CHAPTER - 2

INSTRUMENTAL METHODS OF ANALYSIS

Introduction, absorption of radiation, UV-Visible Spectrophotometer: Instrumentation and application, IR Spectrophotometer: Instrumentation and application, Thermal methods of analysis-TGA, DTA, DSC, Sensors: Oxygen and Glucose sensor, Cyclic Voltammetry for redox system.

3.1 INTRODUCTION

Analytical instrumentation plays an important role in the production and evaluation of new products and in the protection of consumers and environment. It is used in checking the quality of raw materials such as substances used in integrated circuit chips, detection and estimation of impurities to assure safe foods, drugs, water and air, process optimization and control, quality check of finished products and research and development. Most of the modern instruments are microprocessor/computer controlled with user friendly software for collection of data, analysis and presentation.

This chapter deals with the different types of analytical instrumental methods that find use in a variety of industries. These include molecular spectroscopic methods, thermal methods of analysis, X-ray diffraction, scanning electron microscope and sensors.

3.2 SPECTROSCOPY

It is the study of interaction of electromagnetic radiation with matter consisting of atoms and molecules. When a substance is irradiated with electromagnetic radiation, the energy of the incident photons may be transferred to atoms and molecules raising their energy from ground state level to excited state. This process is known as absorption and the resultant spectrum is known as absorption spectrum. The process of absorption can occur only when the energy difference between the two levels E is exactly matched by the energy of the incident photons as given by the equation

$$\mathbf{E = h\upsilon = hc/\lambda}$$

where **h** is Planck's constant(6.63×10^{-34}Js), **υ** is the frequency of incident radiation, **c** is the velocity of light and **λ** is the wavelength of the incident radiation. The excited state atoms and molecules then relax to the ground state by spontaneous emission of radiation. The frequency of the radiation emitted depends on E.

The energy changes that occur in atoms and molecules during interaction with different regions of electromagnetic radiation are given below.

Radiation absorbed	Energy of the radiation (J/mole)	Effect on the atoms/molecules	Applications

γ-radiation	$> 10^9$	Change in nuclear configuration	Used for cancer radiotherapy.
X- radiation	10^7- 10^9	Change in core electron distribution	Chemical crystallography, qualitative and quantitative analysis.
Ultraviolet and Visible radiation	10^5-10^7	Change in valence shell electron distribution.	In qualitative and quantitative analysis.
Infra red rays	10^3-10^5	Change in the vibrational and rotational energy levels	Detection of functional groups in compounds, calculation of force constant, bond length, etc., and in quantitative analysis
Microwave radiation	10-10^3	Change in rotational energy levels	Calculation of force constant, bond length , bond angle, etc.
Radio frequency	10^{-3} - 10	Changes in nuclear and electron spin in the presence of external magnetic field.	Detection of proton environment and paramagnetic ions.

3.2.1 UV-Visible spectroscopy

The UV –Visible spectroscopy is also known as electronic absorption spectroscopy as molecules absorb radiation resulting in transitions between electronic energy levels. Absorption of radiation in the UV (wavelength range 190-400nm) and visible (wavelength 400–800nm) regions result in transitions between electronic energy levels. The principle of electronic transitions and the instruments required to record electronic transitions are common for both the regions. The electronic transition occurs based on *Franck Condon* principle which states that electronic transition takes place so rapidly that a vibrating molecule does not change its inter-nuclear distance appreciably during the transition.

Polyatomic organic molecules, according to molecular orbital theory, have valence shell electronic energy structure as shown in **Fig 3.1**.

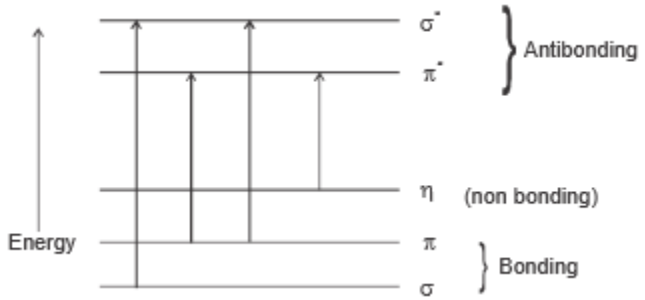


Fig.3.1 Valence shell electronic structure of polyatomic molecules and possible electronic transitions

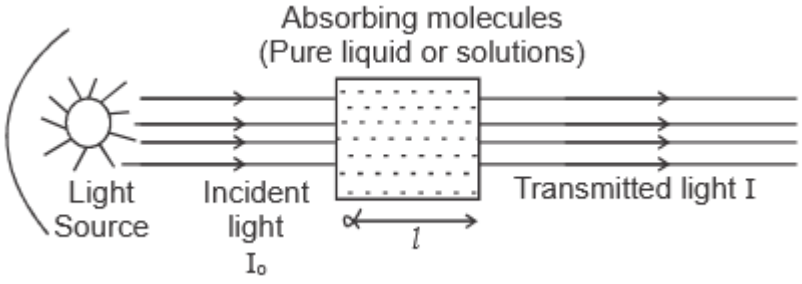
In most of the organic molecules, the bonding and non-bonding molecular orbitals are filled, and the anti-bonding orbitals are vacant. The various electronic transitions that can take place include (i) σ-σ^* (ii) n-σ^* (iii) π-$\pi^*$and (iv) n-π^*. The relative energy changes involved in these transitions are in the increasing order n-π^* < π-π^* ~ n-σ^* << σ-σ^*.

n-π^*, π-$\pi^*$and n-σ^* transitions account for the absorption in 200 – 800 nm region of the electromagnetic spectrum. On the other hand, σ-σ^* transition occur in vacuum UV region below 200 nm.

3.2.2 Laws of Absorption

The fraction of the photons absorbed by the molecule at a given frequency depends on

1. The nature of the absorbing molecules
2. The concentration of the molecules (C). The higher the molar concentration, the higher is the absorption of photons.
3. The length of the path of the radiation through the substance or the thickness of the absorbing medium. Larger the path length (in cm), larger is the number of molecules exposed and greater is the probability of photons being absorbed.



Lambert's law

When a monochromatic beam of radiation passes through an absorbing medium, the intensity of the transmitted radiation decreases exponentially with the thickness of the absorbing medium. The law is expressed as

$$\mathbf{I_t = I_o 10^{-kx}} \qquad \mathbf{(1)}$$

I_t and I_o are the intensities of the transmitted and incident beams of radiations, x is the thickness of the absorbing medium and k is a constant.

Beer's law

When a monochromatic beam of radiation passes through an absorbing medium, the intensity of the transmitted radiation decreases exponentially with the concentration of the absorbing substance. The law is expressed as

$$\mathbf{I_t = I_o 10^{-k'C}} \qquad \mathbf{(2)}$$

where C is the molar concentration of the absorbing substance and k' is another constant.

Beer-Lambert's law

When a beam of monochromatic radiation is passed through a transparent absorbing medium, the decrease in the intensity of radiation is directly proportional to the concentration of the absorbing substance and the thickness of the absorbing medium.

$$\mathbf{-\frac{dI}{I} = kC\ dx}$$

where I is the intensity of radiation, ***C*** is the molar concentration of the absorbing species, **x** is the thickness of the absorbing medium and **k** is the proportionality constant. If $\mathbf{I_o}$ is the intensity of incident radiation and **I** is the intensity of transmitted radiation, after passing through a path length (thickness) of ***l*** cm in the solution, and upon integrating the above equation, between the limits $I = I_o$ when x= 0 and I= I at x= *l*, we get,

$$\int_{Io}^{I} \frac{dI}{I} = -\ kC \int_{0}^{l} dx$$

$$\mathbf{\ln \frac{I}{Io} = - \ kCl}$$

$$2.303 \log \frac{\mathbf{I}}{\mathbf{Io}} = \mathbf{-kCl}$$

$$\log \frac{Io}{I} = \frac{kC\,l}{2.303} \quad \text{or}$$

$$\log \frac{Io}{I} = \varepsilon\, C l \quad \textbf{(where } \varepsilon = k/2.303\textbf{)}$$

ε is the molar absorptivity or molar extinction coefficient, and $\log I / I_o = A$ which is known as the **absorbance** of the material.

$$A = \varepsilon\, C\, l \qquad \textbf{(3)}$$

Thus absorbance **A,** also known as **optical density,** is directly proportional to (i) the concentration **C** of the absorbing species and (ii) the path length ***l*** and has no units. Eq. (3) is the mathematical expression for Lambert's Beer law.

ε is defined as the absorbance of the solution of unit molar concentration (1M) placed in a cell of path length one cm**.** If **C** is expressed in mol dm^{-3}, then the unit for **ε** is $dm^3\ mol^{-1}\ cm^{-1}$.

Limitations of Beer –Lambert's law

Beer-Lambert's law is strictly valid only in dilute solutions. For dilute solutions, a linear relationship is exhibited by a plot of absorbance (A) as a function of concentration of the absorbing substance (C), as shown in **Fig 3.2**.

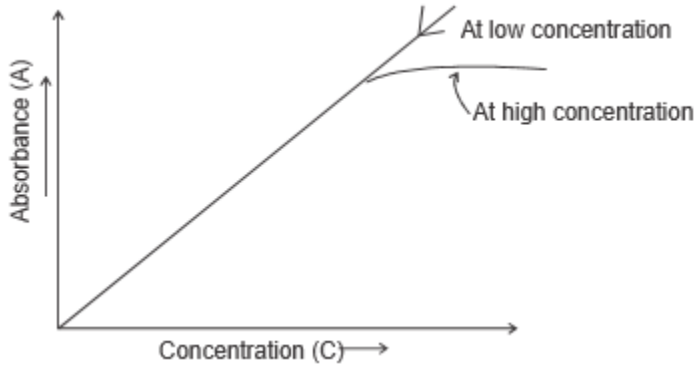


Fig 3.2 Plot of Absorbance versus Concentration

(i) Real deviations occur at higher concentration of the absorbing species. At higher concentrations (>10^{-3}M), there is a change in the refractive index of the solution.
(ii) Chemical deviations occur when there is more than one absorbing species present in the solution. When the absorbing molecules associate or dissociate in the solution, there is a change in the number of absorbing species.
(iii) Instrumental deviation occurs due to changes in absorptivity of the species as a function of instrumental bandwidth.

Transmittance T

Transmittance is defined as the "fraction of the incident light that is transmitted by a given species".

$$\mathbf{T = \frac{I}{Io}}$$

where I is the intensity of transmitted light and I_o is the intensity of incident light.

(Absorbance) $\mathbf{A = \varepsilon C l}$

$$\mathbf{\log \frac{Io}{I} = \varepsilon C l}$$

$$\mathbf{A = - \log T}$$

$$\mathbf{A = \log \frac{1}{T} = \varepsilon C l} \qquad \mathbf{(4)}$$

Transmittance T is expressed as % T.

3.2.3 Instrumentation of UV-Visible spectrophotometer

The instrument used to record the spectra of molecules is called a spectrometer. The sophisticated double beam recording UV-Visible spectrophotometer covers the entire wavelength range of 190 - 800 nm. The basic components are

1. Source of radiation
2. Monochromator
3. Sample cell
4. Detector
5. Display/ Recorder

The block diagram of ultraviolet and visible spectrophotometer is shown in **Fig 3.3**

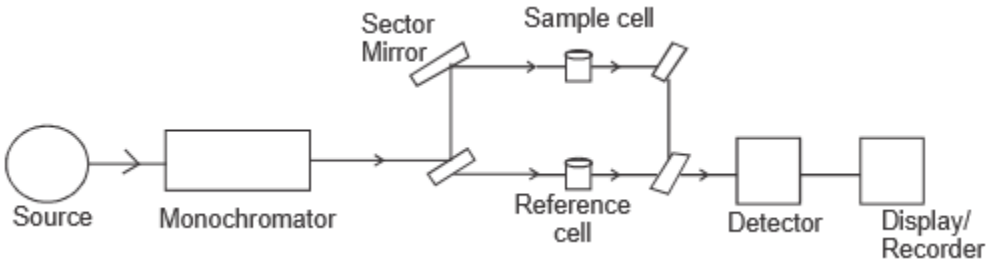


Fig 3.3 Block diagram of UV-Visible spectrophotometer

1. **Radiation source**: Hydrogen discharge lamp or deuterium lamp is used as UV radiation source. For visible light, tungsten filament lamp is used.

2. **Monochromator**: It disperses the polychromatic radiation from the source to a narrow range of wavelength. For UV and visible light, quartz prism or a grating is used. Two types of prisms, namely 60^o Cornu quartz prism and 30^o Littro prisms are employed. For visible light, a glass prism can be used.

3. **Sample holder (cells or cuvettes)**: Sample containers should be transparent to UV and visible radiation. Cuvettes made of quartz are used for both UV and Visible region, whereas for visible light, glass cuvettes are used. Standard path length of these cuvettes is usually 1 cm.

4. **Sector mirror**: The monochromatic beam of radiation is split into two parallel beams by the sector mirrors which pass through the sample and reference cells and reach the detector.

5. **Solvents for UV region:** Electronic absorption spectra are usually recorded for solutions. Solvent used should absorb in the same region as the solute. Solvents used in the UV and visible region are water, methyl alcohol, ethyl alcohol, chloroform, hexane, etc. 95% ethyl alcohol is the most widely used solvent in UV region since it is a polar solvent, cheaper and transparent up to 210 nm.

6. **Detectors:** Photovoltaic cells or photo emissive cells or the more sensitive photomultiplier tubes are used to convert the incident photons into electric current.

7. **Display/Recorder**: The wavelength drive of the recorder and display unit are synchronized so that the detector signal converted into the transmittance or absorbance units is recorded as a function of wavelength of the incident beam of radiation.

In UV-visible spectrometer, a beam of light is split into two equal halves. One half of the beam (sample beam) is directed towards the sample cell containing the solution of the compound being analyzed and the other half (reference beam) through the reference cell that contains only the solvent. The instrument is so designed that it can compare the intensities of both the beams at each wavelength of the region 190-800 nm. If the compound absorbs light at a particular wavelength, the intensity of the sample beam, I will be less than the intensity of the reference beam I_o. An output graph, which is a plot of the wavelength (λ) versus the absorbance (A) at each wavelength obtained, is known as absorption spectrum.

Characteristics of UV and Visible spectra

1. λ_{max} value is the wavelength at which absorption maximum occurs and is different for different molecules.
2. ε value (molar absorptivity) for a given concentration of the compound is related to the height of the absorption band.

The λ_{max} and ε value depend upon the concentration and structure of the molecule and therefore used in characterization and in quantitative estimation of a compound. Unsaturated groups having n or π electrons are essentially responsible for absorption and these fragments are known as chromophores. Simple chromophores such as C=C, C≡C, C≡N, N=N, C=O undergo n-π^*

transitions in the short wavelength regions of UV light. Saturated groups containing hetero atoms which modify the absorption of the chromophores are called auxochromes - e.g. -OH, -Cl,-OR, NR_2, etc. UV visible spectrum of benzene in ethanol is shown below.

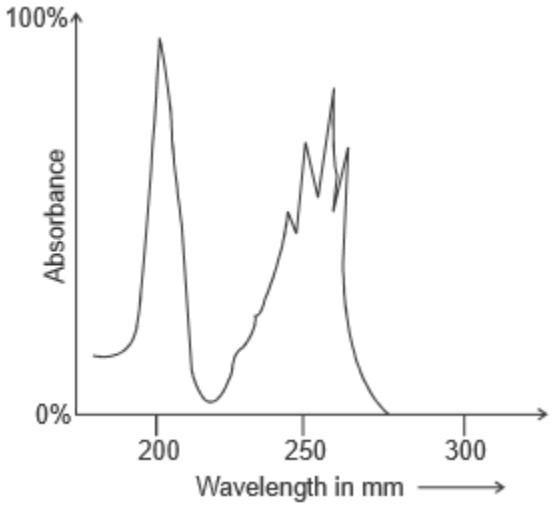


Fig.3.4. Electronic absorption spectrum of a solution of benzene in hexane (λ_{max} = 225 nm)

3.2.4 Applications of UV-Visible Spectrophotometry

1. Qualitative Analysis

(a) UV-Visible spectra aids in the identification of unknown organic samples.

Observation	Possible conclusion
Absorption below 200 nm	Molecules contain only σ bonds or lone pairs or isolated double bonds. E.g. $CH_2=CH_2$ (λ_{max}=180nm) Presence of conjugated double bonds is indicated by an increase in λ_{max} E.g. butadiene ($CH_2=CH-CH=CH_2$) absorbs at 210 nm. Long chain conjugated molecules such as polyenes, carotenes, etc absorb in the visible region with very high ε value.
Strong absorption between 200 and 250 nm (ε=1000)	Presence of aromatic ring. E.g. benzene (λ_{max} = 250 nm)
Weak absorption near 300 nm	Carbonyl compound (containing C=O)

(b) Purity check**:** ε value is used in the identification of the substance. The magnitude of ε value depends on the chemical nature of the absorbing substance and the wavelength of incident light (λ).The purity of the sample can be checked by comparing the ε values of the test sample with the standard sample. Deviations show the presence of impurity or adulteration in the test sample.

2. Quantitative analysis - Many organic compounds and inorganic complexes may be determined by direct absorbance measurement values using the Lambert's Beer law.

$$\mathbf{A = \varepsilon C \mathit{l}}$$

A plot of Absorbance (A) vs. C the concentration gives a linear plot.

3. Determination of dissociation constants of weak acids and bases from the change in absorption spectra with pH.

4. Study of kinetics of chemical reactions.

5. Study of electronic structure of molecules such as vitamins, detecting steric hindrance, etc.

3.2.5 IR SPECTROSCOPY

Defnition

It is the spectroscopy which deals with the infrared region(700nm to 1000μm) of the electromagnetic spectrum with a longer wave length and lower frequency than visible light.

Principle

IR spectra is produced by the absorption of energy by a molecule in the infrared region and the transitions occur between vibrational levels. Hence IR spectroscopy is also known as vibrational spectroscopy
It is divided into three regions.

(i) Near IR – 12500 to 4000 cm^{-1}
(ii) IR – 4000 to 670 cm^{-1}
(iii) Far IR – 670 to 50 cm^{-1}

The most useful IR region lies between 4000 to 670 cm^{-1}

Theory of IR absorption

IR radiation does not have sufficient energy to induce electronic transitions like UV-Visible spectroscopy. It causes only vibrational and rotational changes. For a molecule to absorb IR radiation, two conditons must be satisfied.

(i) There must be change in the net dipole moment of the molecule during the vibration.

(ii) The energy of the IR radiation must match th energy difference between two vibrational levels.

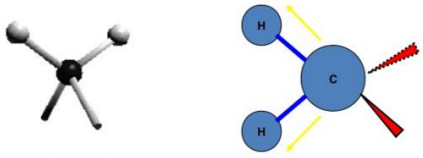
The bonds of a molecule experience various types of vibrations. The atoms are not stationary and fluctuate continuously. Vibrational motions are defined by stretching and bending modes. There are two types of vibrations.

(i) Stretching Vibration – Symmetric and Asymmetric

(ii) Bending Vibration – (a) Inplane bending – Rocking, Scissoring,
(b) Outplane bending – Wagging and Twisting.

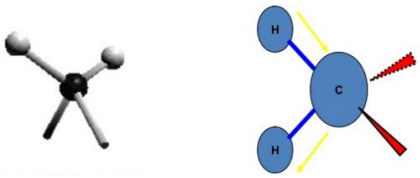
a) Symmetrical stretching:

2 bonds increase or decrease in length simultaneously.



b) Asymmetrical stretching

in this, one bond length is increased and other is decreased.



a) In plane bending

i. Scissoring:

- This is an in plane blending
- 2 atoms approach each other
- Bond angles are decrease

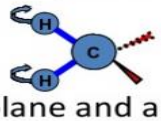
ii. Rocking:

- Movement of atoms take place in the same direction.

b) Out plane bending

i. Wagging:

- 2 atoms move to one side of the plane. They move up and down the plane.



ii. Twisting:

- One atom moves above the plane and another atom moves below the plane.

3.2.6 Instrumentation

The basic components of an infrared spectrophotometer are as follows.

(i) Source – The most common sources used are the Nernst glower and th eglobar. The Nernst glower is a tube made up of zirconium, yttrium and thorium.The globar is a cylindrical rod made up of SiC. Both need to be heated to 1000^0C to 1800^0C to emit IR radiation.

(ii) Sample cells and Sampling techniques – The sample cells are made up of Nacl and it is transparent to IR light. Gaseous samples are taken in a 10 cm long cell. Liquid samples are placed between two discs of Nacl. Solid samples are made into a mull by grinding with Nujol (mineral oil) or a pellet by grinding with KBr pellets.

(iii) Solvents – The solvent should be transparent to IR light and must dissolve the sample completely. Eg., CCl_4, CS_2, etc.

(iv) Monochromator – The monochromator separates polychromatic radiation into individual wavelengths. Eg., NaCl, LiF, CaF_2, etc.

(v) Detectors – They convert the light signal into electrical signal. Photovoltaic cells, photoconductive cells, bolometers, thermocouples, etc are used as detectors.

(vi) Amplifier / Recorder – The electrical signal is amplified and converted to percentage transmittance as a function of wavenumber and recorded.

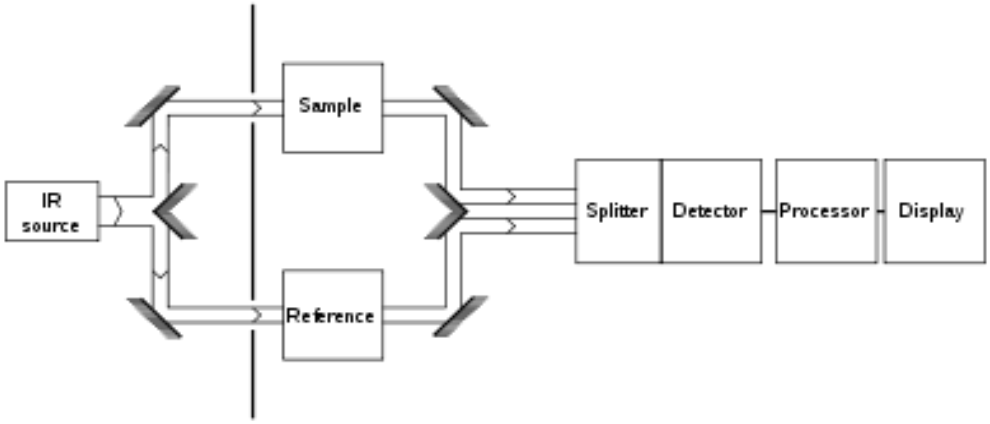


Fig 3.5 Block diagram of IR spectrophotometer

3.2.7 Application

(i) Identification of functional group and structure elucidation

Entire IR region is divided into group frequency region and fingerprint region. Range of group frequency is 4000-1500 cm^{-1} while that of finger print region is 1500-400 cm^{-1}.
In group frequency region, the peaks corresponding to different functional groups can be observed. According to corresponding peaks, functional group can be determined.
Each atom of the molecule is connected by bond and each bond requires different IR region so characteristic peaks are observed. This region of IR spectrum is called as finger print region of the molecule. It can be determined by characteristic peaks.

(ii) Identification of substances

IR spectroscopy is used to establish whether a given sample of an organic substance is identical with another or not. This is because large number of absorption bands is observed in the IR spectra of organic molecules and the probability that any two compounds will produce identical spectra is almost zero. So if two compounds have identical IR spectra then both of them must be samples of the same substances.
IR spectra of two enatiomeric compound are identical. So IR spectroscopy fails to distinguish between enantiomers.
For example, an IR spectrum of benzaldehyde is observed as follows.
C-H stretching of aromatic ring- 3080 cm^{-1}
C-H stretching of aldehyde- 2860 cm^{-1} and 2775 cm^{-1}
C=O stretching of an aromatic aldehyde- 1700 cm^{-1}
C=C stretching of an aromatic ring- 1595 cm^{-1}
C-H bending- 745 cm^{-1} and 685 cm^{-1}

(iii) Studying the progress of the reaction

Progress of chemical reaction can be determined by examining the small portion of the reaction mixure withdrawn from time to time. The rate of disappearance of a characteristic absorption band of the reactant group and/or the rate of appearance of the characteristic absorption band of the product group due to formation of product is observed.

(iv) 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 compound.

(v) 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 I_0/I_t of peaks for standard and test sample is compared. This is called base line technique to determine the quantity of the substance.

Limitation

a. Molecular weight cannot be predicted.
b. It is frequently non- adherence to Beers law of complexity spectra.
c. IR spectroscopy does not provide information of relative position of different functional group on a molecule.

3.3 THERMAL ANALYSIS

Thermal analysis includes a group of techniques which monitors the change in physical properties such as weight, temperature or enthalpy of a sample material as a function of temperature. The sample is subjected to a programmed heating from an initial lower temperature to a final higher temperature at a specified heating rate during the analysis. The most commonly used techniques include thermogravimetry (TG), differential thermal analysis (DTA) and differential scanning calorimetry (DSC). Thermal analysis has been used to determine the physical and chemical properties of polymers, electronic circuit boards, geological materials, etc. Thermal events that may occur in the sample as it is undergoing a change in temperature include phase transitions, melting, sublimation/volatilization, decomposition, glass transition in polymers, oxidation/reduction, etc. The summary of thermal analysis techniques is given below.

Technique	Quantity measured	Typical applications
Thermogravimetric analysis (TGA)	Change in weight of the sample is recorded as a function of temperature.	Thermal stability of a substance and compositional analysis of alloys and mixtures and corrosion studies.
Differential thermal analysis (DTA)	Temperature difference between a sample substance and the reference material is measured as a function of temperature when subjected to a controlled temperature programme.	Generation of phase diagrams and study of phase transitions of a solid sample, thermal stability and characterization of polymers
Differential scanning calorimetry (DSC)	Difference in energy inputs into a sample substance and a reference material is measured as a function of temperature when subjected	Reaction kinetics, purity analysis of drugs

	to a controlled temperature programme.	

The thermal analysis instrumentation consists of four components

(i) The furnace which is controlled by the computer and a temperature sensor and has a controlled atmosphere such as air or inert gases (N_2, He, Ar).
(ii) The sample and its container
(iii) The sensors for measuring temperature and sample properties
(iv) The computer, data collection and processing equipment and a display device for the results

3.4 THERMOGRAVIMETRY (TG)

Definition: Thermogravimetry is a technique in which a change in weight of the sample is recorded as a function of temperature.

Principle: The weight of the sample is continuously monitored as a function of temperature when the sample is heated at a controlled heating rate of 10-20^{o}C/minute. When the temperature is increased from ambient to 1200^{o}C, the sample may undergo dehydration, decomposition or volatilization which results in direct weight loss. The online plot of sample weight versus temperature is called a TG thermogram.

Instrumentation: The major component of TG is the thermobalance or thermogravimetric analyzer for measuring the mass. It includes a thermobalance and a microprocessor controlled tubular furnace. **Fig 3.5** shows the instrumentation of TG.

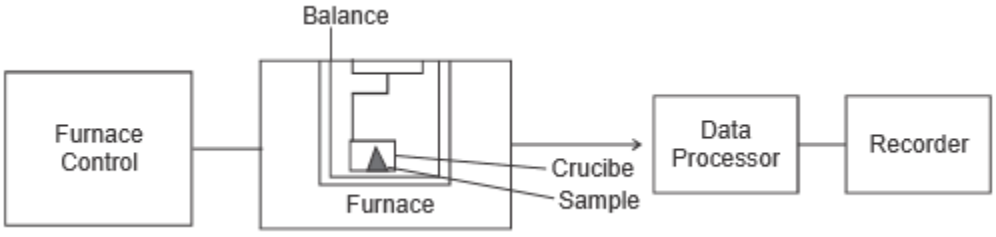


Fig 3.5 Block diagram of TG apparatus

1. **Sample**: A solid sample of 5-50 mg is placed in a platinum crucible (sample container) and connected to a sensitive microbalance. The sensitive microbalance can detect a weight change of 1µg of the sample.

2. **Thermobalance**: The balance is placed inside the tubular furnace. A thermocouple, located immediately below the crucible, monitors the furnace temperature. The temperature of the furnace is accurately controlled and programmed for any change by the microprocessor.
3. The **null point** balance is used in TG. When there is a **change in the weight** of the sample, the balance beam will deviate from its usual position. A sensor detects the deviation and

initiates a force that will restore the balance to the null position. The restoring force is proportional to the change in weight. The atmosphere inside the furnace can be controlled by using inert gases such as nitrogen, helium or argon or reactive gases such as oxygen, hydrogen, etc.

4. **Data processor and recorder:** The balance assembly measures the initial weight of the sample and continuously monitors changes in sample weight as heat is applied to the sample inside the furnace. The furnace data and balance data are collected during the experiment and sent to the computer for manipulation. The computer records the TG curve.

The thermogram obtained for calcium oxalate monohydrate is shown in **Fig 3.6** and the various thermal reactions that occur when calcium oxalate monohydrate is heated from 30^{o}C to about 1000 ^{o}C is summarized in Table 3.1. The horizontal portions or plateaus indicate regions where there is no weight loss and the curved portion or downward steps indicate regions of weight loss.

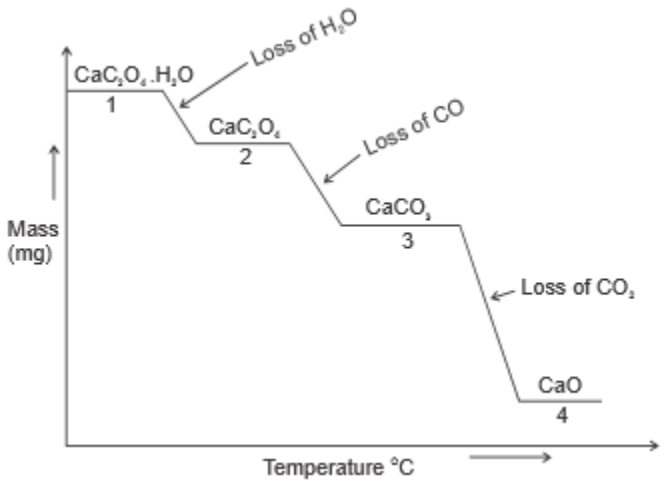


Fig 3.6 TG curve for decomposition of $CaC_2O_4.H_2O$

Table 3.1 Summary of thermal reactions in the decomposition of calcium oxalate monohydrate

Temperature range in ^{o}C	Thermal reaction	Change in mass
30 - 130	1st plateau region. $CaC_2O_4.H_2O$ is thermally stable	No change in mass
130 - 190	1st downward step. $CaC_2O_4.H_2O \longrightarrow CaC_2O_4 + H_2O$	Loss of water of crystallization and there is decrease in mass

190 - 400	2^{nd} plateau region. Anhydrous CaC_2O_4.is thermally stable	No change in mass
400 - 470	2^{nd} downward step. CaC_2O_4. $\longrightarrow$ $CaCO_3$ + CO	Decrease in mass due to loss of CO.
470 - 700	3^{rd} plateau $CaCO_3$ is thermally stable.	No change in mass
700 - 840	3^{rd} downward step $CaCO_3$ $\longrightarrow$ $CaO + CO_2$	Decrease in mass due to loss of CO_2
840 - 1000	4^{th} plateau CaO is thermally stable	No change in mass. The residue obtained is CaO.

The themogram obtained for $CuSO_4.5H_2O$ is shown in the **Fig 3.7** and thermal events are summarized in the Table 3.2.

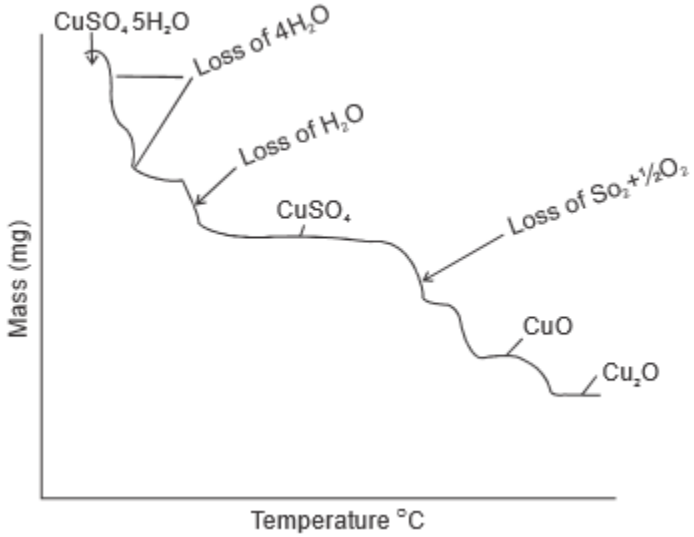


Fig.3.7 TG curve for $CuSO_4.5H_2O$

Table 3.2 Summary of thermal reactions in the decomposition of $CuSO_4.5H_2O$

Temperature range in ^oC	**Thermal reaction**	**Change in mass**
30 - 90	1^{st} plateau region. $CuSO_4.5H_2O$ is thermally stable	No change in mass
90 - 150	1^{st} downward step. $CuSO_4.5H_2O$ → $CuSO_4$. H_2O+3 H_2O	Loss of water of crystallization and there is decrease in mass

150 - 200	2nd plateau region. $CuSO_4.H_2O$ is thermally stable	No change in mass
200 - 275	2nd downward step. $CuSO_4.H_2O \longrightarrow CuSO_4 + H_2O$	Decrease in mass due to loss of H_2O
275 - 700	3rd plateau anhydrous $CuSO_4$ is thermally stable.	No change in mass
700 - 900	3rd downward step. $CuSO_4 \longrightarrow CuO+SO_2+1/2O_2$	Decrease in mass due to decomposition
900 - 1000	4th plateau CuO is thermally stable	No change in mass.
1000 - 1100	4th downward step $2CuO \longrightarrow Cu_2O+1/2O_2$.	Reduction of CuO to Cu_2O and there is decrease in mass.

Applications of TGA

1. In the analysis of thermal decomposition of inorganic salts and complexes which are used as catalysts, semiconductors and fine chemicals.
2. The decomposition temperature of commodity plastics and rubber are investigated by TGA. Each kind of polymer has a characteristic thermogram and can be used for identification purposes**. Fig 3.8** shows the thermogram of some common polymers. The thermal stability of polymers deceases in the order PTFE < LDPE < PMMA < PVC. PVC shows two-stage decomposition.

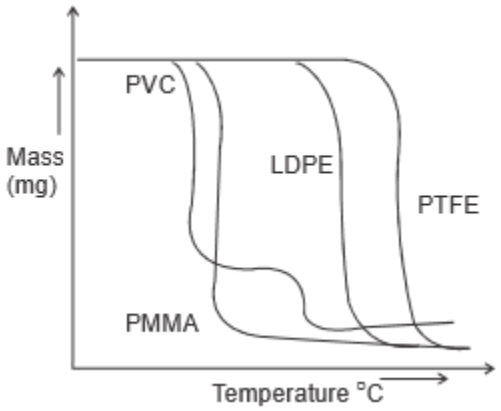


Fig.3.8 TG curve for the determination of thermal stability of polymers.

3. TGA of pharmaceuticals, coal and minerals is useful in the study of complex thermal reactions.
4. In the determination of composition of alloys and mixtures. E.g. determination of a mixture of calcium and strontium as their carbonates. Both undergo decomposition with evolution of CO_2. But the decomposition of $CaCO_3$ occurs in the temperature range 650-850^oC whereas $SrCO_3$ decomposes in the higher range 950-1150^oC.
5. In qualitative analysis of compounds.
6. In studying the oxidation of alloys.

3.5 DIFFERENTIAL THERMAL ANALYSIS (DTA)

Definition: Differential thermal analysis is a technique in which the temperature difference (ΔT) between the sample and an inert reference material is measured as a function of sample temperature when both are heated uniformly.

Principle: When the sample undergoes any transition like melting, dehydration, decomposition etc., there is liberation or absorption of energy by the sample with the corresponding deviation of its temperature from that of the reference. When the sample does not undergo any physical or chemical change, both the sample and the inert reference material are at the same temperature and ΔT is zero. If any endothermic or exothermic reaction occurs in the sample, the temperature of the sample decreases or increases and causes a difference in temperature (ΔT) between the sample and reference. A plot of ΔT vs. T gives the DTA thermogram.

Instrumentation: The instrument consists of a microprocessor controlled furnace, data processor and recorder and a facility to control the atmosphere. A block diagram of the DTA instrument is shown in **Fig 3.9.**

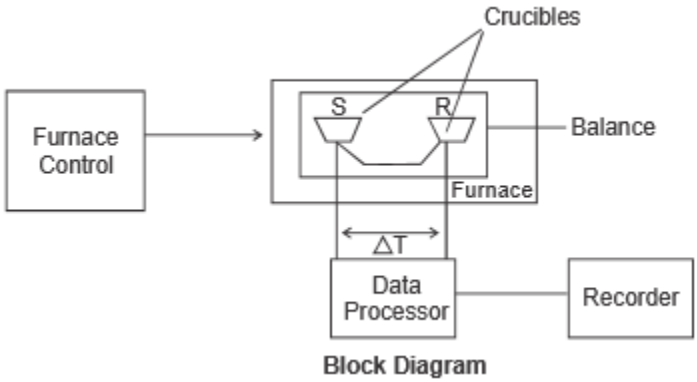


Fig.3.9 Block diagram of the DTA instrument

1. **Sample holder assembly:** The solid sample and the reference material (usually an inert substance) like alumina of 10 mg is placed in a platinum crucible (sample container) and connected to a sensitive microbalance. The temperature of the sample and reference is measured by an individual thermocouple.

2. **Microprocessor controlled furnace**: The whole sample holder assembly is placed inside the furnace. The sample and the reference are heated at the same heating rate from ambient to 1500^oC. A temperature programmer or furnace control maintains a constant heating rate of 1^oC/min -100^oC/min.

3. **Facility to control atmosphere**: Sample and reference chamber are designed to permit the circulation of inert gases such as nitrogen or reactive gases such as oxygen or air.

4. **Data processor and recorder**: The difference in temperature (ΔT) between the sample and the reference (S and R) thermocouples is continuously measured. After amplification, the difference in signal is recorded on the y-axis. The temperature of the furnace is measured by an independent thermocouple and recorded on the x axis. The balance and furnace data collected is sent to the PC for manipulation and a DTA plot of (ΔT) vs. T is obtained.

An idealized DTA curve is shown in **Fig 3.10.** Peak 1 is an exothermic peak and peak 2 is an endothermic peak. Endothermic peaks signify changes in crystallinity or dehydration reactions while exothermic curves results due to chemical reactions such as oxidation.

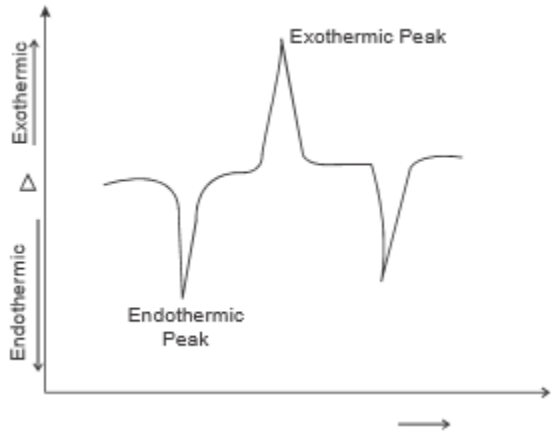


Fig.3.10 Idealized DTA curve.

Applications of DTA

1. **Qualitative analysis of materials**: DTA measurements provide a rapid method for the finger printing of minerals, clays and polymeric materials. E.g. **Fig 3.11** shows the DTA thermogram of calcium oxalate monohydrate in flowing air (O_2) obtained by increasing the temperature at a rate of 8^oC/min. It contains two endothermic peaks and one exothermic peak. The decomposition and oxidation reactions are shown below.

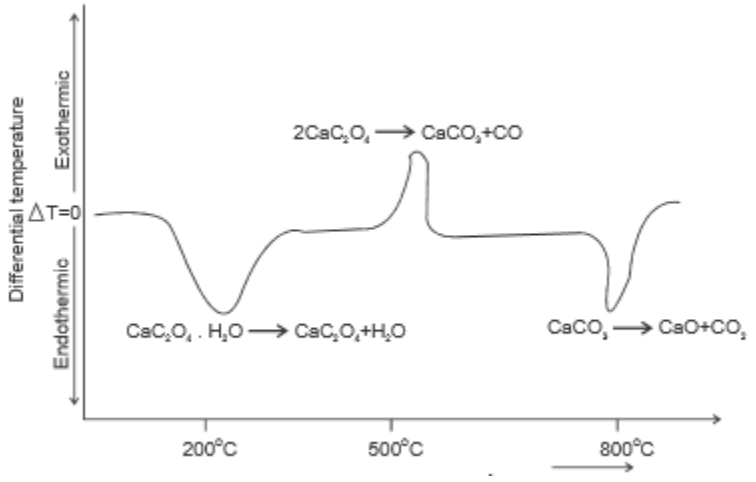


Fig 3.11 DTA curve of calcium oxalate monohydrate in O_2 atmosphere

2. DTA provides information regarding processes like fusion, dehydration, oxidation, reduction, adsorption and solid state reactions and in generation of phase diagram and the study of phase transitions.
3. It provides an accurate way of determining the melting and boiling points for organic compounds.
4. It has been widely used to study and characterization of polymers and qualitative analysis of polymer mixture. **Fig 3.12** is the DTA curve which illustrates the various types of transitions that occurs during heating of a polymer.

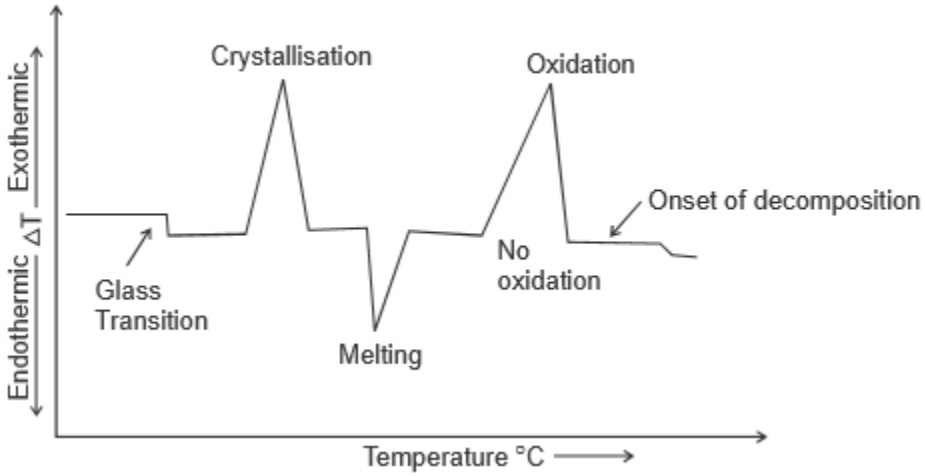


Fig 3.12 DTA thermogram of polymer

3.6 DIFFERENTIAL SCANNING CALORIMETRY (DSC)

Definition: DSC is a thermal method in which the difference in heat flow into the sample and the reference material is measured as a function of linear increase or decrease of the sample temperature.

Principle: DSC measures the difference in heating power (heat flow) required to keep the temperature of a sample and a reference material the same**.** The sample and the reference are heated by separate electrical heaters exactly at the same rate. Whenever the sample undergoes an endothermic or exothermic reaction, there is a change in sample temperature with respect to the reference temperature. The power to the sample heater is modified so that the difference in temperature between the sample and the reference, $\Delta T = 0$. The difference in power supplied to the sample and the reference represents the energy change or enthalpy change (ΔH) in the sample.

Instrumentation: DSC instrumentation is the same as DTA. DSC is a calorimetric method in which the difference in heat energy (ΔH) is recorded as a function of sample temperature. The block diagram of DSC instrument is shown in **Fig 3.13.**

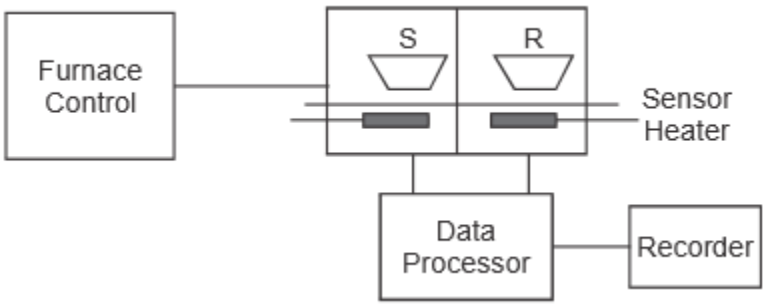


Fig 3.13 Block diagram of DSC instrument

The main components are a microprocessor controlled furnace provided with individual heater and thermocouples for measuring the temperature of sample and reference, data processor and recorder, and a facility for atmosphere control.

1. **Sample holder assembly:** The solid sample and the reference material (usually an inert substance) like alumina of 10 mg is placed in a separate platinum crucible (sample container) and connected to a sensitive microbalance. The sample and the reference are heated separately and the temperature of the sample and reference is measured by individual thermocouple. The thermocouples measure the difference in heat flow.

2. **Microprocessor controlled furnace**: The whole sample holder assembly is placed inside the furnace. The sample and the reference are heated at the same heating rate from

ambient to 700^{o}C. A temperature programmer or furnace control maintains a constant heating rate of 1^{o}C/min -100^{o}C/min.

3. **Facility to control atmosphere**: Sample and reference chamber are designed to permit the circulation of inert gases such as nitrogen or reactive gases such as oxygen or air.

4. **Data processor and recorder**: The difference in heat flow (ΔH) between the sample and the reference (S and R) thermocouples is continuously measured. After amplification, the difference in signal is recorded on the y-axis. The temperature of the furnace is measured by an independent thermocouple and recorded on the x axis. The balance and furnace data collected is sent to the PC for manipulation and the DSC thermogram which is a plot of ΔH vs. T is obtained. A typical DSC thermogram for the decomposition of calcium oxalate monohydrate is shown in **Fig 3.14.**

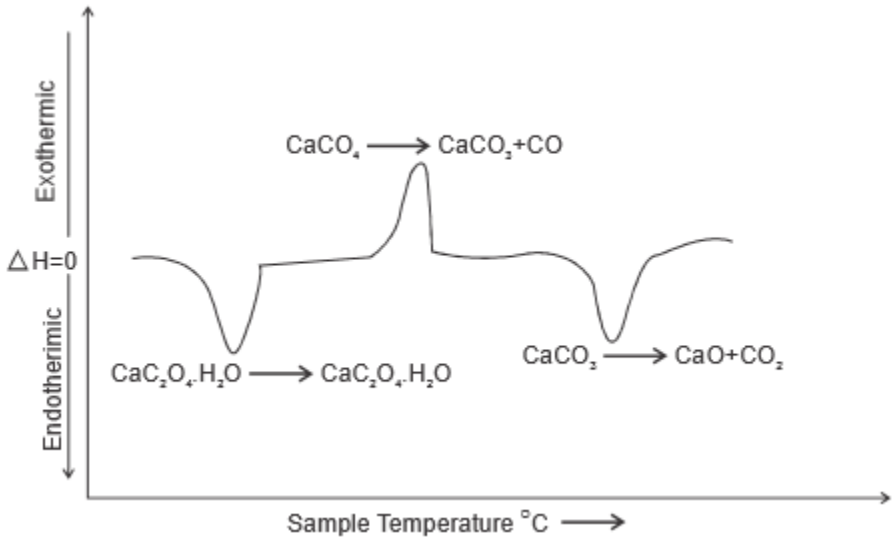


Fig 3.14 DSC thermogram for the decomposition of calcium oxalate monohydrate

Applications of DSC

1. DSC is highly useful in the study of phase transitions and temperature induced changes that occur at very low temperatures in the case of polymers, biological samples, pharmaceutical samples, etc.
2. It finds widespread application in calculating the enthalpy of transitions such as enthalpy of melting, enthalpy of crystallization, enthalpy of fusion, etc. of polymeric materials.
3. DSC also provides accurate method of determining melting, boiling and decomposition points for organic compounds.
4. It is used for the determination of purity of drug samples.

3.7 SENSORS

Devices used to detect changes in physical and chemical quantities are called sensors.

3.8 OXYGEN SENSORS

An oxygen sensor or lambda sensor is an electronic device that measures the proportion of oxygen (O_2) in the gas or liquid being analysed.

1.CLARKE ELECTROCHEMICAL OXYGEN SENSOR

An electrochemical oxygen analyzer is based on electrochemical reduction of O_2 at a negatively polarized electrode. This electrode is known as "Clark Type" after their inventor, Dr. Leland Clark. The Clark electrode consists of a silver anode and a platinum cathode in contact with 0.01M KCl electrolyte solution **(Fig.3.15**). It is covered at the tip by a semi-permeable membrane usually polypropylene membrane or Teflon membrane which is permeable to gases but impermeable to the electrolyte. The sensor is placed in contact with the sample solution containing dissolved oxygen. O_2 diffuses through the membrane and comes into contact with the cathode. The silver/ silver chloride (Ag/AgCl) anode provides electrons for the cathode reaction. Silver at the anode undergoes oxidation. A potential difference of 1.5V is applied between the two electrodes, the cathode being sufficiently polarized (-0.6V) to reduce all of the oxygen. The current in the circuit is proportional to the quantity of gas migrating across the membrane and measures the oxygen concentration in the solution.

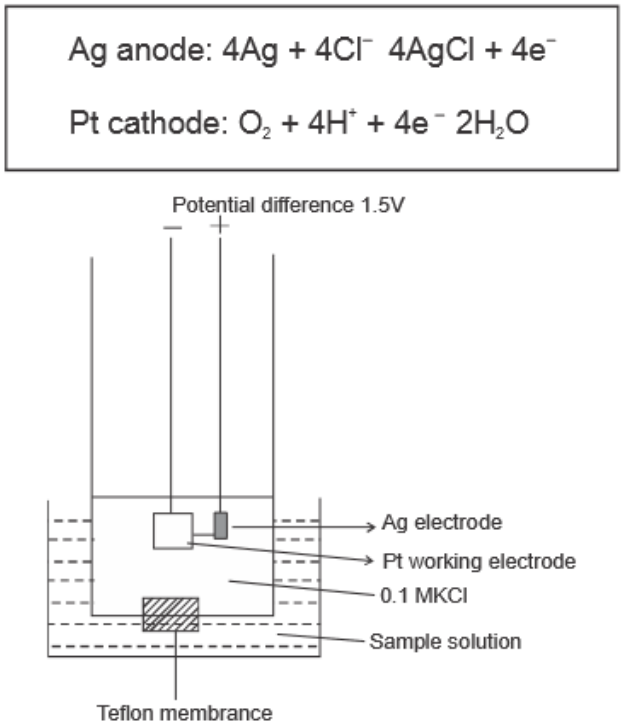


Fig.3.15 Clarke electrochemical O_2sensor.

Limitations:
It is susceptible to damage in the presence of acidic gases such as HCl, H_2S, SO_2 etc.,

2. PARAMAGNETIC OXYGEN SENSOR

Oxygen, nitric oxide and nitrogen dioxide are unique among the ordinary gases in that they are paramagnetic; that is they are attracted into a magnetic field. Oxygen is several times more paramagnetic than nitric oxide or nitrogen dioxide. This property of oxygen is taken advantage in gaseous oxygen analyzers.

In the cell, two glass spheres filled with nitrogen gas are suspended with strong metal **(Fig.3.16**). At first, the spheres are kept in balance in a homogeneous magnetic field. When oxygen molecules having a large magnetic susceptibility flow there, the molecules are pulled toward the stronger magnetic field zone and the spheres are moved away from the zone. The resulting deviation of the spheres is detected with the light source, reflecting mirror and light receiving element, and a current is supplied through the feedback loop to control so that the spheres can return to the initial balanced state. The current flowing through the feedback loop is proportional to oxygen concentration. Thus, oxygen concentration is converted into an electric signal.

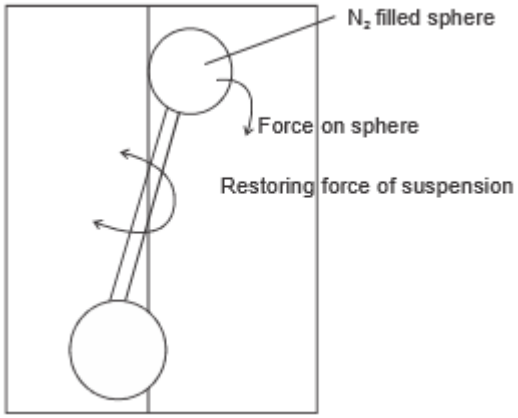


Fig.3.16 Paramagnetic oxygen sensor

3. ZIRCONIA OXYGEN SENSORS

Solid state potentiometric oxygen sensors typically use an oxygen ion conducting material as electrolyte. Yttria stabilized zirconia (YSZ) has been the material of choice due to its ionic nature and better stability in harsh environments. A doping level of 6–12 mol% of yttria stabilizes zirconia in the cubic phase, a more favourable phase for ionic conduction at high temperatures. High temperature electrochemical sensor consists of a cell made up of YSZ which acts as a solid electrolyte.

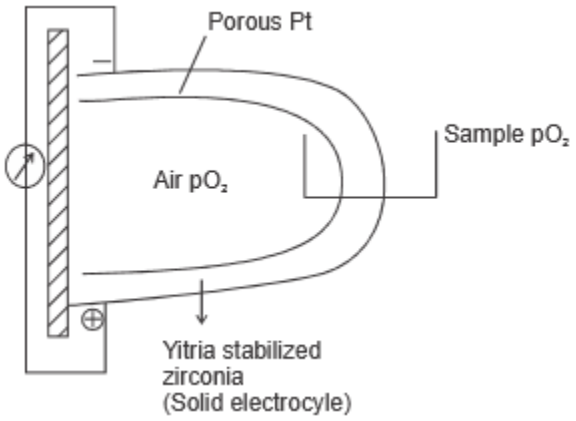


Fig.3.17 Zirconia or high temperature oxygen sensor

Fig. 3.17 shows a schematic diagram of a thimble type YSZ based oxygen sensor. It consists of a ceramic tube made up of YSZ coated with ultra thin layer of porous platinum on the inner and outer surfaces to act as anode and cathode electrodes. At high temperature (above 450^oC), openings in the crystal lattice permits the movement of oxygen ions(O^{2-}). As long as partial pressures are equal on both the sides, the movement of ions into the lattice is random and no net flow within the lattice. When a sample gas containing O_2 is introduced on one side, oxygen ions migrates through the crystal lattice to form a concentration gradient from the higher O_2 partial pressure to the lower pressure inside (reference air). The concentration gradient which determines the amount of O_2 in the sample gas is determined by Nernst equation expression. At a high temperature, the potential difference across the cell is given by Nernst equation.

$$E = \frac{RT}{4F} \log_e \frac{P_1}{P_2}$$

where

E is the potential difference (volts)

R is the gas constant (8.314 J mol^{-1} K^{-1})

T is the absolute temperature (K)

F is the Faraday constant (96500C)

P_1 & P_2 are the partial pressures of the oxygen on either side of the zirconia tube.

Thus if oxygen partial pressure at one side is known and the temperature of the sensor is controlled, the potential difference measured enables us to determine the unknown partial pressure of oxygen in the test sample.

Applications of oxygen sensors

1.To measure the exhaust gas concentration of oxygen for IC engines in automobiles and other vehicles.

2.To measure the partial pressure of oxygen in the breathing gas used by the deep sea divers.

3.Used in anesthesia monitors, respirators and oxygen concentrators.

3.9 GLUCOSE SENSORS

Sensitive and selective glucose sensors are not only relevant for use in blood sugar monitoring, but also in the food industry, bio-processing and in the development of renewable, sustainable fuel cells. The first amperometric enzyme glucose sensor was developed in 1973 in which the anodic production of hydrogen peroxide was analysed.
The enzyme glucose 1-oxidase (GOx) is the main catalytic component used in enzymatic biosensors. It is the 'ideal enzyme' for glucose oxidation, owing to a relatively high selectivity, sensitivity and stability, compared to other enzymatic materials. The key component of the large protein molecule is the redox centre, flavin adenine dinucleotide (FAD). The flavin group is reduced on interaction with glucose, thus producing the redox product of glucolactone, in the first step.

$$GOx(FAD) + \text{glucose GOx} \longrightarrow (FADH_2) + \text{glucolactone}$$

The cofactor is regenerated by reacting with oxygen, leading to the formation of hydrogen per oxide.

$$GOx(FADH_2) + O_2 \longrightarrow GOx(FAD) + H_2O_2$$

$$H_2O_2 \longrightarrow 2H^+ + O_2 + 2\,e^-$$

Glucose concentration is determination by direct measurement of the hydrogen peroxide produced. Hydrogen peroxide is oxidised at a catalytic platinum (Pt) anode. the electrode easily recognises the number of electron transfers and this electron flow is proportional to to the number of blood glucose present in the blood Sensing of hydrogen peroxide at the anodic potential of 0.6V vs. SCE (Saturated calomel electrode), gave a current directly proportional to the glucose concentration. This method offers a more simple, precise and selective approach to blood glucose testing. The oxygen is regenerated in the oxidation of the peroxide, thus replenishing the oxygen electron mediator.

3.10 CYCLIC VOLTAMMETRY

Definition

Cyclic voltammetry (CV) is an electrochemical method which measures the current that develops in an electrochemical cell under conditions where voltage is in excess of that predicted by the Nernst equation. CV is performed by cycling the potential of a working electrode, and measuring the resulting current.

Explanation

In cyclic voltammetry, experiment the working electrode potential is ramped linearly versus time. Unlike in linear sweep voltammetry, after the set potential is reached in a CV experiment, the working electrode's potential is ramped in the opposite direction to return to the initial potential. These cycles of ramps in potential may be repeated as many times as desired. The current at the working electrode is plotted versus the applied voltage (i.e., the working electrode's

potential) to give the cyclic voltammogram trace. Cyclic voltammetry is generally used to study the electrochemical properties of an analyte in solution.

In cyclic voltammetry, the electrode potential ramps linearly versus time in cyclical phases(**fig 3.18**) . The rate of voltage change over time during each of these phases is known as the experiment's scan rate (V/s). The potential is applied between the working electrode and the reference electrode while the current is measured between the working electrode and the counter electrode. These data are plotted as current (*i*) vs. applied potential (*E*, often referred to as just 'potential'). In Figure 2, during the initial forward scan (from t_0 to t_1) an increasingly reducing potential is applied; thus the cathodic current will, at least initially, increase over this time period assuming that there are reducible analytes in the system. At some point after the reduction potential of the analyte is reached, the cathodic current will decrease as the concentration of reducible analyte is depleted. If the redox couple is reversible then during the reverse scan (from t_1 to t_2) the reduced analyte will start to be re-oxidized, giving rise to a current of reverse polarity (anodic current) to before. The more reversible the redox couple is, the more similar the oxidation peak will be in shape to the reduction peak. Hence, CV data can provide information about redox potentials and electrochemical reaction rates.
For instance, if the electron transfer at the working electrode surface is fast and the current is limited by the diffusion of analyte species to the electrode surface, then the peak current will be proportional to the square root of the scan rate. This relationship is described by the Cottrell equation. In this situation, the CV experiment only samples a small portion of the solution, i.e., the diffusion layer at the electrode surface.

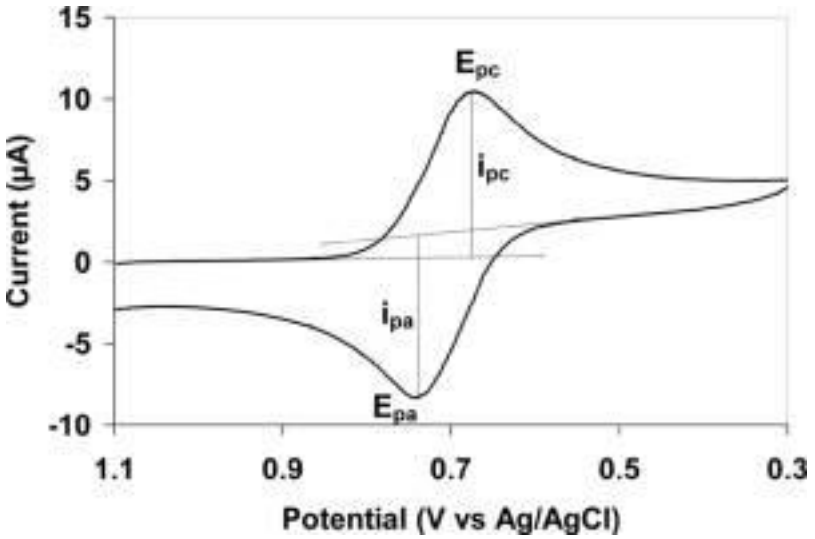


Fig.3.18 Cyclic Voltammetry

Typical cyclic voltammogram where i_{pc} and i_{pa} show the peak cathodic and anodic current respectively for a reversible reaction.

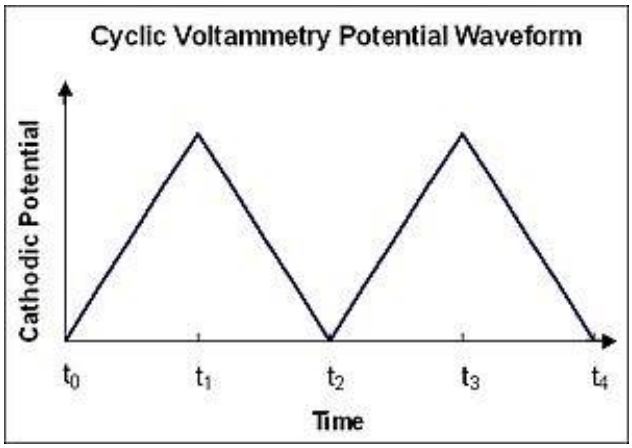


Fig 3.19 Cyclic voltammetry waveform

In cyclic voltammetry, the electrode potential ramps linearly versus time .

Advantages

1. A technique usually used to acquire qualitative information about electrochemical reactions
2. Offers a rapid location of redox potentials of the electroactive species
3. Can be used to get valuable kinetic information of electrode reaction
4. Electrode reaction usually involve e-transfer reaction which is influenced by electrode potential
5. Mass transfer (diffusion) in some cases can control over-all electrode reaction
6. It is a technique to control electrode's potential and measure resultant current.

Disadvantage

1. The effects of slow heterogeneous electron transfer and chemical reactions cannot be separated. If both of these effects are present, then the rate constants for these processes can only be calculated using simulation methods.

2. There is a background charging current throughout the experiment of magnitude nC_{dl} (where C_{dl} is the capacitance of the interface at the working electrode). This restricts the detection limit to about 10^{-5} M. In addition, the ratio of the peak faradaic current to the charging current decreases with increasing n (since i_p is proportional to $n^{1/2}$), and this places an upper limit on the value of n that can be used.

Application

1. Cyclic Voltammetry can be used to study qualitative information about electrochemical processes under various conditions, such as the presence of intermediates in oxidation-reduction reactions, the reversibility of a reaction.

2. CV can also be used to determine the electron stoichiometry of a system, the diffusion coefficient of an analyte, and the formal reduction potential, which can be used as an identification tool.

3. In addition, because concentration is proportional to current in a reversible, Nernstian system, concentration of an unknown solution can be determined by generating a calibration curve of current vs. concentration.

Questions

Part – A

1. Define Beer – lambert's law.
2. What are the limitations of Beer – lambert's law.
3. What is UV-Visible spectrophotometer?
4. What is the sources for UV and visible light in UV-Visible spectrophotometer?
5. What are the requirements of solvents used in UV-Visible spectrophotometer?
6. Give two applications of UV-Visible spectrum.
7. State the principle of IR spectrophotometer.
8. Mention the detectors used in IR spectrophotometer.
9. Give any two applications of IR spectrophotometer.
10. What is the principle of thermogravimetry?
11. Define the principle of DTA.
12. State the principle of DSC.
13. Give two applications of TG.
14. Mention two applications of DTA.
15. State the principle of DSC.
16. Give two applications of DSC.
17. What are sensors? Mention its type.

18. Mention the different type of oxygen sensor.
19. Mention the application of oxygen sensors.
20. Distinguish DSC and DTA.
21. What is glucose sensor?
22. Define cyclic voltammetry.
23. Mention the advantages of cyclic voltammetry.
24. Give the applications of cyclic voltammetry.

Part-B

1. Explain the laws of absorption. Derive the mathematical expression of beer-Lambert's law. Give its limitations.
2. Explain the instrumentation of UV-Visible spectrophotometer with a neat block diagram.
3. Explain the instrumentation of IR spectrophotometer with a neat block diagram.
4. Explain the principle, instrumentation and applications of thermogravimetry.
5. Explain the principle, instrumentation and applications of Differential thermal analysis.
6. Explain the principle, instrumentation and applications of differential scanning calorimeter.
7. What are sensors? Explain the different types of oxygen sensors.
8. What are biosensors? Explain glucose sensor.
9. Explain the working principle of cyclic voltammetry with a neat diagram.