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INSTRUMENTAL CHEMICAL ANALYSIS:

BASIC PRINCIPLES AND TECHNIQUES

PREFACE

This manual has been prepared for the final year undergraduate students to educate the basics in the instrumental chemical analysis and techniques. It explains the classification of instruments based on the interactions of the molecules with the matter and energy, the principles involved in the individual technique and their applications in various fields. As this work is intended for quick and easy learning all the descriptions are kept at concise and simple, though appropriate references are given for advanced and detailed descriptions.

We hope that this could be a good initiative and guidance for the students who as a part of their study programme should pursue with short research works. We strongly advice the students to go through this manual completely before handling the instruments as this may give some confidence and familiarisation over the techniques.

The details explained in this manual have been collected from various sources cited at the reference section and any suggestions or modifications for this manual from the staff members and students are welcome.

Good Luck!
DR D RAJARATHNAM

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1. INTRODUCTION

The need of the sophisticated analytical instruments and determinations using them is almost a routine process for the modern chemical laboratories. It has been a vast expanding area of knowledge as the instrument and computer manufacturers are producing analytical machines, which are in ever-increase of power and scope. Further, all the manual techniques in the line of the analytical studies had steadily been transferred to the instrumental techniques. Basically, chemical analysis can be divided into three broad categories as given below, which are almost invariably applied to major areas such as Fundamental Research, Product Development, Product Quality Control, Monitoring & Control of Pollutants, Medical & Clinical Studies, etc:

QUALITATIVE ANALYSIS:
Chemical analysis which just identifies one or more species present in a sample

QUANTITATIVE ANALYSIS:
Chemical analysis which finds out the total amount of the particular species present in a sample

STRUCTURAL ANALYSIS:
Chemical analysis which helps in finding the spatial arrangement of atoms in a molecule and the presence or position of certain organic functional groups in a given compound

In addition, *'Surface Analysis'*, plays an important role in material studies to obtain surface related physical properties such as topography, depth profiling, orientation of molecules, etc.

Chemical analysis has some basic steps like, choice of method, sampling, preliminary sample treatment, separations, final measurement and assessment of results. It is with the first step viz. choice of method, care should be exercised to select the proper instrument to carry out fruitful analysis. A wrong selection at this point will lead to a meaningless analysis. Selection of the instrument is such important criteria!

In order to compete with this type of situation some basic knowledge of instruments and analytical techniques are required. This may give the person the ability, with some confident, to choose and operate a varied range of instruments which would be much required at the advanced research laboratories. The following pages will give an insight into the theory, principles and applications of various analytical instruments.

2. CLASSIFICATION OF THE ANALYTICAL TECHNIQUES

In a broad sense the techniques for the chemical analysis can be classified as follows. Though this classification doesn't include few other techniques like radiochemical analyses, bioanalytical methods and some of the physical methods, it is more than sufficient to start with, since it covers almost all our Departmental analytical equipment under common pool:

ANALYSIS THROUGH SPECTROSCOPY

ANALYSIS THROUGH CHROMATOGRAPHY

ANALYSIS THROUGH THERMAL ENERGY

ANALYSIS THROUGH X-RAY TECHNIQUES

ANALYSIS THROUGH MICROSCOPY

ANALYSIS THROUGH ELECTROCHEMICAL TECHNIQUES

ANALYSIS THROUGH MISCELLANEOUS TECHNIQUES

This classification is based on the interactions of molecules with various forms of energy like electro-magnetic radiation, heat (thermal energy) and with matters like electrons. Each technique has specific principle, mode of operation, advantages and disadvantages.

3. ANALYSIS THROUGH SPECTROSCOPY

3.1 Processes in Spectroscopy

The interaction of the light (electro-magnetic radiation) with a substance and the subsequent energy transfer ends with three main processes namely:

Absorption:

The process by which the energy of the light (in the form of photons) is transferred to the atom or molecule raising them from the ground state to an excited state

Fluorescence:

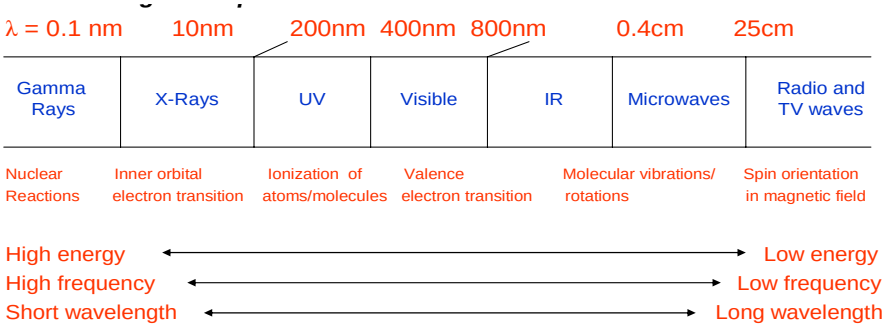
The absorbed energy is rapidly lost to the surroundings by collisions within the system and relax back to the ground state. Sometimes the energy is not lost in this way but is re-emitted a few milli seconds later, which is referred as fluorescence

Emission:

If the substances (atoms or molecules) are heated to high temperatures (in a flame or in an electric discharge) the electrons are exited to higher energy levels. Later, they relax to the ground state with the emission of radiation, the magnitude of which is more or less equivalent to absorbed energy

Most of the analytical techniques are based on the light interactions with the substances and utilise any of the above three associated processes. Substances interact with light differently at various wavelengths and hence different types of analysis & instruments. The entire spectrum of light can be represented as below. Since, light has both electrical and magnetic components, this representation is referred as an **'Electro-Magnetic Spectrum'**:

Fig: 1



The following is short comparison between Ultra Violet (UV), Visible (Vis) and Infra Red (IR) ranges for the energy, frequency and wavelength:

Energy: UV > Vis > IR
Frequency: UV > Vis > IR
Wavelength: UV < Vis < IR

The symbol for the wavelength is "lambda" (λ) and the unit is either nanometer (nm) or micrometer (or micron, μm). The symbol for frequency is "nu" (μ) and the unit is either hertz or sec^{-1}. A parameter closely related to frequency is the wave number, which has the symbol "nu bar" (υ) and the unit is cm^{-1}.

There are two levels by which the substances can interact with the light as, atomic level and molecular level and hence the corresponding techniques:

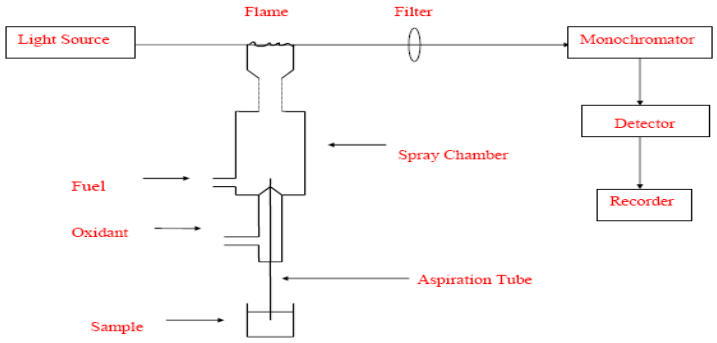
3.1.1 Atomic Level

3.1.1a Atomic Absorption Spectroscopy (AAS):

Principle: The sample is vaporized by aspiration of solution into a flame or evaporation from electrically heated surface (temperature range: 1800 – 3100^0 K). At this condition where the individual atoms co-exist, a beam of light is passed through them. The atoms will absorb in the visible and ultraviolet region resulting in changes in electronic structure (excited state). So, the resultant light beam coming out of the sample will be missing the light in the corresponding wave length, which is a measure of the characteristics of the sample.

Instrumentation: Sources emitting radiation characteristic of element of interest (hollow - cathode lamp), flame or electrically heated furnace, monochromator, detector (photomultiplier) and recorder. The following is the simplified outline of the instrumentation:

Fig: 2



Applications: This is the most widely used technique for the quantitative determination of metals at trace levels (0.1 to 100ppm), which present in various materials. It utilizes Beer - Lambert Law for the analysis and a standard curve is obtained by plotting absorbance *vs* concentration of the samples taken. The usual procedure is to prepare a series of standard solutions over a concentration range suitable for the sample to be analysed. Then, the standards and the samples are separately aspirated into the flame, and the absorbances are read from the instrument. The plot will give the useful linear range and the concentrations of the samples can be found out from the plot.

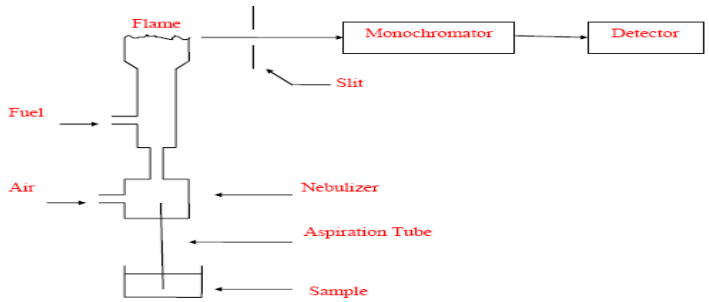
Disadvantages: Sample must be in solution or at least volatile. Individual source lamp and filters needed for each element, since, each metal has its own characteristic absorption.

3.1.1b Atomic Emission Spectroscopy (AES):

Principle: This is simply called as 'Flame Photometry', and measures the atoms excited by a ***flame*** (temperature range: 2000 – 3100^0 K) and not by light source as in the atomic absorption case. After excitation, atoms will readily lose the gained energy and revert back to the ground state and the emission occurs. It is that emission that actually being measured. The wavelengths of the emitted light will almost be similar as those that were absorbed in the atomic absorption, since exactly the same energy transitions occur, except in the order of reverse!

Instrumentation: A simple flame photometer consists of burner, nebulizer, monochromator, detector and recorder. The following is the simplified figure:

Fig: 3



Applications: It is used exclusively in the quantitative determination of metals in solution, especially alkali and alkaline earth in the given samples. The principle is like that described for atomic absorption. Qualitative determination is also possible as each element emits its own characteristic line spectrum.

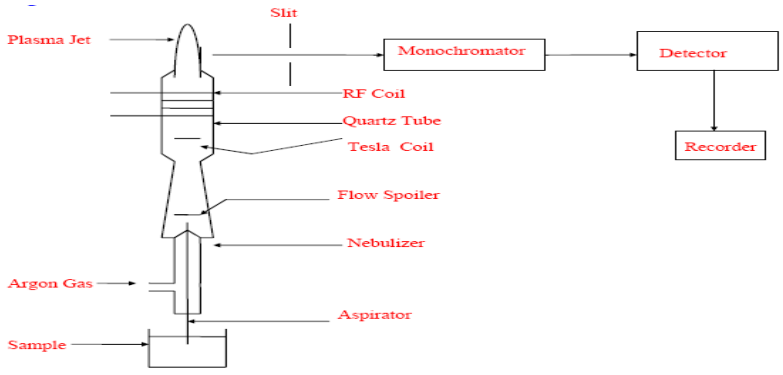
Disadvantages: Intensity of emission is very sensitive to changes in flame temperature. Usually, spectral interference and self-absorption are also encountered which affects the precision of the measurement. Further, a linear plot of absorbance against concentration is not always obtained.

3.1.1c Plasma Emission Spectroscopy:

Principle: Mostly referred as Inductively Coupled Plasma (ICP) Emission Spectroscopy, is also an atomic emission technique, most closely related to the preceded flame photometry except that the atoms and ions present in the sample are excited in high temperature gas plasma. Since the plasma provides very high temperature and hence the energy, almost all the atoms present in the sample can be excited with this technique ending up with high efficiency (a hotter source increases both atomization efficiency and excitation efficiency). Thus, the emissions from the atoms would be more intense and even very small concentrations of metals/metal ions can be detected and accurately measured.

Instrumentation: This is basically an emission spectrometer comprising nebulizer, RF coil, ICP Source (Argon plasma), monochromator, detector and recorder.

Fig: 4



A plasma source or jet is a flame-like system of ionized, very hot flowing argon gas. At high temperatures (≈ 6000 K) a gas such as argon will contain a high proportion of ions and free electrons constituting plasma (This ionisation is initiated by "Tesla" coil). Additional energy may be supplied to the electrons in the plasma by the application of an external electromagnetic field through RF coil. By collisions between the electrons and other species in the plasma this additional energy is uniformly distributed. As the collisions increase, the energy transfer becomes more efficient, which leads to a substantial temperature enhancement to a range of 8000 - 10000 K. It is the temperature at which the samples are introduced and analysed.

Applications: Similar to atomic emission spectroscopy but it covers very widespread for both qualitative and quantitative analysis of metals and some non-metals too, at trace levels. Because of the high temperature and homogeneity of the source, it offers better signal stability and hence the analytical precision. The technique when utilising an optical emission detector is termed as Inductively Coupled Plasma – Optical Emission Spectrometer (***ICP-OES***) and if it utilises a mass spectrometer (***refer section 9.6***) as detector then it is termed as Inductively Coupled Plasma – Mass Spectrometer (***ICP-MS***).

Disadvantages: Samples require dissolution before analysis. Instrumentation is complex and requires high operator's skill and is very expensive.

3.1.1d Fluorometry: Atomic Fluorescence

This technique is not widely used though its counterpart - the molecular fluorescence is applied well to the analytical studies. The principle of atomic fluorescence is that when atoms are elevated to higher energy levels, they sometimes return to the ground state through a pathway, which has several intermediate electronic states, before reaching to the actual ground state. Such series of fall through the electronic levels accompany by light emission - which is atomic fluorescence. The intensity of this emitted light is measured at right angles to the incident light and related to concentration. Uses are similar to AAS and AES.

3.1.2 Molecular Level

3.1.2a Ultraviolet - Visible Spectroscopy (**UV/Vis**):

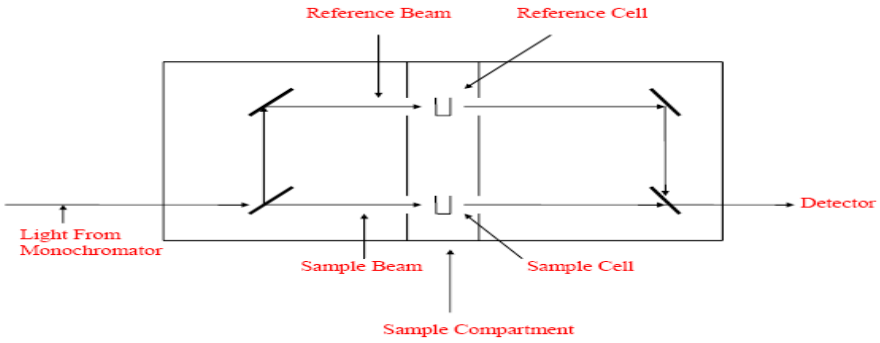
Principle: It involves the absorption of electromagnetic radiation by the substances in the ultraviolet and visible regions of the spectrum. This will result in changes in the electronic structure of ions and molecules through the excitations of bonded and non-bonded electrons.

Instrumentation: It consists of a dual light source viz., tungsten lamp for visible range and deuterium lamp for ultraviolet region, grating monochromator, photo-detector, mirrors and glass or quartz cells.

NOTE: For measurements to be made under visible region both glass and quartz cells can be used. For the measurements under ultraviolet region, only quartz cell should be used, since, glass cells absorb ultraviolet rays.

There are two types of instrumental designs for this technique as single beam and double beam spectrophotometers. However double beam spectrophotometers are widely used and following is the outline of the instrument:

Fig: 5



Applications: It is the most widely used technique for quantitative molecular analysis, for this Beer-Lambert law is applied. Sometimes it is used in conjunction with other techniques such as NMR, IR, etc., in the identification and structural analysis of organic compounds. For qualitative analysis it provides valuable information through the absorption spectrum which is unique for a given compound.

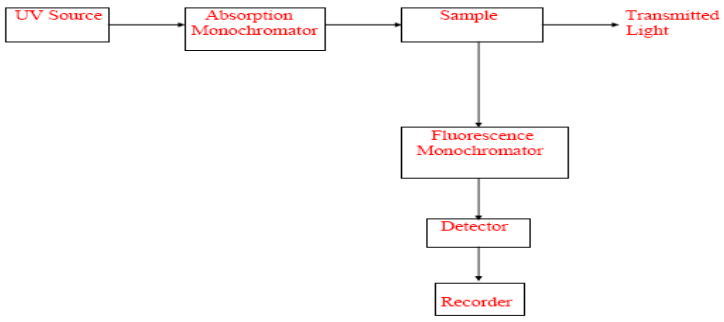
Disadvantages: Samples should be in solution. Mixture of substances poses difficult to analyse and requires prior separation. Interference from the sample's matrix makes the measurement difficult.

3.1.2b Fluorometry: Molecular Fluorescence

Principle: This technique utilises the phenomenon of molecular fluorescence, the theory behind this is exactly the same that has been discussed under atomic fluorescence but through the excitation of bonded electrons. Here, most often the irradiating light is in the range of ultraviolet and visible.

Instrumentation: The instrumental set-up comprises of a UV/Visible source, two monochromators, detector and recorder. The fluorescence exhibited by the sample is measured at right angles to the incident beam. The following is the basic set-up:

Fig: 6



Applications: The applications of this technique are limited and it offers quantitative estimations of those compounds like benzene and fused benzene ring systems. Inorganic metals can also be analysed by the ability of them to form complexes with the ligands. It finds uses in the analysis of foods for vitamin content, since vitamins like riboflavin, niacin, etc., exhibit fluorescence. Only limited compounds show the fluorescence hence this technique is relatively free of any interference and is very sensitive.

Disadvantages: The application is very limited as relatively a few substances exhibit flourescence.

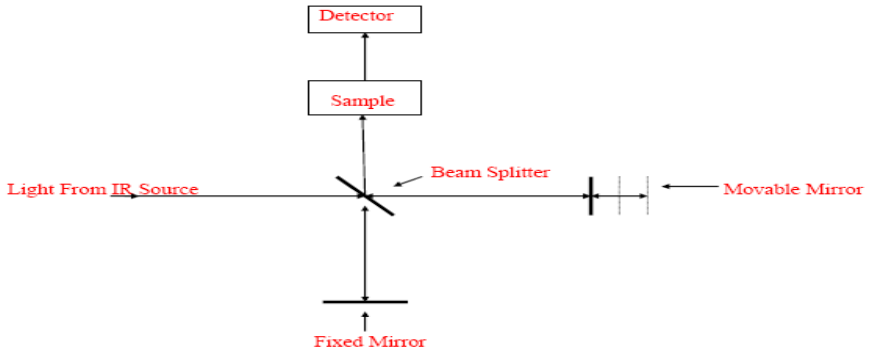
3.1.2c Fourier Transform Infrared Spectroscopy (**FT-IR**):

Principles: It involves the absorption of electromagnetic radiation in the infrared region of the spectrum which results in changes in the *vibrational energy* of molecule. Since, usually all molecules will be having vibrations in the form of stretching, bending, etc., the absorbed energy will be utilised in changing the energy levels associated with them. It is a valuable and formidable tool in identifying organic compounds which have polar chemical bonds (such as OH, NH, CH, etc.) with good charge separation (strong dipoles).

Instrumentation: It was originally designed as a double beam spectrophotometer comprising IR source (red hot ceramic material), grating monochromator, thermocouple detector, cells made of either sodium chloride or potassium bromide materials, etc. In this process the light is dispersed by the monochromator. But, this type of basic design for IR measurements has been outdated. Instead a newer technique termed ***Fourier Transform-Infrared (FT-IR)*** has been in practice. This technique utilises a single beam of un-dispersed light and has the instrument components similar to the previous one.

In FT-IR, the un-dispersed light beam is passed through the sample and the absorbances at all wavelengths are received at the detector simultaneously. A computerized mathematical manipulation (known as "Fourier Transform") is performed on this data, to obtain absorption data for each and every wavelength. To perform this type of calculations interference of light pattern is required for which the FT-IR instrumentation contains two mirrors, one fixed and one moveable with a beam splitter in between them. Before scanning the sample a reference or a blank scanning is required. The following is the simplified design of the instrument:

Fig: 7



Applications: It finds extensive use in the identification and structural analysis of organic compounds, natural products, polymers, etc. The presence of particular functional group in a given organic compound can be identified. Since every functional group has unique vibrational energy, the IR spectra can be seen as their fingerprints.

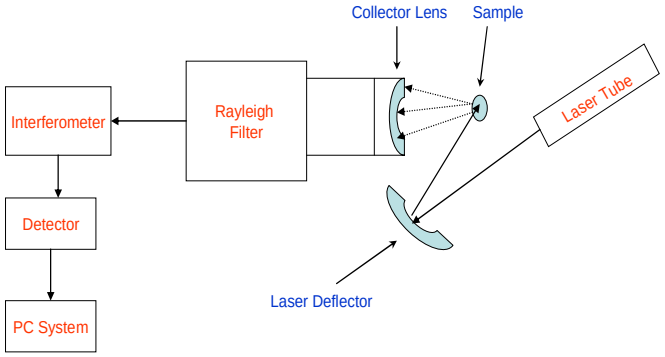
Disadvantages: Samples containing mixture of substances can not be analysed. Since the sample holders and beam splitter, are made of moisture sensitive materials like sodium chloride or potassium bromide (KBr), special cells are required for aqueous samples (e.g. KRS-5, ZnSe, etc.). Water is a bad solvent for IR spectral works.

3.1.2d Fourier Transform Raman Spectroscopy (FT-Raman):

Principle: This technique is complementary to FT-IR and is a scattering technique, whereby a laser beam (near-IR region) is directed to the sample and the scattered radiation is collected. Most of the scattered radiation has the same wavenumber as that of the incident laser beam, however a fraction will be having a different wavenumber. This is the Raman signal and characteristic of particular functional group. Raman spectroscopy finds applications in identifying organic compounds containing non-polar bonds such as carbon - carbon double bonds or aromatic rings (weak dipoles).

Instrumentation: The instrumentation comprises of exciting laser normally in near-IR region, Rayleigh filter, beam splitter, detector, etc. Data collection and processing are akin to IR including the Fourier transformations.

Fig: 8



Applications: The applications are similar to FT-IR and gives useful information on the non-polar bonds, i.e. bonds with null or reduced dipole moment. Water is a good solvent for FT-Raman.

Disadvantages: Signal strength is normally weak, and liquid samples give poor signals. Heat sensitive samples can't be analyzed, since local heating will damage the samples. Dark colored samples can't be analyzed.

3.1.2e Microwave Spectroscopy:

This technique is actually an extension to IR spectroscopy. Microwave region lies at the far infra-red region of the electromagnetic spectrum and its absorption by molecules give rise to change in the *rotational energies* of the molecules. In IR spectroscopy, the molecules are subjected to changes in vibrational energies; the energy required for making changes at rotational levels is lesser than that for vibrational levels. Though the principles are same to that of IR, the instrumentation is slightly different and it requires samples in ***gaseous state*** for the analysis. Its applications are limited to smaller and simpler molecules since larger molecules will have interactions between the rotational energy levels within the molecule through various bonds they have. Besides qualitative analysis, this technique can be applied for conformational analysis of simpler compounds (study of stereo chemistry of the compounds).

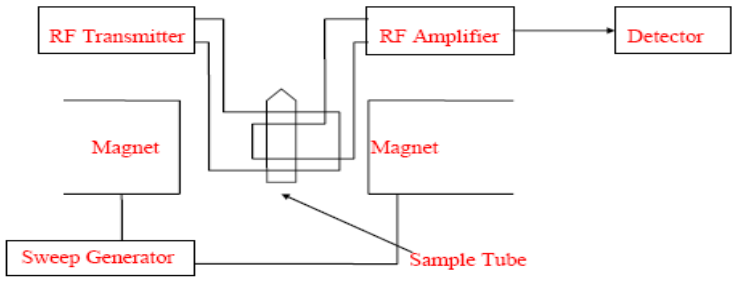
3.1.2f Nuclear Magnetic Resonance Spectroscopy (NMR):

Principle: In NMR substances absorb energy in the radio frequency region of the electro-magnetic spectrum under influence of a strong magnetic field. It is a well known fact that the nuclei of the atoms bonded to each other in molecules spin on an axis like a top. Since nuclei are positively charged, this spin will create a small magnetic field. If an external magnetic field is applied to these nuclei this magnetic field will split into two energy levels. The energy difference is very small and corresponds to radiofrequency energy which is unique for every molecule and will give the information regarding the nature of the compounds and the presence of various functional groups and their environment.

Since this technique is mostly measures the spinning of the hydrogen nuclei (almost all the organic compounds contain hydrogen atoms!), it is sometimes referred as ***Proton Magnetic Resonance (PMR)*** spectroscopy.

Instrumentation: The instrumentation for this technique includes powerful magnet, radio-frequency signal generator, amplifier, detector, etc. The following is the outline of the instrument:

Fig: 9



Applications: The application lies mostly in the identification and structural analysis of organic compounds and thus, it is mostly a tool for qualitative analysis. It gives valuable information regarding the position of the functional groups in a molecule and provides distinguished spectra for the isomer. Much precise information on the structure of the compounds can be obtained using the same technique with other magnetic nuclei like C^{13}, O^{17}, the instrumentation being the same except that the sweep of the magnetic field is varied.

Disadvantages: Very expensive and the instrumentation is complex and needs exceptional skills to operate. Its sensitivity ranges from moderate to poor, however, can get clear information using C^{13} or O^{17} NMR. The usage of the solvents is limited and in most of the situations deuterated solvents are required.

3.1.2g Electron Spin Resonance Spectroscopy (ESR):

The basic principle of electron spin resonance spectroscopy is that, electrons always have a spin and thus have a magnetic moment. Thus, the magnetic resonance theory applies to electrons too like that of nuclei, as in NMR. Especially this technique is of high value when it comes to the compounds which contain odd electrons, i.e. those substances which have paramagnetic behaviour (if electrons are paired as in bonded orbital then their mutual spinning will cancel each other and there will be no response for the applied magnetic field,

whereas, if it is unpaired then it can align with the applied magnetic field and the feasibility of getting ESR spectra is higher). Thus, the principle and the instrumentation are much similar to that of NMR technique. It is also referred as, ***Electron Magnetic Resonance (EMR)*** or ***Electron Paramagnetic Resonance (EPR)*** spectroscopy.

It is mostly used as a potential technique to study the formation and lifetime of free radicals, which are the major intermediates in most of the organic reactions. Another important application is in the estimation of trace amounts of paramagnetic ions, particularly in biological works like, Mn^{2+}, Mg^{2+}, etc.

4. ANALYSIS THROUGH CHROMATOGRAPHY

The technique through which the chemical components present in complex mixtures are separated, identified and determined is termed as chromatography. This technique is widely used like spectroscopy and is a very powerful tool not only for analytical methods but also for preparative methods. Compounds of high grade purity can be obtained by this method. Chromatography can be simply defined as follows:

"It is the technique in which the components of a mixture are separated based upon the rates at which they are carried or moved through a stationary phase (column) by a gaseous or liquid mobile phase".

Based on the mobile phase this technique can be simply classified into two categories as: ***Liquid Chromatography*** and ***Gas Chromatography***. The column which holds the stationary phase (which in the form of small particles of the diameter of the order in microns), plays unique role in these processes. Usually silica is the base material for producing this phase.

4.1 LIQUID CHROMATOGRAPHY (LC/HPLC)

Principle: Early liquid chromatography was carried out in long glass columns with wide diameter. The diameters of the stacked particles inside the column were of the order of 150-200 microns range. Even then, the flow rates (eluent time) of the mobile phase with the analyte were very slow and separation times were long - often several hours!. With the advent of latest technology the particle diameters were reduced as small as to 10 microns with replacement of glass columns with steel ones. The flow rate of the mobile phase was improved by applying high pressure to the column using pumps and hence the performance was improved. This development led the instrument to be mostly called as "***High-Performance Liquid Chromatography"*** or ***"High-Pressure Liquid Chromatography" (HPLC)***. Though HPLC retains major of the credits to the analytical side, the earlier one of simple Liquid Chromatography still finds applications in the preparative purposes.

The HPLC technique can be divided into four main categories depending on the nature of the processes that occur at the columns as follow:

4.1.1 High-Performance Adsorption Chromatography: Here the analyte species (components to be analysed) are adsorbed onto the surface of a polar packing. The stationary phase consists of finely divided solid particles packed inside a steel tube. If the component mixture is eluted through this tube with the mobile phase, different components present in the mixture adsorb to different degrees of strength and they become separated as the mobile phase moves steadily through the column. The nature of the adsorption involves the interaction of polar molecules with a very polar solid stationary phase. The stationary phase could be silica gel or alumina. This method is extensively used for the separations of relatively non-polar, water-insoluble organic compounds (since polar molecules will be adsorbed on to the column momentarily). One particular application is in resolving isomeric mixtures such as *meta*- and *para*-substituted benzene derivatives.

4.1.2 High-Performance Partition Chromatography: It is the most widely used liquid chromatographic procedures to separate most kinds of organic molecules. Here the components present in the analyte mixture distribute (or partition) themselves between the mobile phase and stationary phase as the mobile phase moves through the column. The stationary phase actually consists of a thin liquid film either adsorbed or chemically bonded to the surface of finely divided solid particles. Of these the latter is considered more important and has a distinct stability advantage. It is not removed from the solid phase either by reaction or by heat and hence it is more popular. It finds wide applications in various fields, viz., pharmaceuticals, bio-chemicals, food products, industrial chemicals, pollutants, forensic chemistry, clinical medicine, etc.

4.1.3 High-Performance Ion-Exchange Chromatography: This method is used to separate mixtures of ions (organic or inorganic), and finds its application mostly in protein separations. The stationary phase consists of very small polymer resin "beads" which have many ionic bonding sites on their surface, termed as Ion Exchange Resins. This resin can be either an anion exchange resin, which possesses positively charged sites to attract negative ions, or a cation exchange resin, which possesses negatively charge sites to attract positive ions. If the analyte mixture which contains mixture of ions is introduced into the column packed with suitable ion-exchange resin, selected ions will be attached or bonded on to the resin, thus being separated from other species that do not bond. Later, these attached ions can be dislodged from the column by repeated elution with a solution that contains an ion that competes for the charged groups on the resin surface, in other words, which has high affinity for the charged sites on the resin than the analyte ions. Thus the analyte ions get exchanged and separated from the column.

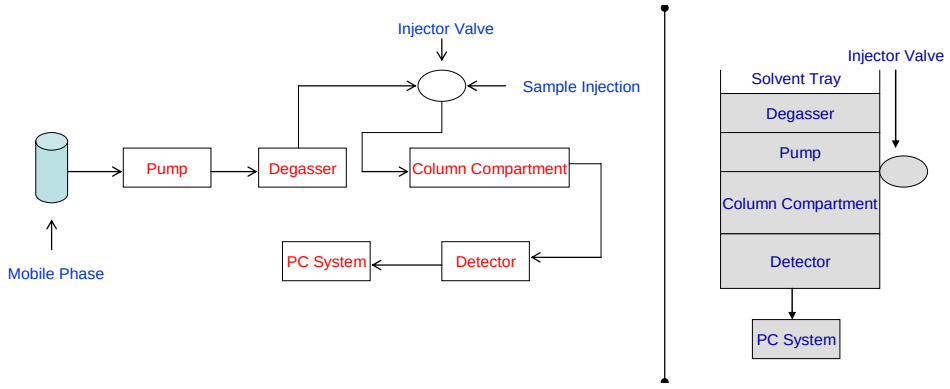
4.1.4 High Performance Size Exclusion Chromatography: This technique is for separating dissolved species on the basis of their size and particularly applicable to high-molecular-weight species like oligomers and polymers to determine their relative sizes and molecular weight distributions. Here, the stationary phase is polymer resin, which contains small pores. If the components to be separated are passed through the column the small sized particles can easily enter into these pores and their mobility is retarded. Whereas the large sized particles, which can't enter into these pores can come out of the column fast and elude first. Thus the separation of various sized particles is possible through variations in the elution time. It is classified into two categories based on the nature of the columns and their packing as:

Gel Filtration Chromatography - which uses hydrophilic packing to separate polar species and uses mostly aqueous mobile phases. This technique is mostly used to identify the molecular weights of large sized proteins & bio-molecules.

Gel Permeation Chromatography - which uses hydrophobic packing to separate nonpolar species and uses nonpolar organic solvents. This technique is used to identify the molecular weights of polymers.

Instrumentation: The basic HPLC system consists of a solvent (mobile phase) reservoir, pump, degasser, injection device, column and detector. The pump draws the mobile phase from the reservoir and pumps it to the column through the injector. At the end of the column (effluent end), a detector is positioned. Mostly UV absorption detector is used. In the case of analytical studies, after the detection the eluents are collected in waste bottles. In the case of preparative studies the eluents are fractionally collected for further studies. Most of the HPLC design will be the same as described for all the four main groups previously described. However, there can be differences in selecting the specific detectors for particular type of analysis, say for example, with ion-exchange chromatography, detectors commonly used are conductivity detectors for obvious reasons. Other important detectors for HPLC separations include refractive index detector, fluorescence detector and mass selective detector. The following is the most generalised outlay of the HPLC system:

Fig: 10



Disadvantages: Column performance is very sensitive, which depends on the method of packing. Further, no universal and sensitive detection system is available.

4.2 GAS CHROMATOGRAPHY (GC)

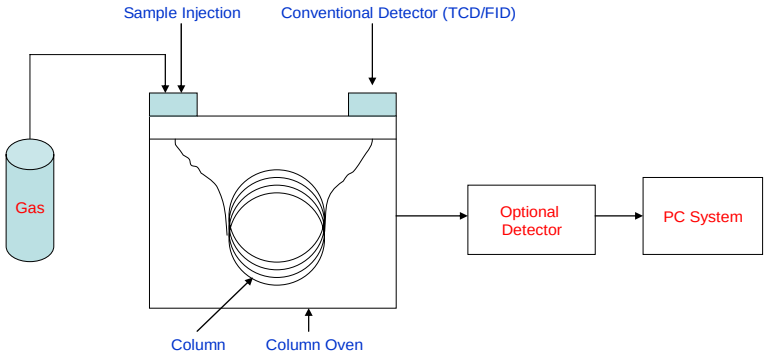
Principle: Here an inert carrier gas (Helium or Nitrogen) acts as the mobile phase. This will carry the components of analyte mixture and elutes through the column. The column usually contains an immobilized stationary phase. The technique can be categorised depending on the type of stationary phase as follow:

Gas Solid Chromatography (GSC) - here the stationary phase is a solid which has a large surface area at which adsorption of components of the analyte takes place. The separation is possible based on the differences in the adsorption power and diffusion of gaseous analyte molecules. The application of this method is limited and is mostly used in the separation of the low-molecular-weight gaseous species like carbon monoxide, oxygen, nitrogen and lower hydrocarbons.

Gas Liquid Chromatography (GLC) - this is the most important and widely used method for separating and determining the chemical components of volatile organic mixtures. Here the stationary phase is a liquid that is immobilized on the surface of a solid support by adsorption or by chemical bonding. The separation of the mixture into individual components is by distribution ratio (partition) of these anayte components between the gaseous phase and the immobilized liquid phase. Because of its wide applications most of the GCs are configured for the GLC technique.

Instrumentation: The instrumentation for GC is different from that of HPLC in that the injection port, column and detector are to be heated to a pre-specified temperature. Since the mobile phase here is a gas (carrier gas) the components present in the analyte mixture should be vaporised, so that it can be effectively carried through the column. The basic instrumentation for GC includes a carrier gas cylinder with regulator, a flow controller for the gas, an injection port for introducing the sample, the column, the detector and the recorder.
An outlay is as follow:

Fig: 11



In the above illustration the injection port, column oven and detector are hot zones. The success of this technique requires the appropriate selection of the column and the temperature conditions at which the column to be maintained throughout the analysis. Basically the columns for GC are classified as analytical columns and preparative columns. The analytical columns are of two types: *packed column and open-tubular or capillary column*. Both differ in the way the stationary phases are stacked inside.

In the instrumentation of GC detectors play unique role. There are a number of detectors, which vary in design, sensitivity and selectivity. Detectors in GC are designed to generate an electronic signal when a gas other than the carrier gas elutes from the column. Few examples and applications of the detectors are:

Thermal Conductivity Detector (TCD) - this operates on the principle that gases eluting from the column have thermal conductivity different from that of the carrier gas. It is the universal detector (detects most of the analytes) and is non-destructive and hence used with preparative GC, but less sensitive than other detectors.

Flame Ionization Detector (FID) - it is one of the important detectors where the column effluent is passed into a hydrogen flame and the flammable components are burned. In this process a fraction of the molecules gets fragmented into charged species as positive and

negative. While positively charged ions are drawn to a collector, negatively charged ions are attracted to positively charged burner head, this creates an electric circuit and the signal is amplified. The FID detector is very sensitive, but destroys the sample by burning. It only detects organic substances that burn and fragment in a hydrogen flame (e.g. hydrocarbons). Hence its usage is restricted for preparative GC and for inorganic substances which do not burn.

Electron Capture Detector (ECD) - this is another type of ionization detector which utilises the beta emissions of a radioactive source, often nickel-63, to cause the ionization of the carrier gas molecules, thus generating electrons which constitute an electrical current. This detector is used for environmental and bio-medical applications. It is especially useful for large halogenated hydrocarbons and hence in the analysis of halogenated pesticide residues found in environmental and bio-medical samples. It is extremely sensitive. It does not destroy the sample and thus may be used for the preparative work.

Nitrogen/Phosphorus Detector (NPD) - the design of the detector is same to that of the FID detector except that a bead of alkali metal salt is positioned just above the flame. It is also known as 'Thermionic Detector'. It is useful for the phosphorus and nitrogen containing pesticides, the organophosphates and carbamates. The sensitivity for these compounds are very high since the fragmentation of the other organic compounds are minimized.

Flame Photometric Detector (FPD) - here a flame photometer is incorporated into the instrument. The principle is that the sulfur or phosphorus compounds burn in the hydrogen flame and produce light emitting species. This detector is specific for organic compounds containing sulphur or phosphorus. It is very selective and very sensitive.

Electrolytic Conductivity Detector (ECD Hall) - this otherwise known as 'Hall detector', converts the eluting gaseous components into ions in liquid solution and then measures the electrolytic conductivity of the solution in a conductivity cell. The conversion to ions is done by chemically oxidizing or reducing the components with a "reaction gas" in a small reaction chamber. This detector is used in the analysis of organic halides and has excellent sensitivity & selectivity, but is a destructive detector.

The recent developments allow the GC to be coupled with other analytical techniques like Infra Red Spectrometry (Gas Chromatography-Infrared Spectrometry, GC-IR) and Mass Spectrometry (Gas Chromatography- Mass Spectrometry, GC-MS). These are termed as 'hyphenated techniques', and are very efficient for qualitative analysis as very accurate and precise information like mass or IR spectrum of the individual sample components are readily obtained as they elute from the GC column. It saves time and reduces the steps involved for a component to be separated and analysed.

Disadvantages: Samples must be volatile and thermally stable below about 400^0 C. No single universal detector is available and most commonly used detectors are non-selective. One should take much care in the analytical steps starting from the selection of the column, the detector and must define the temperatures of all the three ports viz., injection port, column oven and detector. An improper programming on these will lead to erratic results.

5. ANALYSIS THROUGH THERMAL ENERGY

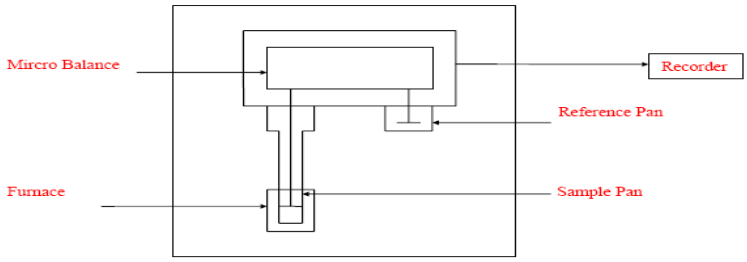
5.1 THERMAL ANALYSIS

The technique of thermal analysis actually comprises of a series of methods, which detect the changes in the physical and mechanical properties of the given substance by the application of heat or thermal energy. The physical properties include mass, temperature, enthalpy, dimension, dynamic characteristics, etc. It finds its application in finding the purity, integrity, crystallinity and thermal stability of the chemical substances under study. Sometimes it is used in the determination of the composition of complex mixtures. This technique has been adopted as testing standard in quality control in the production field, process control and material inspection. It is applied in wide fields, including, polymer, glass, ceramics, metals, explosives, semiconductors, medicines and foods. The following are the popular methods under this technique:

5.1.1 Thermogravimetric Analysis (TGA):

In this technique the change in sample weight is measured while the sample is heated at a constant rate (or at constant temperature), under air (oxidative) or nitrogen (inert) atmosphere. This technique is effective for quantitative analysis of thermal reactions that are accompanied by mass changes, such as evaporation, decomposition, gas absorption, desorption and dehydration. The following is the simplified diagram for the instrumentation:

Fig: 12



The micro-balance plays a significant role, during measurement the change in sample mass affects the equilibrium of the balance. This imbalance is fed back to a force coil, which generates additional electromagnetic force to recover equilibrium. The amount of additional electromagnetic force is proportional to the mass change. During the heating process the temperature may go as high as 1500^0 C inside the furnace.

5.1.2 Thermomechanical Analysis (TMA):

Thermomechanical analysis is the measurement of a material's behaviour, ie. expansion or contraction, when temperature and a load is applied. A scan of dimensional changes related to temperature (at constant load) or load (at constant temperature) provides valuable information about the sample's mechanical properties. One of the most important applications of TMA is the characterization of composite and laminate materials, through the study of glass transition temperature and the expansion coefficient. Another application is in the quantitative measurement of extension and contraction observed in textile fibres, thin films and similar materials.

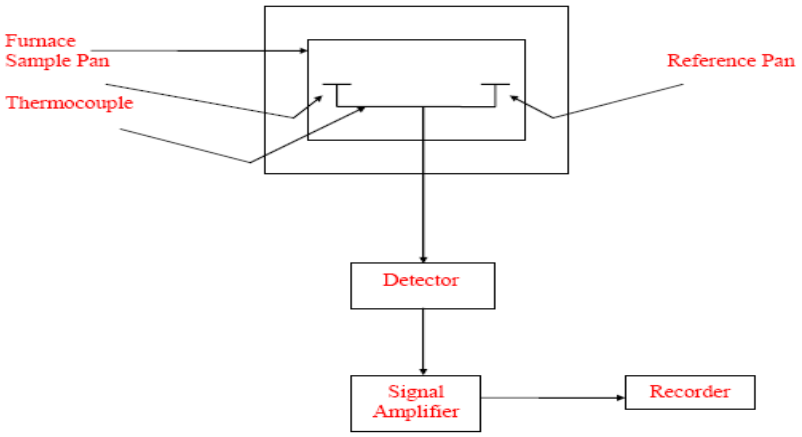
5.1.3 Differential Thermal Analysis (DTA):

This technique measures the temperature difference between a sample and a reference material as a function of temperature as they are heated or cooled or kept at a constant temperature (isothermal). Here the sample and reference material are simultaneously heated

or cooled at a constant rate. Reaction or transition temperatures are then measured as a function of the temperature difference between the sample and reference. It provides vital information of the materials regarding their endothermic and exothermic behaviour at high temperatures. It finds most of its applications in analysing and characterising clay materials, ceramic, ores, etc.

5.1.4 Differential Scanning Caloriemeter (DSC):
This technique is more or less similar to DTA except that it measures the amount of heat absorbed or released by a sample as it is heated or cooled or kept at constant temperature (isothermal). Here the sample and reference material are simultaneously heated or cooled at a constant rate. The difference in temperature between them is proportional to the difference in heat flow (from the heating source i.e. furnace), between the two materials. It is the well-suited technique in the detection and further studies of liquid crystals. This technique is applied to most of the polymers in evaluating the curing process of the thermoset materials as well as in determining the heat of melting and melting point of thermoplastic polymers, glass transition temperature (T_g.), endothermic & exothermic behaviour. Through the adjunct process of isothermal crystallization it provides information regarding the molecular weight and structural differences between very similar materials. The instrumentation is exactly similar to that of DTA except for the difference in obtaining the results.

Fig: 13 (DSC/DTA)

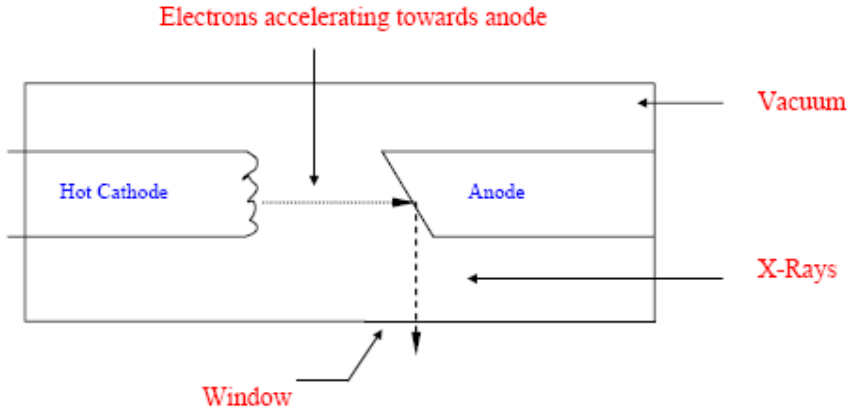


6. ANALYSIS THROUGH X-RAY TECHNIQUES

6.1 Generation of X-Rays: When metals, like copper, molybdenum, tungsten, etc., are bombarded directly with a stream of high energy electrons or radioactive particles, X-rays (wavelengths of order 0.1-100Å) are emitted because of the transitions involving K-Shell and L-Shell electrons. This can be simply expressed as follows:

A cathode in the form of a metal wire when electrically heated gives off electrons. If a positive voltage, in the form of an anode (target comprised of the metals mentioned above), is placed near these electrons, the electrons are accelerated toward the anode. Upon striking the anode, the electrons transfer their energy to the metallic surface, which then gives off X-ray radiation. This is referred as ***primary X-rays***.
The following is the schematic diagram for the process:

Fig: 14



Note: The wavelength of the emitted X-ray is characteristic of the element being bombarded. Hence with some modifications this process can be used as a tool for qualitative and quantitative elemental analysis by measuring the wavelength and emission intensity of the X-rays respectively. This forms the basis for Electron Probe Microanalysis! **(Ref: Sec 7.1)**

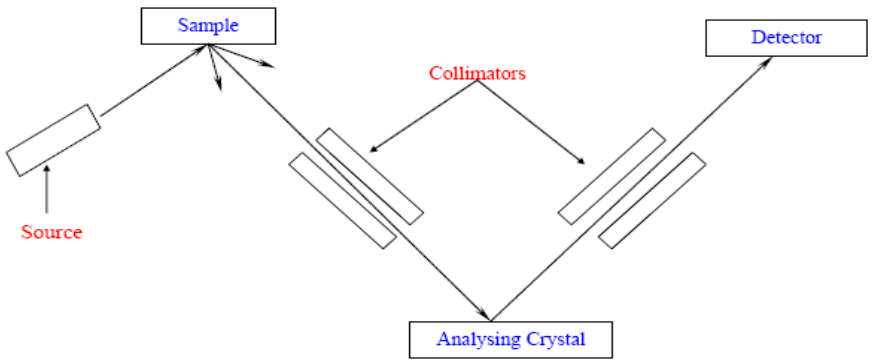
6.2 X-Ray Fluorescence Spectrometry (XFS):

Principle: When a sample is placed in a beam of primary X-rays, part of it will be absorbed and the atoms get excited, by the ejection of electrons present in K and L shells. While relaxing they re-emit X-rays of characteristic wavelength. This re-emitted X-rays are called ***secondary or fluorescent X-rays*** and hence the name for this technique. Since, the wavelength of the fluorescence is characteristic of the element being excited, measurement of the wavelength and intensity enables to carry out the qualitative and quantitative analyses.

Instrumentation: It comprises of a source for primary X-rays, collimators, analyzing crystal and detector.

Applications: It is one of the non-destructive methods in the elemental analysis of solid or liquid samples for major and minor constituents. Most of the elements in the periodic table, both metals and nonmetals, respond to this technique. Detection limit is between 10 to 100 ppm. One of the significant uses of this method is in the medical field in identifying and determining the sulfur in protein.

Fig: 15



Disadvantages: The sensitivity gets affected for elements with lower atomic numbers, particularly elements with atomic number lower than 15 are difficult to analyze. The sensitivity is also limited by matrix absorption, secondary fluorescence and scattering of the particles. Instruments are often large, complicated and costly.

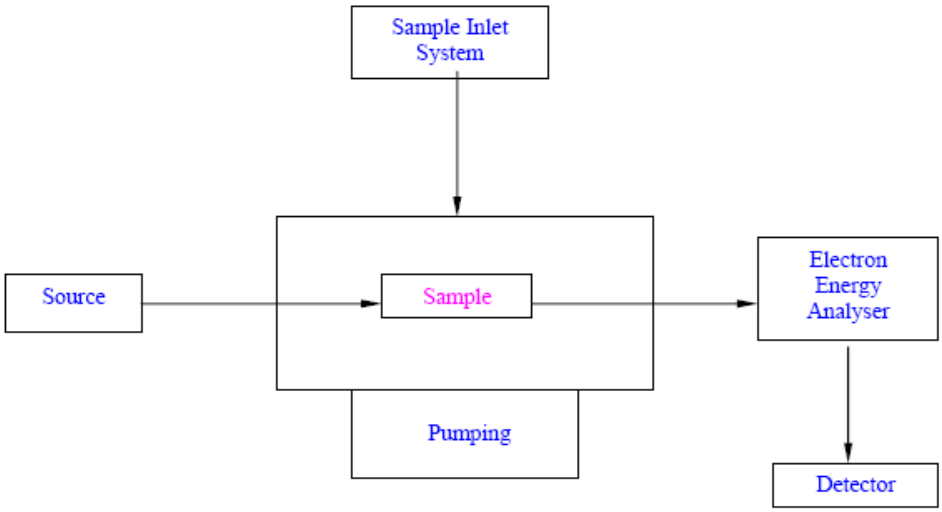
6.3 X-Ray Photo-emission Spectrometry (XPS):

Principle: When a primary X-ray beam of precisely known energy impinges on sample atoms, inner shell electrons are ejected and the *energy of the ejected electrons* is measured. The difference in the energy of the impinging X-ray and the ejected electrons gives the binding energy (E_b) of the electron to the atom. Since, this binding energy of the emitted electron depends on the energy of the electronic orbit and the element it can be used to identify the element involved. Further, the chemical form or environment of the atom affects the binding energy to a considerable extent to give rise to some *chemical shift*, which can be used to identify the valence state of the atom and its exact chemical form. This technique is mostly referred as **Electron Spectroscopy for Chemical Analysis (ESCA).**

An associated process with this method is that when the electron is ejected from the inner orbital a vacancy is left with. Hence, another electron from the outer orbits may fall to fill the vacancy and by doing so emits X-ray fluorescence. The energy of this X-ray fluorescence is sometimes transferred to a second electron to make it to be ejected. This second electron thus emitted is termed as ***Auger electron*** and the method ***Auger Spectroscopy*** (after French Physicist *Pierre Auger*). Its applications are more or less similar to ESCA and both the methods are used in conjunction since in both cases the energies involved are similar.

Instrumentation: It consists of a radiation source for primary X-rays, monochromator, the energy analyzer (to resolve the electrons generated from the samples by energy) and detector to measure the intensity of the resolved electrons. The analysis is done in high vacuum.

Fig: 16



Applications: It is mainly used for surface analysis, especially in the qualitative identification of the elements in a sample. Based on the chemical shifts, the chemical environment around the atoms can also be estimated. This measurement is useful in determining the valence states of the atoms present in various moieties in a sample. Quantitative measurements can be made by determining the intensity of the ESCA lines of each element.

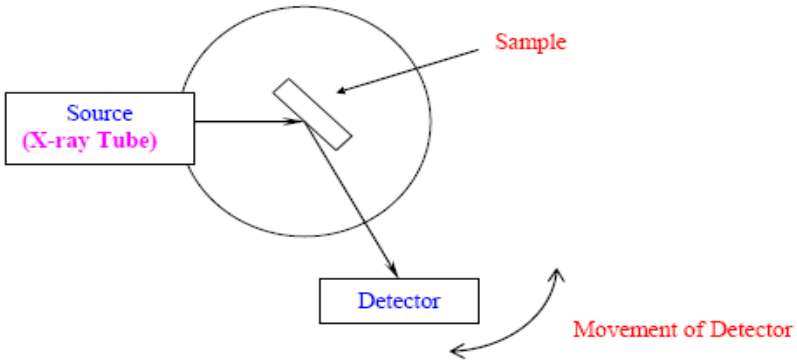
Disadvantages: High vacuum is necessary for the system to avoid the low energy electrons to be collided with other impurities, which may result in low sensitivity. It is not possible to detect the impurities at the ppm or ppb levels. The whole instrumentation is highly complicated.

6.4 X-Ray Diffractometry (XRD):

Principle: In this technique the primary X-rays are made to fall on the sample substance under study. Because of its wave nature, like light waves, it gets diffracted to a certain angle. This angle of diffraction, which differs from that of the incident beam, will give the information regarding the crystal nature of the substance. The wavelength of the X-rays can be varied for the application by using a grating plate.

Instrumentation: It consists of X-ray tube for the source, monochromator and a rotating detector.

Fig: 17



Applications: The diffraction of X-rays is a good tool to study the nature of the crystalline substances. In crystals the ions or molecules are arranged in well-defined positions in planes in three dimensions. The impinging X-rays are reflected by each crystal plane. Since the spacing between the atoms and hence the planes can't be same or identical for any two chemical substances, this technique provides vital information regarding the arrangement of atoms and the spacing in between them and also to find out the chemical compositions of crystalline substances. The sample under study can be of either a thin layer of crystal or in a powder form. Since, the power of a diffracted beam is dependent on the quantity of the corresponding crystalline substance, it is also possible to carry out quantitative determinations.

7. ANALYSIS THROUGH MICROSCOPY

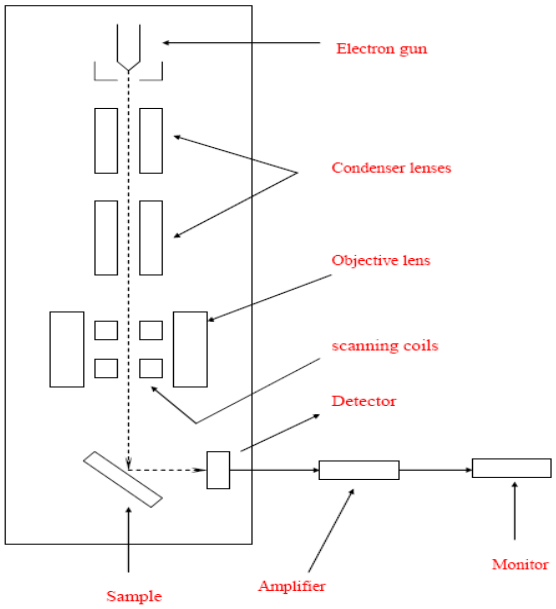
The techniques described here are not for the simple, ordinary optical microscopes which use light for the magnification. These employ electron beam and mechanical probes to magnify the surfaces under study.

7.1 Scanning Electron Microscopy (SEM):

Principle: In this technique, an electron beam is focused onto the sample surface kept in a vacuum by electro-magnetic lenses (since electron possesses dual nature with properties of both particle and wave an electron beam can be focused or condensed like an ordinary light) The beam is then rastered or scanned over the surface of the sample. The scattered electron from the sample is then fed to the detector and then to a cathode ray tube through an amplifier, where the images are formed, which gives the information on the surface of the sample.

Instrumentation: It comprises of a heated filament as source of electron beam, condenser lenses, aperture, evacuated chamber for placing the sample, electron detector, amplifier, CRT with image forming electronics, etc.

Fig: 18



Applications: Scanning electron microscopy has been applied to the surface studies of metals, ceramics, polymers, composites and biological materials for both topography as well as compositional analysis. An extension (or sometimes conjunction to SEM) of this technique is **Electron Probe Micro Analysis (EPMA)**, where the emission of X-rays, from the sample surface, is studied upon exposure to a beam of high energy electrons. Depending on the type of detectors used this method is classified in to two as: Energy Dispersive Spectrometry (EDS) and Wavelength Dispersive Spectrometry (WDS). This technique is used extensively in the analysis of metallic and ceramic inclusions, inclusions in polymeric materials, diffusion profiles in electronic components.

Disadvantages: The instrumentation is complicated and needs high vacuum for the optimum performance.

7.2 Transmission Electron Microscopy (TEM):

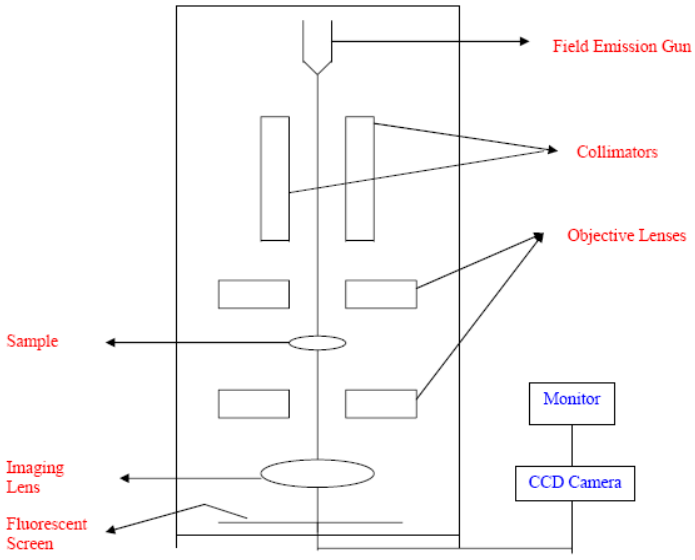
Principle: In this technique, a beam of high-energy electrons (typically 100 - 400keV) is collimated by magnetic lenses and allowed to pass through a specimen under high vacuum. The transmitted beam and a number of diffracted beams can form a resultant diffraction pattern, which is imaged on a fluorescent screen kept below the specimen. The diffraction pattern gives the information regarding lattice spacing and symmetry of the structure under consideration. Alternatively, either the transmitted beam or one of the diffracted beams can be made to form a magnified image of the sample on the viewing screen as bright-and dark-field imaging modes respectively, which give information about the size and shape of the micro-structural constituents of the material. High-resolution image, that contains information about the atomic structure of the material, can be obtained by recombining the transmitted beam and diffracted beams together.

Instrumentation: It comprises of a tungsten filament or LaB_6 or a field emission gun as source of electron beam, objective lens, imaging lens, CCD camera, monitor, etc.

Applications: Transmission electron microscopy is used to study the local structures, morphology, and dispersion of multi-component polymers, cross sections & crystallization of metallic alloys and semiconductors, microstructure of composite materials, etc. The instrument can be extended to include other detectors like Energy Dispersive Spectrometer (EDS) or Energy Loss Spectrometer (ELS) to study about the local chemistry of the material similar to SEM technique.

Disadvantages: The instrumentation is complicated and needs high vacuum. Sample preparation is very time consuming. Some materials, especially polymers, are sensitive to electron beam irradiation which results in the loss of crystallinity and/or mass.

Fig: 19



7.3 Scanning Probe Microscopy (SPM):

The scanning probe microscopy is a general term for a wide variety of microscopic techniques, which measure the morphology and properties of surfaces on the atomic scale. This includes the following:

Scanning Tunneling Microscopy (STM) – which studies the surface topography and electronic structure, *Atomic Force Microscopy (AFM)* – which studies the surface topography, surface hardness and elastic modulus, *Lateral Force Microscopy (LFM)* – which studies the relative frictional properties, *Scanning Thermal Microscopy (SThM)* – which studies the thermal conductivity, *Magnetic Force & Electric Force Microscopies (MFM & EFM)* – which study the magnetic and electric properties.

The techniques of STM and AFM are discussed below, since these are widely used:

Principle: The general principle for all the scanning probe microscopes is that a sharper probe (or a very fine tip) is used to scan the surface of the sample with much lower force and obtain the topography and morphology information.

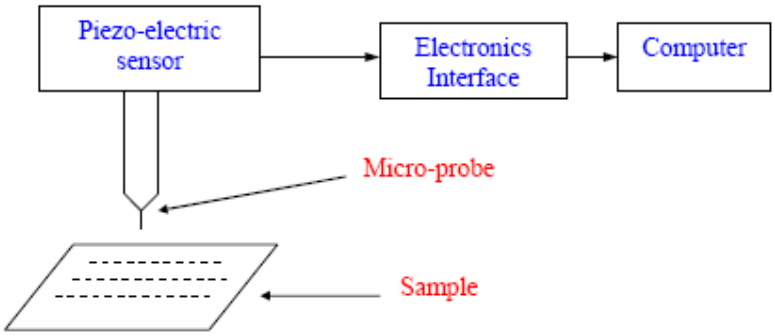
Scanning tunneling microscope: When a sharp tip made of a *conducting material* is brought close to a *conducting sample*, overlapping of the electron clouds between the two surfaces will occur. If a potential is given between them a current of electrons is formed, which is often referred as *"tunneling" current*, and the effect is known as *"tunneling" effect*. This effect is largely depended on the distance between the tip and the sample material. Hence, if the scanning tip is controlled by a high precision motion device made of piezo-electric material, the distance between the tip and the sample can be measured during a scanning through a feedback loop control of the piezo-electric element. By this way the sample can be scanned with sub-angstrom precision.

Atomic force microscope: This technique operates by measuring the forces between the sample and the tip, and the sample need not be a conducting material. Here, the tip is brought close enough to the sample surface to detect the *repulsive force* between the atoms of the tip material and the sample. The probe tip is mounted at the end of a cantilever of a low spring constant and the tip-to-sample spacing is held fixed by maintaining a constant and very low force on the cantilever. Hence, if the tip is brought close to the sample surface, the repulsive force will induce a bending of the cantilever. This bending can be detected by a laser beam, which is reflected off the back of the cantilever. Thus by monitoring the deflection of the cantilever, the surface topography of the sample can be tracked. Since the force maintained on the cantilever is in the range of inter-atomic forces (about 10^{-9} Newton), this technique derived the name *"atomic force" microscopy*.

AFM operates at two modes:
Repulsive or contact mode – which detects the repulsive forces between the tip and sample;
Attractive or non-contact mode – which detects the van der waals forces that act between the tip and sample.

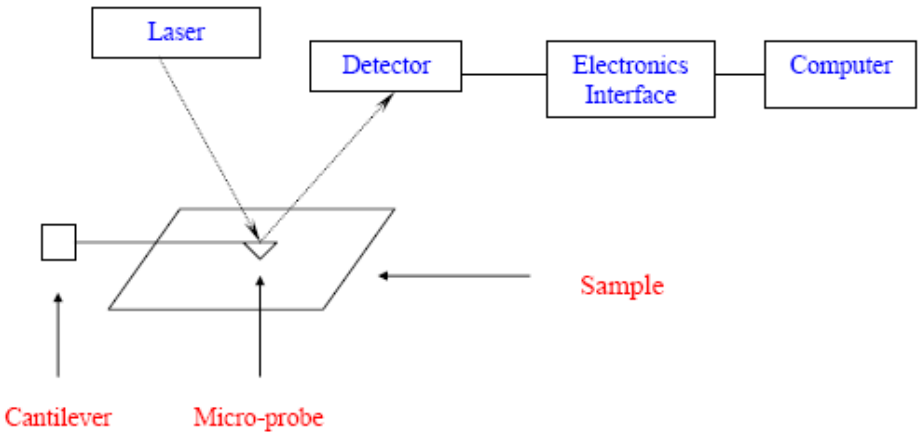
Instrumentation:
Scanning tunneling microscope: It mainly consists of a scanner, probe motion sensor composed of piezo-electric material, micro probe, etc.

Fig: 20



Atomic force microscope: It mainly consists of a scanner, cantilever, laser source, photo-diode detector, micro-probe, etc.

Fig: 21



Applications: Both STM and AFM find applications widely in material sciences especially for surface studies on a nano scale range. While STM finds its applications in the characterization of surface structure (including the electronic structure), AFM finds its applications in measuring the hardness of materials. Sometimes, AFM can be used in the study of "depth profile" of the deposited oxide layer on to a material.

Disadvantages: A limitation to STM is that it can study only the conducting samples, since the technique is based on the tunneling current between two conducting areas. Hence, it doesn't lend itself to the study of non-conducting materials. In fact, the AFM had been developed to encounter this problem. These methods require special sample preparation techniques, which are tedious, like, thin sectioning, electo-polishing, various mechanical cutting and polishing techniques, etc.

8. ANALYSIS THROUGH ELECTRO - CHEMICAL TECHNIQUES

Though there are several techniques under this title like Potentiometry, Amperometry, Conductometry, Electrogravimetry, Coulometry, etc., two important techniques are discussed below, which find wide applications.

8.1 POLAROGRAPHY

Principle: This technique involves the measurement of the current flowing in an electrolysis cell due to the oxidation - reduction reactions (redox reactions) of the analyte substance present in the solution. This redox reaction usually occurs at the surface of one of the electrodes. The current that produced in this way is directly proportional to the concentration of the components under study.

Instrumentation: The system comprises of three electrodes:

Working electrode - where the oxidation or reduction processes of the analyte of interest occur. It consists of liquid mercury flowing through a very narrow-bore capillary tube and is called "dropping mercury electrode" and abbreviated as DME.

Reference electrode - this electrode is silver-silver chloride (Ag/AgCl) type electrode and is crucial for the precise control of the potential of the working electrode.

Auxillary electrode - the use of this electrode is to carry the bulk of the current and counters the process that occurs at the working electrode thus making it free from any disturbances except to maintain the redox reaction. Hence, this electrode is also called as "counter electrode". It is placed in a separate chamber with a fritted glass disc allowing electrical contact with the rest of the cell, but not allowing diffusion of undesirable species to the working electrode.

The sample is placed in a glass container with a medium, which consists of high concentrations of electrolyte. This excess electrolyte helps in bringing down the potential of the electrode process to the desired range and eliminates the interference caused by unwanted complexations and other reactions within the system. This is also referred as *"background electrolyte"*.

Applications: It is widely used for the quantitative and qualitative determination of metals and metal complexes as well as organic compounds in trace levels.

Disadvantages: The measurements with this technique are very sensitive to solution composition, dissolved oxygen and capillary characteristics. Further, impurities if any, present in the background electrolyte also affect the sensitivity.

8.2 ELECTROPHORESIS

Principle: This technique is actually a separation process by applying an electric field. The principle is that under the influence of an electric field dissolved ions present in a solution will migrate at varied rates and direction in a column or a surface. At this instance two events take place:

1. ions of opposite charge will migrate in different directions and become separated.

2. ions of like charge while migrating in the same direction, become separated due to different migration rates.

Factors influencing migration rates are *charge values* and *different mobility*. Further, the mobility of an ion is dependent on the size and shape of the ion, as well as the nature of the medium through which it migrates. The medium used in most of the cases is either cellulose or gel. This technique is sometimes referred as *"Zone or Capillary Electrophoresis"*.

Instrumentation: The materials needed are cellulose or polymeric gel as a supporting medium, enclosed tank with electrodes and buffer reservoirs and dc power supply.

Applications: This technique finds application in the qualitative characterization of biologically active materials, where, charged amino acids and other biomolecules need to be separated. Thus, analysis of protein and nucleic acid using this method becomes very popular.
This is highly useful for clinical and forensic work, where small amounts of complex samples may be involved.

Disadvantages: This technique gives less precision results for quantitative works hence application in this aspect is restricted. Mobility is very sensitive to supporting medium, so selection of the medium is very important.

9. ANALYSIS THROUGH MISCELLANEOUS TECHNIQUES

9.1 TOTAL ORGANIC CARBON ANALYZER (TOC)

The element carbon is the most common form that can be found everywhere. Its measurement at trace levels is very important, especially, in the fields of Environmental Pollution, Pharmaceuticals, Industrial Effluents, etc., to maintain the threshold limits of the concerned contaminants. Among these, monitoring the organic compounds in the environment is important, since inorganic carbon constitutes to only a lesser extent having its presence with carbonates, bicarbonates and dissolved carbon dioxide. Hence, it is customary to find out the presence of carbon as Total Organic Carbon, which represents the quantity of carbon present in water as organic matter, either dissolved or suspended.

The principle of the TOC is very simple which involves complete oxidation of carbonaceous materials to carbon dioxide and water by catalytic combustion or by chemical oxidation. The released carbon dioxide is measured using an IR detector since this molecule strongly absorbs in the IR region. In cases, wherein the measurement of inorganic carbon is necessitated then it is done within the same instrument by purging air through the sample placed in acid solution. This will create the formation of carbon dioxide and water from the inorganic carbonates and bicarbonates.

9.2 ELEMENTAL ANALYZER

This technique determines the presence of the elements like Carbon, Hydrogen, Nitrogen and Sulphur in a given substance and gives the result as percentage amount of these atoms against the total weight. Since this technique specifically determines these four elements this instrument is also called as *"CHN/S Analyzer"*. Most of the organic compounds are made up of these four elements and oxygen, hence after determining the former elements percentage weight of oxygen can be easily calculated.

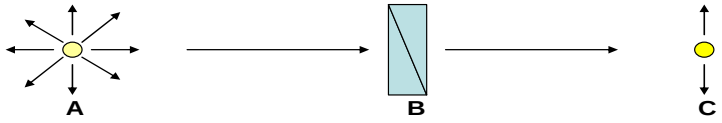
In this technique the substance under study is combusted under oxygen stream in a furnace at high temperatures. The end products of the combustion would be mostly the oxides of the concerned elements in the form of gases. These are then separated and carried to the detector using inert gases like helium or argon.

It is one of the few analytical techniques that give a clear quantitative measurement of the carbon, hydrogen, nitrogen and sulphur. It finds applications in almost every field of chemistry like in the analysis of organics (especially to find out the molecular formula of a newly synthesized compound), polymers, pharmaceuticals, energy (fuels), environmental studies, etc.

9.3 POLARIMETRY

Principle: In the preceding section under spectroscopy (***Section 3***) interaction of light with the substances leading to absorption, emission and fluorescence has been dealt. Here the phenomena concerns with the rotation of the plane of the *plane polarised light* when it is passed through the samples which lacks symmetry (e.g. sugar), these substances which are asymmetric in nature are said to be optically active substances. A plane polarised light is that which essentially has its vibration in only one direction or one plane as shown below:

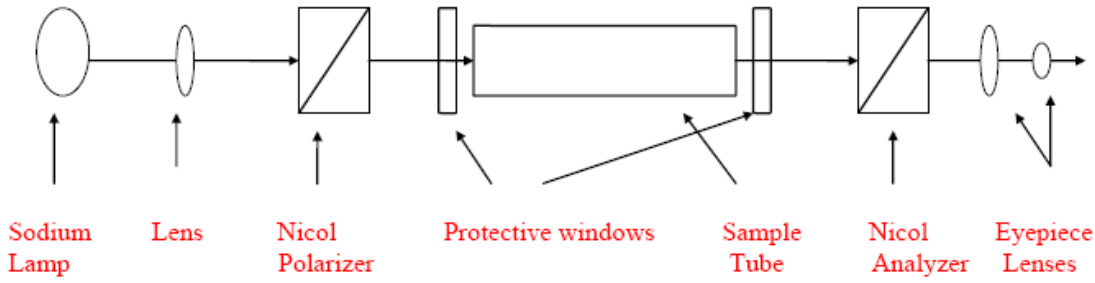
Fig: 22



In the above illustration "A" refers to the light waves propagating in all directions or planes (multidirectional), "B" refers to the Nicol prism which cuts all the planes of light and allows the light to come out with vibration in only one plane or direction (unidirectional) and "C" is the resultant light which is said to be a plane polarised light. It is the light, which is utilised for the measurement of the optical activity of a compound. The plane of this light will be rotated to certain extend depending on the nature of the compound under study and forms the basis of this technique.

Instrumentation: It comprises of a sodium lamp, nicol polarizer, sample tube, nicol analyzer and an eyepiece.

Fig: 23



If, the rotation of the plane by a compound is in the clockwise then it is said to be *dextro*, if it is in the anticlockwise then it is said to be *laevo*. For any compound the rotation depends on the concentration and the length of the sample tube and importantly on the temperature too.

Applications: It is mainly used as a quantitative tool. It finds extensive application in the analysis of sugar. In pharmaceutical industry it is used for the measurement of concentration of optically active drugs.

Disadvantages: It requires the samples only in solution form. The sample tube, after filling with the sample solution, should be free of bubble or any free particle, otherwise the light path will be affected and hence the accuracy. The sample holder should be thermostatted, since the optical activities of the substances vary with temperature.

9.4 UV/VISIBLE SPECTROPOLARIMETRY

Principle: In the preceded section the rotation of the plane of the polarized light by optically active compounds has been discussed. In practice, it is produced by combining two plane-polarized light waves of identical frequency moving through the same region of the space in the same direction, to produce a resultant wave, which is also linearly polarized. If these two waves of identical amplitude are orthogonal, combining them can lead to linearly polarized or circularly polarized light depending on the phase difference between the two waves.

*Optical Rotatory Dispersion **(ORD)**:*

In polarimetry, the rotation of the plane of linearly polarized light (two combined plane-polarized waves with no phase difference) by optically active substances is studied *at **a fixed wavelength***. In spectropolarimetry it is studied over ***a range of wavelength*** whereby the dependence of the rotation on the selected wavelength range is measured and is termed as Optical Rotatory Dispersion. The instrumentation is similar to a polarimeter except for a tunable wavelength source. ORD measures the **differences in refraction of the polarized light waves** through the sample resulting in a rotation of the plane.

Circular Dichroism ***(CD)**:* If the two plane-polarized orthogonal light waves are 90^0 out of phase then the resultant gives a circularly polarized light. It can either be *right circularly polarized (d-component)* or *left circularly polarized (l-component)* depending on the direction of the phase shifting. If these two oppositely polarized circular waves are combined the resultant would be a linearly plane-polarized light. CD measures the **differences in absorbance of the polarized light waves** by the sample resulting in an ellipticity, when the two circularly polarized light waves are combined after passing through the sample (Fig: 24a).

*Linear Dichroism **(LD)**:* The linearly polarized light can either be made parallel or perpendicular to the orientation axis using a polarizer and, if this is passed through the sample it will give the information regarding the orientation of the molecules present in the sample. LD measures the **differences in absorbance of the linearly polarized light waves** (Fig: 24b).

Fig: 24a

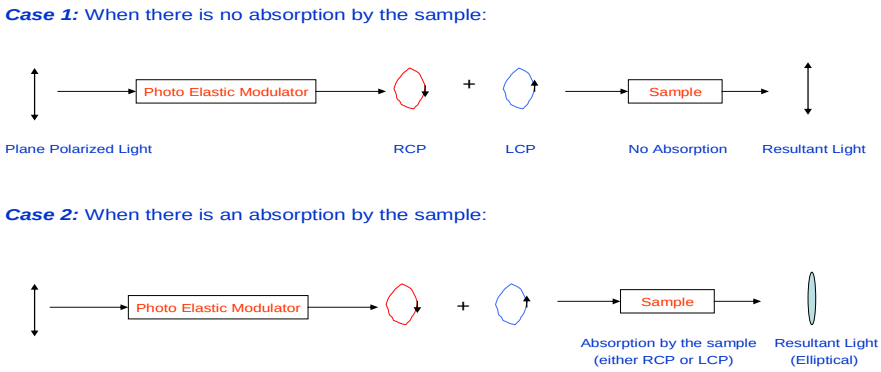
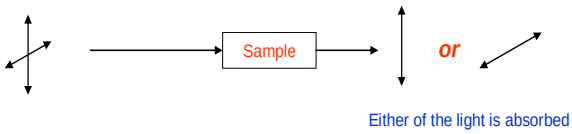
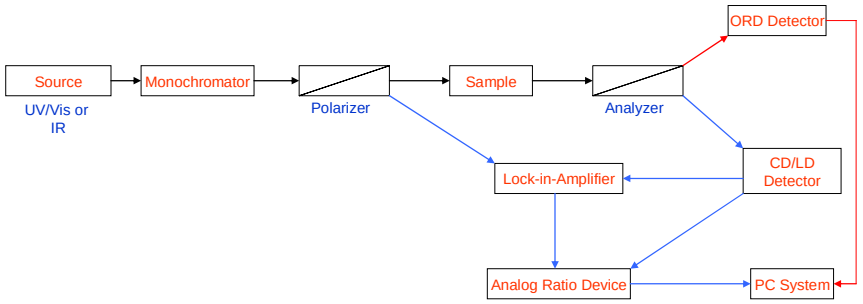


Fig: 24b



Instrumentation: Normally the instrumentation of a spectropolarimeter is so designed to accommodate both the functions of ORD and CD. It consists of a source (covering UV to visible), monochromator, polarizer, analyzer, detectors, lock-in amplifier, display device, etc.

Fig: 24c



Applications: Both CD and ORD measurements are employed in fundamental studies to provide structural information in the area of stereochemistry and conformation about optically active compounds like, amino acids, proteins, etc. LD measurement is mostly employed to study the molecular orientations in the stretched polymer films.

Disadvantages: Only optically active compounds and surface oriented thin films can be analyzed.

9.5 VIBRATIONAL CIRCULAR/LINEAR DICHROISM (VCD/VLD)

Principles: The phenomenon of CD and LD if occurs in the Infrared region which corresponds to the vibrational frequencies of the molecules then the techniques are termed as Vibrational Circular Dichroism (VCD) and Vibrational Linear Dichroism (VLD).

Instrumentation: VCD spectrometer is constructed using the same detection scheme as described for the UV/Vis CD spectrometer described above, except for the optical components suitable for infrared region. Thus normally a VCD accessory is combined with a FT-IR spectrometer.

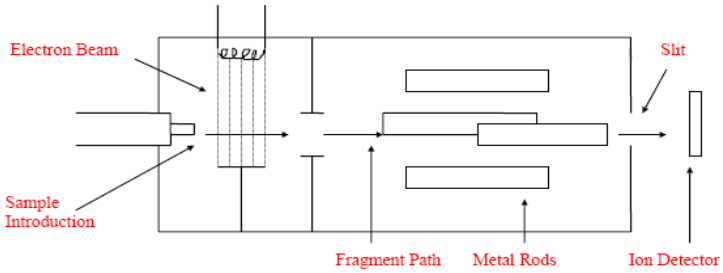
9.6 MASS SPECTROMETRY

Principle: In this technique chemical substances are bombarded with high energy electron beam causing total destruction and fragmentation of the molecules in the sample. This fragmentation results in small charged "pieces" or fragments of the molecules, which, because of their charge are made to move through a magnetic field. The magnetic field

affects each fragments differently based on their *mass* and *charge* that they carry and thus they are separated.

Instrumentation: It consists of a source of high-energy electrons, ion accelerator, magnets, detector, recorder and high vacuum pumping system. In the instrumentation the entire path of the fragments, including the inlet system must be evacuated. The reason is to avoid collisions of both the electron beam and the sample ions with contaminating foreign particles which would otherwise affect the sensitivity and hence the results. For this technique there are two different instrument designs as Magnetic Sector Mass Spectrometer and Quadrupole Mass Spectrometer. The former is the older version which uses strong magnets to focus the fragmented ions to the detector plate, whereas, the latter uses four short parallel metal rods; wherein two vertical rods are connected to the positive pole and the other two to the negative pole of a variable power source. This will create a variable electric field and is used to focus the fragments on to the detector slit. This quadrupole instrument is newer and more popular because of its compact design and also it provides a faster scanning capability. The following is the illustration for the ***quadrupole mass spectrometer***:

Fig: 25



Applications: It is widely used in conjunction with IR, UV and NMR in the identification and structural analysis of organic compounds. It is also possible to determine the trace impurities in a wide range of inorganic materials. It is well suited for gas analysis and is used by the petroleum industry for both the qualitative and quantitative analysis of hydrocarbon distillates and other petrochemicals. It is invaluable in the analysis of both terrestrial and extra-terrestrial atmospheres, the latter being achieved by instruments with light-weight components. The mass spectrometer has been used as a detector in gas chromatography (GC-MS), in liquid chromatography (HPLC-MS), in thermogravimetry (TGA-MS) and in inductively coupled plasma spectrometry (ICP-MS).

Disadvantages: The instrumentation is complex and difficult to maintain.

9.7 LASER LIGHT SCATTERING SYSTEM

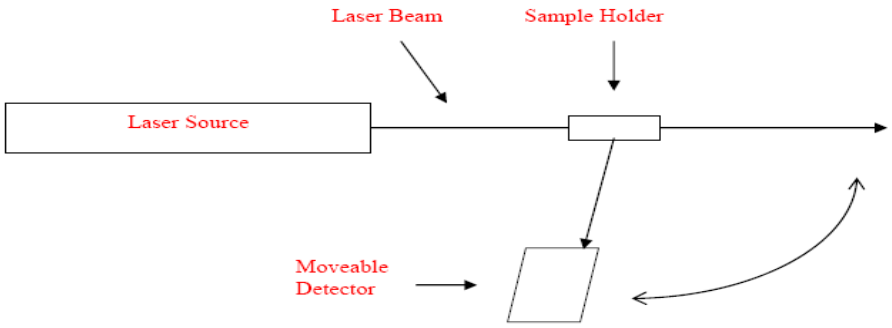
When light (usually a laser beam) is shined or focused on a solution containing the macromolecule of interest (polymers, proteins, etc.), it will scatter and provide the information about molecular structure and motion in the solution.

Principle: Interaction of light (electro-magnetic radiation) with matter results in two processes, viz. absorption (which forms the basis of spectroscopy) and scattering. When a beam of light falls upon a matter, the electric field associated with the light polarizes the electron cloud of the atoms. Thus a dipole is induced in the molecules, which oscillates with the electric field. These oscillating electron clouds then serve as secondary sources of light

and emit light in various directions (scattering). The scattered light has almost the same wave length as the incident light (also referred as ***Elastic Scattering*** or ***Rayleigh Scattering***) and merely redirected from the incident beam. It is the intensity of the scattered light and its angular distribution which give the information regarding the nature of the molecules under study.

Instrumentation: It consists of a laser source, thermostatted sample holder, movable photo diode detector, correlator, etc.

Fig: 26



Applications: Usually the instrument operates at two modes viz. ***Static Light Scattering*** mode and ***Dynamic Light Scattering*** mode.

Static Light Scattering – this mode measures the intensity and angular dependence of the scattered light (i.e. Elastic or Rayleigh scattering), which gives the information on molecular mass, radius of gyration and second virial coefficient (interaction of solvents with the substances under study). Thus it is helpful in characterizing macromolecules and their associations.

Dynamic Light Scattering – this mode measures the fluctuations in the intensity of the scattered light caused by the continuous motion of the particle (Brownian motion). Continuous movement of particles in the medium will cause the frequency of the scattering light to change (Doppler shift) which is referred as *Quasi-Elastic Light Scattering (QELS)* and the technique *Photon Correlation Spectroscopy*. The measurement gives the information on the translational diffusion coefficient and the polydispersity of the sample, which are useful in determining the macromolecule size and their aggregations.

10. SUMMARY

The following is the summary of the foregone descriptions:

TECHNIQUES	APPLICATIONS
SPECTROSCOPY	
Atomic Absorption and Emission Spectroscopy **(AAS/AES)**	To analyse alkali and alkaline earth metals in dilute solution, natural liquids, and extracts at trace levels
Ultraviolet-Visible Spectroscopy **(UV/Vis)**	To analyse molecular (organic) and ionic species capable of absorbing at UV or Visible wavelengths in dilute solutions
Fourier Transform Infrared Spectroscopy **(FT-IR)**	To analyse only molecular compounds (organic compounds, natural products, polymers, etc.)
Fourier Transform Raman Spectroscopy **(FT-Raman)**	To analyse molecular (organic) compounds which are not responding well in the IR region and hence, it is an alternate to IR
Nuclear Magnetic Resonance Spectroscopy **(NMR)**	To identify and characterize the organic and inorganic compounds
Microwave Spectroscopy	To analyse simple gaseous molecules in Far IR region, to study their stereo chemistry
Electron Spin Resonance Spectroscopy **(ESR)**	To study the formation and life time of the free radicals formed in organic reactions and also finds applications in biological works
Molecular Flourescence Spectroscopy	To study the molecular and ionic compounds in dilute solutions capable of giving flourescence, finds applications in vitamin analyses
CHROMATOGRAPHY	
High Performance Liquid Chromatography **(HPLC)**	To separate and analyse complex mixtures or solutions which include liquids and solids of both organic and inorganic origins
Gas Chromatography **(GC)**	To separate and analyse mixtures of volatile organic compounds, solvent extracts and gases
THERMAL ANALYSIS	
Thermogravimetric Analysis **(TGA)**	To study the mass changes of materials like polymers, glasses, ceramics, etc., such as evaporation, decomposition, gas absorption, de-sorption, dehydration, etc.
Thermomechanical Analysis **(TMA)**	To study the expansion coefficient of composite and laminate materials
Differential Thermal Analysis **(DTA)**	To study the exothermic and endothermic behaviour of clay materials, ceramics, ores, etc.
Differential Scanning Caloriemetry **(DSC)**	To study the glass transition temperature, curing process of the thermoset polymers and heat of melting of thermoplastic polymers.

X-RAY TECHNIQUES	
X-Ray Fluorescence **(XRF)** Spectrometry and X-Ray Photo-emission Spectrometry **(XPS)**	To identify the elements and their valence states present in the surface of the materials
X-Ray Diffractometry **(XRD)**	To study the crystalline properties of solid substances
MICROSCOPY	
Scanning Electron Microscopy **(SEM)**	To study the topography, electronic structure and compositions of metals, ceramics, polymers, composites and biological materials
Transmission Electron Microscopy **(TEM)**	To study the local structures, morphology, and dispersion of multicomponent polymers, cross sections and crystallizations of metallic alloys, semiconductors, microstructure of composites, etc.
Scanning Probe Microscopy **(SPM)**	To study the hardness and topography of materials like ceramics, polymers, composites, etc., on a nano-scale range
ELECTRO-CHEMICAL TECHNIQUES	
Polarography	To study and determine metals, metal complexes and organic compounds in trace levels
Capillary Electrophoresis **(CE)**	To study and characterize biologically active compounds like proteins, amino acids and other bio-molecules
MISCELLANEOUS TECHNIQUES	
Total Organic Carbon Analyzer **(TOC)**	To monitor pollutants in environmental studies by determining the carbon contents of the trace compounds
Elemental Analyzer **(CHN/S)**	To estimate percentage compositions of elements like carbon, hydrogen, nitrogen and sulphur present in newly synthesised organic compounds, pharmaceuticals, etc.
Polarimetry	To analyse and quantitate optically active compounds like sugar
UV/Visible Spectropolarimetry (Circular Dichroism **(CD)** and Optical Rotatory Dispersion **ORD)**)	To get the structural information of optically active compounds like, amino acids, proteins, etc.
Vibrational Circular Dichroism **(VCD)** and Vibrational Linear Dichroism **(VLD)**	Same as above but in the IR region. VLD measurement is employed to study the molecular orientations of thin polymer films
Mass Spectrometry **(MS)**	To identify the organic compounds. Often used as detectors with HPLC and GC
Laser Light Scattering System **(LLIS)**	In the study of macromolecules like polymers, gels, proteins, etc., for determining molecular mass & size and their associations

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