STUDY MATERIAL FOR B.Sc MICROBIOLOGY

BIO INSTRUMENTATION

SEMESTER – II

ACADEMIC YEAR 2023-24

INDEX

UNIT I

BUFFER SOLUTION

Buffer Solution is a water solvent-based solution which consists of a mixture containing a weak acid and the conjugate base of the weak acid, or a weak base and the conjugate acid of the weak base. They resist a change in pH upon dilution or upon the addition of small amounts of acid/alkali to them.

The Ph of Buffer Solutions shows minimal change upon the addition of a very small quantity of strong acid or strong base. They are therefore used to keep the Ph at a constant value.

What is Buffer Solution?

The buffer solution is a solution able to maintain its Hydrogen ion concentration (pH) with only minor changes on the dilution or addition of a small amount of either acid or base. Buffer Solutions are used in fermentation, food preservatives, drug delivery, electroplating, printing, the activity of enzymes, blood oxygen carrying capacity need specific hydrogen ion concentration (pH).

Solutions of a weak acid and its conjugate base or weak base and its conjugate acid are able to maintain pH and are buffer solutions.

Types of Buffer Solution

The two primary types into which buffer solutions are broadly classified into are acidic and alkaline buffers.

Acidic Buffers

As the name suggests, these solutions are used to maintain acidic environments. Acid buffer has acidic pH and is prepared by mixing a weak acid and its salt with a strong base. An aqueous solution of an equal concentration of acetic acid and sodium acetate has a pH of 4.74.

- pH of these solutions is below seven
- These solutions consist of a weak acid and a salt of a weak acid.
- An example of an acidic buffer solution is a mixture of sodium acetate and acetic acid (pH = 4.75).

Alkaline Buffers

These buffer solutions are used to maintain basic conditions. Basic buffer has a basic Ph and is prepared by mixing a weak base and its salt with strong acid. The aqueous solution of an equal concentration of ammonium hydroxide and ammonium chloride has a Ph of 9.25.

- The Ph of these solutions is above seven

- They contain a weak base and a salt of the weak base.
- An example of an alkaline buffer solution is a mixture of ammonium hydroxide and ammonium chloride (Ph = 9.25).
- Acid and Base
- Ph Scale and Acidity
- Ph and Solutions

Mechanism of Buffering Action

In solution, the salt is completely ionized and the weak acid is partly ionized.

- $CH_3COONa \rightleftharpoons Na^+ + CH_3COO^-$
- $CH_3COOH \rightleftharpoons H^+ + CH_3COO^-$

On Addition of Acid and Base

1. On addition of acid, the released protons of acid will be removed by the acetate ions to form an acetic acid molecule.

$H^+ + CH_3COO^-$ (from added acid) $\rightleftharpoons CH_3COOH$ (from buffer solution)

2. On addition of the base, the hydroxide released by the base will be removed by the hydrogen ions to form water.

$HO^- + H^+$ (from added base) $\rightleftharpoons H_2O$ (from buffer solution)

Preparation of Buffer Solution

If the dissociation constant of the acid (pKa) and of the base (pKb) are known, a buffer solution can be prepared by controlling the salt-acid or the salt-base ratio.

As discussed earlier, these solutions are prepared by mixing of weak bases with their corresponding conjugate acids, or by mixing weak acids with their corresponding conjugate bases.

An example of this method of preparing buffer solutions can be given by the preparation of a phosphate buffer by mixing HPO_4^{2-} and $H_2PO_4^-$. The pH maintained by this solution is 7.4.

Henderson-Hasselbalch Equation

Preparation of Acid Buffer

Consider an acid buffer solution, containing a weak acid (HA) and its salt (KA) with a strong base(KOH). Weak acid HA ionizes, and the equilibrium can be written as-

$HA + H_2O \leftrightharpoons H^+ + A^-$

Acid dissociation constant = Ka = [H+] [A–]/HA Taking, negative log of RHS and LHS:

$$-\log Ka = -\log [H+] - \log \left(\frac{[A^-]}{[HA]} \right)$$

$$pKa = pH - \log \left(\frac{[salt]}{[acid]} \right)$$

pH of acid buffer = pKa + ([salt]/[acid])

The equation is the Henderson-Hasselbalch equation, popularly known as the Henderson equation.

Preparation of Base Buffer

Consider base buffer solution, containing a weak base (B) and its salt (BA) with strong acid.

pOH, can be derived as above,

• pOH of a basic buffer = pKb + log ([salt]/[acid])

• pH of a basic buffer = pKa – log ([salt]/[acid]) Significance of Henderson Equation Henderson Equation can be used to:

Equation Henderson Equation can be used to:

1. Calculate the pH of the buffer prepared from a mixture of the salt and weak acid/base.
2. Calculate the pKa value.
3. Prepare buffer solution of needed pH.

Limitations of Henderson-Hasselbalch Equation

The Henderson – Hassel Balch equation cannot be used for strong acids and strong bases.

pH Maintenance

In order to understand how buffer solutions maintain a constant pH, let us consider the example of a buffer solution containing sodium acetate and acetic acid.

In this example, it can be noted that the sodium acetate almost completely undergoes ionization whereas the acetic acid is only weakly ionized. These equilibrium reactions can be written as:

- $CH3COOH \rightleftharpoons H+ + CH3COO–$
- $CH3COONa \rightleftharpoons Na+ + CH3COO–$

When strong acids are added, the H+ ions combine with the CH3COO– ions to give a weakly ionized acetic acid, resulting in a negligible change in the pH of the environment.

When strongly alkaline substances are introduced to this buffer solution, the hydroxide ions react with the acids which are free in the solution to yield water molecules as shown in the reaction given below.

$CH_3COOH + OH^- \rightleftharpoons CH_3COO^- + H_2O$

Therefore, the hydroxide ions react with the acid to form water and the pH remains the same.

Uses of Buffer Solutions

There exists a few alternate names that are used to refer buffer solutions, such as pH buffers or hydrogen ion buffers.

An example of the use of buffers in pH regulation is the use of bicarbonate and carbonic acid buffer system in order to regulate the pH of animal blood.

Buffer solutions are also used to maintain an optimum pH for enzyme activity in many organisms.

The absence of these buffers may lead to the slowing of the enzyme action, loss in enzyme properties, or even denature of the enzymes. This denaturation process can even permanently deactivate the catalytic action of the enzymes.

Normality

Normality in chemistry is one of the expressions used to measure the concentration of a solution. It is abbreviated as 'N' and is sometimes referred to as the equivalent concentration of a solution. It is mainly used as a measure of reactive species in a solution and during titration reactions or particularly in situations involving acid-base chemistry.

As per the standard definition, normality is described as the number of gram or mole equivalents of solute present in one litre of a solution. When we say equivalent, it is the number of moles of reactive units in a compound. Normality Formula

- Normality = Number of gram equivalents × [volume of solution in litres]-1
- Number of gram equivalents = weight of solute × [Equivalent weight of solute]-1
- N = Weight of Solute (gram) × [Equivalent weight × Volume (L)]
- N = Molarity × Molar mass × [Equivalent mass]-1
- N = Molarity × Basicity = Molarity × Acidity

Normality is often denoted by the letter N. Some of the other units of normality are also expressed as eq L-1 or meq L-1. The latter is often used in medical reporting.

How to Calculate Normality?

There are certain tips that students can follow to calculate normality.

1. The first tip that students can follow is to gather information about the equivalent weight of the reacting substance or the solute. Look up the textbook or reference books to learn about the molecular weight and the valence.
2. The second step involves calculating the no. of gram equivalent of solute.
3. Students should remember that the volume is to be calculated in litres.
4. Finally, normality is calculated using the formula and replacing the values.

Calculation of Normality in Titration

Titration is the process of gradual addition of a solution of a known concentration and volume with another solution of unknown concentration until the reaction approaches its neutralization. To find the normality of the acid and base titration:

N1 V1 = N2 V2 Where,

- N1 = Normality of the Acidic solution
- V1 = Volume of the Acidic solution
- N2 = Normality of the basic solution
- V3 = Volume of the basic solution

Normality	Molarity
Also known as equivalent concentration.	Known as molar concentration.
It is defined as the number of gram equivalent per litre of solution.	It is defined as the number of moles per litre of solution.
It is used in measuring the gram equivalent in relation to the total volume of the solution.	It is used in measuring the ratio between the number of moles in the total volume of the solution.
The units of normality are N or eq L-1	The unit of molarity is M or Moles L-1

Uses of Normality

Normality is used mostly in three common situations:

- In determining the concentrations in acid-base chemistry. For instance, normality is used to indicate hydronium ions (H3O+) or hydroxide ions (OH–) concentrations in a solution.
- Normality is used in precipitation reactions to measure the number of ions which are likely to precipitate in a specific reaction.
- It is used in redox reactions to determine the number of electrons that a reducing or an oxidizing agent can donate or accept.

Limitations in Using Normality

Many chemists use normality in acid-base chemistry to avoid the mole ratios in the calculations or simply to get more accurate results. While normality is used commonly in precipitation and redox reactions there are some limitations to it.

These limitations are as follows:

- It is not a proper unit of concentration in situations apart from the ones that are mentioned above. It is an ambiguous measure and molarity or molality are better options for units.
- Normality requires a defined equivalence factor.
- It is not a specified value for a particular chemical solution. The value can significantly change depending on the chemical reaction. To elucidate further, one solution can actually contain different normalities for different reactions.

Molality	Molarity
It is the number of moles of solute per kilogram of solvent.	It is the number of moles of solute per kilogram of solvent.
Change in temperature will not affect this unit of concentration.	The unit will change with change in temperature.
Its unit is mol/kg.	Its unit is mol/litre.
Denoted by the letter (m).	Denoted by the letter (M).

Definition: Molarity of a given solution is defined as the total number of moles of solute per litre of solution. The molality of a solution is dependent on the changes in physical properties of the system such as pressure and temperature as unlike mass, the volume of the system changes with the change in physical conditions of the system. Molarity is represented by M, which is termed as molar. One molar is the molarity of a solution where one gram of solute is dissolved in a litre of solution. As we know, in a solution, the solvent and solute blend to form a solution, hence, the total volume of the solution is taken.

Molarity Formula:

The equation for calculating molarity is the ratio of the moles of solute whose molarity is to be calculated and the volume of solvent used to dissolve the given solute.

$M=n/V$

Here, M is the molality of the solution that is to be calculated n is the number of moles of the solute V is the volume of solution given in terms of Litres

Example 1:

A solution is prepared by bubbling 1.56 grams of hydrochloric acid in water. Here, the volume of the solution is 26.8 mL. Calculate the molarity of the solution.

Solution:

The chemical formula of hydrochloric acid = HCl

The chemical formula for Water = H_2O

The molecular weight of HCl = $35.5 \times 1 + 1 \times 1 = 36.5$ moles/gram

The molecular weight of $H_2O = 1 \times 2 + 16 \times 1 = 18$ moles/gram

Given, mass of hydrochloric acid in the solution = 1.56 g The number of moles of hydrochloric acid = nT=mass in grams/ molecular weight $nT=1.5636.5=4.27\times10-2$mole

Now, given volume of the solution = 26.8 mL Expressing the volume in terms of litres, $volume=26.81000=2.68\times10-2$Litre

Now, we calculate the molarity of the solution using the formula given above.

Molarity=Number of moles of element / volume of solution in litres

$Molality=4.27\times10-22.68\times10-2=1.59M$ The molarity of the solution is 1.59 M.

pH Definition pH is defined as the negative logarithm of H+ ion concentration. Hence the meaning of the name pH is justified as the power of hydrogen.

We know that all the acids and bases do not react with the same chemical compound at the same rate. Some react very vigorously, some moderately while others show no reaction. To determine the strength of acids and bases quantitatively, we use a universal indicator which shows different colours at different concentration of hydrogen ion in solution. Generally, the value of pH of acids and bases are used to quantitatively determine their strength.

pH Chemistry

A pH scale is a tool for measuring acids and bases. The scale ranges from 0-14: Litmus paper is an indicator used to tell if a substance is an acid or a base. The color of the paper matches up with the numbers on the pH scale to indicate what kind of substance is being tested. For example, Vinegar is an acid and measures 2.4 on the pH scale.

A healthy pH balance plays an significant role in your overall well-being, and doctors and scientists usually agree on this. The pH level, or possible level of hydrogen, in your body is determined by the food and type of drink you consume. The pH is the concentration of the hydrogen ions. This calculation is based on a 0 to 14 scale.

pH of Acids and Bases – Red Litmus Test pH Abbreviation pH may be seen as an abbreviation of the power of hydrogen-or, more fully, the concentration of hydrogen ion in a liquid.

Mathematical definition of pH is a little less intuitive but more useful in general. It states that the pH equals the negative logarithmic value of the concentration of hydrogen ion (H+) **pH = -log [H+]**

A water source ‘s pH value is a function of its acidity, or alkalinity. The pH level is a function of the hydrogen atom activity, as the hydrogen activity is a reasonable indicator of the water’s acidity or alkalinity. As seen below, the pH scale ranges from 0 to 14 when 7.0 is neutral. It is said that water with a low pH is acidic and that water with a high pH is basic, or alkaline. Pure water should have a pH of 7.0 however, due to pollutants in the water, water supplies and precipitation appear to be slightly acidic.

In view of the defined scale, pH is a decided value, like the temperature. This means that the pH of water is not a physical parameter that can be measured either as a fixed, or in a quantity. Rather, it’s a nearby number of 0 and 14 that characterizes how acidic or basic a body of water is in a logarithmic scale. The smaller the amount, the more acidic the water will be. The higher the list, the more basic it is.

Importance of pH

- Only a narrow range of pH change can be sustained by a living organism, any further change in pH can make the living difficult. For example: in the case of acid rain, the pH of water is less than 7. As it flows into a river, it lowers the pH of river water which makes the survival of aquatic life difficult.

- We know that our stomach contains hydrochloric acid which helps in the digestion of food. When the stomach produces too much of hydrochloric acid during indigestion, we feel a lot of pain and irritation.
- Hence, we generally use antacids or a mild base which increases the pH of the acidic stomach and thus decreases the pain.
- Bacteria present in our mouth sometimes lower the pH of our mouth by producing acids through degradation of the food particle. Hence, we are instructed to clean our mouths with toothpaste (which are generally basic) to prevent their decay by maintaining the pH.
- We experience a lot of pain in case of bee-sting as the bee injects the methanoic acid through its sting. Hence, we are generally advised to apply baking soda or other mild bases on the surface as it helps in maintaining the pH of the surface.

pH Meter

Exactly 100 years ago, Carlsberg 's director of chemistry, Soren Sorensen, developed a vital diagnostic tool for measuring acidity, thus helping to detect digestive, respiratory and metabolic disorders. The invention of Sorensen was the pH scale.

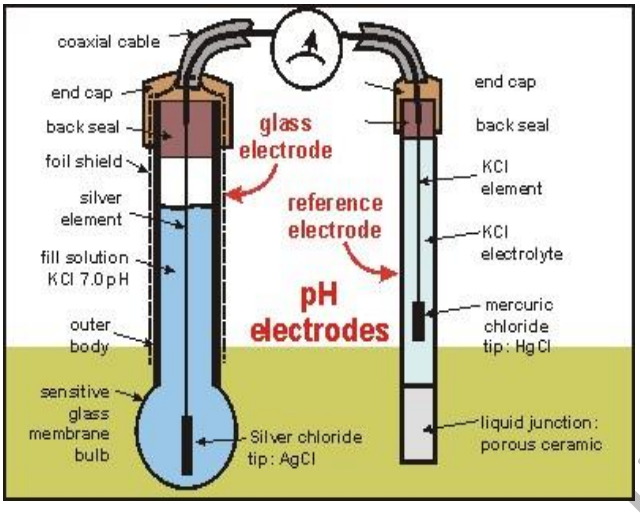
A pH meter measures essentially the electro-chemical potential between a known liquid inside the glass electrode (membrane) and an unknown liquid outside. Because the thin glass bulb allows mainly the agile and small hydrogen ions to interact with the glass, the glass electrode measures the electro-chemical potential of hydrogen ions or the potential of hydrogen. To complete the electrical circuit, also a reference electrode is needed. Note that the instrument does not measure a current but only an electrical voltage, yet a small leakage of ions from the reference electrode is needed, forming a conducting bridge to the glass electrode. A pH meter must thus not be used in moving liquids of low conductivity (thus measuring inside small containers is preferable).

The pH meter measures the electrical potential (follow the drawing clock-wise from the meter) between the mercuric chloride of the reference electrode and its potassium chloride liquid, the unknown liquid, the solution inside the glass electrode, and the potential between that solution and the silver electrode. But only the potential between the unknown liquid and the solution inside the glass electrode change from sample to sample.

So all other potentials can be calibrated out of the equation.

The calomel reference electrode consists of a glass tube with a potassium chloride (KCl) electrolyte which is in intimate contact with a mercuric chloride element at the end of a KCL element. It is a fragile construction, joined by a liquid junction tip made of porous ceramic or similar material. This kind of electrode is not easily 'poisoned' by heavy metals and sodium.

The glass electrode consists of a sturdy glass tube with a thin glass bulb welded to it. Inside is a known solution of potassium chloride (KCl) buffered at a pH of 7.0. A silver electrode with a silver chloride tip makes contact with the inside solution. To minimise electronic interference, the probe is shielded by a foil shield, often found inside the glass electrode.



Most modern pH meters also have a thermistor temperature probe which allows for automatic temperature correction, since pH varies somewhat with temperature.

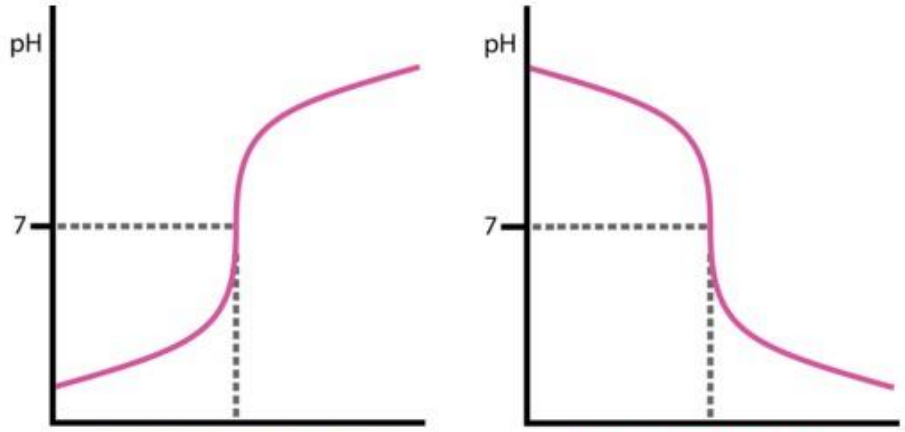
Caring for a pH meter depends on the types of electrode in use. Study the manufacturer's recommendations. When used frequently, it is better to keep the electrode moist, since moisturising a dry electrode takes a long time, accompanied by signal drift. However, modern pH meters do not mind their electrodes drying out provided they have been rinsed thoroughly in tap water or potassium chloride. When on expedition, measuring sea water, the pH meter can be left moist with sea water. However for prolonged periods, it is recommended to moist it with a solution of potassium chloride at pH=4 or in the pH=4.01 acidic calibration buffer. pH meters do not like to be left in distilled water.

Note that a pH probe kept moist in an acidic solution, can influence results when not rinsed before inserting it into the test vial. Remember that a liquid of pH=4 has 10,000 more hydrogen ions than a liquid of pH=8. Thus a single drop of pH=4 in a vial measuring 400 drops of pH=8 really upsets measurements. Remember also that the calibration solutions consist of chemical buffers that try to keep pH levels constant, so contamination of your test vial with a buffer is really serious.

Titration Curves

As base is added to acid at the beginning of a titration, the pH rises very slowly. Nearer to the equivalence point, the pH begins to rapidly increase. If the titration is a strong acid with a strong base, the pH at the equivalence point is equal to 7. A bit past the equivalence point, the rate of change of the pH again slows down. A titration curve is a graphical representation of the pH of a solution during a titration. The figure below shows two different examples of a strong

acid-strong base titration curve. On the left is a titration in which the base is added to the acid and so the pH progresses from low to high. On the right is a titration in which the acid is added to the base. In this case, the pH starts out high and decreases during the titration. In both cases, the equivalence point is reached when the moles of acid and base are equal and the pH is 7. This also corresponds to the color change of the indicator.



Titration curve of a strong acid (HCl) with a strong base (NaOH)

Titration curve of a strong base (NaOH) with a strong acid (HCl)

A titration curve shows the pH changes that occur during the titration of an acid with a base. On the left, base is being added to acid. On the right, acid is being added to base. In both cases, the equivalence point is at pH 7. Titration curves can also be generated in the case of a weak acid-strong base titration or a strong acid-weak base titration. The general shape of the titration curve is the same, but the pH at the equivalence point is different. In a weak acid-strong base titration, the pH is greater than 7 at the equivalence point. In a strong acid-weak base titration, the pH is less than 7 at the equivalence point.

Autoclave

Autoclaves provide a physical method for disinfection and sterilization. They work with a combination of steam, pressure and time. Autoclaves operate at high temperature and pressure in order to kill microorganisms and spores.

They are used to decontaminate certain biological waste and sterilize media, instruments and lab ware. Regulated medical waste that might contain bacteria, viruses and other biological material are recommended to be inactivated by autoclaving before disposal.

Autoclave Procedure

Wear personal protective equipment:

- Lab coat
- Eye protection
- Closed-toe shoes
- Heat-resistant gloves to remove items, especially hot glassware

Packaging and Loading

- Only designated individuals should be allowed to set and/or change parameters for the autoclaves.
- Before using the autoclave, check inside for any items left by the previous user that could pose a hazard.
- Clean the drain strainer before loading the autoclave.
- Always place items in a secondary container.
- Do not overload or package bags too tightly. Leave sufficient room for steam circulation. If necessary, place container on its side to maximize steam penetration and avoid entrapment of air.
- Use only autoclavable bags to package waste.
- Do not allow bags to touch the interior walls of the autoclave to avoid melting of plastic.
- Ensure sufficient liquid is packed with contents of autoclave bags if dry.
- Place soiled glassware and lab ware in secondary containers and autoclave them in the solids cycle. Do not fill containers more than 2/3 full with liquids. Loosen caps or use vented closures.
- In case of clean glassware and wrapped instruments, lay them in a secondary container before autoclaving in wrapped goods cycle.
- For secondary containment, use autoclave trays made out of polypropylene, polycarbonate or stainless steel. The trays should have a solid bottom and sides to contain the contents and catch spills.
- Choose appropriate cycle for the material. Incorrect selection of cycle may damage the autoclave, cause liquid to boil over or bottles to break.
- Start your cycle and fill out the autoclave user log. A completed cycle usually takes between 1 to 1.5 hours.
- Check chamber/jacket pressure gauge for minimum pressure of 20 pounds per square inch (psi).
- Close and lock door.
- Check temperature for 250^{o}F (121^{o}C) every load.
- Do not attempt to open the door while autoclave is operating.

Hot Air Oven is commonly used for dry heat sterilization. Dry heat sterilization is a method of controlling microorganisms. It employs higher temperatures in the range of 160-180°C and requires exposures time up to 2 hour, depending upon the temperature employed.

The benefit of dry heat includes good penetrability and non-corrosive nature which makes it applicable for sterilizing glasswares and metal surgical instruments. It is also used for sterilizing non-aqueous thermostable liquids and thermostable powders.

Dry heat destroys bacterial endotoxins (or pyrogens) which are difficult to eliminate by other means and this property makes it applicable for sterilizing glass bottles which are to be filled aseptically). Dry heat kills by Oxidation, Protein Denaturation and toxic effects of elevated levels of electrolytes.

Hot Air Oven, which is usually used for the dry heat sterilization is consists of the following parts:

- An insulated chamber surrounded by an outer case containing electric heaters.
- A fan
- Shelves
- Thermocouples
- Temperature sensor
- Door locking controls

How to operate a Hot Air Oven?

- Articles to be sterilized are first wrapped or enclosed in containers of cardboard, paper or aluminium.
- Then, the materials are arranged to ensure uninterrupted air flow.
- Oven may be pre-heated for materials with poor heat conductivity.
- The temperature is allowed to fall to 40°C, prior to removal of sterilized material.

Advantages

- This treatment kills the bacterial endotoxin, not all treatments can do this.
- Protective of sharps or instruments with a cutting edge (fewer problems with dulling of cutting edges).
- Dry heat sterilization by Hot Air Oven does not leave any chemical residue.
- Eliminates “wet pack” problems in humid climates.

Disadvantages

- Plastic and rubber items cannot be dry-heat sterilized because temperatures used (160–170°C) are too high for these materials.
- Dry heat penetrates materials slowly and unevenly.
- And the Oven requires a continuous source of electricity.

Safety Guidelines

• Before placing in Hot Air Oven

i) Dry glass wares completely

ii) Plug test tubes with cotton wools

iii) Wrap glass wares in kraft papers. Do not overload the oven. Overloading alters heat convection and increases the time required to sterilize.

- Allow free circulation of air between the materials.
- The material used for wrapping instruments and other items must be porous enough to let steam through but tightly woven enough to protect against dust particles and microorganisms.

Incubator

Incubator is a device used to grow and maintain microbiological cultures or cell cultures. The incubator maintains optimal temperature, humidity and other conditions such as the CO (CO2) and oxygen content of the atmosphere inside. Incubators are essential for a lot of experimental work in cell biology, microbiology and molecular biology and are used to culture both bacterial as well as eukaryotic cells.

Louis Pasteur used the small opening underneath his staircase as an incubator. Incubators are also used in the poultry industry to act as a substitute for hens. This often results in higher hatch rates due to the ability to control both temperature and humidity. Various brands of incubators are commercially available to breeders.

The simplest incubators are insulated boxes with an adjustable heater, typically going up to 60 to 65 °C (140 to 150 °F), though some can go slightly higher (generally to no more than 100 °C). The most commonly used temperature both for bacteria such as the frequently used E. coli as well as for mammalian cells is approximately 37 °C (99 °F), as these organisms grow well under such conditions. For other organisms used in biological experiments, such as the budding yeast Saccharomyces cerevisiae, a growth temperature of 30 °C (86 °F) is optimal.

More elaborate incubators can also include the ability to lower the temperature (via refrigeration), or the ability to control humidity or CO2 levels. This is important in the cultivation

of mammalian cells, where the relative humidity is typically >80% to prevent evaporation and a slightly acidic pH is achieved by maintaining a CO2 level of 5%.

Laminar flow cabinet

A laminar flow cabinet a carefully enclosed bench designed to prevent contamination of semiconductor wafers, biological samples, or any particle sensitive device. Air is drawn through a HEPA filter and blown in a very smooth, laminar flow towards the user. The cabinet is usually made of stainless steel with no gaps or joints where spores might collect.

Such hoods exist in both horizontal and vertical configurations, and there are many different types of cabinets with a variety of airflow patterns and acceptable uses. NSF49 is the commonly accepted regulatory standard for these cabinets.

Laminar flow cabinets may have a UV-C germicidal lamp to sterilize the shell and contents when not in use. (It is important to switch this light off during use, as it will quickly give any exposed skin sunburn and may cause cataracts.)

BOD incubator is the most versatile and reliable low temperature incubator which is designed to maintain at 20°C, necessary for **Biological Oxygen Demand/Biochemical Oxygen Demand** (BOD) determination. BOD incubators provide controlled temperature conditions for accelerated tests and exposures. The biological oxygen demand (BOD) is an empirical test in which standardized laboratory procedures are used to determine the relative oxygen requirements of microbes in wastewaters, effluents, and polluted waters and in simple words, It is a chemical process that determines how fast biological organisms use up oxygen in a body of water or it measures the oxygen required for the biochemical degradation of organic material (carbonaceous demand) and the oxygen used to oxidize inorganic materials, such as sulfides and ferrous iron The seeding and dilution procedures provide an estimate of the BOD at pH 6.5 to 7.5.

Overview of BOD Methodology

Biochemical oxygen demand (BOD), sometimes referred to as biological oxygen demand, is a quantitative expression of microbe's ability to deplete the oxygen content of a wastewater. This depletion takes place due to the microbes consuming organic matter in the water via aerobic respiration. This type of respiration uses oxygen as an electron acceptor, and the organic material being consumed provides the energy source. This organic matter also undergoes oxidation without the aid of microbes, which can be measured using the chemical oxygen demand (COD) procedure.

The method consists of placing a sample in a full, airtight bottle and incubating the bottle under specified conditions for a specific time. Dissolved Oxygen (DO) (amount of oxygen present in water body) is measured initially and after incubation. The BOD is computed from the difference between the initial and final DO. High levels of BOD in streams cause the dissolved oxygen (DO) content of the water to drop. It is this DO that fish, and zooplankton use to survive. If the DO drops to below a critical level the ecology of the stream could begin to die off as well. This condition can lead to an increase in anaerobic bacteria (species that can live in the absence of

oxygen) that leads to the production of foul-smelling, and possibly toxic gases. These gases may include methane, hydrogen sulphide, and ammonia. If this water were to enter into groundwater sources one can imagine the potential problems with toxicity to humans.

Applications

- In measuring waste loadings to treatment plants and in evaluating the BOD-removal efficiency of such treatment systems.
- It measures the molecular oxygen utilized during a specified incubation period for the biochemical degradation of organic material (carbonaceous demand) and the oxygen used to oxidize inorganic material such as sulfides and ferrous iron.
- It measures the amount of oxygen used to oxidize reduced forms of nitrogen (nitrogenous demand) unless their oxidation is prevented by an inhibitor.

Lyophilizer

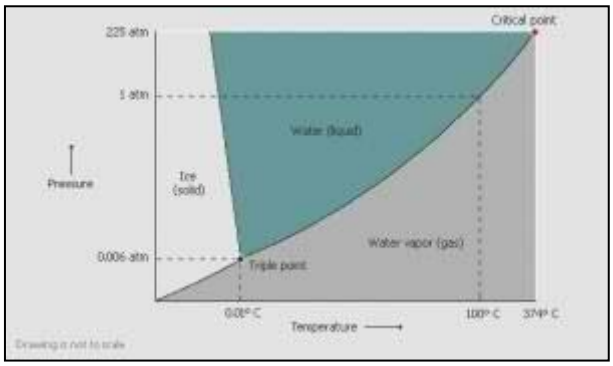
Lyophilizer and **freeze dryer** are synonymous names for the same equipment. A lyophilizer executes a water removal process typically used to preserve perishable materials, to extend shelf life or make the material more convenient for transport. Lyophilizers work by freezing the material, then reducing the pressure and adding heat to allow the frozen water in the material to sublimate.

A Lyophilizer's 3 Primary Stages

A lyophilizer functions in three phases, with the first and most critical being the freezing phase. Proper lyophilization, otherwise known as freeze drying, can reduce drying times by 30%.

Freezing Phase

A lyophilizer uses various methods to freeze the product. Freezing can be done in a freezer, a chilled bath (shell freezer), or on a shelf in the lyophilizer. The lyophilizer cools the material below its triple point to ensure that sublimation, rather than melting, will occur. This preserves the material's physical form.



A lyophilizer most easily freeze dries large ice crystals, which can be produced by slow freezing or annealing. However, with biological materials, when crystals are too large they may break the cell walls, and that leads to less-than-ideal freeze drying results. To prevent this, the freezing is done rapidly. For materials that tend to precipitate, annealing can be used. This process involves fast freezing, then raising the product temperature to allow the crystals to grow.

Primary Drying (Sublimation) Phase

A lyophilizer's second phase is primary drying (sublimation), in which the pressure is lowered and heat is added to the material in order for the water to sublimate. The lyophilizer's vacuum speeds sublimation. The lyophilizer's cold condenser provides a surface for the water vapor to adhere and solidify. The condenser also protects the vacuum pump from the water vapor. About 95% of the water in the material is removed in this phase. Primary drying can be a slow process. Too much heat can alter the structure of the material.

Secondary Drying (Adsorption) Phase

A lyophilizer's final phase is secondary drying (adsorption), during which the ionically-bound water molecules are removed. By raising the temperature higher than in the primary drying phase, the bonds are broken between the material and the water molecules. Freeze dried materials retain a porous structure. After the lyophilizer completes its process, the vacuum can be broken with an inert gas before the material is sealed. Most materials can be dried to 1-5% residual moisture.

A biosafety cabinet (BSC)

A biosafety cabinet (BSC)—also called a biological safety cabinet or microbiological safety cabinet—is an enclosed, ventilated laboratory workspace for safely working with materials contaminated with (or potentially contaminated with) pathogens requiring a defined biosafety level. Several different types of BSC exist, differentiated by the degree of biocontainment required. BSCs first became commercially available in 1950

The primary purpose of a BSC is to serve as a means to protect the laboratory worker and the surrounding environment from pathogens. All exhaust air is HEPA-filtered as it exits the biosafety cabinet, removing harmful bacteria and viruses. This is in contrast to a laminar flow clean bench, which blows unfiltered exhaust air towards the user and is not safe for work with pathogenic agents. Neither are most BSCs safe for use as fume hoods. Likewise, a fume hood fails to provide the environmental protection that HEPA filtration in a BSC would provide. However, most classes of BSCs have a secondary purpose to maintain the sterility of materials inside (the product).

Centrifuge

A centrifuge is a device used to separate components of a mixture on the basis of their size, density, the viscosity of the medium, and the rotor speed.

- The centrifuge is commonly used in laboratories for the separation of biological molecules from a crude extract.
- In a centrifuge, the sample is kept in a rotor that is rotated about a fixed point (axis), resulting in strong force perpendicular to the axis.
- There are different types of centrifuge used for the separation of different molecules, but they all work on the principle of sedimentation. Relative Centrifugal Force (RCF)
- Relative centrifugal force is the measure of the strength of rotors of different types and sizes.
- This is the force exerted on the contents of the rotor as a result of the rotation.
- RCF is the perpendicular force acting on the sample that is always relative to the gravity of the earth.
- The RCF of the different centrifuge can be used for the comparison of rotors, allowing the selection of the best centrifuge for a particular function.

The formula to calculate the relative centrifugal force (RCF) can be written as: **RCF (g Force)= 1.118 × 10-5 × r × (RPM)2** where r is the radius of the rotor (in centimeters), and **RPM** is the speed of the rotor in rotation per minute.

Centrifuge Rotors

Rotors in centrifuges are the motor devices that house the tubes with the samples. Centrifuge rotors are designed to generate rotation speed that can bring about the separation of components in a sample. There are three main types of rotors used in a centrifuge, which are:

1. Fixed angle rotors

- These rotors hold the sample tubes at an angle of 45° in relation to the axis of the rotor.
- In this type of rotor, the particles strike the opposite side of the tube where the particles finally slide down and are collected at the bottom.
- These are faster than other types of rotors as the pathlength of the tubes increases.
- However, as the direction of the force is different from the position of the tube, some particles might remain at the sides of the tubes.

2. Swinging bucket rotors/ Horizontal rotors

- Swinging bucket rotors hold the tubes at an angle of 90° as the rotor swings as the process is started.
- In this rotor, the tubes are suspended in the racks that allow the tubes to be moved enough to acquire the horizontal position.

- In this type of rotors, the particles are present along the direction or the path of the force that allows the particles to be moved away from the rotor towards the bottom of the tubes.
- Because the tubes remain horizontal, the supernatant remains as a flat surface allowing the deposited particles to be separated from the supernatant. 3. Vertical rotors
- Vertical rotors provide the shortest pathlength, fastest run time, and the highest resolution of all the rotors.
- In vertical rotors, the tubes are vertical during the operation of the centrifuge.

UNIT – II

Colorimetry

Colorimetry is the field of determining the concentration of a coloured compound in a solution. A colorimeter, also known as a filter photometer, is an analytical machine that acts as the tool quantify a solutions concentration by measuring the absorbance of a specific wavelength of light.

Colorimeters are used for a wide range of applications across the chemical and biological fields including, but not limited to, the analysis of blood, water, nutrients in soil and foodstuffs, determining the concentration of a solution, determining the rates of reaction, determining the growth of bacterial cultures and laboratory quality control.

Colorimeter Principles

Colorimeters are used to detect colour and determine the solutions concentration, i.e. when a wavelength is passed through a sample, some of the light is absorbed and some passes through. It is the wavelengths of light that pass through that are detected.

By knowing which wavelengths have passed through, the detector can also work out which coloured wavelengths were absorbed. If the solution to be tested is colourless, a common procedure is to introduce a reagent that reacts with the solution to produce a coloured solution. The results are compared against known standards.

The colorimeter uses the Beer-Lambert law to detect the absorbance of the wavelength. Beer-Lamberts law is commonly written as:

$A = \varepsilon cl$

Where, A is the absorbance, Ɛ (epsilon) is the molar absorptivity, c is the concentration of the solution and l is the length that the light passes through (also known as the mean free path). Aside from this, if there is a continual changing of the solution, i.e. it is a reaction, then % of transmittance against time is generally used.

To measure concentrations, the amount of light absorbed is dependent upon the amount of solute (also known as the analyte as it is the species being measured) in the solution- a higher concentration of dissolved solute means that more light will be absorbed, and vice versa, hence, the concentration can be backed out from the absorption of specific wavelengths.

The Colorimeter Itself

A colorimeter is composed of many parts. Aside from using a known standard solution, alongside both known concentrations and unknown concentrations, there are many vital components to a colorimeter.

As the principles are based around light, a light source is required and usually takes the form of a filament lamp. Other components include an adjustable aperture to let the light

through, coloured filters to filter specific wavelengths of light, a cuvette to hold the solution (commonly made of quartz), a photodetector to measure the transmitted light and a meter to quantify the values into a readable output.

The coloured filters are chosen to select the wavelength in which the dissolved solute will absorb the most. For most experiments the common wavelength range is between 400 and 700 nm, but when some analytes absorb in the ultraviolet range (less than 400 nm) then modification of the colorimeter is generally required. This normally takes the form of removing the filament lamp and replacing it with light-emitting diode(s) of a specific colour.

The output can be either analogue or digital in nature and, depending of the principle used, will give either an absorbance (0-infinity logarithmic output) or a %transmittance (0-100%) readout. The ideal output for an absorbance measurement is between 0 and 2, but it is desirable to have a reading between 0 and 1, as above 1 the results can become unreliable due to the scattering of light. The readout is usually in the form of a spectrum. Most calorimeters will require calibration, which is the solvent alone and not the measurable contents with the solvent- i.e. a standard or 'blank' solution. The calibration allows the absorbance of the solvent to be measured, also known across many instruments as the background noise. Once measured, the solvent absorption values are removed from any future readings, allowing the absorbance (or %transmittance) to be calculated (and plotted on a spectrum) for the desired analyte(s) without noise interference.

Spectrometry

Spectrometry is the measurement of the interactions between light and matter, and the reactions and measurements of radiation intensity and wavelength. In other words, spectrometry is a method of studying and measuring a specific spectrum, and it's widely used for the spectroscopic analysis of sample materials. Mass spectrometry is an example of a type of spectrometry, and it measures masses within a chemical sample through their mass-to-charge ratio. This is usually done by ionising particles with a shower of electrons, then passing them through a magnetic field to separate them into different stages of deflection. Once the particles are separated, they're measured by an electron multiplier, and we can identify the makeup of the sample through the weight of each ion's mass. Typically, scanning electron microscopes offer options for spectrometry based on the application.

The practical uses of mass spectronomy include isotope dating and protein characterisation. Independent roving space exploration robots such as the Mars Phoenix Lander also carry mass spectrometers for the analysis of foreign soils.

Spectroscopy

Spectroscopy is the study of the absorption and emission of light and other radiation by matter. It involves the splitting of light (or more precisely electromagnetic radiation) into its constituent wavelengths (a spectrum), which is done in much the same way as a prism splits light

into a rainbow of colours. In fact, old style spectroscopy was carried out using a prism and photographic plates.

Modern spectroscopy uses diffraction grating to disperse light, which is then projected onto CCDs (chargecoupled devices), similar to those used in digital cameras. The 2D spectra are easily extracted from this digital format and manipulated to produce 1D spectra that contain an impressive amount of useful data.

Recently, the definition of spectroscopy has been expanded to also include the study of the interactions between particles such as electrons, protons, and ions, as well as their interaction with other particles as a function of their collision energy.

How Spectroscopy is Used

Far from being a specialised, unique field, spectroscopy is integral to a variety of disciplines. While it provided a theoretical backing to early quantum research in radiation and atomic structure, it also has a staggering number of other applied uses; magnetic resonance imaging (MRI) and X-ray machines utilise a form of radio-frequency spectroscopy, we measure the unique makeup and physical properties of distant astral bodies through their spectra and wavelength, and it's even used to test doping in sports.

The types of spectroscopy can also be distinguished by the nature of the interaction between the energy and the material. Examples include:

Astronomical Spectroscopy

This type of spectroscopy is chiefly concerned with the analysis of objects in space. From simple spectroscopic analysis of an astronomical object, we can measure the spectrum of electromagnetic radiation and determine its wavelength. This can tell us about the object's chemical composition (as a factor of their spectra and mass), temperature, distance and speed (using a function of their wavelength and the speed of light).

Absorption Spectroscopy

Absorption spectroscopy involves the use of spectroscopic techniques that measure the absorption of radiation in matter. We can determine the atomic makeup of a sample by testing for the absorption of specific elements across the electromagnetic spectrum.

Biomedical Spectroscopy

Biomedical spectroscopy is a type of spectroscopy that's used in biomedical science. For example, magnetic resonance spectroscopy (a specialised technique associated with magnetic resonance imaging) is often used to diagnose and study chemical changes in the brain that can cause anything from depression to physical tumours, as well as analyse the metabolic structure of muscle. This works by mapping a spectrum of wavelengths in the brain that correspond to the known spectrum, and carefully analysing patterns and aberrations in those patterns.

Energy-Dispersive X-Ray Spectroscopy

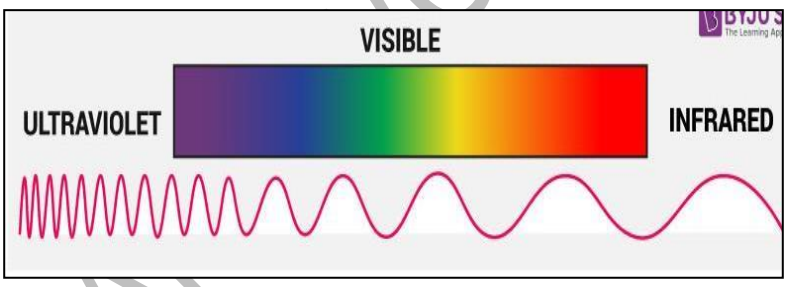
Energy dispersive X-ray spectroscopy (otherwise known as EDS/EDX) is used for the identification and quantification of elements found in a sample. This technique is used by the Phenom ProX Desktop SEM. It can also be used in conjunction with Transmission Electron Microscopy (TEM) and Scanning Transmission Electron Microscopy (STEM) to create spatially-resolved elemental analysis in areas as small as a few nanometres in diameter.

Ultraviolet-visible (UV-Vis) spectrophotometry is a technique used to measure light absorbance across the ultraviolet and visible ranges of the electromagnetic spectrum. When incident light strikes matter it can either be absorbed, reflected, or transmitted. The absorbance of radiation in the UV-Vis range causes atomic excitation, which refers to the transition of molecules from a low-energy ground state to an excited state.

Before an atom can change excitation states, it must absorb sufficient levels of radiation for electrons to move into higher molecular orbits. Shorter bandgaps typically correlate to absorption of shorter wavelengths of light. The energy required for molecules to undergo these transitions, therefore, are electrochemically-specific. A UVVis spectrophotometer can use this principle to quantify the analytes in a sample based on their absorption characteristics.

IR Spectroscopy

An IR spectrum is essentially a graph plotted with the infrared light absorbed on the Y-axis against. frequency or wavelength on the X-axis. An illustration highlighting the different regions that light can be classified into is given below.



IR Spectroscopy detects frequencies of infrared light that are absorbed by a molecule. Molecules tend to absorb these specific frequencies of light since they correspond to the frequency of the vibration of bonds in the molecule.

The energy required to excite the bonds belonging to a molecule, and to make them vibrate with more amplitude, occurs in the Infrared region. A bond will only interact with the electromagnetic infrared radiation, however, if it is polar.

The presence of separate areas of partial positive and negative charge in a molecule allows the electric field component of the electromagnetic wave to excite the vibrational energy of the molecule.

The change in the vibrational energy leads to another corresponding change in the dipole moment of the given molecule. The intensity of the absorption depends on the polarity of the bond. Symmetrical non-polar bonds in

N≡N and O=O do not absorb radiation, as they cannot interact with an electric field.

Check ⇒ NMR Spectroscopy

Regions of the Infrared spectrum

Most of the bands that indicate what functional group is present are found in the region from 4000 cm-1 to 1300 cm-1. Their bands can be identified and used to determine the functional group of an unknown compound.

Bands that are unique to each molecule, similar to a fingerprint, are found in the fingerprint region, from 1300 cm-1 to 400 cm-1. These bands are only used to compare the spectra of one compound to another.

Samples in Infrared Spectroscopy

The samples used in IR spectroscopy can be either in the solid, liquid, or gaseous state.

- Solid samples can be prepared by crushing the sample with a mulling agent which has an oily texture. A thin layer of this mull can now be applied on a salt plate to be measured.
- Liquid samples are generally kept between two salt plates and measured since the plates are transparent to IR light. Salt plates can be made up of sodium chloride, calcium fluoride, or even potassium bromide.
- Since the concentration of gaseous samples can be in parts per million, the sample cell must have a relatively long pathlength, i.e. light must travel for a relatively long distance in the sample cell.

Thus, samples of multiple physical states can be used in Infrared Spectroscopy.

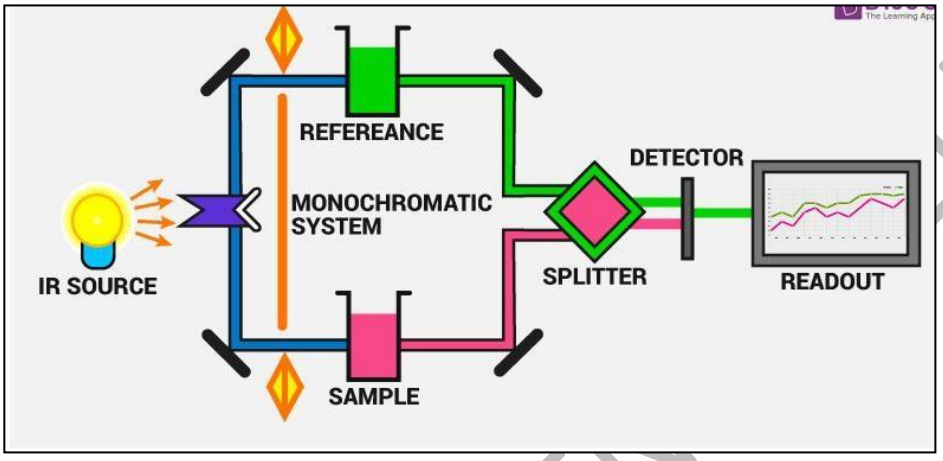
Principle of Infrared Spectroscopy

The IR spectroscopy theory utilizes the concept that molecules tend to absorb specific frequencies of light that are characteristic of the corresponding structure of the molecules. The energies are reliant on the shape of the molecular surfaces, the associated vibronic coupling, and the mass corresponding to the atoms.

For instance, the molecule can absorb the energy contained in the incident light and the result is a faster rotation or a more pronounced vibration.

IR Spectroscopy Instrumentation

The instrumentation of infrared spectroscopy is illustrated below. First, a beam of IR light from the source is split into two and passed through the reference ant the sample respectively. Now, both of these beams are reflected to pass through a splitter and then through a detector. Finally, the required reading is printed out after the processor deciphers the data passed through the detector.



Thus, IR spectroscopy involves the collection of absorption information and its analysis in the form of a spectrum. To learn more about similar topics, download the free BYJU's app from the Google Play store.

Raman Spectroscopy

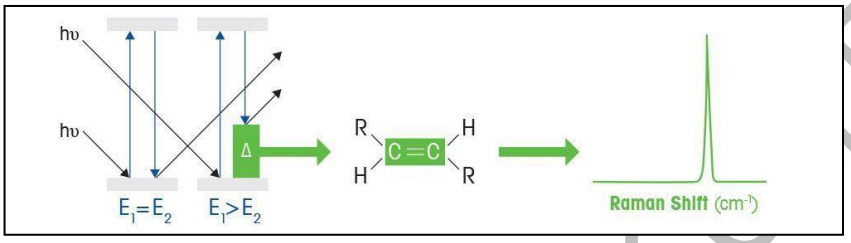
Raman spectroscopy is a molecular spectroscopic technique that utilizes the interaction of light with matter to gain insight into a material's make up or characteristics, like FTIR. The information provided by Raman spectroscopy results from a light scattering process, whereas IR spectroscopy relies on absorption of light. Raman spectroscopy yields information about intra- and inter-molecular vibrations and can provide additional understanding about a reaction. Both Raman and FTIR spectroscopy provide a spectrum characteristic of the specific vibrations of a molecule ("molecular fingerprint') and are valuable for identifying a substance.

The Raman Spectroscopy Principle

When light interacts with molecules in a gas, liquid, or solid, the vast majority of the photons are dispersed or scattered at the same energy as the incident photons. This is described as elastic scattering, or Rayleigh scattering. A small number of these photons, approximately 1 photon in 10 million will scatter at a different frequency than the incident photon. This process is called inelastic scattering, or the Raman effect, named after Sir C.V. Raman who discovered this and was awarded the 1930 Nobel Prize in Physics for his work. Since that time, Raman has been utilized for a vast array of applications from medical diagnostics to material science and reaction analysis. Raman allows the user to collect the vibrational signature of a molecule, giving insight into how it is put together, as well as how it interacts with other molecules around it.

Raman Scattering Process

The Raman Scattering Process, as described by quantum mechanics, is when photons interact with a molecule, the molecule may be advanced to a higher energy, virtual state. From this higher energy state, there may be a few different outcomes. One such outcome would be that the molecule relaxes to a vibrational energy level that is different than that of its beginning state producing a photon of different energy. The difference between the energy of the incident photon and the energy of the scattered photon is the called the Raman shift.



When the change in energy of the scattered photon is less than the incident photon, the scattering is called Stokes scatter. Some molecules may begin in a vibrationally excited state and when they are advanced to the higher energy virtual state, they may relax to a final energy state that is lower than that of the initial excited state. This scattering is called anti-Stokes.

Raman Spectroscopy Basics

How Does Raman Spectroscopy Work?

Unlike FTIR Spectroscopy that looks at changes in dipole moments, Raman looks at changes in a molecular bonds polarizability. Interaction of light with a molecule can induce a deformation of its electron cloud. This deformation is known as a change in polarizability. Molecular bonds have specific energy transitions in which a change of polarizability occurs, giving rise to Raman active modes. As an example, molecules that contain bonds between homonuclear atoms such as carbon-carbon, sulfur-sulfur, and nitrogen-nitrogen bonds undergo a change in polarizability when photons interact with them. These are examples of bonds that give rise to Raman active spectral bands, but would not be seen or difficult to see in FTIR.

Because Raman is an inherently weak effect, the optical components of a Raman Spectrometer must be well matched and optimized. Also, since organic molecules may have a greater tendency to fluoresce when shorter wavelength radiation is used, longer wavelength monochromatic excitation sources, such as solid-state laser diodes that produces light at 785 nm, are typically used.

Key Raman Spectroscopy Applications

Raman spectroscopy is used in industry for a variety of applications, including:

- Crystallization Processes
- Polymorphism Identification

- Polymerization Reactions
- Hydrogenation Reactions
- Chemical Synthesis
- Bio-catalysis and Enzymatic Catalysis
- Flow Chemistry
- Bioprocess Monitoring
- Synthesis Reactions
- Quality by Design

X-ray spectroscopy is a x technique that detects and measures photons, or particles of light, that have wavelengths in the X-ray portion of the electromagnetic spectrum. It's used to help scientists understand the chemical and elemental properties of an object.

There are several different X-ray spectroscopy methods that are used in many disciplines of science and technology, including archaeology, astronomy and engineering. These methods can be used independently or together to create a more complete picture of the material or object being analyzed.

How X-ray spectroscopy works

When an atom is unstable or is bombarded with high-energy particles, its electrons transition from one energy level to another. As the electrons adjust, the element absorbs and releases high-energy X-ray photons in a way that's characteristic of atoms that make up that particular chemical element. X-ray spectroscopy measures those changes in energy, which allows scientists to identify elements and understand how the atoms within various materials interact.

There are two main X-ray spectroscopy techniques: wavelength-dispersive X-ray spectroscopy (WDXS) and energy-dispersive X-ray spectroscopy (EDXS). WDXS measures the X-rays of a single wavelength that are diffracted by a crystal. EDXS measures the X-ray radiation emitted by electrons stimulated by a high-energy source of charged particles.

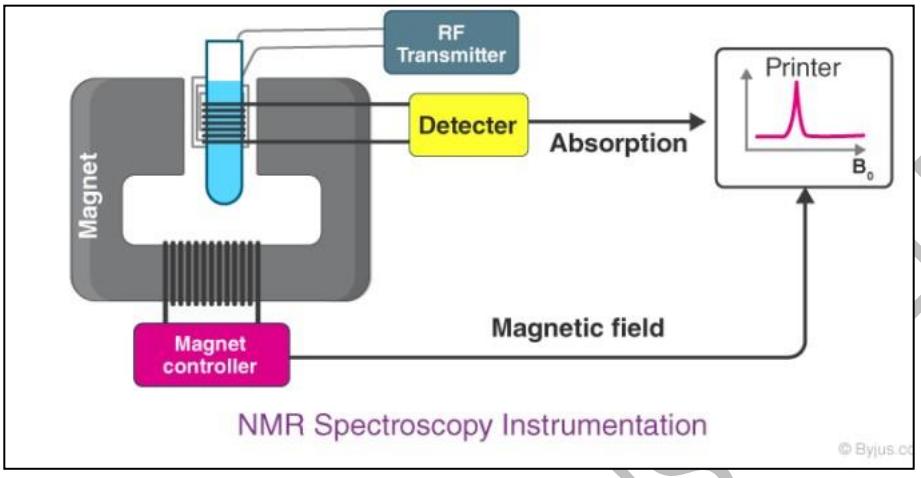
In both techniques, how the radiation is dispersed indicates the atomic structure of the material and therefore, the elements within the object being analyzed.

NMR Spectroscopy

NMR Spectroscopy is abbreviated as **Nuclear Magnetic Resonance spectroscopy.**

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.

Zeeman first observed the strange behaviour 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.



It is a research technique that exploits the magnetic properties of certain atomic nuclei. The NMR spectroscopy determines the physical and chemical properties of atoms or molecules.

It relies on the phenomenon of nuclear magnetic resonance and provides detailed information about the structure, dynamics, reaction state, and chemical environment of molecules.

Basis of NMR Spectroscopy

Nuclear Magnetic Resonance (NMR) was first detected experimentally at the end of 1945, nearly concurrently with the work groups Felix Bloch, Stanford University and Edward Purcell, Harvard University. The first NMR spectra was first published in the same issue of the Physical Review in January 1946. Bloch and Purcell were jointly awarded the 1952 Nobel Prize in Physics for their research of Nuclear Magnetic Resonance Spectroscopy.

Nuclear magnetic resonance (NMR) spectroscopy is a crucial analytical tool for organic chemists. The research in the organic lab has been significantly improved with the aid of the NMR. Not only can it provide information on the structure of the molecule, it can also determine the content and purity of the sample. Proton (1H) NMR is one of the most widely used NMR methods by organic chemists. The protons present in the molecule will behave differently depending on the surrounding chemical environment, making it possible to elucidate their structure.

NMR Spectroscopy Principle

- All nuclei are electrically charged and many have spin.
- Transfer of energy is possible from base energy to higher energy levels when an external magnetic field is applied.
- The transfer of energy occurs at a wavelength that coincides with the radio frequency.

- Also, energy is emitted at the same frequency when the spin comes back to its base level.
- Therefore, by measuring the signal which matches this transfer the processing of the NMR spectrum for the concerned nucleus is yield.

NMR Spectroscopy Working

- Place the sample in a magnetic field.
- Excite the nuclei sample into nuclear magnetic resonance with the help of radio waves to produce NMR signals.
- These NMR signals are detected with sensitive radio receivers.
- The resonance frequency of an atom in a molecule is changed by the intramolecular magnetic field surrounding it.
- This gives details of a molecule's individual functional groups and its electronic structure.
- Nuclear magnetic resonance spectroscopy is a conclusive method of identifying monomolecular organic compounds.
- This method provides details of the reaction state, structure, chemical environment and dynamics of a molecule.

Chemical Shift in NMR Spectroscopy

A spinning charge generates a magnetic field that results in a magnetic moment proportional to the spin. In the presence of an external magnetic field, two spin states exist; one spin up and one spin down, where one aligns with the magnetic field and the other opposes it.

Chemical shift is characterized as the difference between the resonant frequency of the spinning protons and the signal of the reference molecule. Nuclear magnetic resonance chemical change is one of the most important properties usable for molecular structure determination. There are also different nuclei that can be detected by NMR spectroscopy, 1H (proton), 13C (carbon 13), 15N (nitrogen 15), 19F (fluorine 19), among many more.

1H and 13C are the most widely used. The definition of 1H as it is very descriptive of the spectroscopy of the NMR. Both the nuts have a good charge and are constantly revolving like a cloud. Through mechanics, we learn that a charge in motion produces a magnetic field. In NMR, when we reach the radio frequency (Rf) radiation nucleus, it causes the nucleus and its magnetic field to turn (or it causes the nuclear magnet to pulse, thus the term NMR).

NMR Spectroscopy Instrumentation

This instrument consists of nine major parts. They are discussed below:

- **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.

NMR Spectroscopy Techniques

1. Resonant Frequency

It refers to the energy of the absorption, and the intensity of the signal that is proportional to the strength of the magnetic field. NMR active nuclei absorb electromagnetic radiation at a frequency characteristic of the isotope when placed in a magnetic field.

2. Acquisition of Spectra

Upon excitation of the sample with a radiofrequency pulse, a nuclear magnetic resonance response is obtained.

It is a very weak signal and requires sensitive radio receivers to pick up.

NMR Spectroscopy Applications

1. NMR spectroscopy is a Spectroscopy technique used by chemists and biochemists to investigate the properties of organic molecules, although it is applicable to any kind of sample that contains nuclei possessing spin.
2. For example, the NMR can quantitatively analyze mixtures containing known compounds. NMR can either be used to match against spectral libraries or to infer the basic structure directly for unknown compounds.
3. Once the basic structure is known, NMR can be used to determine molecular conformation in solutions as well as in studying physical properties at the molecular level such as conformational exchange, phase changes, solubility, and diffusion.

UNIT – III

Chromatography

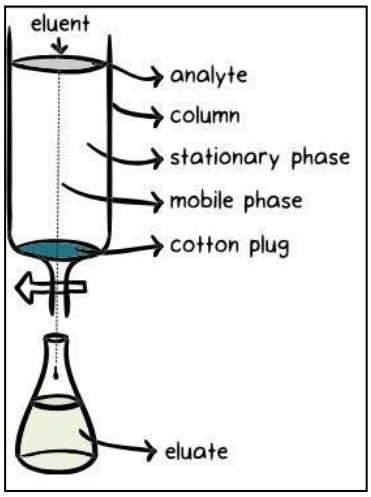
- Chromatography is an important biophysical technique that enables the separation, identification, and purification of the components of a mixture for qualitative and quantitative analysis.
- The Russian botanist Mikhail Tswett coined the term chromatography in 1906.
- The first analytical use of chromatography was described by James and Martin in 1952, for the use of gas chromatography for the analysis of fatty acid mixtures.
- A wide range of chromatographic procedures makes use of differences in size, binding affinities, charge, and other properties to separate materials.
- It is a powerful separation tool that is used in all branches of science and is often the only means of separating components from complex mixtures. Principles
- Chromatography is based on the principle where molecules in mixture applied onto the surface or into the solid, and fluid stationary phase (stable phase) is separating from each other while moving with the aid of a mobile phase.
- The factors effective on this separation process include molecular characteristics related to adsorption (liquid-solid), partition (liquid-solid), and affinity or differences among their molecular weights.
- Because of these differences, some components of the mixture stay longer in the stationary phase, and they move slowly in the chromatography system, while others pass rapidly into the mobile phase, and leave the system faster.

Three components thus form the basis of the chromatography technique.

1. **Stationary phase:** This phase is always composed of a “solid” phase or “a layer of a liquid adsorbed on the surface solid support”.

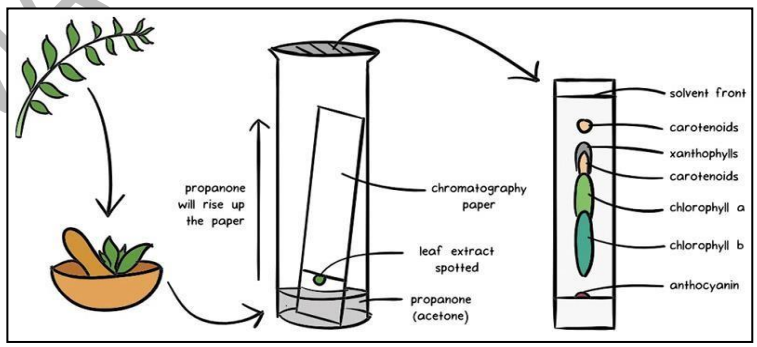
2. Mobile phase: This phase is always composed of “liquid” or a “gaseous component.”

3. Separated molecules : The type of interaction between the stationary phase, mobile phase, and substances contained in the mixture is the basic component effective on the separation of molecules from each other.



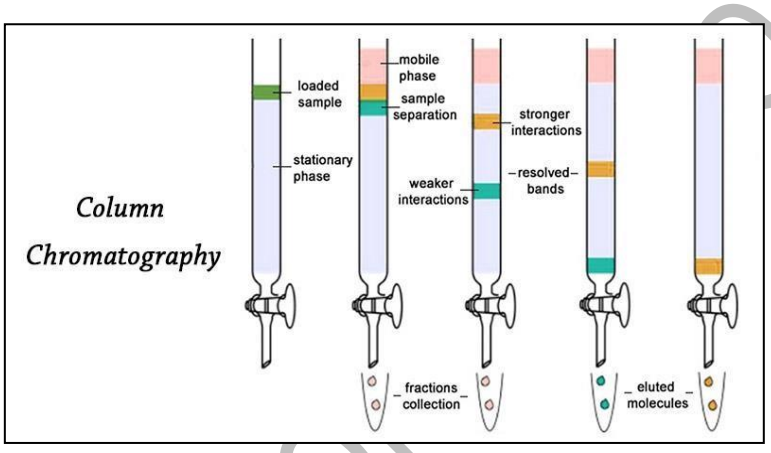
Types of Chromatography

1. Column chromatography
2. Ion-exchange chromatography
3. Gel-permeation (molecular sieve) chromatography
4. Affinity chromatography
5. Paper chromatography
6. Thin-layer chromatography
7. Gas chromatography (GS)
8. Dye-ligand chromatography
9. Hydrophobic interaction chromatography
10. Pseudo affinity chromatography



Column Chromatography

- Column chromatography is a technique in which the substances to be separated are introduced onto the top of a column packed with an adsorbent, passed through the column at different rates that depend on the affinity of each substance for the adsorbent and for the solvent or solvent mixture, and are usually collected in solution as they pass from the column at different times.
- It is a solid – liquid technique in which the stationary phase is a solid & mobile phase is a liquid or gas.
- It was developed by the American chemist D.T Day in 1900 while M.S. Tswett, the Polish botanist, in 1906 used adsorption columns in his investigations of plant pigments.



There are two forms of column chromatography.

1. Liquid chromatography (LC)
2. Gas chromatography (GC)

The most widely used forms of column chromatography are:

- Adsorption chromatography
- Partition chromatography
- Ion exchange chromatography
- Gel chromatography

A typical column chromatographic system using a gas or liquid mobile phase consists of the following components:

A stationary phase:

• Chosen to be appropriate for the analytes to be separated.

A column:

- In liquid chromatography these are generally 25- 50 cm long and 4mm internal diameter and made of stainless steel whereas in gas chromatography they are 1-3m long and 2-4mm internal diameter and made of either glass or stainless steel.
- They may be either of the conventional type filled with the stationary phase, or of the microbore type in which the stationary phase is coated directly on the inside wall of the column.

A mobile phase and delivery system:

- Chosen to complement the stationary phase and hence to discriminate between the sample analytes and to deliver a constant rate of flow into the column.

An injector system:

- To deliver test samples to the top of the column in a reproducible manner.

A detector and chart recorder:

- To give a continuous record of the presence of the analytes in the eluate as it emerges from the column.
- Detection is usually based on the measurement of a physical parameter such as visible or ultraviolet absorption or fluorescence.
- A peak on the chart recorder represents each separated analyte.

A fraction collector: For collecting the separated analytes for further biochemical studies.

Steps

A. Preparation of the Column

- The column mostly consists of a glass tube packed with a suitable stationary phase.
- A glass wool/cotton wool or an asbestos pad is placed at the bottom of the column before packing the stationary phase.
- After packing, a paper disc kept on the top, so that the stationary layer is not disturbed during the introduction of sample or mobile phase.

There are two types of preparing the column, they are:

1. Dry packing / dry filling

In this the required quantity of adsorbent is poured as fine dry powder in the column and the solvent is allowed to flow through the column till equilibrium is reached.

2. Wet packing / wet filling

In this, the slurry of adsorbent with the mobile phase is prepared and is poured into the column. It is considered as the ideal technique for packing.

- Before using column, it should be washed properly and dried.
- The column should also be free from impurity and uniformly filled with the stationary phase.

B. Introduction of the Sample

- The sample which is usually a mixture of components is dissolved in minimum quantity of the mobile phase.
- The entire sample is introduced into the column at once and get adsorbed on the top portion of the column.
- From this zone, individual sample can be separated by a process of elution.

C. Elution

- By elution technique, the individual components are separated out from the column.
- It can be achieved by two techniques:
- **Isocratic elution technique:** Same solvent composition or solvent of same polarity is used throughout the process of separation.

  Eg. Use of chloroform alone.
- **Gradient elution technique:** Solvents of gradually ↑ polarity or ↑ elution strength are used during the process of separation.

  E.g. initially benzene, then chloroform, then ethyl acetate then chloroform

D. Detection of Components

- If the compounds separated in a column chromatography procedure are colored, the progress of the separation can simply be monitored visually.
- If the compounds to be isolated from column chromatography are colorless.
- In this case, small fractions of the eluent are collected sequentially in labelled tubes and the composition of each fraction is analysed by TLC.

Factors affecting Column Efficiency

Dimensions of the column, Particle size of the adsorbent, Nature of the solvent, Temperature of the column, Pressure

Its major application includes:

- Separation of mixture of compounds.
- Removal of impurities or purification process.
- Isolation of active constituents.
- Isolation of metabolites from biological fluids.
- Estimation of drugs in formulation or crude extracts.

Ion Exchange Chromatography

- Chromatography is the separation of a mixture of compounds into its individual components based on their relative interactions with an inert matrix.
- Ion exchange chromatography (or ion chromatography) is a process that allows the separation of ions and polar molecules based on their affinity to ion exchangers.
- The principle of separation is thus by reversible exchange of ions between the target ions present in the sample solution to the ions present on ion exchangers.
- In this process two types of exchangers i.e., cationic and anionic exchangers can be used.

1. Cationic exchangers possess negatively charged group, and these will attract positively charged cations.

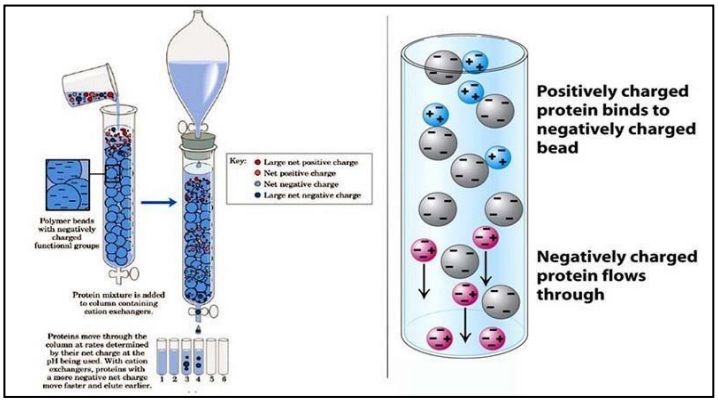
These exchangers are also called “Acidic ion exchange” materials, because their negative charges result from the ionization of acidic group.

2. Anionic exchangers have positively charged groups that will attract negatively charged anions. These are also called “Basic ion exchange” materials.

- Ion exchange chromatography is most often performed in the form of column chromatography. However, there are also thin-layer chromatographic methods that work basically based on the principle of ion exchange.

Principle

- This form of chromatography relies on the attraction between oppositely charged stationary phase, known as an ion exchanger, and analyte.
- The ion exchangers basically contain charged groups covalently linked to the surface of an insoluble matrix.
- The charged groups of the matrix can be positively or negatively charged.
- When suspended in an aqueous solution, the charged groups of the matrix will be surrounded by ions of the opposite charge.
- In this “ion cloud”, ions can be reversibly exchanged without changing the nature and the properties of the matrix.



Instrumentation

Typical IC instrumentation includes: pump, injector, column, suppressor, detector and recorder or data system.

1. Pump

The IC pump is considered to be one of the most important components in the system which has to provide a continuous constant flow of the eluent through the IC injector, column, and detector.

2. Injector

Sample introduction can be accomplished in various ways. The simplest method is to use an injection valve. Liquid samples may be injected directly and solid samples need only to be dissolved in an appropriate solvent. Injectors should provide the possibility of injecting the liquid sample within the range of 0.1 to 100 ml of volume with high reproducibility and under high pressure (up to the 4000 psi).

3. Columns

Depending on its ultimate use and area of application, the column material may be stainless steel, titanium, glass or an inert plastic such as PEEK. The column can vary in diameter from about 2mm to 5 cm and in length from 3 cm to 50 cm depending on whether it is to be used for normal analytical purposes, microanalysis, high speed analyses or preparative work.

Guard column is placed anterior to the separating column. This serves as a protective factor that prolongs the life and usefulness of the separation column. They are dependable columns designed to filter or remove particles that clog the separation column

4. Suppressor

The suppressor reduces the background conductivity of the chemicals used to elute samples from the ion exchange column which improves the conductivity measurement of the ions being tested. IC suppressors are membrane-based devices which are designed to convert the ionic eluent to water as a means of enhancing the sensitivity.

5. Detectors

Electrical conductivity detector is commonly use.

6. Data system

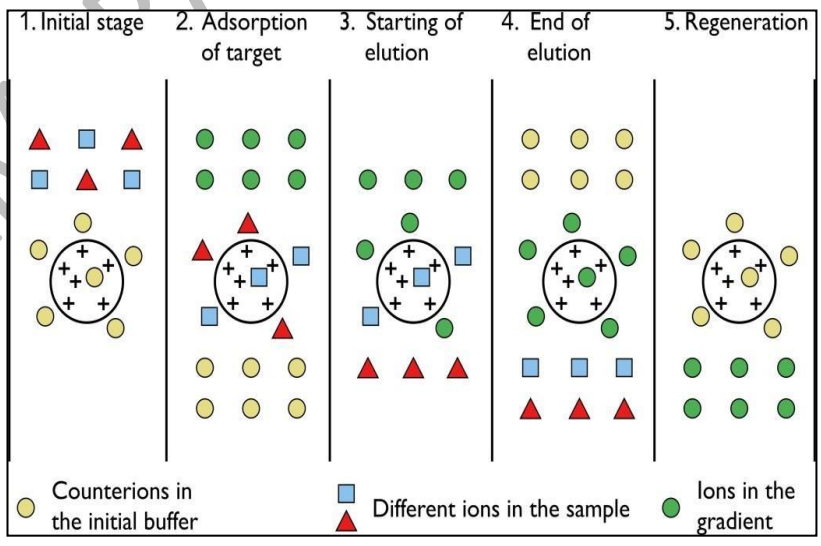
In routine analysis, where no automation is needed, a pre-programmed computing integrator may be sufficient.

For higher control levels, a more intelligent device is necessary, such as a data station or minicomputer.

Procedure

Ion exchange separations are carried out mainly in columns packed with an ion-exchanger.

- These ionic exchangers are commercially available. They are made up of styrene and divinyl benzene. Example. DEAE-cellulose is an anionic exchanger, CMcellulose is a cationic exchanger.
- The choice of the exchanger depends upon the charge of particle to be separated. To separate anions “Anionic exchanger” is used, to separate cations “Cationic exchanger” is used.
- First the column is filled with ion exchanger then the sample is applied followed by the buffer. The trisbuffer, pyridine buffer, acetate buffer, citrate and phosphate buffers are widely used.
- The particles which have high affinity for ion exchanger will come down the column along with buffers.
- In next step using corresponding buffer separates the tightly bound particles.
- Then these particles are analysed spectroscopically.

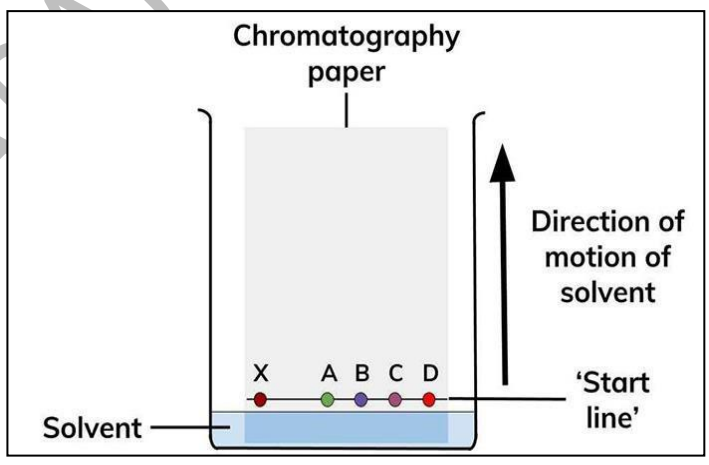


Application

- An important use of ion-exchange chromatography is in the routine analysis of amino acid mixtures.
- The 20 principal amino acids from blood serum or from the hydrolysis of proteins are separated and used in clinical diagnosis.
- This is most effective method for water purification. Complete deionization of water (or) a non-electrolyte solution is performed by exchanging solute cations for hydrogen ions and solute anions for hydroxyl ions. This is usually achieved by method is used for softening of drinking water.
- In the analysis of products of hydrolysis of nucleic acids. In this way, information is gained about the structure of these molecules and how it relates to their biological function as carriers of hereditary information.
- Chelating resins are used to collect trace metals from seawater.
- To analyze lunar rocks and rare trace elements on Earth.

Paper Chromatography

- Paper chromatography (PC) is a type of a planar chromatography whereby chromatography procedures are run on a specialized paper.
- PC is considered to be the simplest and most widely used of the chromatographic techniques because of its applicability to isolation, identification and quantitative determination of organic and inorganic compounds.
- It was first introduced by German scientist Christian Friedrich Schonbein (1865).



Types

1. Paper Adsorption Chromatography

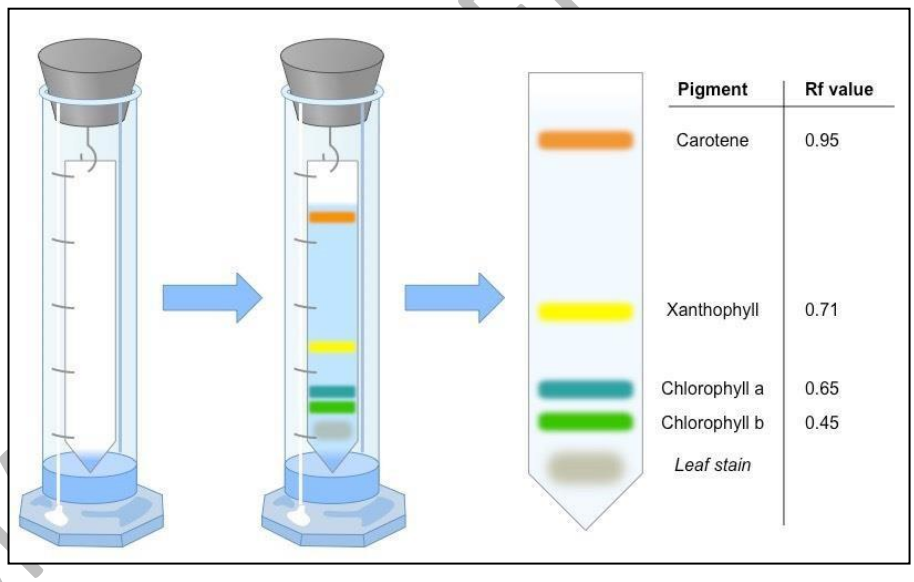
Paper impregnated with silica or alumina acts as adsorbent (stationary phase) and solvent as mobile phase.

2. Paper Partition Chromatography

Moisture / Water present in the pores of cellulose fibers present in filter paper acts as stationary phase & another mobile phase is used as solvent In general paper chromatography mostly refers to paper partition chromatography.

Principle

The principle of separation is mainly partition rather than adsorption. Substances are distributed between a stationary phase and mobile phase. Cellulose layers in filter paper contain moisture which acts as stationary phase. Organic solvents/buffers are used as mobile phase. The developing solution travels up the stationary phase carrying the sample with it. Components of the sample will separate readily according to how strongly they adsorb onto the stationary phase versus how readily they dissolve in the mobile phase.



Instrumentation

1. Stationary phase & papers used
2. Mobile phase
3. Developing Chamber
4. Detecting or Visualizing agents

1. Stationary Phase and Papers

- Whatman filter papers of different grades like No.1, No.2, No.3, No.4, No.20, No.40, No.42 etc
- In general the paper contains 98-99% of α-cellulose, 0.3 – 1% β -cellulose.

Other modified papers

- Acid or base washed filter paper
- Glass fiber type paper.
- Hydrophilic Papers – Papers modified with methanol, formamide, glycol, glycerol etc.
- Hydrophobic papers – acetylation of OH groups leads to hydrophobic nature, hence can be used for reverse phase chromatography.
- Impregnation of silica, alumna, or ion exchange resins can also be made.

2. Paper Chromatography Mobile Phase

- Pure solvents, buffer solutions or mixture of solvents can be used.

Examples-

Hydrophilic mobile phase

- Isopropanol: ammonia : water 9:1:2
- Methanol : water 4:1
- N-butanol : glacial acetic acid : water 4:1:5

Hydrophobic mobile phases

- dimethyl ether: cyclohexane kerosene : 70% isopropanol
- The commonly employed solvents are the polar solvents, but the choice depends on the nature of the substance to be separated.
- If pure solvents do not give satisfactory separation, a mixture of solvents of suitable polarity may be applied.

3. Chromatographic Chamber

- The chromatographic chambers are made up of many materials like glass, plastic or stainless steel. Glass tanks are preferred most.
- They are available in various dimensional size depending upon paper length and development type.
- The chamber atmosphere should be saturated with solvent vapor.

Steps

In paper chromatography, the sample mixture is applied to a piece of filter paper, the edge of the paper is immersed in a solvent, and the solvent moves up the paper by capillary action. The basic steps include:

1. Selection of Solid Support

Fine quality cellulose paper with defined porosity, high resolution, negligible diffusion of sample and favouring good rate of movement of solvent.

2. Selection of Mobile Phase

Different combinations of organic and inorganic solvents may be used depending on the analyte.

Example. Butanol: Acetic acid: Water (12:3:5) is suitable solvent for separating amino-acids.

3. Saturation of Tank

The inner wall of the tank is wrapped with the filter paper before solvent is placed in the tank to achieve better resolution.

4. Sample Preparation and Loading

If solid sample is used, it is dissolved in a suitable solvent. Sample (2-20ul) is added on the base line as a spot using a micropipette and air dried to prevent the diffusion.

5. Development of the Chromatogram

Sample loaded filter paper is dipped carefully into the solvent not more than a height of 1 cm and waited until the solvent front reaches near the edge of the paper. Different types of development techniques can be used:

Ascending Development

- Like conventional type, the solvent flows against gravity.
- The spots are kept at the bottom portion of paper and kept in a chamber with mobile phase solvent at the bottom.

Descending Type

- This is carried out in a special chamber where the solvent holder is at the top.
- The spot is kept at the top and the solvent flows down the paper.
- In this method solvent moves from top to bottom so it is called descending chromatography.

Ascending – Descending Development

- A hybrid of above two techniques is called ascending-descending chromatography.
- Only length of separation increased, first ascending takes place followed by descending.

Circular / Radial Development

- Spot is kept at the centre of a circular paper.
- The solvent flows through a wick at the centre & spreads in all directions uniformly.

6. Drying of Chromatogram

After the development, the solvent front is marked and the left to dry in a dry cabinet or oven.

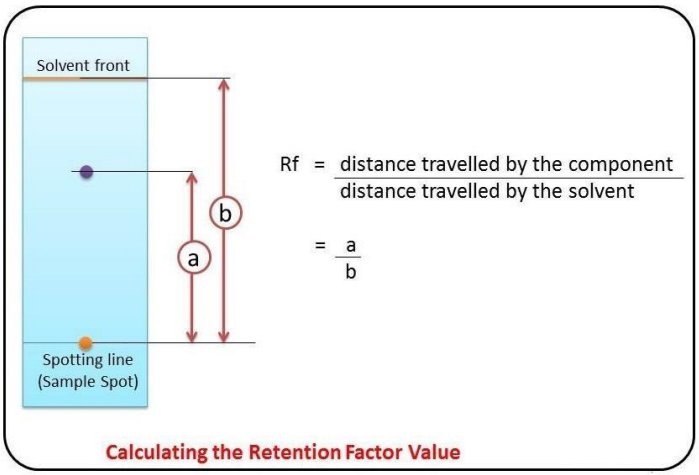
7. Detection

Colourless analytes detected by staining with reagents such as iodine vapour, ninhydrin etc.

Radio labeled and fluorescently labeled analytes detected by measuring radioactivity and florescence respectively.

Rf Values

Some compounds in a mixture travel almost as far as the solvent does; some stay much closer to the base line. The distance travelled relative to the solvent is a constant for a particular compound as long as other parameters such as the type of paper and the exact composition of the solvent are constant. The distance travelled relative to the solvent is called the Rf value. Thus, in order to obtain a measure of the extent of movement of a component in a paper chromatography experiment, “Rf value” is calculated for each separated component in the developed chromatogram. An Rf value is a number that is defined as distance travelled by the component from application point.

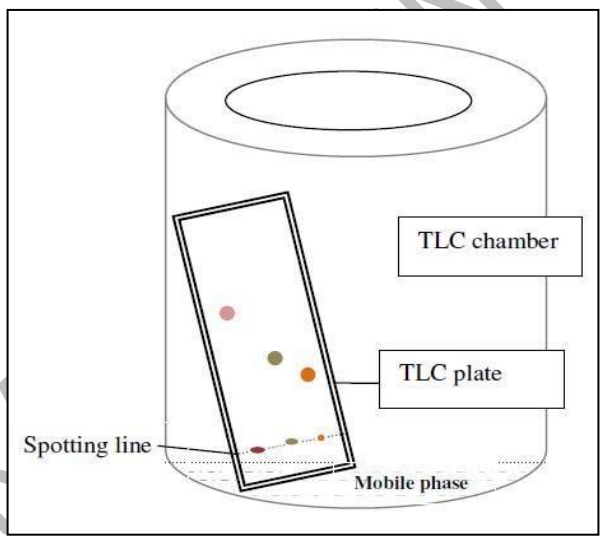


Application

- To check the control of purity of pharmaceuticals,
- For detection of adulterants,
- Detect the contaminants in foods and drinks,
- In the study of ripening and fermentation,
- For the detection of drugs and dopes in animals & humans
- In analysis of cosmetics
- Analysis of the reaction mixtures in biochemical labs.

Thin Layer Chromatography (TLC)

Thin Layer Chromatography can be defined as a method of separation or identification of a mixture of components into individual components by using finely divided adsorbent solid / (liquid) spread over a plate and liquid as a mobile phase.



Principle

- Thin-layer chromatography is performed on a sheet of glass, plastic, or aluminium foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminium oxide (alumina), or cellulose. This layer of adsorbent is known as the stationary phase.
- After the sample has been applied on the plate, a solvent or solvent mixture (known as the mobile phase) is drawn up the plate via capillary action. Because different analytes ascend the TLC plate at different rates, separation is achieved.
- It is thus based on the principle of adsorption chromatography or partition chromatography or combination of both, depending on adsorbent, its treatment and

nature of solvents employed. The components with more affinity towards stationary phase travels slower. Components with less affinity towards stationary phase travels faster.

- Once separation occurs, the individual components are visualized as spots at a respective level of travel on the plate. Their nature or character is identified by means of suitable detection techniques.

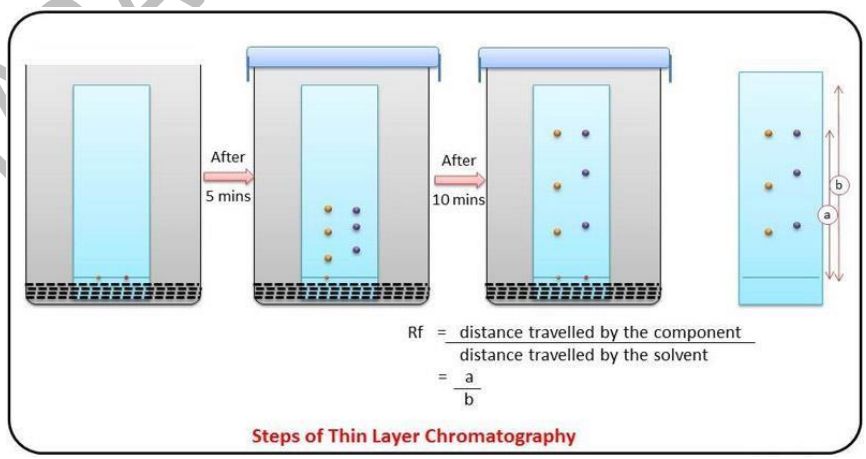
Components

TLC system components consists of:

1. **TLC plates**, preferably ready made with a stationary phase: These are stable and chemically inert plates, where a thin layer of stationary phase is applied on its whole surface layer. The stationary phase on the plates is of uniform thickness and is in a fine particle size.
2. **TLC chamber**- This is used for the development of TLC plate. The chamber maintains a uniform environment inside for proper development of spots. It also prevents the evaporation of solvents, and keeps the process dust free.
3. **Mobile phase**- This comprises of a solvent or solvent mixture The mobile phase used should be particulate-free and of the highest purity for proper development of TLC spots. The solvents recommended are chemically inert with the sample, a stationary phase.
4. **A filter paper**- This is moistened in the mobile phase, to be placed inside the chamber. This helps develop a uniform rise in a mobile phase over the length of the stationary phase.

Procedure

The stationary phase is applied onto the plate uniformly and then allowed to dry and stabilize. These days, however, ready-made plates are more commonly used.



Steps of Thin Layer Chromatography

1. With a pencil, a thin mark is made at the bottom of the plate to apply the sample spots.
2. Then, samples solutions are applied on the spots marked on the line in equal distances.
3. The mobile phase is poured into the TLC chamber to a levelled few centimetres above the chamber bottom.
4. A moistened filter paper in mobile phase is placed on the inner wall of the chamber to maintain equal humidity (and also thereby avoids edge effect).
5. Now, the plate prepared with sample spotting is placed in TLC chamber so that the side of the plate with the sample line is facing the mobile phase. Then the chamber is closed with a lid.
6. The plate is then immersed, such that the sample spots are well above the level of mobile phase (but not immersed in the solvent) for development.
7. Sufficient time is given for the development of spots.
8. The plates are then removed and allowed to dry.
9. The sample spots are then seen in a suitable UV light chamber, or any other methods as recommended for the given sample.

Some common techniques for visualizing the results of a TLC plate include

1. UV light
2. Iodine Staining: is very useful in detecting carbohydrates since it turns black on contact with Iodine
3. KMnO4 stain (organic molecules)
4. Ninhydrin Reagent: often used to detect amino acids and proteins

Retention Factor (Rf) Value

1. The behaviour of a compound on a TLC is usually described in terms of its relative mobility or Rf value.
2. Rf or Retention factor is a unique value for each compound under the same conditions.
3. The Rf for a compound is a constant from one experiment to the next only if the chromatography conditions below are also constant:
4. solvent system
5. adsorbent
6. thickness of the adsorbent
7. amount of material spotted

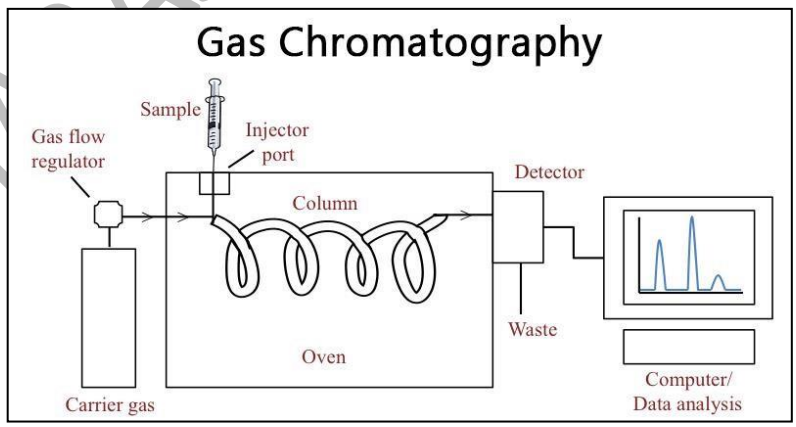
8. temperature
9. Since these factors are difficult to keep constant from experiment to experiment, relative Rf values are generally considered.
10. Relative Rf means that the values are reported relative to a standard.

Application

1. Identify compounds present in a given mixture

2. Determine the purity of a substance.

- Analyzing ceramides and fatty acids
- Detection of pesticides or insecticides in food and water
- Analyzing the dye composition of fibers in forensics
- Assaying the radiochemical purity of radiopharmaceuticals
- Identification of medicinal plants and their constituents 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

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 non-volatile 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.
- The process is repeated in each plate as the sample is moved toward the outlet.
- 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.

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, N2, H, 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.
- N2 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 micro syringe 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 2500
- Swage 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 9000 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 2500

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.

Procedure Step 1: Sample Injection and Vapourization

1. A small amount of liquid sample to be analyzed is drawn up into a syringe.
2. The syringe needle is positioned in the hot injection port of the gas chromatograph and the sample is injected quickly.

3. The injection of the sample is considered to be a "point" in time, that is, it is assumed that the entire sample enters the gas chromatograph at the same time, so the sample must be injected quickly.
4. The temperature is set to be higher than the boiling points of the components of the mixture so that the components will vaporize.
5. The vaporized components then mix with the inert gas mobile phase to be carried to the gas chromatography column to be separated.

Step 2: Separation in the Column

- Components in the mixture are separated based on their abilities to adsorb on or bind to, the stationary phase.
- A component that adsorbs most strongly to the stationary phase will spend the most time in the column
- (will be retained in the column for the longest time) and will, therefore, have the longest retention time (Rt). It will emerge from the gas chromatograph last.
- A component that adsorbs the least strongly to the stationary phase will spend the least time in the column (will be retained in the column for the shortest time) and will, therefore, have the shortest retention time (Rt). It will emerge from the gas chromatograph first.
- If we consider a 2-component mixture in which component A is more polar than component B then:

1. component A will have a **longer retention time** in a polar column than component B
2. component A will have a **shorter retention time** in a non-polar column than component B

Step 3: Detecting and Recording Results

1. The components of the mixture reach the detector at different times due to differences in the time they are retained in the column.
2. The component that is retained the shortest time in the column is detected first. The component that is retained the longest time in the column is detected last.
3. The detector sends a signal to the chart recorder which results in a peak on the chart paper. The component that is detected first is recorded first. The component that is detected last is recorded last.

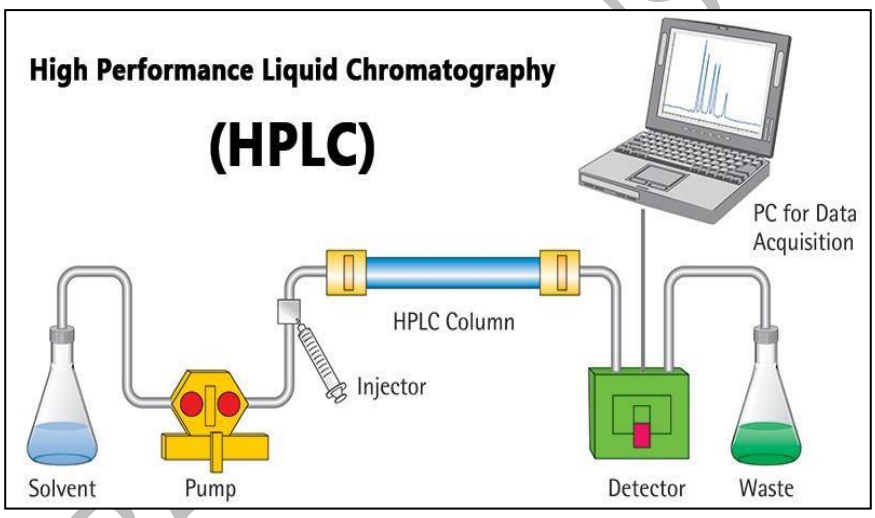
Application

- GC analysis is used to calculate the content of a chemical product, for example in assuring the quality of products in the chemical industry; or measuring toxic substances in soil, air or water.
- Gas chromatography is used in the analysis of:
    a) air-borne pollutants
    b) performance-enhancing drugs in athlete's urine samples
    c) oil spills
    d) essential oils in perfume preparation
- GC is very accurate if used properly and can measure picomoles of a substance in a 1 ml liquid sample, or parts-per-billion concentrations in gaseous samples.
- Gas Chromatography is used extensively in forensic science. Disciplines as diverse as solid drug dose (pre-consumption form) identification and quantification, arson investigation, paint chip analysis, and toxicology cases, employ GC to identify and quantify various biological specimens and crime-scene evidence.

High-Performance Liquid Chromatography (HPLC)

- The samples are also required to be salt-free; they should not contain ions.
- Very minute amounts of a substance can be measured, but it is often required that the sample must be measured in comparison to a sample containing the pure, suspected substance known as a reference standard.
- High performance liquid chromatography or commonly known as HPLC is an analytical technique used to separate, identify or quantify each component in a mixture.
- The mixture is separated using the basic principle of column chromatography and then identified and quantified by spectroscopy.
- In the 1960s the column chromatography LC with its lowpressure suitable glass columns was further developed to the HPLC with its high-pressure adapted metal columns.
- HPLC is thus basically a highly improved form of column liquid chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres. Principles
- The purification takes place in a separation column between a stationary and a mobile phase.
- The stationary phase is a granular material with very small porous particles in a separation column.

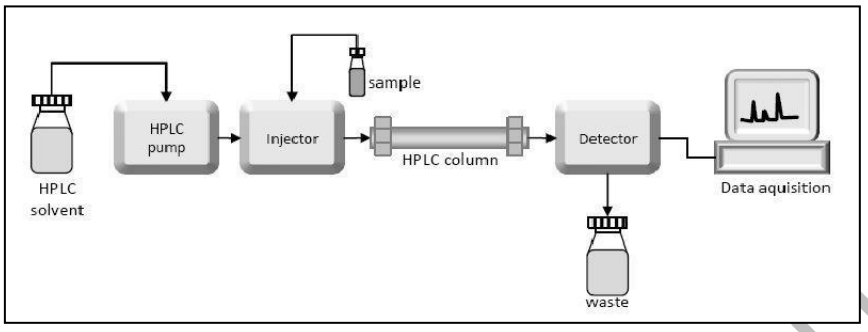
- The mobile phase, on the other hand, is a solvent or solvent mixture which is forced at high pressure through the separation column.
- Via a valve with a connected sample loop, i.e. a small tube or a capillary made of stainless steel, the sample is injected into the mobile phase flow from the pump to the separation column using a syringe.
- Subsequently, the individual components of the sample migrate through the column at different rates because they are retained to a varying degree by interactions with the stationary phase.
- After leaving the column, the individual substances are detected by a suitable detector and passed on as a signal to the HPLC software on the computer.
- At the end of this operation/run, a chromatogram in the HPLC software on the computer is obtained.
- The chromatogram allows the identification and quantification of the different substances.



Instruments The Pump

- The development of HPLC led to the development of the pump system.
- The pump is positioned in the most upper stream of the liquid chromatography system and generates a flow of eluent from the solvent reservoir into the system.
- High-pressure generation is a “standard” requirement of pumps besides which, it should also to be able to provide a consistent pressure at any condition and a controllable and reproducible flow rate.

- Most pumps used in current LC systems generate the flow by back-and-forth motion of a motor-driven piston (reciprocating pumps). Because of this piston motion, it produces "pulses".



Injector

- An injector is placed next to the pump.
- The simplest method is to use a syringe, and the sample is introduced to the flow of eluent.
- The most widely used injection method is based on sampling loops.
- The use of the autosampler (auto-injector) system is also widely used that allows repeated injections in a set scheduled-timing.

Column

- The separation is performed inside the column.
- The recent columns are often prepared in a stainless steel housing, instead of glass columns.
- The packing material generally used is silica or polymer gels compared to calcium carbonate.
- The eluent used for LC varies from acidic to basic solvents.
- Most column housing is made of stainless steel since stainless is tolerant towards a large variety of solvents.

Detector

- Separation of analytes is performed inside the column, whereas a detector is used to observe the obtained separation.
- The composition of the eluent is consistent when no analyte is present. While the presence of analyte changes the composition of the eluent. What detector does is to measure these differences.
- This difference is monitored as a form of an electronic signal. There are different types of detectors available.

Recorder

- The change in eluent detected by a detector is in the form of an electronic signal, and thus it is still not visible to our eyes.
- In older days, the pen (paper)-chart recorder was popularly used. Nowadays, a computer-based data processor (integrator) is more common.
- There are various types of data processors; from a simple system consisting of the in-built printer and word processor while those with software that are specifically designed for an LC system which not only data acquisition but features like peak-fitting, baseline correction, automatic concentration calculation, molecular weight determination, etc.

Degasser

The eluent used for LC analysis may contain gases such as oxygen that are non-visible to our eyes.

- When gas is present in the eluent, this is detected as noise and causes an unstable baseline.
- Degasser uses special polymer membrane tubing to remove gases.
- The numerous very small pores on the surface of the polymer tube allow the air to go through while preventing any liquid to go through the pore.

Column Heater

The LC separation is often largely influenced by the column temperature.

- In order to obtain repeatable results, it is important to keep consistent temperature conditions.
- Also for some analysis, such as sugar and organic acid, better resolutions can be obtained at elevated temperatures (50 to 80°C).
- Thus columns are generally kept inside the column oven (column heater).

Application

The HPLC has developed into a universally applicable method so that it finds its use in almost all areas of chemistry, biochemistry, and pharmacy.

- Analysis of drugs
- Analysis of synthetic polymers
- Analysis of pollutants in environmental analytics
- Determination of drugs in biological matrices
- Isolation of valuable products

- Product purity and quality control of industrial products and fine chemicals
- Separation and purification of biopolymers such as enzymes or nucleic acids
- Water purification
- Pre-concentration of trace components
- Ligand-exchange chromatography
- Ion-exchange chromatography of proteins
- High-pH anion-exchange chromatography of carbohydrates and oligosaccharides

Electrophoresis

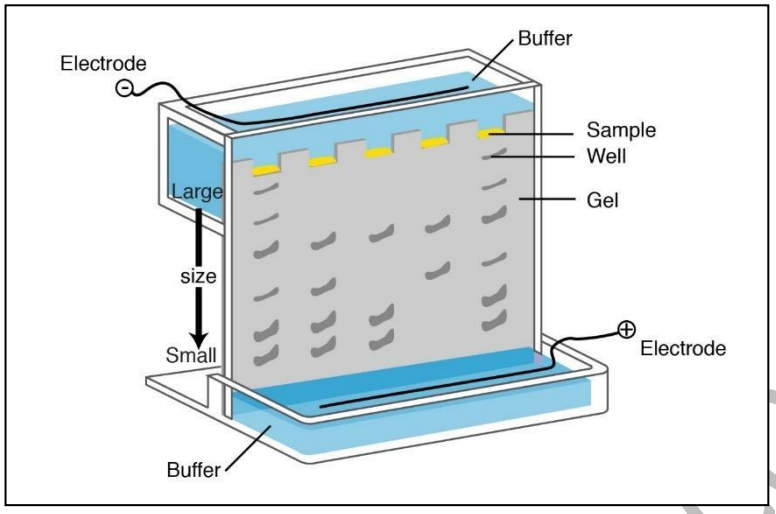
Electrophoresis is a very broadly used technique which, fundamentally, applies electric current to biological molecules, whether they're usually DNA, they can be protein or RNA, too...and separates these fragments into pieces which are larger or smaller. It is used in a variety of applications. Everything from forensics for determining the identity of individuals that may have been involved in a crime, by linking their DNA pattern, their electrophoresis pattern, to one that's in a database.

The whole basis by which the human genome was done is by something called capillary electrophoresis, by separating DNA into shorter pieces and then running them on these electrophoresis gels which allow the patterns of As, Cs, Ts, and Gs to be elucidated. They are also very important in protein research, and then genetic mutation research, because when proteins or DNA are mutated, they are frequently longer or shorter, and they therefore show up on an electrophoresis gel differently than normal, so many diagnostic tests are still done using electrophoresis, so it is a very widely used basic research technique, was very important for the understanding of gene and protein function, but it's now gotten into the area of clinical diagnostics and forensics as well.

Electrophoresis is usually done in what looks like a box which has a positive charge at one end and a negative charge at the other. And as we all learned in basic physics, when you put a charged molecule into an environment like that, the negative molecules go to the positive charge, and vice versa. In looking at proteins in a gel, in one of these boxes, you usually take the entire protein, and you're looking at the entire length of the protein and seeing how big it is, and the bigger it is, the shorter it will migrate into the gel, so that the small proteins will end up at the bottom of the gel, because they have migrated the farthest, and the biggest ones will wind up staying at the top.

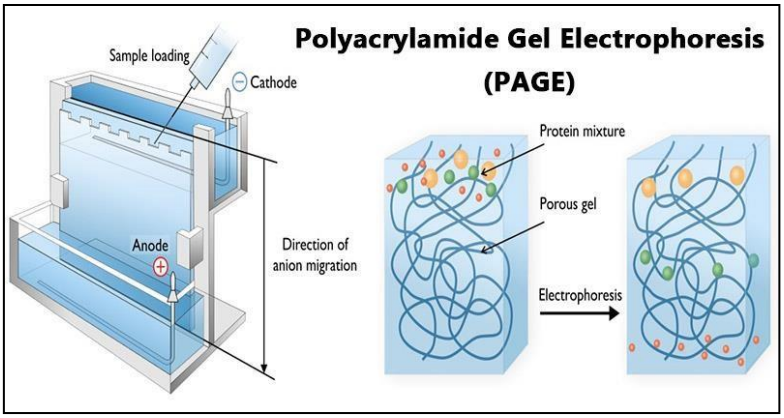
In the case of DNA, DNA is a very long molecule, so you wouldn't want to run, for the most part, a whole DNA molecule from a cell onto a gel. It is just so big that it would never get into the gel, so what scientists do, and what people do in classrooms these days, is to chop up that DNA using things like inscription enzymes, which chop up the DNA into more manageable pieces in a

reproducible way. And then those pieces, depending on how big the pieces are, migrate more or less into the gel father down the box from top to the bottom.



Polyacrylamide Gel Electrophoresis (PAGE)

- Electrophoresis through agarose or polyacrylamide gels is a standard method used to separate, identify and purify biopolymers, since both these gels are porous in nature.
- Polyacrylamide gels are chemically cross-linked gels formed by the polymerization of acrylamide with a crosslinking agent, usually N,N'methylenebisacrylamide.
- The reaction is a free radical polymerization, usually carried out with ammonium persulfate as the initiator and N,N,N',N'-tetramethylethylendiamine (TEMED) as the catalyst.
- Polyacrylamide gel electrophoresis (PAGE) is a technique widely used in biochemistry, forensic chemistry, genetics, molecular biology and biotechnology to separate biological macromolecules, usually proteins or nucleic acids, according to their electrophoretic mobility.
- The most commonly used form of polyacrylamide gel electrophoresis is the Sodium dodecyl sulphate Polyacrylamide gel electrophoresis (SDS- PAGE) used mostly for the separation of proteins.



Principle

SDS-PAGE (Polyacrylamide Gel Electrophoresis), is an analytical method used to separate components of a protein mixture based on their size. The technique is based upon the principle that a charged molecule will migrate in an electric field towards an electrode with opposite sign. The general electrophoresis techniques cannot be used to determine the molecular weight of biological molecules because the mobility of a substance in the gel depends on both charge and size.

The proteins being covered by SDS are negatively charged and when loaded onto a gel and placed in an electric field, it will migrate towards the anode (positively charged electrode) are separated by a molecular sieving effect based on size. After the visualization by a staining (protein-specific) technique, the size of a protein can be calculated by comparing its migration distance with that of a known molecular weight ladder (marker).

Requirements

- Acrylamide solutions (for resolving & stacking gels).
- Isopropanol / distilled water.
- Gel loading buffer.
- Running buffer.
- Staining, destaining solutions.
- Protein samples
- Molecular weight markers.

The equipment and supplies necessary for conducting SDS-PAGE includes:

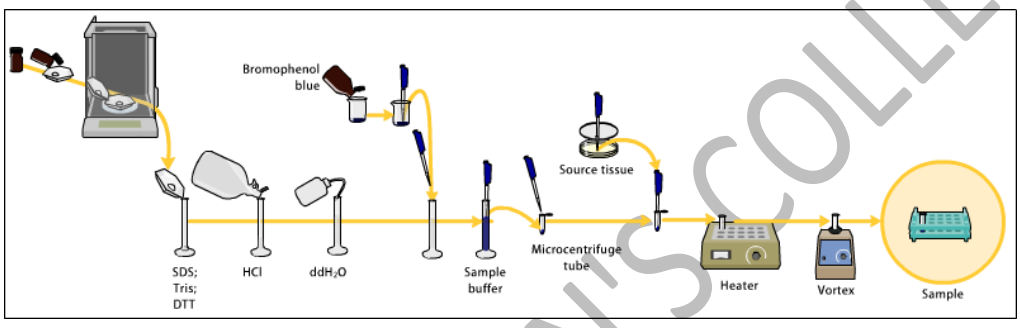
- An electrophoresis chamber and power supply.
- Glass plates (a short and a top plate).
- Casting frame
- Casting stand
- Combs

Steps

1. Sample preparation

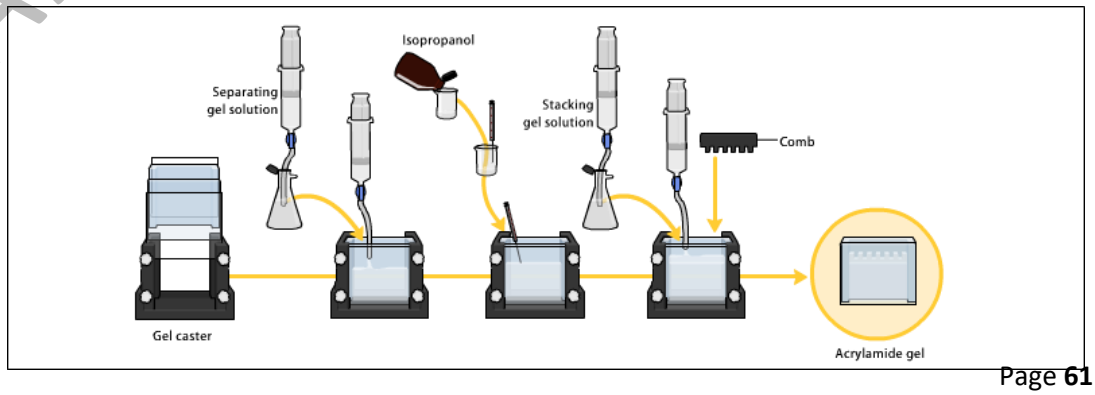
- Samples may be any material containing proteins or nucleic acids.
- The sample to analyze is optionally mixed with a chemical denaturant if so desired, usually SDS for proteins or urea for nucleic acids.

- SDS is an anionic detergent that denatures secondary and non–disulfide–linked tertiary structures, and additionally applies a negative charge to each protein in proportion to its mass. Urea breaks the hydrogen bonds between the base
- pairs of the nucleic acid, causing the constituent strands to anneal. Heating the samples to at least 60 °C further promotes denaturation.
- A tracking dye may be added to the solution. This typically has a higher electrophoretic mobility than the analytes to allow the experimenter to track the progress of the solution through the gel during the electrophoretic run.



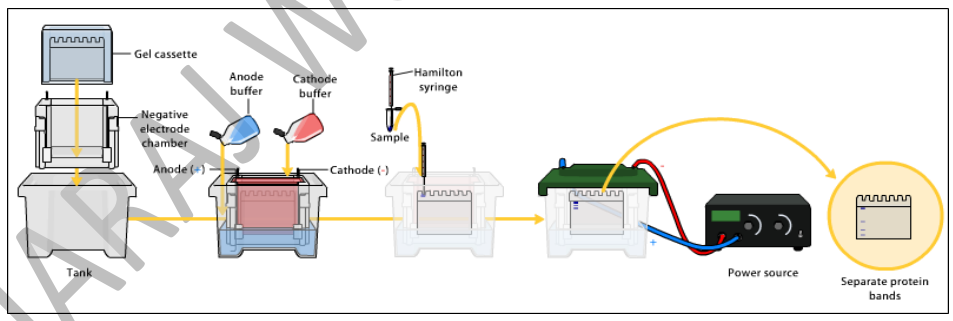
2. Preparation of polyacrylamide gel

- The gels typically consist of acrylamide, bisacrylamide, the optional denaturant (SDS or urea), and a buffer with an adjusted pH.
- The ratio of bisacrylamide to acrylamide can be varied for special purposes, but is generally about 1 part in 35. The acrylamide concentration of the gel can also be varied, generally in the range from 5% to 25%.
- Lower percentage gels are better for resolving very high molecular weight molecules, while much higher percentages of acrylamide are needed to resolve smaller proteins,
- Gels are usually polymerized between two glass plates in a gel caster, with a comb inserted at the top to create the sample wells.
- After the gel is polymerized the comb can be removed and the gel is ready for electrophoresis.



3. Electrophoresis

- Various buffer systems are used in PAGE depending on the nature of the sample and the experimental objective.
- The buffers used at the anode and cathode may be the same or different.
- An electric field is applied across the gel, causing the negatively charged proteins or nucleic acids to migrate across the gel away from the negative and towards the positive electrode (the anode).
- Depending on their size, each biomolecule moves differently through the gel matrix: small molecules more easily fit through the pores in the gel, while larger ones have more difficulty.
- The gel is run usually for a few hours, though this depends on the voltage applied across the gel.
- After the set amount of time, the biomolecules will have migrated different distances based on their size.
- Smaller biomolecules travel farther down the gel, while larger ones remain closer to the point of origin.
- Biomolecules may therefore be separated roughly according to size, which depends mainly on molecular weight under denaturing conditions, but also depends on higher-order conformation under native conditions.



4. Detection

- Following electrophoresis, the gel may be stained (for proteins, most commonly with Coomassie Brilliant Blue or autoradiography; for nucleic acids, ethidium bromide; or for either, silver stain), allowing visualization of the separated proteins, or processed further (e.g. Western blot).
- After staining, different species biomolecules appear as distinct bands within the gel.

- It is common to run molecular weight size marker of known molecular weight in a separate lane in the gel to calibrate the gel and determine the approximate molecular mass of unknown biomolecules by comparing the distance travelled relative to the marker.

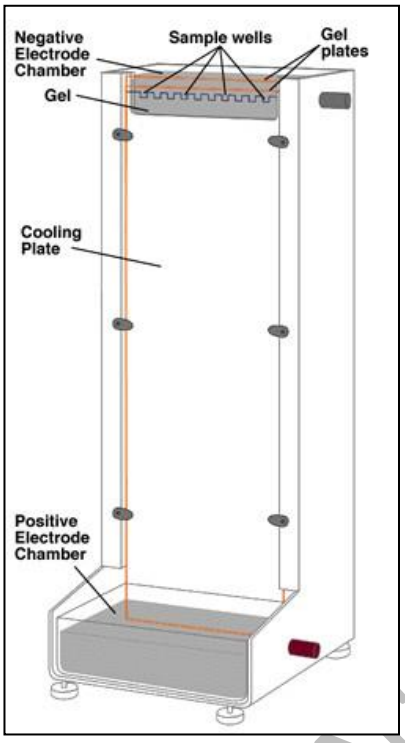
Application

- Measuring molecular weight.
- Peptide mapping.
- Estimation of protein size.
- Determination of protein subunits or aggregation structures.
- Estimation of protein purity.
- Protein quantitation.
- Monitoring protein integrity.
- Comparison of the polypeptide composition of different samples.
- Analysis of the number and size of polypeptide subunits.
- Post-electrophoresis applications, such as Western blotting.
- Staining of Proteins in Gels with Coomassie G-250 without Organic Solvent and Acetic Acid.
- Pouring and Running a Protein Gel by reusing Commercial Cassettes.
- Selective Labelling of Cell-surface Proteins using CyDye DIGE Fluor Minimal Dyes.
- Detection of Protein Ubiquitination.

Horizontal and Vertical Gel Systems - The Vertical Slab Gel System

A typical vertical apparatus used for sequencing is shown in the figure below. This system shows the components common to all vertical slab systems. The gel is cast between two glass plates, separated by spacers, typically less than 2mm thick. The gel is mounted in the system so that the top is in contact with the negative electrode chamber, and the bottom is in contact with the positive electrode chamber. Unlike the horizontal system, the only connection between the buffer chambers is through the gel.

This allows precise and reproducible control of the voltage gradient. Because of the high resistance of the thin gel, the apparatus must have provisions for cooling. In the system shown, the front of the gel cassette is exposed to the air, while the back of the gel is held against a metal plate which dissipates heat rapidly. In some systems, the upper buffer chamber extends almost to the bottom of the gel, and the upper buffer is used for cooling. The relatively small amount of current carried through the gel means that buffer recirculation is generally not required.



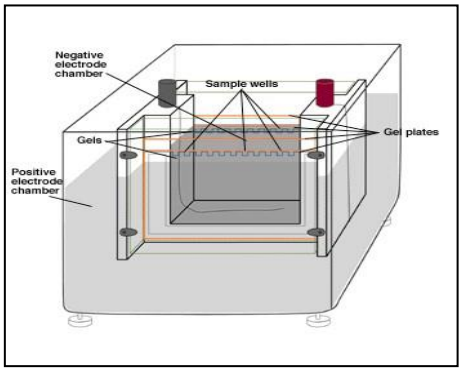
The figure shows a standard "mini" gel apparatus. Such gels—generally 10cm x 10cm or smaller—have become the standard for many applications because of their ease of preparation and handling and short run times. As with the "full size" system the gels are cast between glass plates, but in the mini-gel system the cassettes are mounted onto a "U" shaped frame so the cassettes themselves form the sides of the negative electrode chamber.

This assembly is placed in a tank of buffer which contains the positive electrode. This means that the gels are effectively submerged in buffer during the run, providing optimal cooling.

In general, vertical slab gels are loaded through the top, under a layer of buffer. The gels are monitored during the run through the front glass plate. The fact that the body of the gel in these systems cannot be accessed until the end of the run can be an inconvenience. Some sample recovery techniques used on horizontal gels are not available for vertical gels. However, the resolution and reproducibility of vertical polyacrylamide gels more than compensate for this.

Casting a vertical slab gel

Vertical gels are cast in a cassette made up of two glass plates separated by spacers which run along the sides of the plates. The bottom of the cassette is sealed by some temporary means (tape, agarose, or a gasket). The gel monomer solution is treated to initiate polymerization, and poured into the cassette. A comb is inserted into the top of the cassette to form the sample wells, and the gel is allowed sufficient time to polymerize. After polymerization, the bottom of the gel is unsealed, and the cassette is mounted in the apparatus.



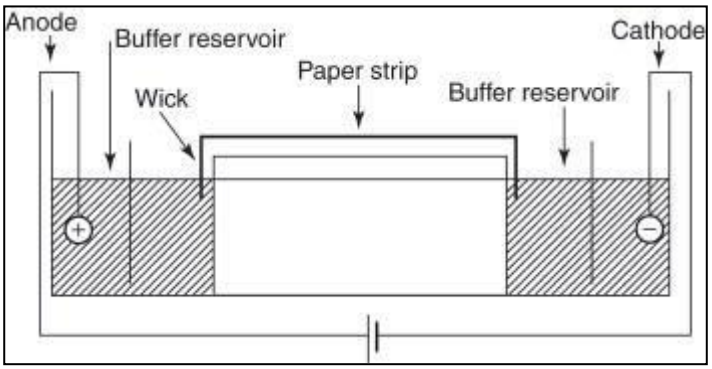
Paper Electrophoresis

This technique is useful for the separation of small charged molecules such as amino acids and small proteins. A strip of filter paper is moistened with buffer and the ends of the strip are immersed into buffer reservoirs containing the electrodes. The samples are spotted in the center of the paper, high voltage is applied, and the spots migrate according to their charges. After electrophoresis, the separated components can be detected by a variety of staining techniques, depending upon their chemical identity.

Electrophoretic techniques have also been adapted to other applications such as the determination of protein isoelectric points. Affinity gels with bio specific properties are used to study binding sites and surface features of proteins. Continuous flow electrophoresis is applied to separations in free solution and has found very useful application in blood cell separation. Recently, High Performance Capillary Electrophoresis (HPCE) has been developed for the separation of many classes of biological molecules.

Paper Electrophoresis (Low Voltage)

Low-voltage paper electrophoresis is the simplest and cheapest form of electrophoresis. A strip of commercially available chromatography paper is soaked in buffer and placed with one end in each buffer reservoir (connecting wicks may be used). It is important that the paper is saturated with the buffer since it is the buffer that conducts the majority of the current. A spot of sample is placed in the center of the strip. When the current is applied ions separate out and migrate toward the attractive electrodes. Paper electrophoresis has several limitations. Only small charged molecules can be reliably separated since many macromolecules adsorb on to the paper, although it is possible to reduce the adsorption by using a buffer that is more alkaline than the isoelectric point of the sample. Paper systems are associated with high electrical resistance causing heating that dries out the paper. Although this method has been largely superseded by gel methods for many samples it is still useful for separation of small molecules such as amino acids, small peptides, nucleotides, and inorganic ions. An important limitation to this technique is that considerable diffusion of small molecules occurs at low voltage.



High-Voltage Paper Electrophoresis

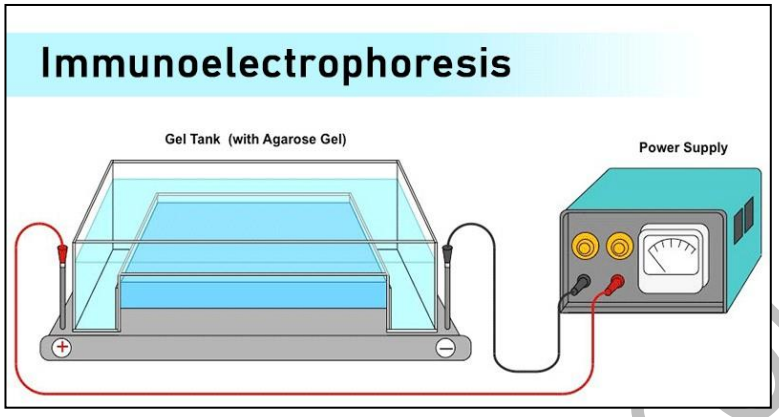
High-voltage PE is a separation technique based on the differences in the electrophoretic mobilities of charged compounds. Commercially available apparatus consist of a high-voltage (1–10 kV) supply that is connected across the opposite ends of an electrophoresis paper. Application of a convenient electric potential induces a current to flow along the sheet of paper, producing a migration of ions. Under certain conditions of pH and current, the extent of migration of the components of a mixture is related to the charges located on the individual chemical species. Therefore, the number of ionizable groups and their pKa values are critical for determining the electrophoretic behaviour. In the case of purines, pyrimidines, and nucleosides, the pKa values of groups such as amino and keto groups are important for electrophoretic separation. Nucleotides can be separated from the other compounds on the basis of the pKa values of their phosphoric acid groups. As would be expected from their difference in negative charge, nucleoside mono-, di- and triphosphates migrate at different speeds toward the anode, and they can be separated easily one from another.

Volatile 'buffers' with a pH ranging from 2.0 to 9.0 are generally utilized in PE. Typically, a formic acid/acetic acid/water mixture at pH 2.0 is used for nucleotides. Noncyclic 5'-nucleotides can be separated from cyclic 3',5'- nucleotides, which are simultaneously present in animal, plant. and microbial tissues, by applying a nonvolatile sodium borate buffer at pH 9.3; indeed borate interacts specifically with the cis-vicinal hydroxyl groups of 5'-nucleotide ribose but not with cyclic nucleotides.

Immunoelectrophoresis

- Immunoelectrophoresis refers to precipitation in agar under an electric field.
- It is a process of a combination of immunodiffusion and electrophoresis.
- An antigen mixture is first separated into its component parts by electrophoresis and then tested by double immuno-diffusion.
- Antigens are placed into wells cut in a gel (without antibody) and electrophoresed. A trough is then cut in the gel into which antibodies are placed.

- The antibodies diffuse laterally to meet diffusing antigens, and lattice formation and precipitation occur permitting determination of the nature of the antigens.
- The term "immunoelectrophoresis" was first coined by Grabar and Williams in 1953.



Principle

When an electric current is applied to a slide layered with gel, the antigen mixture placed in wells is separated into individual antigen components according to their charge and size. Following electrophoresis, the separated antigens are reacted with specific antisera placed in troughs parallel to the electrophoretic migration and diffusion is allowed to occur. Antiserum present in the trough moves toward the antigen components resulting in the formation of separate precipitin lines in 18-24 hrs, each indicating reaction between individual proteins with its antibody.

Procedure

1. Agarose gel is prepared on a glass slide put in a horizontal position.
2. Using the sample template, wells are borne on the application zone carefully.
3. The sample is diluted 2:3 with protein diluent solution (20µl antigen solution +10 µl diluent).
4. Using a 5 µl pipette, 5 µl of control and sample is applied across each corresponding slit (Control slit and Sample slit).
5. The gel is placed into the electrophoresis chamber with the samples on the cathodic side, and electrophoresis runs for 20 mins/ 100 volts.
6. After electrophoresis completes, 20 µl of the corresponding antiserum is added to troughs in a moist chamber and incubated for 18- 20 hours at room temperature in a horizontal position.
7. The agarose gel is placed on a horizontal position and dried with blotter sheets.

8. The gel in saline solution is soaked for 10 minutes and the drying and washing repeated twice again.
9. The gel is dried at a temperature less than 70°C and may be stained with protein staining solution for about 3 minutes followed by decolorizing the gel for 5 minutes in distaining solution baths.
10. The gel is dried and results evaluated.

Results

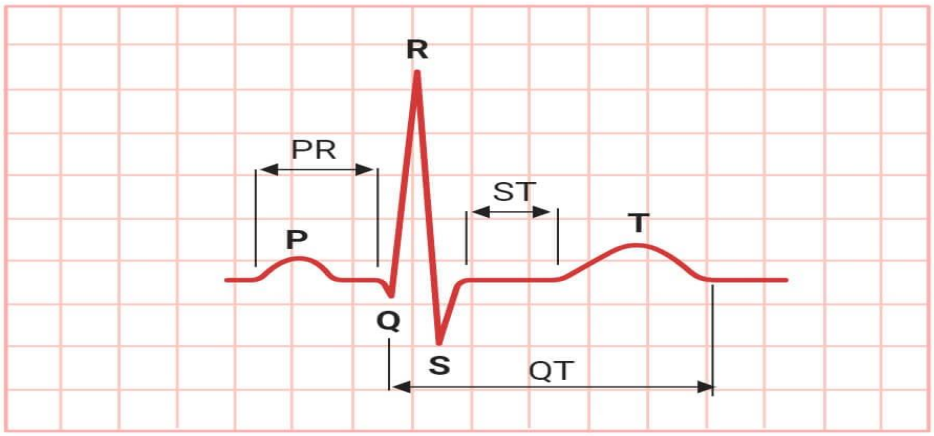
1. The presence of elliptical precipitin arcs represents antigen-antibody interaction.
2. The absence of the formation of precipitate suggests no reaction.
3. Different antigens (proteins) can be identified based on the intensity, shape, and position of the precipitation lines.

Application

1. The test helps in the identification and approximate quantization of various proteins present in the serum. Immunoelectrophoresis created a breakthrough in protein identification and in immunology.
2. Immunoelectrophoresis is used in patients with suspected monoclonal and polyclonal gammopathies.
3. The method is used to detect normal as well as abnormal proteins, such as myeloma proteins in human serum.
4. Used to analyze complex protein mixtures containing different antigens.
5. The medical diagnostic use is of value where certain proteins are suspected of being absent (e.g., hypogammaglobulinemia) or overproduced (e.g., multiple myeloma).
6. This method is useful to monitor antigen and antigen-antibody purity and to identify a single antigen in a mixture of antigens.
7. Immunoelectrophoresis is an older method for qualitative analysis of M-proteins in serum and urine.
8. Immunoelectrophoresis aids in the diagnosis and evaluation of the therapeutic response in many disease states affecting the immune system.

UNIT – IV

Electrocardiogram, commonly known as ECG or EKG is a medical test report indicating the heart's electrical activity and rhythm during repeated cardiac cycles.



Electrocardiogram
(ECG)

Electrocardiogram (ECG)

It is printed on grid paper called the ECG strip or ECG tracing. The process of producing an electrocardiogram is termed electrocardiography. It is a non-invasive medical procedure performed by recording the cardiac impulses on the surface of our body using specialized electrodes over specific regions of our body.

The main purpose of ECG is to detect and diagnose any underlying cardiovascular issues or structural abnormalities in the heart. Electrocardiography is one of the most commonly prescribed tests by cardiologists to obtain information about the patient's cardiac electrical functioning.

Instrumentation

ECG test is done using an ECG machine containing a set of electrodes connected to a central signal processor through lead wires and a monitor and a printer to display and print the ECG.

Typically 10 electrodes, that receive, collect and transmit the electric potentials from our body (biopotentials), are present in a standard 12-lead ECG-producing machine. The biopotentials collected by the electrodes are carried by lead wires to the central impulse processing machine. The central ECG machine amplifies the received biopotential, filters the signal, and processes it to produce ECG waves in specific intervals and segments. The processed signals are displayed on the monitor and are printed on grid papers to produce an ECG.

Principle of Electrocardiogram

The ECG is based on the cardiac action potential and cardiac conduction. The SA node generates the cardiac action potential which is relayed down the cardiac conduction pathway

resulting change in the membrane voltage (potential) across the membrane of cardiomyocytes. This change in cardiac action potential results in the continuous running of the cardiac cycle.

The cardiac impulse transmits through the heart, spreads around the surrounding tissue, and finally reaches the skin of our body. This electric impulse in the skin is received by the electrodes of the ECG machine and processed to produce an ECG.

Interpretation of ECG

An ECG is analyzed and interpreted by trained medical personnel only. While reading an ECG the P wave, QRS complex, T wave, PR interval, QT interval, PR segment, and ST segment are mainly focused. The elevation and depression of each wave, the duration of the waves, and the duration of the segments and intervals are studied. Additionally, the heart rate, rhythm, and axis on an ECG are also studied. Based on a collective result of all these various components, an ECG report is interpreted.

A normal ECG will show the following results:

1. **Heart Rate:** Normal heart rate of 60 to 100 beats per minute
2. **Heart Rhythm:** Heart rhythm will be consistent and even
3. **PR Interval:** 0.12 to 0.20 seconds
4. **QRS Duration:** 0.06 to 0.10 seconds
5. **QT Interval:** 0.40 seconds
6. **ST Segment:** 0.08 seconds
7. **P-wave**

- Upright, uniform, and consistent before each QRS complex.
- P duration < 0.12 seconds
- P amplitude < 2.5 mm

8. **T-wave:** Upright in lead I, II, V3 to V6, and inverted in aVR.

Application of ECG

- Diagnosing arrhythmias and related cardiovascular diseases.
- Studying the heart's electrical activity and the cardiac cycle of the heart.
- Assessing heart diseases and heart attacks.
- It is used to evaluate the progress after medication or cardiac surgery.
- It is coupled with ECHO to access the overall structural and functional health of the heart and valves.

- Used to check how the heart performs under stress.

EMG

Electromyography (EMG) measures muscle response or electrical activity in response to a nerve's stimulation of the muscle. The test is used to help detect neuromuscular abnormalities. During the test, one or more small needles (also called electrodes) are inserted through the skin into the muscle. The electrical activity picked up by the electrodes is then displayed on an oscilloscope (a monitor that displays electrical activity in the form of waves). An audio-amplifier is used so the activity can be heard. EMG measures the electrical activity of the muscle during rest, slight contraction and forceful contraction. Muscle tissue does not normally produce electrical signals during rest. When an electrode is inserted, a brief period of activity can be seen on the oscilloscope, but after that, no signal should be present.

After an electrode has been inserted, you may be asked to contract the muscle, for example, by lifting or bending your leg. The action potential (size and shape of the wave) that this creates on the oscilloscope provides information about the ability of the muscle to respond when the nerves are stimulated. As the muscle is contracted more forcefully, more and more muscle fibers are activated, producing action potentials.

A related procedure that may be performed is nerve conduction study (NCS). NCS is a measurement of the amount and speed of conduction of an electrical impulse through a nerve. NCS can determine nerve damage and destruction, and is often performed at the same time as EMG. Both procedures help to detect the presence, location, and extent of diseases that damage the nerves and muscles.

EEG

Electroencephalography is a technique that was first developed in 1929 by a German scientist named Hans Berge. Electroencephalography can be defined as a technique to record and intercept the activity of the brain with the help of brain waves. The nerve cells of the brain generate electrical waves which when recorded and interpreted give the idea of the brain's activity. The graph generated by electrical waves of the brain is known as an electroencephalograph, whereas the device that is used to study and record the electrical impulse of nerve present in the brain is known as the electroencephalogram. The data interpreted as electroencephalograph is also known as EEG.

Electroencephalography is used as the diagnostic tool for conditions such as epilepsy, brain tumor, head injury, sleeping disorder, cerebral infection, and brain death. The most widespread use of encephalography has been recorded in the case of epilepsy. It is also a crucial diagnosis in case of brain death because it allows healthcare professionals to maintain the vitality of the organs in case of brain death of a donor.

The nerves of the brain communicate through electrical signals EEG or electroencephalograph is a graphical representation of the recorded electrical signal of the rain. EEG helps understand brain activity. EEG is used to diagnose the following conditions

1. Brain tumor
2. Seizures
3. Epilepsy
4. Encephalitis (inflammation of the brain)
5. Encephalopathy (dysfunction of the brain as an effect of various diseases)
6. Neurodegenerative disease like Alzheimer.

EEG Test Procedure

It is a simple procedure that includes the following steps: the first patient is laid down then two steps of electrodes are attached to either side of the brain. These electrodes are the part of electroencephalograms, they are used to detect neural electrical impulses. Electrodes are connected through a wire to the amplifier, where it sends the message, and waves get amplified enough to be sent to the computer, and the computer deciphers the electrical signals and the graph is plotted on the computer screen. This graph is known as an electroencephalograph. Now, this complete setup is called an electroencephalogram, and the procedure is known as electroencephalography.

Risk Associated

Only a small part of the population has the risk of seizure due to EEG. The signal-to-noise ratio in EEG tests is poor, hence there are relatively large numbers of subjects required to gain a piece of correct pathological information by EEG.

Mechanism of Electroencephalography

Electroencephalography works on the principle of volume conduction. Volume conduction refers to the mechanism of measuring various electrical potentials generated from a distant source. The human brain consists of several millions of neurons, these neurons maintain common resting potential and when needed generates an action potential. These potentials are generated and maintained by manipulating the concentration of ions inside and outside of the neuron. During any activity a large number of ions are expelled from the neurons, ions with similar charges repel each other. This repulsion causes a push action, that is ions push each other causing the formation of waves. Thus this wave represents the activity of the human brain. When the wave generated by the virtue of repulsion of ions reaches the electrodes, it pushes the electrons of the electrode. The electrode is made of metal that can easily push or pull electrons (ions). The difference between the voltages of push or pull between electrodes can be measured by a voltmeter. When these voltages are recorded over time this gives us the EEG wave.

Types of Wave in EEG

EEG can be divided into 2 two main components, rhythmic activity, and transients. The rhythmic activity of the brain can be divided into bands according to frequency. The frequency ranges from less than 4 Hz to 32 Hz. The frequency bands can be divided as follows-

1. Delta
2. Theta
3. Alpha
4. Beta
5. Gamma
6. Mu

Delta Waves- These waves range from 1 to less than 4 Hz. These waves are pathologically important for the detection of subcortical lesions, diffuse lesions, deep midline lesions, and metabolic encephalopathy hydrocephalus. It has the highest amplitude and slowest wave propagation. It is generally generated during discontinuous attention tasks.

Theta Wave- These range from 4 to 7 Hz. it is more common in young children. These waves are pathologically important for the detection of focal subcortical lesions, deep midline disorders, metabolic encephalopathy, and minor cases of hydrocephalus. Theta waves are also associated with the repression of an elicited response.

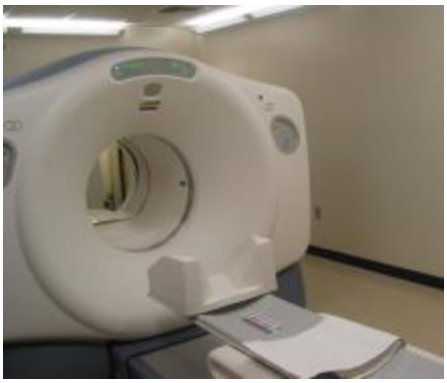
Alpha Waves- These waves range from 8 to 15 Hz. they are abundantly found in the posterior region of the brain. Hence these waves are also named the posterior basic rhythm. It is also associated with the inhibition of the elicited response. They are pathological determinants of the coma.

Beta Waves- These range from 16 to 31 Hz, they are predominantly found in the frontal region of the brain. They have symmetrical distribution, these waves have a low amplitude. These waves are associated with high thinking and anxious behaviour. These waves are pathologically important for the detection of Dup15q syndrome.

Gamma Waves- These waves have frequencies up to 30 Hz max. Gamma waves decrease are associated with a decline in cognitive ability, but it still needs evidence research to prove the hypothesis to be used as a diagnostic measurement.

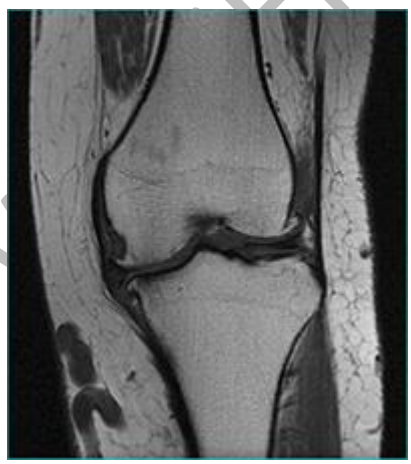
Mu Waves- This has a frequency range of 8 to 12 Hz. These are predominantly found in the sensorimotor cortex. They are associated with the diagnosis of autism.

MRI



Magnetic Resonance Imaging (MRI) is a non-invasive imaging technology that produces three dimensional detailed anatomical images. It is often used for disease detection, diagnosis, and treatment monitoring. It is based on sophisticated technology that excites and detects the change in the direction of the rotational axis of protons found in the water that makes up living tissues.

How does MRI work?



MRI of a knee.

MRIs employ powerful magnets which produce a strong magnetic field that forces protons in the body to align with that field. When a radiofrequency current is then pulsed through the patient, the protons are stimulated, and spin out of equilibrium, straining against the pull of the magnetic field. When the radiofrequency field is turned off, the MRI sensors are able to detect the energy released as the protons realign with the magnetic field. The time it takes for the protons to realign with the magnetic field, as well as the amount of energy released, changes depending on the environment and the chemical nature of the molecules. Physicians are able to tell the difference between various types of tissues based on these magnetic properties.

To obtain an MRI image, a patient is placed inside a large magnet and must remain very still during the imaging process in order not to blur the image. Contrast agents (often containing the element Gadolinium) may be given to a patient intravenously before or during the MRI to increase the speed at which protons realign with the magnetic field. The faster the protons realign, the brighter the image.

What is MRI used for?

MRI scanners are particularly well suited to image the non-bony parts or soft tissues of the body. They differ from computed tomography (CT), in that they do not use the damaging ionizing radiation of x-rays. The brain, spinal cord and nerves, as well as muscles, ligaments, and tendons are seen much more clearly with MRI than with regular x-rays and CT; for this reason MRI is often used to image knee and shoulder injuries.

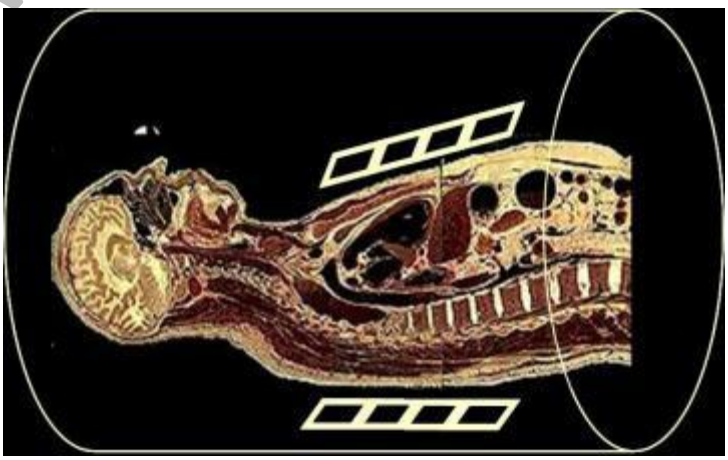
In the brain, MRI can differentiate between white matter and grey matter and can also be used to diagnose aneurysms and tumors. Because MRI does not use x-rays or other radiation, it is the imaging modality of choice when frequent imaging is required for diagnosis or therapy, especially in the brain. However, MRI is more expensive than x-ray imaging or CT scanning.

One kind of specialized MRI is functional Magnetic Resonance Imaging (fMRI.) This is used to observe brain structures and determine which areas of the brain “activate” (consume more oxygen) during various cognitive tasks. It is used to advance the understanding of brain organization and offers a potential new standard for assessing neurological status and neurosurgical risk.

Are there risks?

Although MRI does not emit the ionizing radiation that is found in x-ray and CT imaging, it does employ a strong magnetic field. The magnetic field extends beyond the machine and exerts very powerful forces on objects of iron, some steels, and other magnetizable objects; it is strong enough to fling a wheelchair across the room. Patients should notify their physicians of any form of medical or implant prior to an MR scan.

When having an MRI scan, the following should be taken into consideration:



- **People with implants, particularly those containing iron,** — pacemakers, vagus nerve stimulators, implantable cardioverter- defibrillators, loop recorders, insulin pumps, cochlear implants, deep brain stimulators, and capsules from capsule endoscopy should not enter an MRI machine.

- **Noise**—loud noise commonly referred to as clicking and beeping, as well as sound intensity up to 120 decibels in certain MR scanners, may require special ear protection.

- **Nerve Stimulation**—a twitching sensation sometimes results from the rapidly switched fields in the MRI.

- **Contrast agents**—patients with severe renal failure who require dialysis may risk a rare but serious illness called nephrogenic systemic fibrosis that may be linked to the use of certain gadolinium-containing agents, such as gadodiamide and others. Although a causal link has not been established, current guidelines in the United States recommend that dialysis patients should only receive gadolinium agents when essential, and that dialysis should be performed as soon as possible after the scan to remove the agent from the body promptly.

- **Pregnancy**—while no effects have been demonstrated on the fetus, it is recommended that MRI scans be avoided as a precaution especially in the first trimester of pregnancy when the fetus' organs are being formed and contrast agents, if used, could enter the fetal bloodstream.

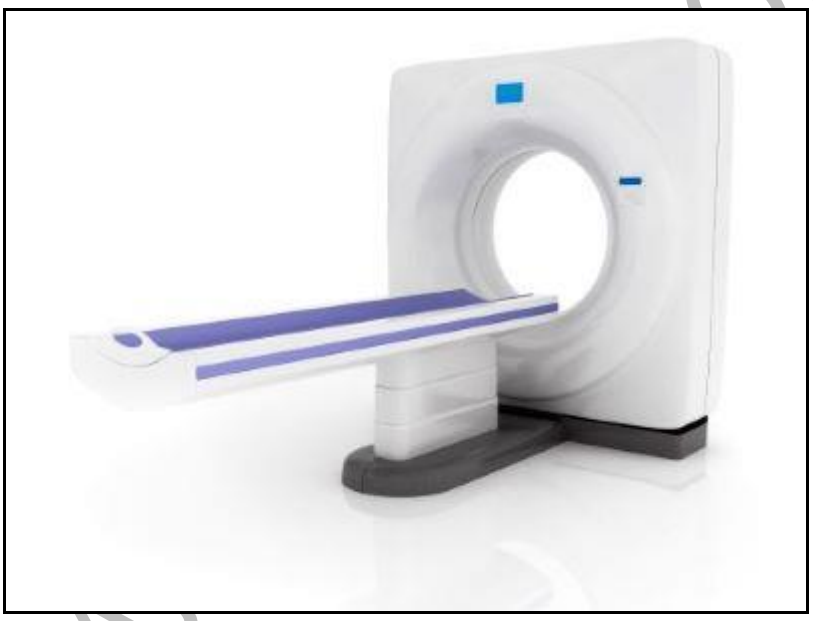
New open MRI machine

- **Claustrophobia**—people with even mild claustrophobia may find it difficult to tolerate long scan times inside the machine. Familiarization with the machine and process, as well as visualization techniques, sedation, and anesthesia provide patients with mechanisms to overcome their discomfort. Additional coping mechanisms include listening to music or watching a video or movie, closing or covering the eyes, and holding a panic button. The open MRI is a machine that is open on the sides rather than a tube closed at one end, so it does not fully surround the patient. It was developed to accommodate the needs of patients who are uncomfortable with the narrow tunnel and noises of the traditional MRI and for patients whose size or weight make the traditional MRI impractical. Newer open MRI technology provides high quality images for many but not all types of examinations

CT

The term "computed tomography," or CT, refers to a computerized x-ray imaging procedure in which a narrow beam of x-rays is aimed at a patient and quickly rotated around the body, producing signals that are processed by the machine's computer to generate cross-sectional images, or "slices." These slices are called tomographic images and can give a clinician more detailed information than conventional x-rays. Once a number of successive slices are collected by the machine's computer, they can be digitally "stacked" together to form a three-dimensional (3D) image of the patient that allows for easier identification of basic structures as well as possible tumors or abnormalities.

How does CT work?



Unlike a conventional x-ray—which uses a fixed x-ray tube—a CT scanner uses a motorized x-ray source that rotates around the circular opening of a donut-shaped structure called a gantry. During a CT scan, the patient lies on a bed that slowly moves through the gantry while the x-ray tube rotates around the patient, shooting narrow beams of x-rays through the body. Instead of film, CT scanners use special digital x-ray detectors, which are located directly opposite the x-ray source. As the x-rays leave the patient, they are picked up by the detectors and transmitted to a computer.

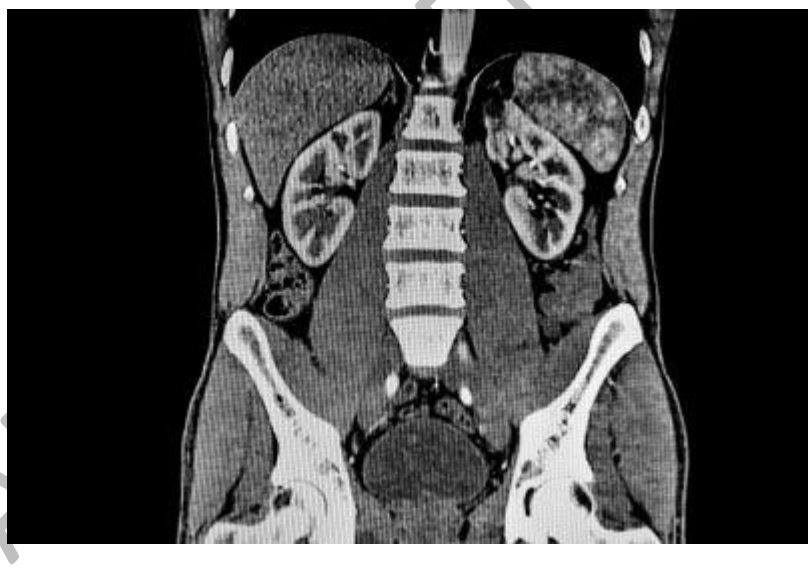
Each time the x-ray source completes one full rotation, the CT computer uses sophisticated mathematical techniques to construct a two-dimensional image slice of the patient. The thickness of the tissue represented in each image slice can vary depending on the CT machine used, but usually ranges from 1-10 millimeters. When a full slice is completed, the image is stored and the motorized bed is moved forward incrementally into the gantry. The x-ray scanning process is then

repeated to produce another image slice. This process continues until the desired number of slices is collected.

Image slices can either be displayed individually or stacked together by the computer to generate a 3D image of the patient that shows the skeleton, organs, and tissues as well as any abnormalities the physician is trying to identify. This method has many advantages including the ability to rotate the 3D image in space or to view slices in succession, making it easier to find the exact place where a problem may be located.

CT scans can be used to identify disease or injury within various regions of the body. For example, CT has become a useful screening tool for detecting possible tumors or lesions within the abdomen. A CT scan of the heart may be ordered when various types of heart disease or abnormalities are suspected. CT can also be used to image the head in order to locate injuries, tumors, clots leading to stroke, hemorrhage, and other conditions. It can image the lungs in order to reveal the presence of tumors, pulmonary embolisms (blood clots), excess fluid, and other conditions such as emphysema or pneumonia. A CT scan is particularly useful when imaging complex bone fractures, severely eroded joints, or bone tumors since it usually produces more detail than would be possible with a conventional x-ray.

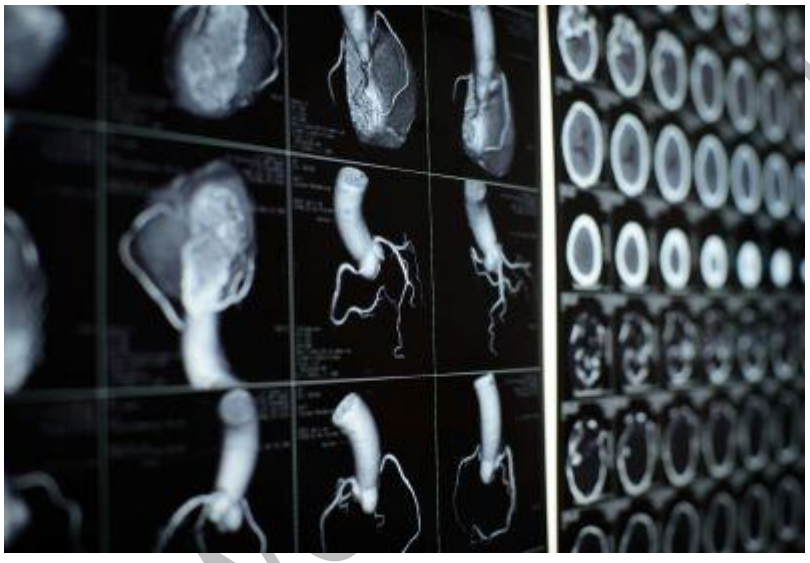
What is a CT contrast agent?



As with all x-rays, dense structures within the body—such as bone—are easily imaged, whereas soft tissues vary in their ability to stop x-rays and therefore may be faint or difficult to see. For this reason, contrast agents have been developed that are highly visible in an x-ray or CT scan and are safe to use in patients. Contrast agents contain substances that can stop x-rays and are therefore more visible on an x-ray image. For example, to examine the circulatory system, an intravenous (IV) contrast agent based on iodine is injected into the bloodstream to help illuminate blood vessels. This type of test is used to look for possible obstructions in blood vessels, including those in the heart. Oral contrast agents, such as barium-based compounds, are used for imaging the digestive system, including the esophagus, stomach, and gastrointestinal (GI) tract.

Are there risks?

CT scans can diagnose possibly life-threatening conditions such as hemorrhage, blood clots, or cancer. An early diagnosis of these conditions could potentially be lifesaving. However, CT scans use x-rays, and all x-rays produce ionizing radiation. Ionizing radiation has the potential to cause biological effects in living tissue. This is a risk that increases with the number of exposures added up over the life of an individual. However, the risk of developing cancer from x-ray radiation exposure is generally small.



A CT scan in a pregnant woman poses no known risks to the baby if the area of the body being imaged isn't the abdomen or pelvis. In general, if imaging of the abdomen and pelvis is needed, doctors prefer to use exams that do not use radiation, such as magnetic resonance imaging (MRI) or ultrasound. However, if neither of those can provide the answers needed, or there is an emergency or other time constraint, CT may be an acceptable alternative imaging option.

In some patients, contrast agents may cause allergic reactions, or in rare cases, temporary kidney failure. IV contrast agents should not be administered to patients with abnormal kidney function since they may induce a further reduction of kidney function, which may sometimes become permanent.

Because children are more sensitive to ionizing radiation and have a longer life expectancy, they have a higher relative risk for developing cancer from such radiation compared with adults. Parents may want to ask the technologist or doctor if their machine settings have been adjusted for children.

PET

A positron emission tomography (PET) scan is an imaging test that can help reveal the metabolic or biochemical function of your tissues and organs. The PET scan uses a radioactive drug called a tracer to show both typical and atypical metabolic activity. A PET scan can often detect the atypical metabolism of the tracer in diseases before the disease shows up on other imaging tests, such as computerized tomography (CT) and magnetic resonance imaging (MRI).

The tracer is most often injected into a vein within your hand or arm. The tracer will then collect into areas of your body that have higher levels of metabolic or biochemical activity. This often pinpoints the location of the disease. The PET images are typically combined with CT or MRI and are called PET-CT or PET-MRI scans.

PET scan is an effective way to help discover a variety of conditions, including cancer, heart disease and brain disorders. Your health care provider can use this information to help diagnose, monitor or treat your condition.

Cancer

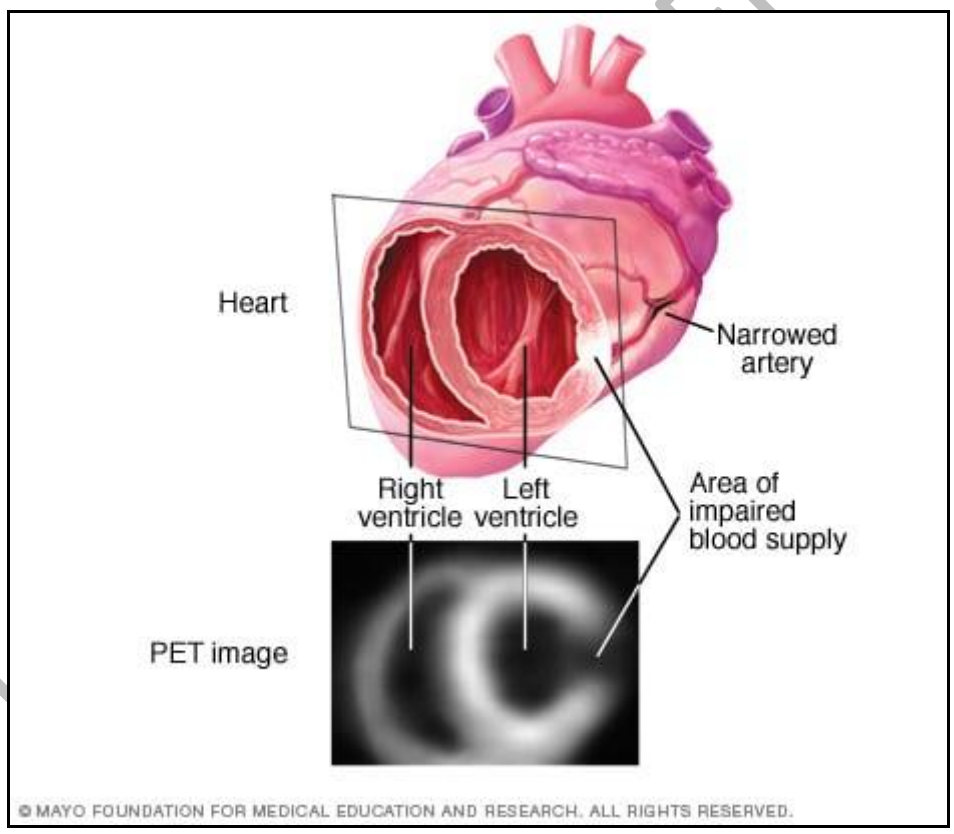
Cancer cells show up as bright spots on PET scans because they have a higher metabolic rate than do typical cells. PET scans may be useful in:

- Detecting cancer.
- Revealing whether your cancer has spread.
- Checking whether a cancer treatment is working.

- Finding a cancer recurrence.

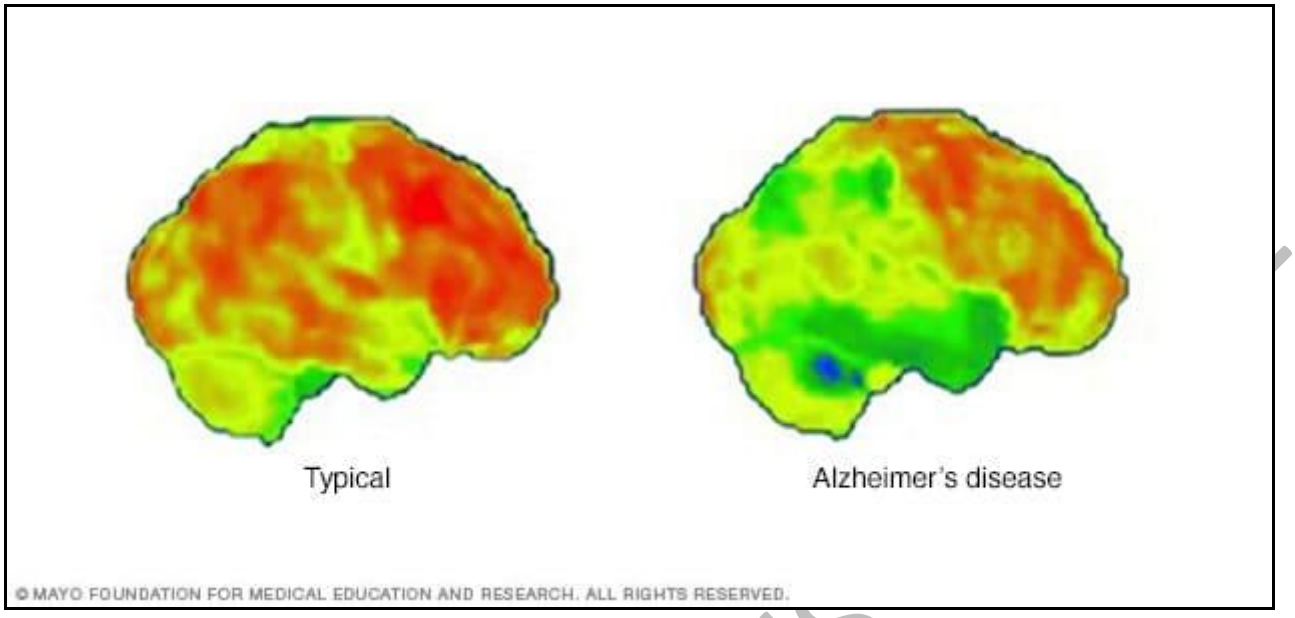
PET scans must be interpreted carefully because noncancerous conditions can look like cancer. Also, some cancers do not appear on PET scans. Many types of solid tumors can be detected by PET-CT and PET-MRI scans, **including:**

- Brain.
- Breast.
- Cervical.
- Colorectal.
- Esophageal.
- Head and neck.
- Lung.
- Lymphatic system.
- Pancreatic.
- Prostate.
- Skin.
- Thyroid.
- Heart disease



PET scans can reveal areas of decreased blood flow in the heart. This information can help you and your health care provider decide, for example, whether you might benefit from coronary artery bypass surgery or a procedure to open clogged heart arteries, called angioplasty.

Brain disorders



PET scans of the brain for Alzheimer's disease

PET scans can be used to check certain brain disorders, such as tumors, Alzheimer's disease and seizures. similar to CT or MRI scanners.

From start to finish, the procedure takes about two hours to complete and typically does not require an overnight hospital stay. When you arrive for your scan, you may be asked to:

- Change into a hospital gown.
- Empty your bladder.

A member of your health care team injects the tracer into a vein in your arm or hand. You may briefly feel a cold sensation moving up your arm. You rest and remain silent in a reclining chair for 30 to 60 minutes while the tracer is absorbed by your body.

During the procedure

When you are ready, you lie on a narrow, padded table that slides into the part of the scanner that looks like a doughnut hole. During the scan you must be very still so that the images aren't blurred. It takes about 30 minutes to complete a PET-CT scan and 45 minutes for a PET-MRI scan. The machine makes buzzing and clicking sounds.

The test is painless. If you're afraid of enclosed spaces, you may feel some anxiety while in the scanner. Be sure to tell the nurse or technologist about any anxiety causing you discomfort. You may be given medicine to help you relax.

After the procedure

After the test you can carry on with your day as usual, unless your provider tells you otherwise. You'll need to drink plenty of fluids to help flush the tracer from your body.

Results

A specialist trained to interpret scan images, called a radiologist, will report the findings to your provider.

The radiologist may compare your PET images with images from other tests you've undergone recently, such as an MRI or CT. Or the PET images may be combined to provide more detail about your condition.

UNIT – V

Spectrofluorometers

- Spectrofluorometers (or fluorescence spectrophotometers) measure the fluorescence signature of an analyte in a sample based on its specific excitation and emission wavelengths.
- The fluorescence signature can be correlated to the concentration level of the analyte in the sample.
- A spectrofluorometer can be used in basic and applied research:
  - biofuels analysis,
  - biotechnology applications,
  - quality control,
  - medical diagnostics,
  - plasma monitoring
  - polymer analysis
  - as a tool in teaching laboratories

Principles

- Fluorescence is an emission phenomenon where an energy transition from a higher to a lower state is accompanied by radiation.
- Only molecules in their excited forms are able to emit fluorescence; thus, they have to be brought into a state of higher energy prior to the emission phenomenon.

Principle

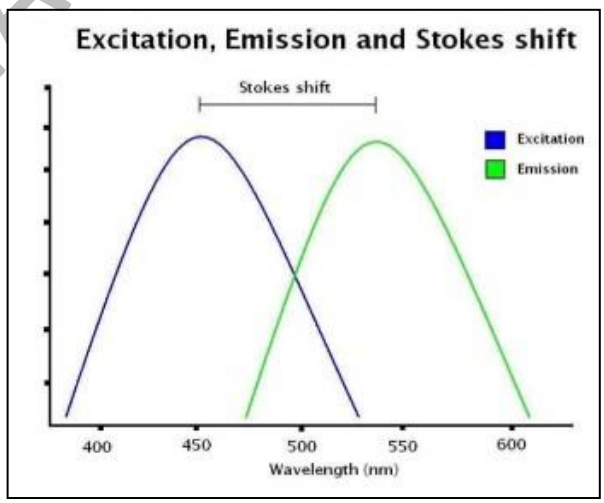
- A molecule in its electronic and vibrational ground state (S0v0) can absorb photons matching the energy difference of its various discrete states.
- The required photon energy has to be higher than that required to reach the vibrational ground state of the first electronic excited state (S1v0).
- The excess energy is absorbed as vibrational energy (v>0), and quickly dissipated as heat by collision with solvent molecules.
- The molecule thus returns to the vibrational ground state (S1v0).
- These relaxation processes are non-radiating transitions from one energetic state to another with lower energy, and are called internal conversion (IC).
- From the lowest level of the first electronic excited state, the molecule returns to the ground state (S0) either by emitting light (fluorescence) or by a non-radiative transition.
- Upon radiative transition, the molecule can end up in any of the vibrational states of the electronic ground state (as per quantum mechanical rules).
- Since radiative energy is lost in fluorescence as compared to the absorption, the fluorescent light is always at a longer wavelength than the exciting light (Stokes shift).
- The emitted radiation appears as band spectrum, because there are many closely related wavelength values dependent on the vibrational and rotational energy levels attained.
- An associated phenomenon in this context is phosphorescence which arises from a transition from a triplet state (T1) to the electronic (singlet) ground state (S0).
- The molecule gets into the triplet state from an electronic excited singlet state by a process called intersystem crossing (ISC).
- The transition from singlet to triplet is quantum-mechanically not allowed and thus only happens with low probability in certain molecules where the electronic structure is favourable.
- Such molecules usually contain heavy atoms.
- The rate constants for phosphorescence are much longer and phosphorescence thus happens with a long delay and persists even when the exciting energy is no longer applied.

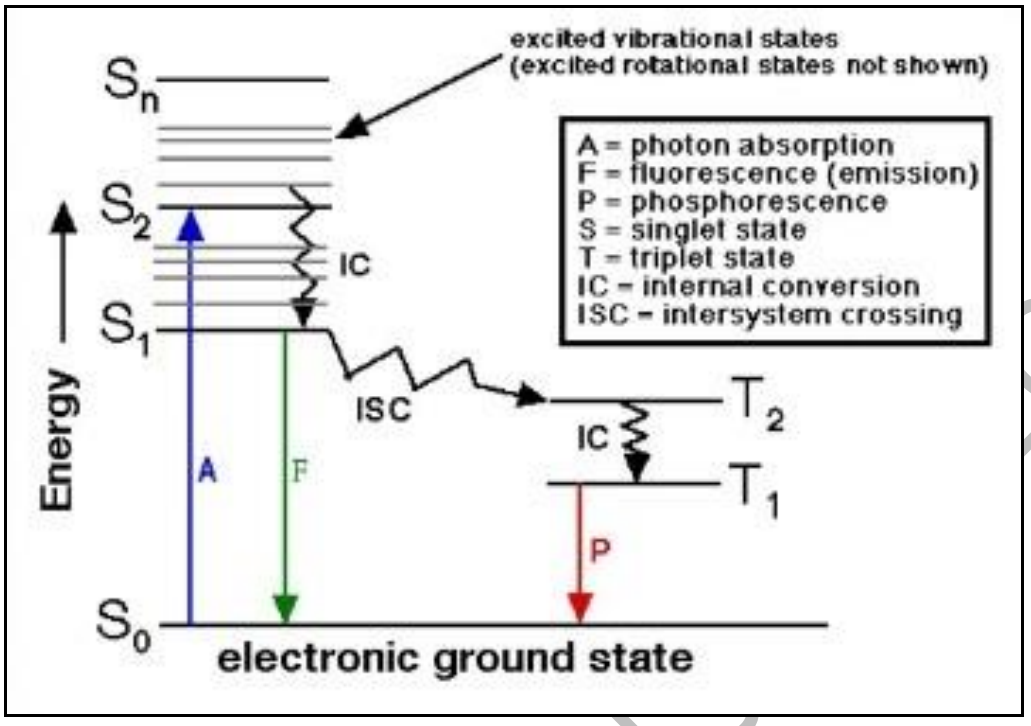
Excitation and Emission of Fluorophores

- The physics behind fluorescence involves the different electronic and vibrational states that fluorophores can exist in.
- An electronic state is divided into multiple vibrational states.
- Photons, that have energies in the ultraviolet to blue-green range of the spectrum can trigger an electronic transition from a lowest vibration in the ground state to one of the vibrational levels in a higher electronic excited state.
- As soon as the energy input from the photon (in other words the excitation) stops, the fluorophore molecule relaxes into the lowest vibrational level of the excited electronic state.
- The fluorophore remains in this state for some time (around 10 nanoseconds, known as the fluorescence lifetime) and then returns to the electronic ground state.
- This return to the ground state is associated with a release of energy, known as fluorescence emission.

Quantum yield

- The number of photons emitted by a fluorophore, relative to the number of photons absorbed, is called the quantum yield. A fluorophore with a large quantum yield, like rhodamine, will display a bright emission.
- The difference between excitation and emission wavelengths is called Stokes shift.
- Stokes' studies of fluorescent substances led to the formulation of Stokes' Law, which states that the wavelength of fluorescent light is always greater than that of the exciting radiation.
- The process that happens between excitation and emission is illustrated using Jablonski diagrams (named after the father of fluorescence spectroscopy, Alexander Jablonski).





Triplet excited state: An excited state in which unpaired electron spins occur.(when the analyte returns from a higher- energy state to a lower- energy state with the opposite spin)

Singlet excited state: An excited state in which all electron spins are paired. (When the analyte returns from a higher- energy state to a lower- energy state with the same spin)

A molecule's fluorescence quantum yield is influenced by external Variables such as:

- Temperature
- viscosity of solvent
- pH
- Increasing temperature generally decreases Φf because more frequent collisions between the molecule and the solvent increases external conversion.
- Decreasing the solvent's viscosity decreases Φf for similar reasons.
- For an analyte with acidic or basic functional groups, a change in pH may change the analyte's structure and, therefore, its fluorescent properties.
- Intrinsic fluorophores are molecules with a natural fluorescence, such as chlorophyll and the aromatic amino acids.
- Extrinsic fluorophores are those that are added to a sample to provide fluorescence, or to change the spectral properties of the sample.
- Examples of extrinsic fluorophores are fluorescein and rhodamine, but there are many more.

- Each fluorophore has its own characteristic properties, such as fluorescence lifetime, intensity, and position of the emission wavelength, thus, each fluorophore will yield a unique fluorescence spectrum.
- A fluorescence spectrum is a plot of the fluorescence intensity as a function of wavelength.
- Fluorescent intensity F is dependent on both intrinsic properties of the compound (fluorescence quantum yield Φf), and on readily controlled experimental parameters including:
  - intensity of the absorbed light I0
  - molar absorption coefficient Ɛ
  - path length of the cell b
  - concentration of the fluorophor in solution c

$$F = \phi I_0(1-e^{-\varepsilon bc})$$

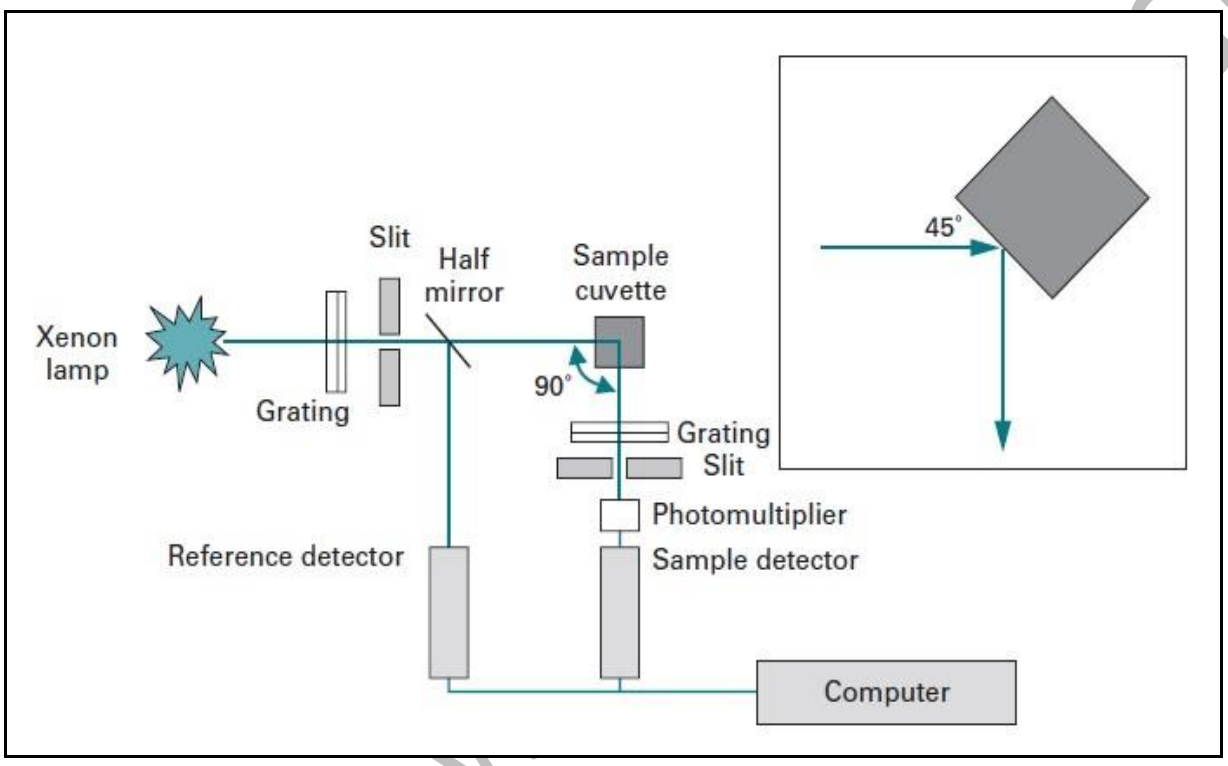
How a spectrofluorometer works?

- The essential components of a spectrofluorometer are a light source, an excitation monochromator, a sample cell/cuvette, an emission monochromator and a detector.
- Fluorometers quantify biological analytes as a function of fluorescence.
- This requires the sample to be bound to a specific fluorescent agent and loaded into the instrument in a cell/cuvette holder.
- The light source sends out light at the excitation wavelength of an analyte in a sample.
- Before it reaches the sample, the light passes through the excitation monochromator, which transmits a wavelength specific to the excitation spectrum of the analyte while blocking other wavelengths.
- The light from the excitation monochromator passes through the sample contained in the sample cell/cuvette holder and excites the analyte (fluorophore).
- Fluorophores absorb light of a distinct excitation wavelength and emit, or fluoresce, light of reduced energy thus a longer wavelength.

How a spectrofluorometer works?

- The emitted light passes through the emission monochromator positioned at a right angle to the excitation light.

- The emission monochromator minimizes light scatter and screens the emission light before it reaches the detector.
- The detector measures the emitted light, displays the fluorescence value and produces the fluorescence signature of the analyte.
- The fluorescence value is proportional to the concentration level of the analyte in the sample.



What can Spectrofluorometer Do?

- It has been used for the direct or indirect quantitative and qualitative analysis by measuring the fluorescent intensity F.
- It is relatively inexpensive and sensitive (the sensitivity of fluorescence is approximately 1,000 times greater than absorption spectrophotometric methods).

Applications of fluorescence spectroscopy

Bioscience

- In the biosciences, one of the most frequent applications of fluorescence spectroscopy is the high precision quantification of DNA and RNA using ethidium bromide as an extrinsic fluorophore.

- Another modern application is SMRT (single molecule real-time) DNA sequencing. In its ability to produce long read single molecules with high accuracy, it is predicted to be central to the next genetic diagnostic revolution.
- Proteins possess three intrinsic fluorophores: tryptophan, tyrosine and phenylalanine, although the latter has a very low quantum yield and its contribution to protein fluorescence emission is thus negligible. The main application for intrinsic protein fluorescence aims at conformational monitoring.

Industrial

- Fluorescence spectroscopy is used in several industrial settings as a fast, noninvasive technique in the assessment of contamination.
- For example, it has been used to detect contaminating organic compounds in groundwater, after hydraulic fracturing for gas exploration.

Applications of fluorescence spectroscopy

Chemical

- An important chemical application of fluorescence spectroscopy can be found in the field of nanoparticle synthesis for potential medical uses, such as drug delivery.

Environmental

- In environmental monitoring, the technique also has wide application.
- High-resolution fluorescence spectroscopy and 3D-excitation emission matrix fluorescence spectroscopy are used to characterize dissolved organic matter in these samples and then based on that, optimize treatment processes for landfill.

Applications of fluorescence spectroscopy

Pharmaceutical

- Spectrofluorometric techniques are also used in the pharmaceutical field to analyze drugs.
- An example is the analysis of co-formulated tablets prescribed as cholesterol medication.

Agricultural

- In agriculture, spectroscopic techniques are also widely applied for instance in the identification of different crop varieties.
- Likewise, total luminescence spectroscopy can be used by tea manufacturers as a quick, affordable and objective alternative to employing trained tea tasters, to discriminate between similar types of tea.

FLAME PHOTOMETER

INTRODUCTION

Flame Photometry is a branch of spectroscopy in which the species are examined in the spectrometer are in the form of atoms.

A Flame Photometer is an instrument used in inorganic chemical analysis to determine the concentration of certain metal ions (mainly are sodium, potassium, calcium, lithium)

Flame photometry is based on measurement of intensity of light emitted when a metal is introduced into the flame.

The wavelength of the colour tells what the metal is [Qualitative]

The color's intensity tells us how much element present [Quantitative]

Flame Photometry is also named as Flame Emission Spectroscopy because of the use of a flame to provide the energy of excitation to atoms introduced into the flame.

Classification of Atomic Spectroscopic Methods:

As mentioned earlier, in atomic spectroscopy, the elements present in a sample are converted to gaseous atoms or elementary ions in a process called atomisation. This process can be accomplished in a number of ways, using any of the following.

- Flame
- Electrothermal (or furnace) method
- Inductively coupled argon plasma
- Direct current argon plasma
- Electric arc

Atomic Spectroscopic Methods-I:

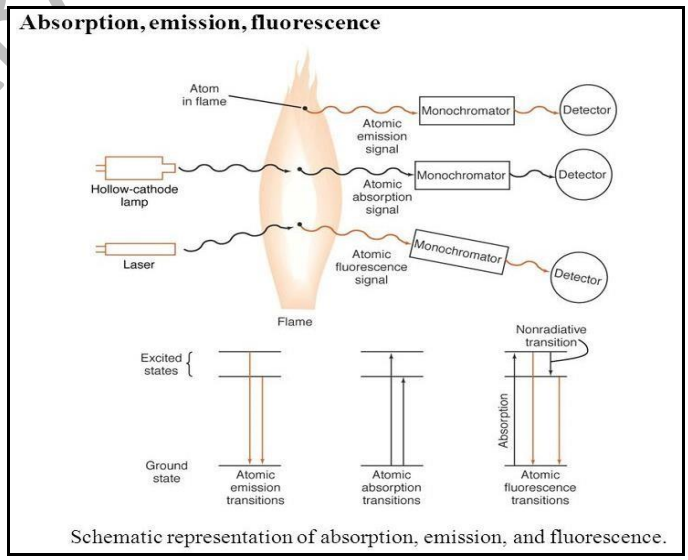
These methods accomplish the atomisation process in different temperature ranges. Once in the vapour phase, the atoms of the elements interact with radiation and provide information about themselves. You would recall from Unit 1 that the interaction of radiation and matter can manifest itself in terms of absorption, emission or fluorescence emission. Accordingly, we have

atomic absorption, emission or fluorescence spectroscopies. Let us learn about the basic principle of these methods.

Atomic Absorption Spectrometry (AAS): In this method, the atomic vapours containing free atoms of an element in the ground state are subjected to a UV-VIS radiation source emitting a characteristic frequency of the element present in atomic vapours. The radiation is absorbed and the intensity of the radiation is attenuated. The absorbed radiation causes excitation of electrons from the ground state to an excited level. The extent of absorption is a quantitative measure of the concentration of the atomic vapours of the element in the ground state. It is an electronic excitation and the energy of transitions lies in the to UV-VIS region of the electromagnetic spectrum.

Atomic Emission Spectrometry (AES): In this method, a sample is normally excited by the thermal energy of a flame, argon plasma or an electrical discharge. The atoms in the sample absorb thermal energy, causing the excitation of the outer orbital electrons. As the excited state is short lived, the excited atoms return back to the ground state after a very short lifetime (typically 10–6 to 10–9 s). This is accompanied by the emission of electromagnetic radiation, normally in the form of light in the UV - VIS region. The wavelength of the emitted radiation and its intensity provide the qualitative and quantitative information about the analyte. The atomic emission spectroscopy employing flame as a means of excitation is called flame photometry or flame emission spectroscopy (FES). It is used for the routine determination of metals, mainly of alkali and alkaline earth metals, though other metals can also be analysed.

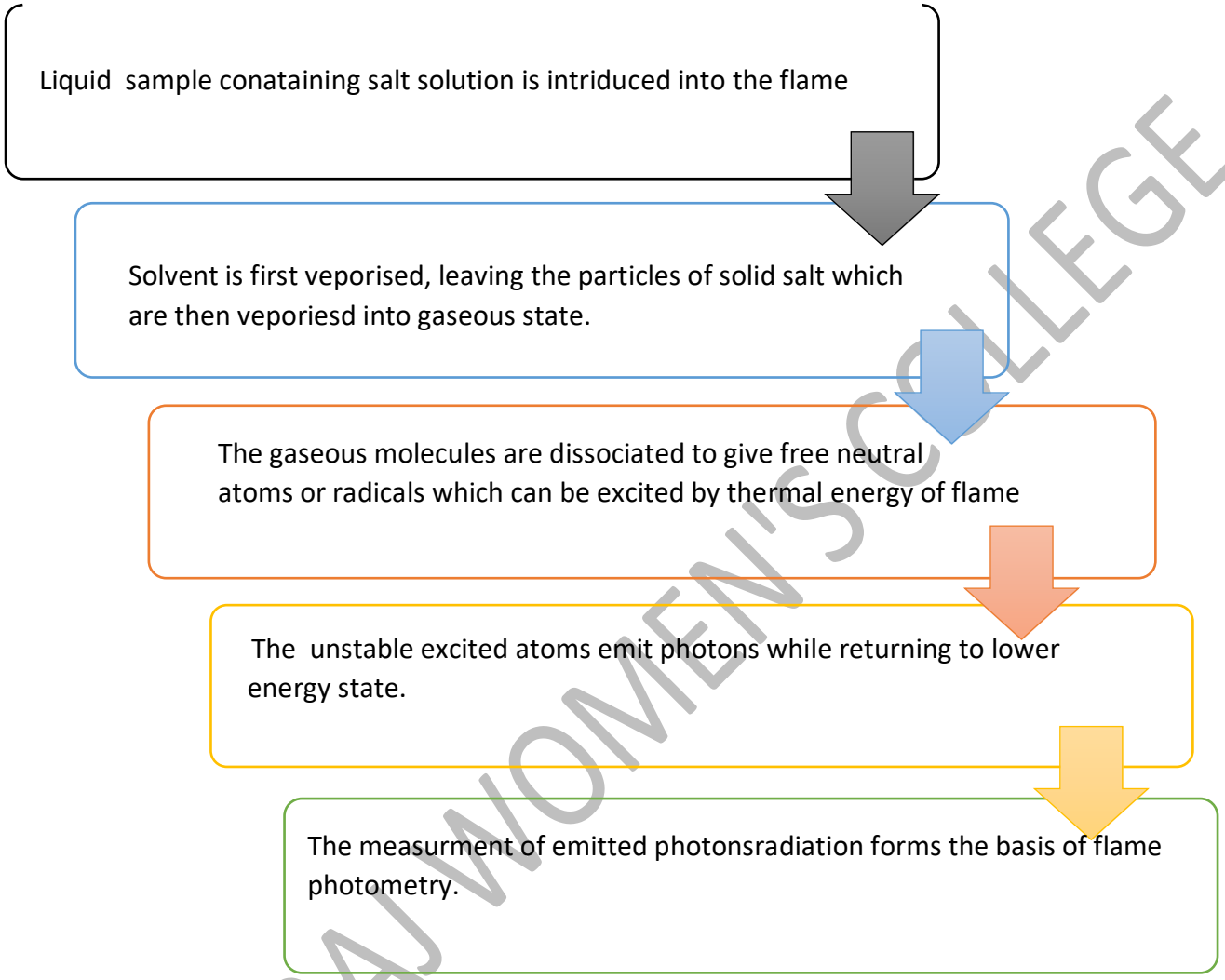
Atomic Fluorescence Spectrometry (AFS): In AFS, the gaseous atoms are excited to higher energy level by absorption of the electromagnetic radiation and the fluorescence emission from these excited atoms is measured. The main advantage of fluorescence detection compared to absorption measurements is the greater sensitivity achievable because the fluorescence signal has a very low background radiation. A schematic representation of the transitions associated with the phenomenon of atomic absorption, atomic emission and atomic fluorescence emission is given in figure:



Schematic representation of absorption, emission, and fluorescence.

GENERAL PRINCIPLES

(simplified version of sequential events:)



:Under constant and controlled conditions, the light intensity of the characteristic wavelength produced by each of the atoms is directly proportional to the number of atoms that are emitting energy, which in turn is directly proportional to concentration of the test sample.

A brief overview of the process:

1. The solvent is first evaporated leaving fine divided solid particles.
2. This solid particles move towards the flame, where the gaseous atoms and ions are produced.
3. The ions absorb the energy from the flame and excited to high energy levels.
4. When the atoms return to the ground state radiation of the characteristic element is emitted.
5. The intensity of emitted light is related to the concentration of the element.

The processes occurring during flame photometer analysis are summarized below:

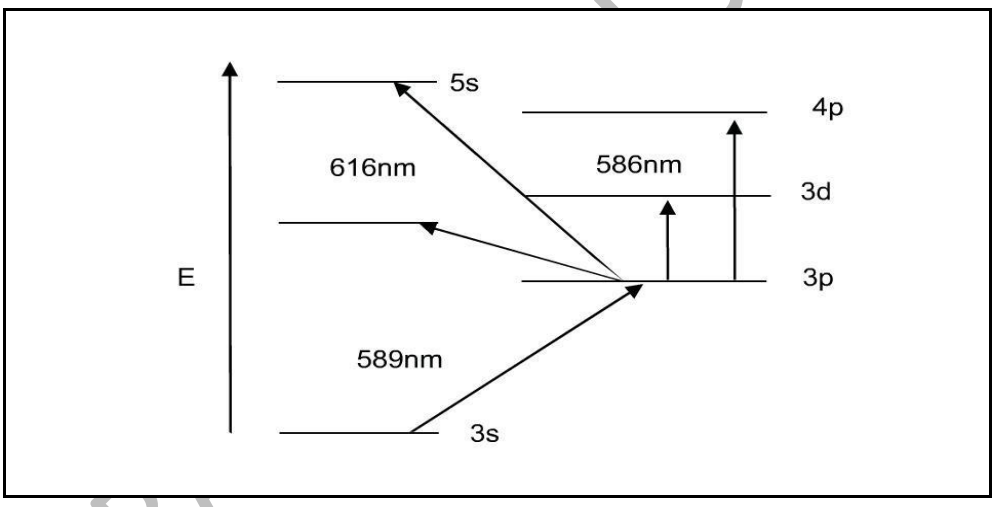
Desolvation: Desolvation involves drying a sample in a solution. The metal particles in the solvent are dehydrated by the flame and thus solvent is evaporated.

Vaporization: The metal particles in the sample are also dehydrated. This also led to the evaporation of the solvent.

Atomization: Atomization is the separation of all atoms in a chemical substance. The metal ions in the sample are reduced to metal atoms by the flame.

Excitation: The electrostatic force of attraction between the electrons and nucleus of the atom helps them to absorb a particular amount of energy. The atoms then jump to the higher energy state when excited.

Emission: Since the higher energy state is unstable the atoms jump back to the ground state or low energy state to gain stability. This jumping of atoms emits radiation with characteristic wavelength. The radiation is measured by the photo detector. The energy level diagram of the sodium atom is shown in figure.



Energy level diagram for atomic sodium

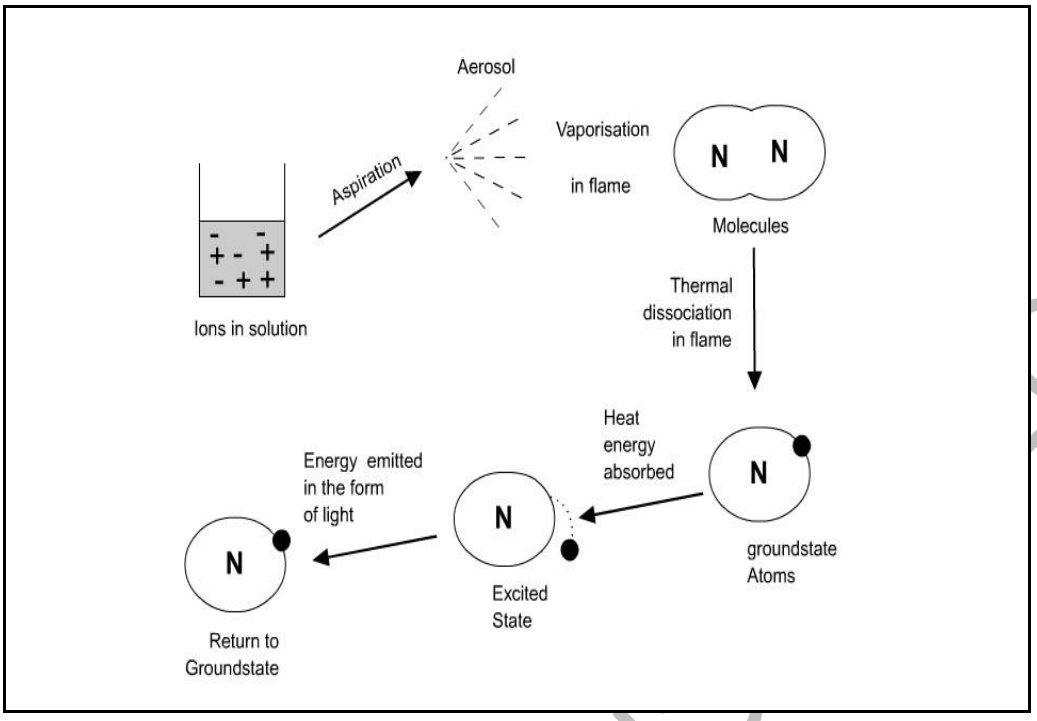
Scheibe-Lomakin equation:

Scheibe-Lomakin equation describes intensity of light emitted with the help of following formula:

I = k × cn Where:

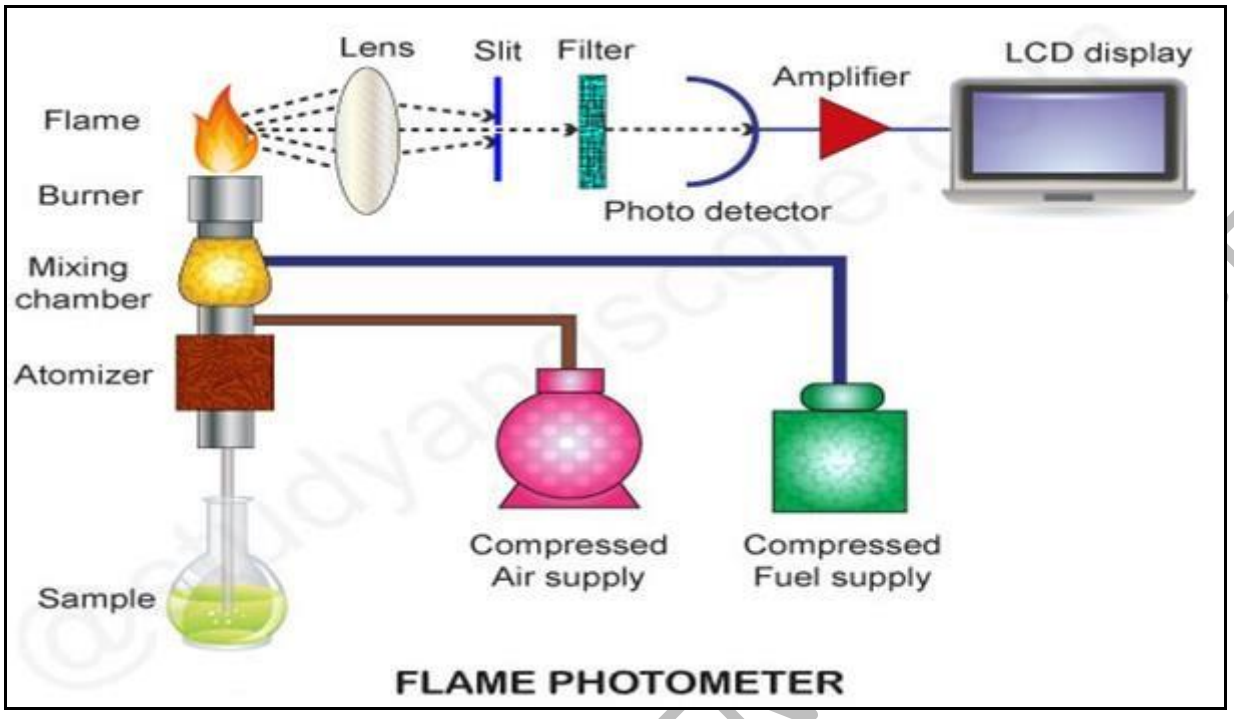
I = Intensity of emitted light c = Concentration of the element k = Proportionality constant

At the linear part of the calibration curve n~1, then I = k × c. In other words, the intensity of emitted light is directly related to the concentration of the sample.



Name of the element	Emitted wavelength range (nm)	Observed colour of the flame
Potassium (K)	766	Violet 
Lithium (Li)	670	Red 
Calcium (Ca)	622	Orange 
Sodium (Na)	589	Yellow 
Barium (Ba)	554	Lime green 

INSTRUMENTATION



The instrument possesses the same basic components as a spectroscopic apparatus has.

However, the major components are:

1. Sample delivery system
2. Source
3. Mirrors
4. Slits
5. Monochromators/Filters
6. Detectors

1. SAMPLE DELIVERY SYSTEM:

There are Three components for introducing the liquid sample.

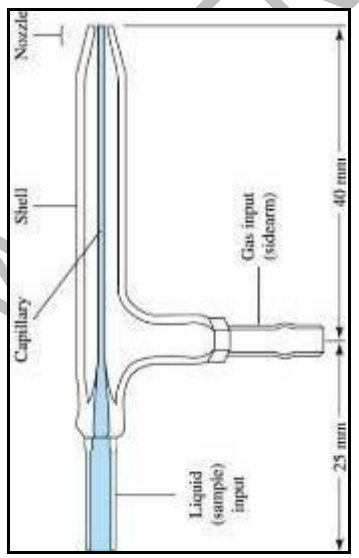
(a) Nebulizer: it breaks up the liquid into small droplets. Nebulization is a conversion of sample to a mist of finely divided droplets using a jet of compressed gas. a flaw carries the sample into atomization region.

Nebuliser is a device used for sample introduction into the flame. The process is called nebulisation and consists of thermal vapourisation and dissociation of aerosol particles at high temperatures producing small particle size with high residence time.

A number of nebulisation methods are available. A few are listed below.

- Pneumatic nebulisation
- Ultrasonic nebulisation
- Electrothermal vapourisation
- Hydride generation (used for certain elements only)

However, we would discuss about the pneumatic nebulisation only. It is the most commonly employed nebulisation method in flame photometers. Pneumatic nebuliser is the most commonly used nebuliser for introducing aqueous/ liquid samples. In this the sample solution is fed or aspirated into the nebuliser which converts liquid into a fine mist, or aerosol which is then fed into the flame. A common type of pneumatic nebuliser is called concentric pneumatic nebuliser, as shown in Fig. 7.9. The concentric pneumatic nebuliser consists of a fine capillary surrounded by concentric tube with a small orifice near one end of the capillary. The capillary is dipped into a solution of the analyte while the outer tube is connected to a high pressure gas supply. The analyte is sucked into the capillary by the high pressure gas stream flowing around the tip of the capillary using the Bernoulli effect. The process is called aspiration. The high velocity gas breaks up the liquid into various sized fine droplets. The other types of the pneumatic nebulisers also work on the same principle.



Concentric type pneumatic nebuliser

(b) Aerosol modifier: it removes large droplets from the stream and allow only smaller droplets.

(c) Flame or Atomizer: it converts the analyte into free atoms.

2. SOURCE/BURNER:

The flame used in in instrument must possess these functions…

-the flame should possess the ability to evaporate the liquid droplets from the sample solution resulting in the formation of solid residues.

-the flame should decompose the compound in the solid residue, resulting in the formation of atoms.

-it must have capability to excite the atoms and cause them to emit radiant energy (emission intensity should be steady over reasonable periods of time 1-2 min.)

: In flame photometry, several burners and fuel-oxidant combinations have been used to produce the analytical flame. Including Mecker, Lundergarph, Total consumption burner, Premix of Laminar air flow burner, Shielded burners, Nitrous Oxide-Acetylene flames.

Sequence of Events in Flame:

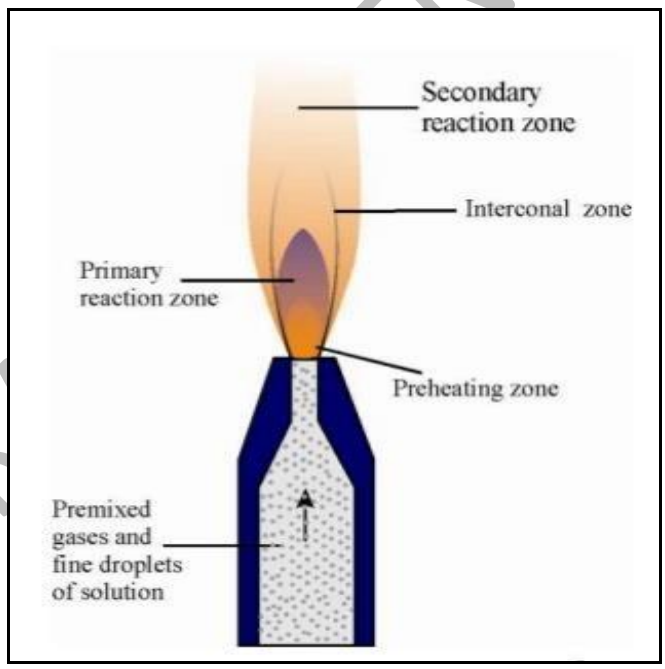
i. The water or solvent is evaporated, leaving minute particles of the dry salt.

ii. The dry salt is vaporized or converted into the gaseous state.

iii. A part or all of the gaseous molecules are dissociated to give free neutral atoms/radicles.

iv. Which are thermally excited or even ionized.

Structure of Flames: Flames are not uniform in composition, length or cross section. The structure of a premixed flame, supported as a laminar flow is shown in Figure:



Schematic structure of a laminar flow flame showing various zones As seen in the figure, the flame may be divided into the following regions or zones.

i) Preheating zones

ii) Primary reaction zone or inner zone

iii) Internal zone

iv) Secondary

v) reaction zone

The first or the innermost region of the flame is the preheating zone where the combustion mixture is heated to the ignition temperature by thermal conduction from the primary reaction zone. The second zone is the primary reaction zone or inner zone. This zone is about 0.1 mm thick at atmospheric pressure and is visible by virtue of its blue green light ascribed to radicals . C2 and CH. There is no thermodynamic equilibrium in this zone and the concentration of ions and free radicals is very high. This region is not used for flame photometry.

Immediately above the primary reaction zone lies the third or interconal zone or the reaction free zone which can extend up to considerable height. The maximum temperature is achieved just above the tip of the inner zone. The higher temperature favours both production of free atoms and maximum excitation for atomic emission spectroscopy. Therefore, this zone is used for flame photometry.

The outermost fourth zone is the secondary reaction zone. Within this zone, the products of the combustion processes are burnt to stable molecular species by the surrounding air.

The shape of an unmixed flame is generally different. The inner zone can still be recognised, but it is very vague and is thickened. A laminar flame makes a strong hissing noise which gets louder when a liquid is atomised into it. We shall now look into the reactions which are taking place when the element is placed in flame.

Reactions in Flames:

The most important reactions occurring in the flame are given below.

i) Dissociation of molecules

$MX \rightarrow M + X$, $K_{mx} = P_m P_x / P_{mx}$

ii) Formation of compounds with flame components

$M + Y \rightarrow MY$, $K_{my} = P_m P_y / P_{my}$

iiI) Formation of compounds with flame components

$M + Y \rightarrow MY$, $K_{my} = P_m P_y / P_{my}$

iv) Ionisation of atoms

$M \rightarrow M^+ + e^-$, $K_P = P_m P / P_m$

In these equations, p is the partial pressure of the species indicated in the subscript. The partial pressure of the flame gas components is much larger than the partial pressure of the given element. It is, therefore, considered constant and included into the equilibrium constant.

The most important of the flame gas components forming compounds with the elements are oxygen, the hydroxyl radical and hydrogen. The most common compounds formed in flames burning with air or oxygen and metal monoxides. For example, a major fraction of alkaline earth elements is present as monoxides unless very fuel rich flames are used. However, alkali metals practically do not form any oxides.

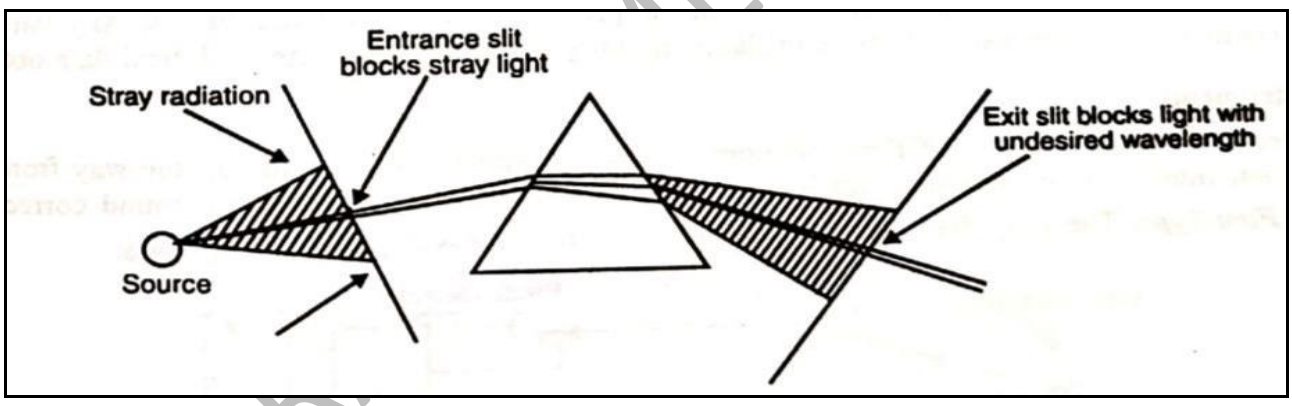
Hydroxide species are present for some alkali and alkaline earth elements in hydrocarbon flames. Sodium forms practically no hydroxide, while the concentration of LiOH molecules often exceeds the atomic lithium concentration by a factor of 10. None of the alkali metal hydroxides emit spectral bands in the visible or UV region; whereas the spectral bands of alkaline earth monohydroxide can be used for the determination of these elements.

3.MIRRORS:

The radiation from the flame is emitted in all direction in space. In order to maximize the amount of radiation used in the analysis, a mirror is located behind the burner to reflect the radiation back to the entrance slit of the monochromator. This mirror is concave and covers as wide angle from the flame as possible.

4.SLITS:

Entrance and exit slits are used before and after the dispersion elements.



The entrance slit cuts out most of the radiation from the surroundings and allows only the radiation from the flame and the mirrored reflection of the flame to enter the optical system. The exit slit is placed after the monochromator and allows only a selected wavelength range to pass through detector.

5.MONOCHROMATORS:

In simple models the monochromator is the prism, but in expensive models, the gratings are used.

Prisms- Quartz is the material most commonly used for making prisms. The reason is that quartz is transparent over the entire region.

Gratings- It employs gratings which is essentially a series of parallel straight lines cut into a plane surface.

(Sometimes optical filter may be used in place of slit and monochromators system. When a filter is kept between the flame and detector, the radiation od desired wavelength from the flame will be entering the detector and be measured. The remaining undesired wavelength will be absorbed by the filter and not measured.)

6.DETECTORS:

The radiation coming from the optical system is allowed to fall on the detector which measure the intensity of radiation falling on it. The detector should be sensitive to radiation of all wavelengths that may be examined. In good flame photometer, the photomultiplier detectors are employed which produce an electrical signal from the radiation falling on them.

INTERFERENCES IN FLAME PHOTOMETRY:

In determining the amount of particular element present, other elements can also affect the result.

1.Spectral Interference:

a) first type of spectral interference arises when two elements may exhibit different spectra but their spectra may partly overlap and both are emitting at some particular wavelength. Ex: Iron line at 3247.28 Å overlaps the copper line at 3247.54Å. This canbe overcome by removing interfering element effect by using calibration curves which are prepared from a solution having similar quantities of that interfering element.

b) The second type occurs if spectral lines of two or more elements are close but their spectra do not overlap. This occurs when filter is used as spectral isolation device, and can be overcome by increasing the resolution of spectral isolation device.

c) The third type occurs between a spectral line and continuous background. Due to high concentration of salts in the sample, especially in salts of alkali and alkaline earth metals. It can be corrected by scanning technique.

Other spectral line Interference example:

a) Na and K mixtures interfere with each other.

Remedy:

1. Extraction of interefering material.

2. Calibration curve of interfering material.

3. Use of gratings instead of prism / filters.

b) Aluminium interferes with emission line of Ca and Mg

c) Orange band of Ca – 543 – 622 nm interference with Na doublet 589 and 589.6nm and Ba line at 553.6nm

4. Ionization Interference:

In some cases, some of the metal atoms may ionize in high temperature flame. e.g., Sodium ion possesses an emission spectrum of its own, with different frequencies from those of the atomic spectrum of sodium. This can be overcome by adding a large quantity of potassium salt to all the solution-unknown and standard. The addition of K prevents theionization of Na.

5. Cation-Cation Interference:

This decreases the signal intensity of the element present in the sample. Ex= Al interference with Ca and Mg. Also Na and K have interference on one another.

6. Cation-Anion Interference:

Anion such as oxalate, phosphate, sulphate, may affect the intensity of radiation emitted by element. For example, Ca in the presence of phosphate ion apparently forms a stable substance, so the calcium signal is depressed because it will not decompose easily.

7. Oxide Formation Interference:

It arises due to the formation of stable oxide with free metal atoms if oxygen is present in the flame. This can be overcome by either using very high temp flames for dissociation or using oxygen free environment.

APPLICATIONS:

1. Qualitative Analysis:

Flame photometry is used to detect elements of groups l and ll of the periodic table. These are Na, K, Li, Mg, Ca etc. Identification is done by Peak matching technique, where at least 3 peaks of emission spectrum should match when sample and standard spectra are recorded.

Flame photometric methods are widely used for the determination of alkali and the alkaline earth metals in samples that are easily prepared as aqueous solutions. Some of these elements can be detected visually by the colour in the flame, e.g. sodium produces yellow flame. However, this method is not very reliable. The best method is to use flame photometer with a filter or monochromator to separate radiation with the wavelengths characteristic of the different metals from other radiations present. If the radiation of the characteristic wavelength is detected, it will indicate the presence of the metal in the sample.

The method to carry out detection of elements by flame photometry is fast, simple and if carried out with care, quite reliable. However, there are some difficulties. It does not provide information about the molecular structure of the compound present in the sample solution. Nonradiating element such as carbon, hydrogen and halides cannot be detected. These can only be determined under special circumstances.

2. Quantitative Analysis:

- Concentration of calcium in serum.
- Concentrationof Na, K, Ca, and K in urine.
- Amount of Ca, Na, K, and Mg in intravenous fluids, ORS.
- Assay of KCl in in syrup.
- Concentration of Lithium in serum for therapeutic drug monitoring.

(The concentration or the amount of elements can be determined by any of the following four methods.)

(a) Direct comparison method

(b) Calibration curve method.

(c) Standard addition method.

(d) Internal standard method.

SCINTILLATION COUNTER

The flash of light that is produced by a transparent material due to the passage of a subatomic particle (electron, ion, alpha particle, or photon) is called scintillation. The scintillation counter is a device that is used to detect radiation by means of a scintillation effect. It is also known as a scintillation detector. Scintillation is a major part of a scintillation detector. A scintillation detector usually consists of the following components

Scintillator: A scintillator is a device that emits light when a high-energy particle hits it. The energy of the emitted pulse of light is directly proportional to the particle that hits the scintillator. This makes it an efficient energy-dispersive radiation detector much used in spectroscopy. The generation of photons occurs in the scintillator as a response to the incident radiation.

Photodetector: A photodetector converts light to an electrical signal in order to process the signal. A photomultiplier tube (PMT), a photodiode or a charged coupled device (CCD) is generally used as a photodetector. Scintillation Counter

How it Works Let us try to understand the principle of the scintillation counter through the following points. When ionizing incident radiation enters the scintillator, it interacts with the material of the scintillator due to which the electrons enter an excited state. Charged particles follow the path of the particle itself. The energy of gamma radiation (uncharged) is converted to a high energy electron either through the photoelectric effect, Compton scattering, or pair-production effect. The excited atoms of the scintillator material gradually undergo de-excitation and emit photons in the visible range of light. This emission is directly proportional to the energy of the incident ionizing particle. The material shines or flows brightly due to fluorescence. Three types of phosphors are used namely:

Inorganic crystals, Plastic phosphors, Organic crystals. The pulse of light emitted by the scintillator hits the photocathode of the photomultiplier and releases at most one photoelectron for each photon. These electrons are accelerated through electrostatic means by applying a voltage potential and are targeted to hit the first dynode, by having enough energy to produce further electrons. These released electrons are called secondary electrons. They strike the second dynode, thereby releasing further electrons. This process occurs in a photomultiplier tube. Each subsequent impact on the dynode releases further electrons, and hence a current amplifying effect occurs on the dynodes. Each subsequent dynode is at a higher potential than the previous one, and so helps in enhancing the acceleration. Likewise, the primary signal is multiplied throughout 10 to 12 stages. At the final dynode, highly sufficient numbers of electrons are present to produce a pulse of high magnitude to develop amplification. This pulse carries information about the energy of the incident ionizing particle. The number of pulses per unit time gives the significance of the intensity of radiation.

Types of Scintillation Counter There are basically two types of scintillators used in nuclear and particle physics. They are plastic or organic scintillators and crystalline or inorganic scintillators. Organic Scintillators Organic scintillators are organic materials that provide photons in the visible part of the spectrum after a charged particle is passed through it. The scintillation mechanism of organic material is different from that of inorganic material. The fluorescence or scintillation in organic materials is produced due to the transition of the energy levels of a single molecule. The fluorescence in organic materials can be observed independently in any of the physical states viz: vapor, liquid, and solid. Inorganic Scintillators Inorganic scintillators are crystals made in high-temperature furnaces. They include lithium iodide (LiI), cesium iodide (CsI), sodium iodide (NaI) and zinc sulfide (ZnS). NaI(TI) (thallium-doped sodium iodide) are highly used inorganic scintillation materials. The iodide present in sodium iodide provides the necessary stopping power (because it has a high Z = 53). The process of scintillation in inorganic materials is normally slower than that of organic materials. The inorganic scintillators have a very high efficiency to detect gamma rays and are also capable of handling high rates of counts.

GEIGER MULLER COUNTER

Difference between Scintillation Counters and Geiger Counters Now, you already know what scintillation counters are but the students must get an understanding of Geiger counters as well to understand major differences between the two. The Geiger counter is used to detect ionizing radiation. Essentially, it is also called GM Counter. GM stands for Geiger-Mueller. The GM Counter is named after Hans Geiger and Walther Mueller, who invented the Geiger-Mueller Tube in 1928. Along with scintillation counters, it is one of the most commonly used instruments to detect and measure radiation. GM tube is a sensing element in a Geiger counter, which is filled with an inert (unreactive) gas like neon, helium or argon at low pressure. This gas is supplied with a high voltage. This gas becomes conductive by ionization from a particle or photon of incident radiation and hence, the Geiger counter can conduct an electric charge very briefly. But with the Townsend discharge or Townsend avalanche, ionization within the tube is significantly amplified, which makes it easy to measure the pulse to be detected.

Then, this pulse is fed into the processing and display electronics. The display method used in the Geiger counter is the number of counts per time unit. Hence, the main difference between Geiger counters and scintillation counters is the difference in the method used to detect and measure ionizing radiation. Scintillation counters use the excitation effect of incident radiation on a scintillating material (materials that exhibit the property of luminescence upon the excitation effect of ionizing radiation) and detect the resulting light pulses with a photodetector to measure ionizing radiation. Whereas, Geiger counters use the ionizing effect on the gas within a GM tube to detect ionizing radiation.

The cost of scintillation counters is relatively higher compared to the Geiger counters which are comparatively quite inexpensive. The size of Geiger counters is smaller than the scintillation counters. But the scintillation counters are considered more sensitive in detecting radiation compared to Geiger counters. This explains the higher accuracy of scintillation counters in comparison to Geiger counters. Scintillation counters have better quantum efficiency to detect and measure ionizing radiation and can also be used to determine the intensity and energy of incident radiation. Compared to Geiger counters, scintillation counters are better because scintillation counters are capable of not only detecting even the tiniest amount of rdiation but also identifying the radioactive isotope in some cases.

AUTORADIOGRAPHY

Autoradiography - Definition, Types and Applications

Autoradiography is a simple and sensitive photochemical technique used to record the spatial distribution of radiolabeled compounds within a specimen or an object. Autoradiography is subdivided into two broad groups, commonly referred to as macro-autoradiography and micro-autoradiography. In the Autoradiography topic, here we will cover the Definition, Types and Applications of Autoradiography.

Localization and detection of molecules with the use of radioactive radiations and producing a photograph on the photographic emulsion is known as autoradiography and the film obtained is called autoradiograph.

There are several radioisotopes that can be used in autoradiography like -

Radioactive isotope	Uses
Calcium - 47	Studying cellular function & bone formation
Carbon - 14	To study the Metabolireaction
Cesium - 137 and Copper - 67	Use in Cancer treatment
Chromium - 51	RBC studies
Cobalt - 57	Pernicious anemia is delecated by it
Cobalt - 60	Sterilization of surgical instruments
Iodine - 131	Treat thyroid disorders
Selenium - 75	Protein studies
Tritium	Drug metabolism
Xenon - 133	Lung ventilation & blood flow

These radioisotopes can emit the following three types of radiations-

(1**) Alpha rays** which consist of 2 neutrons and 2 protons and in fact charged helium atoms. Polonium-210 is an alpha rays emitter.

(2) **Beta rays** are the electrons emitted by nuclei. These are emitted from C14, P32

(3) **Gamma rays** are electromagnetic rays that resemble X-rays. CO60 is a gamma emitter and it has the highest penetration power.

When this β-particle or γ-rays passed through photographic emulsion the silver ions of the emulsion are converted to silver atoms.

Types of autoradiography

Direct autoradiography-

In this sample is placed with intimate contact with film and produces black spots on the photographic film. Although this technique is not used for high-energy particles like β-rays and γ-rays because they pass through the film so they are detected by indirect method.

Indirect autoradiography-

In this technique emitted energy is converted to light by use of a scintillator or by fluorography. Scintillators are the molecules that have the capability to luminescence by any ionizing radiation. So every sample used in this process is coated with a scintillator. The radioactive emissions transfer their energy to the scintillator molecules, which generates photon and they are exposed to the photographic emulsion.

Two general methods by which autoradiography is done are in-vivo autoradiography. At first, molecules are radiolabeled inside the living tissues, and tissue is removed, processed, and visualized. In-vitro autoradiography tissues are isolated from specimen and slide-mounted and then incubated with radioligand and visualized. Any emissions passing through the photographic emulsion are absorbed by the screen and converted to light, effectively superimposing a photographic image upon the direct autoradiographic films.

A single hit by an alpha particle or gamma rays can produce hundreds of silver atoms, but a single hit by a photon of light produces only a single silver atom. Measurement of radioactivity is done by the Geiger Muller counter and the Scintillation counter.

Application of autoradiography -

Localization of a radioactive substance, which is either bound to a receptor or enzyme or hybridized with the nucleic acid.

PET and SPECT for three-dimensional localization of the radiation source.

Quantitative analysis of macro autographs.

Analyze the length and number of DNA.