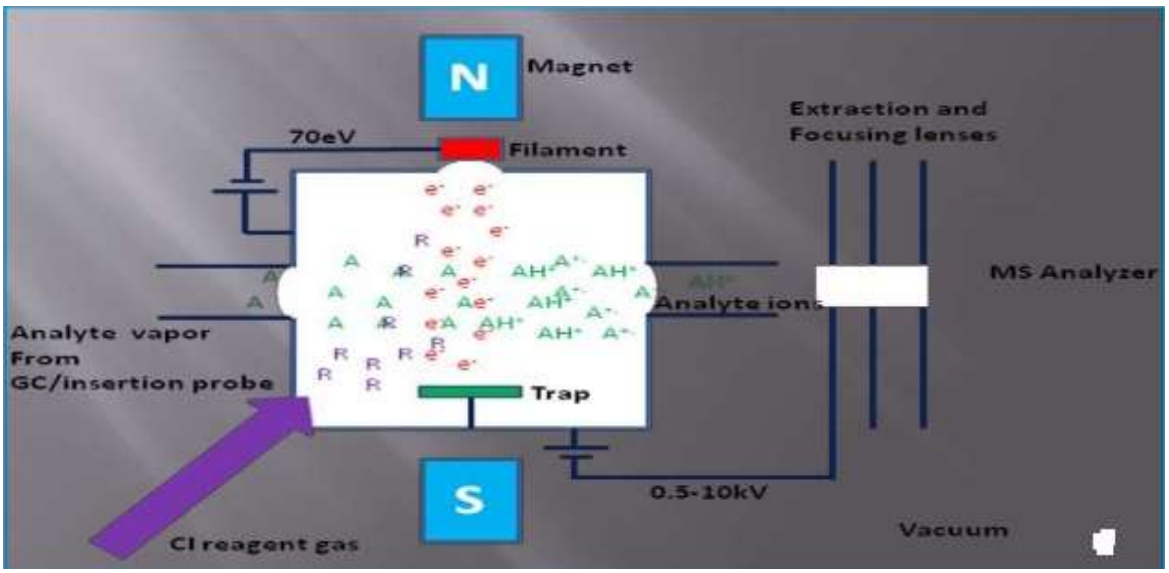
- Due to the electron impact the sample is broken into positive or negative ions.
- The energetic electron beam is emitted by a electrically heated tungsten or rhenium which are then accelerated by the potential difference of 70eV.
- Collision between ions and molecules may also result in ion with higher m/z values than the molecular ion. Where M+ is a radical cation which gives molecular weight

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



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.

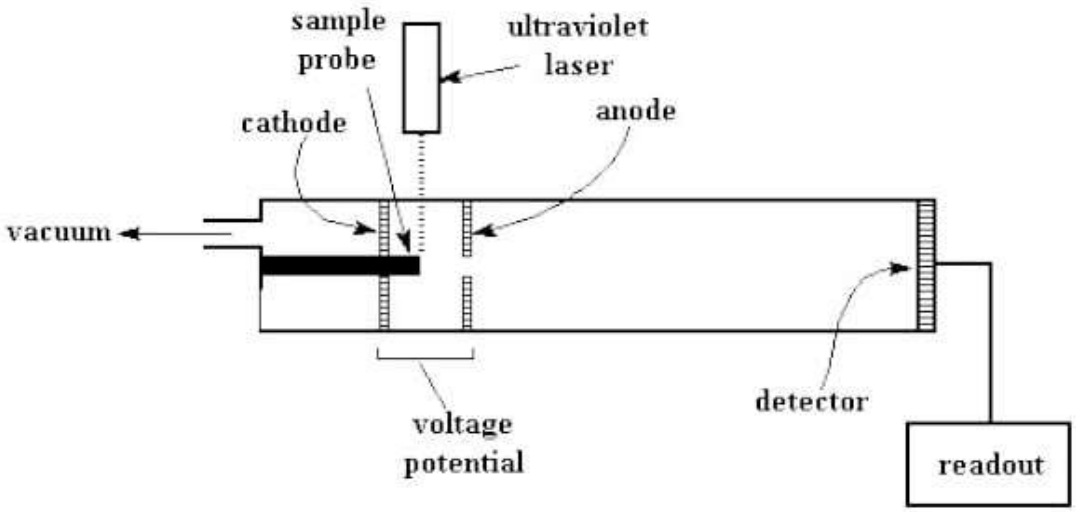


API:

- 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)

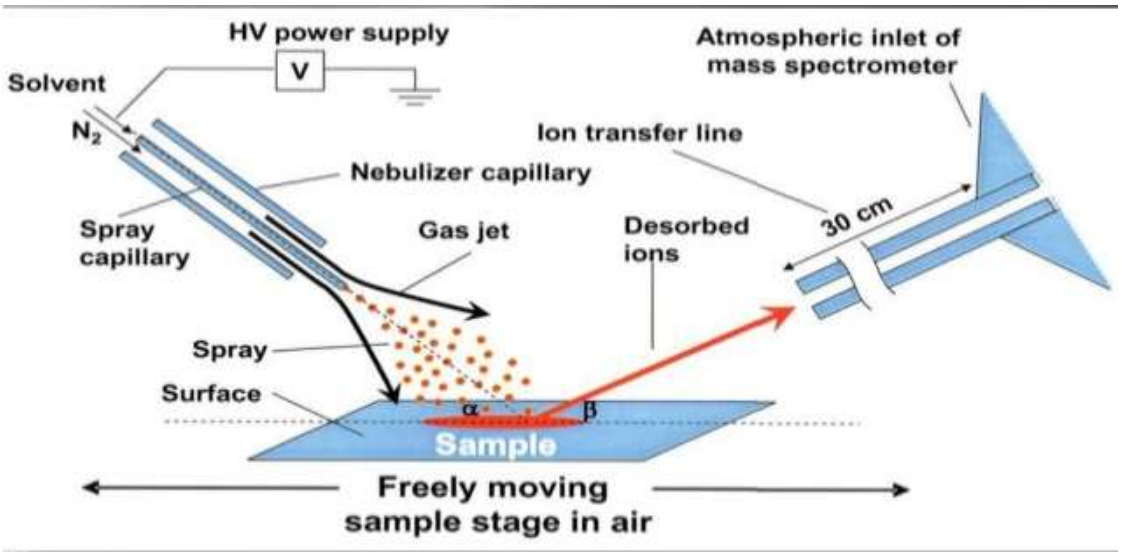
a) **MALDI:**

- 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) **ESI:**

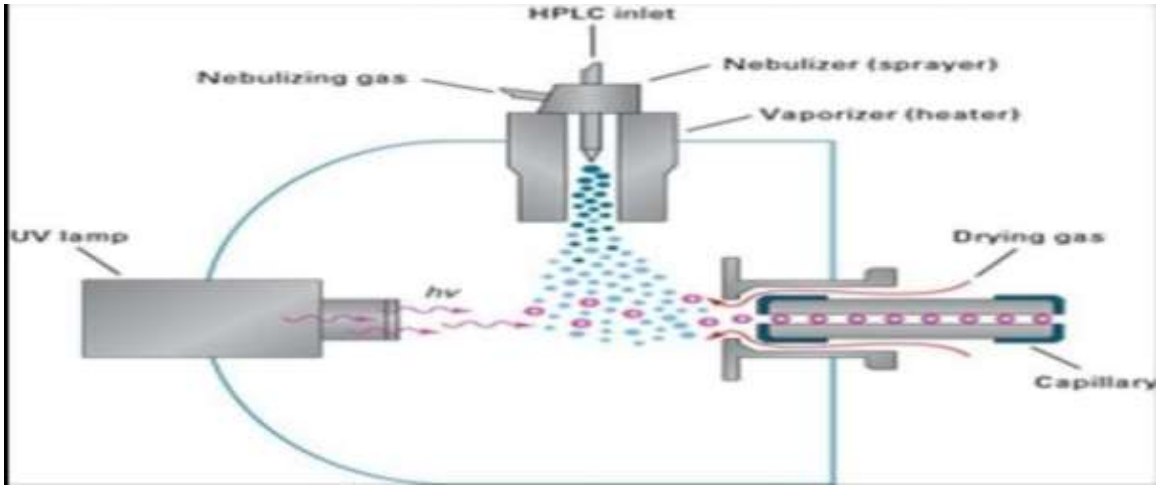
- It operates at atmospheric pressure. A sample solution is sprayed from a small pore into electric field in the presence of flow of warm nitrogen to assist desolvation.
- The droplets thus formed evaporates in the region of vacuum maintained at high pressure to form ions. The increased pressure causes 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) **APPI:**

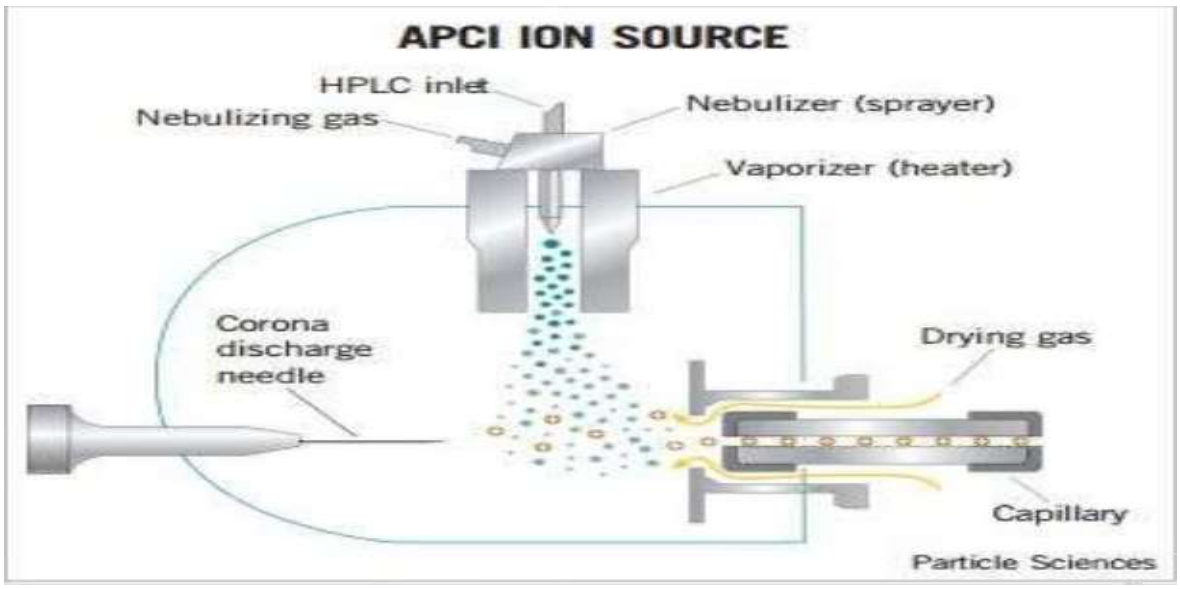
- A mixture of the analyte and the solvent i.e. a liquid solution is first vaporized with the help of nebulizing gas N2.

- 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.
-



d) APCI:

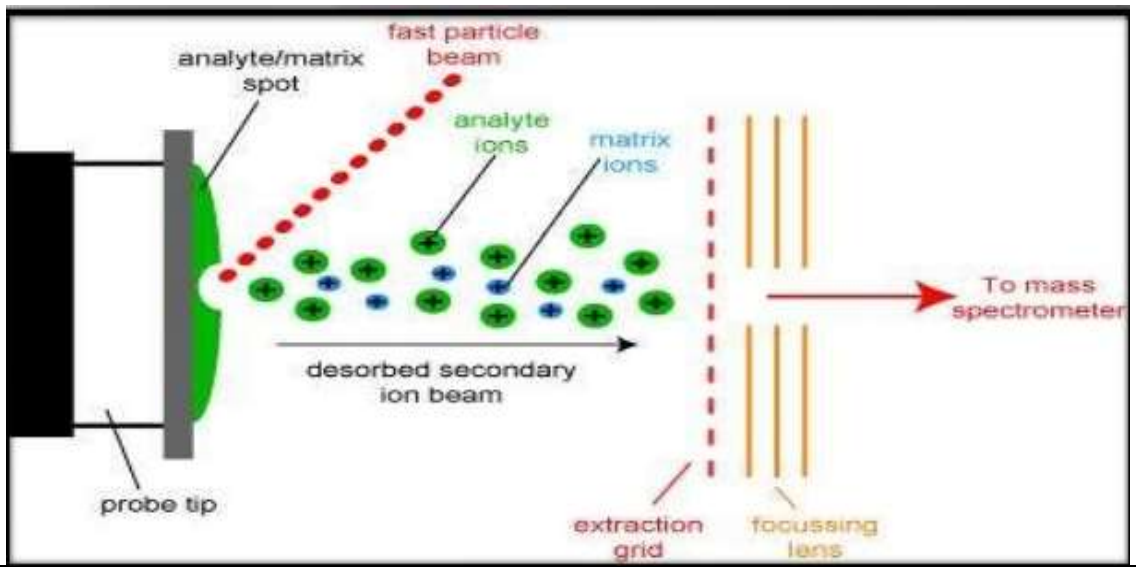
- The corona discharge produces primary ions in this technique.
- The nebulized sample via high speed nitrogen gas is displaced to a quartz tubing called as desolvation chamber.
- In desolvation chamber these droplets are converted to mixture of compound which are subsequently carried to a corona discharge 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.:

Willard, Hobart, et al., "Instrumental Methods of Analysis" 7th Edition, CBS, 1986 (Pg. No. 468-476).

Course Faculty

Verified by HoD

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 | **II/IV**

Course Name with Code : **Instrumental Methods of Analysis 19BTD08**

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

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

Topic of Lecture: MS – Mass Spectrometry and Applications of molecular mass

Introduction :

- Mass spectrometry is a powerful analytical technique used to quantify known materials, too identify unknown compounds within a sample and to elucidate the structure and chemical properties of different molecules.
- The complete process involves the conversion of the sample into gaseous ions, with or without fragmentation which are then characterized by their mass to charge ratios (m/z) and relative abundances.
- This technique basically studies the effect of ionizing energy on molecules. It depends upon chemical reactions in the gas phase in which sample molecules are consumed during the formation of ionic and neutral species.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on working and instrumentation of MS.
- Prerequisite knowledge on how the sample is ionized through the beam and detected.
- Prerequisite knowledge on the technique of finding the unknown samples.

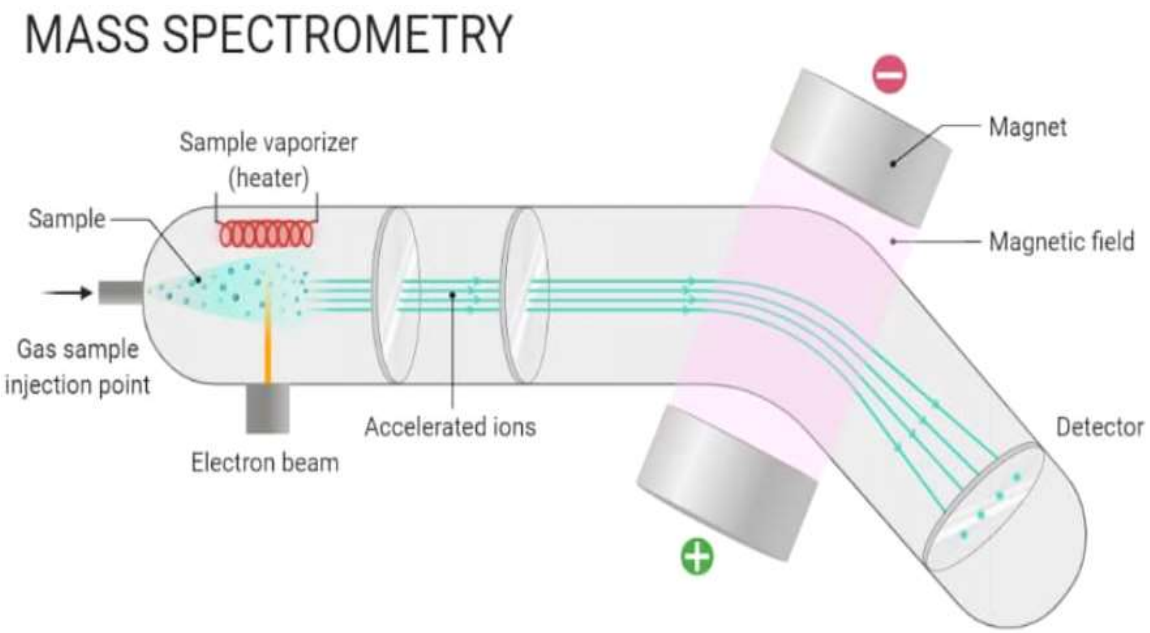
Detailed content of the Lecture:

- Mass Spectrometry (MS) is an analytical chemistry technique that helps identify the amount and type of chemicals present in a sample by measuring the mass-to-charge ratio and abundance of gas-phase ions.
- In this instrumental technique, the sample is converted to rapidly moving positive ions by electron bombardment and charged particles are separated according to their masses.
- A mass spectrum is a plot of relative abundance against the ratio of mass/charge (m/e).
- These spectra are used to determine the elemental or isotopic signature of a sample, the masses of particles and of molecules, and to elucidate the chemical structures of molecules and other chemical compounds.

PRINCIPLE:

- In this technique, molecules are bombarded with a beam of energetic electrons.
- The molecules are ionized and broken up into many fragments, some of which are positive ions. Each kind of ion has a particular ratio of mass to charge, i.e. m/e ratio (value).
- For most ions, the charge is one, and thus, the m/e ratio is simply the molecular mass of the ion.
- The ions pass through magnetic and electric fields to reach the detector where they are detected and signals are recorded to give mass spectra.

INSTRUMENTATION:



WORKING:

- In a typical procedure, a sample, which may be solid, liquid, or gas, is ionized, for example by bombarding it with electrons.
- This may cause some of the sample's molecules to break into charged fragments. These ions are then separated according to their mass-to-charge ratio, typically by accelerating them and subjecting them to an electric or magnetic field:
- Ions of the same mass-to-charge ratio will undergo the same amount of deflection.
- The ions are detected by a mechanism capable of detecting charged particles, such as an electron multiplier. Results are displayed as spectra of the relative abundance of detected ions as a function of the mass-to-charge ratio.
- The atoms or molecules in the sample can be identified by correlating known masses (e.g. an entire molecule) to the identified masses or through a characteristic fragmentation pattern.

APPLICATIONS:

- Environmental monitoring and analysis (soil, water, and air pollutants, water quality, etc.).
- Geochemistry – age determination, soil, and rock composition, oil and gas surveying.
- Chemical and Petrochemical industry – Quality control .
- Identify structures of biomolecules, such as carbohydrates, nucleic acids.
- Sequence biopolymers such as proteins and oligosaccharides.
- Determination of the molecular mass of peptides, proteins, and oligonucleotides.

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

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

Willard, Hobart, et al., "Instrumental Methods of Analysis" 7th Edition, CBS, 1986 (Pg. No. 465-468).

Course Faculty

Verified by HoD

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 | **II/IV**

Course Name with Code : **Instrumental Methods of Analysis 19BTD08**

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

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

Topic of Lecture: EPR – Basic concept and Theory

Introduction :

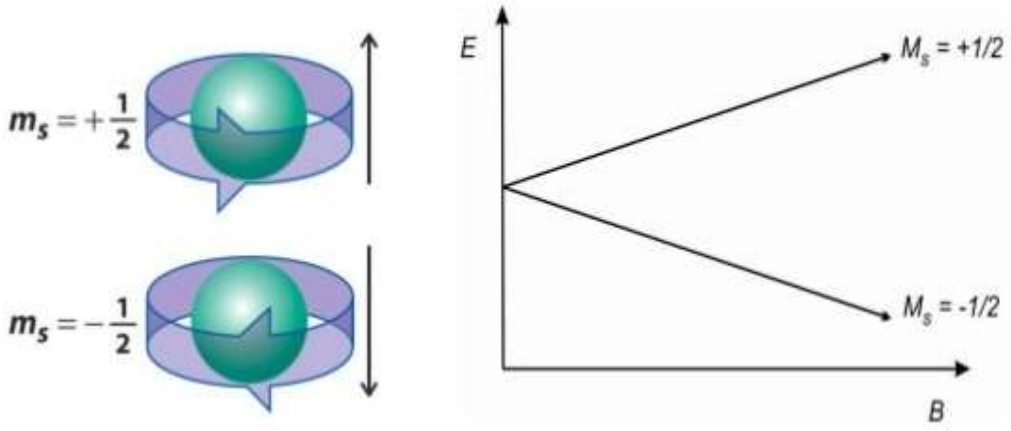
- Electron Paramagnetic Resonance (EPR) or Electron Spin Resonance (ESR) spectroscopy is a method for studying materials with unpaired electrons.
- The basic concepts of EPR are analogous to those of nuclear magnetic resonance (NMR) but the spins excited are those of the electrons instead of the atomic nuclei.
- EPR is particularly useful for studying metal complexes and organic radicals.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on the technique of EPR.
- Prerequisite knowledge on changes and spin of electrons.
- Prerequisite knowledge on the energy levels of electron when on electromagnetic field.

Detailed content of the Lecture:

- The unpaired electrons are excited to a high energy state under the magnetic field by the absorption of microwave.
- The excited electron changes its direction of spin and relaxes into the ground state by emitting phonons.
- Microwave absorption is measured as a function of the magnetic field by ESR spectroscopy.
- A chemical species with an odd number of electrons exhibits characteristic magnetic properties much like the nucleus.
- The spinning action of an unpaired electron generates a magnetic moment μ.
- If an intense magnetic field is applied, the electron assumes orientations aligned with (lower energy $-\mu H_o$) or against (higher energy $+\mu H_o$) the field.
- An electron in a magnetic field is able to absorb energy of the proper frequency $\Delta E = h\nu$ which will catapult it from lower to higher energy level



Energy levels of an electron in a magnetic field

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

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

Willard, Hobart, et al., "Instrumental Methods of Analysis" 7th Edition, CBS, 1986 (Pg. No. 499-500).

Course Faculty

Verified by HoD

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 | **II/IV**

Course Name with Code : **Instrumental Methods of Analysis 19BTD08**

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

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

Topic of Lecture: EPR – Instrumentation and g - values

Introduction :

- It is a branch of absorption spectroscopy in which radiation frequency in microwave region (300 MHz to 3000 GHz) is absorbed by paramagnetic substance to induce transition between magnetic energy level of electron with unpaired spin.
- Magnetic energy splitting is done by applying a static magnetic field.
- Instead of radiowaves in NMR, microwaves is used in ESR.
- It is based on the fact that an electron is a charged particle and it spins around its axis and this causes it to act like a tiny bar magnet.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on instrumentation and working of EMR.
- Prerequisite knowledge on the type of behavior of electron.
- Prerequisite knowledge on nuclei of atoms in a molecule or sample.

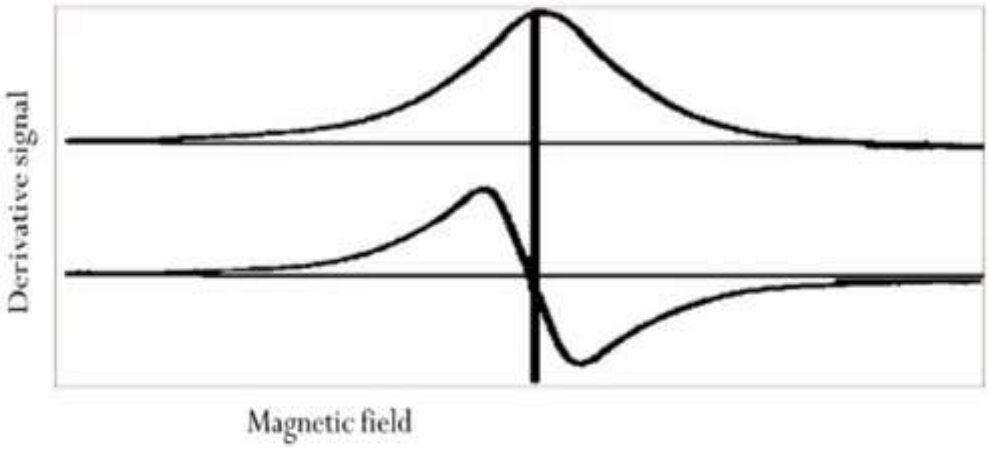
Detailed content of the Lecture:

EMR:

- 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:

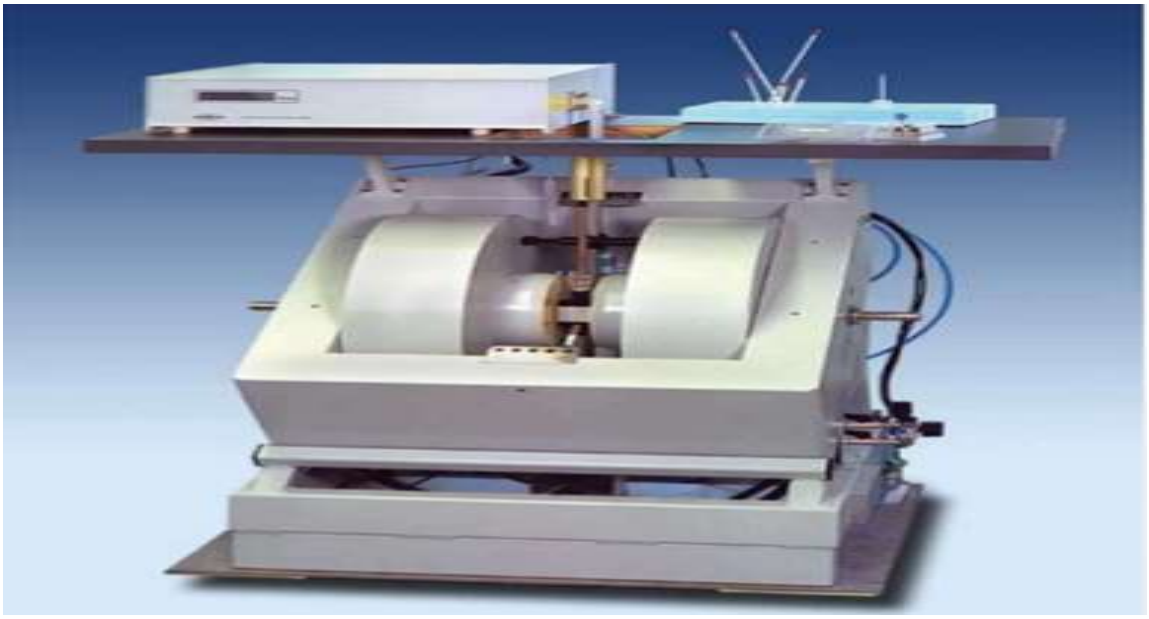
a) Atoms having odd number of electrons.
b) Ions having partly filled inner electron shells
c) Free radicals having unpaired electrons

- 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.

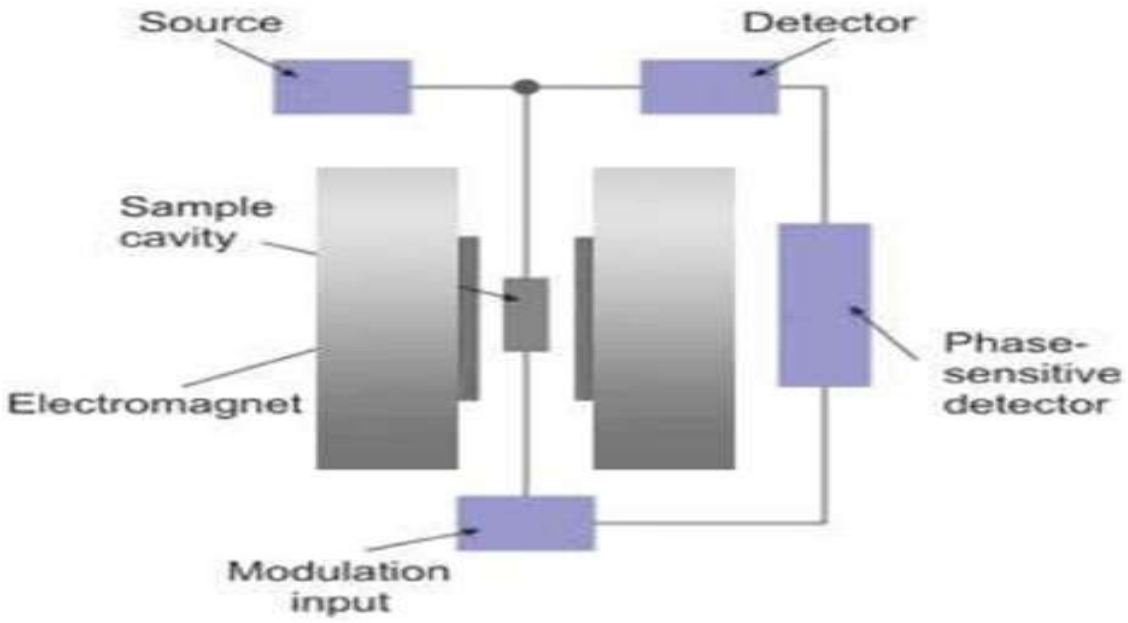


INSTRUMENTATION:

- 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.

g-values:

POSITION OF THE SIGNAL

Already mentioned g value gives the position of the signal.

Actually g is not a constant. It is a tensor quantity- changes with environment.

Many systems show g values close to that of free e-, but deviations are also common.

Deviations in the order±0.05 may be the mixing of low lying e.s with the g.s

- **g values for the d metal ions (3d) ranges from 0.2 – 8.**
- **The wide range is attributed to many reasons.**
  - **L-S coupling**
  - **Crystal field Splitting**
  - **Presence of inherent magnetic field in the crystal.**
  - **But L-S coupling and oxidation state of the metal ion make the g value characteristic**

Reference used

- When the operating frequency of the instrument is not known precisely then DPPH radical is used as standard.
- It gives five extremely sharp peaks with intensity ratio 1:2:3:2:1 (in solid state one sharp line)
- $g = 2.0036[1-\Delta H/H]$
- ΔH – diff between std and sample
- H – sample field

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

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

Willard, Hobart, et al., "Instrumental Methods of Analysis" 7th Edition, CBS, 1986 (Pg. No. 501-502).

Course Faculty

Verified by HoD

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 | **II/IV**

Course Name with Code : **Instrumental Methods of Analysis 19BTD08**

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

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

Topic of Lecture: General description of chromatography

Introduction :

- The feature that distinguishes chromatography from most other physical and chemical methods of separation is that two mutually immiscible phases are brought into contact; one phase is stationary and the other mobile.
- A sample introduced into a mobile phase is carried along through a column containing a distributed stationary phase.
- When both phases are properly chosen, the sample components are gradually separated into bands in the mobile phase.
- At the end of process, separated components emerge in order of increasing interaction with the stationary phase.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on different separation techniques.
- Prerequisite knowledge on how samples are analysed based on the nature of compound.
- Prerequisite knowledge on how separation of compounds takes place using chromatography.

Detailed content of the Lecture:

- The separation column is the heart of the chromatograph. It provides versatility in the types of analyses that can be performed.
- This versatility, due to the wide choice of materials for the stationary and mobile phases makes it possible to separate molecules that differ only slightly in their physical and chemical properties.
- The mobile phase can be a gas or a liquid, whereas the stationary phase can be only a liquid or a solid.
- When the separation involves predominantly a simple partitioning between two immiscible liquid phases, one stationary phase and the other mobile the process is called LLC (Liquid-Liquid Chromatography).
- The analytes interacting most strongly with the stationary phase will take longer to pass through the system than those with weaker interactions.
- These interactions are usually chemical in nature, but in some cases physical interactions can also be used.
-

Components:

Mobile phase: a solvent that flows through the supporting medium.

Stationary phase: a layer or coating on the supporting medium that interacts with the analytes.

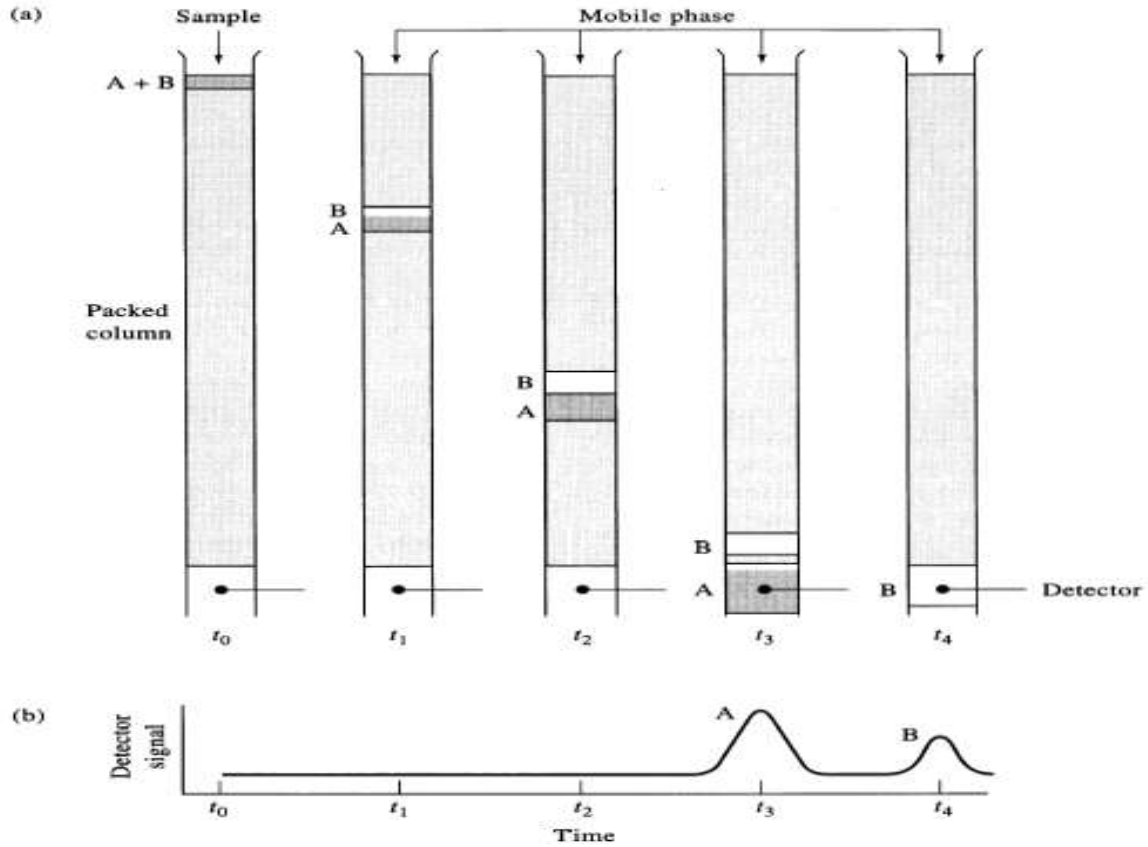
Supporting medium: a solid surface on which the stationary phase is bound or coated.

Classification based on Mobile Phase:

- Gas (GC)
- Water (LC)
- Organic solvent (LC)
- Supercritical fluid (SCFC)

Classification based on Attractive Forces:

- Adsorption
- Ion Exchange
- Partition
- Size Exclusion



- **Separation and flow of sample; b) Detection of sample**

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

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

Willard, Hobart, et al., "Instrumental Methods of Analysis" 7th Edition, CBS, 1986 (Pg. No. 513-514).

Course Faculty

Verified by HoD

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 | **II/IV**

Course Name with Code : **Instrumental Methods of Analysis 19BTD08**

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

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

Topic of Lecture: Band broadening and optimization of column performance

Introduction :

- Various processes take place on a column during a chromatographic separation that contribute to the peak variance, σ^2 or band broadening.
- Theories of band spreading in liquid and gas chromatography are nearly identical. Plate height expresses in simple terms the extent of band broadening and the factors that affect the broadening.
- It's the function of thermodynamic and kinetic processes within the column.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on optimization of column for separation.
- Prerequisite knowledge on band broadening for sample detection.
- Prerequisite knowledge on chromatography.

Detailed content of the Lecture:

Band Broadening: Band Broadening is a major problem because it effects the resolution of solutes that have similar retention time.

- The peak width increases with the square root of column length. Therefore, we just cannot make a column longer to obtain a 'better' separation.
- Theory of Band Broadening van Deemter Equation Theoretical studies of zone broadening in the 1950s by Dutch chemical engineers led to the van Deemter equation, which can be written in the form

$$H = B/u + C_S u + C_m u$$

Where, B – longitudinal diffusion
CS–mass transfer coefficient in mobile phase
CM-mass transfer coefficient in stationary phase
u– velocity of mobile phase

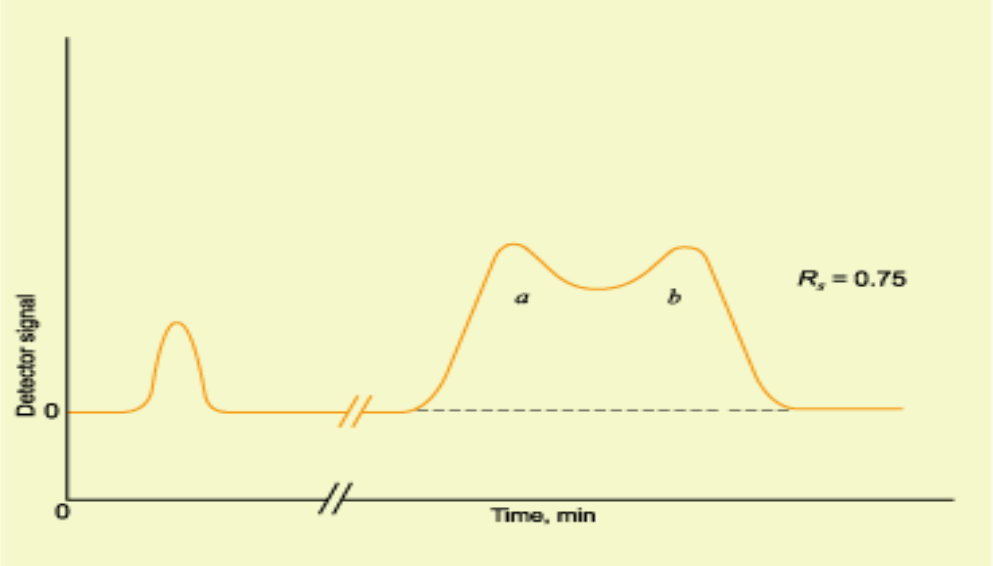
Methods for reducing band broadening:

- Small packing diameter (of stationary phase).
- Small column diameter.
- For liquid stationary phase- thickness of the layer should be minimized.
- Optimum flow-rate of mobile phase.
- Optimum temperature.
- Variation in solvent composition.

Optimisation of column performance:

Optimisation of chromatographic separations is achieved by varying the experimental conditions of the run until the components of the mixture are separated cleanly in a reasonable amount of time. There are two aspects to achieving good separation:

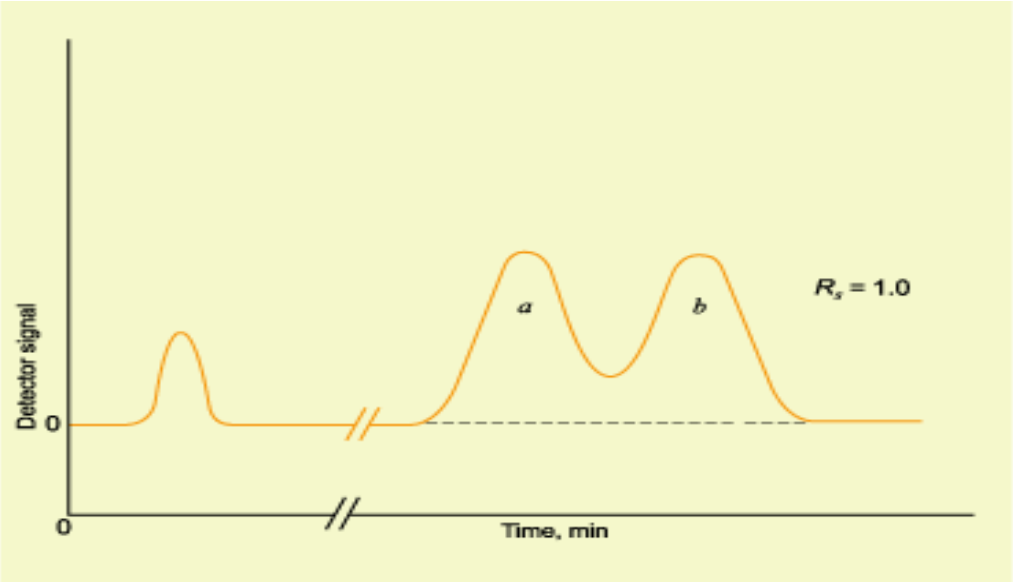
- The components in the mixture need to migrate or travel down the column at sufficiently different rates.
- The peaks for the components need to be relatively sharp and uniform (as components migrate, they tend to broaden or spread out such that they can overlap each other, thereby compromising detection/accuracy).



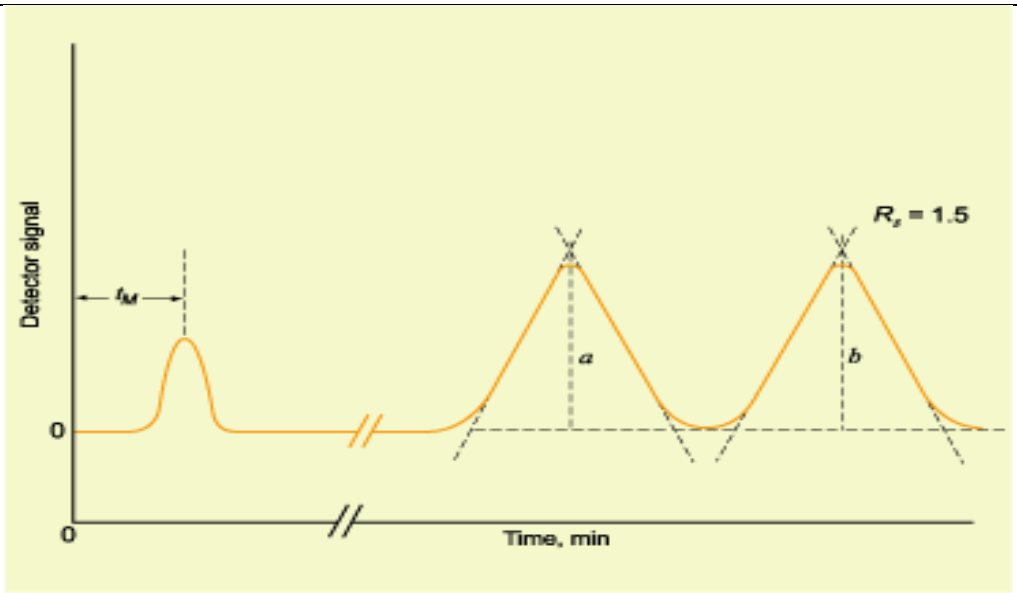
A poor separation of components of A and B

Optimisation has two aims:

- Reduction of zone broadening (the component moves through the column as a zone which is detected by the detector and translated into a peak).
- Altering the migration rates of the components.



An improvement in resolution leads to a partial separation of peaks



A further improvement in resolution has led to separation of peaks

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

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

Willard, Hobart, et al., "Instrumental Methods of Analysis" 7th Edition, CBS, 1986 (Pg. No. 525-527).

Course Faculty

Verified by HoD

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 | **II/IV**

Course Name with Code : **Instrumental Methods of Analysis 19BTD08**

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

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

Topic of Lecture: Liquid chromatography

Introduction :

- Liquid chromatography is a technique used to separate a sample into it's individual parts.
- This separation occurs based on the interactions of the sample with the mobile and stationary phases.
- Since there are many stationary / mobile phases combinations that can be employed when separating a mixture, there are several different types of chromatography that are classified based on the physical states of those phases.
- Liquid-solid column chromatography, the most popular chromatography technique which features a liquid mobile phase which slowly filters down through the solid stationary phase bringing the separated components with it.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on compound separation technique for purification process.
- Prerequisite knowledge on identifying of compounds from the mixture.
- Prerequisite knowledge on knowing the types of mobile phases.

Detailed content of the Lecture:

- Components within a mixture are separated in a column based on each component's affinity for the mobile phase.
- So, if the components are of different polarities and a mobile phase of a distinct polarity is passed through the column, one component will migrate through the column faster than the other.
- Because molecules of the same compound will generally move in groups, the compounds are separated into distinct bands within the column.
- Also, the efficacy of the separation is dependent on the nature of the adsorbent solid used and the polarity of the mobile phase solvent.

Types of chromatography:

1. Normal phase
2. Reverse phase and
3. Flash

Other varieties of LC include:

1. Partition
2. Liquid-Solid
3. Ion exchange
4. Size-exclusion

5. Affinity
6. Chiral

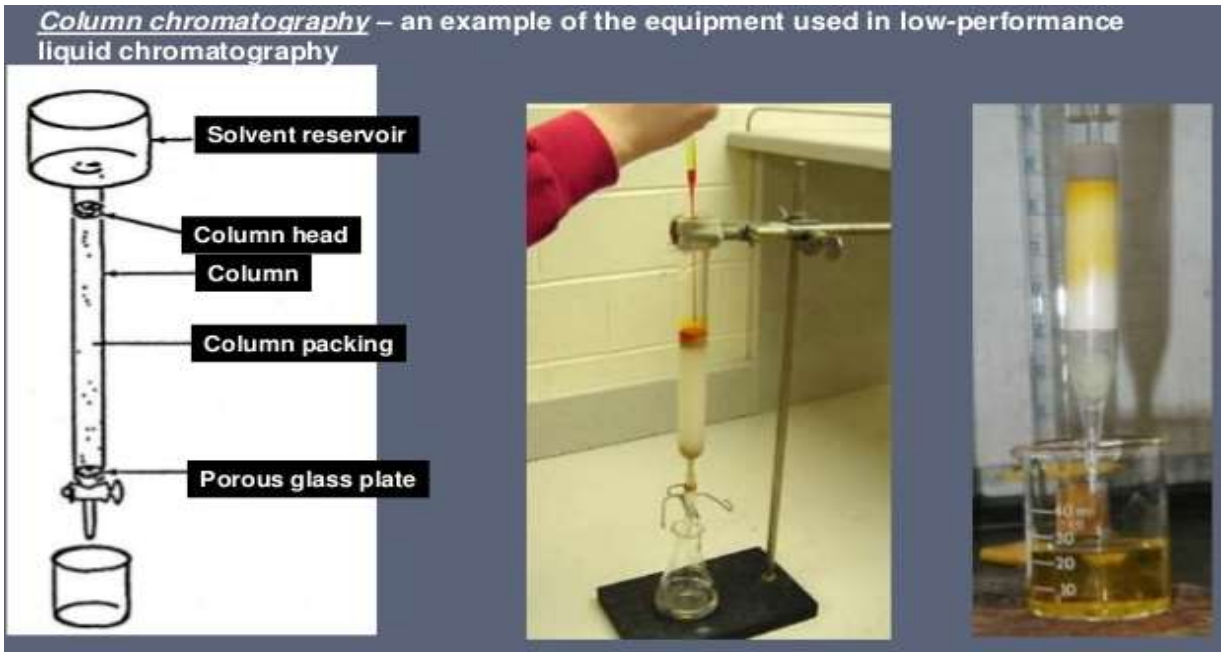


Figure demonstrating the column chromatography

Video Content / Details of website for further learning (if any):
https://chem.libretxts.org/bookshelves/Analytical_Chemistry/Supplemental_Modules_(Analytical_Chemistry)/Instrumental_Analysis/Chromatography/Liquid_Chromatography

Important Books/Journals for further learning including the page nos.:
Willard, Hobart, et al., "Instrumental Methods of Analysis" 7th Edition, CBS, 1986 (Pg. No. 580-585).

Course Faculty

Verified by HoD

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 | **II/IV**

Course Name with Code : **Instrumental Methods of Analysis 19BTD08**

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

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

Topic of Lecture: Partition chromatography

Introduction :

- Partition chromatography technique is defined as the separation of components between two liquid phases i.e original solvent and film of solvent used in column.
- The separation of the components from the sample mixture is carried out by the process of partition of the components between 2 phases.
- Both phases are in liquid form. In this process, the immiscible solid surface coated with the liquid surface on the stationary phase is in the mobile phase.
- The mobile phase moves from the stationary phase and components get separated. The separation depends on different partition coefficient.

Prerequisite knowledge for Complete understanding and learning of Topic:

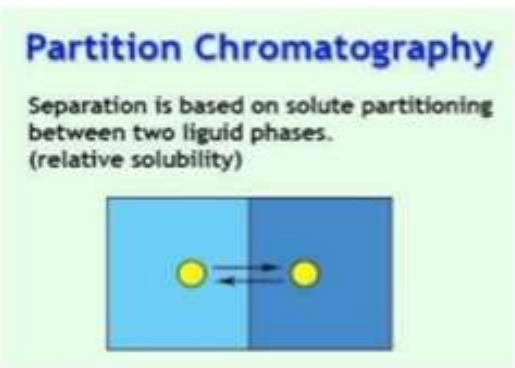
- Prerequisite knowledge on separation of compound using partition chromatography.
- Prerequisite knowledge on various types of partition chromatography.
- Prerequisite knowledge on the applications of chromatography techniques.

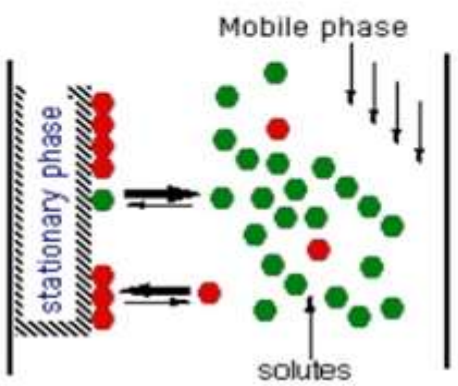
Detailed content of the Lecture:

- Partition chromatography is one of the types of chromatography introduced in the 1940s by Richard Laurence Millington Synge and Archer Martin.

Partition Chromatography

• Method of separation in which the components present in the mixture get distributed more likely into two liquid phases because of differences in partition coefficients during the flow of mobile phase in the chromatography column.



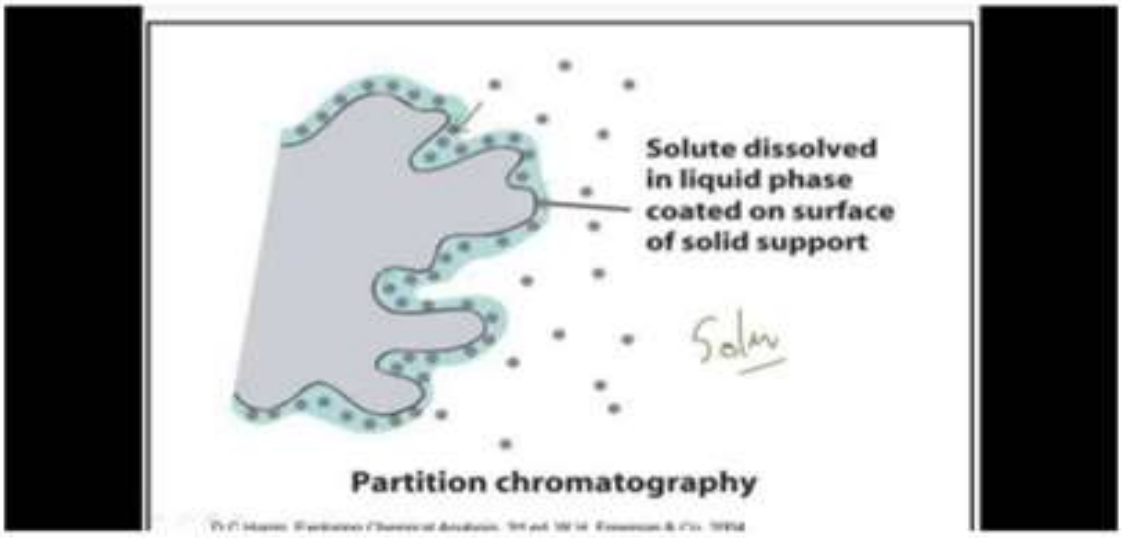


Partition Chromatography

- The most widely used type of **HPLC** is partition chromatography .
- The **stationary phase** is a second liquid that is **immiscible** with the **liquid mobile phase**.
- Earlyr in PC used **liquid liquid column** but in modern LC systems **liquid bonded phase column** is used.
- In **liquid liquid chromatography** the liquid was held in place by **physical adsorption** but in **liquid bonded phase column** system, attached by **chemical bonding** resulting in highly stable packing insoluble in the mobile phase. Bonded - phase columns are also compatible with **gradient elution technique**

Partition Chromatography Principle

- Separation of components of given sample occurs due to partition of components between two liquid phases.
- Stationary phase is coated with a liquid which is immiscible in mobile phase.
- Stationary phase immobilizes the liquid surface and makes it stationary phase.
- The mobile phase passes over the stationary phase and separate out.
- The separation depends on the relative solubility in the stationary liquid layer because of different partition coefficient, different component of sample are separated.



TYPES:

- Liquid-Liquid
- Gas-Liquid

LLC:

Liquid - Liquid Chromatography

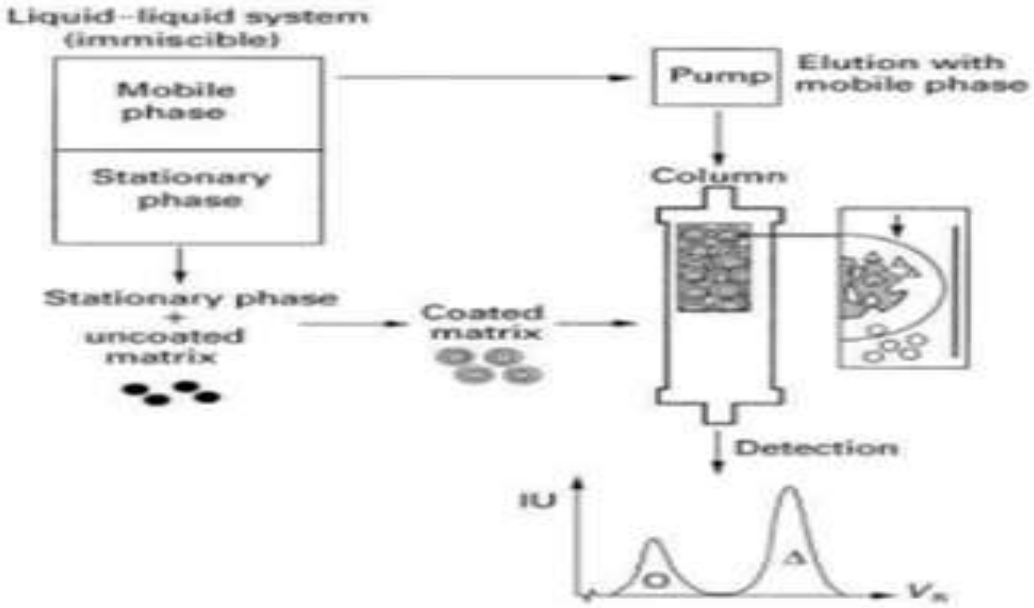
Partition or liquid-liquid chromatography (LLC): A powerful separation technique which is used for the separation and analysis of acids and proteins.

The basis of LLC is the distribution of sample molecules between two immiscible liquid phases, a stationary phase and a mobile phase (Figure 1).

In conventional LLC, the stationary phase is mechanically held to a support by adsorption.

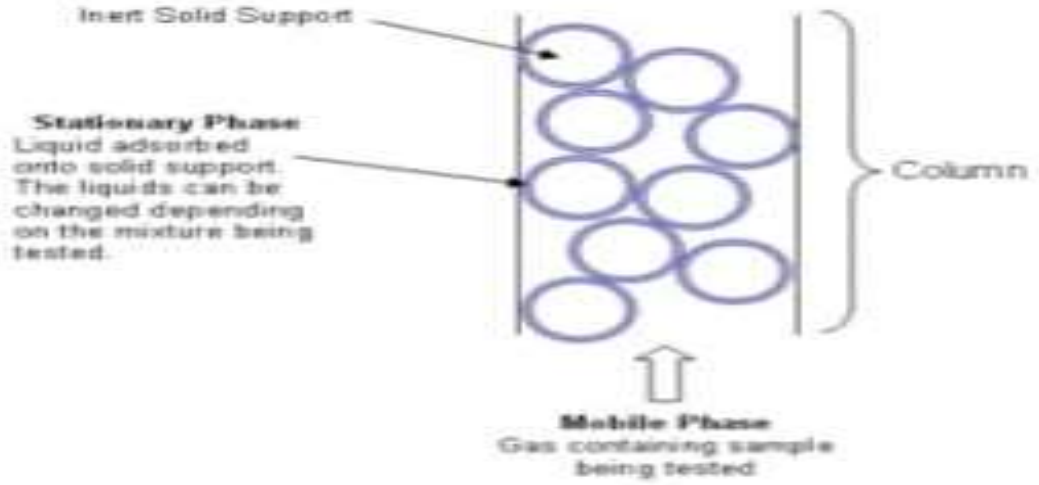
Employs liquid mobile and stationary phases.

Uses small particles with molecules bonded to their surface to give a thin film that has liquid like properties.



GLC:

- In gas-liquid chromatography the mobile phase is an unreactive gas, such as nitrogen (carrier gas) and the stationary phase comprises of a small amount of non-volatile liquid held on a finely divided inert solid support.
- The components of vaporize samples are fractionated due to partition between a gaseous mobile phase and a liquid stationary phase held in column.



APPLICATIONS:

- Used for final purification natural extracts, synthetic mixtures and biological matrices.
- It is also used for fractionization of complex crude extracts. Eg: petroleum fractions.
- Determination of water quality.
- Separation of aroma molecules of wine.
- Determination of pesticide residue.

Video Content / Details of website for further learning (if any):
https://lab-training.com/2021/03/26/partition-chromatography/

Important Books/Journals for further learning including the page nos.:
Willard, Hobart, et al., "Instrumental Methods of Analysis" 7th Edition, CBS, 1986 (Pg. No. 611-613).

Course Faculty

Verified by HoD

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 | **II/IV**

Course Name with Code : **Instrumental Methods of Analysis 19BTD08**

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

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

Topic of Lecture: Adsorption and Ion exchange chromatography

Introduction :

- Adsorption chromatography is the oldest types of chromatography technique. It makes use of a mobile phase which is either in liquid or gaseous form.
- The mobile phase is adsorbed onto the surface of a stationary solid phase.
- Adsorbent – A substance which is generally porous in nature with a high surface area to adsorb substances on it's surface by intermolecular forces is called adsorbent. Some of the commonly used adsorbents are silica gel H, silica gel G, cellulose, alumina, etc.
- Ion chromatography separates ions and polar molecules based on their affinity to the ion exchanger.
- It works on almost any kind of charged molecule – including large proteins, small nucleotides and amino acids.

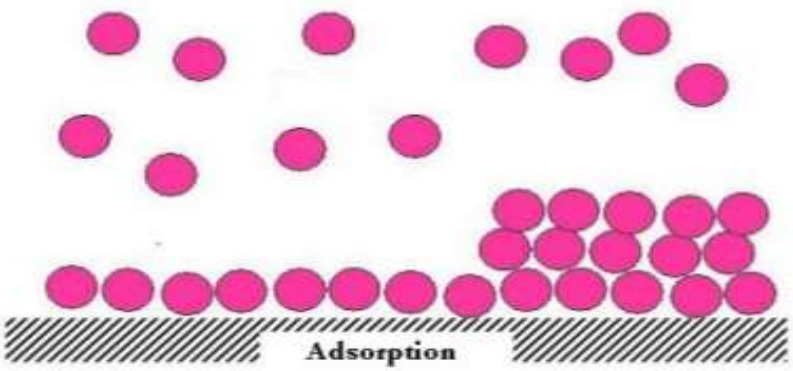
Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on different types of materials used in chromatography.
- Prerequisite knowledge on differences in chromatography techniques.
- Prerequisite knowledge on process of separation of ions and molecules.

Detailed content of the Lecture:

ADSORPTION CHROMATOGRAPHY:

- It is a type of chromatography in which a mobile liquid or gaseous phase is adsorbed onto the surface of a stationary solid phase.
- The equilibration between the mobile and stationary phase accounts for the separation of different solutes.

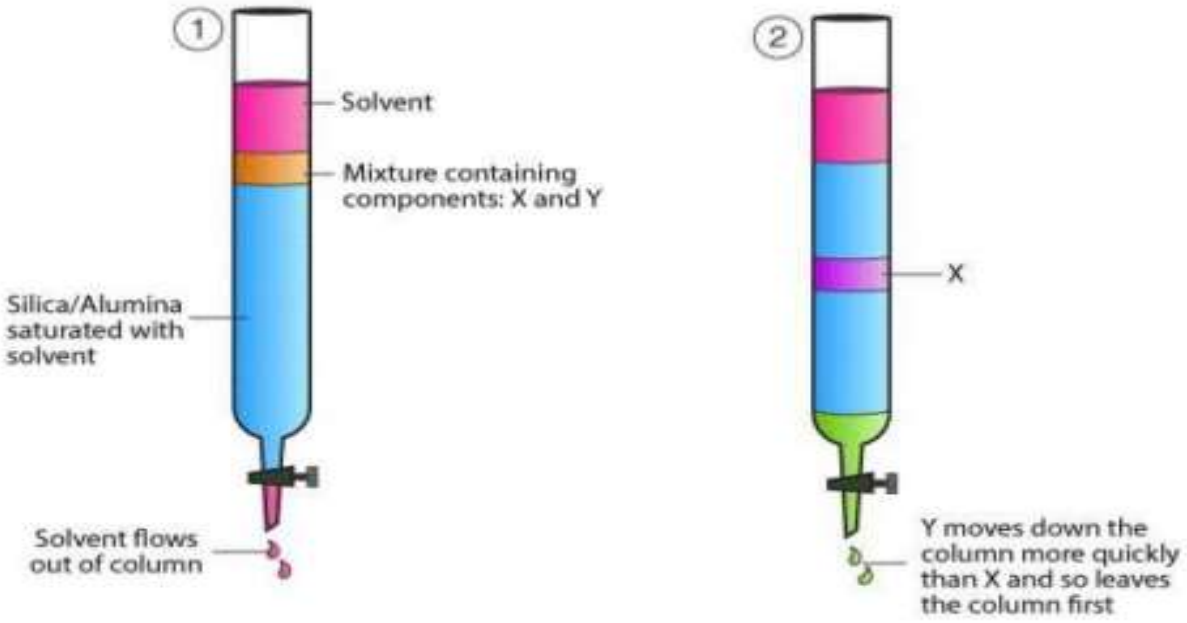


Adsorption chromatography

PRINCIPLE:

- In this chromatography, seperation of components of a mixture takes place by the adsorption efficiency of the sample.
- The most strongly adsorbed component forms the topmost band.
- the least adsorbed component forms the lowermost band on the adsorbent column.
- Degree of separation depends upon the separation of surface area of adsorbent.

WORKING:



PREPARATION OF THE COLUMN:

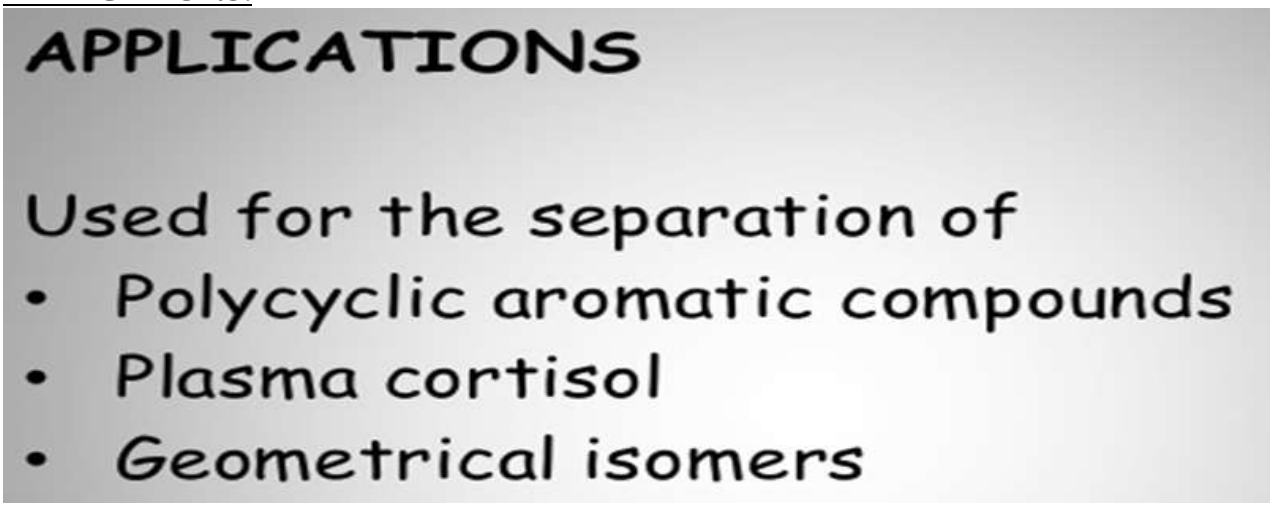
DRY PACKING

- The powdered sample is poured inside the tube (both the sample and the stationary column remain dry)

WET PACKING

- The bottom of the tube is closed
- The column is filled with solvent
- This method is known as wet packing

APPLICATIONS:



ION EXCHANGE CHROMATOGRAPHY:

- Ion exchange chromatography may be defined as the reversible exchange of ions in the solution with ions electrostatically bound to some sort of insoluble matrix or a stationary phase."
- This technique is extremely useful in the separation of charge compounds like proteins differing by only one charged amino acid.
- In Ion exchange chromatography technique one can choose whether to bind the substance of interest and allow the contamination to pass through the column and vice versa.

Ion exchange chromatography

PRINCIPLE:

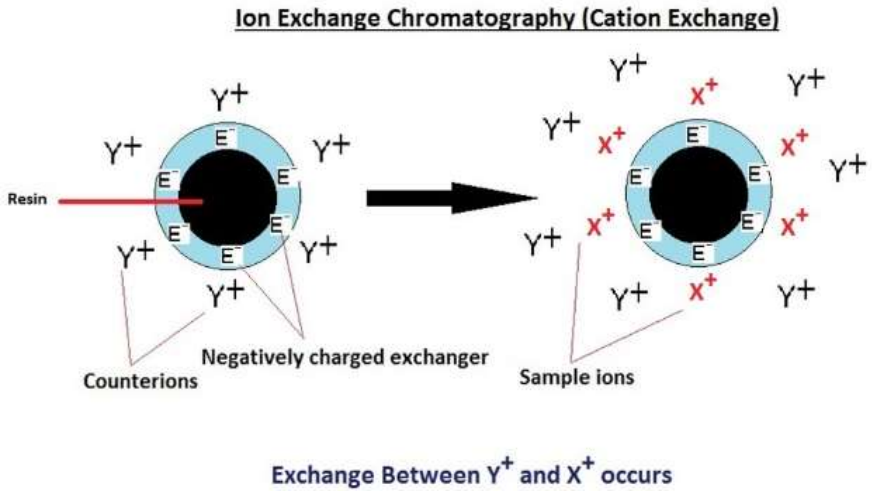
- Ion exchange chromatography relies on the attraction between oppositely charged stationary phase, known as an ion exchanger, and analyte.
- The ion exchanger consists of an inert support medium coupled covalently to positive (anion exchanger) or negative (cation exchanger) functional groups.
- To these covalently bound functional groups the oppositely charged ions are bounded (mobile counter ion), which will be exchanged with like charge ions in the sample having charge magnitude more than the ions bounded to the matrix.
- Thus if anion exchange chromatography is performed, negatively charged sample components will interact more with the stationary phase and will be exchanged for like charged ions already bounded to the matrix.

WORKING:

- Consider a column having E - Y+ cation exchanger in which E - is negative charged exchanger and Y+ is the mobile counter ion.
- Let X+ be the cation in the sample having charge greater than Y+.
- The X+ ion can exchange sites with the counter ion Y+ with satisfying the following relationship;

$$E^- Y^+ + X^+ \rightarrow E^- X^+ + Y^+.$$

- The remaining neutral and negatively charged particles



Bounded interest of ion (X+) can now be eluted by either of the two ways;

1. By adding a component M+ having magnitude of charge more than that of X+ so that M+ will replace X+ and X+ will be eluting out.
2. By changing pH of the solvent (mobile phase so that X+ have no charge and is then unbounded from the matrix and can be eluted out.

APPLICATIONS:

- Softening of hard water
- Demineralization of water
- To analyze base composition of nucleic acid
- To concentrate the metal ions in the sample
- To measure the additives in food and drug sample

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

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

Willard, Hobart, et al., "Instrumental Methods of Analysis" 7th Edition, CBS, 1986 (Pg. No. 617-621; 633-640).

Course Faculty

Verified by HoD

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 | **II/IV**

Course Name with Code : **Instrumental Methods of Analysis 19BTD08**

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

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

Topic of Lecture: Size-exclusion and Affinity chromatography

Introduction :

- In this regard, SEC enables obtaining information about how much a sample contains of respective molecular weights.
- It separates compounds of a mixture sample on the basis of their molecular size.
- Affinity chromatography is a separation based technique of specific binding interaction between an immobilized ligand and it's binding partner.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on separation of molecules based on size.
- Prerequisite knowledge on knowing the technique for sample purification process.
- Prerequisite knowledge on binding interaction of molecules based separation using affinity chromatography.
- Prerequisite knowledge on macromolecular binding process and difference in their principle.

Detailed content of the Lecture:

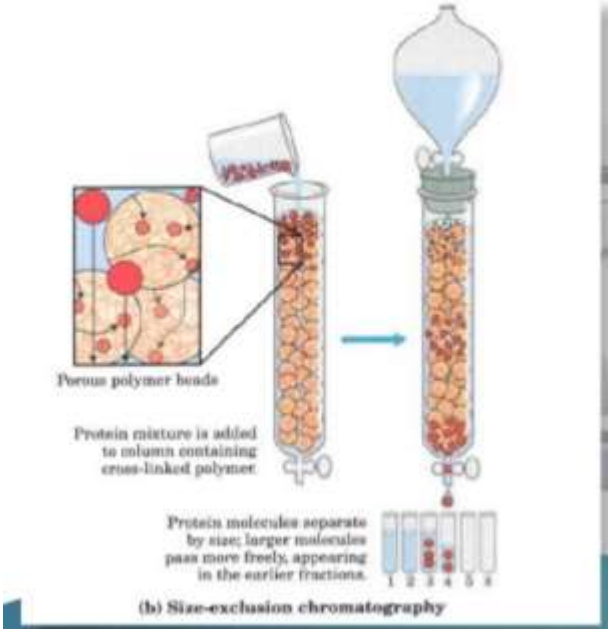
SIZE-EXCLUSION CHROMATOGRAPHY:

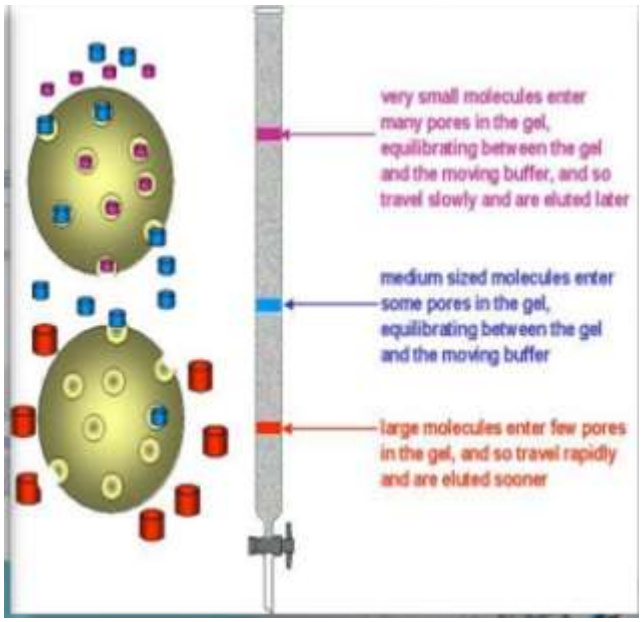
INTRODUCTION:

- Size-exclusion chromatography (SEC), also called gel-filtration or gel-permeation chromatography uses porous particles to separate molecules of different sizes.
- It is generally used to separate biological molecules and to determine molecular weights and molecular weight distributions of polymers.
- It is usually applied to large molecules or macromolecular complexes such as proteins and industrial polymers.

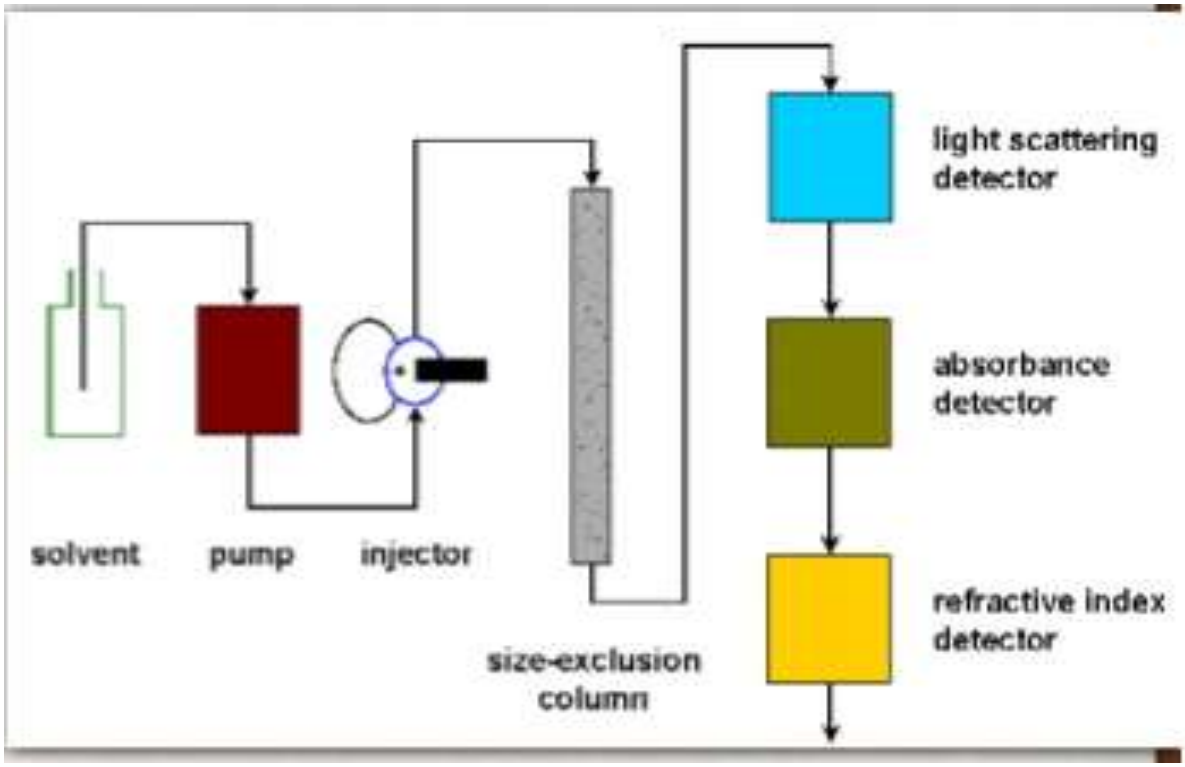
PRINCIPLE:

- A mixture of molecules dissolved in liquid is applied to a chromatography column which contains a solid support in the form of microscopic spheres or beads.
- The mass of beads within the column is often referred to as the column bed.
- The beads acts as 'traps' and function to filter small molecules which become temporarily trapped within the pores.





WORKING:



COMPONENTS:

1. Stationary phase
2. Mobile phase
3. Columns
4. Pump
5. Detectors

APPLICATIONS:

- Proteins fractionation.
- Purification.
- Molecular weight determination.
- Separation of sugar, proteins, peptides, rubbers and others on the basis of their size.
- This technique can be determine the quaternary structure of purified proteins.
- SEC is a widely used technique for the purification and analysis of synthetic and biological polymers, such as protein, polysaccharides and nucleic acid.

- Various species of RNA and viruses have been purified using agarose gels.

AFFINITY CHROMATOGRAPHY:

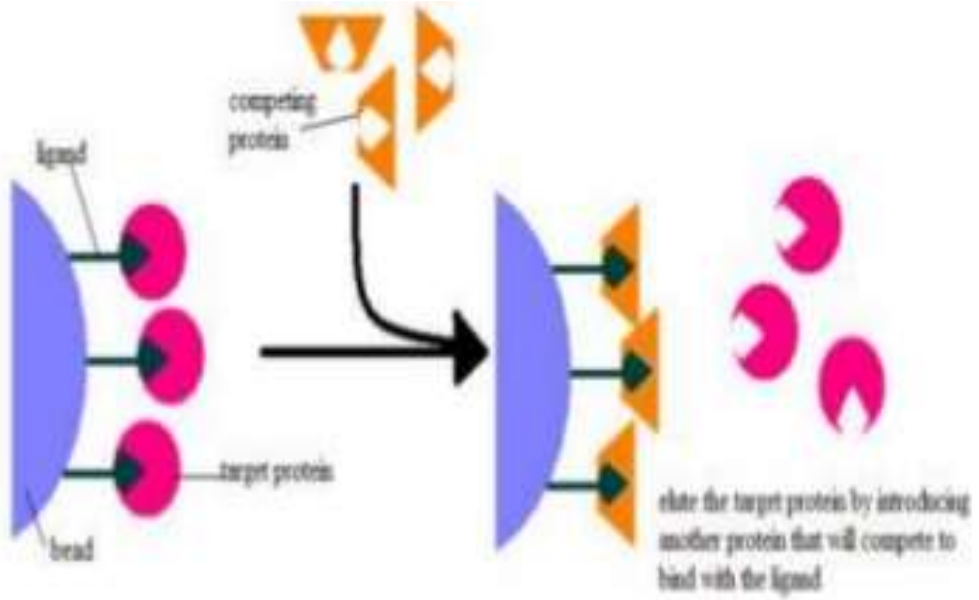
Affinity Chromatography is essentially a sample purification technique, used primarily for biological molecules such as proteins.

It is a method of separating a mixture of proteins or nucleic acids (molecules) by specific interactions of those molecules with a component known as a ligand, which is immobilized on a support. If a solution of, say, a mixture of proteins is passed over (through) the column, one of the proteins binds to the ligand on the basis of specificity and high affinity (they fit together like a lock and key).

The other proteins in the solution wash through the column because they were not able to bind to the ligand.

PRINCIPLE:

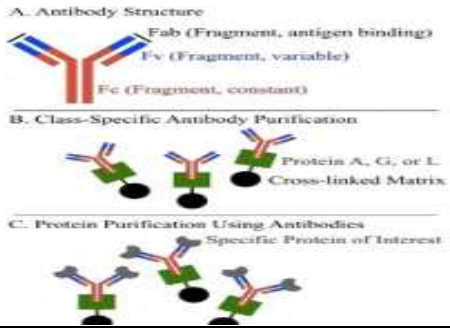
- Affinity chromatography is one of the most diverse and powerful chromatographic methods for purification of a specific molecule or a group of molecules from complex mixtures
- It is based on highly specific biological interactions between two molecules such as interactions between enzyme and substrate,receptor and ligand,or antibody and antigen.
- These interactions which are typically revesible are used for purification by placing one of the interacting molecules referred to as affinity ligand onto a solid matrix to create a stationary phase while a target molecule is in the mobile phase.
- Many of the commonly used ligands coupled to affinity matrices are now commercially available and are ready to use.

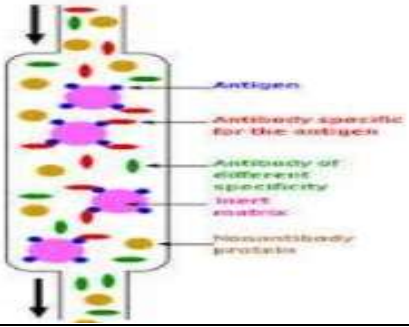


Ion-exchange chromatography

APPLICATIONS

- 1)It is used for isolation and purification of all biological macromolecule.
-
- 2)It is used to purify nucleic acid,antibodies,enzymes.etc
- 3)To notice which biological compounds bind to a particular substance.
- 4)to reduce a amount of substance in a mixture





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

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

Willard, Hobart, et al., "Instrumental Methods of Analysis" 7th Edition, CBS, 1986 (Pg. No. 644-652).

Course Faculty

Verified by HoD

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 | **II/IV**

Course Name with Code : **Instrumental Methods of Analysis 19BTD08**

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

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

Topic of Lecture: Principles of GC and applications

Introduction :

- It is a process of separating compounds from the given crude drug by using a gaseous mobile phase.
- It involves a sample being vaporized and injected onto the head of the chromatographic column.
- The sample is transported through the column by the flow of inert, gaseous mobile phase.
- The column itself contains a liquid stationary phase which is adsorbed onto the surface of an inert solid.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on how the sample is injected and finally identified using GC.
- Prerequisite knowledge on the technique and principle behind GC.
- Prerequisite knowledge on knowing how to utilize the instrument for research purpose.

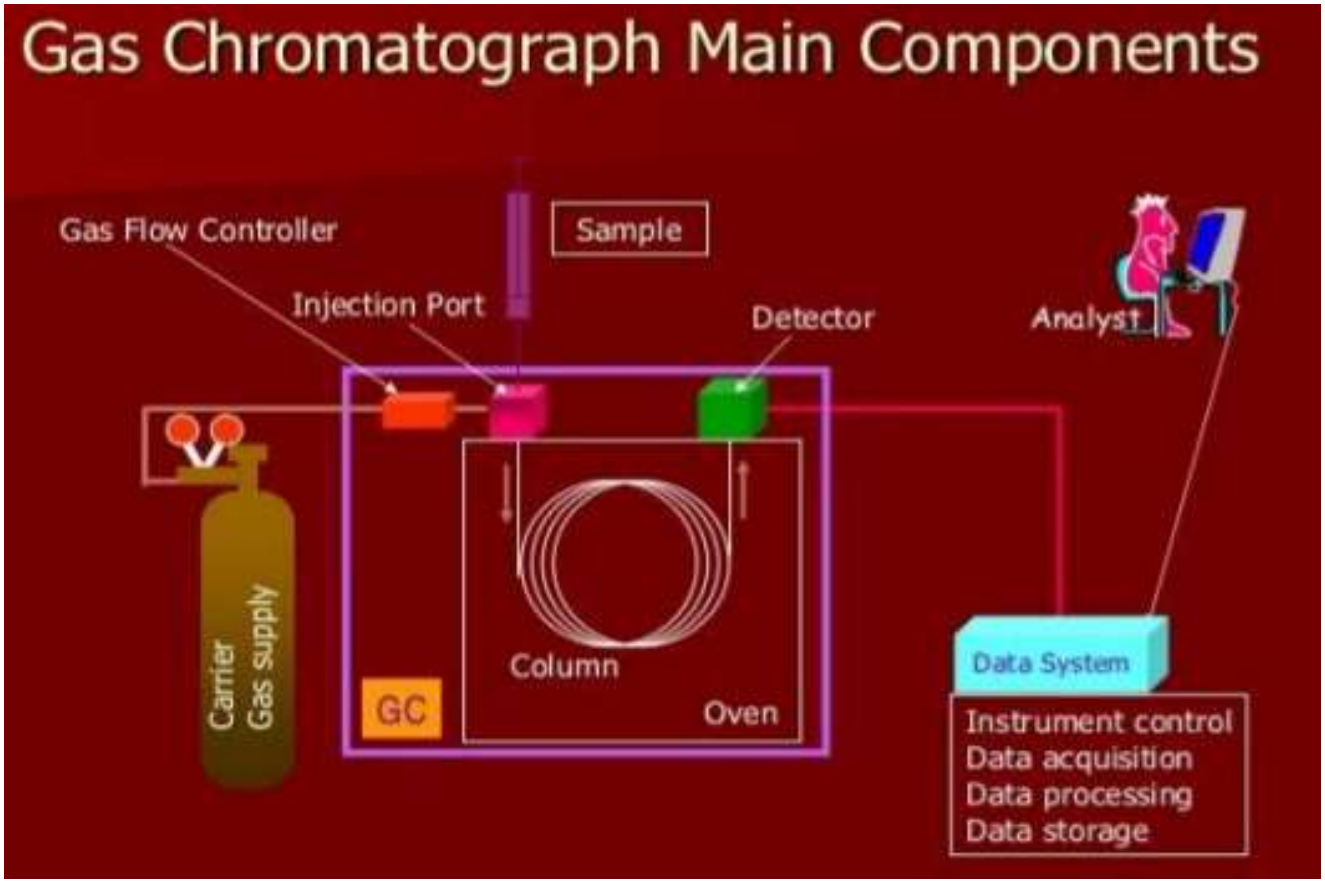
Detailed content of the Lecture:

- When a mixed solution sample is injected into GC the compounds cpntained in the sample including the solvent components are heated and vaporized within the sample injection unit.
- With GC system, the mobile phase referred to as the carrier gas always flows in sequence from the sample injection unit to the column and then to the detector.
- The target compounds that were vaporized in the sample injection unit are transported by the carrier gas to the column.
- Once in the column, the mixture of compounds is separated into the various components and the amount of each compound is then measured by the detector.
- The detector converts the amount of each compound into an electrical signal and sends these signals to a data processing point.
- The data obtained enables determination of the compounds contained in the sample and in what amoiunts.
- Two major types: Gas-solid and Gas-liquid.

PRINCIPLE:

- The principle of separation in GC is "partition."
- The mixture of component to be separated is converted to vapour and mixed with gaseous mobile phase.
- The component which is more soluble in stationary phase travel slower and eluted later. The component which is less soluble in stationary phase travels faster and eluted out first.
- No two components has same partition coefficient conditions. So the components are separated according to their partition coefficient.
- Partition coefficient is "the ratio of solubility of a substance distributed between two immiscible liquids at a constant temperature.'

INSTRUMENTATION:



WORKING:

- Fill the syringe with sample.
- Record the setting i.e., column temperature, detector temperature and injection port temperature.
- Introduce sample into the injection port by completely inserting the needle into the rubber septum. Note down the injection time.
- The sample gets vapourized due to higher temperature of injection port and is swept into column by carrier gas.
- This sample components now get distributed between the gas and stationary liquid phase depending upon their solubilizing tendencies.
- The components with minimal solubility move faster and those with maximum solubility travel slowly.
- The components leaving the column activate detector and recorder to give a plot.

APPLICATIONS:

- Qualitative Analysis – by comparing the retention time or volume of the sample to the standard / by collecting the individual components as they emerge from the chromatograph and identifying these compounds by other methods like UV, IR, NMR.
- Quantitative Analysis- area under a single component elution peak is proportional to the quantity of the detected component/response factor of the detectors.

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

https://www.ssi.shimadzu.com/products/gas-chromatography/fundamental-guide-to-gas-chromatography/what -is-gas-chromatography.html

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

Willard, Hobart, et al., "Instrumental Methods of Analysis" 7th Edition, CBS, 1986 (Pg. No. 396-399).

Course Faculty

Verified by HoD

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 | **II/IV**

Course Name with Code : **Instrumental Methods of Analysis 19BTD08**

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

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

Topic of Lecture: HPLC – Instrumentation and applications

Introduction :

- It is column chromatography.
- It is Liquid Chromatography.
- It is modified form of gas chromatography, it is applicable for both Volatile as well as non-volatile compound.
- It can mainly divided by two types
  1. Normal phase HPLC
  2. Reversed Phase HPLC
- It is having a high resolution and separation capacity.
- It is used as qualitative as well as quantitative analysis.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on learning the principle of HPLC.
- Prerequisite knowledge on studying the importance and applications part of HPLC.
- Prerequisite knowledge on how the sample and the reference compound is detected with respect to R_t (Retention time).

Detailed content of the Lecture:

- High performance liquid chromatography (HPLC) is a chromatographic technique used to separate a mixture of compounds in analytical chemistry and biochemistry with the purpose of identifying, quantifying or purifying the individual components of the mixture.

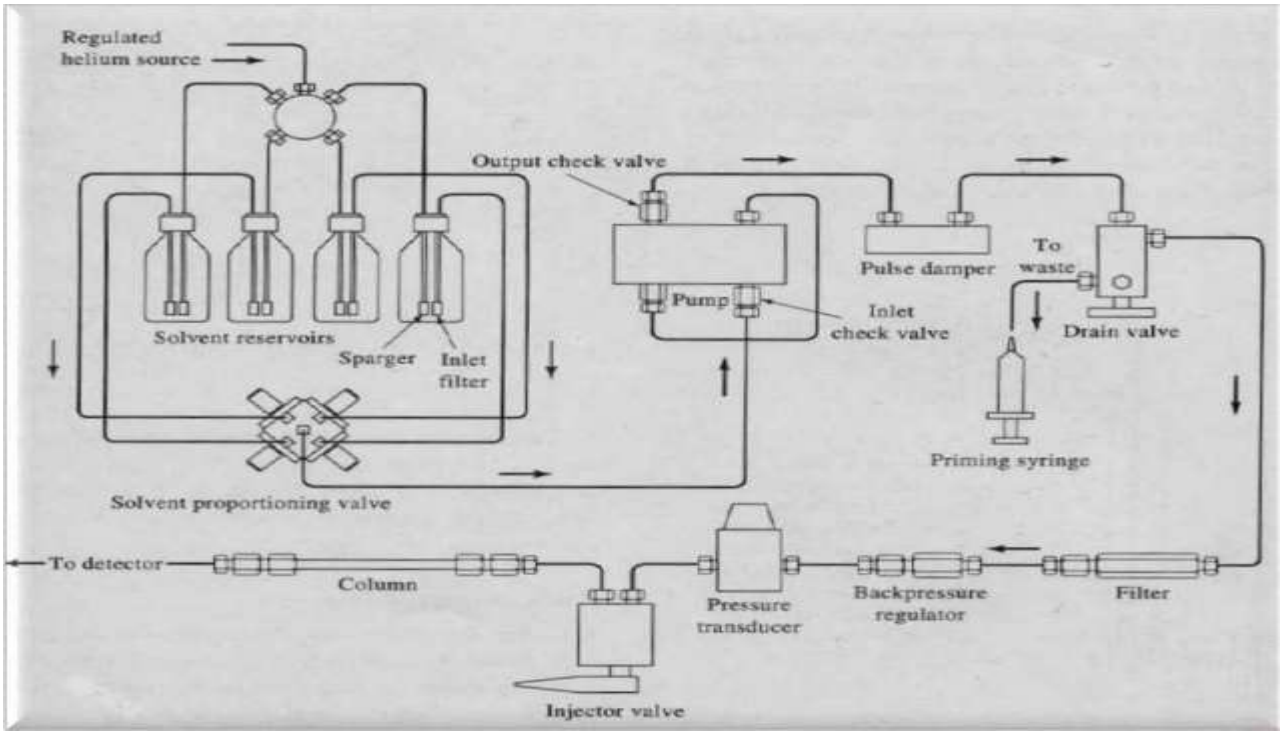
PRINCIPLE:

- High Performance Liquid Chromatography [HPLC] is principle is based on adsorption as well as partition chromatography is depending on the nature of stationary phase, if stationary phase is solid principle is based on adsorption chromatography and if stationary phase is liquid principle is based on partition chromatography.
- It is important for determination of volatile and non-volatile compounds.
- It is important for determination qualitative and quantitative analysis.
- It is important for determination of Retention Time (the time is required, after sample injection maximum angle peak reaches to detector).

INSTRUMENTATION:

1. Solvent storage bottle
2. Gradient controller and mixing unit
3. De-gassing of solvents

4. Pump
5. Pressure gauge
6. Pre-column
7. Sample introduction system
8. Column
9. Detector
10. Recorder



APPLICATIONS:

- Drug Discovery
- Clinical Analysis
- Proteomics
- Identification of Bile Acid Metabolite
- Clinical Applications
- Biochemical Genetics
- qualitative and quantitative analysis and Therapeutic Drug Monitoring

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

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

Willard, Hobart, et al., "Instrumental Methods of Analysis" 7th Edition, CBS, 1986 (Pg. No. 400-411).

Course Faculty

Verified by HoD

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 | **II/IV**

Course Name with Code : **Instrumental Methods of Analysis 19BTD08**

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

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

Topic of Lecture: Capillary electrophoresis and applications

Introduction :

- The differential movement or migration of ions by attraction or repulsion in an electric field or it describes migration of charged particles or molecules under the influence of electric field.
- Purpose for carrying out electrophoresis:
  1. To determine the number, amount and mobility of components in a given sample or to separate them.
  2. To obtain information about the electrical double layers surrounding the particles.
  3. Determination of molecular weight of proteins and DNA sequence.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on understanding the movement of ions in electric field.
- Prerequisite knowledge on knowing the instrumentation and working of CE.
- Prerequisite knowledge on learning of different types of CE.

Detailed content of the Lecture:

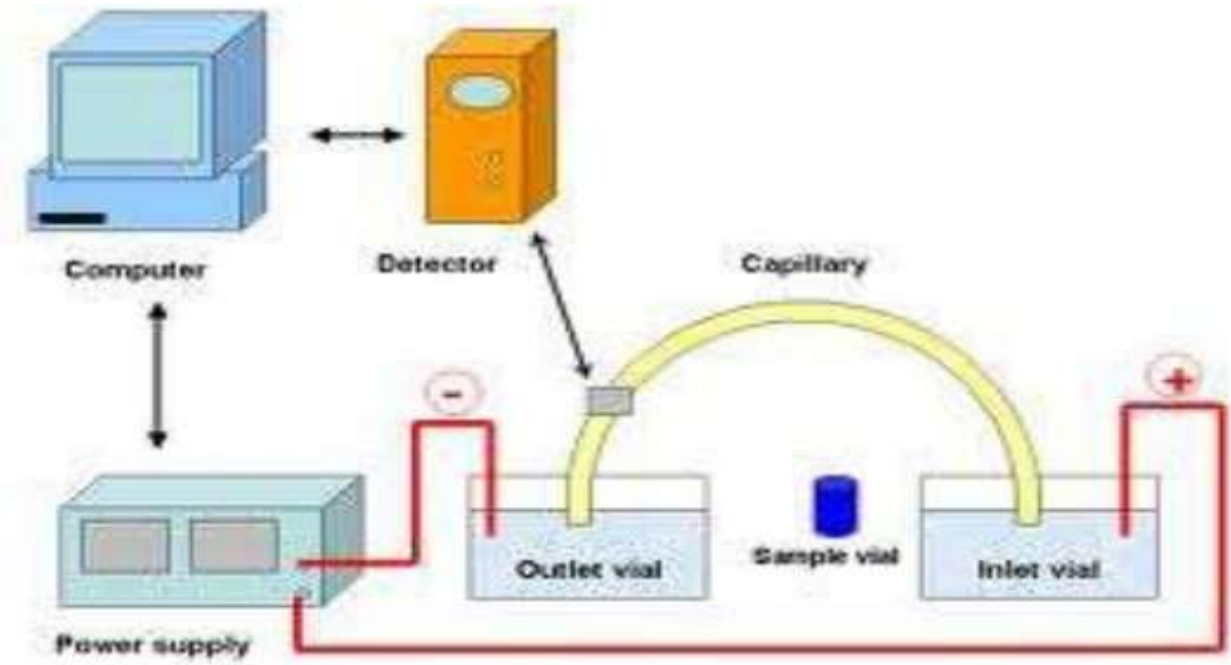
DEFINITION:

- These kind of separations are facilitated by the use of high voltages, which may generate electro-osmotic and electro-phoretic flow of buffer solutions and ionic species, respectively within the capillary.

PRINCIPLE:

- Capillary electrophoresis is an analytical technique that separates ions based on their electrophoretic mobility with the use of an applied voltage.
- The electrophoretic mobility is dependent upon the charge of the molecule, the viscosity, and the atom's radius.
- The rate at which the particle moves is directly proportional to the applied electric field i.e. the greater the field strength, the faster the mobility & vice versa.
- Neutral species are not affected, only ions move with the electric field. If two ions are the same size, the one with greater charge will move the fastest.
- For ions of the same charge, the smaller particle has less friction and overall faster migration rate.
- Capillary electrophoresis is used most predominately because it gives faster results and provides high resolution separation.
- It is a useful technique because there is a large range of detection methods available.

INSTRUMENTATION:



Modes Of CE:

a) Capillary Zone electrophoresis (CZE).
b) Capillary gel electrophoresis (CGE).
c) Capillary isoelectric focusing (CIEF).
d) Capillary isotachophoresis (CITP).

APPLICATIONS:

- Genetic Analysis.
- Analysis of Pharmaceuticals.
- Pharmaceuticals with Chiral Centers (Enantiomers).
- Counter-ion analysis in drug discovery.
- Protein Characterization.

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

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

Willard, Hobart, et al., "Instrumental Methods of Analysis" 7th Edition, CBS, 1986 (Pg. No. 530-532).

Course Faculty

Verified by HoD

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 **II/IV**

Course Name with Code : **Instrumental Methods of Analysis 19BTD08**

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

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

Topic of Lecture: Electrochemical cells- Electrode potential

Introduction :

- An electrochemical cell is a device capable of either generating electrical energy from chemical reactions or using electrical energy to cause chemical reactions.
- These devices are capable of converting chemical energy into electrical energy or vice versa.
- The standard electrode potential of an electrode can be defined as the potential difference that arises between the electrode and the electrolyte under standard conditions.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite Knowledge on electrode potential and salt bridges.
- Prerequisite Knowledge on main key features on cathode and anode.
- Prerequisite Knowledge on half-cells and types of electrochemical cells.

Detailed content of the Lecture:

- The electrochemical cells which generate an electric current are called voltaic cells or galvanic cells and those that generate chemical reactions, via electrolysis for example, are called electrolytic cells.
- A common example of a galvanic cell is a standard 1.5 volt cell meant for consumer use.
- A battery consists of one or more cells, connected in parallel, series or series-and-parallel pattern.

Galvanic Cell

A galvanic cell, or voltaic cell is an electrochemical cell that derives electrical energy from spontaneous redox reactions taking place within the cell. It generally consists of two different metals connected by a salt bridge, or individual half-cells separated by a porous membrane.

Primary cell:

A primary cell is a Galvanic battery that is designed to be used once and discarded, in contrast to a secondary cell (rechargeable battery), which can be recharged with electricity and reused.

Secondary cell:

A secondary cell, commonly referred to as a rechargeable battery, is an electrochemical cell that can be run as both a galvanic cell and as an electrolytic cell. This is used as a convenient way to store electricity, when current flows one way the levels of one or more chemicals build up (charging), while it is discharging they reduce and the resulting electromotive force can do work.

Fuel cell:

A fuel cell is an electrochemical cell that converts the chemical energy from a fuel into electricity through an electrochemical reaction of hydrogen fuel with oxygen or another oxidizing agent.

Electrode potential, E, in chemistry or electrochemistry, according to an IUPAC definition, is the electromotive force of a cell built of two electrodes:

- on the left-hand side of the cell diagram is the standard hydrogen electrode (SHE), and
- on the right-hand side is the electrode in question.

The SHE is defined to have a potential of 0 V, so the signed cell potential from the above setup is

$$E_{cell} = E_{right} - E_{left}(SHE) = E_{electrode} - 0\ V = E_{electrode}.$$

Video Content / Details of website for further learning (if any):
https://byjus.com/chemistry/electrochemical-cell/

Important Books/Journals for further learning including the page nos.:
Willard, Hobart, et al., "Instrumental Methods of Analysis". VIIth Edition, CBS, 1986. (Pg. No. 561-562).

Course Faculty

Verified by HoD

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 **II/IV**

Course Name with Code : **Instrumental Methods of Analysis 19BTD08**

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

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

Topic of Lecture: Potentiometry and reference electrode

Introduction :

- Potentiometer working can be explained when the potentiometer is understood. It is defined as a three-terminal resistor having either sliding or rotating contact that forms an adjustable voltage divider.
- In order to use the potentiometer as a rheostat or variable resistor, it should have only two terminals with one end and the wiper.
- One of the electrodes is a reference electrode whose potential is known and the other electrode is the test electrode.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on basic concepts in potentiometer.
- Prerequisite Knowledge on the potentiometric measurements.
- Prerequisite Knowledge on types of electrodes.

Detailed content of the Lecture:

- The potentiometer consists of L which is a long resistive wire and a battery of known EMF V whose voltage is known as driver cell voltage.
- Assume a primary circuit arrangement by connecting the two ends of L to the battery terminals.
- One end of the primary circuit is connected to the cell whose EMF E is to be measured and the other end is connected to galvanometer G.
- This circuit is assumed to be a secondary circuit.
- The working principle depends on the potential across any portion of the wire which is directly proportional to the length of the wire that has a uniform cross-sectional area and current flow is constant. Following is the derivation of used to explain the potentiometer working principle:

$$V=IR \text{ (Ohm's law)}$$

Where,
I: current
R: total resistance
V: voltage

$$R=\rho L/A$$

$$V=I\,\rho L/A$$

Where,
ρ: resistivity
A: cross-sectional area
With ρ and A constant, I is constant too for a rheostat.

$$L\rho/A=K$$

$$V=KL$$

$$E=L\rho x/A=Kx$$

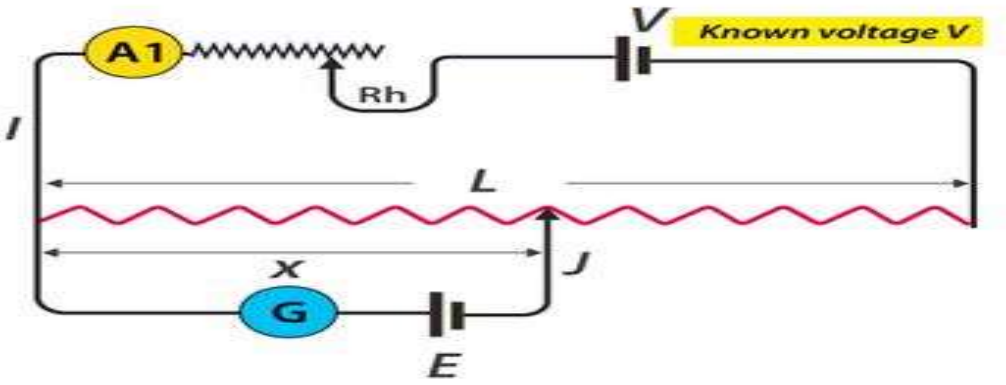
Where,
x: length of potentiometer wire
E: cell with Lower EMF
K: constant

- The galvanometer G has null detection as the potential difference is equal to zero and there is no flow of current. So, x is the length of the null point.
- Unknown EMF can be found by knowing x and K.

$$E=L\rho x/A=Kx$$

- Since the EMF has two cells, let L1 be the null point length of the first cell with EMF E1 and L2 be the null point length of the second cell with EMF E2.

$$E1/E2=L1/L2$$



Video Content / Details of website for further learning (if any):
https://byjus.com/physics/potentiometer-working/

Important Books/Journals for further learning including the page nos.:
Willard, Hobart, et al., "Instrumental Methods of Analysis". VIIth Edition, CBS, 1986. (Pg. No. 573).

Course Faculty

Verified by HoD

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 | **II/IV**

Course Name with Code : **Instrumental Methods of Analysis 19BTD08**

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

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

Topic of Lecture: Ion selective and molecular selective electrodes

Introduction :

- An ion-selective electrode (ISE), also known as a specific ion electrode (SIE), is a transducer (or sensor) that converts the activity of a specific ion dissolved in a solution into an electrical potential.
- The voltage is theoretically dependent on the logarithm of the ionic activity according to the Nernst equation.
- Ion selective electrodes are used in analytical chemistry where measurements of ionic concentration in an aqueous solution are required.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite Knowledge on techniques used in ISE.
- Prerequisite Knowledge on how a thin membrane is used for binding that creates a potential difference in the electrodes.
- Prerequisite Knowledge on differences between ion and molecular electrodes.

Detailed content of the Lecture:

Detailed content of the Lecture:

- The voltage is theoretically dependent on the logarithm of the ionic activity, according to the Nernst equation.
- Ion-selective electrodes are used in analytical chemistry and biochemical/biophysical research, where measurements of ionic concentration in an aqueous solution are required.

Glass membrane

- Glass membranes are made from an ion-exchange type of glass (silicate or chalcogenide).
- This type of ISE has good selectivity, but only for several single-charged cations; mainly H^+, Na^+, and Ag^+.
- Chalcogenide glass also has selectivity for double-charged metal ions, such as Pb^{2+}, and Cd^{2+}.
- The glass membrane has excellent chemical durability and can work in very aggressive media.
- A very common example of this type of electrode is the pH glass electrode.

Ion-exchange resin membrane

- Ion-exchange resins are based on special organic polymer membranes which contain a specific ion-exchange substance (resin).
- This is the most widespread type of ion-specific electrode.

- Usage of specific resins allows preparation of selective electrodes for tens of different ions, both single-atom or multi-atom.
- They are also the most widespread electrodes with anionic selectivity. However, such electrodes have low chemical and physical durability as well as "survival time".
- An example is the potassium selective electrode, based on valinomycin as an ion-exchange agent.

Enzyme Electrodes

- Enzyme electrodes definitely are not true ion-selective electrodes but usually are considered within the ion-specific electrode topic.
- Such an electrode has a "double reaction" mechanism - an enzyme reacts with a specific substance, and the product of this reaction (usually H+ or OH−) is detected by a true ion-selective electrode, such as a pH-selective electrodes.
- All these reactions occur inside a special membrane which covers the true ion-selective electrode, which is why enzyme electrodes sometimes are considered as ion-selective.
- An example is glucose selective electrodes.

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

https://chem.libretexts.org/Bookshelves/Analytical_Chemistry/Supplemental_Modules_(Analytical_Chemistry)/Analytical_Sciences_Digital_Library/JASDL/Courseware/Analytical_Electrochemistry%3A_Potentiometry/03_Potentiometric_Theory/03_Ion-Selective_Electrodes

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

Willard, Hobart, et al., "Instrumental Methods of Analysis". VIIth Edition, CBS, 1986. (Pg. No. 573).

Course Faculty

Verified by HoD

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 | **II/IV**

Course Name with Code : **Instrumental Methods of Analysis 19BTD08**

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

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

Topic of Lecture: Instrument for potentiometric studies

Introduction :

- A potentiometer is an instrument for measuring voltage or 'potential difference' by comparison of an unknown voltage with a known reference voltage.
- If a sensitive indicating instrument is used, very little current is drawn from the source of the unknown voltage.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on potentiometry and it's techniques.
- Prerequisite Knowledge on the applications of instrument with working.

Detailed content of the Lecture:

- In this circuit, the ends of a uniform resistance wire R1 are connected to a regulated DC supply VS for use as a voltage divider.
- The potentiometer is first calibrated by positioning the wiper (arrow) at the spot on the R1 wire that corresponds to the voltage of a standard cell so that R 2 R 1 = cell voltage V S
- A standard electrochemical cell is used whose emf is known (e.g. 1.0183 volts for a Weston standard cell).
- The supply voltage VS is then adjusted until the galvanometer shows zero, indicating the voltage on R2 is equal to the standard cell voltage.
- An unknown DC voltage, in series with the galvanometer, is then connected to the sliding wiper, across a variable-length section R_3 of the resistance wire.
- The wiper is moved until no current flows into or out of the source of unknown voltage, as indicated by the galvanometer in series with the unknown voltage.
- The voltage across the selected R_3 section of wire is then equal to the unknown voltage. The final step is to calculate the unknown voltage from the fraction of the length of the resistance wire that was connected to the unknown voltage.
- The galvanometer does not need to be calibrated, as its only function is to read zero or not zero. When measuring an unknown voltage and the galvanometer reads zero, no current is

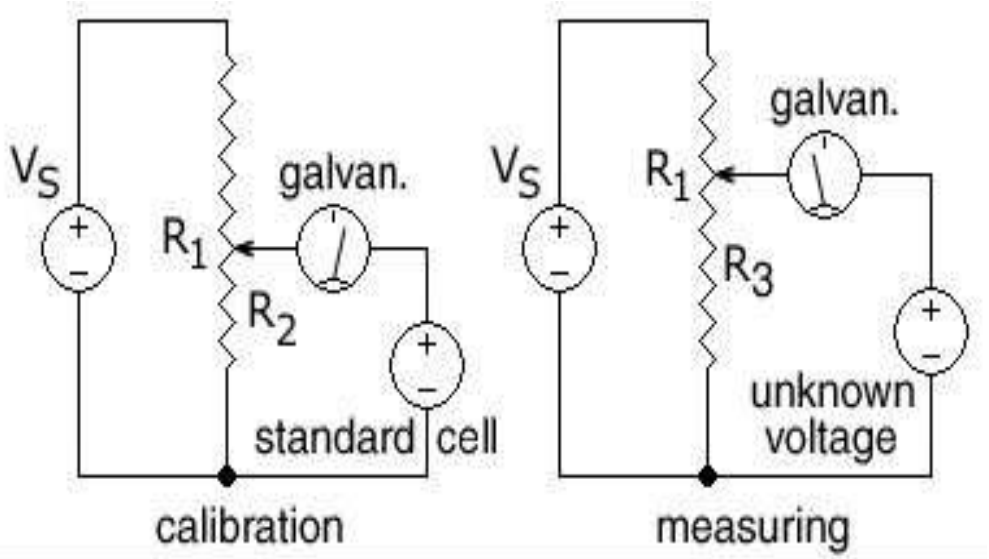
drawn from the unknown voltage and so the reading is independent of the source's internal resistance, as if by a voltmeter of infinite resistance.

- Because the resistance wire can be made very uniform in cross-section and resistivity, and the position of the wiper can be measured easily, this method can be used to measure unknown DC voltages greater than or less than a calibration voltage produced by a standard cell without drawing any current from the standard cell.

- If the potentiometer is attached to a constant voltage DC supply such as a lead–acid battery, then a second variable resistor (not shown) can be used to calibrate the potentiometer by varying the current through the R1 resistance wire.

- If the length of the R1 resistance wire is AB, where A is the (-) end and B is the (+) end, and the movable wiper is at point X at a distance AX on the R3 portion of the resistance wire when the galvanometer gives a zero reading for an unknown voltage, the distance AX is measured or read from a pre-printed scale next to the resistance wire.

- The unknown voltage can then be calculated: $V_U = (\text{Calibration Cell Voltage}) AX/AB$.



Video Content / Details of website for further learning (if any):
https://instrumentationtools.com/potentiometer-working-principle-animation/

Important Books/Journals for further learning including the page nos.:
Willard, Hobart, et al., "Instrumental Methods of Analysis". VIIth Edition, CBS, 1986. (Pg. No. 574-579).

Course Faculty

Verified by HoD

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 **II/IV**

Course Name with Code : **Instrumental Methods of Analysis 19BTD08**

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

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

Topic of Lecture: Voltametry – principle and types

Introduction :

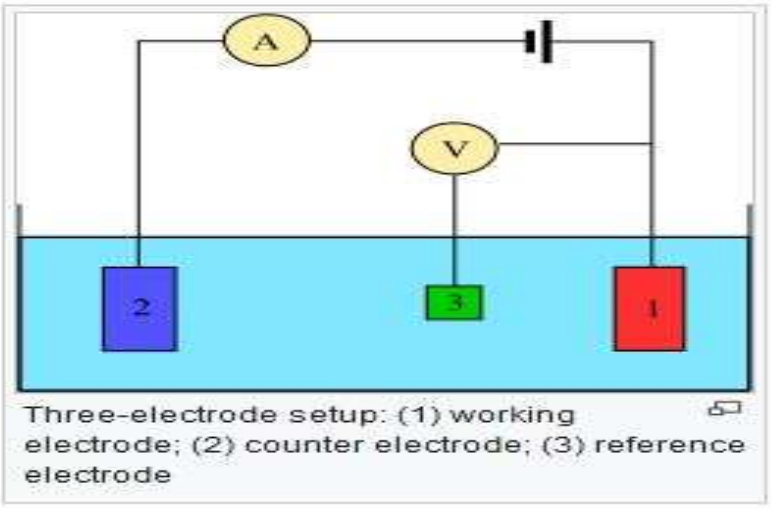
- Voltammetry is a category of electroanalytical methods used in analytical chemistry and various industrial processes.
- In voltammetry, information about an analyte is obtained by measuring the current as the potential is varied.
- The analytical data for a voltammetric experiment comes in the form of a voltammogram which plots the current produced by the analyte versus the potential of the working electrode.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on voltameter.
- Prerequisite Knowledge on various types of voltammetry.

Detailed content of the Lecture:

- In voltammetry, information about an analyte is obtained by measuring the current as the potential is varied.
- The analytical data for a voltammetric experiment comes in the form of a voltammagram which plots the current produced by the analyte versus the potential of the working electrode
- Data analysis requires the consideration of kinetics in addition to thermodynamics, due to the temporal component of voltammetry.
- Idealized theoretical electrochemical thermodynamic relationships such as the Nernst equation are modeled without a time component.
- While these models are insufficient alone to describe the dynamic aspects of voltammetry, models like the Tafel equation and Butler–Volmer equation lay the groundwork for the modified voltammetry relationships that relate theory to observed results.



Three-electrode setup: (1) working electrode; (2) counter electrode; (3) reference electrode

Video Content / Details of website for further learning (if any):
https://sites.chem.colostate.edu/diverdi/C477/experiments/electrochemistry_cyclic_voltammetry/science%20references/voltammetry%20-%20Skoog.pdf

Important Books/Journals for further learning including the page nos.:
Willard, Hobart, et al., "Instrumental Methods of Analysis". VIIth Edition, CBS, 1986. (Pg. No. 633).

Course Faculty

Verified by HoD

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 | **II/IV**

Course Name with Code : **Instrumental Methods of Analysis 19BTD08**

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

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

Topic of Lecture: Cyclic and pulse voltametry

Introduction :

- Cyclic Voltammetry (CV) is an electrochemical technique which measures the current that develops in an electrochemical cell under conditions where voltage is in excess of that predicted by the Nernst equation.
- CV is performed by cycling the potential of a working electrode, and measuring the resulting current.
- Differential Pulse Voltammetry requires a computer-controlled or programmable potentiostat. DPV provides excellent high sensitivity.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on voltameter.
- Prerequisite Knowledge on types of voltammetry.

Detailed content of the Lecture:

Cyclic Votammetry:

- A CV system consists of an electrolysis cell, a potentiostat, a current-to-voltage converter, and a data acquisition system.
- The electrolysis cell consists of a working electrode, counter electrode, reference electrode, and electrolytic solution.
- The working electrode's potential is varied linearly with time, while the reference electrode maintains a constant potential.
- The counter electrode conducts electricity from the signal source to the working electrode.
- The purpose of the electrolytic solution is to provide ions to the electrodes during oxidation and reduction.
- A potentiostat is an electronic device which uses a dc power source to produce a potential which can be maintained and accurately determined, while allowing small currents to be drawn into the system without changing the voltage.
- The current-to-voltage converter measures the resulting current, and the data acquisition system produces the resulting voltammogram.

Differential Pulse voltammetry

- The method uses pulse waveforms so that ASV takes longer and interference from oxygen can becomes significant.
- Thus, it is advisable to remove oxygen from solutions by purging them with nitrogen for a few minutes.

Video Content / Details of website for further learning (if any): https://chem.libretexts.org/Bookshelves/Analytical_Chemistry/Supplemental_Modules_(Analytical_Chemistry)/Instrumental_Analysis/Cyclic_Voltammetry
Important Books/Journals for further learning including the page nos.: Willard, Hobart, et al., "Instrumental Methods of Analysis". VIIth Edition, CBS, 1986. (Pg. No. 634-639)

Course Faculty

Verified by HoD

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 | **II/IV**

Course Name with Code : **Instrumental Methods of Analysis 19BTD08**

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

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

Topic of Lecture: Applications of voltametry
Introduction : • Voltammetry is a category of electroanalytical methods used in analytical chemistry and various industrial processes. • In voltammetry, information about an analyte is obtained by measuring the current as the potential is varied
Prerequisite knowledge for Complete understanding and learning of Topic: • Basic knowledge on voltameter. • Prerequisite Knowledge on knowing the applications of voltametry.
Detailed content of the Lecture: • Cyclic voltammetry is a versatile method for scientific investigation and innovation due to the fact that most processes involve electron transfer, which makes them be able to be monitored by this technique. • Cyclic voltammetry is generally used to study the electrochemical properties of an analyte in solution or of a molecule that is adsorbed onto the electrode • Its uses cover characterization, synthesis, mechanisms, and analysis. • In all applications, the technique can work well with a large variety of compounds including organic, inorganic, polymer, films, and semiconductors, among others. • Furthermore, the method operates satisfactorily whether in a direct or an indirect approach. • As an analytical tool, it plays an important role in not only chemistry but also other involving areas.
Video Content / Details of website for further learning (if any): https://www.intechopen.com/books/voltammetry/cyclic-voltammetry-and-its-applications
Important Books/Journals for further learning including the page nos.: Willard, Hobart, et al., "Instrumental Methods of Analysis". VIIth Edition, CBS, 1986. (Pg. No. 540).

Course Faculty

Verified by HoD

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 | **II/IV**

Course Name with Code : **Instrumental Methods of Analysis 19BTD08**

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

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

Topic of Lecture: Scanning probe microscopes

Introduction :

- Scanning probe microscope (SPM) is a branch of microscopy that forms images of surfaces using a physical probe that scans the specimen.
- The precursor of AFM, the SPM was developed by Gerd Binnig and Heinrich Rohrer in the early 1980s at IBM research – Zurich.

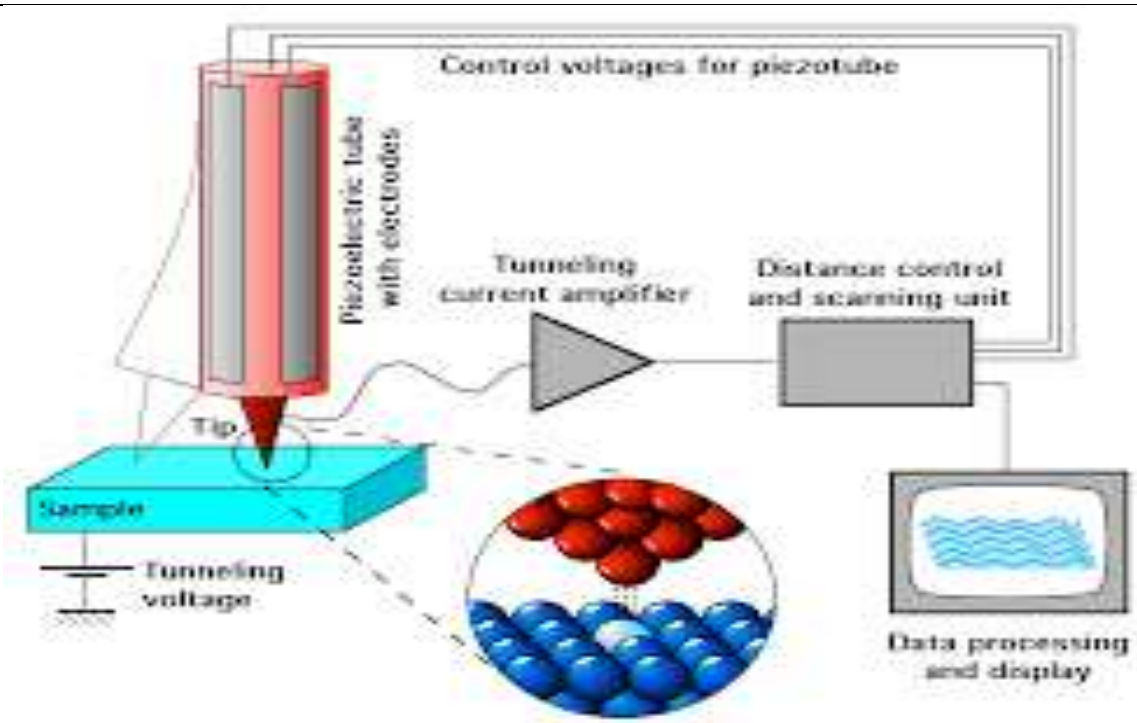
Prerequisite knowledge for Complete understanding and learning of Topic:

- Prerequisite knowledge on optical instruments
- Prerequisite Knowledge on Engineering Physics, Engineering Chemistry, and in Biological science**.**

Detailed content of the Lecture:

Scanning Probe Microscopes

- The latest type of microscope is the Scanning Probe microscope.
- These microscopes did not use light or electrons which had limitations on their outreach.
- There are various types of SPC.
- The most widely used is the Atomic probe microscope which uses a nano-scale ‘finger’ or a tip which is almost the size of an atom.
- This atomically thin needle like structure is run on sample surfaces and an image is recreated based on its interaction with the surface.
- Another type is the Magnetic Force microscope which forms images based on the change in the magnetic field on an atomic level.
- The Scanning Tunneling Microscope which recreates images based on the change in electrical energy between the needle and the atomic surface.



Video Content / Details of website for further learning (if any):
https://blog.byjus.com/seeing-atoms/

Important Books/Journals for further learning including the page nos.:
Willard, Hobart, et al., "Instrumental Methods of Analysis". VIIth Edition, CBS, 1986. (Pg. No. 545)

Course Faculty

Verified by HoD

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 | **II/IV**

Course Name with Code : **Instrumental Methods of Analysis 19BTD08**

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

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

Topic of Lecture: AFM and STM instrumental details and applications.

Introduction :

- Atomic force microscopy or scanning force microscopy is a very-high-resolution type of scanning probe microscopy, with demonstrated resolution on the order of fractions of a nanometer, more than 1000 times better than the optical diffraction limit.

Prerequisite knowledge for Complete understanding and learning of Topic:

- Basic knowledge on concepts of instrument working.
- Prerequisite knowledge on types of microscopes.

Detailed content of the Lecture:

Instrumentation:

- Scanning Tunneling Microscope works, a sharp tip is raster-scanned over a surface using a feedback loop to adjust parameters needed to image a surface.
- Unlike Scanning Tunneling Microscopes, the Atomic Force Microscope does not need a conducting sample. Instead of using the quantum mechanical effect of tunneling, atomic forces are used to map the tip-sample interaction.
- Often referred to as scanning probe microscopy (SPM), there are Atomic Force Microscopy techniques for almost any measurable force interaction – van der Waals, electrical, magnetic, thermal. For some of the more specialized techniques, modified tips and software adjustments are needed.
- In addition to Angstrom-level positioning and feedback loop control, there are 2 components typically included in Atomic Force Microscopy: Deflection and Force Measurement.
- AFM Probe Deflection
- Traditionally, most Atomic Force Microscopes use a laser beam deflection system where a laser is reflected from the back of the reflective AFM lever and onto a position-sensitive detector. AFM tips and cantilevers are typically micro-fabricated from Si or Si3N4. Typical tip radius is from a few to 10s of nm.

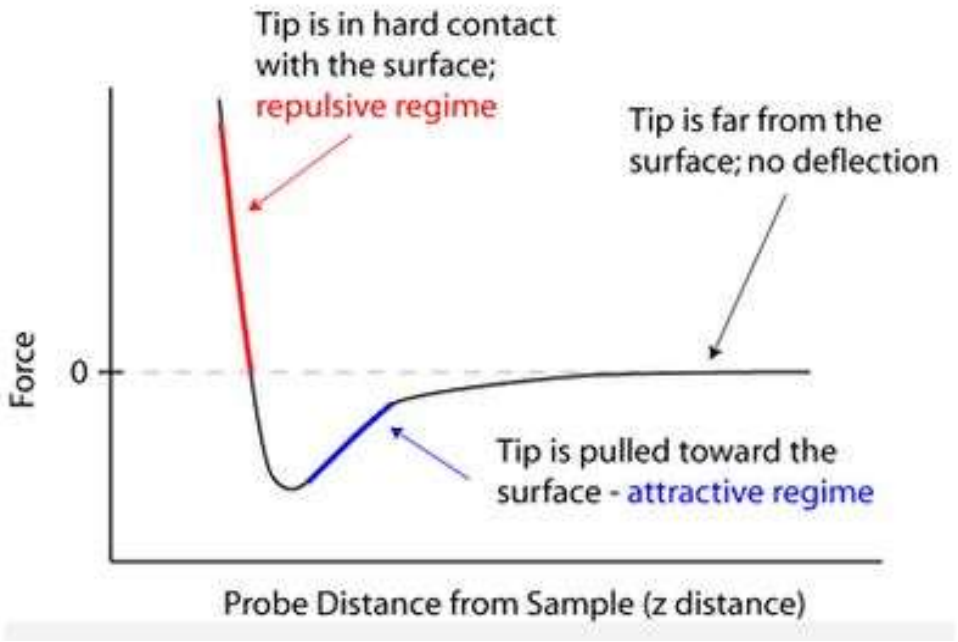
Measuring Forces

- Because the Atomic Force Microscope 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.

Hooke's law gives:

$$F = -kz$$

Where,
F is the force, k is the stiffness of the lever, and z is the distance the lever is bent.



Applications

• Digitally image a topographical surface
• Determine the roughness of a surface sample or to measure the thickness of a crystal growth layer
• Any sample like ceramic material, human cells or individual molecules of DNA.
• In biological applications: Image non-conducting surfaces such as proteins and DNA

- Study Unfolding Of Proteins
- Imagining Of Biomolecules
- Force Measurements In Real Solvent Environments
- Antibody-Antigen Binding Studies
- Ligand-Receptor Binding Studies

Binding Forces Of Complimentary DNA Strands

Video Content / Details of website for further learning (if any):
https://www.microscopemaster.com/atomic-force-microscope.html

Important Books/Journals for further learning including the page nos.:
Willard, Hobart, et al., "Instrumental Methods of Analysis". VIIth Edition, CBS, 1986. (Pg. No. 546).

Course Faculty

Verified by HoD