Bachelor of Science
(B.Sc. – CBZ)

Analytical Methods in Chemistry
(DBSZDS101T24)

Self-Learning Material
(SEM 1)

Jaipur National University
Centre for Distance and Online Education

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PREFACE

Welcome to the world of analytical chemistry! In this book, we delve into the fascinating realm of analytical methods used in chemistry. Analytical chemistry is a cornerstone of modern science, playing a vital role in various fields such as pharmaceuticals, environmental monitoring, forensics, and materials science.

This book serves as a comprehensive guide to the principles, techniques, and applications of analytical methods in chemistry. Our aim is to provide students, researchers, and professionals with a solid foundation in analytical chemistry, empowering them to tackle real-world challenges with confidence and precision.

Throughout this book, readers will explore a wide range of analytical techniques, including spectroscopy, chromatography, electrochemistry, and mass spectrometry, among others. Each chapter is designed to offer clear explanations, practical examples, and hands-on exercises to enhance understanding and proficiency in analytical methods.

Additionally, this book emphasizes the importance of critical thinking, problem-solving, and experimental design in analytical chemistry. We encourage readers to approach each topic with curiosity and creativity, fostering a deeper appreciation for the analytical process and its impact on scientific discovery and innovation.

Whether you are a student embarking on your academic journey, a researcher seeking to expand your analytical toolkit, or a professional navigating the complexities of analytical challenges, this book is intended to be your trusted companion. We hope it inspires you to explore the vast and dynamic field of analytical chemistry and equips you with the knowledge and skills to excel in your pursuits.Thank you for joining us on this exciting journey through the world of analytical methods in chemistry. Let's embark on this adventure together and unlock the mysteries of the molecules that shape our world.

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UNIT -I

OPTICAL METHODS OF ANALYSIS

Learning Objective

- Explain various elements comprising a spectrophotometer.
- Define absorbing entities like chromophores and auxochromes.
- Identify substances present in water, wastewater, soil, and air samples through ultraviolet-visible spectrophotometry.
- Discuss flame photometry and atomic absorption spectrophotometry.

1.1 OPTICAL METHODS

Numerous techniques employed in Modern day science specially chemistry and biochemistry rely on the interaction between the analyte and light passing by it.Aalterations in color or light intensity, luminescence, fluorescence, changes in the rotation of polarized light, or light scattering can give an overview about the sample's properties.

Moreover, optical methods stand out as extensively utilized instrumental techniques in environmental chemistry. These approaches gauge outcomes of the interfaceamong electromagnetic radiation and the type of substances.

This spectrum of radiation encompasses X-rays, visible light, and radio waves. Within optical methods of investigation, examine emission, absorption, scattering, or modifications in radiation properties.

The measurement of these effects yields various types of optical methods, offering avenues for identifying and quantifying one or more constituents within a sample.

1.2 INFRARED SPECTROSCOPY

Infrared Spectroscopy involves examining how infrared light interacts with a molecule, which can be assessed through absorption, emission, or reflection. This technique finds widespread

applications in both organic and inorganic chemistry, primarily serving chemists in identifying functional groups within molecules. IR Spectroscopy specifically detects and measures the vibrations of atoms within the sample to be analyzed.

- Using this method, functional groups within molecules can be identified. Typically, stronger bonds and lighter atoms exhibit higher stretching frequencies (wavenumbers) during vibration.
- Infrared segment of electromagnetic spectrum is typically categorized into 3 divisions: near-infrared (NIR), mid-infrared (MIR), and far-infrared (FIR), denoted in relation to visible spectrum.
- Near-infrared range, with higher energy levels, spans approx. 14000-4000 cm^{-1} (0.8-2.5μm wavelength), capable of stimulating overtone or harmonic vibrations.
- In contrast, mid-infrared region, ranging approx.. 4000-400 cm^{-1}(2.5-25μm), is suitable for investigating essential vibrations and their associated rotational-vibrational structures.
- Lastly, far-infrared category, 400-10 cm^{-1}(25-1000μm), positioned togetherin the microwave region, entails lower energy levels and is conducive to rotational spectroscopy.
- It's worth noting that the nomenclature and classifications of these subregions are conventional, loosely grounded in relative molecular or electromagnetic attributes.

1.3 THEORY

- In IR spectroscopy distinctive Frequencies based on structure are absorbed by molecules. These absorptions are resonant frequencies, meaning that the bond or group that is vibrating and the absorbed radiation have the same frequency.
- The molecular potential energy surfaces' morphology, the atoms' masses, and the related vibrancy coupling all influence the energies.
- “IR active” is molecule’s vibrational modewhen connected to modifications in the permanent dipole.
- A molecule possesses various vibrational modes, each representing a distinct way in which it can vibrate. For linear molecules, the number of vibrational modeis given by 3N - 5,

while for nonlinear molecules it's 3N - 6, where N denotes the total number of atoms (also referred to as vibrational degrees of freedom).

- For instance, in the case of H_2O, a nonlinear molecule, the calculation yields $3 \times 3 - 6 = 3$ vibrational modes.

Simple diatomic molecules, comprising only one bond, exhibiting vibrational band. Symmetrical diatomic molecules, such as N_2, do not display this band in the infrared (IR) spectrum but rather in the Raman spectrum, being symmetric and diatomic molecules CO captivateIR spectrum.

Complex molecules with multiple bonds exhibit more intricate vibrational spectra, leading to numerous peaks in their IR spectra.

An illustration of this intricacy can be found in the CH_2 group, frequently encountered in organic compounds. This group exhibits six unique vibrational modes: symmetric stretching, antisymmetric stretching, scissoring, rocking, wagging, and twisting.

1.4 IR SPECTROPHOTOMETER

An IR spectrophotometer serves as an analytical instrument for identifying materials, particularly organic polymers. It operates by measuring the relative energy of infrared radiation as it passes through a sample, with data recorded as a function of the IR radiation's wavelength.

As a result, variations in the IR absorption spectra will be reflected in the chemical structures of various samples, making sample identification possible.

FTIR spectrophotometer/FTIR spectrometeris used to concurrently gather spectrum data of a material, in contrast to a dispersive spectrometer.

This is accomplished by employing an interferometer to obtain the interferogram, sometimes denoted to as the raw signal format, which can subsequently be converted into the sample's infrared spectrum using a Fourier transform method.

Greater signal-to-noise ratio, excellent resolution, higher throughput, and a short wavelength limit are only a few of the benefits that follow. FTIR spectrometers have application in diverse industries such as petrochemical, pharmaceutical, and environmental.

1.5 PRINCIPLE

Infrared Spectroscopy involves the examination of infrared light interacting with a molecule.Photon energies falling within the infrared (IR) range, typically ranging from 1 to 15 kcal/mole, lacking the capacity to excite electrons directly. However, they are capable of inducing vibrational excitement in covalently bonded atoms and groups.

Besides the simple rotation of groups around single bonds, molecules demonstrate a range of vibrational movements inherent to their component atoms.

Nearly all organic compounds captivateIR radiation that aligns with these vibrational frequencies.

IR, akin to other spectrometers in principle, enable chemists to procure absorption spectra unique to the molecular structure of compounds.

The primary output of infrared spectroscopy is an infrared spectrum, portraying the measured infrared intensity against the wavelength (or frequency) of light.

Infrared (IR) spectroscopy detects atomic vibrations, aiding in the identification of functional groups.

Typically, strong bonds and lighter atoms exhibit higher stretching frequencies during vibration.

1.6 INSTRUMENTATION OF INFRARED (IR) SPECTROSCOPY

The following are the components that make up the IR spectrometer(Fig 1.1)

1. Radiation sources
2. Substance sampling
3. Monochromators
4. Detectors
5. Recorder

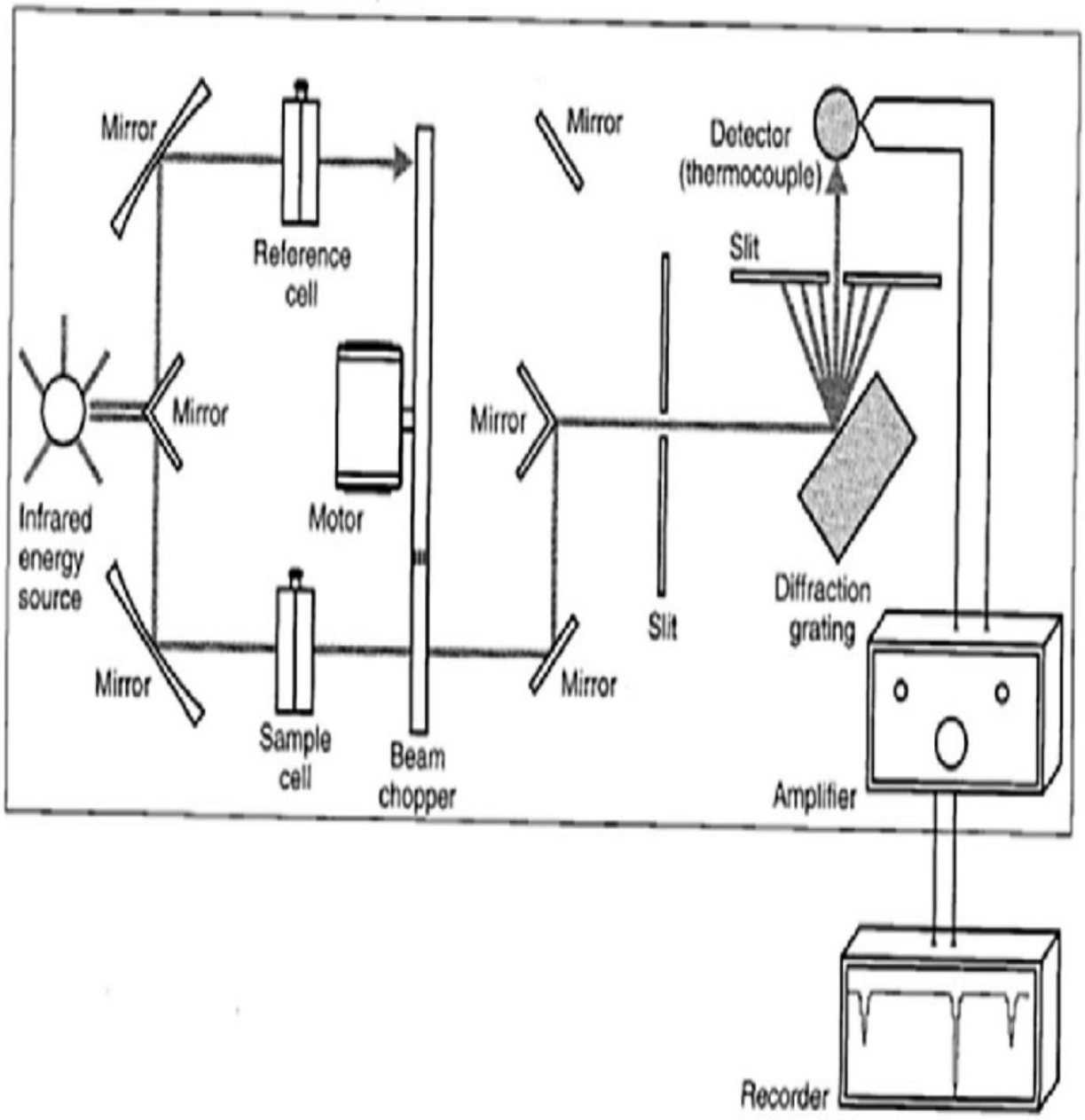


Fig 1.1A schematic diagram of the classical dispersive IR spectrophotometer

1.7 IR RADIATION SOURCES

For an infrared device to operate effectively, the radiant energy source must be consistent, sufficiently strong to be detectable, and capable of covering the required wavelength range of infrared radiation.

Here are some examples of different IR radiation sources.

1. Nernst glower
2. Fluorescent light
3. The Mercury Arc
4. tungsten lamp
5. Glober Source
6. Nichrome wire

1.8 SAMPLE CELL AND SAMPLING OF SUBSTANCES

Possible to illustrate solid, liquid, or gas samples using IR spectroscopy.

i. Solid - Various techniques, such as the pressed pellet method, solid dissolution, solid film deposition, mulling process, among others, are utilized for the preparation of solid samples..

ii. Liquid: Alkali halide liquid sample cells can be used to store samples. It is not possible to utilize aqueous solvents.

iii. Gas: Gas sampling is comparable to liquid sampling.

MONOCHROMATORS

Prisms and filters.Sodium chloride, potassium bromide, or calcium iodide are used to make prisms.

Diffraction gratings are composed of alkali halides, and filters are composed of lithium fluoride.

DETECTORS

Detectors serve to gauge the concentration of unabsorbed infrared radiation.

Various detectors including thermocouples, bolometers, thermistors, Golay cells, and pyroelectric detectors are employed for this purpose..

RECORDERS

IR spectrum is documented in recorders

1.9 IR ANALYSIS

QUALITATIVE ANALYSIS

IR spectroscopy proves to be a valuable tool for compound identification, particularly in discerning functional groups. Group frequencies, representing vibrations associated with specific functional groups, aid in structural analysis.

By comparing the vibrational frequencies observed in an IR spectrum to those present in reference databases, it becomes feasible to identify the functional groups within a molecule.

For instance, consider the IR spectrum of formaldehyde. With its C=O functional group and C-H bond, the observed values can be cross-referenced with stored data for formaldehyde. Typically, a molecule featuring a C=O stretch exhibits an IR band around 1700 cm^{-1}, while the CH_2 bend tends to manifest around 1400 cm^{-1}.

It's crucial to acknowledge that these values can be influenced by other functional groups present in the molecule.

A higher value around 1700 cm^{-1} signifies a substantial dipole moment change. Stretching vibrations, being more energetically demanding than bending modes, typically entail higher frequencies.

The fingerprint region, spanning from 1400-650 cm^{-1}, is unique to each molecule and is challenging to attribute specific values(Fig 1.2).

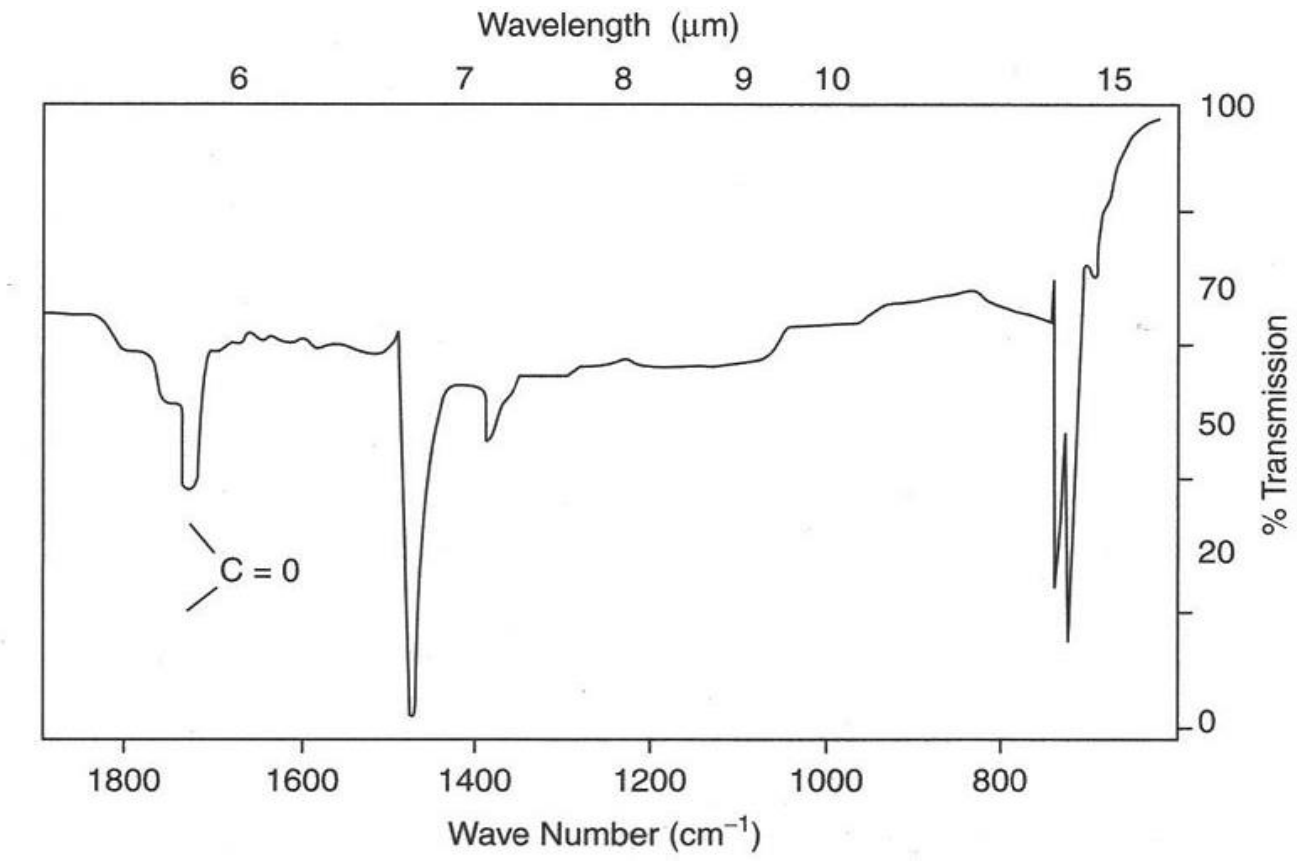


Figure 1.2: IR Spectrum of Formaldehyde

1.10 QUANTITATIVE ANALYSIS

Quantitative analysis can be functional with the application of infrared spectroscopy, however it isn't always as precise as other analytical techniques like liquid and gas chromatography.

1.11 INFLUENCE FACTORS OF IR

• Isotope Effects: Observations indicate that replacing an atom with an isotope has a negligible impact on k, the rate constant, but influences ν due to alterations in mass. This is the effect of reduced mass on rotational behavior.

•Effect of solvent: Polarity of a solvent exerts a significant influence on the IR spectra of organic compounds through solvent-compound interactions, termed as solvent effects.

• When introducing a compound having n, pi, and pi* orbitals into a polar solvent, the solvent selectively stabilizes these 3 orbitals to varying degrees. The most substantial stabilization occurs for the n orbital, followed by the pi* orbital, with the least effect on the pi orbital.

Consequently, the spectra of n→pi* transitions shift towards the blue end, indicating shorter wavelengths and higher energies, as the polar solvent widens the energy gap between the n and pi* orbitals. Conversely, the spectra of pi→pi* transitions shift towards the red end, indicating stretched wavelengths and inferior energies, as the polar solvent reduces the energy difference among the n and pi* orbitals.

1.12 SOLICITATIONS OF INFRARED (IR) SPECTROSCOPY

- It has proved extremely important of domains including:
- protein characterisation;
- Nano scale semiconductor analysis
- Compound identification; • Gaseous, liquid, or solid sample analysis;
- Quantitative analysis
- From the IR spectra, information on the composition and functional groups of molecules can be inferred.
- Gaining knowledge about molecular interactions

1.13 PRECATIONS

- Prevent inhaling anything poisonous.
- Very poisonous substances include phosgene, hydrochloric acid, and hydrofluoric acid.
- It is important to make sure your work area has adequate ventilation while utilizing solvents that contain halogenated hydrocarbons.
- Combustible and volatile materials and solvents may catch fire due to the spectrometer's internal infrared source.

1.14 SELF ASSESSMENT

1. What is the primary principle behind infrared (IR) spectroscopy?
2. How does IR spectroscopy help in identifying functional groups in organic compounds?
3. Can you explain the concept of wavenumber in IR spectroscopy?
4. What are some common applications of IR spectroscopy in various fields?
5. How do isotope effects and solvent effects influence IR spectra?

UNIT - 2

FLAME ATOMIC ABSORPTION SPECTROPHOTOMETRY

Upon completion of this unit, student will gain proficiency in:

- Explaining the origin, types, and characteristics of atomic spectra.
- Distinguishing between atomic and molecular spectra.
- Describing the structure and properties of a flame.
- Understanding the principle behind flame atomic absorption spectrophotometry.

2.1 ORIGIN AND CHARACTERISTICS OF ATOMIC SPECTRA

- The color observed is a result of radiation emission by gaseous atoms or ions in an excited state.
- In the mid-19^{th} century, Bunsen and Kirchhoff demonstrated that the optical radiation produced from flames is distinct to the elements introduced into the burning flame.
- Furthermore, the researchers had established that the intensity of emitted radiation is contingent upon the quantities of elemental species present.
- Consequently, the wavelength and intensity of emitted radiation serve as indicators for identifying and quantifying elements within a sample.

2.2 ORIGIN OF ATOMIC SPECTRUM

The origin of atomic spectrum lies in the concept of quantized energy levels within an atom, which electrons can occupy based on their energy. The lowest energy level is termed as the ground state, while higher energy levels are known as excited states.

On the contrary, in the case where electron moves from a higher energy level to a lower state, it emits radiation with a wavelength that matches the energy gap between the levels. The range of wavelengths of radiation absorbed or emitted by an atom, along with their respective intensities, forms its atomic spectrum..

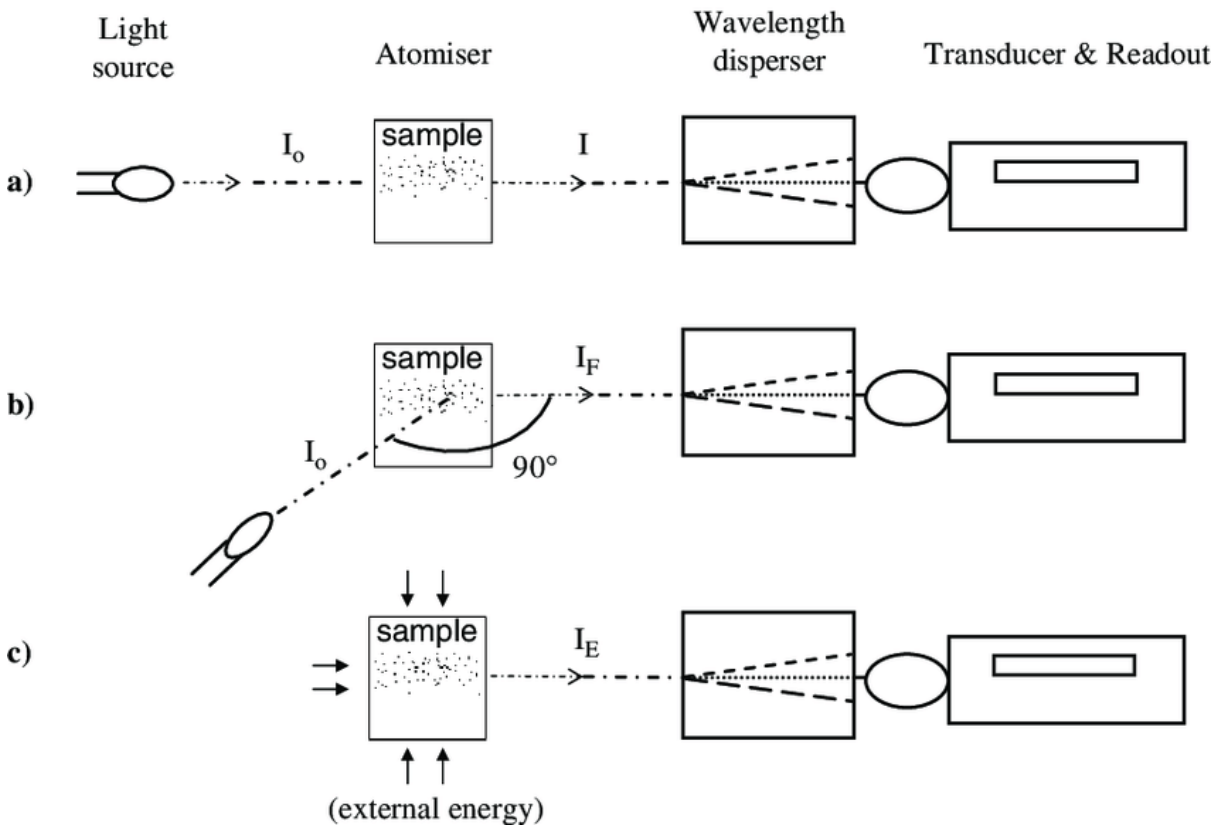


Fig. 2.1: Graphic illustration of transitions in atomic (a) absorption (b) emission and (c) fluorescence emission spectroscopy

2.3 Atomic Absorption Spectrometry: This technique involves exposing atomic absorption encompassing free atoms of an element in their ground level to a UV-Vis radiation source producing a typical frequency corresponding to the element existing in the atomic vapors. A portion of the radiation is absorbed by the atoms, resulting in a decrease in the intensity of the radiation (Fig. 2.1).

Atomic Emission Spectrometry (AES): In AES, an unknown sample is commonly energized by thermal energy generated from a flame, argon plasma, or an electrical discharge. This thermal energy causes the atoms within the sample to absorb it, resulting in the excitation of their outer orbital electrons.

Atomic Fluorescence Spectrometry (AFS): AFS entails the excitation of gaseous atoms to higher energy levels through the preoccupation of electromagnetic radiation, followed by the measurement of fluorescence emission from these excited atoms. Compared to absorption measurements, fluorescence detection offers greater sensitivity due to the minimal background radiation associated with the fluorescence signal.

2.4 CHARACTERISTICS OF ATOMIC SPECTRUM:

Molecular spectra typically exhibit broad features, comprising numerous closely spaced lines that form what is known as a band spectrum. In contrast, atomic spectra are characterized by extremely sharp lines specific to the atomic species. Hence, atomic spectra are referred to as line spectra (Fig. 2.2).

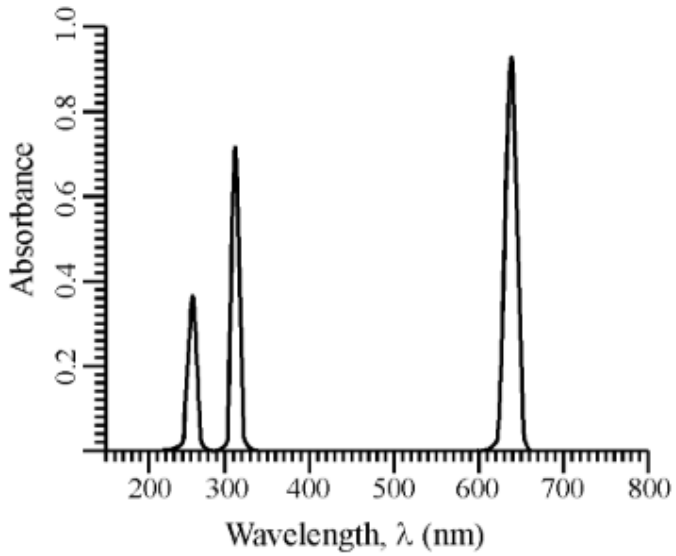


Fig. 2.2: Representation of a typical atomic spectrum.

2.5 FLAME AND ITS STRUCTURE:

- The conversion of elements within a sample into gaseous atoms or elementary ions is achieved through a process known as atomization.
- This process can be carried out using a flame, furnace, plasma, or electric arc..

- There are two common types of flames utilized in atomic spectroscopy.
- In one type, the fuel and oxidant, resulting in what are known as premixed due to their laminar hood characteristics.
- Structure of Premixed Flame: Flames exhibit non-uniformity in composition, length, and cross-section.

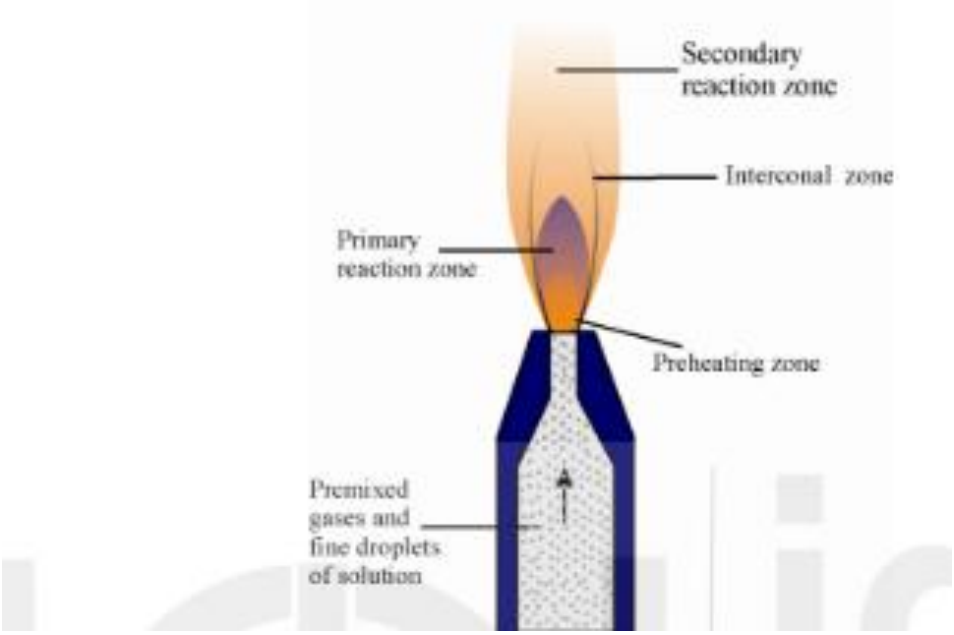


Fig 2.1 A schematic structure of a premixed or laminar flame

The laminar flame can be segmented into the subsequent four regions or zones:

i) Preheating zone

ii) Primary reaction zone

iii) Interconal zone

iv) Secondary reaction zone

2.6 PRINCIPLE OF FLAME ATOMIC ABSORPTION SPECTROPHOTOMETRY:

In flame atomic absorption spectrophotometry, atomization occurs as the sample solution is aspirated on a flame, converting the analyte into gaseous phase. The atomic vapors absorb radiation, resulting in a decrease in intensity.

The extent of absorption serves as a quantifiable degree of ground state atoms in the vapors. Investigation is conducted by associatingpragmatic absorption with that obtained from appositetypical samples of the analyte under similar tentative conditions, typically utilizing a calibration curve method.

2.7 COMPONENTS

A typical flame atomic absorption spectrophotometer comprises the following components (Fig 2.3):

A. Radiation source

B. Atom reservoir (flame)

C. Monochromator

D. Detector

E. Readout device

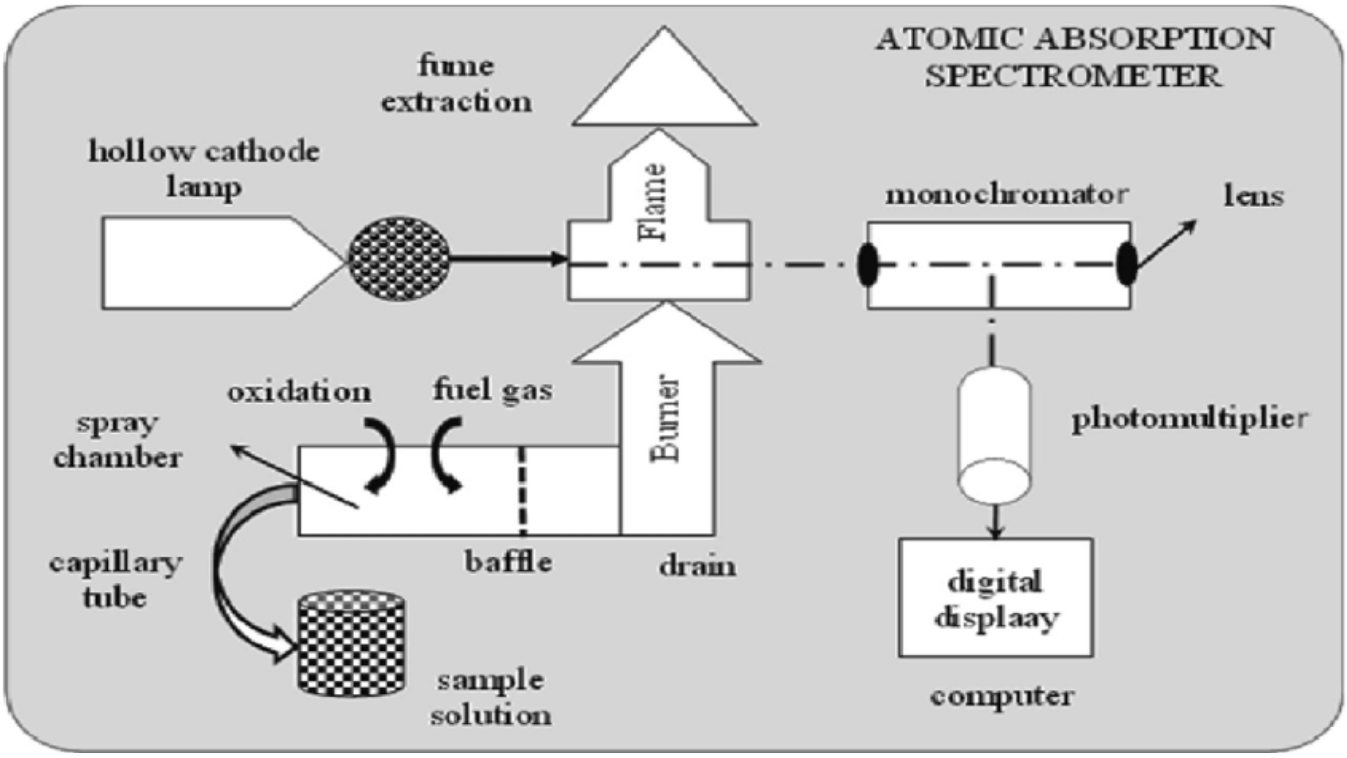


Fig 2.3 Schematic diagram of an atomic absorption spectrophotometer showing its basic components

2.8 RADIATION SOURCES:

In all commercially available flame atomic absorption spectrophotometers, a radiation source is employed that emits the characteristic spectrum of the element being analyzed (Fig 2.4).

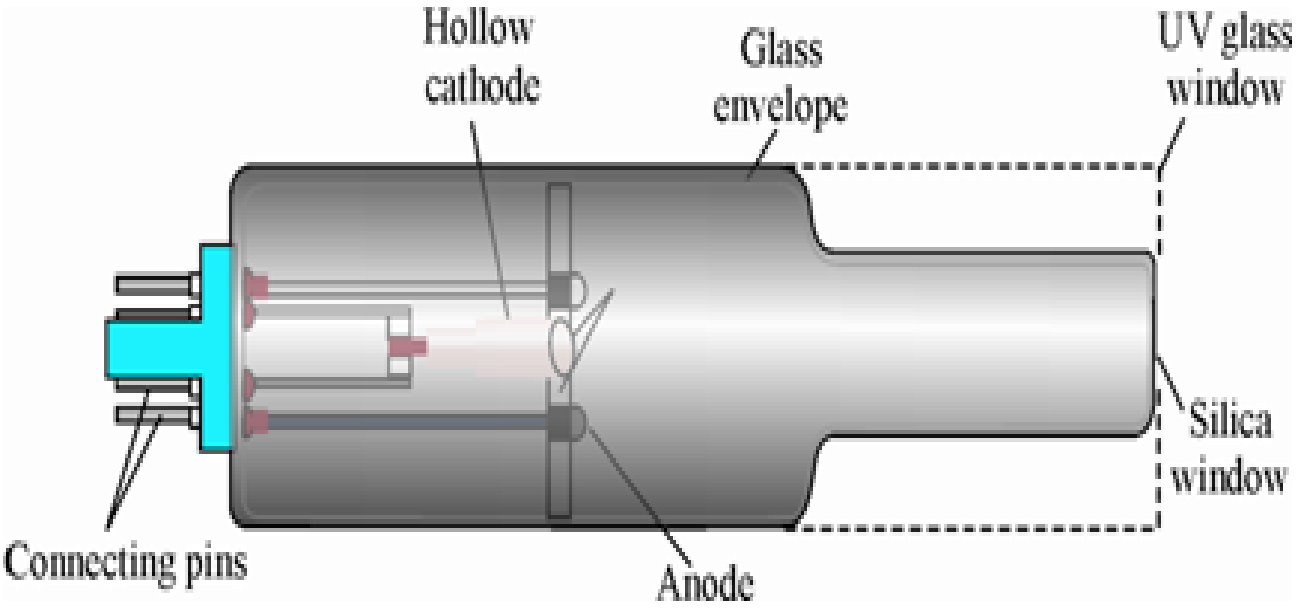


Fig 2.4 Schematic diagram of hollow cathode lamp illustrating different components

- **Electrodeless Discharge Lamp (EDL):** This lamp houses a small quantity of a volatile component, such as a halide, along with neon, within a quartz tube under vacuum conditions.
- **Atomizers:** Atomizers play a crucial role in delivering a small portion of the sample into the optical path and transforming it into free neutral ground state atoms.
- **Flame Atomizer**: The flame atomizer assembly comprises two primary components: a nebulizer and a burner. During a typical flame atomization process, sample solutions are nebulized using a nebulizer, solution form is drawn in and converted into a fine mist or aerosol.
- **Nebulizer**: This device is utilized for introducing the sample into the flame. It facilitates the thermal vaporization and dissociation of aerosol particles at elevated temperatures, resulting in the generation of small particle sizes(Fig 2.5).

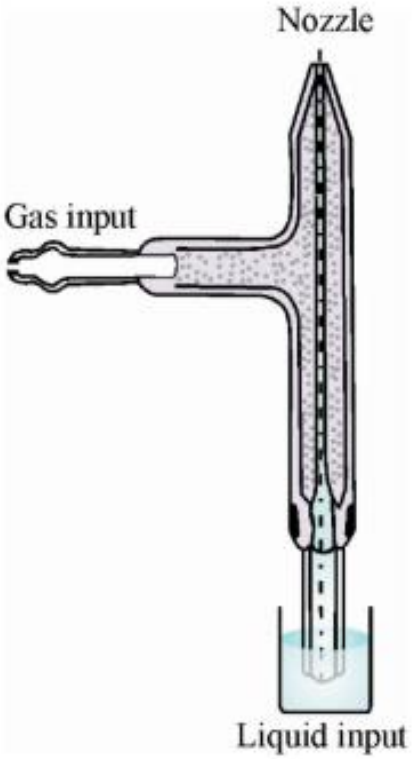


Fig 2.5 Concentric type pneumatic nebulizer.

Burners: The two primary types of nebulizer burners employed in atomic absorption spectrophotometers are the premix nebulizer-burner system (Fig 2.6) and the total consumption burner.

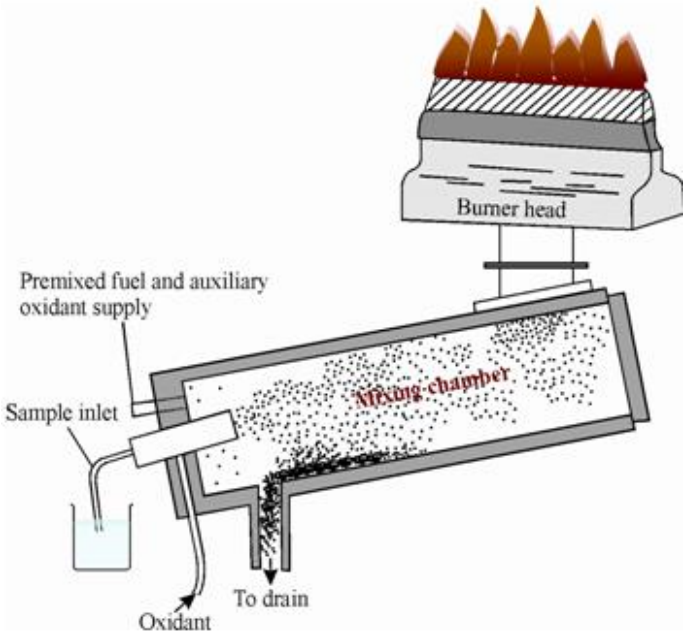


Fig 2.6: Schematic diagram of premix nebuliser burner system used in AAS

Two types of atomic absorption spectrophotometers are as follows:

Single Beam Atomic Absorption Spectrophotometer:

This instrument comprises a hollow cathode lamp (HCL) as a radiation source(Fig 2.9):

- a flame for atomization,
- a monochromator,
- a photomultiplier detector,
- a recording system.

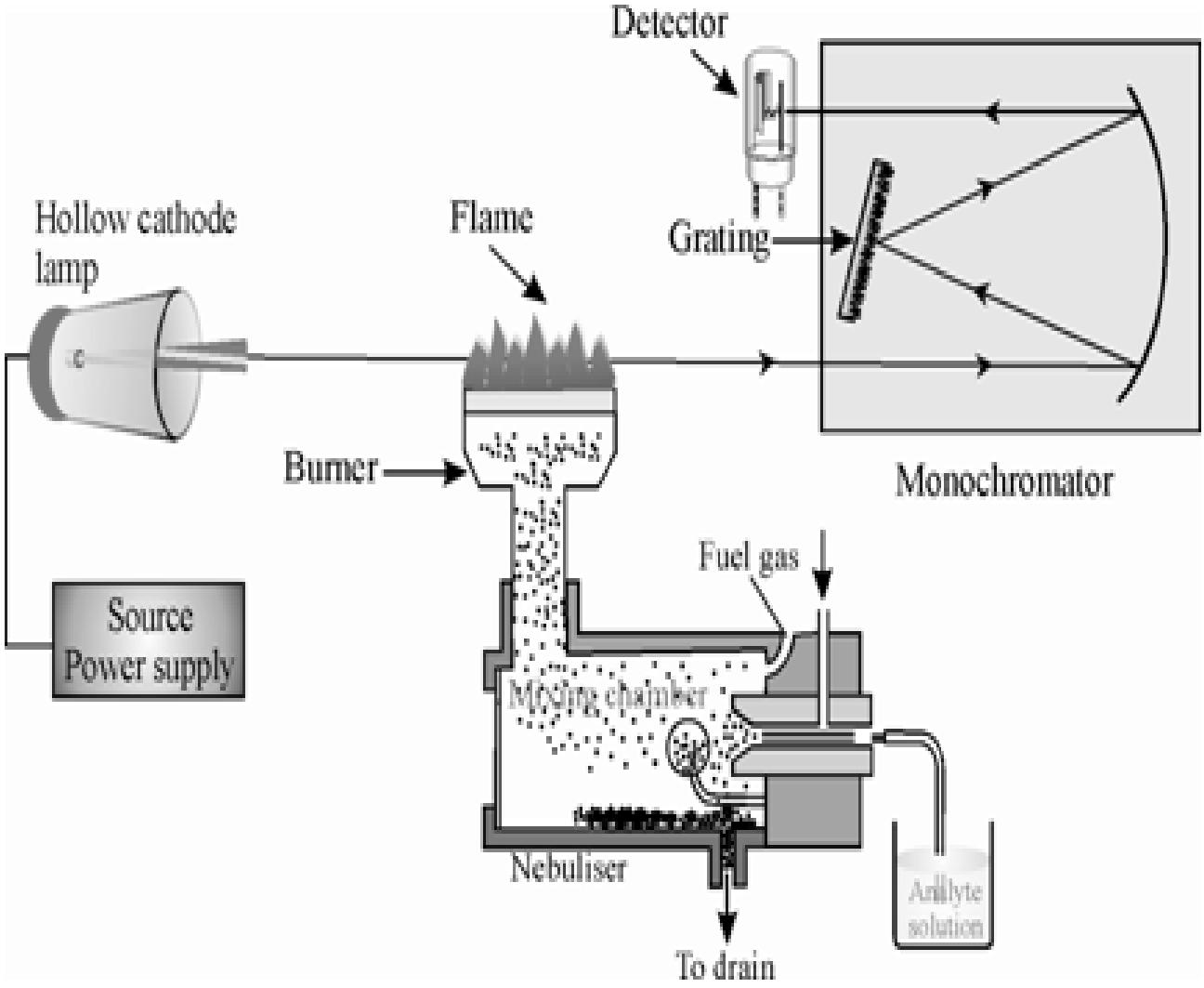


Fig 2.7: Single beam atomic absorption spectrophotometer

2.9 Double Beam Flame Atomic Absorption Spectrophotometer:

- In this setup, two beams are initially separated, then recombined using a half-silvered mirror before passing through the monochromator.
- Subsequently, the ratio between the reference and sample signals are amplified and transmitted to the readout display and recorder.

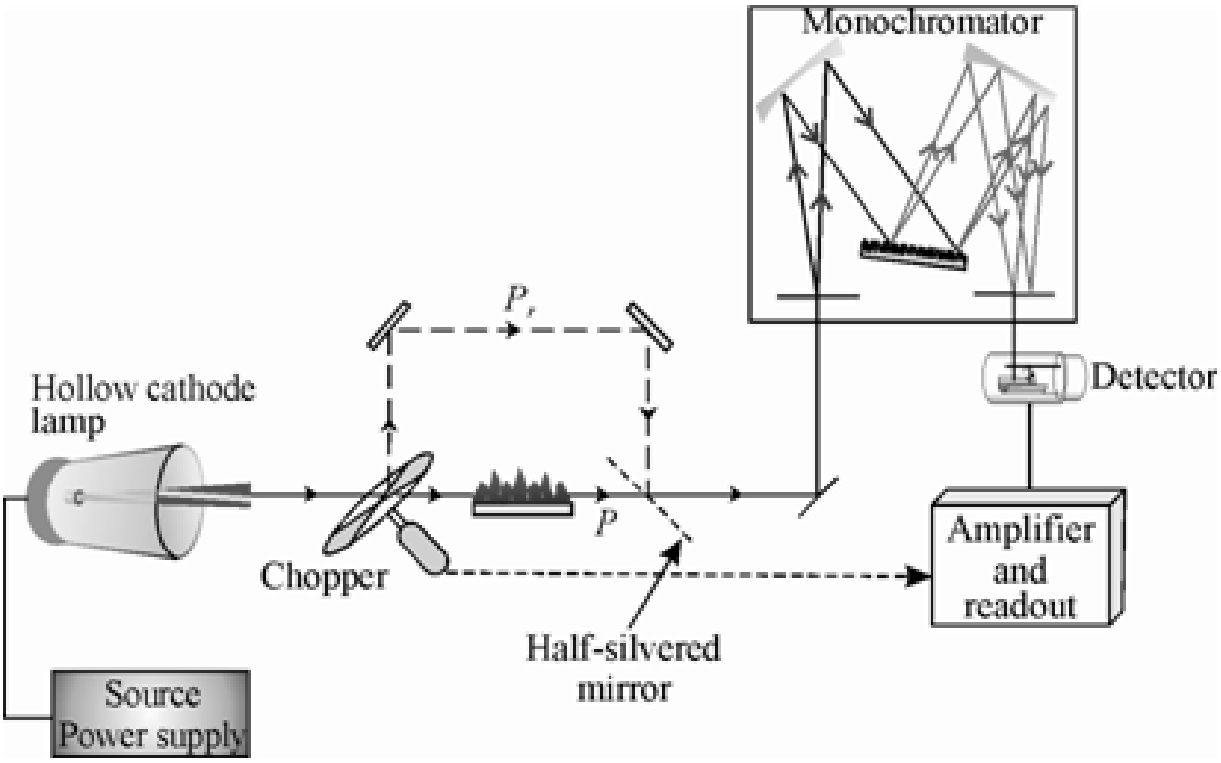


Fig 2.8 Double beam atomic absorption spectrophotometer

2.10 DATA ANALYSIS

- Analyzing data in atomic absorption spectrometry (AAS) involves several steps, including method and wavelength selection to achieve optimal results.
- Background correction is crucial in AAS data analysis, with two widely used methods: deuterium and Zeeman background correction.

2.11 APPLICATIONS:

- AAS is extensively applied across various sectors, from mining and pharmaceuticals to environmental monitoring and agriculture.
- Given the toxicity of many heavy metals, their avoidance is imperative which can be detected by the AAS.

- AAS plays a vital role in quality control processes for pharmaceuticals, ensuring products are free from catalysts like palladium or platinum.

2.12 SELF ASSESSMENT

- Explain the origin of flame atomic absorption spectrum.
- What information can be obtained from the spectrum?
- Give a description about the commonly used flame in flame spectrometry.
- List the main parts of AAS instrument and give the function of each one of them
- Describe hollow cathode lamp and explain the principle of its working
- Describe the calibration method used in AAS

UNIT- 3

OPTICAL METHODS OF ANALYSIS-II

Objective

After completion of unit student will be able to

- Able to categorize the spectroscopic techniques,
- characterize electromagnetic radiation.
- Identify and link different electromagnetic terms such as wavelength, frequency, wave number, etc.
- Beer-Lambert's Law

3.1 Origin of spectra

The term “spectroscopy” is now used to describe a variety of significant optical techniques, most of which involve studying the energy changes that occur in nuclei, atoms, or molecules during the emission, absorption, or scattering of electromagnetic radiation. In absorption spectrophotometry, electromagnetic energy is absorbed; in emission spectrophotometry, it is emitted. In contrast, scattering yields turbidimetry, nephelometry, and Raman spectroscopy data.

It has been discovered that spectroscopy is a useful instrument that may be used to swiftly resolve several challenging problems in chemistry and other scientific fields. Numerous domains, including structure identification, chemical and functional group identification, qualitative and quantitative analysis, thermodynamic property determination, and pollution analysis, employ spectroscopic approaches.

Government rules pertaining to air pollutants have been multiplying recently, requiring the development of sensitive, quick, and targeted techniques for a range of chemical substances.

Spectrophotometry is the most effective single instrument for solving the problem. In spectrophotometry, measurements are made at a fixed, predetermined wavelength of the light as well as the amount of energy absorbed by a chemical system as a function of wavelength.

The interaction of electromagnetic radiation with the quantized energy levels of matter is a common feature of all spectroscopic techniques. The transitions between various states of energy are caused by the emission or absorption of radiation at a specific frequency. The purpose of spectroscopy is to quantify the relative quantities of radiant energy emitted or absorbed at a given frequency and to correlate these variations with the composition and quantity of different substances.

For everyday work, the most commonly used analytical approach for quantitative analysis of trace components is still probably UV-visible spectroscopy. Thus, dealing with UV-visible absorption techniques and using them in pollution research is one of the applications.

3.2 Interaction of Radiation with matter

Electromagnetic radiation interacts with matter through multiple ways in all optical modalities. It is best to start with a brief overview of electromagnetic radiation and its properties.

Electromagnetic radiation (EMR) is composed of two distinct forms:

(i) A Ray of discrete particles known as photons (or quanta)

(ii) A kind of energy known as waves which travel across space at very high speeds.

The wave nature of EMR provides the best interpretation for a variety of visual phenomena.

As illustrated in Fig. 3.1, electromagnetic radiation can be broadly defined “as wave motion with electric and magnetic displacement at right angles to each other and to the radiation's direction of propagation”.

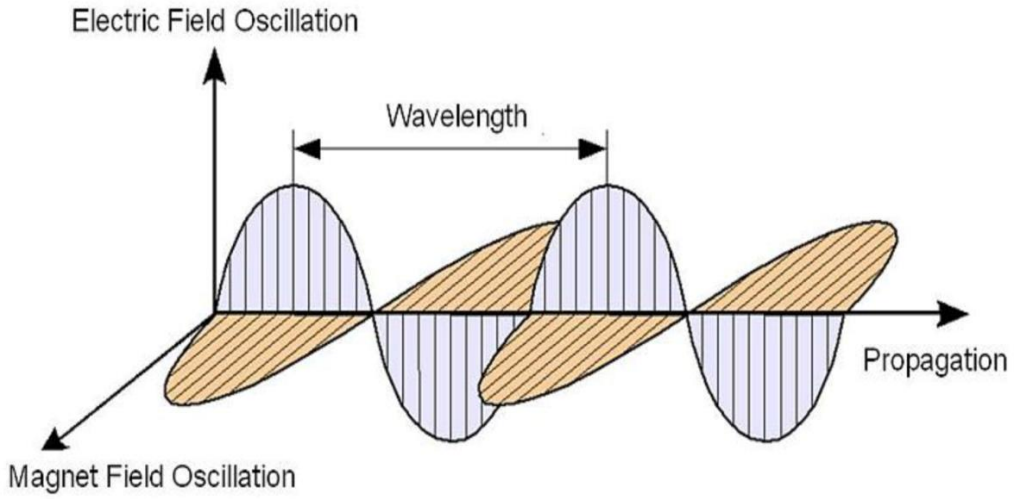


Figure 3.1: Electromagnetic spectrum

Only the electric displacement needs to be taken into account for optical phenomena. It is convenient to represent these waves using parameters like velocity, frequency, wavelength, and wave number in order to characterize many of the features of electromagnetic radiation.

3.3 Velocity

The rate at which the wave front passes through a medium is known as the electromagnetic wave's velocity. All electromagnetic waves in vacuum have the same velocity, which is represented by the symbol c and is equal to 2.998 x 108 m s^{-1}. On the other hand, velocity depends on both frequency and medium composition. However, radiation velocity reaches its maximum and becomes frequency independent in vacuum(Fig 3.1).

3.4 Frequency

It refers to the distance between (two) adjacent crests or troughs. It is designated by λ (lambda). The units of wavelength depend upon the region of the spectrum. In ultraviolet and visible region Angstrom(Å), Nanometer (nm) are widely used.

3.5 Wavelength

The term "wave number" refers to the quantity of waves per unit length. Wavelength and wave number are reciprocals. Wave number is denoted by the symbol$\bar{\nu}$ (nu bar). Reciprocal centimeters (cm^{-1}) are the standard unit of measurement for wave numbers.

3.6 Wave Number

The number of waves (or cycles) that pass a location in space in a unit of time is known as its frequency. The Hertz unit of measurement is one cycle per second (1Hz). The symbol for frequency is ν (nu). Regardless of the media that radiation passes through, frequency is set by the source and never changes.

3.7 Interaction of Radiation with Matter

There may be an energy exchange when atoms or molecules of matter come into contact with electromagnetic radiation. The system might take in energy and transition from the ground state, or lower energy state, to the excited state, or higher energy state, or E_2. Alternatively, energy can be lost and a system that starts in the higher energy level E_2 can descend to the lower energy state E_1. The energy transfer is quantized.

Energy difference (ΔE) between these two states can be calculated by using the following formula.

$$\Delta E = E_2 - E_1 = h\nu$$

This equation can be related to λ and ν

$$\Delta E = hc/\lambda = hc\bar{\nu}$$

h= Plank's Constant

ν = Frequency of electromagnetic radiation

h= 6.626×10^{-34} J s (Jules second)

Energy changes are shown in figure 3.2

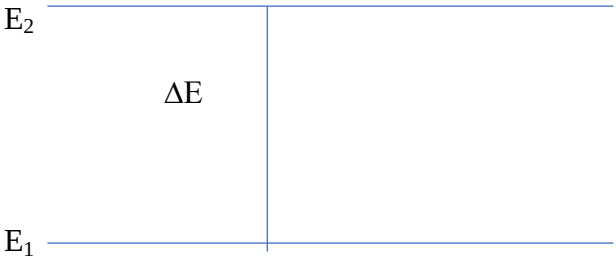


Figure 3.2: Energy level diagram

There are multiple zones within the electromagnetic radiation spectrum (Fig. 3.3). The boundaries of these areas are established based on several techniques and types of information obtained from electromagnetic radiation contact. The various spectral regions, together with an approximation of their wavelength ranges and the sorts of transitions involved.

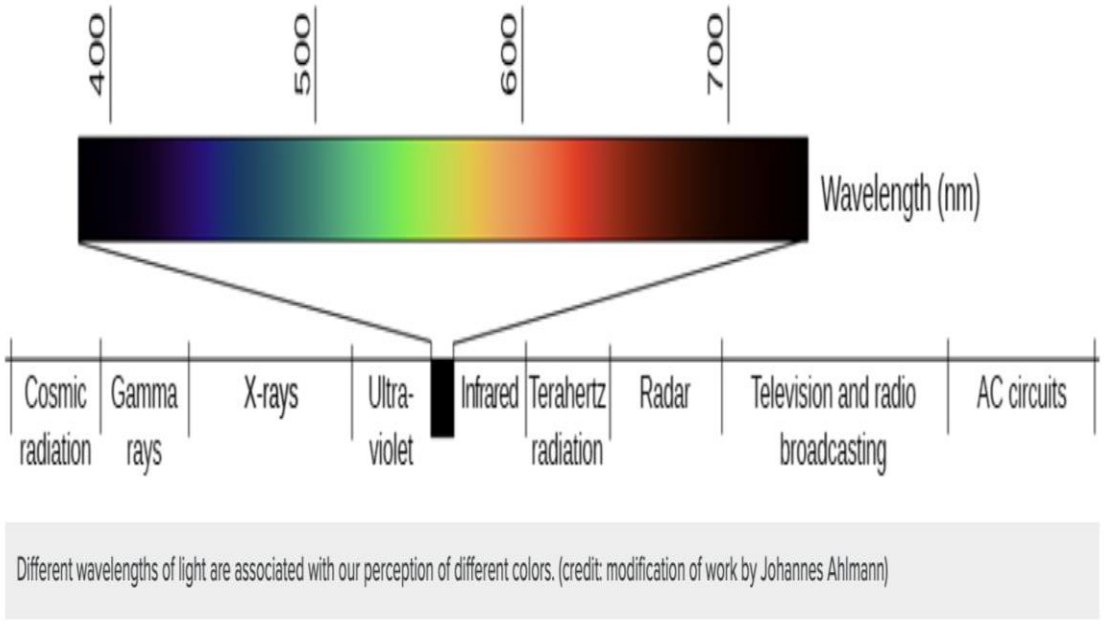


Figure 3.3: Electromagnetic spectrum of the light

3.8 Fundamental laws of Spectroscopy and selection rules

In the previous subsection we had discussed about the spectroscopic methods rely on energy changes brought about by EMR interaction with matter. As a result, they can be categorized according to the energy transformations (nuclear, electrical, vibrational, rotational, etc.) that take place throughout the transition. The kind of mechanism (e.g., emission, absorption, or scattering) involved in the transition can also be used to classify materials. Another way to categorize spectroscopic techniques is based on the electromagnetic radiation's spectrum region of interest. γ-ray, X-ray, microwave, electron spin resonance, visible, infrared, ultraviolet, and nuclear magnetic resonance techniques are among them.

To tackle the issues of structural clarification, quantitative estimation, or measurement of such parameters as the equilibrium constant, dipole moment, etc., we can select an appropriate spectroscopic method. Inner shell (K and L shell) electron transitions are typically brought on by X-rays. Metallurgy has been the field in which X-rays have been employed the most, although they can also be used to analyze metals, minerals, liquids, glassware, and ceramics. They can be used to quantify the thickness of an extremely thin layer of tin plating and to ascertain the crystal structure.

Electronic transitions in atoms and molecules are the subject of ultraviolet-visible spectroscopy. Its primary application is in the quantitative analysis of materials belonging to several classes, including inorganic, organic, and biochemical compounds. This method's application is crucial for conducting chemical analyses of environmental sample material in clinical laboratories.

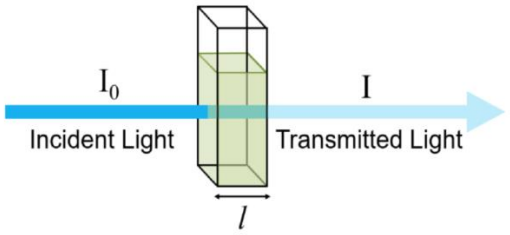
The molecular structure can be ascertained by the use of infrared spectroscopy. Compounds can be identified by determining their functional groups. Quantitative analysis is an additional option. Another method that makes use of this frequency range is Raman spectroscopy, which is supplementary to infrared (IR) spectroscopy and utilized for similar purposes.

Although they are relatively new techniques, electron spin resonance (ESR) and nuclear magnetic resonance (NMR) spectroscopy are very helpful in elucidating structures, especially of organic

molecules. With these techniques, an externally applied strong magnetic field splits the energy levels. With these basic backdrops in place, we will now focus our discussion on the absorption approach, paying particular attention to techniques that include the ultraviolet-visible area of electromagnetic radiation.

3.9 Validity of Beer-Lambert's Law

THEORY:



$$\log (I_o / I_t) = A = \varepsilon c l$$

Where,

$I_o$and $I_t$are the incident and transmitted intensity

A=Absorbance

c= Concentration

l=path length of light

A solution's concentration can be quantitatively calculated using Beer-Lambert's law by measuring the amount of light the solution absorbs in a cell with a specified path length.

3.10 UV Visible spectrometry

UV-Vis spectroscopy, an analytical technique, quantifies the absorption or transmission of UV or visible light wavelengths by a sample in comparison to a known reference or some times against a blank sample.

The property is influenced by the sample's composition, offering insights into its contents and concentrations. Photon, the fundamental unit of light, is integral to this method.

The wavelength of light and its energy exhibit an inverse relationship, with shorter wavelengths possessing higher energy and longer wavelengths carrying less energy.

A substance's electrons must be propelled to a higher energy state with a specific quantity of energy in order for absorption to be perceived. Different amounts of energy are required by an electron in different bonding situations in order to transfer it to a higher energy state. This clarifies the reason behind usage of different substances absorbing light at different wavelengths. Human eye can see visible light with wavelengths ranging from around 380 nmwhich is violet, to 780 nmwhich is red.

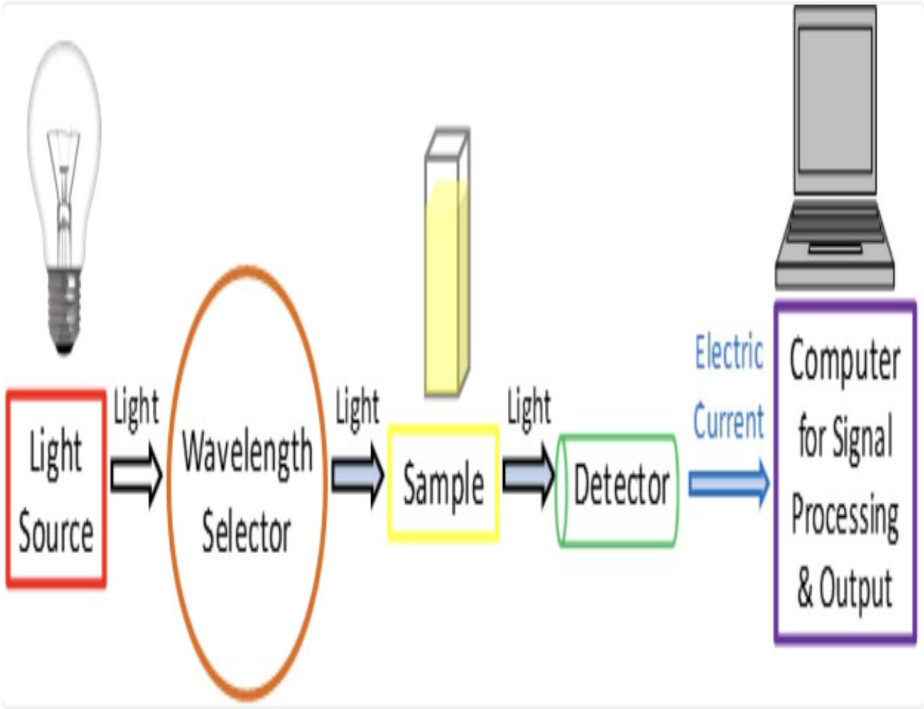


Figure 3.4: A schematic diagram of the important component of a UV-Vis spectrophotometer. (Credit: Dr. Justin Tom)

3.11 Principle

Lambert's Law states that “each unit length of absorbing material through which light passes absorbs the same fraction of entering light”. It was first proposed by Bouguer and then by Lambert. This mathematical relationship is based on the transmission of monochromatic light by homogeneous absorbing media.

Beer's Law: Beer (1852) altered Lambert's law by considering the impact of the absorbing species' concentration under a fixed path length (b). According to his indication, "the power of a monochromatic radiant beam decreases proportionately to the beam's power multiplied by the change in the concentration of absorbing substance in the path." In the same way as Lambert's - Beer's law has a mathematical expression. When a single absorbing substance of concentration c is subjected to a parallel monochromatic radiation beam spanning any thickness of solution, and c is altered by a small amount dc to c + dc, the transmitted power changes as follows:

$$A=\in bc$$

Where$\in$ (Epsilon) is called molar absorptivity denoted by cm^{-1} mol^{-1} dm^3.

3.12 Self-Assessment

1. Provide the Relationship between Path length, absorbance, importance of material of cuvette.
2. Provide the frequency in Hz and the wave number in cm-1 for radiation with a wavelength of 4000 A.
3. What is relation between Absorbance and transmittance

UNIT- 4
ULTRAVIOLET-VISIBLE SPECTROPHOTOMETRY

Objective

After completion of unit student will be able to

- Enumerate the parts of a spectrophotometer
- Define absorbing species, such as auxochromes and chromophores.
- Identify chemicals in water, waste waters, soil, and air samples using ultraviolet-visible spectrophotometry.

In the field of ultraviolet-visible spectrophotometry, one can identify and quantify the concentration of one or more components in a solution by measuring the amount of light that is absorbed, either UV or visible. The main benefit of employing this discipline as an instrumental technique is the ability to determine substance traces in an easy-to-understand manner, something that is not achievable with traditional procedures. This is among the earliest instrumental analysis techniques. Previously in the development of thephysico-chemical method of investigation, light sources such as artificial or natural white light were employed. Simple instruments were utilized for the measurements, and in the initial stages of the technique's development, the color intensity of the solution (Figure 4.1).

However, photometers have superseded the human eye in measuring color intensity; the devices created in this way are referred to as photometers or colorimeters. In order to select a spectral band for measuring color, filters were added to colorimeters. Spectrophotometer are devices that were later developed that had the ability to pick a certain wavelength. Accuracy of standard colorimetric techniques is limited to approximately 1 percent. It is necessary to employ spectrophotometric techniques for increased precision.

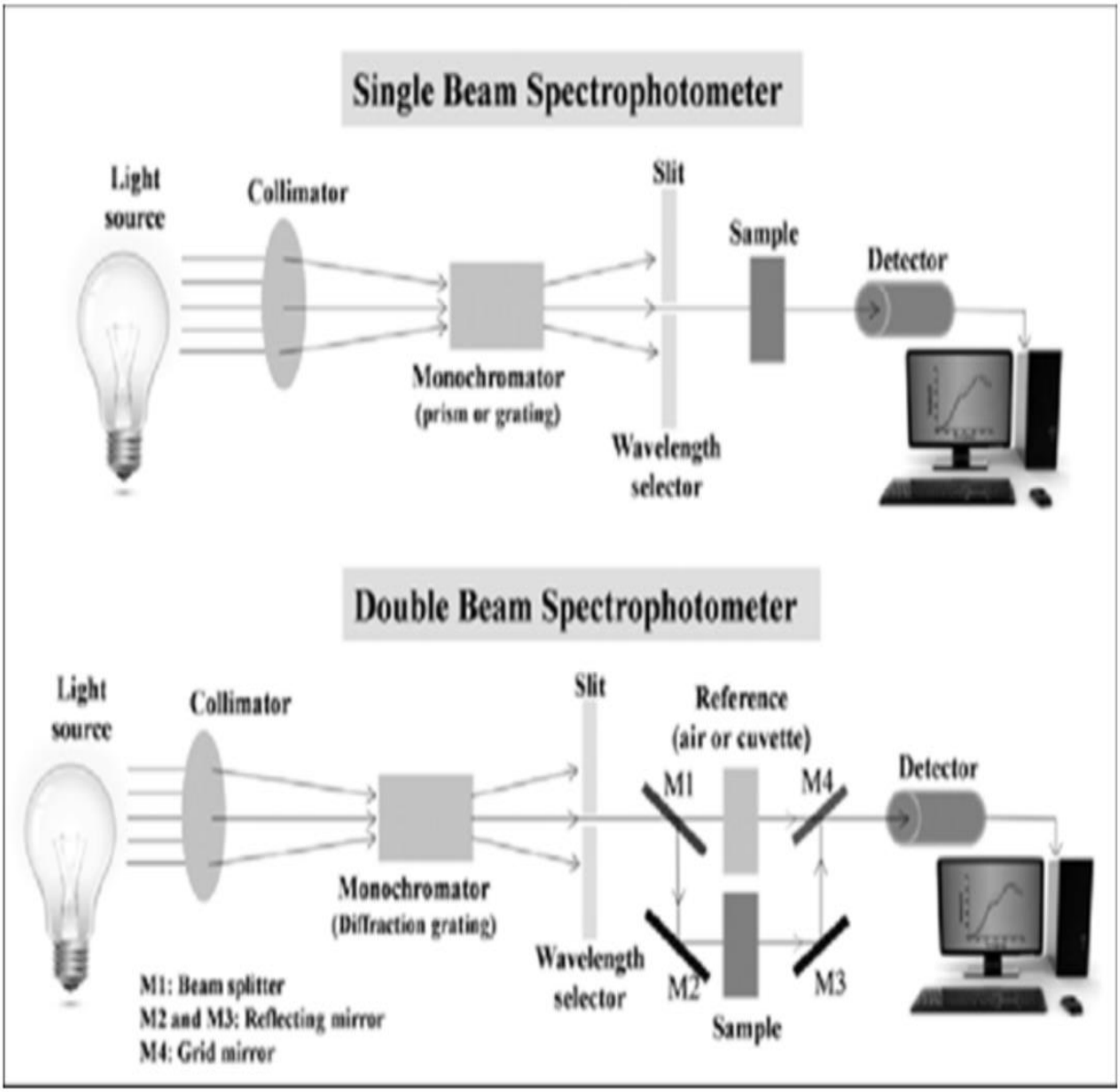


Figure 4.1: Single and Double beam Spectrophotometer

Enough radiant power must be produced over the wavelength range where absorption is to be evaluated by a suitable radiation source. A wavelength selector uses a monochromator or filters the source's light to arrive at the correct wavelength. In contrast to spectrophotometry, which uses a monochromator in the ultraviolet, visible, and infrared regions, filter photometry uses filters primarily in the visible range.

The solvent or sample put within the cuvette can pass through the chosen wavelength according to a signal indicator may read the signal produced by the detector once it has determined the strength of the radiation being sent. We now provide a more thorough description of these parts.

4.1 SOURCES: Low-pressure deuterium discharge lamps or hydrogen are the typical sources of radiation in the ultraviolet spectrum (180-350 nm).

The key function of these lamps is to use a mechanical aperture between the cathode and the anode to keep the discharge on a limited route. The lamp's brightness is increased by using deuterium instead of hydrogen.

The source of radiation in the visible and near-infrared range (325 nm-3μm) is typically an incandescent light bulb with a filament made of tungsten wire.

A vacuum or inert gas-filled sealed glass bulb encloses the coil of wire filament. Radiation is released when an electric current heats the filament.

4.2 SELECTION OF WAVELENGTH

Selecting a certain range of wavelengths from the source is the aim of a wavelength selector.

Radiation sources with nearly constant emission across a broad frequency range include hydrogen discharge tubes and tungsten lamps.

Filters can be used to separate specific wavelength ranges narrow spectral areas from a continuous spectrum.

To generate a monochromatic radiation at the desired frequency, though, a monochromator is needed. Filters can isolate a limited range, and a monochromator with a prism or grating as the dispersing device can accomplish a further narrower range (monochromatic).

4.3 FILTERS

A colored piece of glass that absorbs light at a certain wavelength more than others is called an absorption filter.

It is known that there are seven distinct colors that make up white light (VIBGYOR) as per Figure 4.2.

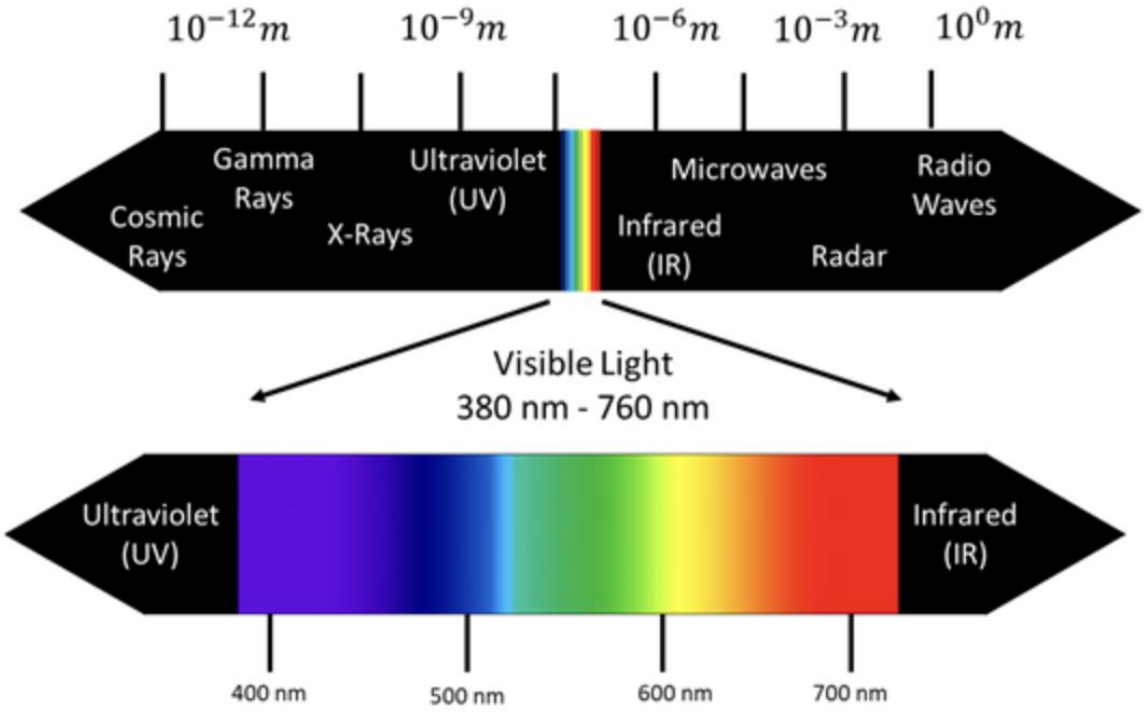


Figure 4.2: White light and VIBGYOR

4.4 MONOCHROMATOR

A device that emits single beam light from a source is called a monochromator. To produce the monochromatic light, a dispersive material—typically a prism or diffraction grator—is employed. By taking use of the wavelength-dependent variation in refractive index that occurs when light travels through glass, a prism divides light into a spectrum. The surface of a diffraction grating is governed with parallel grating lines.

The reflected light splits into a spectrum due to the serrated grating lines(Fig 4.3).

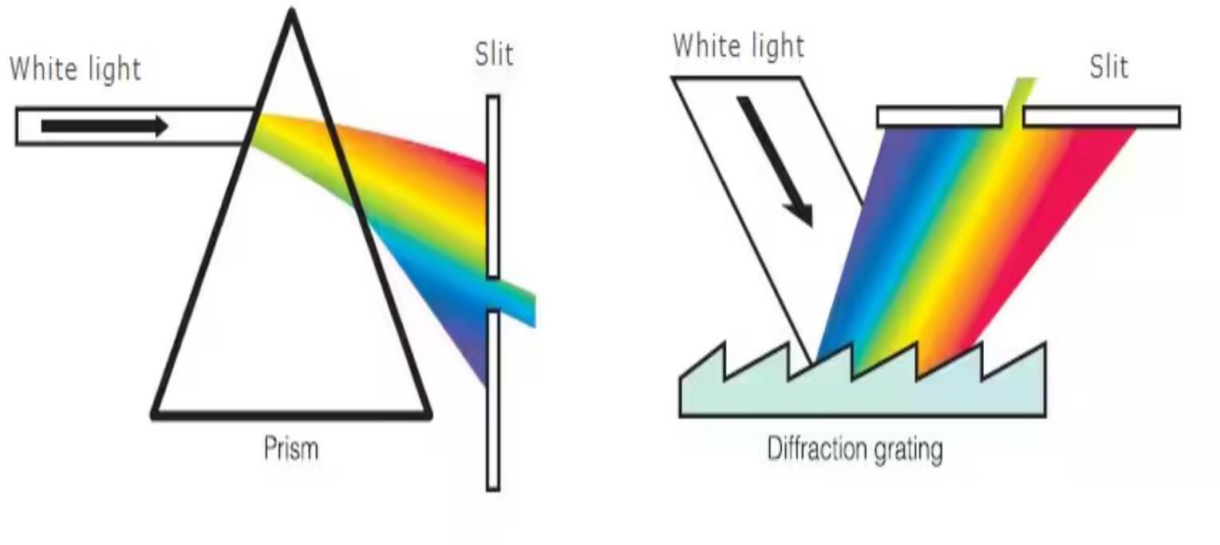


Figure 4.3: Monochromatic light emission

4.5 CUVETTE

One component, often known as the sample beam, moves through the cuvette containing the sample to be studied in a clearly transparent solution. Reference beams, or second beams, move through a set of cuvettes that contain only solvent. Containers for reference and sample solutions must be clear and there should no mark or finger prints present while holding it during the experiments (Figure 4.4).

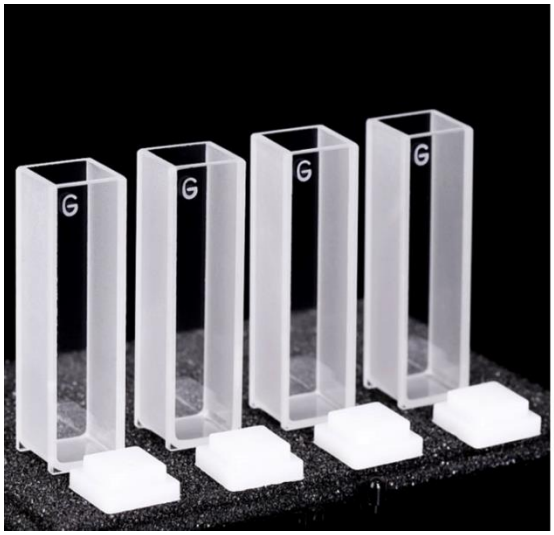


Figure 4.4: Cuvette containing the sample to be analysed by spectrophotometer

4.6 DETECTOR

One type of multichannel photon detector is the linear photodiode array. These detectors are capable of measuring every component of a scattered radiation beam at the same time. Many tiny silicon photodiodes made on a single silicon chip make up a linear photodiode array. On a chip, photodiodes can range from 64 to 4096 sensor components; however, the most typical number is 1024. A switch and a storage capacitor are also present for every diode. One can progressively scan each diode-capacitor circuit in turn (Fig 4.5).

Photomultiplier tube with its protective covering removed. The sensitivity of the detector depends on the photocathode substance used.

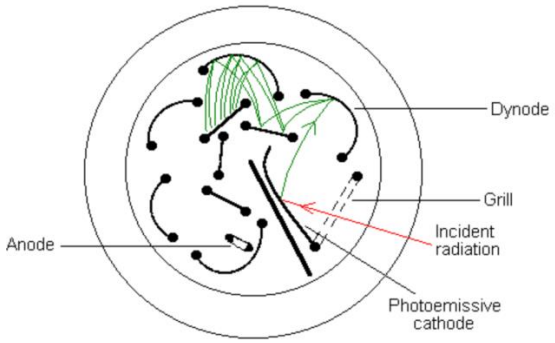


Figure 4.5: Photomultiplier diode

4.7 CHARGED COUPLED DEVICES

Similar to diode array detectors, Charge-Coupled Devices (CCDs) are made up of an array of photocapacitors rather than diodes. The intensity of the reference beam, denoted I0, should experience minimal or no absorption, whereas the sample beam's intensity is denoted I. In a brief amount of time, the spectrophotometer automatically analyzes each wavelength component. This method works well for assessing structural alterations or molecular structure in addition to concentration. Examining changes in vibrational and structural energy levels both before and after an interaction with a substrate or molecule is another possible application for it.

Figure 4.6: Spectrophotometer Device

4.8 APPLICATION

- To identifyany organic and inorganic species are present in a solutionspectroscopy is been utilized by industries worldwide (Fig 4.6).

- It is employed to determine the unknown solution's concentration.

- For the purpose of determining structure in conjunction with additional information such as bands and functional group.

- The instrument can be employed in the study of chemical kinetics, which deals with the emergence and removal of functional groups.

- Isomers are studied using UV-visible spectroscopy; for example, in geometric isomerism, the trans-species absorb a higher wavelength than the cis-species with a larger molar absorptivity (or "ε") value.

- It facilitates the identification of conjugation or its presence.

- Drug analysis is done in clinics and hospitals using UV-vis spectroscopy.

- The petrochemical industries employ it.

- Water quality control labs use UV-visible spectroscopy.

- The forensic labs employ it.

- In the study of chemical and biological plants, UV-visible spectroscopy has another significant use.

- Maximum absorption is utilized for qualitative analysis, and Lambert Beer's law is employed for quantitative analysis.

4.9 Self-Assessment

1. What causes a negative UV absorption value?
2. Why are samples in solid form unsuitable for UV-vis spectroscopy?
3. Why is ethanol an excellent solvent forUV spectroscopy but not for infrared spectroscopy?

UNIT - 5

THERMAL METHODS OF ANALYSIS

Objective

Upon completing this unit students will be able to:

- Describing the fundamental thermal methods of analysis.
- Explaining the principle behind Thermogravimetric Analysis (TGA).
- Detailing the experimental setup employed in TGA.
- Interpreting analytical information derived from TGA curves for organic and inorganic compounds

Analytical methods that rely on detecting changes in chemical or physical properties of materials upon heating are termed thermal methods. These methods offer valuable insights into various phenomena:

• Thermal decomposition of solids and liquids.

• Chemical reactions between solids, or between solids and gases.

• Material specification, purity assessment, and identification.

• Material adsorption behavior assessment.

• Analysis of phase transitions.

5.1 Thermogravimetric Analysis (TGA) involves measuring the weight of a material as it is heated under controlled conditions. This technique provides essential data on weight changes during heating, facilitating the determination of reaction stoichiometry.

5.2 Differential Thermal Analysis (DTA) and Differential Scanning Calorimetry (DSC) both involve measuring the temperature difference (ΔT) between the sample and an inert reference material as a function of temperature under controlled conditions. DSC, similar to DTA, exhibits endothermic signals during melting and endothermic or exothermic signals during material decomposition

The schematic diagram below illustratesthe fundamental components of an instrument utilized for thermal methods(Fig 5.1).

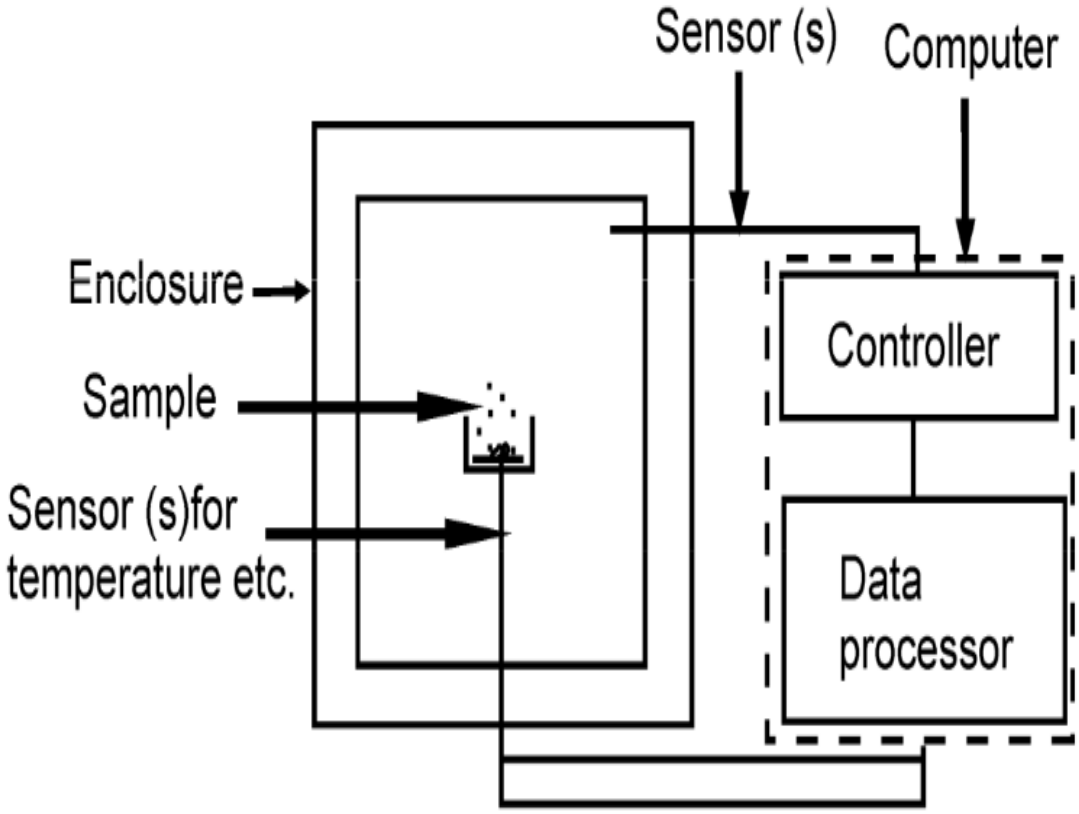
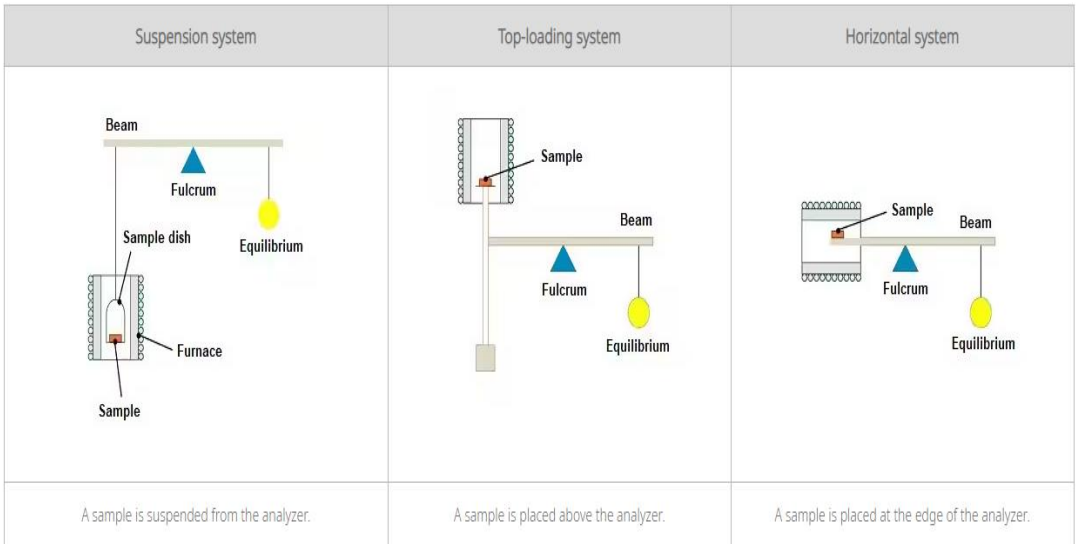


Fig 5.1: Schematic of a typical instrument used to measure thermal properties

Thermogravimetric Analysis (TGA), also known as thermal gravimetric analysis based on thermal methods wherein the mass of a sample is continuously monitored over time as the temperature of material changes.

The method of measurement provides valuable insights into a range of chemical processes, including chemisorption, thermal breakdown, and solid-gas reactions like oxidation and reduction, as well as physical phenomena including phase transitions, absorption, and desorption.

5.3 TYPES OF THERMOGRAVIMETRY:



5.4 PRINCIPLE OF TGA

Thermogravimetric analysis (TGA) involves subjecting the sample to controlled heating within a controlled environment, such as air, N_2, CO_2, He, Ar, etc., at a controlled rate. The change in the sample's weight is monitored either as a function of temperature or time. The sample's initial weight is known, and the temperature is steadily increased at a constant rate. Changes in weight are recorded at different time intervals. The resulting plot of weight change against temperature is known as a thermogravimetric curve or thermogram, which embodies the core principle of TGA.

5.5 TGA CURVE

Thermogravimetric instruments employ a programmed precision balance, often referred to as a thermobalance, to measure the temperature increase. The outcomes are presented as a graph of changes in mass versus temperature/time, denoted as thermogravimetric curves or TG curves.

5.6 INSTRUMENTATION

The device utilized in thermogravimetry (TG) is called a thermobalance, which consists of several essential components. These are designed to offer the required flexibility to generate valuable analytical data in the form of TGA curves(Fig 5.2).

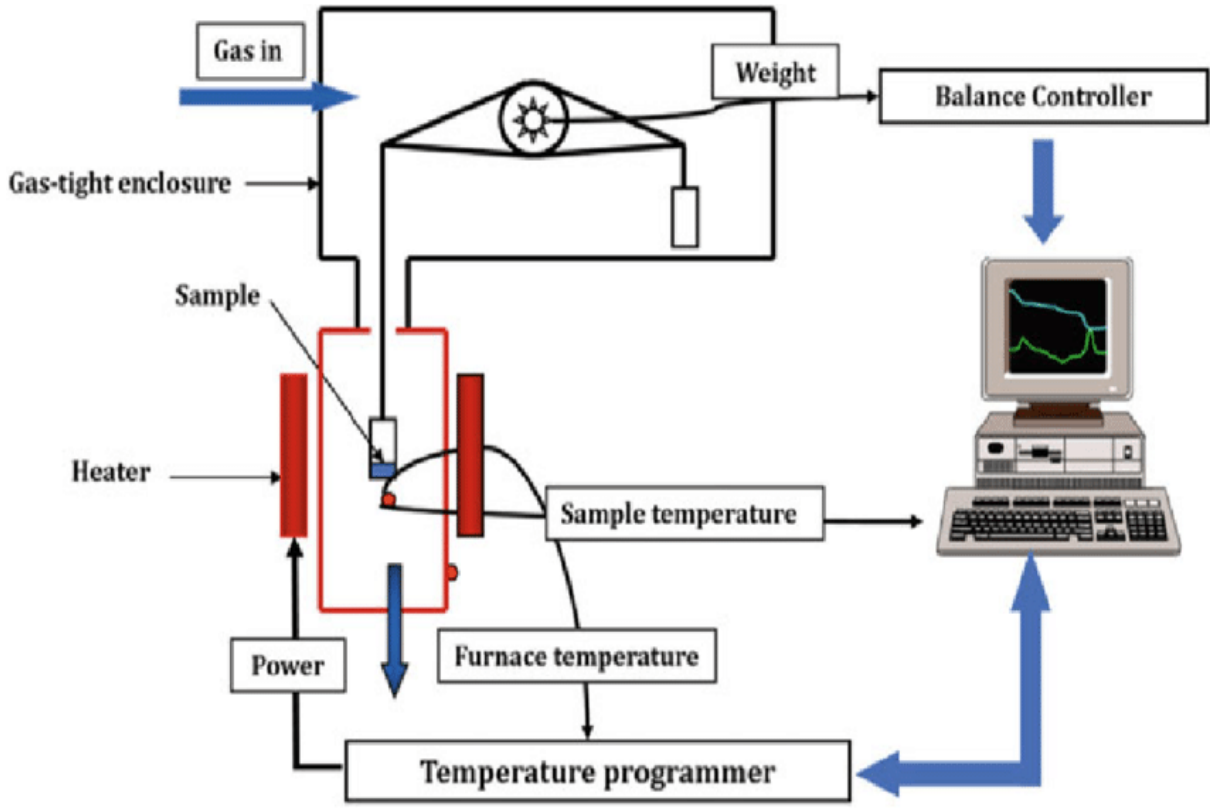


Fig 5.2 Block diagram of a Thermobalance.

5.7 BASIC COMPONENTS OF A TYPICAL THERMOBALANCE INCLUDE

i) **Balance**: A precision instrument for measuring the mass of the sample.

ii) **Furnace**: The heating device responsible for heating the sample.

iii) **Temperature measurement and control unit (Programmer)**: Controls and monitors the temperature of the furnace.

iv) **Recorder**: An automatic recording unit that records changes in mass and temperature.

5.8 ADDITIONAL COMPONENTS:

- **Sample Holder:** The shape, size, and material of sample holder influencesthe resolution and shape of peak of DTA. To enhance resolution, the size of the holder and the amount of sample should be minimized. Sample holders are typically made of ceramic, quartz, stainless steel and in few cases platinum.
- **Recording Balance:** This balance should be sensitive along with the accuracy is demanded.The result should be reproducible for the mechanical and electrical properties. It should be unaffected by vibrations and easy to operate.
- **Furnace and Furnace Temperature Controller:** The type of furnace and temperature control depends on the temperature range required for heating the sample.
- **Temperature Sensor/Thermocouple:** For temperatures up to 1100°C, Chromel or alumel thermocouples are used, while for temperatures up to 1750°C, thermocouples made of platinum or rhodium alloys are utilized (Fig 5.3)**.**

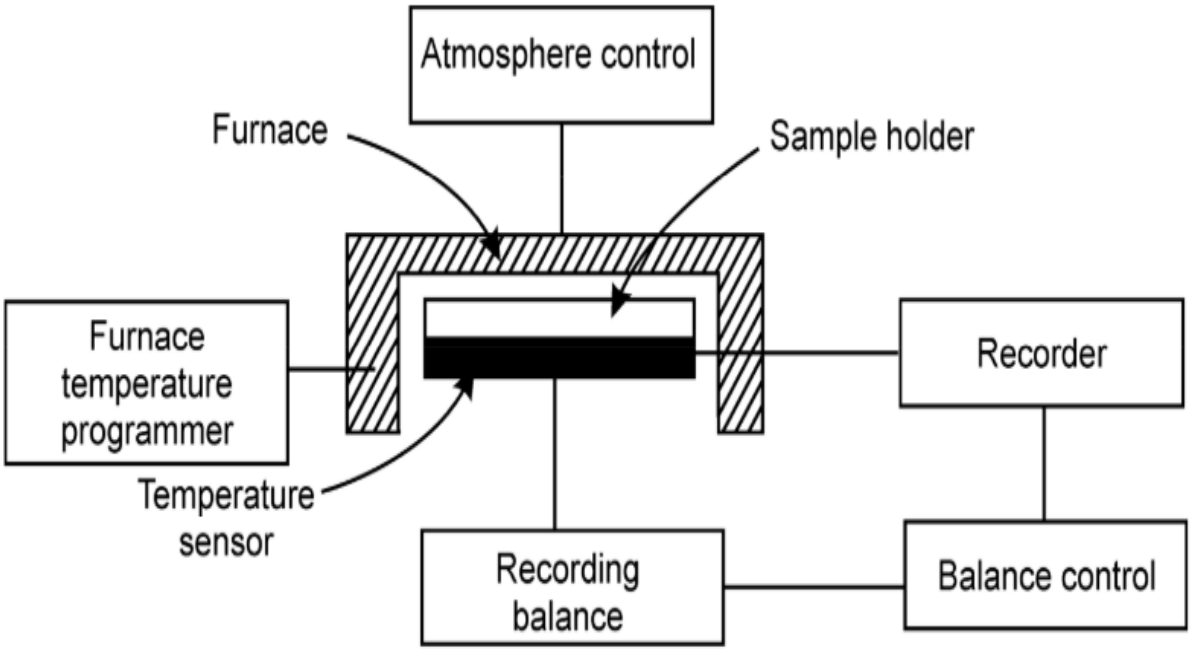


Fig 5.3: Thermobalance

Recording Balance:

- A microbalance is used to record changes in the mass of a sample or substance. An ideal microbalance should have the following characteristics:
- Accurate results those can be reproduciblecontrols the changes in sample mass under ideal atmospheric conditions and temperatures.
- Send out electronic impulses so that a recorder may capture large-scale changes.
- Deliver rapid response to changes in mass.

5.9 FACTORS AFFECTING THERMOGRAVIMETRIC CURVES:

- Heating Rate: Faster heating rates lead to decomposition at higher temperatures, while slower rates result in lower decomposition temperatures.

- Instrumental Factors: Furnace atmosphere influences decomposition temperatures; for instance, calcium carbonate decomposes at higher temperatures in a CO_2 gas atmosphere compared to N_2 gas.

- Other Factors: Particle size of the sample, source of the sample, amount of sample, and size and shape of the crucible.

5.10 APPLICATIONS OF THERMOGRAVIMETRIC ANALYSIS:

1. Characterization of materials through decomposition pattern analysis.
2. Study of degradation mechanisms and reaction kinetics.
3. Determination of organic and inorganic content in samples.
4. Investigation of thermal stability of materials like polymers.
5. Determination of correct drying temperatures for precipitates in gravimetry.
6. Study of oxide reactions.
7. Determination of moisture, ash, and volatile matter in various samples.

5.11 SOURCES OF ERRORS IN TGA

Several sources of error in TGA can lead to inaccuracies in temperature and mass data. Correct placement and careful handling of the thermobalance may help mitigate some errors. Examples include the buoyancy effect and condensation on the balance suspension.

5.12 INTERPRETING RESULTS FROM TGA INSTRUMENTS

- TGA is crucial for material characterization, offering insights into stability and composition under thermal stress.
- The TGA curve, depicting mass change over time or temperature, serves as a unique fingerprint revealing a material's thermal behavior and stability.
- Quality control and assurance involve testing a product's resistance to heat and identifying decomposition points to ensure adherence to industry standards.

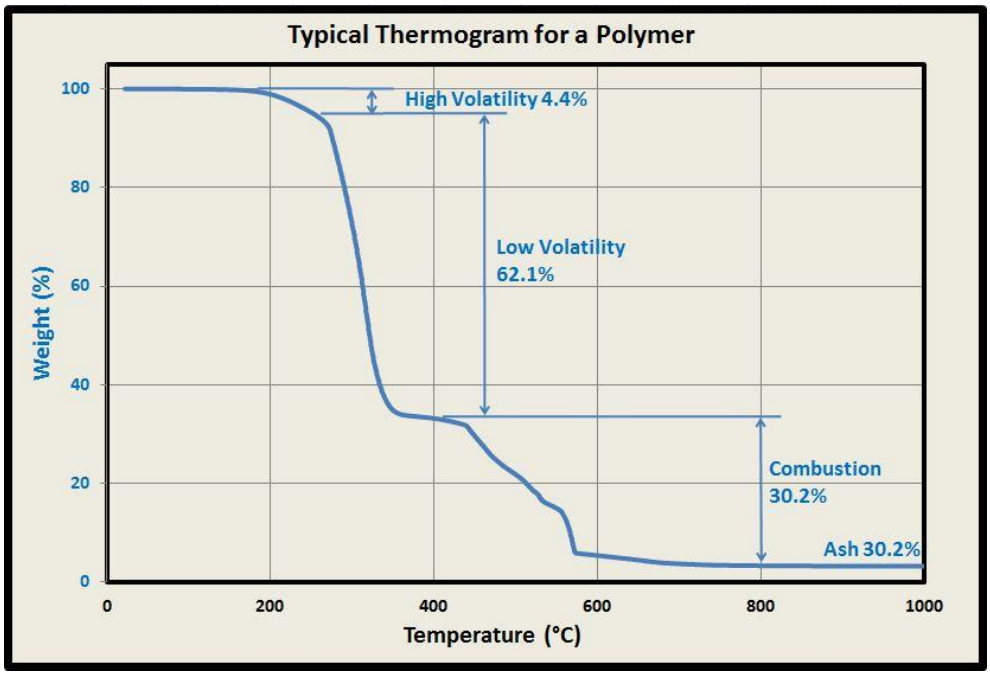


Fig5.4: Thermogravimetric Curves

5.13 INTERPRETING TGA CURVES

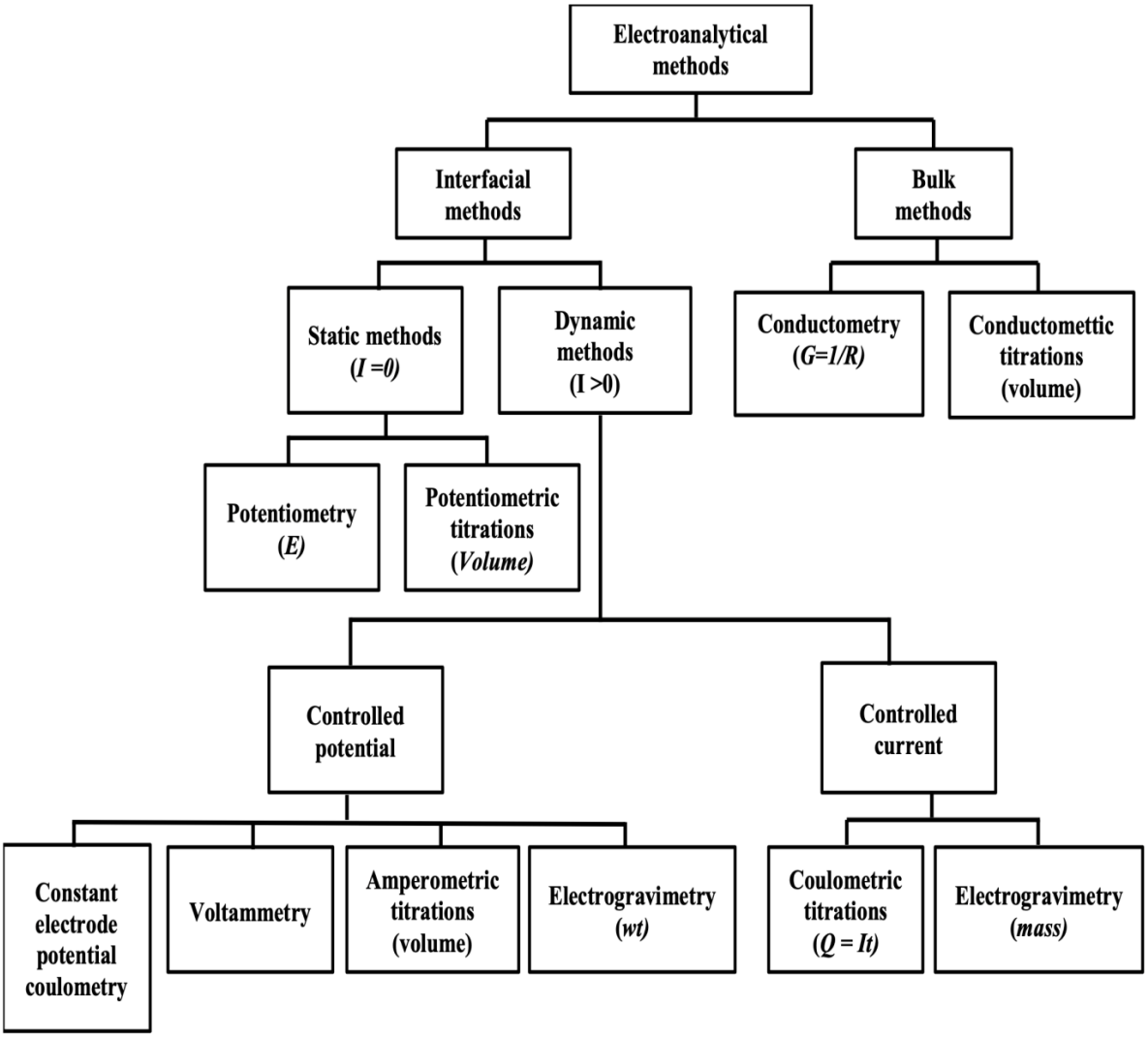
- Interpreting TGA curves requires a nuanced approach, demanding a solid grasp of the technique alongside careful attention to the intricacies within the data.
- Artifacts in TGA refer to distortions in data that stem not from the sample's behavior, but from external influences.
- These artifacts can significantly impact results if left unaddressed. One prevalent artifact is the buoyancy effect, where alterations in the density of the surrounding gas induced by heating can simulate a change in sample mass.
- This effect typically manifests as an apparent weight gain, typically falling within the range of 50 µg to 200 µg.
- To uphold data integrity, it's imperative to meticulously correct for these buoyancy effects, often accomplished through automatic blank curve subtraction, which compensates for these discrepancies.

5.14 WHY TO USE TECHNIQUES FOR ELECTROANALYSIS?

Compared to other analytical techniques, electroanalytical methods have the following advantages:

1. Electrochemical analysis makes it possible to determine an element's many oxidation states in a solution in addition to its overall concentration.
2. Exceptionally low detection limits and a wealth of characterization data, including chemical kinetics data, can be obtained using electroanalytical techniques.
3. Specific to a species' specific redox state, such as Ce^{3+} versus Ce^{4+}
4. It's inexpensive.
5. Quickness

5.15 CLASSIFICATION OF ELECTROANALYTICAL METHODS



Any species that has the ability to oxidize or reduce at an electrode is said to be electroactive. By adjusting the working electrode's potential, we may choose which electroactive species react and which do not. It is known that metal electrodes are polarizable, meaning that tiny currents can readily alter their potentials. When a reference electrode, like calomel, has a relatively constant potential without a noticeable current passing across it, it is considered nonpolarizable.

5.16 pH METRIC METHODS

It uses a scale of 0 to 14 to measure pH. A substance's pH is determined by the ratio of hydrogen ions (H+) to hydroxyl ions (OH-). A substance is acidic if its [H+] concentration is higher than its [OH-] concentration. There is a pH of less than 7. If the concentrations of [H+] and [OH-] are the same, the material is neutral. There is a pH of 7. If there is less [OH-] than [H+] in the substance, it is basic. There is a pH greater than Zygmunt Klemensiewicz (1886–1963), German chemist Fritz Haber (1868–1934), who won the Nobel Prize in 1909, presented the idea of a glass electrode for the first time. The modern electronic pH meter was created by American chemist Arnold Beckman in 1934 (1900–2004).

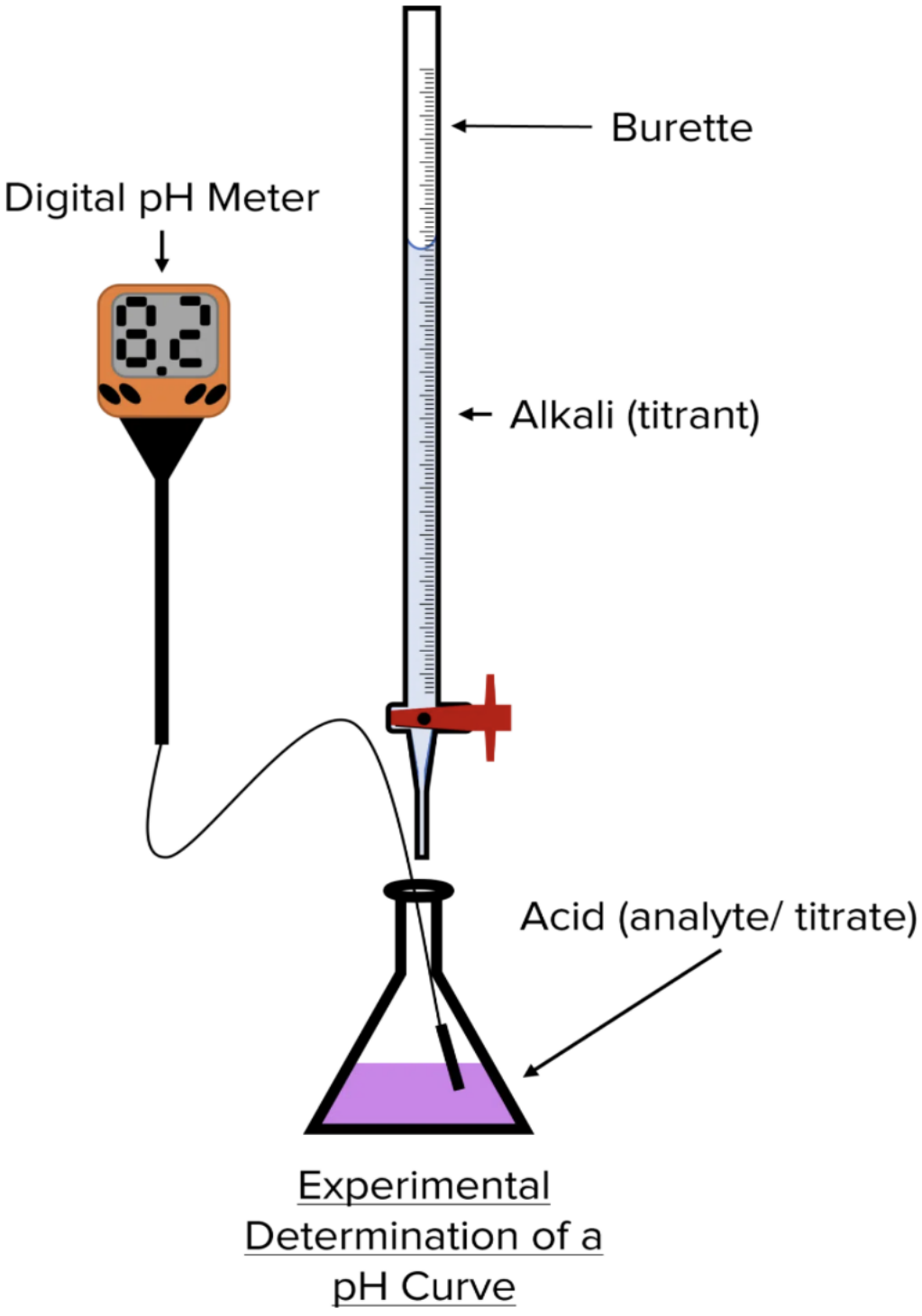


Figure 5.5: pH metric method

5.17 POTENTIOMETRIC TITRATION

A reference electrode, or E_{ref}, is a half-cell with a known potential that is unaffected by changes in the analyte solution's content and stays constant at constant temperature. The reference electrode in potentiometric observations is always denoted as the left-hand electrode. Caldel electrodes and silver/silver chloride electrodes are examples of reference electrodes.The potential of a sensor electrode fluctuates in response to changes in analyte concentration. The majority of indicator electrodes used in potentiometry respond selectively.

Two types of indication electrodes are metallic electrodes and membrane electrodes.

A salt bridge, which keeps the components of the analyte solution from combining with the reference electrode, is the third part of a potentiometric cell.

At either end of the liquid connections, a potential forms across the liquid junctions at the last point of salt bridge.

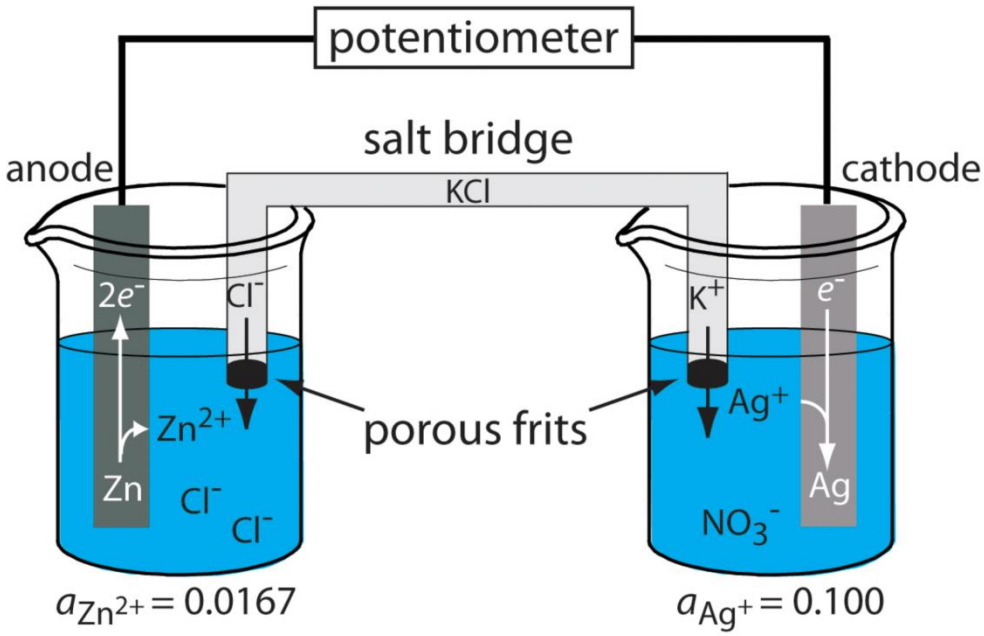


Figure 5.6: Titration method on the basis of Potentiometry

5.18 CONDUCTOMETRIC TITRATION

At constant temperature, the kind and quantity of charge carriers dictate the electrical conductivity of aqueous electrolyte solutions. differences in reagent systems' ionic composition are linked to characteristic differences in conductivity. These can serve as markers of the color change point in the conductometric titration. The conductivity sensor measures the change in conductivity in various titrations.

Figure 5.7: Sample analysis by conductometric titration

5.19 TECHNIQUE USED FOR DETERMINATION OF EQUIVALENCE POINT

The standard solution is often added using a burette, which is a graduated tube. The material to be determined is titrated, which is the process of adding the standard solution until the reaction is almost finished. The theoretical (or stoichiometric) end point, also known as the equivalency point, is the point at which this happens. A physical change, such as the slight pink color formed by potassium permanganate in the standard solution, or, more frequently, the addition of an auxiliary reagent known as an indicator, signal the completion of the titration. Alternatively, another physical measurement may be employed. The indication should provide a distinct appearance once the drug and standard solution have almost finished reacting.

A reaction needs to meet the following requirements in order to be used in titrimetric analysis.

1 A straightforward reaction that can be described by a chemical equation must occur; the material to be ascertained and the reagent must react entirely in stoichiometric or comparable amounts.

2. Reaction time ought to be fairly quick. (The majority of ionic reactions meet this requirement.) A catalyst may occasionally need to be added in order to speed up a reaction.

3. At the equivalency point, the solution's chemical or physical characteristics must change.

4. A suitable equivalency point can often be reached by monitoring the titration process and measuring the following:

(a) the potential between an indicator electrode and a reference electrode

(b) the change in the solution's electrical conductivity

(c) the current that flows through the titration cell between an indicator electrode and a depolarized reference electrode at a suitable applied.

There are four primary classes of reactions used in titrimetric analysis. Since the first three of these rely on the arrangement of ions, there is no change in oxidation state involved. However, the fourth type of events, known as oxidation-reduction reactions, entails an electron transfer or a modification of the oxidation state.

1. Acidimetry and alkalimetry, also known as neutralization reactions. These consist of titrating free bases, or those generated from weak acid salts through hydrolysis, using a standard acid (acidimetry) and titrating free acids, or those generated from weak acid salts through hydrolysis,

using a standard base (alkalimetry). Water is created by the reactions of hydrogen and hydroxide ions.

Titrations in non-aqueous media must be included under this area as well.

5.20: Self-Assessment

1. What measures the change in conductivity in various titrations.
2. State the classification of electroanalytical methods.
3. Write the interpretion of results from TGA instruments.

CHAPTER - 6

SEPARATION TECHNIQUES

Objective

- Identify the principles behind a particular separation technique.
- Identify an appropriate separation technique to separate a mixture basedon the physical properties

6.1 Extraction Principles- Solvent extraction has been a vital separation technique since the early days of the Manhattan Project, when scientists separated uranyl nitrate into diethyl ether to purify the uranium used in the first reactors. Solvent extraction, commonly referred to as liquid-liquid extraction, is a technique used in both industrial and scientific settings. However, because to the high cost of components and the increasing challenge and cost of disposing of the mixed waste produced by the vast amounts of solvents required, current laboratory trends are moving away from this technology. Because separatory funnels must be used for many extractions, the process is also often labor-intensive. However, solvent extraction remains a powerful separation method that should be taken into consideration.

The technique of using a solvent to remove a solute from a liquid mixture on a selective basis is known as solvent extraction. It is a partitioning procedure that uses the uneven distribution of the solute (A) between two immiscible solvents—typically water (aq) and an organic liquid (org)—as a separating technique:

$$A_{aq} W A_{org}$$

The solute may exist in a liquid or solid state. Water, a water-miscible solvent, or a water-immiscible solvent can all be used as the extraction solvent, but it must be insoluble in the liquid mixture's solvent. varied solvents exhibit different solubilities for different solutes. As a result, the characteristics of the solute, the liquid combination, and other needs of the experimental protocol will all influence the extraction solvent selection. Although different solvent combinations can be used, water and a nonpolar organic liquid, such as hexane or diethyl ether, are the solvents in many applications. In most cases, one of the solvents (water, for example) already contains the solute to be extracted together with any contaminants or interfering analytes that need to be separated.

This technique added a nonpolar organic solvent and thoroughly mixing the two, commonly by shaking in a separatory funnel. Each solvent is finely dispersed into the other by shaking, and after standing for a few minutes, the mixture will split into two different layers. The bottom layer will form from the more dense solvent. Because the solute and any concomitant impurities or analytes have varying solubilities in the two solvents, separation is achieved. For instance, the solute may stay more readily in the aqueous phase, but the analyte or contaminants dissolve more readily in the organic phase. From the aqueous layer, the analyte and impurities are removed and transferred to the organic layer. Alternatively, the impurities may remain in the aqueous layer as the solute becomes more soluble in the organic solvent and moves from it into the organic layer.

6.2 DISTRIBUTION COEFFICIENT

The partition coefficient, or distribution coefficient, Kd, describes the various solubilities of a solute in the solvent combinations of an extraction system. The solute's solubility in one solvent in comparison to its solubility in another is represented by the coefficient, an equilibrium constant. The concentration of solute in one phase directly correlates with the concentration of solute in the other phase after equilibrium is reached. This can be mathematically stated as:

where Kd is a constant and [Aorg] and [Aaq] represent the solute concentrations in the organic and aqueous phases, respectively. Since the concentrations are usually given in g/g or moles/kg (molality) units, the constant has no units. These solubilities typically indicate the solute's saturation concentrations in each solvent. The coefficient is dependent on temperature, but not by a constant factor because the solubilities change with temperature. A listing of solvent extraction methods with distribution coefficients and laboratory conditions for carrier-free tracers may be found in Wahl and Bonner's (1951).

When a solute in a hexane/water system has a distribution coefficient of 90, it indicates that, at saturation conditions, the solute is 90 times more soluble in hexane than in water; yet, some water still carries a little quantity of the solute. The solute is selectively dissolved in one solvent by solvent extraction, but it is not entirely extracted from the other solvent. A higher coefficient would suggest that following extraction, a smaller amount of solute would remain in the water but be

more widely dispersed in hexane. To quantitatively extract a solute from a liquid combination, solvent extraction techniques sometimes involve several extractions.

The distribution law statement is simply a very helpful approximation; it is neither thermodynamically strict nor situations in which the solute is participating in a chemical process in either phase, such as dissociation or association. Consider dimerization in the organic phase, for instance:

$$2A_{org} W(A)_{2,org}$$

Where the distribution ratio, D, is an alternate form of the distribution coefficient expressed by:

$$D=([A_{org}]_{monomer}+[A_{org}]_{dimer})/[A_{aq}]$$

or

$$D=([A_{org}]+2[(A)_{2,org}])/[A_{aq}]$$

Because the concentration of the monomer that represents the dimeric form of the solute is twice that of the concentration of the dimer:

$$[A_{org}]_{dimer}=2[(A)_{2,org}]$$

Substitution of K_d produces:

$$D=K_d(1+2K_2[A_{org}])$$

Where K_2 is the dimerization con stant, $K_2=[(A)_{2,org}]/[A_{org}]^2$.
Because dimerization decreases the concentration of the monomer, the species that takes part directly in the phase partition, the overall distribution increases

6.3 Extraction Technique- Although there is a lot of literature on extraction procedures, this list just includes a few of the many sources. Irving and Williams (1961), Lo et al. (1983), and Dean (1995) provide in-depth discussions of the solvent extraction hypothesis. A great place to find out about recent developments in this topic is the journal Solvent Extraction and Ion Exchange. Korkisch (1969) offers a helpful overview of the fundamentals of solvent extraction. The following talks about a metallic element in solution that is a cation that is removed by a nonpolar solvent:
An element found in an aqueous solution is transformed into a soluble compound in an organic solvent by the process of solvent extraction. The organic solvent and water must combine nearly exactly. Shaking the aqueous solution containing the organic solvent (extractant) in a separating funnel allows the element to be extracted into the organic phase. After the aqueous and organic phases in the funnel have had time to separate, the organic extract is removed from contact with the aqueous layer.

This is a single-stage batch extraction method applied when Kd is relatively large. A sufficient difference in the distribution coefficients of the metal ions to be separated is necessary for a straightforward separation. The separation factor, which is the ratio of the distribution coefficients of two different elements, is comparable to ion exchange.

This ratio establishes the separability of two components via liquid-liquid extraction. Separations can only be finished when this ratio shows a number that differs from unity; they are also clean, fast, and easy to perform in cases where one of the distribution coefficients is very large and the other is very tiny (high separation factor).

Fractionation or continuous extraction techniques must be used in extractions when the separation factor is close to unity. With the latter methods, different fractions are distributed, transferred, and recombined a sufficient number of times to achieve separation. A continuous counter-current flow of both phases or an immiscible solvent flow through the solution are

used in continuous extraction. Continuous extraction involves either adding fresh solvent constantly from a reservoir or stripping the wasted solvent and recycling it by distillation. In

6.4 **Continuous counter-current extraction**, the two liquid phases are made to flow in opposition to one another. This method is generally applied to large-scale separations.

Choosing an appropriate organic solvent is one of the most crucial factors to take considered when using liquid-liquid extraction procedures. In addition to the previously indicated requirement that it be almost immiscible with water, a high solubility of the extracted component in the solvent is necessary for achieving a satisfactory separation. It must also demonstrate the ability to extract one component of a solution over another, i.e., it must be selective. Phase diagrams can be used to evaluate a solvent's selectivity for a particular component, however this method is not often employed in analytical chemistry. The main issue is just that there aren't nearly enough phase diagrams in the literature. As a result, selecting an extractant is determined by either semi-empirical or experiential factors. However, polar compounds are often extracted from nonpolar media using polar solvents, and vice versa. The selectivity of the solvent will undoubtedly be impacted by the interactions between the solute and the solvent. A solute will be soluble in a certain solvent if it can be dissolved in it easily. Selectivity and solubility are influenced by the creation of hydrogen bonds between the solute and solvent.

The recovery of the solute from the organic extract is nearly as crucial as the extractant's selectivity. If the solute is thermally stable and nonvolatile, recovery can be accomplished by distilling or evaporating the solvent. However, the idea of back extraction, also known as stripping, is applied more commonly than this approach. In stripping, the organic extract is treated with an aqueous solution containing a reagent, which induces the extracted solute to flow quantitatively into the aqueous layer.

6.5 Qualitative and Quantitative aspects of Solvent Extraction:

6.5.1 Extraction from metal ion aqueous Solution

The process of forming a chelate, or closed ring structure, between the chelating agent and the metal ion to be extracted is how extraction works in this class. such as the extraction of iron with cupric chloride in carbon tetrachloride and uranium with 8-hydroxyquinoline in chloroform, etc.The elimination of metal ions is significantly aided by selective ion exchange. More rapid sorption kinetics and a high resin capacity for metal ions like lead

(Pb^{2+}), copper (Cu^{2+}), etc. were offered by the newly developed resins. Moreover over studies suggest that electroplating can also aid in removal of metal ion like nickel, zinc, manganese, lead etc.

6.5.2 Extraction of Organic Species from aqueous and non-aqueous media liquid-liquid extraction

Organic component s within a mixture can be separated via liquid-liquid extraction or by acid –base extraction. A liquid-liquid extraction moves salts and other water-soluble contaminants into the aqueous phase or an organic substance to an organic solvent.This procedure involves adding an aqueous solution to a separator funnel, then adding an organic solvent that isn't soluble in water.Due to its greater solubility in the organic phase relative to the aqueous phase, the organic chemical partitions into the organic phase when thoroughly combined. The solubility of the organic component in each phase determines its partitioning between the two layers. When the solute's chemical potential is the same in both phases, equilibrium is reached. The ratio of the sample's concentration in the organic layer divided by its concentration in the aqueous phase is known as the partition coefficient for a solute, or K. Large partition coefficient solutes are more likely to be removed into the layer of organic solvent. Smaller partition coefficient solutes are more likely to go into the aqueous phase.

6.5.3Acid-Base Extraction

One kind of liquid-liquid extraction that uses the acid-base characteristics of organic substances is called acid-base extraction. Most organic molecules are normally neutral, but when they interact with salt, they become ionic and are important in the extraction of organic acids or bases from organic phases into aqueous phases. Thus, this kind of procedure makes use of these characteristics to change the solute's solubility by converting it into its salt form that is soluble in water. For the procedure to work, there must be a significant difference in the solubility of the organic component and its salt. Acid-base extraction can also be used to separate two weak acids or bases with a significant pKa difference. In terms of the acids, the comparatively stronger acid to produce a salt.Next, during the extraction process, the salt is drawn into the aqueous phase. For weak bases, the procedure is the same: a weak acid is used to neutralise the relatively stronger base to a salt.

6. 6 Self-Assessment

1. What is principle involved in solvent extraction?

2. Explain counter current extraction

3. Explain extraction of organic species in aqueous solution

Chapter - 7
CHROMATOGRAPHY

Learnig Objectives:

- Understand the process of chromatography,
- Describe different practical methods ,
- Use and learn the application of

7.1 Definition

Chromatography a type of seperation techniques based on the polarity, adsoprtion and absoprtion properties of of the analyte. This technique uses a solid/fluid stationary phase on to which the crude is seprated in the presence of mobile phase (liquid /gaeous). The following molecules with regard to adsorption (liquid-solid), partition (liquid-solid), and affinity to the stationary phase are the ones that could influence the separation process. As a result, some of the mixture's components remain in the stationary phase for a longer period of time, while others enter the mobile phase quickly and exit the system quickly due to their short retention times.

Three elements serve as the foundation for the chromatography technique based on this methodology.

Stationary phase: phase on which moleules are to be seperated. it may be solid or liquid (a layer of a liquid adsorbed on the surface a solid support).

- Mobile phase: inert liquid or a gaseous component
- Anaylte: Mixture of the component to be analyzed
- Elution: process of washing out a column using a appropriate solvent
- Eluate: Fluid existing in the column
- Eluent: Fluid entering the column

7.2 Develoment of Chromatogram

- Frontal chromatogram is a binary separation method where component with the least retention separated from the rest.
- An elution chromatogram uses a column to monitor the solutes' migration across the system once they are added, and then solute detection. Displacement chromatogram

comprises sample placement on to the column which further displace more strongly adsorbed molecule form the original mixture.

7.3 Types of Chromatography

7.3.1 Ion Exchange chromatography

The basis of ion-exchange chromatography is the electrostatic interactions that occur between the protein's charged amino group and matrix, which is the soil support system that is countercharged to the amino group in the protein. Proteins are separated from the column by adjusting the buffer solution's ionic strength, pH, or ion salt concentration.

There are two types of ion chromatography: anion exchangers and cation exchangers.

Anion- exchange chromatography:Used for the separation for negatively charged molecules as the pH for chromatography is more than the pI

Sr. No	Name	Type	Functional group
1	DEAE Cellulose (Anion exchanger)	Weakly basic	DEAE (Diethylaminoethyl)
2	QAE Sephadex (Anion exchanger)	Strongly basic	QAE (Quaternary aminoethyl)
3	Q Sepharose (Anion exchanger)	Strongly basic	Q (Quaternary ammonium)
4	CM- Cellulose (Cation exchanger)	Weakly acidic	CM (Carboxymethyl)
5	SP Sepharose (Cation exchanger)	Strongly acidic	SP (Sulfopropyl)
6	SOURCE S (Cation exchanger)	Strongly acidic	S (Methyl sulfate)

Fig 1: commonly used ion exchangers

7.3.2 Gas- Liquid Chromatography (GLC)

Gas Chromatography or Gas- Liquid Chromatography is a complex chromatography that uses gaseous (Helium and Nitrogen) mobile phase for separation. Mobile phase is passed through a column under high pressure.

GC exploits volatile properties of the molecules to be analyzed. Upon vaporization the molecules enters the gaseous mobile phase and dispersed between mobile phase, and stationary phase (Soild /liquid matrix) leading to separation of the molecules.

Advantages:

- Gas chromatography is a simple, multifaceted, highly sensitive

- Separates very little amounts of analysts
- Quick investigation and swift equilibrium.
- High Precision and accuracy

Applications:

- Used for identification and separation in quality management.
- Purity control, environmental, and pharmaceutical analysis
- Quantitative examination of primary and trace components

7.3.3 Gel Permeation Chromatography (GPC)

It is also known as Size exclusion chromatographythat efficiently separates colloidal particles or high molecular weight on the basis of size commonly in the presence of organic solvents . It was developed by 1955 by Lathe and Ruthven.

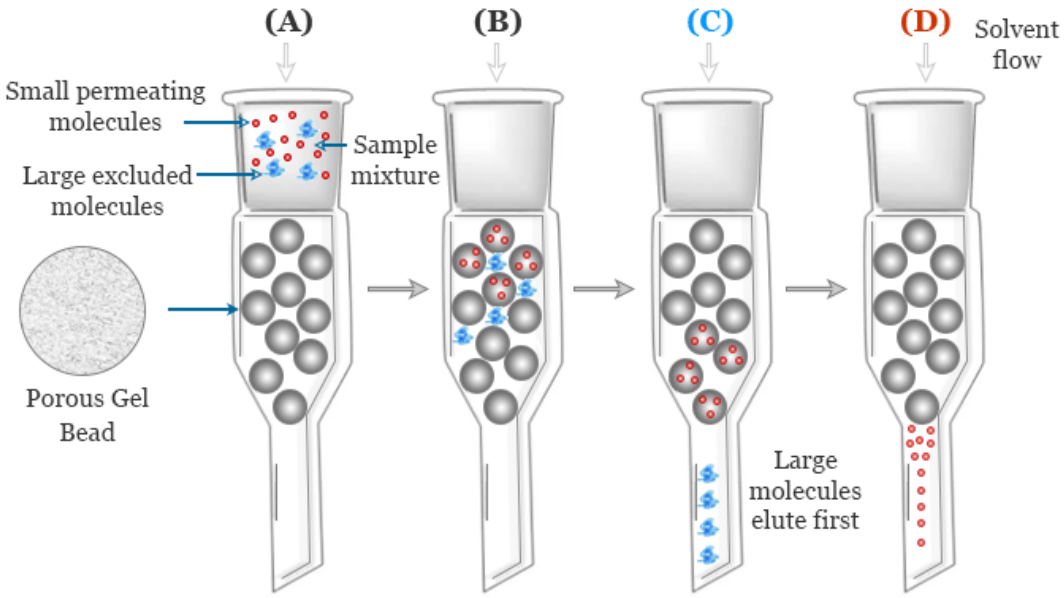


Figure 7.1 : Sechamtic diagram reprenting Gel Permeation Chromatography

Methodology:

The column (stationary phase) consists of inert molecules with small pores(Figure 7.1).

The analyte consisting of different sized molecules are passed.

Large sized molecules are passed through spaces between porous particles, and move rapidly through inside the column while smaller molecules as compared to porous column diffuses into pores leave the column with proportionally longer retention times .

Agraose, Sephadeks G, dextran, polyacrylamide are also used as column materials .

Advantages:

- Shorter retention time.
- Precise and narrow band separation.
- Highly sensitivity.
- No sample loss.
- The small amount of mobile phase required.

Application:

- GPC aid in purification of enzymes, polysaccharides, nucleic acids, proteins, and other biological macromolecules.
- It efficiently aid in refolding of denatured proteins by careful control of changing buffer conditions.

7.3.4 Thin Layer Chromatography (TLC)

Thin layer chromatography is type of planer chromatography. It was developed by Schraiber invented in 1939.

Principle:

TLC is based on the adsorption concept.Adsorption **involves the analytical separation based on the interaction of the adsorbent (inert liquid fixed on soild support) and adsorbate** (gas or liquid) which gets separated when it passes over the adsorbent.

Method:

Sample mixture a placed in samll amount at the bottom of TLC plate (a chemically stable and inert plates support)

This plates further palced in TLC chambers contating in an mobile phase (a solvent with same polarity of molecules to be separated) to control the retention mechanism by surface deactivation.

The Rf value is used to depict the results, just like in paper chromatography

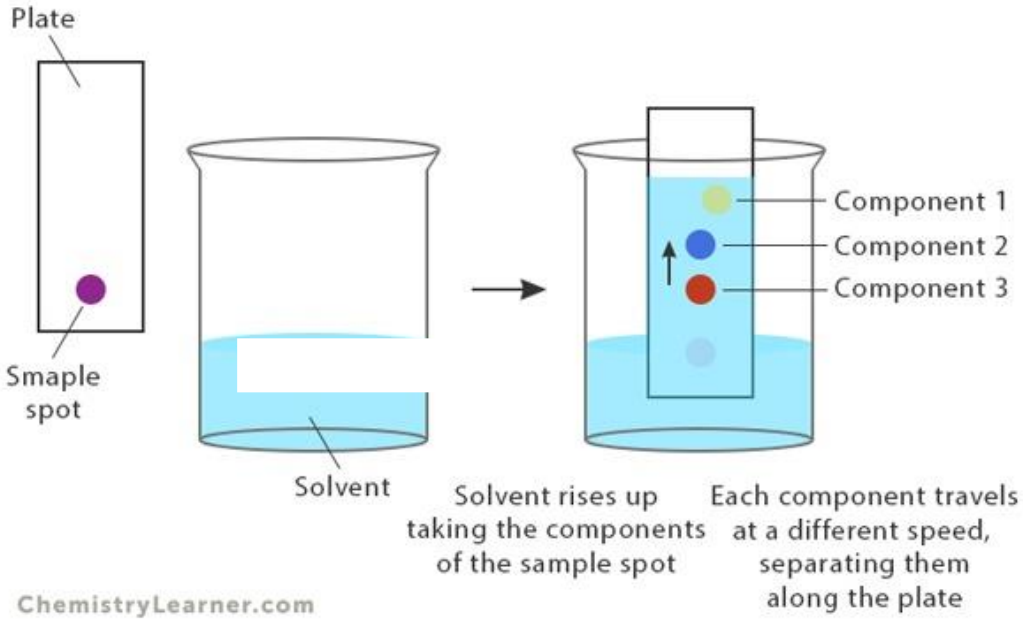


Figure 7.2 : Sechamtic diagram reprenting Thin Layer Chromatography

Advantages

- High sample throughput
- low sample requirnmnet
- Multiple sample analysis at the same time
- Minimal laboratory resources requirnemnt.

Applications

- It is employed in the separation of all types' macro and micromolecules.
- Used in the identification and purification of materials.

7.3.5 High performance Liquid chromatography (HPLC)

High performance Liquid chromatography is an advanced type of TLC which is rapid and can separate a wide range of samples

Methodology:

- To separate components within a mixture, the method uses a staionary phase (soild) and a mobile phase (Liquid/gas).
- The mobile phase is supplied throughout the system by a high-pressure pump.

- Moleculeswith a high affinity for the mobile phase migrate through the column more rapidly while rest retain with the stationary phase.
- After the analytes are separated, a detector calculates their concentration, which may then be examined using peaks generated by electrical signals(Figure 7.3).

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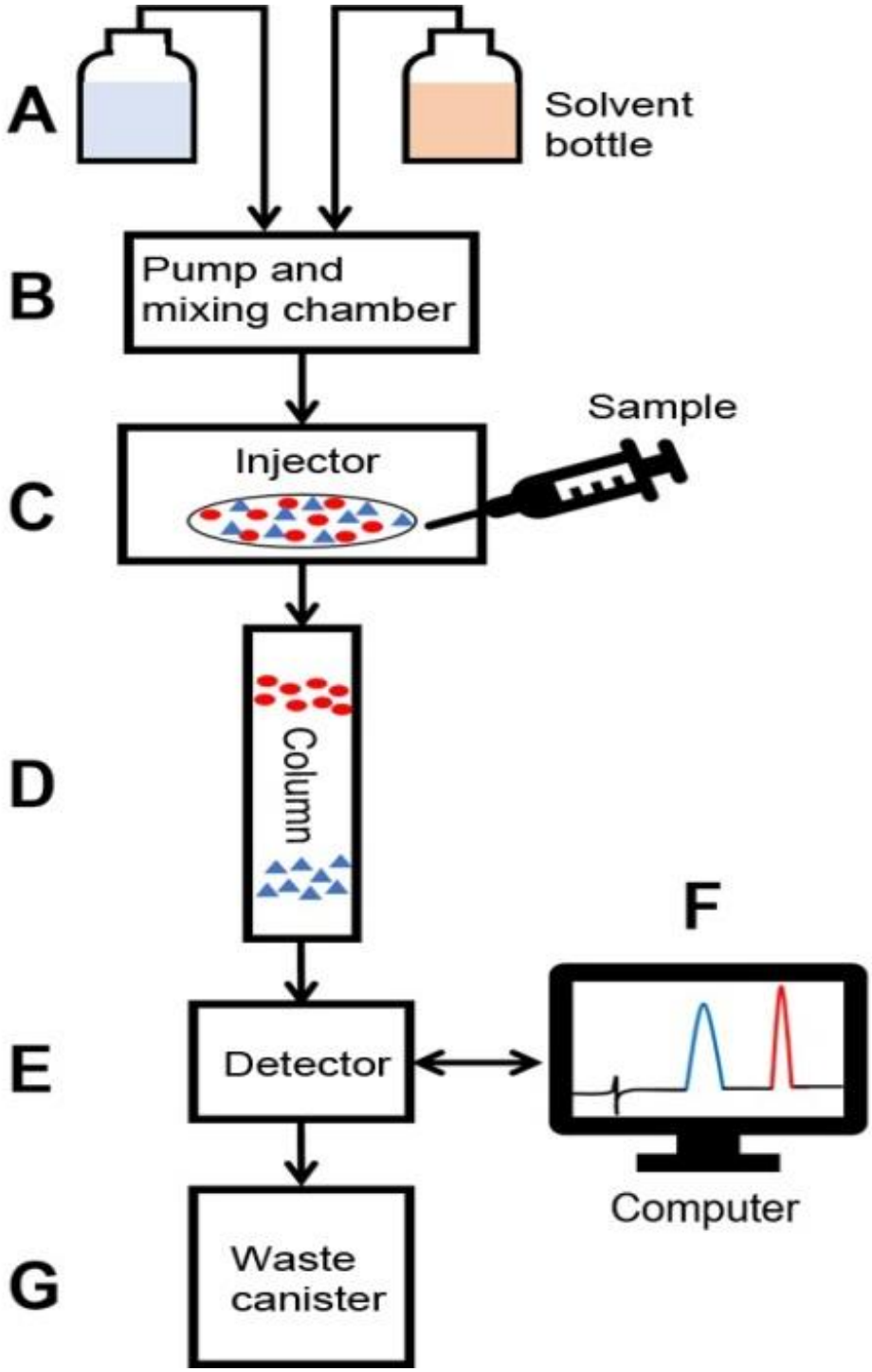

Figure 7.3: Sechamtic diagram reprenting HPLC

Advantages:

- Analytical precision and accuracy are increased through simultaneous sample and standard processing, which eliminates the need for an internal standard.
- Decreased maintenance expenses, as well as a decrease in the time and expense of each analysis
- Work with a range of samples and prepare them simply.
- Low persample mobile phase usage.
- Low contamination detection

Applications:

- An established for determining and purifying various medicines in the pharmaceutical business.
- Food and drug analysis includes herbal medicine quantification, vitamin analysis, water soluble food dye analysis, and pesticide analysis in fruits, vegetables, and other foods.
- Fingerprint analysis, detection of misuse substances, poisons, adulterations, chemical weapons, and illicit drugs are all forensic services.
- Environmental analysis and cosmetology

7.3.6 Stereoisomeric Sepration analysis :

Stereoisomers are isomers which have same molecular formula but different strucuture

The enantiomers are mirror images and non-superimposable.while The diastereomers are non-mirror images as well as non-superimposable.

Enantiomeric excess (**ee**) is a measurement of purity chiral compound. It reflects the degree to in which an enantiomer is present in higher amount as compared to other. on the othe hand The diastereomeric excess is the defines the excess of the diastereomer in a mixture of diastereomers.

$C_4H_6O_6$ is an excellent example of tartaric acid's diastereomers and enantiomers

Nuclear magnetic resonance

To ascertain the precise arrangement of stereoisomers, a chemical investigation known as nuclear magnetic resonance spectroscopy is employed. R or S enantiomers, cis or trans alkenes, and R,R or R,S diastereomers are a few examples. By integrating the area under the

peak that corresponds to a particular stereoisomer in a mixture, NMR may estimate the optical purity of the combination. When a chiral shift agent (a reagent molecule that can form a gas phase complex via noncovalent interactions with the analyte ion) is inserted into dry dimethyl sulfoxide (DMSO) such as tris with a nucleus other than hydrogen or carbon, the accuracy of integration can be increased.

7.4 Self Assisment

1. Define Stereoisomer's.
2. Give the application of High Performance Chromatography.
3. Explain in detail Thin Layer Chromatography.
4. What is chiral shift agent?

GLOSSARY

Absorption: The process by which atoms, molecules, or ions take up or assimilate energy from electromagnetic radiation, typically leading to an increase in their internal energy levels.

Accuracy: The closeness of a measured value to the true or accepted value of a quantity.

Analyte: The substance or component of interest in a sample that is being analyzed.

Atomic Absorption Spectroscopy (AAS): An analytical technique that measures the absorption of electromagnetic radiation by atoms in the gaseous state, often used for determining the concentration of elements in a sample.

Detector: A device that detects and measures the signals generated during an analytical experiment, such as the absorption of radiation or the emission of light.

Electrochemistry: The branch of chemistry that deals with the study of chemical reactions involving the transfer of electrons between reactants, often used in analytical methods such as voltammetry and potentiometry.

Precision: The degree of reproducibility or consistency of measurements obtained from multiple repetitions of an experiment or analysis.

Quantitative Analysis: The determination of the amount or concentration of a substance in a sample, often expressed as a numerical value.

Spectroscopy: The study of the interaction between matter and electromagnetic radiation, used to characterize the structure, composition, and properties of materials.

Standard Solution: A solution of known concentration used for calibrating analytical instruments and quantifying the concentration of analytes in samples.

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