DMI COLLEGE OF ENGINEERING

DEPARTMENT OF ELECTRICAL AND ELECTRONICS ENGINEERING

ANALYTICAL METHODS INSTRUMENTATION

OBT751-Question Bank

UNIT I

SPECTROMETRY

TWO MARKS:

1. Mention the parameters or properties of electromagnetic radiation?

i) Wavelength

ii) Frequency

iii) Velocity

iv) Wave number

v) Amplitude

2. Define wavelength?

It is the distance between the two successive peaks of waves.

3. Define frequency?

It is the number of waves per second.

Frequency=speed of light/wavelength

4. Define transmittance and absorbance?

Transmittance:

It is the ratio of the radiant power transmitted by a sample to the radiant power incident on the sample.

Transmittance T=P/Po

Absorbance:

It is the negative logarithm of transmittance.

Absorbance A=-log10 T

5. Define wave number?

It is the number of waves spread in a length of one centimeter. It is the reciprocal of

6. State Beer's law.

The dependence of radiant power on the concentration of absorbing species can be given by Beer's law.

In p0/p=k''C

Where p0 is the radiant power at c=0 and

p is the radiant power at c=c and

c is the concentration of absorbing species

7. State the process involved in AAS.

The AAS phenomenon can be divided into two major processes

1. The production of free atoms from the sample
2. The absorption of radiation from an external source by these atoms

8. Name the types of detectors used for IR spectrometry.

1. Thermal detectors 2. Photon detectors

9. Define spectroscopy.

Spectroscopy is the measurement and interpretation of radiation

Emitted, scattered or absorbed by different atoms, molecules & other chemical species

10. Name the different types of spectrophotometers.

1. UV_ visible spectrophotometers.
2. Infrared spectrophotometers.
3. FIFR spectrophotometers.
4. Atomic absorption spectrophotometers.
5. Flame emission spectrophotometers

11. What are the light sources used for AAS?

    1. Hollow cathode lamp

    2. Electrode less discharge lamp

12. Give any two applications of flame emission spectrometry.

    1. FES is used in the determination of trace metals in liquid samples.

    2. FES finds wide application in agricultural and environmental analysis, industrial analysis of ferrous metal, alloy as well as glasses.

13. Specify the classification of IR region of spectrum.

    1. Photographic region

    2. Very near IR region (overtone region)

    3. near IR region (vibration rotation region)

    4. Far IR region (rotation region)

14. Name the instruments used in IR spectrometry.

    1. IR radiation sources

    2. Monochromators

    3. Sample cells

    4. Detectors.

15. Name few IR radiation sources.

    1. Incandescent source

    2. Nernst glower

    3. Globar source

    4. Mercury arc.

16. Give the advantages of grating monochromators?

    1. Gratings can be made with materials like aluminum which are not affected by Moisture.

2. Grating monochromators can be used over wide wavelength ranges

17. Give 4 different techniques used for sampling of solids.

1. Solids run in solution

2. Solid film techniques

3. Null techniques

4. Pressed pellet technique

18. Name two different types of IR spectrophotometers?

1. Optical null method

2. Radio recording method

3. Attenuated total reflection method

4. Fourier transforms IR spectrophotometers

19. Give the advantages and disadvantages of Fourier transform IR spectrometers.

Advantages:

1. FIFR methods are faster than dispersive instruments and hence especially useful in situations that require fast repetitive scanning.

2. FIFR provides increased energy throughput.

Disadvantages:

1. It is expensive than sequential dispersive instruments

2. For the precise movement of the mirror computer is also needed.

20. Specify the major design requirements of monochromators.

1. Simplicity

2. Resolution

3. Spectral range

4. Purity of exiting radiation

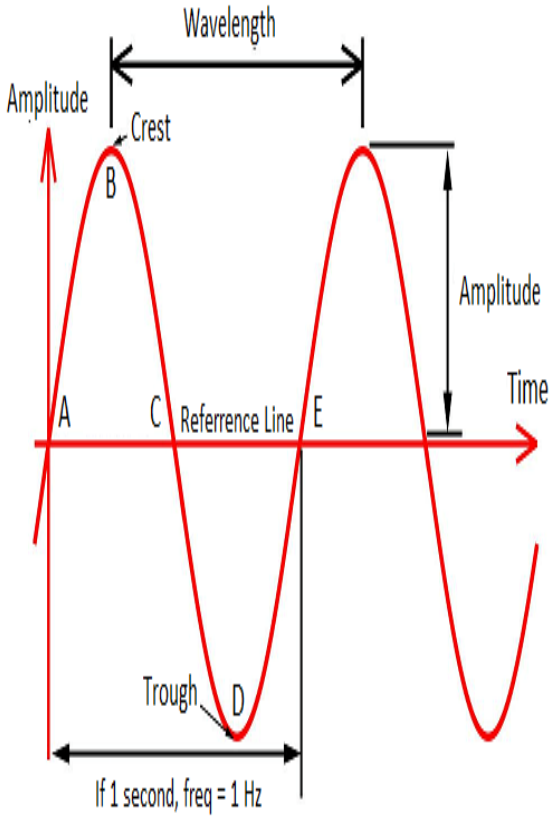
5. Dispersion

PART-B

1. Explain in detail about Wave properties in EMR.

EMR travels in the form of waves & has 3 characteristic properties:

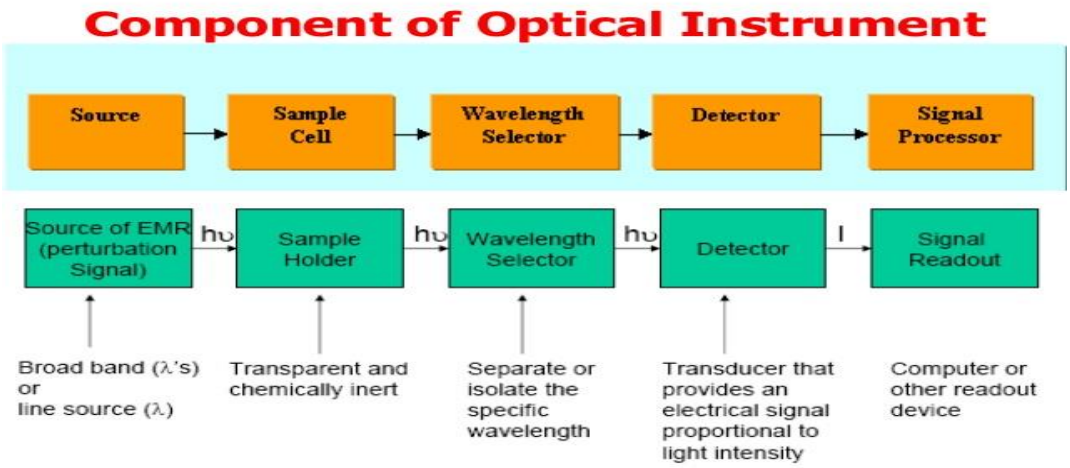
1. Wavelength (λ)
2. wavenumber ($\tilde{V}$)
3. Frequency (V)



2. Explain the detail about the components of optical instruments.

Optical instruments are the devices which process **light wave to enhance an image** for more clear view.

Use of an optical instruments, such as a magnifying lens or any complicated device like microscope or telescope usually makes things bigger and helps us to see in a more detailed manner.



3. Discuss in detail the different types and properties of electromagnetic radiation.

   Electromagnetic radiation (EMR) is a form of energy that is produced by oscillating electric and magnetic disturbance, or by the movement of electrically charged particles traveling through a vacuum or matter.

   Radiation Types

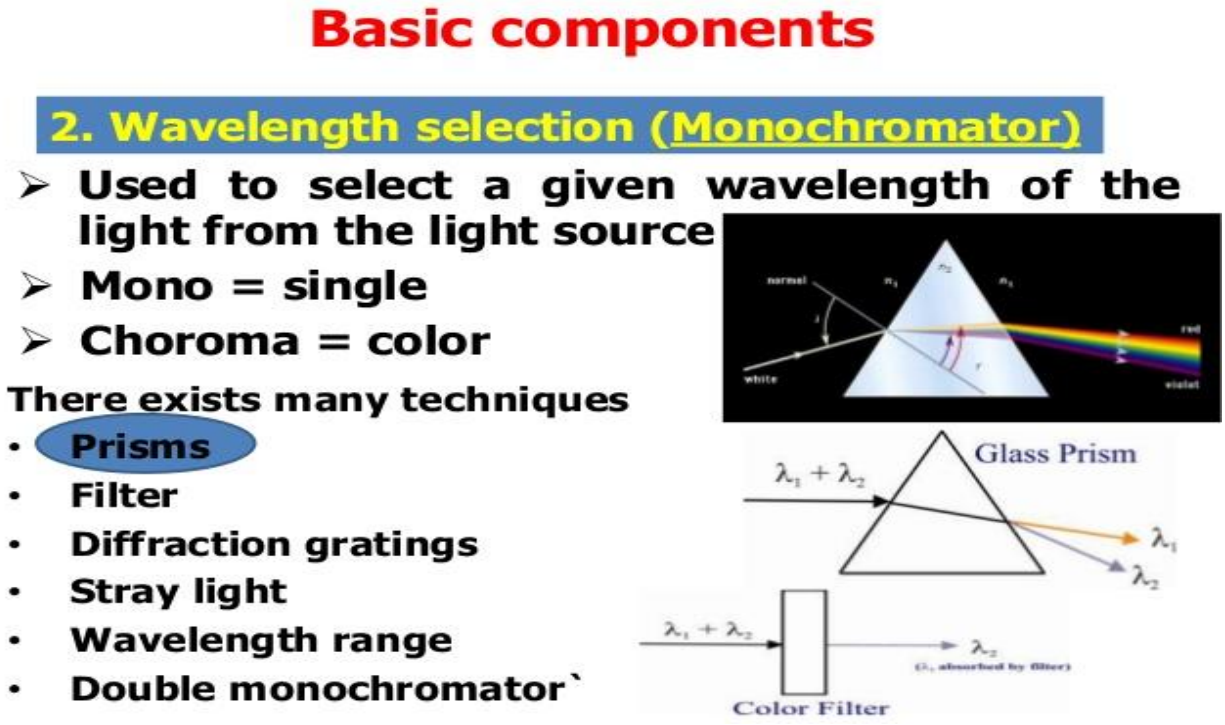
   Infrared radiation

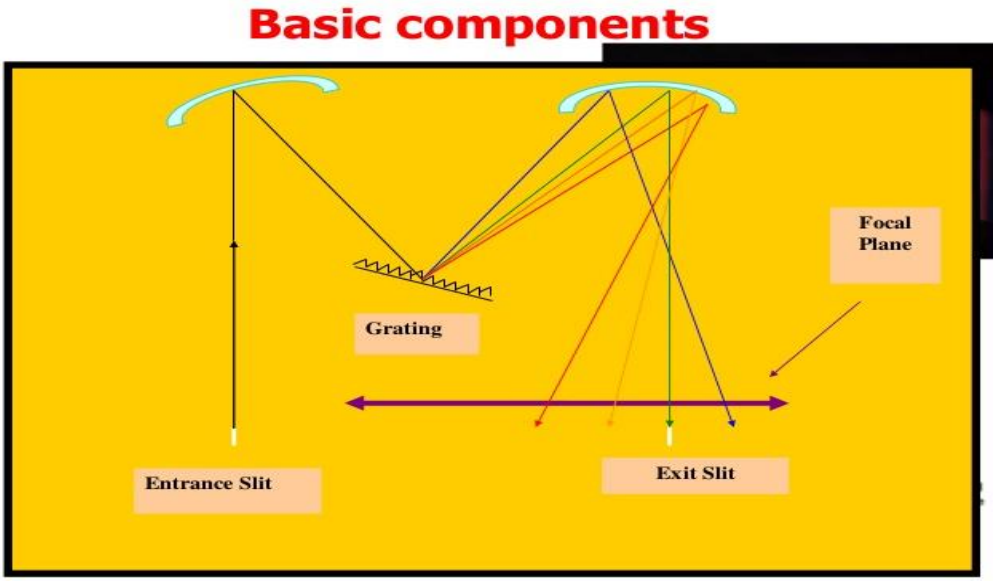
   Visible light

   Interference

   Wave-Particle Duality

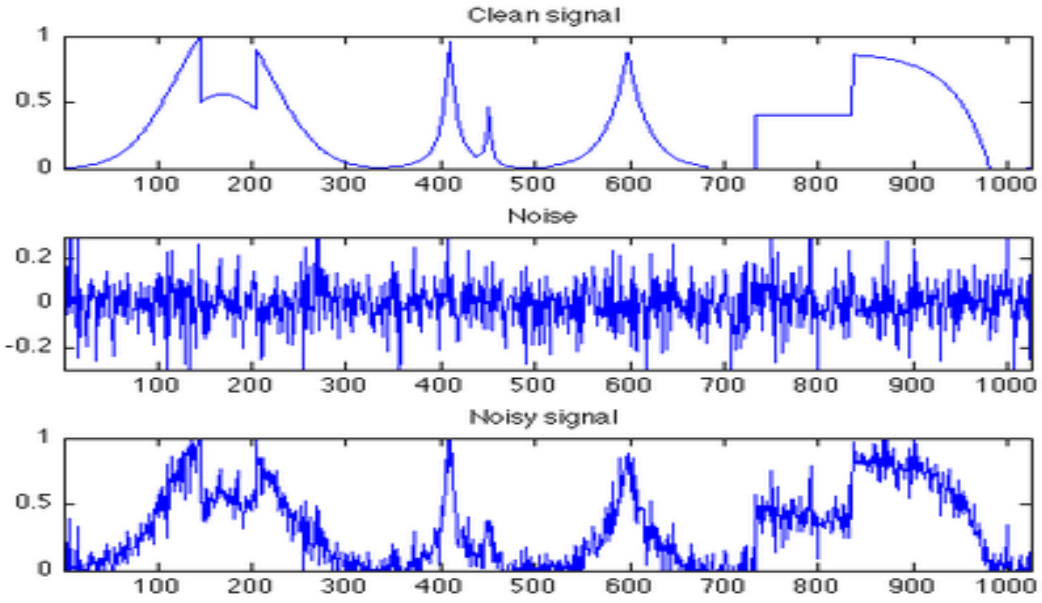
4. Explain about Monochromator.





5. Write short notes on enhancement of signal to noise.

Components picking up radio or TV signals (essentially acting as antennae) can convert those signals to voltage or current fluctuations.The causes of noise can be from the circuit itself, an imperfect design or layout, noise generated by faulty components or loose connections, or switches in related circuits or in switching power supplies that feed the circuit.



UNIT-2

MOLECULAR SPECTROSCOPY

PART-A

1. Explain Beers Law (May/June 2012).

Beer's law states that the absorbance is directly proportional to the concentration of a soluson, you plot absorbance versus concentration, the resulting graph yields a straight line.

2. Explain the term chromophore and give two examples.(May/June 2012)

A chromophore is the part of a when a molecule absorbs certain wavelengths of visible light and transmits or reflects others. The chromophore is a region in the molecule where the energy difference between two different molecular orbitals falls within the range of the visible spectrum Examples:Lycopene, Beta carotene, azo dyes

3. What is Lambert's (May/June 2014) law?

When a beam of light is allowed to pass through a transparent medium, the rate of decrease of intensity(I) with the thickness(1) of medium is detect proportional to the intersity -dl/d "KI Or Itle"

4. What is absorbance?

The absorbance(A) is the logarithm to the base of the reciprocal of the transmittance.

5. What is absorptivity, Explain?

A= log 1/T)--log Tor log 10/It

Absorptivity (a) is the ratio of the absorbance to the product of the concentration and length of optical path It is a constant characteristic of (a= Abc) substance and wavelengh. The alternate of this term is extinction coefficient or absorbance index.

6. What are the reasons for deviation from Beer Law? Deviation from the Beer's law are there reported the as resultant curve is concave upwards or concave downwards. The factors involved in deviation from Beer's law may be chemical & instrumental

7. Define colorimeter?

Any instrument used for measuring absorption in the visible region is generally called colorimeter.

8 Define spectrophotometer.

The instrument which measures the ratio or a function of the two, of the radiant power of two electromagnetic beam over a large wavelength region.

9. Define monochromators.A monochromators used to isolates band of interest of wavelengths. It allows the light of the required wavelength to pass through but absorb the light of other wavelengths. It contains entrance slit, dispensing elements and exit slit.

10. What is Detector and what are the detectors used in visible spectroscopy?

Detector is used for measuring the radiant energy transmitted through the sample. There are three types of photo devices used 1) photovoltaic cell 2) phototube and Photomultiplier tubes.

11. What is meant of single and double beam spectrophotometer?

Single beam have only one light path. Involve three controls: wavelengh zero adjustment and 100 per cent adjustment. The double-beam design provides two equivalent paths for radiation, both originating with the same source. One of these beams passes through the sample and other through reference. The two beams are measured separately, ether by duplicate detector or rapidly alterating use of the same detector.

12. What are the application of IR spectroscopy?

To estimation of organic compounds, inorganic compounds, geometrical isomerism, presence ofwater in the samples, shape of symmetry of a molecules, determination of purity etc.

13. Discum about the sources of AA spectroscopy.

The most successful line spectra source for AA is the hollow-cathode Lamp.

14. What are the applications AA?

1)It is useful in the determination of a large number of metals, specially a trace levels.

2) It is widely used in such field as water and pharmaceutical analysis and in metallurgy.

15. Mention the basic components of instruments that measure transmittance or absorbance. A stable light source, Monochromator, Sample containers for sample and solvent, A radiation detector, A signal indicator.

16. State the advantages of spectroscopy?

More rapid and less time consuming • Gives more information • Requires small amount of the compound to be anlysed Precise and reliable More selective and sensitive • Continuous operation is often possible.

17. Explain molecular spectroscopy.

This is deals with the interaction of electromagnetic radiation with molecules. The results in transition between rotational and vibrational energy levels in addition to electronic transition. Molecular spectra extend from the visible through infrared into the microwave region.

18. Define transmittance. It is the ratio of the radiant power transmitted by the sample (It) to the radiant power incident on the sample (10), both being measured at the same spectral position and with the same slit width. This transmittance T is defined by IU .

19. Discuss atomic absorption.

This is most powerful technique for the quantitative determination of trace metals in liquids. e.g total sodium content of a water. The sample should be gaseous state and volatilization of liquids or solid followed by the dissociation of molecules to give free atoms.

20. What are amphiprotic compounds? Give examples.

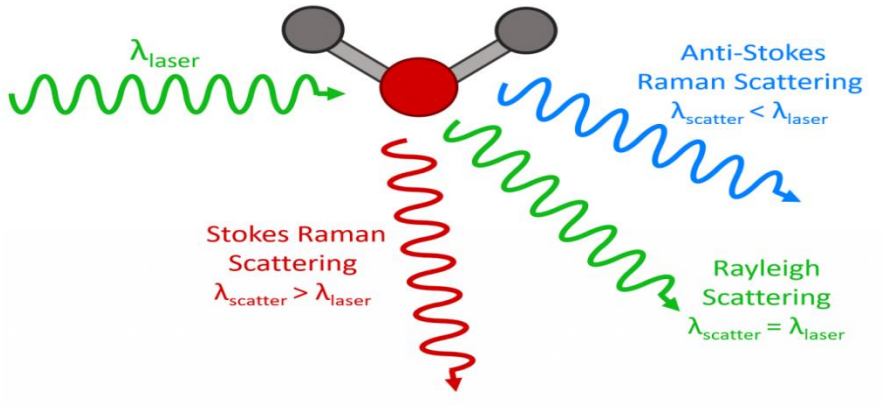
Can act as both acid or base. example: amino acids, water, proteins.

PART-B

1. Explain the theory ,Instrumentation & applications of Raman Spectroscopy?

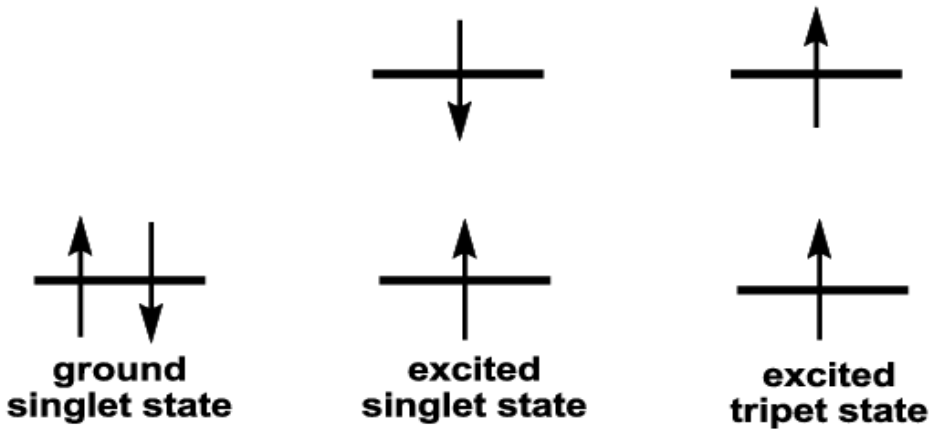
Raman spectroscopy is an analytical technique where **scattered light is used to measure the vibrational energy modes of a sample.**

- Raman spectroscopy can provide both chemical and structural information, as well as the identification of substances through their characteristic Raman ‘fingerprint’.
- Raman spectroscopy extracts this information through the **detection of Raman scattering** from the sample.



2. Explain in detail about Fluorescence and Phosphorescence?

- Fluorescence is the process whereby a molecule in the lower of two electronic states (generally the ground state) is excited to a higher electronic state by radiation whose energy corresponds to an allowed absorption transition, followed by the emission of radiation as the system decays back to the original state.
- The decay process can follow several pathways.
- If the decay is back to the original lower state, the process is called resonance fluorescence and occurs rapidly, in about one nanosecond.
- Phosphorescence is related to fluorescence in terms of its general mechanism but involves a slower decay.
- It occurs when a molecule whose normal ground state is a singlet is excited to a higher singlet state, goes to a vibrationally excited triplet state via either an intersystem crossing or a molecular collision, and subsequently, following vibrational relaxation, decays back to the singlet ground state by means of a forbidden transition.
- The result is the occurrence of a long lifetime for the excited triplet state; several seconds up to several hours are not uncommon.



3. What are the applications of IR spectroscopy?

Infrared spectroscopy is widely used in industry as well as in research. It is a simple and rele technique for measurement, quality control and dynamic measurement. It is also employed

in forense analyses in civil and criminal analyses. Some of the major applications of IR spectroscopy are as follows:

1. Identification of functional group and structure ducidation Endre IR region is divided into group frequency region and fingerprint region. Range of group frequency is 4000-1500 cm while that of finger print region is 1500-400 am In group frequency regon, the peaks corresponding to different functional groups can be observed.

2. Identification of substances IR spectroscopy is used to establish whether a given sample of an organic substance is identical with another or not.

3. Studying the progress of the reaction Progress of chemical reaction can be determined by examining the small portion of the reaction mixture withdrawn from time to time.

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

5.Quantitative analysis

The quantity of the substance can be determined either in pure form or as a mixure of two or more compounds. In this, characteristic peak corresponding to the drug substance is chosen and log 10 of peaks for standard and test sample is compared. This is called base line technique to determine the quantity of the substance.

OTHER APPLICATIONS

Determination of unknown contaminates in industry using FTIR Determination of cell wall of mutare & wild type plant verities using FTIR

Biomedical studies of human hair to identify disease state Identify color &taste component of the system Determine atmospheric pollutants from atmosphere itself It is also used in forensic analysis in both criminal and civil case, example in identifying the polymer degradation and determining the blood alcohol content.

4. Derive the Beer's law and discuss the reasons for derivation of Beer's law?

This relationship is a linear for the most part. However, under certain circumstances the Beer relationship gives a non-linear relationship. These deviations from the Beer Lambert law can be classified into three categories: Real Deviations: These are fundamental deviations due to the limitations of the law itself. Chemical Deviations:

These are deviations observed due to specific chemical species of the sample which is being analyzed. Instrument Deviations: These are deviations which occur due to how the absorbance measurements are made 1- Real Deviation

Beer Law and Lambert law is capable of describing absorption behavior of solutions containing relatively low amounts of solutes dissolved in it (<10-3M) When the concentration of the analyte in the solution is high (>10-3M), the analyte begins to behave differently due to interactions with the solvent and other solute molecules and at times even due to hydrogen bonding interactions. It is also possible that the concentration is so high, that the molecules create a screen for other molecules thereby shadowing them from the incident light 2- Chemical Deviations Chemical deviations occur due to chemical phenomenon involving the analyte molecules due to association, dissociation and interaction with the solvent to produce a product with different absorption characteristics. For example, phenol red undergoes a resonance transformation when moving from the acidic form (yellow) to the basic form (red). Due to this resonance, the electron distribution of the bonds of molecule changes with the pH of the solvent in which it is dissolved.

5. With the neat diagram explain the important components of Infrared Spectroscopy?

INSTRUMENTATION

SOURCE → SAMPLE → MONOCHROMATOR → DETECTOR → READOUT

- Nernst Glower
- Globar
- Incandescent wire source
- Hg Arc

Grating
Filter

Thermal D
Thermocouple
Thermopile
Thermister
Bolometer
Pyroelectric D

Recorder

1. IR radiation source

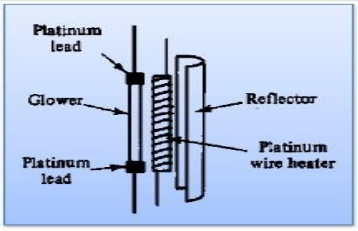
Be continuous over the wavelength range used.

Cover a wide wavelength range.

Be constant over long periods of time.

It provide maximum radiation about 7100cm^{-1} (1.4 μ)

its lifetime depends on the operating temperature and the care taken in handling it



UNIT-III

MAGNETIC RESONANCE SPECTROSCOPY AND MASS SPECTROMETRY

PART-A

1. What is NMR spectroscopy?

Is one of the most powerful tool, based on the measurement of absorption of electromagnetic radiation in the radio-frequency region of roughly 4 to 900 MHz.

2. Compare NMR with UV, Visible and IR absorption spectroscopy.

In contrast to UV, Vis and IR absorption, nuclei of atoms rather than outer electrons are involved in the absorption process.

3. What are the uses of NMR spectroscopy?

A powerful tool available to chemists and biochemists for elucidating the structure of chemical species. The technique is also useful for the quantitative determination of absorbing species.

4. List the types of NMR spectroscopy.

Two general types of spectrometers are currently in use, continuous-wave(CW) and pulsed or Fourier-Transform(FT-NMR).

5. What are the various types of NMR spectra?

Wide line spectra, high resolution spectra.

6. List the factors that decide the type of NMR spectra?

Kind of instrument used -type of nucleus involved -the physical state of the sample -the environment of the analyte nucleus and the purpose of the data collection.

7. Define wide line spectra of NMR.

Wide line spectra are those in which the bandwidth of the source of the lines is large enough that the fine structure due to chemical environment is obscured.

8. List the uses of wide line NMR spectra.

Are useful for the quantitative determination of isotopes and for studies of the physical environment of the absorbing species.

9. Define high-resolution spectra.

Most NMR spectra are high resolution and are collected by instruments capable of differentiating between very small frequency differences of 0.01 ppm or less.

10. What are the two types of relaxation processes important in NMR spectroscopy? (Nov/Dec 2015)

Spin-lattice or longitudinal relaxation and spin-spin or transverse relaxation.

11. Define relaxation time.

Is the measure of the average life time of the nuclei in the higher-energy state.

12. What is meant by free induction decay?

In Fourier Transform NMR, **free induction decay** (FID) is the observable NMR signal generated by non-equilibrium nuclear spin magnetization precessing about the magnetic field(conventionally along z).

13. What is a NMR spectrum?

The NMR spectrum is a plot of the intensity of NMR signals Vs Magnetic Field (Frequency) in reference to TMS.

14. List the components of NMR instrument.

Sample holder, Permanent magnet, magnetic coils, sweep generator, radio frequency transmitter and radio frequency receiver and read out systems.

15. Name some solvents used in NMR spectroscopy.

The following solvents are normally used in NMR in which hydrogen is replaced with deuterium.

- CCl_4- carbon tetrachloride,
- CS_2- carbon disulfide, D_2O- deuterium oxide,
- $CDCl_3$ –Deuteriochlorofor& C_6D_6 - HexaDeutriobenzene.

16. Define Chemical shift.

A chemical shift is defined as the difference in parts per million (ppm) between the resonance frequency of the observed proton and tetramethylsilane (TMS) hydrogens.

17. Name the reference compound mostly used in TMS.

TMS (tetramethylsilane) is the most reference compound in NMR, it is set at

18. List the factors affecting chemical shift.

Electronegative groups –magnetic anisotropy–hydrogenbondingof. π electrons

19. What is n+1 rule?

The multiplicity of signal is calculated by using n+1 rule. This is one of the rule to predict the splitting of proton signals. This is considered by the nearby hydrogen nuclei. Therefore, n = number of protons in the nearby nuclei.

20. Define spin-spin coupling (splitting).

The interaction between the spins of neighboring nuclei in a molecule may cause the splitting of NMr spectrum. This is known as spin-spin coupling or splitting. The splitting pattern is relted to the number of equivalent H-atom at the nearby nuclei.

21. List the rules for spin-spin coupling.

- Chemically equivalent protons do not show spin-spin coupling.
- Only non equivalent protons couple.
- Protons on adjacent carbons normally will couple.
- Protons separated by four or more bonds will not couple.

22. Define coupling constant.

The distance between the peaks in a given multiplet is a measure of the splitting effect known as the coupling constant. It is denoted by the symbol J, Expressed in Hz.Coupling constants are the measure of the effectiveness of spin-spin coupling and very useful in 111 NMR of complex structures.

23. Define NOE.

NOE: Nuclear Over hauser Effect, caused by dipolar coupling between nuclei. The local field at one nucleus is affected by the presence of another nucleus. The result is a mutual modulation of resonance frequencies. The intensity of the interaction is a function of the distance between the nuclei according to the following equation

$$I = A\,(1/r^6)$$

I - intensity
A - scaling constant
r - internuclear distance

24. Give the general applications of NMR spectroscopy.

- NMR is used in biology to study the biofluids, cells, per fused organs and biomacromolecules such as Nucleic acids (DNA, RNA), carbohydrates, proteins and peptides. And also labeling studies in biochemistry.

16 MARK

1) With a neat diagram explain any two types of industrial analyzers.

2) Draw and explain the schematic representation of the method of measurement of dust concentration in

stack.

3) Draw and explain the schematic diagram of a typical NO-NO2 analyzer.

4) Explain with neat diagram the method for measuring CO levels.

5) Explain anyone type of oxygen analyzer used in industrial application.

6) Explain any two types of hydrogen sulphide analyzer.

7) With a neat sketch explain IR analyzers and its types.

8) Write short notes about effects of air pollutants.

9) Explain the measurement of sulphur dioxide and carbon monoxide.

10) Explain the measurement of hydrocarbons and nitrogen oxide.

UNIT IV

TWO MARKS

1. Define chromatography. (NOV/DEC-2010)

Chromatography is a physical or chemical methods of separation in which two mutually immiscible phases are brought into contact; one is stationary and other is mobile. A sample introduced into a mobile phase undergoes repeated interactions through the column. At the end of the process, separated components emerge in the order of increasing with the stationary phase.

2. What are the different chromatography techniques? (DEC- 2009)

Gas chromatography

1.Gas-chromatography 2.Gas-solid

Liquid chromatography

1. Liguid-liquid 2.Liquid-solid

3. What is the use of chromatography ? (DEC-2009)

The position of the peak in the time axis are used to identify the components of the sample, the areas under the peaks provide a quantitative measure of the amount of each component.

4. Define selectivity. (NOV-2010)

Selectivity is a measure of the preference of a stationary phase for one solute over another and is expressed as =K1 / K2

where K1 and K2 are distribution co efficient for two different solutes.

5. Define distribution constant. (NOV-2010)

Distribution constant or partition ratio can be defined as

K=CS/CM

where CS is the molar concentration of the solute in the stationary phase and CM is its molar concentration in the mobile phase.

6. Define retention time. (NOV/DEC 2010)

The time it takes after sample injection for theanalyse peak to reach the detector is called the retention time.

7. What is the advantage of HPLC? (DEC-2009)

Its sensitivity ready adaptability to accurate quantitative determination, its suitability to non volatile species or thermally fragile ones and applicability to substances like amino acids, drugs, pesticides, antibiotics, steroids, metal species etc....

8. List out some of the ideal characteristics of a detector. (NOV-2010)

a. Adequate sensitivity

b. Good stability and reproducibility

c. Linear response of the solutes over several orders of magnitude

d. Temperature from room temperature to 4000C

e. Short response time independent of flow rate

f. High reliability and ease of use.

9. Define dead time. (DEC-2009)

The dead time(tm(or)t0) is the time required for a molecule of a mobile phase to pass through column.

10. What are the requirements for pumping system in HPLC? (APRIL/MAY -2011)

- The generation of power up to 600 psi.
- Moderate flow rate of ml/min.
- pulse free output.
- corrosion resistant components.
- pump should have small hold up volume.

11. Name any four detectors used in liquid chromatography (APRIL/MAY- 2011)

- Ultraviolet- visible spectra photo metric detector.
- Fluorescence detector.
- refractive index detectors.
- electrochemical detectors.

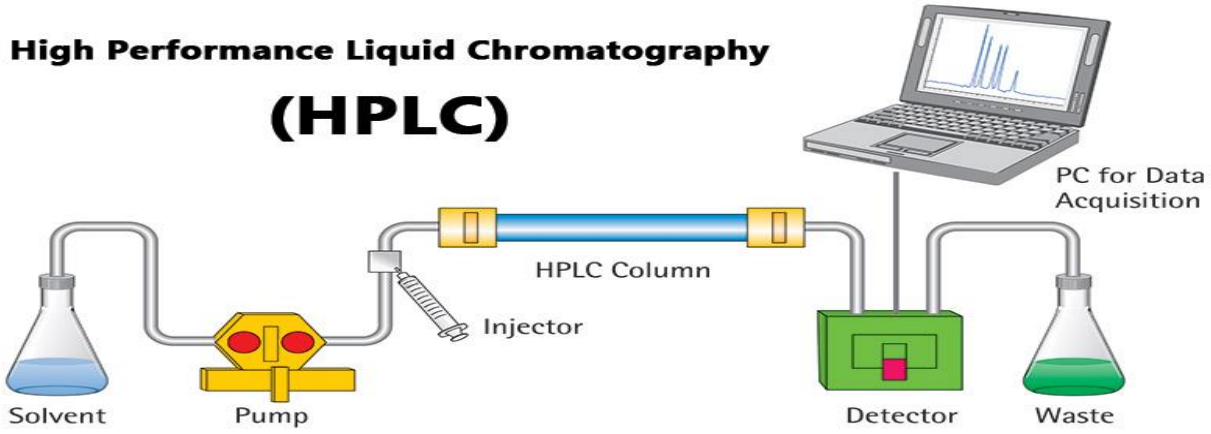
12. What are the pumps used in HPLC? (APRIL/MAY-2011)

- Reciprocating piston pumps
- Syringe type pumps
- Constant pressure pump

16 MARKS

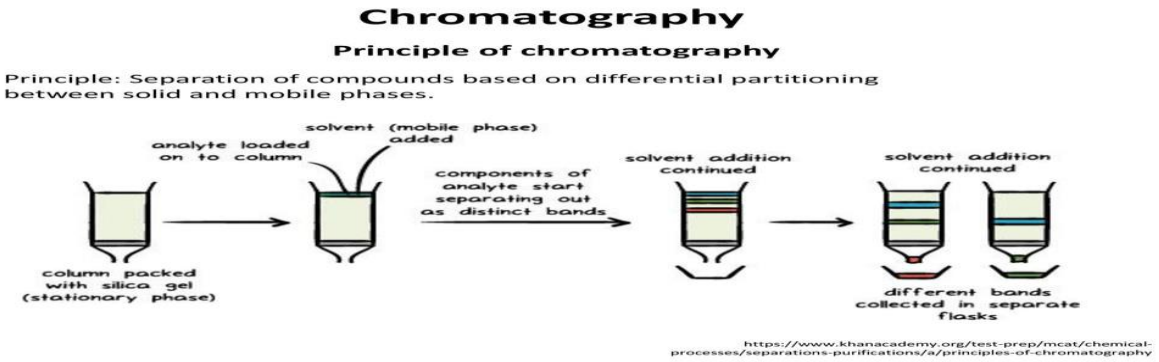
1. Illustrate with a block diagram, the operation of a HPLC? (NOV/DEC-2010) (APRIL/MAY – 2011)

- Normal phase chromatography is also used in the liquid-liquid chromatography method called high pressure liquid chromatography, or HPLC, which also has a polar and non-polar stationary and mobile phase.
- HPLC uses pumps to pass pressurized liquid solvents and samples through columns filled with solid adsorbent materials.
- HPLC is a popular method for urine testing for performance enhancing drugs, purity testing in pharmaceuticals and cleanliness testing in manufacturing.



2. Explain the operation of any two detector used in gas chromatography. (DEC-2009)

Chromatography is the technique for the **separation, purification, and testing of compounds.'Chromatography' is an analytical technique commonly used for separating a mixture of chemical substances into its individual components, so that the individual components can be thoroughly analyzed.**

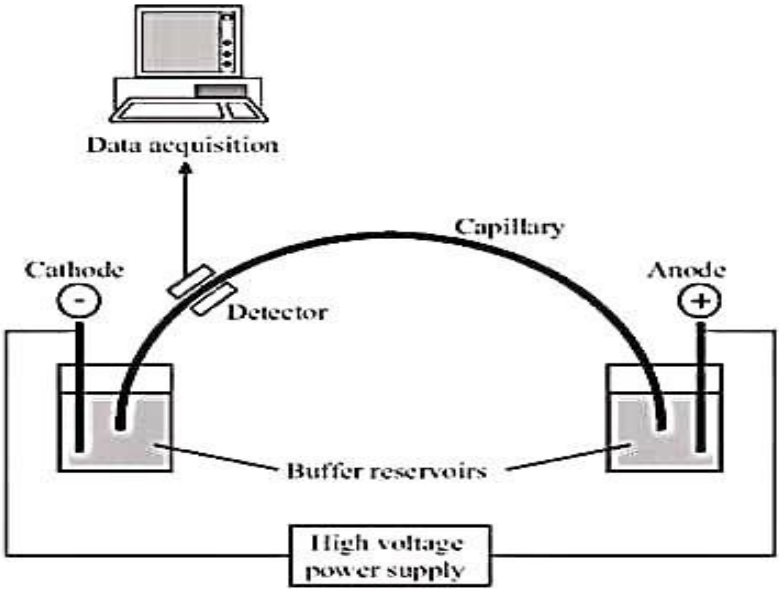


3. With a neat diagram explain the various stages of capillary Electrophoresis and label the main instruments. (NOV/DEC -2010)

Capillary Electrophoresis

Capillary Electrophoresis (CE) is one of the possible methods to analyse complex samples. In High Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC) the separating force is the difference in affinity of the sample components to a stationary phase, and or difference in boiling point. With both techniques the most important factor is the polarity of a sample component. In CE the separating force is the difference in charge to size ratio. Not a flow through the column, but the electric field will do the separation.

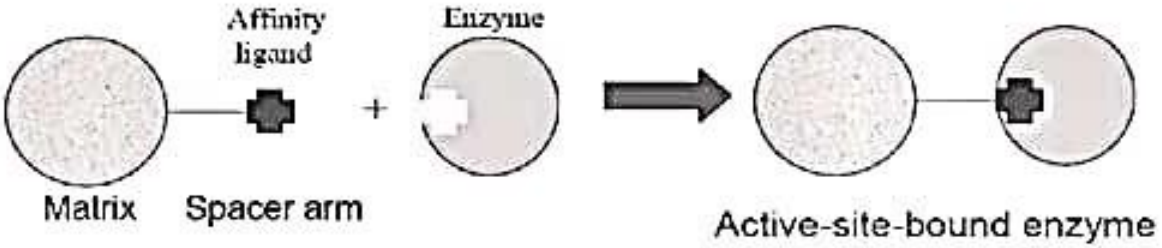
In Capillary Electrophoresis a capillary is filled with a conductive fluid at a certain pH value. This is the buffer solution in which the sample will be separated. A sample is introduced in the capillary, either by pressure injection or by electro kinetic injection. A high voltage is generated over the capillary and due to this electric field (up to more than 300 V/cm) the sample components move (migrate) through the capillary at different speeds. Positive components migrate to the negative electrode, negative components migrate to the positive electrode. When you look at the capillary at a certain place with a detector you will first see the fast components pass, and later on the slower components.



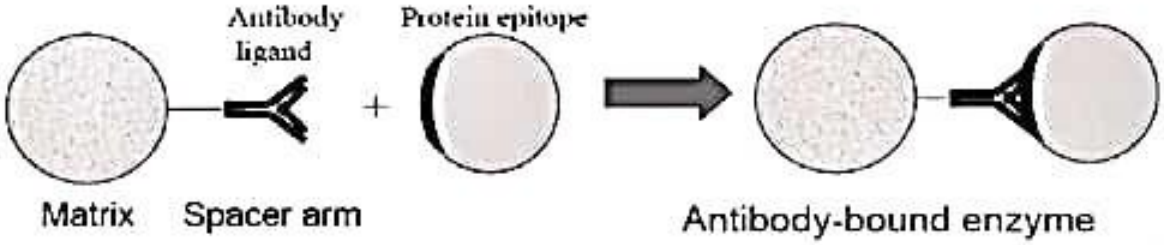
5. With a neat diagram, discuss the types of detectors used in Affinity chromatography.

Affinity chromatography

- Substrate analogue affinity chromatography



- Immunoaffinity chromatography



Chromatography is a method for separating the components of a mixture by differential adsorption between a stationary phase and a mobile (moving) phase

based material) and a mobile gas (most often Helium).The stationary phase is adhered to the inside of a small-diameter glass tube (a capillary column) or a solid matrix inside a larger metal tube (a packed column). It is widely used in analytical chemistry; though the high temperatures used in GC make it unsuitable for high molecular weight biopolymers or proteins (heat will denature them), frequently encountered in biochemistry, it is well suited for use in the petrochemical, environmental monitoring, and industrial chemical fields. It is also used extensively in chemistry research.

LIQUID CHROMATOGRAPHY

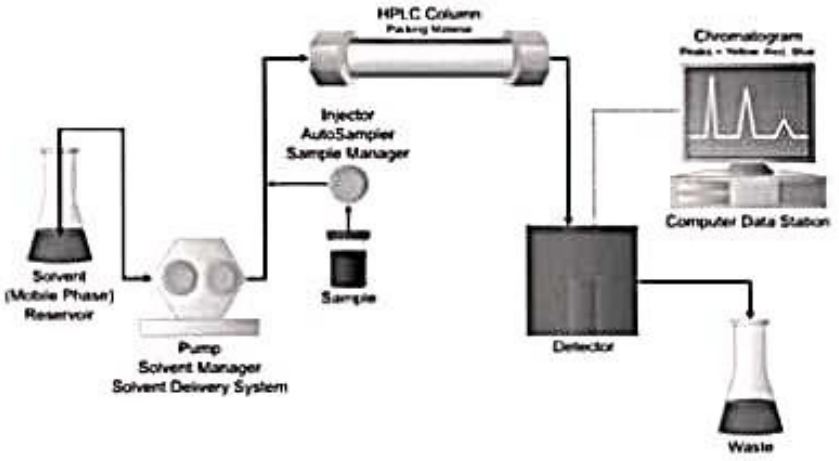
Liquid chromatography (LC) is a separation technique in which the mobile phase is a liquid. Liquid chromatography can be carried out either in a column or a plane. Present day liquid chromatography that generally utilizes very small packing particles and a relatively high pressure is referred as high performance liquid chromatography (HPLC). In the HPLC technique, the sample is forced through a column that is packed with irregularly or spherically shaped particles or a porous monolithic layer (stationary phase) by a liquid (mobile phase) at high pressure. HPLC is historically divided into two different sub-classes based on the polarity of the mobile and stationary phases. Technique in which the stationary phase is more polar than the mobile phase (e.g. toluene as the mobile phase, silica as the stationary phase) is called normal phase liquid chromatography (NPLC) and the opposite (e.g. water-methanol mixture as the mobile phase and C18 = octadecylsilyl as the stationary phase) is called reversed phase liquid chromatography (RPLC). Ironically the "normal phase" has fewer applications and RPLC is therefore used considerably more.

Specific techniques which come under this broad heading are listed below. It should also be noted that the following techniques can also be considered fast protein liquid chromatography if no pressure is used to drive the mobile phase through the stationary phase.

AFFINITY CHROMATOGRAPHY

Affinity chromatography is based on selective non-covalent interaction between an analyte and specific molecules. It is very specific, but not very robust. It is often used in biochemistry in the purification of proteins bound to tags. These fusion proteins are labeled with compounds such as His-tags, biotin or antigens, which bind to the stationary phase specifically. After purification, some of these tags are usually removed and the pure protein is obtained. Affinity chromatography often utilizes a biomolecule's affinity for a metal (Zn, Cu, Fe, etc.). Columns are often manually prepared. Traditional affinity columns are used as a preparative step to flush out unwanted biomolecules. However, HPLC techniques exist that do utilize affinity chromatography properties. Immobilized Metal Affinity Chromatography (IMAC) is useful to separate aforementioned molecules based on the relative affinity for the metal (i.e. Dionex IMAC). Often these columns can be loaded with different metals to create a column with a targeted affinity.

HIGH PERFORMANCE LC



UNIT V ELECTRO ANALYSIS AND SURFACE MICROSCOPY

TWO MARKS

1. What is an electrochemical cell?
A DC electrochemical cell converts chemical energy into electrical energy.

2. State Fataday's law
Faraday's law, states that the amount of chemical reaction that occurs at an electrode is proportional to the current, called a *faradaic current.*

3. Mention the three mechanisms that bring mass transfer in an electrochemical cell.
Three mechanisms bring about this mass transfer:
- ✓ *convection,*
- ✓ *migration,* and
- ✓ *diffusion.*

4. What is the potential of an electrochemical cell?
The potential of an electrochemical cell is the difference between the potential of one of the electrodes and the potential of the other.This potential is a measure of an electrode's electron energy.

5. What is Standard Hydrogen Electrode ?
It is a reference electrode against which the electrode potentials of all electrodes are measured. The Standard Hydrogen Electrode is often abbreviated to SHE, and its standard electrode potential is declared to be 0 at a temperature of 298K. This is because it acts as a reference for comparison with any other electrode.

6. Define : Electrode Potential
An *electrode potential is defined as the potential of a cell in which* the electrode under investigation is the right-hand electrode and the SHE is the left-hand electrode . Electrode potentials could more properly be called *relative electrode potentials* .

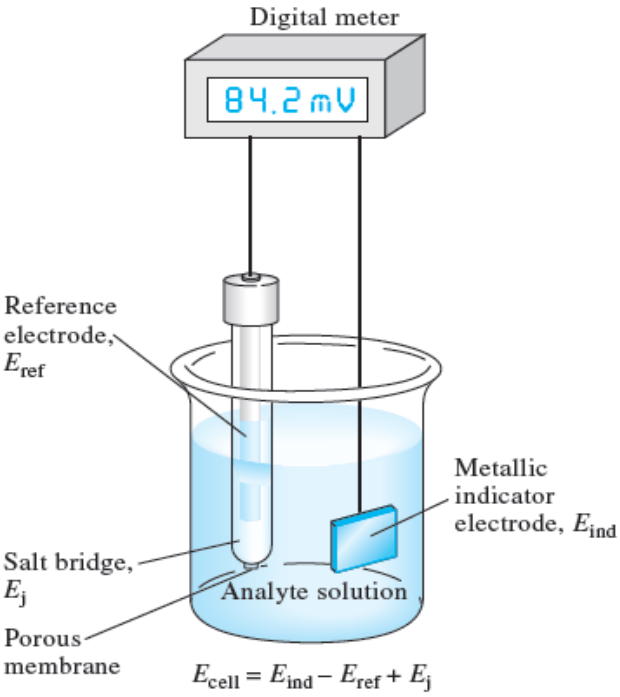
7. What is Potentiometry?
Potentiometry is one of the methods of electro analytical chemistry. It is usually employed to find the concentration of a solute in solution. In potentiometric measurements, the potential between two electrodes is measured using a high impedance voltmeter.

8. What is calomel reference electrode?
Calomel reference electrodes consist of mercury in contact with a solution that is saturated with mercury(I) chloride (calomel) and that also contains a known concentration of potassium chloride. Calomel half-cells can be represented as follows: $Hg|Hg_2Cl_2(sat'd),KCl(xM)\|$
where *x represents the molar concentration of potassium chloride* in the solution.

9. Mention the components of potentiometric methods.

- ✓ *indicator electrode,*
- ✓ *a reference electrode, and*
- ✓ *a potential measuring device.*

10. Draw the potentiometric cell.



11. What are the advantages of ISFETs over membrane electrodes?
Ruggedness, small size, inertness toward harsh environments, rapid response, and low electrical impedance. In contrast to membrane electrodes, ISFETs do not require hydration before use and can be stored indefinitely in the dry state.

12. What are the two types of Membrane materials used in Gas Permeable Membranes?

a. Microporous and

b. homogeneous.

13. Where did biosensors find applications?

Determination of biological and biochemical compounds such as enzymes, DNA, antigens, antibodies, bacteria, cells, and whole samples of animal and plant tissue.

14. What is Voltammetry?

Voltammetry is an electrochemical technique in which a varying potential is applied to a working electrode in an electrochemical system, and the corresponding current is measured.

15. What is Amperometry?

In amperometry, current proportional to analyte concentration is monitored at a fixed potential.

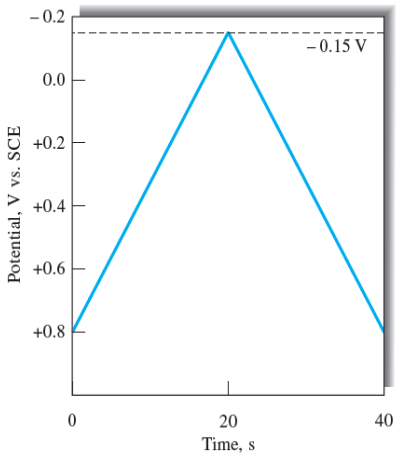
16. What is the purpose of Voltammetry?

Voltammetry is widely used by inorganic, physical, and biological chemists for nonanalytical purposes, including

a. Fundamental studies of oxidation and reduction processes in various media,
b. adsorption processes on surfaces, and
c. electron-transfer mechanisms at chemically modified electrode surfaces.

17. What is cyclic voltammetry?

In *cyclic voltammetry (CV), the current response of a small stationary* electrode in an unstirred solution is excited by a triangular voltage waveform, such as that shown in Figure below:



18. Write the Randles- Sevcik equation?

$$i_p = 2.686 \times 10^5 n^{3/2} A c D^{1/2} v^{1/2}$$

where i_p *is the peak current (A),*

A is the electrode area (cm^2),

D is the diffusion coefficient (cm^2/s),
c is the concentration (mol/cm^3), and
v is the scan rate (V/s).

19. What are the two important pulse techniques in pulse voltammetry?
The two most important pulse techniques are
a. *Differential- pulse voltammetry and*
b. *square-wave voltammetry.*

20. What is a surface?
A *surface is defined as the boundary layer between a solid,* or sometimes a liquid, and a vacuum, a gas, or a liquid.

16 MARK

1) With a neat diagram explain types of Electrochemical cells.

An electric conductor, the electrode metal and an ionic conductor, electrolyte solution, form an interface at which electrode process occurs. An electrochemical cell contains two electrodes (anode and cathode); a liquid-liquid junction separates two electrodes. Anode is the electrode where oxidation occurs. Cathode is the electrode where reduction occurs.

Zn (s) + Br^2 (aq) → Zn^2 + (aq) + 2Br − (aq)

- Reduction Half-reaction: Br^2 (aq) + 2e− → 2Br − (aq)
- Oxidation Half-reaction: Zn (s) → Zn^2 + (aq) + 2e−

Metal-Metal ion

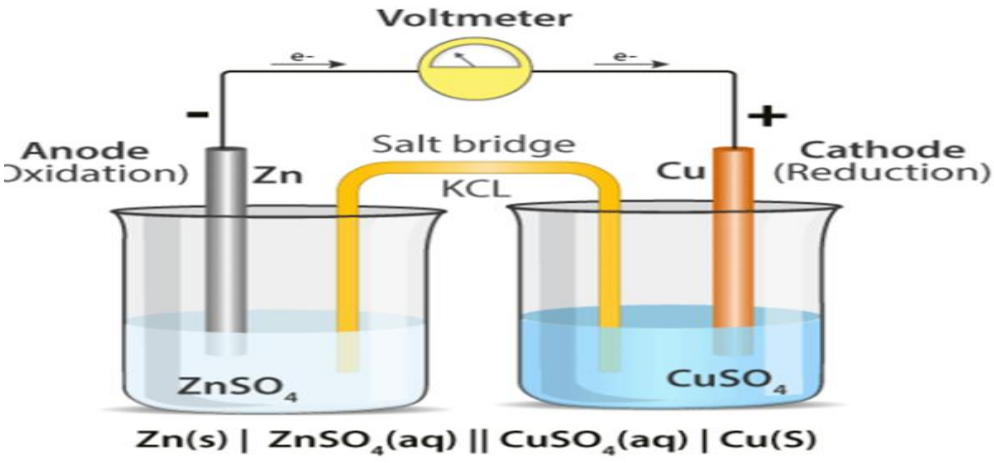
Ion-Ion (redox)

Gas

Pt/Fe", Fe"

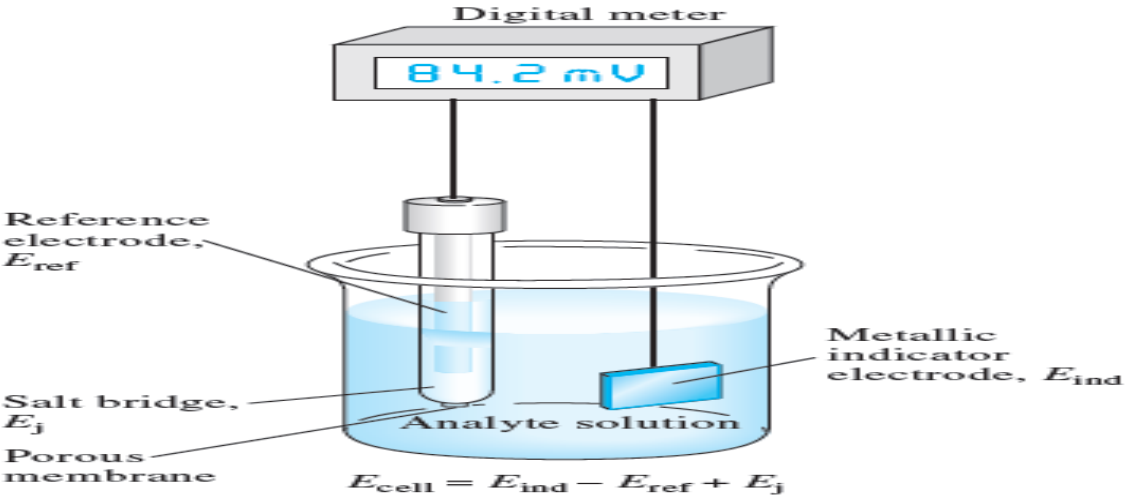
P/H_1 H

Metal-imsoluble salt Hg/Hg.CL/KC



2) Draw and explain the schematic representation of the method of Potentiometry.

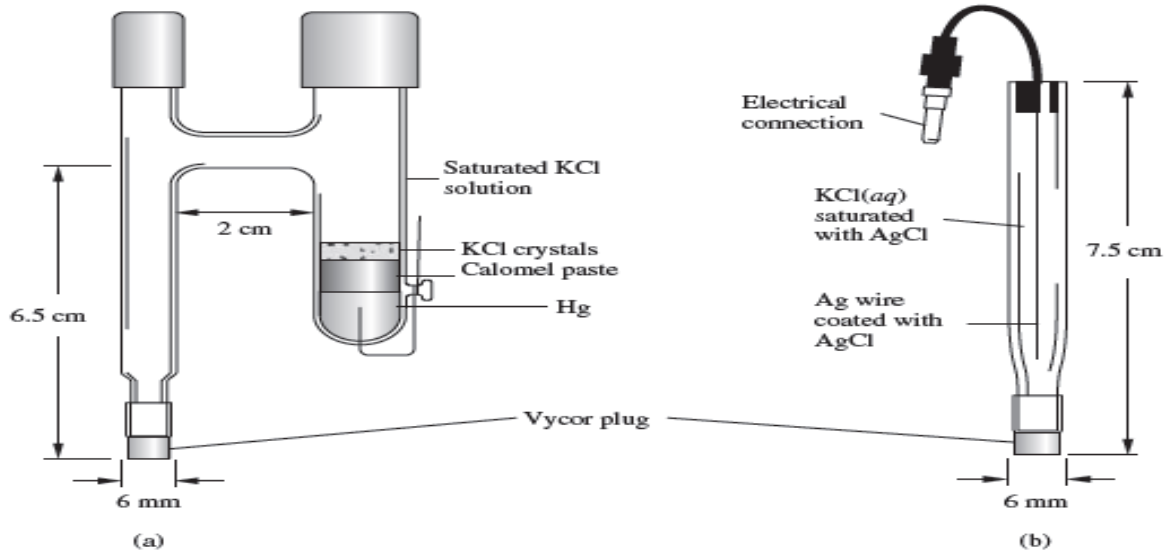
- Potentiometric methods of analysis are based on measuring the potential of electrochemical cells without drawing appreciable current.
- More recently, measurements of the potential of ion-selective electrodes have been used to determine concentrations of a large number of ions.
- Such electrodes are relatively free from interference and provide a rapid and convenient means for quantitative estimations of numerous important anions and cations.
- The equipment required for potentiometric methods is simple and inexpensive and includes an indicator electrode, a reference electrode, and a potential measuring device.



3) Draw and explain the schematic diagram of a Saturated Calomel Electrode.

- The electrode potential for this half-cell is determined by the reaction and depends on the chloride concentration x. Thus, the KCl concentration must be specified in describing the electrode.
- The saturated calomel electrode (SCE) is widely used because of the ease with which it can be prepared.
- Compared with the other calomel electrodes, however, its temperature coefficient is significantly larger.

$$Hg|Hg_2Cl_2(sat'd),KCl(xM)\|$$



4) Write about the applications of Electrochemical cells.

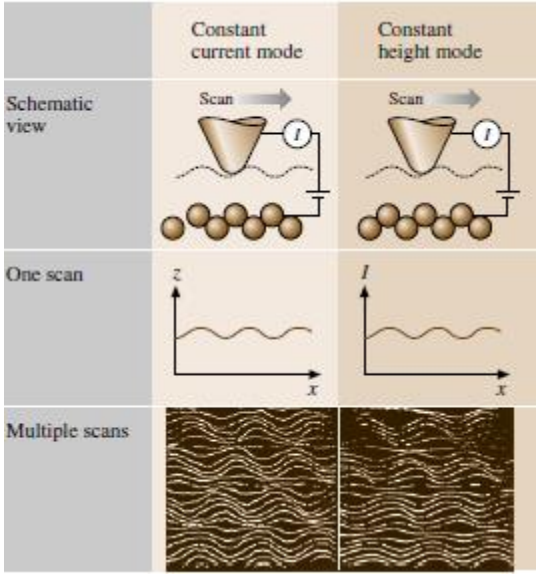
APPLICATIONS OF ELECTROCHEMICAL CELLS

- Electrolytic cells are used in the electro-refining of many non-ferrous metals. They are also used in the electro-winning of these metals.
- The production of high-purity lead, zinc, aluminium, and copper involves the use of electrolytic cells.

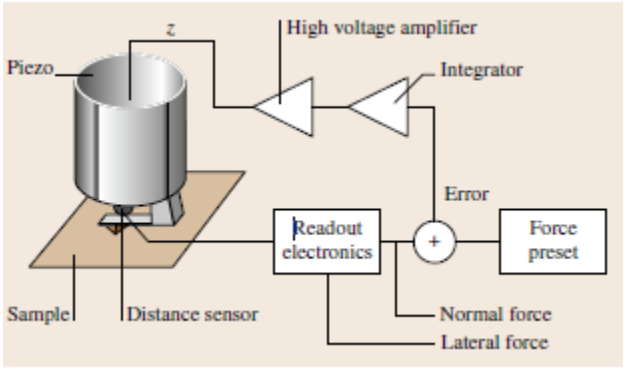
- Metallic sodium can be extracted from molten sodium chloride by placing it in an electrolytic cell and passing an electric current through it.
- Many commercially important batteries (such as the lead-acid battery) are made up of Galvanic cells.
- Fuel cells are an important class of electrochemical cells that serve as a source of clean energy in several remote locations.

5) Explain about STM and AFM.

The principle of the STM is straightforward.A sharp metal tip (one electrode of the tunnel junction) is brought close enough (0.3–1 nm) to the surface to be investigated (the second electrode) to make the tunneling current measurable at a convenient operating voltage (10mV–1 V). The tunneling current in this case varies from 0.2 to 10 nA. The tip is scanned over the surface at a distance of 0.3–1 nm, while the tunnelling current between it and the surface is measured. The STM can be operated in either the constant current mode or the constant height mode (Fig. 21.1). The left-hand column of Fig. 21.1 shows the basic constant current mode of operation. A feedback network changes the height of the tip *z* to keep the current constant. The displacement of the tip, given by the voltage applied to the piezoelectric drive, then yields a topographic map of the surface.
The visualization and interpretation of images from AFMs is intimately connected to the processing of these images. An ideal AFM is a noise-free device that images a sample with perfect tips of known shape and has perfect linear scanning piezos. In reality, AFMs are not that ideal. The scanning device in an AFM is affected by distortions. The distortions are both linear and nonlinear. Linear distortions mainly result from imperfections in themachining of the piezotranslators causing crosstalk from the *z*-piezo to the *x*- and *y*-piezos, and vice versa.



STM



AFM