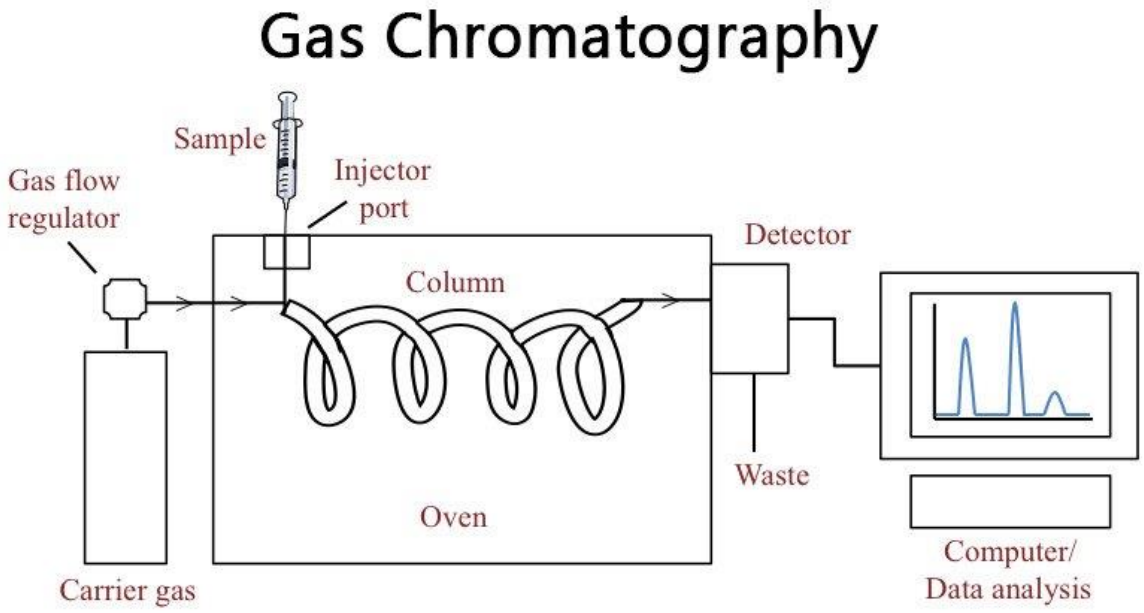
Name of Experiment: Gas Chromatography (GC)

Goal of Experiment: to separate the chemical components of a sample mixture and then detect them to determine their presence or absence and/or how much is present.

Introduction:

What is gas chromatography?

- Gas chromatography differs from other forms of **chromatography** in that the mobile phase is a gas and the components are separated as vapors.
- It is thus used to separate and detect small molecular weight compounds in the gas phase.
- The sample is either a gas or a liquid that is vaporized in the injection port. **The mobile phase for gas chromatography is a carrier gas**, typically helium because of its low molecular weight and being chemically inert.
- The pressure is applied and the mobile phase moves the analyte through the column. The separation is accomplished using a column coated with a stationary phase.



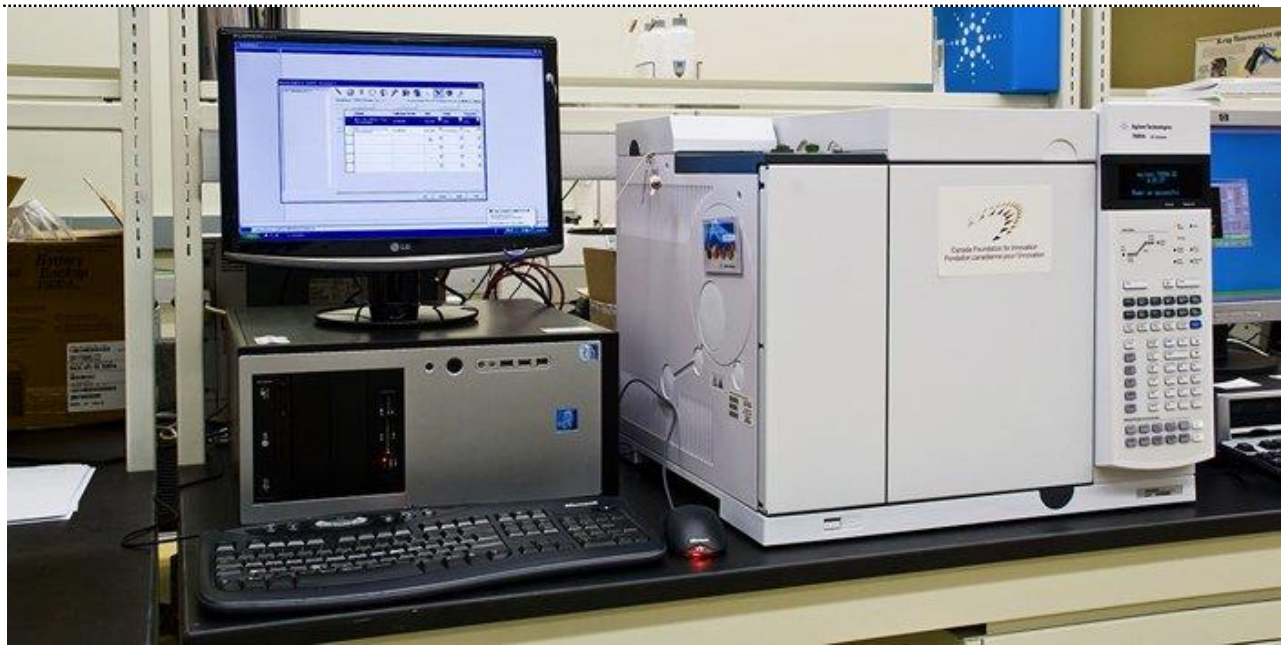
Principle of Gas chromatography (how does gas chromatography work):

The equilibrium for gas chromatography is partitioning, and the components of the sample will partition (i.e. distribute) between the two phases: the stationary phase and the mobile phase.
Compounds that have a greater affinity for the stationary phase spend more time in the column and thus elute later and have a longer **retention time (Rt)** than samples that have a higher affinity for the mobile phase.
Affinity for the stationary phase is driven mainly by intermolecular interactions and the polarity of the stationary phase can be chosen to maximize interactions and thus the separation.

Ideal peaks are Gaussian distributions and symmetrical, because of the random nature of the analyte interactions with the column.

- The separation is hence accomplished by partitioning the sample between the gas and a thin layer of a nonvolatile liquid held on a solid support.
- A sample containing the solutes is injected into a heated block where it is immediately vaporized and swept as a plug of vapor by the carrier gas stream into the column inlet.
- The solutes are adsorbed by the stationary phase and then desorbed by a fresh carrier gas.
- Each solute will travel at its own rate through the column.
- Their bands will separate into distinct zones depending on the partition coefficients, and band spreading.
- The solutes are eluted one after another in the increasing order of their kd, and enter into a detector attached to the exit end of the column.
- Here they register a series of signals resulting from concentration changes and rates of elution on the recorder as a plot of time versus the composition of carrier gas stream.
- The appearance time, height, width, and area of these peaks can be measured to yield quantitative data.

Parts of Gas chromatography



Gas chromatography is mainly composed of the following parts:

1. **Carrier gas in a high-pressure cylinder with attendant pressure regulators and flow meters**

- Helium, N_2, H_2, Argon are used as carrier gases.
- Helium is preferred for thermal conductivity detectors because of its high thermal conductivity relative to that of most organic vapors.
- N_2 is preferable when a large consumption of carrier gas is employed.

- Carrier gas from the tank passes through a toggle valve, a flow meter, (1-1000 ml/min), capillary restrictors, and a pressure gauge (1-4 atm).
- Flow rate is adjusted by means of a needle valve mounted on the base of the flow meter and controlled by capillary restrictors.
- The operating efficiency of the gas chromatograph is directly dependant on the maintenance of constant gas flow.

2. Sample injection system

- Liquid samples are injected by a microsyringe with a needle inserted through a self-scaling, silicon-rubber septum into a heated metal block by a resistance heater.
- Gaseous samples are injected by a gas-tight syringe or through a by-pass loop and valves.
- Typical sample volumes range from 0.1 to 0.2 mL.

3. The separation column

- The heart of the gas chromatography is the column which is made of metals bent in U shape or coiled into an open spiral or a flat pancake shape.
- Copper is useful up to 250 ^{0}C
- Swege lock fittings make column insertion easy.
- Several sizes of columns are used depending upon the requirements.

4. **Liquid phases**

- An infinite variety of liquid phases are available limited only by their volatility, thermal stability and ability to wet the support.
- No single phase will serve for all separation problems at all temperatures.

Non-Polar – Parafin, squalane, silicone greases, apiezon L, silicone gum rubber. These materials separate the components in order of their boiling points.
Intermediate Polarity – These materials contain a polar or polarizable group on a long non-polar skeleton which can dissolve both polar and non-polar solutes. For example. diethyl hexyl phthalate is used for the separation of high boiling alcohols.
Polar – Carbowaxes – Liquid phases with a large proportion of polar groups. Separation of polar and non-polar substances.
Hydrogen bonding – Polar liquid phases with high hydrogen bonding e.g. Glycol.
Specific purpose phases – Relying on a chemical reaction with solute to achieve separations. e.g $AgNO_3$ in glycol separates unsaturated hydrocarbons.

5. **Supports**

- The structure and surface characteristics of the support materials are important parameters, which determine the efficiency of the support and the degree of separation respectively.
- The support should be inert but capable of immobilizing a large volume of liquid phase as a thin film over its surface.
- The surface area should be large to ensure the rapid attainment of equilibrium between stationary and mobile phases.
- Support should be strong enough to resist breakdown in handling and be capable of packed into a uniform bed.
- Diatomaceous earth, kieselguhr treated with Na_2CO_3 for 900 ^{0}C causes the particle fusion into coarser aggregates.
- Glass beads with a low surface area and low porosity can be used to coat up to 3% stationary phases.

- Porous polymer beads differing in the degree of cross-linking of styrene with alkyl-vinyl benzene are also used which are stable up to 250 ^{0}C

6. **Detector**

- Detectors sense the arrival of the separated components and provide a signal.
- These are either concentration-dependent or mass dependant.
- The detector should be close to the column exit and the correct temperature to prevent decomposition.

7. **Recorder**

- The recorder should be generally 10 mv (full scale) fitted with a fast response pen (1 sec or less). The recorder should be connected with a series of good quality resistances connected across the input to attenuate the large signals.
- An integrator may be a good addition.

1. Name of Experiment: UV-Visible Spectroscopy

2. Goal of Experiment

- ✓ Learn UV-VIS Spectrophotometer analysis method principle and operation,
- ✓ Learn UV-VIS spectrophotometer's structure.
- ✓ Identify compounds by UV-VIS (learn how to how to quantify preservative in food).

3. Introduction

3.1 Definition of UV-Vis Spectroscopy

- **Spectroscopy**

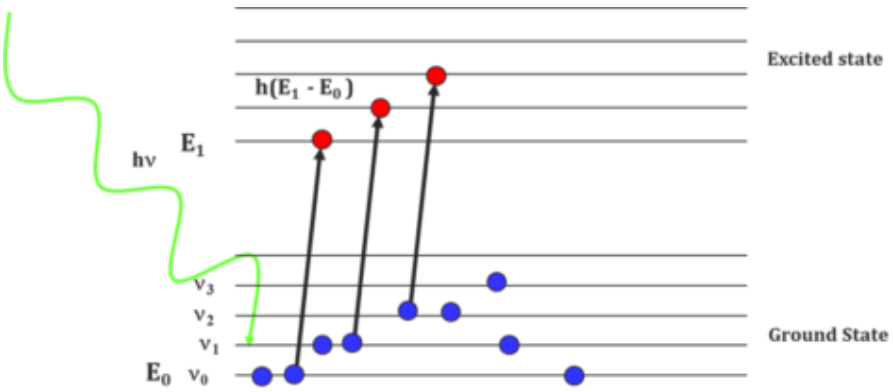
It is the branch of science that deals with the study of interaction of matter with light, or it is the branch of science that deals with the study of interaction of electromagnetic radiation with matter.

- **UV-Vis Spectroscopy** (or Spectrophotometry) is a quantitative technique used to measure how much a chemical substance absorbs light. This is done by measuring the intensity of light that passes through a sample with respect to the intensity of light through a reference sample or blank.

3.2 Principle of UV-Visible

The Principle of UV-Visible Spectroscopy is based on the absorption of ultraviolet light or visible light by chemical compounds, which results in the production of distinct spectra.

- Basically, spectroscopy is related to the interaction of light with matter.
- As light is absorbed by matter, the result is an increase in the energy content of the atoms or molecules.
- When ultraviolet radiations are absorbed, this results in the excitation of the electrons from the ground state towards a higher energy state.



- Molecules containing π-electrons or non-bonding electrons (n-electrons) can absorb energy in the form of ultraviolet light to excite these electrons to higher anti-bonding molecular orbitals.
- The absorption of ultraviolet light by a chemical compound will produce a distinct spectrum which aids in the identification of the compound.

4. Instrumentation of UV Spectroscopy

- **Light Source**

Tungsten filament lamps and Hydrogen-Deuterium lamps are most widely used and suitable light source as they cover the whole UV region.

Tungsten filament lamps are rich in red radiations; more specifically they emit the radiations of 375 nm, while the intensity of Hydrogen-Deuterium lamps falls below 375 nm.

- **Monochromator**

✓ Monochromators generally is composed of prisms and slits.

✓ Most of the spectrophotometers are double beam spectrophotometers.

✓ The radiation emitted from the primary source is dispersed with the help of rotating prisms.

✓ The various wavelengths of the light source which are separated by the prism are then selected by the slits such the rotation of the prism results in a

series of continuously increasing wavelength to pass through the slits for recording purpose.

- ✓ The beam selected by the slit is monochromatic and further divided into two beams with the help of another prism.

- **Sample and reference cells**

- ✓ One of the two divided beams is passed through the sample solution and second beam is passé through the reference solution.
- ✓ Both sample and reference solution are contained in the cells.
- ✓ These cells are made of either silica or quartz **cuvettes**. Glass can't be used for the cells as it also absorbs light in the UV region.

- **Detector**

- ✓ Generally two photocells serve the purpose of detector in UV spectroscopy.
- ✓ One of the photocell receives the beam from sample cell and second detector receives the beam from the reference.
- ✓ The intensity of the radiation from the reference cell is stronger than the beam of sample cell. This results in the generation of pulsating or alternating currents in the photocells.

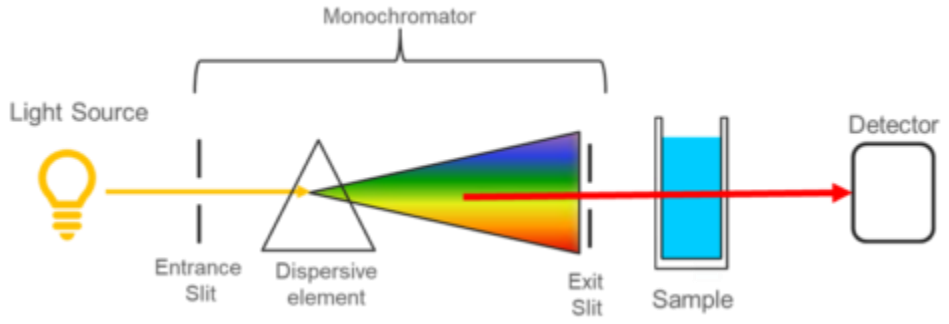
- **Amplifier**

- ✓ The alternating current generated in the photocells is transferred to the amplifier.
- ✓ The amplifier is coupled to a small servometer.
- ✓ Generally current generated in the photocells is of very low intensity, the main purpose of amplifier is to amplify the signals many times so we can get clear and recordable signals.

- **Recording devices**

✓ Most of the time amplifier is coupled to a pen recorder which is connected to the computer.

✓ Computer stores all the data generated and produces the spectrum of the desired compound.



5. Applications of UV Spectroscopy

- **Qualitative & Quantitative Analysis:**

  – It is used for characterizing aromatic compounds and conjugated olefins.

  – It can be used to find out molar concentration of the solute under study.

- **Detection of Impurities**

It is one of the best methods for determination of impurities in organic molecules. Additional peaks can be observed due to impurities in the sample and it can be compared with that of standard raw material. By also measuring the absorbance at specific wavelength, the impurities can be detected.

- **Structure elucidation of organic compounds**

It is useful in the structure elucidation of organic molecules, such as in detecting the presence or absence of unsaturation, the presence of hetero atoms.

- UV absorption spectroscopy can be used for the **quantitative determination of compounds** that absorb UV radiation.
- UV absorption spectroscopy can characterize those types of compounds which absorbs UV radiation thus used in qualitative determination of compounds. Identification is done by comparing the absorption spectrum with the spectra of known compounds.
- This technique is used to detect the presence or absence of functional group in the compound. Absence of a band at particular wavelength regarded as an evidence for absence of particular group.
- Kinetics of reaction can also be studied using UV spectroscopy. The UV radiation is passed through the reaction cell and the absorbance changes can be observed.

- Molecular weights of compounds can be measured spectrophotometrically by preparing the suitable derivatives of these compounds using **Beer's law**.

6. Experiment: Food preservative content

Benzoic acid and sorbic acid are two common kinds of food preservatives. Benzoic acid has an aromatic structure and has K absorption band and B absorption band at wavelengths of 228nm and 272nm. Sorbic acid has α, β unsaturated carbonyl structure, and there is a K absorption band of $\pi \rightarrow \pi^*$ transition at a wavelength of 255nm.Therefore, according to their UV absorption spectrum characteristics can be qualitatively identified and quantitatively determined.

- ***Qualitative analysis of preservative***

Take the purified and diluted ether extract (or Sprite diluted aqueous solution), use a 1cm absorption **cuvette**, with diethyl ether (or distilled water) as a **reference**, UV absorbance spectrum at wavelength of 210~310nm, according to the absorption peak wavelength, absorption intensity and absorbance spectra of benzoic acid and sorbic acid standard samples were compared to determine the type of preservative in the sample.

1. Name of Experiment: Atomic Absorption spectrophotometry (AAS)

2. Goal of Experiment

- ✓ Learn atomic absorption Spectrophotometer analysis method principle and operation,
- ✓ Learn atomic absorption spectrophotometer's structure.
- ✓ Identify ***elements*** by atomic absorption spectrophotometry

3. Introduction

3.1 Definition of Atomic Absorption Spectrophotometry

- **Spectrophotometry**

Spectrophotometry is defined as the measurement of the intensity of light at selected wavelength and widely used method of quantitative and qualitative analysis in the chemical and biological sciences.

- **Atomic Absorption Spectrophotometry**

The atomic absorption spectrophotometer is used to measure concentration by detecting absorption of electromagnetic radiation by atom rather than by molecules. It is a very common technique for detecting **metals** and **metalloids** in sample. Widely used in clinical laboratories to measure elements such as aluminum, calcium, copper, lead, lithium, magnesium, zinc, and other metals.

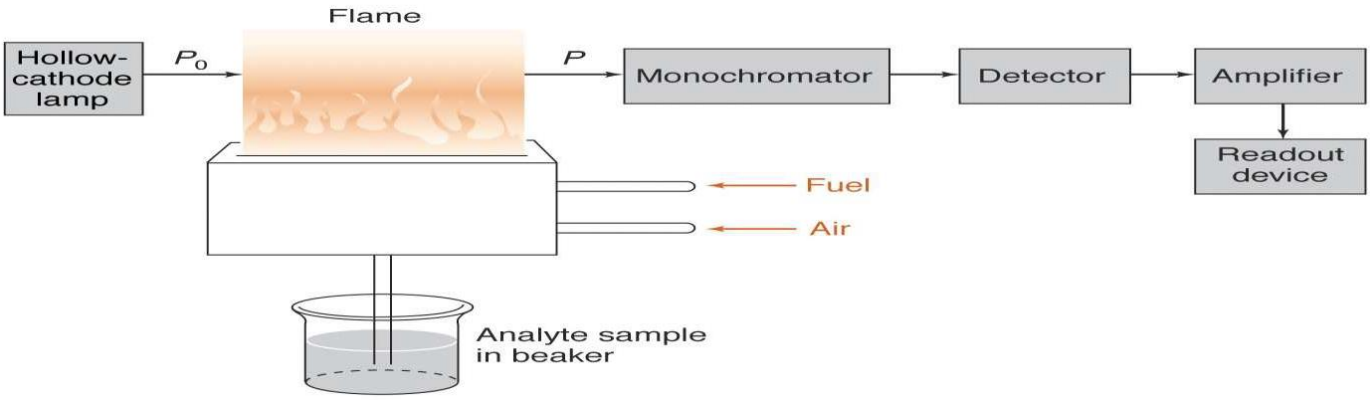
- **Invention**
  - ✓ Introduced in 1955 by Alan Walsh in Australia
  - ✓ First commercial atomic absorption spectrometer was introduced in 1959. Used for mining, medical treatment &agriculture

4. Principle of AAS

- Atomic absorption is an absorption spectrophotometric technique in which a metallic atom in the sample absorbs light of a specific wavelength.
- The element is not appreciably excited in the flame, but is merely dissociated from its chemical bonds (atomized) and placed in an unexcited or ground state (neutral atom).
- This ground state atom absorbs radiation at a very narrow bandwidth corresponding to its own line spectrum.
- A hollow cathode lamp with the cathode made of the material to be analyzed is used to produce a wavelength of light specific for the atom.
- Thus, if the cathode were made of sodium, sodium light at predominantly 589 nm would be emitted by the lamp.
- When the light from the hollow cathode lamp enters the flame, some of it is absorbed by the ground-state atoms in the flame, resulting in a net decrease in the intensity of the beam from the lamp.
- This process is referred to as atomic absorption.
- Concentration measurements are usually determined from a working curve after calibrating the instrument with standards of known concentration.

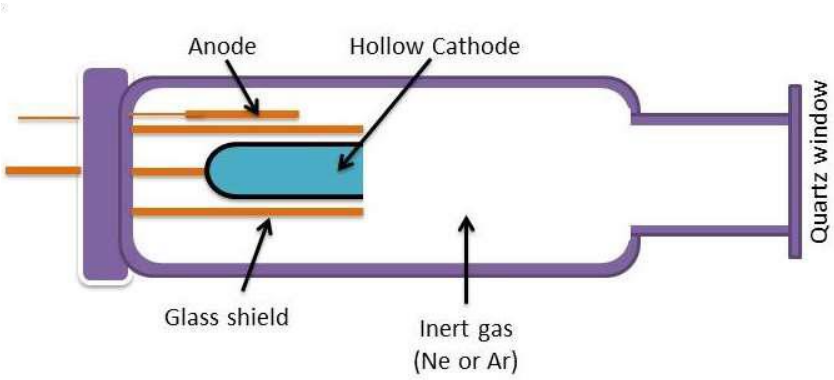
5. Instrumentation of Atomic Absorption Spectrophotometry

- Light source
- Chopper
- Atomizer
- Monochromators
- Detector
- Amplifier
- Read out device

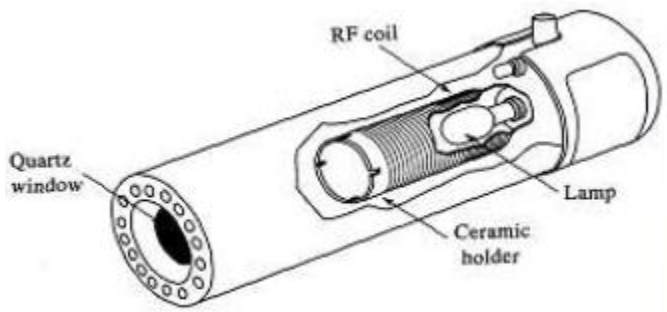


Light Source

- Hollow cathode Lamp

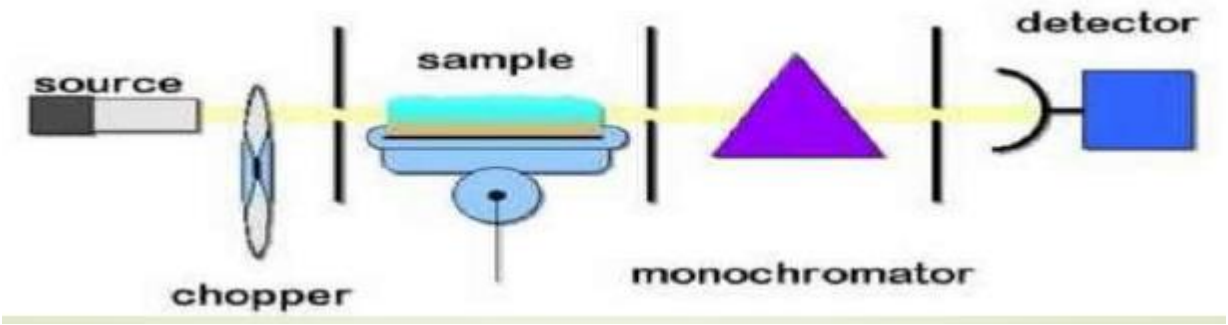


- Electrodeless Discharge Lamp



Chopper

A rotating wheel is interposed between the hollow cathode lamp and flame. It is interposed to break the ***steady light*** coming from the lamp into ***pulsating light*** which is used to measure the intensity of light absorbed by elements without interference by radiation from the flame itself.



Atomizer

- Atomization is separation of particles into individual molecules and breaking molecules into atoms .This is done by exposing the analyte to high temperatures in a *flame* or *graphite furnace*.
- Atomizer converts the *liquid* into *small droplets* which are easily vaporized.

Monochromators

- It is used to separate out all of the thousands of lines. Without a good monochromator, detection limits are severely compromised.
- A monochromator is used to select the specific wavelength of light which is absorbed by the sample, and to exclude other wavelengths. The selection of the specific light allows the determination of the selected element in the presence of others.
- Types of monochromators:
  - Prism monochromator
  - Grating monochromator

Detector

- The light selected by the monochromator is directed onto a detector that is typically a *photomultiplier tube*, whose function is to convert the light signal into an electrical signal proportional to the light intensity.
- The processing of electrical signal is fulfilled by a signal amplifier.

Photomultiplier Tubes

- Components
- Made of a glass vacuum tube
- Photocathode
- Several dynodes
- One anode

Working

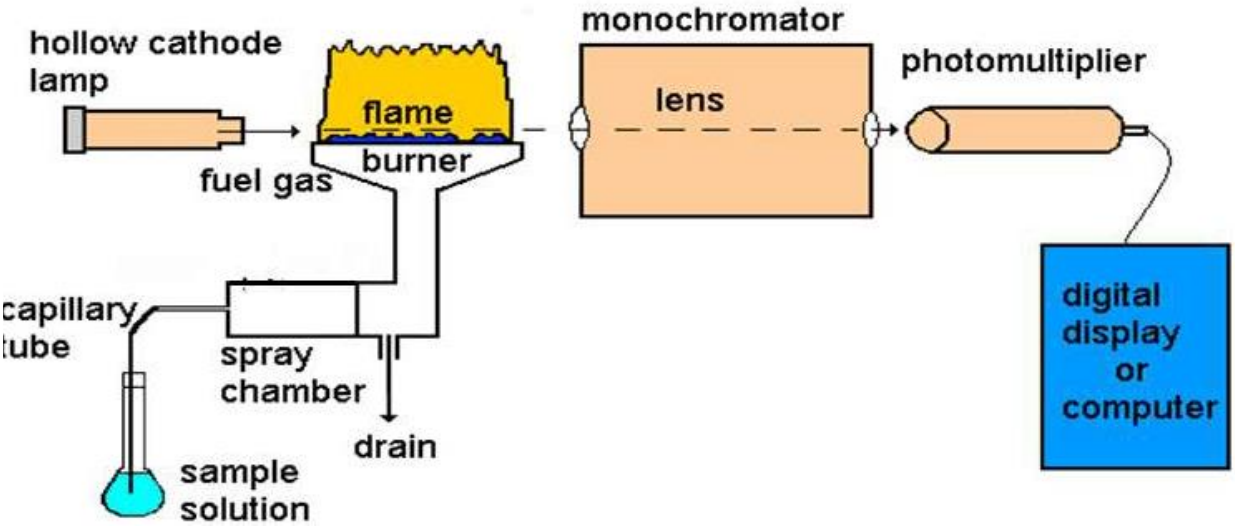
• The atoms of the solid are converted to gaseous state in the atomizer.

• Radiation of specific wavelength is emitted by the hollow cathode lamp onto the gaseous atoms in the atomizer.

• The monochromator focuses the specific wavelengths onto the detector

• The detector finds the amount of light absorbed.

• The concentration of atoms in the sample is directly proportional to the absorbance.



Sample preparation

- Dilution – sample is diluted in distilled water, acids or organic solvent
- Decomposition – isolation of required element from the sample by heating with/without a reagent
  - Wet/acid decomposition (300°C)
  - Dry ashing (400-500°C) - destroying the combustible portion of the sample. Oxidising agents may be used
  - Microwave decomposition (100-200°C)
  - sample decomposed at high pressures in a Teflon container
- Calibration curve must be prepared using different concentrations of the sample

Applications

• Level of metals could be detected in tissue samples like Aluminum in blood and Copper in brain tissues
• Presence of metals as an impurity or in alloys could be found easily
• Determination of elements in the agricultural and food products
• Determination of lead in petrol
• Determination of calcium and magnesium in cement

1. Name of Experiment: Energy and Electromagnetic Spectrum (EMS)

2. Goal of Experiment

- ✓ Understanding of the similarity and differences between electromagnetic waves
- ✓ Understanding of the relationship between electromagnetic waves and the range of wavelengths or frequency over which electromagnetic radiation extends.

3. Introduction

3.1 Definition of Electromagnetic Spectrum

It is a range of energies that electromagnetic radiation can comprise, including radio, microwaves, infrared, visible, ultraviolet, X-rays, and gamma rays; since electromagnetic radiation energy is proportional to the frequency and inversely proportional to the wavelength, the spectrum can also be specified by ranges of frequencies or wavelengths.

$$\nu = \frac{c}{\lambda}$$

Where ν is the frequency, *C* speed of wave (3 x 10^8 $m.s^{-1}$) and λ wavelength.

The electromagnetic (EM) spectrum is a classification of the Sun's radiation. Scientists have identified solar energy as a spectrum of many different wavelengths of electromagnetic rays. Waves of energy are called electromagnetic (EM) **because** they have oscillating electric and magnetic fields as shown in Fig.1. Scientists classify them by their frequency or wavelength, going from high to low frequency (short to long wavelength).

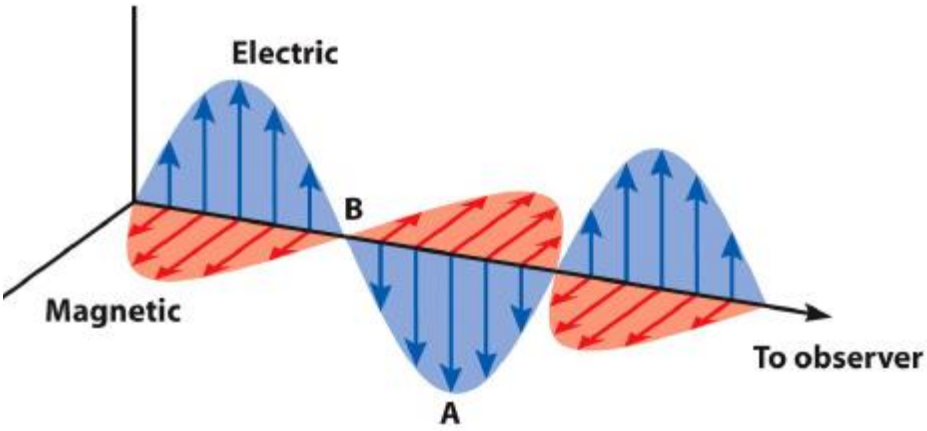


Fig. 1 Electromagnetic wave.

3.2 Uses of EMS

- **Radio waves** are used for communication such as television and radio. Radio waves are transmitted easily through air. They do not cause damage if absorbed by the human body, and they can be reflected to change their direction. These properties make them ideal for communications. Have the longest wavelengths and lowest frequencies of all the electromagnetic waves.
- **Microwaves** have the shortest wavelengths and the highest frequency of the radio waves. Used in oven, waves transfer energy to the water in the food causing them to vibrate which in turn transfers energy in the form of heat to the food.
- **Radar** (Radio Detection and Ranging) used to find the speed of an object by sending out radio waves and measuring the time it takes them to return.
- **Infrared (**below red) shorter wavelength and higher frequency than microwaves.
- **Visible light** shorter wavelength and higher frequency than infrared rays. Electromagnetic waves we can see.

Longest wavelength= red light

Shortest wavelength=violet (purple) light

- **Ultraviolet rays**
  - ✓ Shorter wavelength and higher frequency than visible light.
  - ✓ Carry more energy than visible light.
  - ✓ Used to kill bacteria (Sterilization of equipment).
  - ✓ Too much can cause skin cancer, use sun block to protect against (UV rays).
  - ✓ Causes your skin to produce vitamin D (good for teeth and bones).
- **X- ray**
  - ✓ Shorter wavelength and higher frequency than UV-rays
  - ✓ Carry a great amount of energy
  - ✓ Too much exposure can cause cancer (lead vest at dentist protects organs from unnecessary exposure)
  - ✓ Can penetrate most matter.
  - ✓ Used by engineers to check for tiny cracks in structures.
  - ✓ Used in medicine

– The rays pass through the cracks and the cracks appear dark on film.

- **Gamma rays**
  - ✓ Shorter wavelength and higher frequency than X-rays
  - ✓ Carry the greatest amount of energy and penetrate the most.
  - ✓ Used in radiation treatment to kill cancer cells.
  - ✓ Can be very harmful if not used correctly.
  - ✓ Exploding nuclear weapons emit gamma rays.

Wavelength and frequency are inversely proportional: As the wavelength increases, the frequency decreases. The inverse proportionality is illustrated in Fig 2. This figure also shows the electromagnetic spectrum, the range of all types of electromagnetic radiation. Each of the various colors of visible light has specific frequencies and wavelengths associated with them, and you can see that visible light makes up only a small portion of the electromagnetic spectrum. Because the technologies developed to work in various parts of the electromagnetic spectrum are different, for reasons of convenience and historical legacies, different units are typically used for different parts of the spectrum. For example, radio waves are usually specified as frequencies (typically in units of MHz), while the visible region is usually specified in wavelengths (typically in units of nm or angstroms).

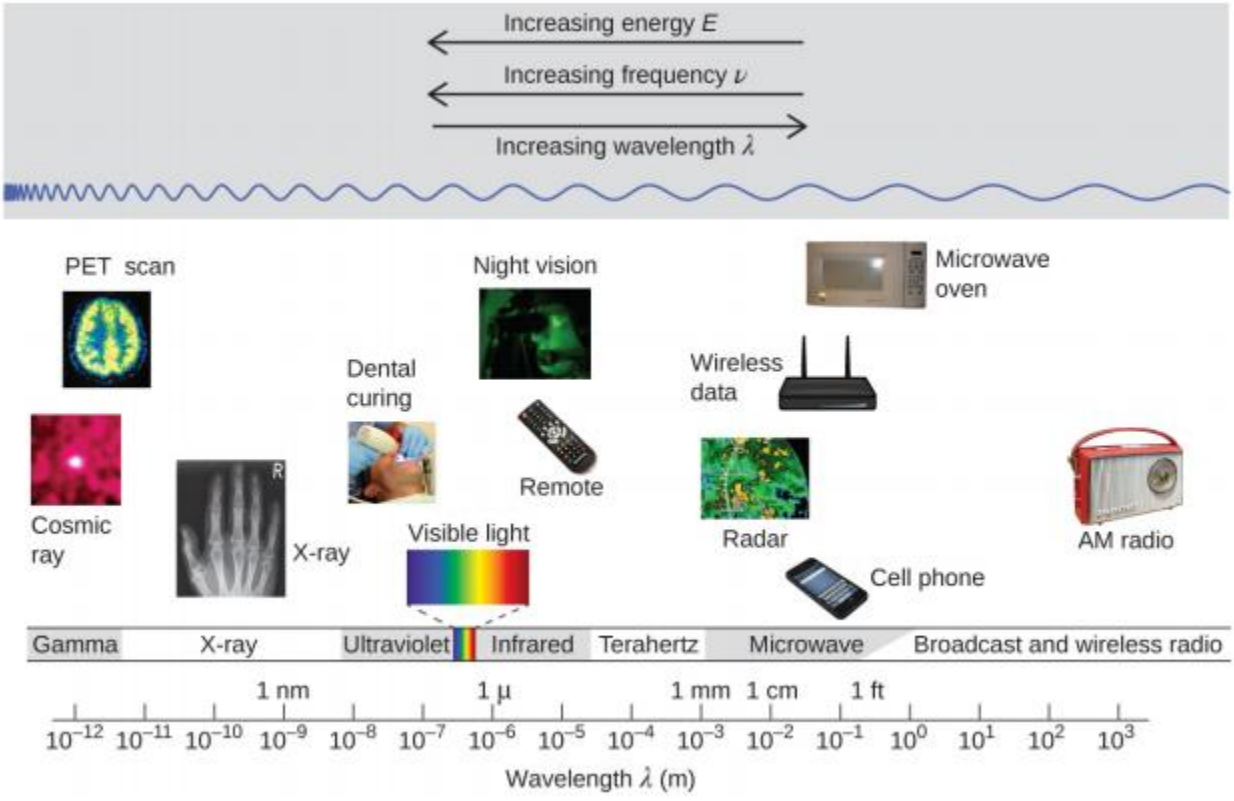


Fig.2 Electromagnetic spectrum

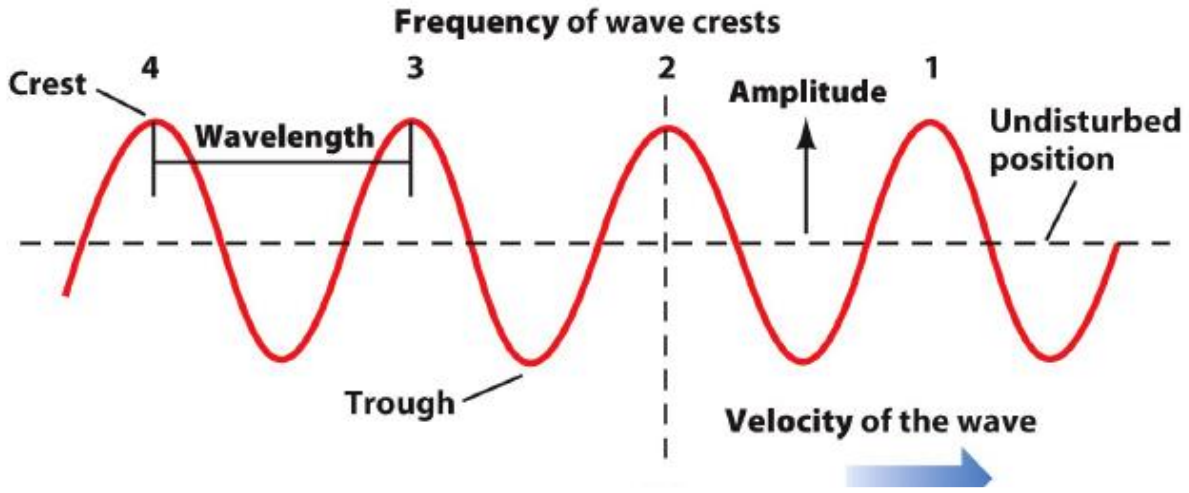


Fig.3 properties of wave

Amplitude: extent of the displacement caused by a wave (for sinusoidal waves, it is one-half the difference from the peak height to the trough depth, and the intensity is proportional to the square of the amplitude)

Frequency (ν): number of wave cycles (peaks or troughs) that pass a specified point in space per unit time (HZ). The unit of frequency, which is the number of cycles per second, s^{-1}

Intensity: property of wave-propagated energy related to the amplitude of the wave, such as brightness of light or loudness of sound

Wavelength (λ): distance between two consecutive peaks or troughs in a wave

Line Spectrum: electromagnetic radiation emitted at discrete wavelengths by a specific atom (or atoms) in an excited state.

Electromagnetic Radiation: energy transmitted by waves that have an electric-field component and a magnetic-field component.

1. Name of Experiment: Nuclear Magnetic Resonance Spectroscopy (NMR)

2. Goal of Experiment

- ✓ Understanding (NMR) Spectroscopy.
- ✓ Understanding how (NMR) spectroscopy works and its application.

3. Introduction

3.1 Definition of Nuclear Magnetic Resonance

Nuclear magnetic resonance (NMR) spectroscopy is the study of molecules by recording the interaction of radiofrequency (Rf) electromagnetic radiations with the nuclei of molecules placed in a ***strong magnetic field***.

3.2 Background

Zeeman first observed the strange behavior of certain nuclei when subjected to a strong magnetic field at the end of the nineteenth century, but the practical use of the so-called **"Zeeman effect"** was only made in the 1950s when NMR spectrometers became commercially available. An NMR instrument allows the molecular structure of a material to be analyzed by observing and measuring the interaction of nuclear spins when placed in a powerful magnetic.

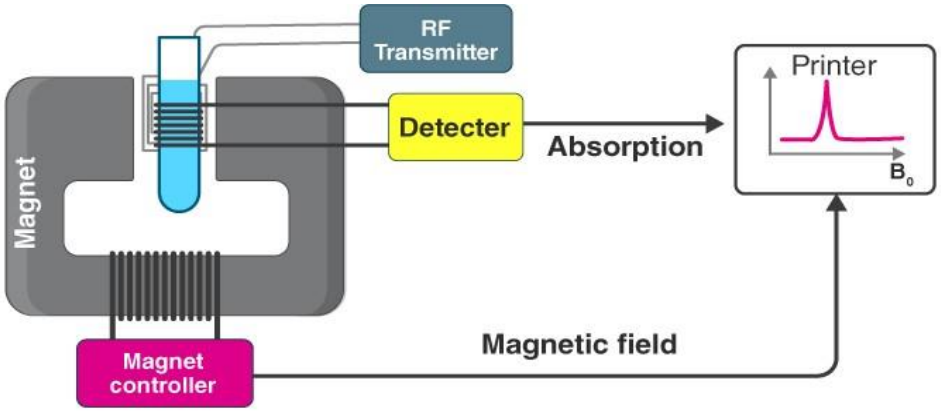


Fig. 1: NMR – Spectroscopy Instrumentation.

As shown in Fig. 1, an NMR is composed of nine basic parts:

- **Sample holder** – It is a glass tube which is 8.5 cm long and 0.3 cm in diameter.
- **Magnetic coils** – Magnetic coil generates magnetic field whenever current flows through it
- **Permanent magnet** – It helps in providing a homogenous magnetic field at **60 – 100 MHZ**
- **Sweep generator** – Modifies the strength of the magnetic field which is already applied.
- **Radiofrequency transmitter** – It produces a powerful but short pulse of the radio waves.
- **Radiofrequency** – It helps in detecting receiver radio frequencies.
- **RF detector** – It helps in determining unabsorbed radio frequencies.
- **Recorder** – It records the NMR signals which are received by the RF detector.
- **Readout system** – A computer that records the data.

3.3 How does an NMR work?

- The principle behind NMR is that many nuclei have spin and all nuclei are electrically charged. If an external magnetic field is applied, an energy transfer is possible between the base energy to a higher energy level (generally a single energy gap).
- The energy transfer takes place at a wavelength that corresponds to radio frequencies and when the spin returns to its base level, energy is emitted at the same frequency.
- The signal that matches this transfer is measured in many ways and processed in order to yield an NMR spectrum for the nucleus concerned.

- A spinning charge generates a magnetic field. The resulting spin-magnet has a magnetic moment **(μ)** proportional to the spin.

- In the presence of an external magnetic field **(B0)**, two spin states exist, **+1/2** and **-1/2**. The magnetic moment of the lower energy +1/2 state is aligned with the external field, but that of the higher energy -1/2 spin state is opposed to the external field. Note that the arrow representing the external field points North.
- The difference in energy between the two spin states is dependent on the external magnetic field strength, and is always very small. The following diagram (Fig.2) illustrates that the two spin states have the same energy when the external field is zero, but diverge as the field increases. At a field equal to **Bx** a formula for the energy difference is given (**I = 1/2** and **μ** is the magnetic moment of the nucleus in the field).

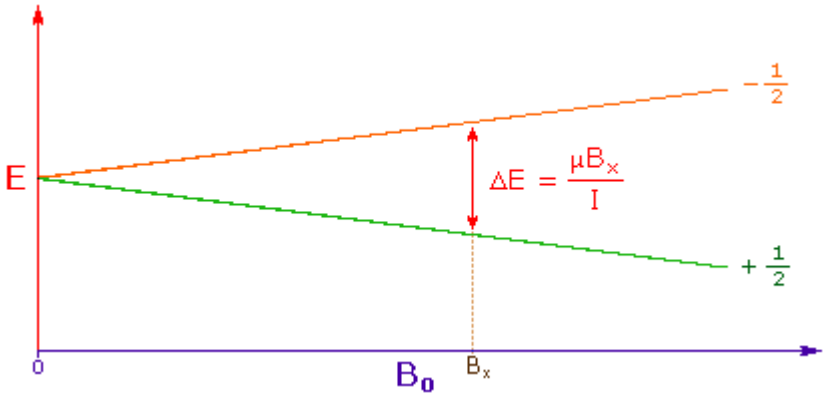
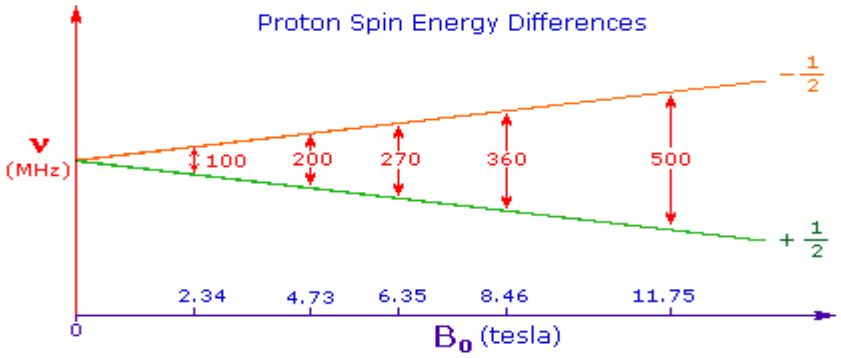


Fig. 2: The two spins and the energy difference.

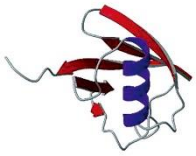
Strong magnetic fields are necessary for NMR spectroscopy. The international unit for magnetic flux is the **tesla (T).** The earth's magnetic field is not constant, but is approximately **4-10 T** at ground level. Modern NMR spectrometers use powerful magnets having fields of **1 to 20 T**. Even with these high fields, the energy difference **(ΔE)** between the two spin states is less than **0.1 cal/mole**. To put this in perspective, recall that infrared transitions involve **1 to 10 kcal/mole** and electronic transitions are nearly 100 time greater. For NMR purposes, this small energy difference **(ΔE)** is usually given as a frequency in units of **MHz (10^6 Hz)**, ranging from **20 to 900 MHz**, depending on the magnetic field strength and the specific nucleus being studied. Irradiation of a sample with radio frequency **(RF)** energy corresponding exactly to the spin state separation of a specific set of nuclei will cause excitation of those nuclei in the +1/2 state to the higher -1/2 spin state. Note that this electromagnetic radiation falls in the radio and television broadcast spectrum. NMR spectroscopy is therefore the energetically mildest probe used to examine the structure of molecules. The nucleus of a hydrogen atom (the proton) has a magnetic moment **μ = 2.7927**, and has been studied more than any other nucleus. The previous diagram may be changed to display energy differences for the proton spin states (as frequencies).



- For spin 1/2 nuclei the energy difference between the two spin states at a given magnetic field strength will be proportional to their magnetic moments. For the four common nuclei noted above, the magnetic moments

are: 1H **μ = 2.7927, 19F μ = 2.6273, 31P μ = 1.1305 & 13C μ = 0.7022**. The following diagram gives the approximate frequencies (**ν**) that correspond to the spin state energy separations for each of these nuclei in an external magnetic field of **2.35 T.** The formula in the colored box shows the direct correlation of frequency (energy difference) with magnetic moment **(h = Planck's constant = 6.626069•10^{-34} Js)**.

3.4 NMR Spectroscopy Applications

	**Analysis of Molecular Structure and Identification of Unknown Chemical Substance** A very wide range of applications including Organic Chemistry, Inorganic Chemistry, Biochemistry, Pharmaceutical Analysis, New Materials, Petrochemistry, etc.
	**Quantitative Analysis** Polymer Chemistry, Quality Control of Synthetic Chemicals, Food Chemistry
	**Analysis of Mixtures** Food Chemistry, Biochemistry, Physiology
	**Dynamics** (chemical reaction speed, identification of binding site, interaction) Organic Chemistry, Inorganic Chemistry, Biochemistry
	**Relaxation Time** (molecular mobility, interatomic distance) Organic Chemistry, Polymer Chemistry

Diffusion Coefficient

(molecular weight, conformation of polymer)

Organic Chemistry, Polymer Chemistry

1. Name of Experiment: Mass Spectroscopy (MS)

2. Goal of Experiment

- ✓ Understanding how an (MS) works.
- ✓ Understanding what an (MS) is used for.

3. Introduction

3.1 Definition of MS

It is an analytical chemistry technique that helps identify the amount and type of chemicals present in a sample by measuring the **mass – to – charge ratio** and abundance of **gas – phase ions**. A mass spectrum is a plot of the ion signal as a function of the mass – to – charge ratio.

3.2 Basic Principle

- Molecules are bombarded with a beam if energetic electrons.
- The molecules are ionized and broken up into many fragments, some of which are positive ions.
- Each kind of ion has a particular value of mass – to – charge ratio $\left(\frac{m}{e}\right)$. For most ions, the charge is one $(e = 1)$ and thus $\left(\frac{m}{e} = m\right)$ where $\boldsymbol{m}$ is the molecular mass of the ion.
- The ions pass through a magnetic field to reach detector where they are detected and signals are recorded to give mass spectra.

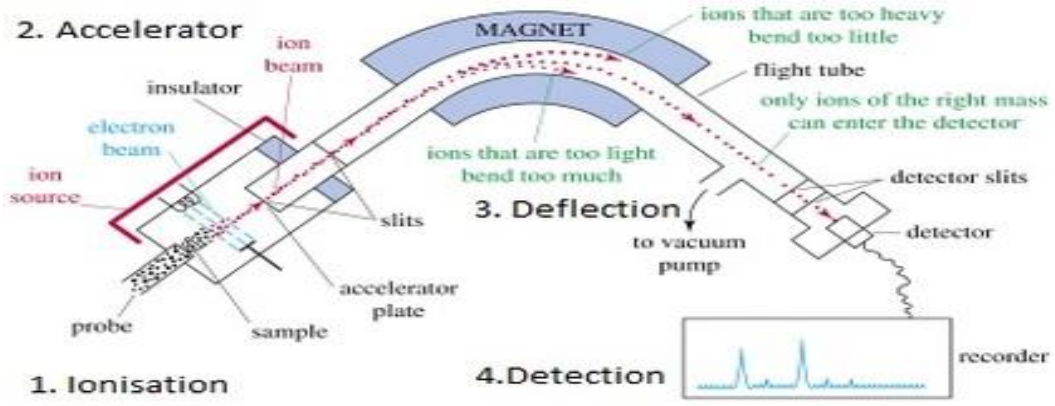


Fig. 1: MS Instrumentation.

3.3 Instrumentation

As shown in Fig. 1, a sample is stored in a large reservoir from which molecules reach ionization chamber at low pressure in a steady stream by a pinhole called **Molecular leak**. Then, the atoms undergo four basic steps:

- **Ionization** – Atoms ionized by knocking one or more electrons off to give positive ions by bombardment with a stream of electrons. Most of the positive ions formed will carry charge of +1.

The following table shows the different ionization methods:

Ionization method	Typical Analytes	Sample Introduction	Mass Range	Method Highlights
Electron Impact (EI)	Relatively small volatile	GC or liquid/solid probe	to 1,000 Daltons	Hard method versatile provides structure info
Chemical Ionization (CI)	Relatively small volatile	GC or liquid/solid probe	to 1,000 Daltons	Soft method molecular ion peak $[M+H]^+$
Electrospray (ESI)	Peptides Proteins nonvolatile	Liquid Chromatography or syringe	to 200,000 Daltons	Soft method ions often multiply charged
Fast Atom Bombardment (FAB)	Carbohydrates Organometallics Peptides nonvolatile	Sample mixed in viscous matrix	to 6,000 Daltons	Soft method but harder than ESI or MALDI
Matrix Assisted Laser Desorption (MALDI)	Peptides Proteins Nucleotides	Sample mixed in solid matrix	to 500,000 Daltons	Soft method very high mass

Table 1: ionization methods

- **Acceleration** – Ions accelerated so that they all have the same kinetic energy. +ve ions pass through 3 slits with voltage in decreasing order. Middle slit carries intermediate and finals at zero volt. All ions are accelerated into a finely forward beam.

- **Deflection** – Ions are deflected by a magnetic field due to difference in their masses. The lighter mass, more they are deflected. It also depends upon the number of +ve charge an ion carrying; the more +ve charge, more it will be deflected. Then, the ions pass through the mass analyzer. There are multiple types of mass analyzer such as:
  - Magnetic sector mass analyzers.
  - Double focusing analyzers.
  - Quadrupole mass analyzers.
  - Time of Flight analyzers. (TOF)
  - Ion trap analyzers.
  - Ion cyclotron analyzers.
- **Detection** – The beam of ions passing through the mass analyzer are detected by detector on the basis of $\left({}^{mass\ no.\ (m)}/_{charge\ no.\ (z)}\right)$. When an ion hits the metal box, charge is neutralized by an electron jumping from metal on to the ion. Faraday cup, Electron Multiplier, Photomultiplier, and Micro Channel Plate different types of used detectors.

3.4 Vacuum System

All mass spectrometers need a vacuum to allow ions to reach the detector without colliding with other gaseous molecules or atoms. If such collisions did occur, the instrument would undergo reduced resolution and sensitivity.

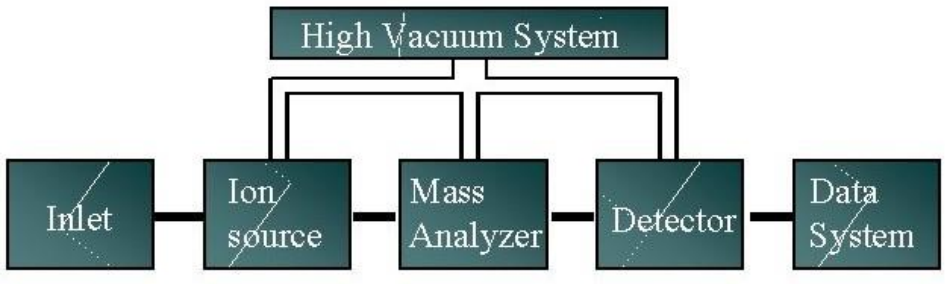


Fig. 2: MS block diagram illustrating the vacuum system

3.5 MS Applications

- ◊ Environmental Monitoring and Analysis (soil, water and air pollution, water quality…etc.).
- ◊ Geochemistry – soil and rock composition, oil and gas surveying, archeological dating, and space exploration.
- ◊ Chemical and Petrochemical industry – quality control, trace pharmaceutical industry, and forensics toxicology.
- ◊ Biotechnology – identify structures of biomolecules, such as carbohydrates, nucleic acids. Also, sequence biopolymers such as proteins.

3.6 The Difference between GC and MS

The mass spectrometry process normally requires a very pure sample while gas chromatography using a traditional detector (e.g., Flame ionization detector) cannot differentiate between multiple molecules that happen to take the same amount of time to travel through the column (i.e., have the same retention time).

A: Mass Spectrometer **B: Portable Mass Spectrometer**

Fig. 3: Different types of MS

1. Name of Experiment: Measurement of pH, EC and TDS

2. Goal of Experiment

- ✓ Learn how to measure pH, EC and TDS

3.1 Electrical Conductivity (EC),

Conductivity (K)

Is a measure of ability of an aqueous solution carry an electric currents. This ability depends on the presence of ions and on the temperature of measurement.

Conductance (G)

Is defined as the reciprocal of resistance.

$$G = \frac{1}{R}$$

The conductance of a solution, *G*, is directly proportional to the electrode surface area, *A*, cm^2, and inversely proportional to the distance between the electrodes, *L*, cm. The constant of proportionality, *k*, such that:

$$G = k\left(\frac{A}{L}\right)$$

The units of *k* are 1/ohm-cm or mho per centimeter.

Electrical conductivity (**EC**) measures how well a substance (usually water) can transmit an electrical current. Small charged particles, called ions, help to carry the electrical charge through a substance. These ions can be positively or negatively charged. The more ions available, the higher the conductivity; fewer ions would result in lower conductivity. **EC** is typically reported in milliSiemans per centimetre (mS/cm) or microSiemans per centimeter (µS/cm).

Things That Affect Electrical Conductivity of Soil

Many things can affect the electrical conductivity of your soil. The most common factors are *temperature, soil type,* its *moisture level, salinity, irrigation, depth of the soil and fertilizer.*

Procedure:

1. Calibrate the EC meter by standard solutions of electrical conductivity.
2. Immerse the electrodes into the sample of water (whose conductivity is to be determined) and wait up to one minute for steady reading.

3. The reading is observed after the indicated value becomes constant.

3.2 Total Dissolved Solids (TDS)

Total dissolved solids (TDS) are the amount of dissolved substances in solution (usually water). This measurement reads all the dissolved inorganic and organic substances in a liquid. Results from this reading are displayed as milligrams per litre (mg/L), parts per million (ppm), grams per litre (g/L), or parts per thousand (ppt).
Measuring TDS is a long process. First, you extract all the water from a soil sample, then evaporate the water and weigh the remaining residue after evaporation. It's much easier to measure the electrical conductivity of substance, and then convert the reading into TDS with a conversion factor.

Something to keep in mind when choosing a conversion factor is that not all dissolved solids conduct electricity. For example, if you measured the conductivity of a glass of water and then add table salt, the conductivity will **go up**. But, if you took that same cup of water, measured the conductivity, and then added **sugar**, the conductivity would **not be affected**. This is **because table salt** breaks apart into charged ions when put into a solution. Sugar does dissolve, but it does not break apart into charged ions. However, if you were to measure the TDS of the two glasses of water they would be affected by the addition of either salt or sugar.

A limit of 500 mg dissolved solids/L is desirable for drinking waters.
Total solids: is the material residue left in the vessel after evaporation of a sample and its subsequent drying in an oven at a defined temperature.
Total solids = total suspended solids + total dissolved solids
Total suspended solids: the portion of total solids retained by a filter of 2μ m.
Total dissolved solids: the portion that passes through the filter of 2μ m or smaller.
Factors affecting separation of suspended from dissolved solids:
1. The pore size.
2. Porosity
3. Thickness of the filter
4. Particle size.
5. Amount of material deposited on the filter.

The theoretical relations between TDS and EC

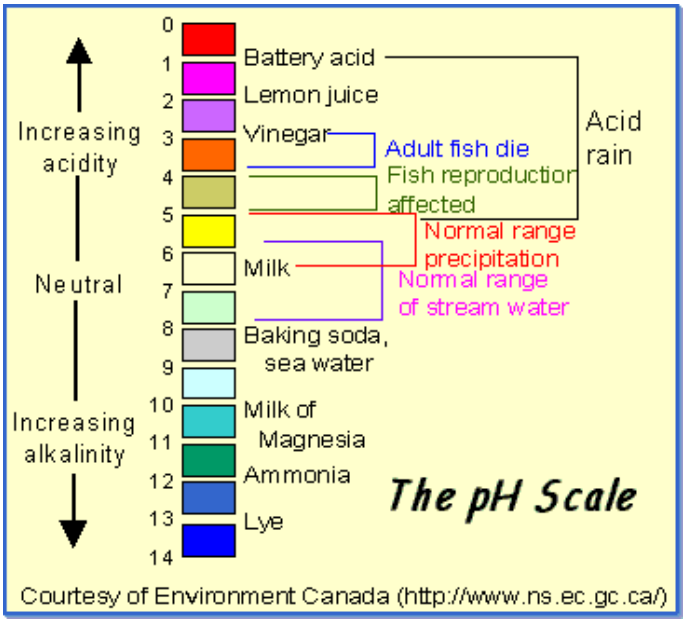
Most common conversion factors between EC and TDS are 0.5 and 0.7. The **0.5** conversion factor is based on how EC and TDS relate to ***sodium chloride***. The **0.7** conversion factor is based on how EC and TDS relate to a mixture of ***sodium sulphate, sodium bicarbonate, and sodium chloride***. To use the conversion factor, simply multiply your EC reading by the conversion factor to calculate the TDS.

In general:

$$TDS\ (mg/L) = 0.6\ EC\ (\mu S/cm)$$

3.3 pH

pH indicates the sample's acidity but is actually a measurement of the potential activity of hydrogen ions (H^+) in the sample. **pH** measurements run on a scale from 0 to 14, with 7.0 considered neutral. Solutions with a **pH** below 7.0 are considered acids.



The intensity of Acidic and basic character of a solution is indicated by ph or hydrogen ion, at a given temperature.

$$H_2O \leftrightarrow H^+ + OH^-$$

The equilibrium constant for this reaction, Kw is the product of H^+ and OH^- concentrations and is equal to 10-14. This relationship may be expressed as

$$[H^+][OH^-] = K_w = 10^{-14}$$

Where $[H^+]$ and $[OH^-]$ are the concentrations of hydrogen and hydroxyl ions respectively.

$$pH + pOH = pK_w$$

In a neutral solution the H^+ concentration is 10^{-7}, so the pH is 7.

Soil **pH affects** the amount of nutrients and chemicals that are soluble in soil water, and therefore the amount of nutrients available to plants. Some nutrients are more available under acid conditions while others are more available under alkaline conditions.

Why is pH Important?

pH is highly **important** and is used to monitor for safe water conditions. Many animals cannot live in a **pH** level below 5 or above 9. Once the normal **pH** range for water has been established, a rise or fall in **pH** can indicate chemical pollution, or acid rain.

pH is important **because** substances such as our stomach acids tend to be at a certain pH in order to work properly. pH is also important **because** it must be at certain levels in order for living organisms to survive.

In short, the less CO_2 in solution, the higher the **pH**.

CO_2, when dissolved in water becomes something called carbonic acid (H_2CO_3). When CO_2 off-gasses (from aeration, splashing or maybe a water feature that agitates water **causing** bubbles to escape), the amount of carbonic acid decreases; so the **pH rises.**

How to measure pH?

Procedure:

1. Calibrate the electrodes with two standard buffer solutions of pH.
2. Immerse the electrodes into the sample of water (whose pH is to be determined) and wait up to one minute for steady reading.

3. The reading is observed after the indicated value becomes constant.