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FOOD ANALYSIS , [redacted]

Chapter 1

Introduction to food anaiysis techniques

Food analysis is the discipline dealing with the development, application and study of analytical procedures for characterizing the properties of foods and their constituents. These analytical procedures are used to provide information about a wide variety of different characteristics of foods, including their composition, structure, physicochemical properties and sensory attributes. This information is critical to our rational understanding of the factors that determine the properties of foods, as well as to our ability to economically produce foods that are consistently safe, nutritious and desirable and for consumers to make informed choices about their diet. This helps to review the basic principles of the analytical procedures commonly used to analyze foods and to discuss their application to specific food components, *e.g.* lipids, proteins, water, carbohydrates and minerals.

Reasons for Analyzing Foods

Foods are analyzed by scientists working in all of the major sectors of the food industry including food manufacturers, ingredient suppliers, analytical service laboratories, government laboratories, and University research laboratories. The various purposes that foods are analyzed are briefly discussed in this section.

1. to abide by Government Regulations and Recommendations
2. to maintain the general quality of the food supply,
3. to ensure the food industry provides consumers with foods that are wholesome and safe,
4. to inform consumers about the nutritional composition of foods so that they can make knowledgeable choices about their diet,
5. to enable fair competition amongst food companies, and to eliminate economic fraud.

There are a number of Government Departments Responsible for regulating the composition and quality of foods, -Food and Drug Administration (FDA),
the United States Department of Agriculture (USDA),
the National Marine Fisheries Service (NMFS) and
the Environmental Protection Agency (EPA).

I. GOVERNMENT REGULATIONS AND RECOMMENDATIONS

1.STANDARDS;Government agencies have specified a number of voluntary and mandatory standards concerning the composition, quality, inspection, and labeling of specific food products.
a.Mandatory Standards **b. Voluntary Standards**

a.Mandatory Standards

i.Standards of Identity. These regulations specify the type and amounts of ingredients that certain foods must contain if they are to be called by a particular name on the food label. For some foods there is a maximum or minimum concentration of a certain component that they must contain, *ex.* peanut butter must be less than 55% fat, ice-cream must be greater than 10% milk fat.

ii.Standards of Quality. Standards of quality have been defined for certain foods (*ex.* canned fruits and vegetables) to set minimum requirements on the color, tenderness, massy and freedom from defects.

iii.Standards of Fill-of-Container. These standards state how full a container must be to avoid consumer deception, as well as specifying how the degree of fill is measured.

b. Voluntary Standards:

Standards of Grade. A number of foods, including meat, dairy products and eggs, are *graded* according to their quality, *e.g.* from standard to excellent. For example meats can be graded as prime, choice, select, standard etc according to their origin, Specification of the grade of a food product on the label is voluntary, but many food manufacturers opt to do this because superior grade products can be sold for a higher price. The government has laboratories that food producers send their products too to be tested to receive the appropriate certification. This service is requested and paid for by the food producer.

2. NUTRITIONAL LABELLING

In 1990, the US government passed the Nutritional Labeling and Education Act (NLEA), making nutritional labeling of foods mandatory for almost all food products By this consumers could make informed choices about their diet. Nutritional labels state the total calorific value of the food, as well as total fat, saturated fat, cholesterol, sodium, carbohydrate, dietary fiber, sugars, protein, vitamins, calcium and iron. The information provided on the label can be used by consumers to plan a nutritious and balanced diet, to avoid over consumption of food components linked with health problems, and to encourage greater consumption of foods that are beneficial to health.

3. Authenticity

The price of certain foods is dictated by the quality of the ingredients that they contain. For example, a packet of premium coffee may claim that the coffee beans are from Columbia, There are many instances in the past where manufacturers have made false claims about the authenticity of their products in order to get a higher price. It is therefore important to have analytical techniques that can be used to test the authenticity of certain food components, to ensure that consumers are not the victims of economic fraud and that competition among food manufacturers is fair.

4.Food Inspection and Grading

The government has a ***Food Inspection and Grading Service*** that routinely analyses the properties of food products to ensure that they meet the appropriate laws and regulations. Hence, both government agencies and food manufacturers need analytical techniques to provide the appropriate information about food properties.

II. FOOD SAFETY ;One of the most important reasons for analyzing foods from both the consumers and the manufacturers' standpoint is **to ensure that they are safe.** A food may be considered to be unsafe because it contains harmful microorganisms (*ex.* Listeria, Salmonella), toxic chemicals (*ex.* pesticides, herbicides) or extraneous matter (*ex.* glass, wood, metal, insect matter). This can be achieved by following **good manufacturing practice** regulations specified by the government for specific food products and by having analytical techniques that are capable of detecting harmful substances. Food manufacturers and government laboratories routinely analyze food products to ensure that they do not contain harmful substances and that the food production facility is operating correctly.

III. QUALITY CONTROL

The food industry is highly competitive and food manufacturers are continually trying to increase their market-share and profits. To do this they must ensure that their products are of *higher quality*, *less expensive*, and more *desirable* than their competitors, whilst ensuring that they are *safe* and *nutritious*. To meet these rigorous standards food manufacturers need analytical techniques to analyze food materials before, during and after the manufacturing process to ensure that the final product meets the desired standards. In a food factory one starts with a number of different raw materials, processes them in a certain manner (*e.g.* heat, cool, mix, dry), packages them for consumption and then stores them. The food is then transported to a warehouse or retailer where it is sold for consumption.They should ensure that they are meeting the specified requirements, and if a problem is detected during the production process, appropriate actions can be taken to maintain final product quality.i

a.Characterization of raw materials: Manufacturers measure the properties of incoming raw materials to ensure that they meet certain minimum standards of quality that have previously been defined by the manufacturer. If these standards are not met the manufacturer rejects the material.. For example, the color of potato chips depends on the concentration of reducing sugars in the potatoes that they are manufactured from: the higher the concentration, the browner the potato chip. Thus it is necessary to have an analytical technique to measure the concentration of reducing sugars in the potatoes so that the frying conditions can be altered to produce the optimum colored potato chip.

i.

b.Monitoring of food properties during processing: It is advantageous for food manufacturers to be able to measure the properties of foods during processing. Thus, if any problem develops, then it can be quickly detected, and the process adjusted to compensate for it. This helps to improve the overall quality of a food and to reduce the amount of material and time wasted there is an increasing tendency in the food industry to use analytical techniques which are capable of rapidly measuring the properties of foods on-line, without having to remove a sample from the process. These techniques allow problems to be determined much more quickly and therefore lead to improved product quality and less waste.

c.Characterization of final product: Once the product has been made it is important to analyze its properties to ensure that it meets the appropriate legal and labeling requirements, that it is safe, and that it is of high quality. It is also important to ensure that it retains its desirable properties up to the time when it is consumed.

d.A system known as Hazard Analysis and Critical Control Point (HACCP) has been developed, whose aim is to systematically identify the ingredients or processes that may cause problems (hazard analysis), assign locations (critical control points) within the manufacturing process where the properties of the food must be measured to ensure that safety and quality are maintained, and to specify the appropriate action to take if a problem is identified.

IV RESEARCH AND DEVELOPMENT

In recent years, there have been significant changes in the preferences of consumers for foods that are healthier, higher quality, lower cost and more exotic. To meet these demands food manufacturers often employ a number of scientists whose primary objective is to carry out research that will lead to the development of new products, the improvement of existing products and the reduction of manufacturing costs. Any scientists working in universities, government research laboratories and large food companies carry out *basic research*. overall properties of foods (*ex.* color, texture, flavor, shelf-life *etc.*), to ascertain the role that each ingredient plays in determining the overall properties of foods, and to determine how the properties of foods are affected by various processing conditions (*ex.* storage, heating, mixing, freezing).

2. Properties analyzed

Food analysts are interested in obtaining information about a variety of different characteristics of foods, including their composition, structure, physicochemical properties and sensory attributes.

a. **Composition.**-The composition of a food largely determines its safety, nutrition, physicochemical properties, quality attributes and sensory characteristics.. Government regulations state that the concentration of certain food components must be specified on the nutritional label of most food products, and are usually reported as specific molecules (*ex.* vitamin A) or types of molecules (*ex.* proteins).

b. Structure-

The structural organization of the components within a food also plays a large role in determining the physicochemical properties, quality attributes and sensory characteristics of many foods. The structure of a food can be examined at a number of different levels:

- *Molecular structure*: Ultimately, the overall physicochemical properties of a food depend on the type of molecules present, their three-dimensional structure and their interactions with each other. It is therefore important for food scientists to have analytical techniques to examine the structure and interactions of individual food molecules.
- *Microscopic structure*: The microscopic structure of a food can be observed by microscope and consists of regions in a material where the molecules associate to form discrete phases, *ex.* emulsion droplets, fat crystals, protein aggregates and small air cells.
- *Macroscopic structure:* This is the structure that can be observed by the unaided human eye, *ex.* sugar granules, large air cells, chocolate chips

All of these different levels of structure contribute to the overall properties of foods, such as texture, appearance, stability and taste. In order to design new foods, or to improve the properties of existing foods, it is extremely useful to understand the relationship between the structural properties of foods and their bulk properties. Analytical techniques are therefore needed to characterize these different levels of structure.

c.Physicochemical Properties

The physiochemical properties of foods (rheological, optical, stability, flavor) ultimately determine their perceived quality, sensory attributes and behavior during production, storage and consumption.

- The *optical properties* of foods are determined by the way that they interact with electromagnetic radiation in the visible region of the spectrum, *ex.* absorption, scattering, transmission and reflection of light. For example, full fat milk has a whiter appearance than skim milk because a greater fraction of the light incident upon the surface of full fat milk is scattered due to the presence of the fat droplets.
- The *rheological properties* of foods are determined by the way that the shape of the food changes, or the way that the food flows, in response to some applied force. For example, margarine should be spreadable

when it comes out of a refrigerator, but it must not be so soft that it collapses under its own weight when it is left on a table.

- The *stability* of a food is a measure of its ability to resist changes in its properties over time. These changes may be chemical, physical or biological in origin. *Chemical stability* refers to the change in the type of molecules present in a food with time due to chemical or biochemical reactions, *ex.* fat rancidity or non-enzymatic browning. *Physical stability* refers to the change in the spatial distribution of the molecules present in a food with time due to movement of molecules from one location to another, *ex.* droplet creaming in milk. *Biological stability* refers to the change in the number of microorganisms present in a food with time, *ex.* bacterial or fungal growth.
- The *flavor* of a food is determined by the way that certain molecules in the food interact with receptors in the mouth (taste) and nose (smell) of human beings. Analytically, the flavor of a food is often characterized by measuring the concentration, type and release of flavor molecules within a food or in the headspace above the food.

Foods must therefore be carefully designed so that they have the required physicochemical properties over the range of environmental conditions that they will experience during processing, storage and consumption, *ex.* variations in temperature or mechanical stress. Consequently, analytical techniques are needed to test foods to ensure that they have the appropriate physicochemical properties.

d. Sensory Attributes

Ultimately, the quality and desirability of a food product is determined by its interaction with the sensory organs of human beings, *ex.* vision, taste, smell, feel and hearingt. Foods are often tested on statistically large groups of untrained consumers to determine their reaction to a new or improved product before full-scale marketing or further development. Alternatively, selected individuals may be trained so that they can reliably detect small differences in specific qualities of particular food products, *ex.* the mint flavor of a chewing gum.

Although sensory analysis is often the ultimate test for the acceptance or rejection of a particular food product, there are a number of disadvantages: it is time consuming and expensive to carry out, tests are not objective, it cannot be used on materials that contain poisons or toxins, and it cannot be used to provide information about the safety, composition or nutritional value of a food.

Choosing an Analytical Technique

. The analytical technique selected depends on the property to be measured, the type of food to be analyzed, and the reason for carrying out the analysis. Often it is necessary to consult scientific and technical publications. There are a number of different sources where information about the techniques used to analyze foods can be obtained:

a.Books.

b.Tabulated Official Methods of Analysis.

c Journals:

d..Equipment and Reagent Suppliers

. Internet:.

f. Developing a New Technique:.

One of the most important factors that must be considered when developing a new analytical technique is the way in which the analyte will be distinguished from the matrix. Most foods contain a large number of different components, and therefore it is often necessary to distinguish **the component being analyzed for ("the analyte") from the multitude of other components surrounding it ("the matrix").** Food components can be distinguished from each other according to differences in their molecular characteristics, physical properties and chemical reactions:

- *Molecular characteristics:* Size, shape, polarity, electrical charge, interactions with radiation.
- *Physical properties:* Density, rheology, optical properties, electrical properties, phase transitions (melting point, boiling point).
- *Chemical reactions:* Specific chemical reactions between the component of interest and an added reagent.

Selecting an Appropriate Technique

Some of the criteria that are important in selecting a technique are listed below:

Precision: A measure of the ability to reproduce an answer between determinations performed by the same scientist (or group of scientists) using the same equipment and experimental approach.
Reproducibility: A measure of the ability to reproduce an answer by scientists using the same experimental approach but in different laboratories using different equipment.
Accuracy: A measure of how close one can actually measure the *true value* of the parameter being measured, *ex.* fat content, or Na concentration.
Simplicity of operation: A measure of the ease with which relatively unskilled workers may carry out the analysis.
Cost: The total cost of the analysis, including the reagents, instrumentation and salary of personnel required to carry it out.
Speed: The time needed to complete the analysis of a single sample or the number of samples that can be analyzed in a given time.
Sensitivity: A measure of the lowest concentration of a component that can be detected by a given procedure.
Specificity: A measure of the ability to detect and quantify specific components within a food material, even in the presence of other similar components, *ex.* fructose in the presence of sucrose or glucose.
Safety: Many reagents and procedures used in food analysis are potentially hazardous *e.g.* strong acids or bases, toxic chemicals or flammable materials.
Destructive/Nondestructive: In some analytical methods the sample is destroyed during the analysis, whereas in others it remains intact.
On-line/Off-line: Some analytical methods can be used to measure the properties of a food during processing, whereas others can only be used after the sample has been taken from the production line.
Official Approval: Various international bodies have given official approval to methods that have been comprehensively studied by independent analysts and shown to be acceptable to the various organizations involved, *ex.* AOAC, AOCS.
Nature of Food Matrix: The composition, structure and physical properties of the matrix material surrounding the analyte often influences the type of method that can be used to carry out an analysis, *ex.* whether the matrix is solid or liquid, transparent or opaque, polar or non-polar.

If there are a number of alternative methods available for measuring a certain property of a food, the choice of a particular method will depend on which of the above criteria is most important. For example, *accuracy* and use of an *official method* may be the most important criteria in a government laboratory which checks the validity of compositional or nutritional claims on food products, whereas *speed* and the ability to make *nondestructive* measurements may be more important for routine quality control in a factory where a large number of samples have to be analyzed rapidly.

CHAPTER 2 ;PRINCIPLES AND METHODS OF FOOD ANALYSIS

In the Quality control analysis two major divisions-Qualitative and quantitative analysis are involved .The procedures aimed at qualitative analysis aim at identifying and determining the approximate amounts of the constituents present in a substance whereas, the procedures of quantitative analysis are concerned with determining the exact amount of constituents present. Therefore for arriving at a complete picture of analysis, the sample must include both qualitative and quantitative analysis.

Various methods have been developed for arriving at the proximate composition of food items either in raw or finished shapes. However, basically these methods can be divided in to four major groups:

1. Gravimetric methods
2. Titrimetric methods
3. Photometric methods
4. Electrical; methods

1. GRAVIMETRIC METHODS :

The gravimetric determinations are entirely based upon the concept of weight. In these procedures, the original substance is weighed, and from it the constituent in which we are interested is isolated and also weighed .From the two weights the amount of the desired constituent is calculated.

(a) Precipitation methods (Ex; Determination of Ca as CaO)
(b) Volatilization methods (Ex; Determination of Moisture)
(c) Electro deposition methods

2. TITRIMETRIC METHODS :

The titrimetric determinations are made by measuring the volume of a standard reagent reacting with the desired constituent in a definite chemical reaction. The amount of the constituent may be obtained from the weight of the original sample and the mill equivalents of the standard solution used in the titration. Precisely, the measurement of the volume of a solution of known composition is required to react quantitatively with the unknown constituent .Also known as volumetric methods of analysis.

(a) Acid –base titrations
(b) Precipitation titrations
(c) Oxidation-reduction titrations
(d) Complexometric Titration

3. PHOTOMETRICMETHODS:

The substance to be determined is converted to a compound which imparts a distinctive color to its solution. The intensity of the color of an unknown solution is compared with standard solution containing known amounts of the colored compound. Such a comparison may provide an estimation of percentage composion.The measurement may also involved the wavelength or the intensity of radiant energy. This may be divided into categories based either on the type of interaction of radiant energy with matter:

(a) Absorption
(b) Emission
(c) Diffraction
(d) Refraction

Or alternatively, in terms of the wavelength range of radiant energy which is involved:

(a) Infrared
(b) Visible (Colorimetric analysis)
(c) Ultra-Violet

i) X-Ray

4. ELECTRICAL METHODS:

Among these methods, can list the measurements of certain basic electrical properties such as potential, conductance, quantity and capacitance. A correlation of these physical measurements with concentrations, for known and unknowns, provides a means for quantitative evaluations .Further these methods depend on the measurement of some fundamental electrical quantity such as:

(a) Voltage
(b) Resistance
(c) Current

OTHER METHODS OF ANALYSIS

1. Macro -analysis : Determinations involving 0.1g or more of sample
2. Semi-Macro analysis : Determinations involving 0.01 to 0.1 g of sample
3. Micro-analysis : Determinations involving 0.001 to 0.01 g of sample
4. Ultra-Microanalysis: Determinations involving less than 0.001g
5. Major constituents: 1.0 percent or more of sample.
6. Minor constituents: 0.01to 1.0percent of sample.
7. Trace Constituents: less than 0.01 per cent of the sample.

CHAPTER -3

Terms and definitions

PREPARATION OF SOLUTIONS

A solution is homogenous mixture of two or more non –reducing substances and has uniform properties like chemical compostion, density refractive index etc. This has to be used for extraction of biomolecules from tissues, their separation, quantitative estimation, purification and even for their physico-chemical characterization.

The dissolved substances in solution are called the solute and the medium in which it is dissolved is known as the solvent. The solvent in a solution is always present in less quantity than the solvent generally used solutions in biochemical laboratory are of solid-liquid and liquid-liquid type.

Solute	Solvent	Example
Solid	Liquid	Tris in water, sugar in water, CuSo4 water, NaOH pellets in water.
Liquid	Liquid	2-mercaptoethanol in water, Alcohol in water,HCl in water,H2SO4 in water, Glycerol in water.

Composition of solution can be expressed in two ways i.e., quantity and concentration.

a) Quantity:It is the amount of any substance (solute)present in the solvent (18 g glucose in 100mL).

b) Concentration: It is the quantity of the solute present in specific amount of solvent (18% Glucose).

Concentration of solutions:

The concentration of the solution that is prepared and used in biological experiments can be expressed in several ways. The most commonly used expressions and the solution preparation are given below.

1.Saturated solution
2.Percent solution
3.Molar solution
4.Molal solution
5.Normal solution
6.Standard solution
7.Parts per million

1.Saturated solution; Solvent it is the liquid which solute goes into solution. **Solute** solid chemical to be dissolved in a solution.The crystalline chemical when dissolved in a liquid, the chemical is referred as the solute and the liquid is called as the solvent. A saturated solution is prepared by continuously dissolving the solute in a solvent until a small amount of crystal is visible in the solution.

Eg Ammonium sulfate crystal is added in a small portion to a fixed amount of water with constant stirring. The addition is continued till some crystals are left undissolved. The clear solution obtained is referred as saturated ammonium sulfate solution.

2. Percent solution;Percent solution is prepared by dissolving a gram of substance in 100ml of solvent. Percent solution is expressed as weight by volume (W/V, if a known weight of solute is dissolved in a solvent) or volume by volume (V/V), a known volume of solvent dissolved in another solvent). A percent **solution (w/v) is** prepared as follows, e.g. preparation of 0.9 percent sodium chloride solution. In this case 0.9 g sodium chloride is dissolved in 100ml of its solution.

Method: weigh exactly 0.9g (900mg) of sodium chloride and mix with 80ml of water until it dissolves completely. Then transfer the contents to a 100ml volumetric flask or a measuring cylinder with stopper. The volume is made upto 100ml with distilled water. The cylinder is stoppered, and mixed properly. It is then transferred to a reagent bottle and labeled.

A percent solution **volume / volume** is prepared as follows, e.g. preparation of 2% HCL solution. Please note that stock HCL provided by the chemical company is of 35 to 38 percent.

V1P1= V2P2

? x 35= 100 x 2

$$= \frac{100 \times 2}{35} = \frac{200}{35}$$

= 5.71ml concentrated HCL make upto 100ml with water

Method: Approximately 50ml water is taken in a 100ml volumetric flask. Concentrated HCL is drawn into the pipette with the help of the rubber bulb. Exactly 5.7ml HCL is transferred to the flask containing water with constant mixing. Then water is added upto the mark. The flask is stoppered and the solution is mixed properly. It is then transferred to a reagent bottle and labeled.

Note : Always add acid to water with mixing. This is important because if water is added to acid, water spurts generating great quantity of heat causing the acid to splash out. The concentrated sulfuric acid is diluted under cold (in ice chest containing ice cubes). Addition of acid is done in small amounts with constants stirring.

3.Molar solution ; A molar solution is one in which a gram molecular weight of the substance is dissolved in liter of its solution. If 1 g molecular weight of the substance is dissolved in a liter of solution it is denoted as '1 M' solution.

Molecular weight of a substance is obtained by adding the atomic weight of the elements in the proportion contained in the compound,

e.g. NaCL :

Atomic weight of Na = 23
Atomic weight of CL =35.5
Molecular weight of NaCL =58.5

H2SO4 :

Atomic weight of H =1
Atomic weight of S =32
Atomic weight of O =16
Molecular weight of H2SO4 =
1x2+32x1+16x4 =2+32+64=98

NaOH :

Atomic weight of Na =23
Atomic weight of O =16
Atomic weigh of H =1
Molecular weight of NaOH =40

If the molecule of a compound is hydrated, the weight of water is also considered.

e.g., COOH-COOH. 2H2O, i.e. C2H2O4, 2H2O

Molecular weight = 12x2+1x2+16x4+2(1x2+16)=24+2+64+36=126
Molecular weight of oxalic acid = 126

During the presentation of molar solution the weight to be taken is calculated according to the following method using the formula, MxSxV

Where M=Molecular weight
S= strength of required solution, (e.g. 0.1M, 0.2M, etc.)
V= volume in liters.

To prepare 100ml of 0. 1M sodium chloride solution

Weight = MxSxV
=58.5x1x100/10=5.85gm

Method: Weigh accurately 5.85g of sodium chloride crystals. Transfer the weighed crystals carefully and completely into a 100ml standard flask through a funnel using distilled water. Dissolve the crystals completely. Add water upto 3/4th, mix. Then add water upto the mark. Stopper the flask, and mix well. Transfer the solution to a clean reagent bottle, label it as 1M sodium chloride with date and initials.

Exercise: Prepare 100ml of 0.2M sodium carbonate solution. In all the cases the molecular weight written on the chemical bottle should be considered.

Using the above stock, 0.1M and 0.02M solutions can be prepared as follows:

The 0.2M solution is diluted 1:1 with water or 1 in 2 with water. The 1:1 dilution is expressed as 1 in 2 dilutions, i.e. 50ml of 0.2M solution to 100ml in a 100ml volumetric flask with deionized water.

For 0.02M solution, dilute the stock 1 in10 with distilled water, i.e. 10ml of 0.2M solution is diluted to 100ml in a 100ml volumetric flask.

To find out the molarity of the liquids the following formula can be used

$$\frac{\%\text{ of purity} \times \text{specific gravity} \times 10}{\text{Molecular weight}}$$

For example

% of purity for H_2SO_4	= 98
Specific gravity	=1.84
Molecular weight	=98.08

$$\frac{98 \times 1.84 \times 10}{98.08}$$

= 18.38M

The molarity of the supplied conc. H_2SO_4 = 18.38M

4. **Molal Solution** :A molal solution is one which is prepared by dissolving one gram – molecular weight of a substance in one thousand grams of its solvent. E.g. 400 g sodium hydroxide is dissolved in *1000 g of* water to get 1 molal solution of sodium hydroxide solution. This type of solution is rarely used.

5. Normal Solutions

A gram equivalent weight of a substance dissolved in one liter of its solution is called 1 normal solution. It is denoted as N,

e.g. if the equivalent weight of a substance is 40 then 40 gm is the gram equivalent wt.

When 40g of that is dissolved in 400ml of water and then made up the volume to 1 liter it is 1 N solution.

Like wise 20 g of it in 1L is 0.5N

80g of that substance in 1L is 2N, and

4g of it in 1L 0.1N solution.

To prepare the normal solution equivalent weight is calculated as follows:Equivalent weight of an acid: Acid is one which has ionizable or replaceable hydrogen ions (H).

If an acid has one replaceable H+ it is called a monobasic acid, A monobasic acid forms one type of salt, , e.g. HCL forms only NaCL. So it is monobasic acid. If 2, it is called a dibasic acid- two types of salts on treating with base , H_2SO_4 forms $NaHSO_4$ and Na_2SO_4 and so it is dibasic.

Equivalent wt. of an acid is = Molecular weight

$$\frac{\text{Molecular weight}}{\text{No. of replaceable H+}}$$

1.e.g. equivalent weight of HCL = 36.5

For monobasic acids molecular weight = equivalent weight

Hence 1 M solution = 1N solution

But, eq. wt. of H_2SO_4 = $\frac{\text{molecular weight}}{2}$

i.e., 1M H_3PO_4= $\frac{\text{Molecular weight}}{3}$

i.e., 1M H_3PO_4= $3NH_3PO_4$

To find out the weight to be taken for a known volume of V liters of acid of strength 'N' of equivalent weight 'E'.

Weight of V liters of N Normal

= ExNxV.

The equivalent weight of a base is taken by molecular weight of the particular base divided by the number of replaceable hydroxyl ions. E.g. NaOH has one replaceable OH ion and hence, equivalent weight of NaOH = Mol.wt/1 = 40/1

Calcium hydroxide $Ca(OH)_2$ has 2 replaceable hydroxyl ions and its equivalent weight

= *Molecular weight*

2

ritten =74/2
=37

The, equivalent weight of salts such as AgNO3 and KMNO4 and K2Cr2O7 are determined by the number of electrons which they give or take during a reaction, e.g. AgNO3 gives out 1 electron. Therefore equivalent weight of AgNO3 is molecular weight divided by 1.

Approximate normality of the liquids can be calculated using the following formula:

$$\frac{\text{\% of purity of the liquid x specific gravity x 10}}{\text{Equivalent weight}}$$

e.g., % of purity for HCL = 37
specific gravity = 1.18kg
equivalent weight = 36.4

$$\frac{37 \times 1.18 \times 10}{36.4}$$

$$= 11.9 \text{ N}$$

The above example shows that the normality of the concentrated HCL in 11.9.
To get 1 N HCL, 10ml of concentrated HCL made up to 100ml with deionised water.

OR

12ml concentrated HCL + 108ml water gives 1N.

Equivalent weight of some common chemicals.
Note: Molecular weight of the chemicals will be given on the reagent bottles.
Preparation of normal solution
Exact normal solutions can be prepared only when a chemical is available in its pure state.
Moreover, correct weighing is possible if chemical does not absorb or lose water on exposure. On the other hand, liquids like acids are not pure as supplied commercially and so an exact solution is possible only to prepare only after titration and by dilution.

Sodium carbonate and oxalic acid are available in the pure form and it is easy to prepare exact normal solution by weight. These are called primary standards. Sodium hydroxide when exposed it absorbs water and liquefies. Sodium carbonate can be used as a primary base whereas oxalic is primary acid. For standardization of other normal solutions these acids or bases can be used.

Preparation of primary standard sodium carbonate solution,
E.g. Prepare 100 ml 0.1 N sodium carbonate solution.Molecular weight of sodium carbonate= 106. Eq wt of sodium carbonate $= \frac{106}{2}$

gm/ L= ExNxV/1000

$$= \frac{0.1 \times 53 \times 100}{1000}$$

Therefore 0.53 gm of sodium carbonate in 100 ml gives 0.1 N solution
Method: Transfer 530 mg of sodium carbonate crystals into a clean 100 ml standard flask. Use a clean funnel. Mix properly to dissolve the chemical. Add distilled water upto the mark. Stopper the flask mix again. Transfer to a clean reagent bottle and label as 0.1 N sodium carbonate solution.
Exercise: Preparer 100ml decinormal (0.1N) oxalic acid solution.
Mol wt of oxalic acid, COOH-COOH 2H2O= 126.067
Equivalent weight of oxalic acid = $\frac{126.067}{2}$

$= 63.033$
$=ESV /1000$
$=63.033 \times 0.1 \times 100/1000$
$=0.6303$

Accurately 0.6303g oxalic acid crystals are weighed and prepared 100ml solutions as done in the previous experiment and filled a clean reagent bottle and labeled.
6. Standard solution

Standard solution refer to the known weight of the chemical substance in a solution in which concentration is expressed in terms of normality or moles or in weights per unit volume. The standar solutions are mainly useful in the biochemical assays.

Uses:

1. **Preparation of standard calibration** graph. Glucose standard solution is used in the estimation of glucose in blood, and urine.
2. **A standard** [single or in duplicate] can also be used to estimate the unknown concentration by comparing the absorbance of standard and test solutions which is measured by using colorimeter.

Concentration of unknown substance = $\frac{T - B}{S - B}$ * concentration of Std.

Where S, T and B are absorbance of standard, test and blank solutions respectively.

3. **Preparation of buffers:** Standard buffer components like acid and its conjugate base are prepared as standard solutions and mixed in different proportion to get different pH.
4. **Standard solutions of glucose**, urea, creatinine, albumin, total protein etc. obtained from distributors and are used to calibrate the autoanalyser.

A **primary standard is a** substance of known high purity which may be dissolved in a known volume of solvent to give a primary standard solution. If stoichiometry is used to establish the strength of a titrant, it is called a secondary standard solution. **A primary standard** is a reference chemical used to measure an unknown concentration of another known chemical. It can be used directly when performing titrations or used to calibrate standard solutions. **Primary standards exhibit distinctive characteristics** that ideally suit them for making accurate and reliable assessments of concentration without the need for specialized equipment. These characteristics include high purity, low reactivity with the air or surrounding environment, low hygroscopicity, large molar mass and a predictable reactivity

The term **secondary standard** can also be applied to a substance whose active agent contents have been found by comparison against a primary standard. Concentrations of standard solutions may be expressed in , or in terms more closely related to those used in specific titrations (as titres).

Examples of primary standards used in redox titrations include pure iron, NaC_2O_4 (sodium oxalate), As_2O_3 (arsenic trioxide), $K_2Cr_2O_7$ (potassium dichromate), $KBrO_3$ (potassium bromate), KIO_3 (potassium iodate) and $KH(IO_3)_2$ (potassium hydrogen iodate

7. **Parts per million (ppm):** Parts per million is a convenient way to express very dilute concentrations. In such case, 1 mg per litre or per 1000 mL is referred to 1 ppm.

CHAPTER 4. SAMPLING AND SAMPLING TECHNIQUES

Analysis of the properties of a food material depends on the successful completion of a number of different steps: planning (identifying the most appropriate analytical procedure), sample selection, sample preparation, performance of analytical procedure, statistical analysis of measurements, and data reporting.

Sample Selection and Sampling Plans

A food analyst often has to determine the characteristics of a large quantity of food material, such as the contents of a truck arriving at a factory, a days worth of production, or the products stored in a warehouse. Ideally, the analyst would like to analyze every part of the material to obtain an accurate measure of the property of interest, but in most cases this is practically impossible. Many analytical techniques destroy the food and so there would be nothing left to sell if it were all analyzed. Another problem is that many analytical techniques are time consuming, expensive or labor intensive and so it is not economically feasible to analyze large amounts of material. It is therefore normal practice to select a fraction of the whole material for analysis, and to assume that its properties are representative of the whole material. Selection of an appropriate fraction of the whole material is one of the most important stages of food analysis procedures, and can lead to large errors when not carried out correctly.

Populations, Samples and Laboratory Samples. It is convenient to define some terms used to describe the characteristics of a material whose properties are going to be analyzed.

Population. The whole of the material whose properties we are trying to obtain an estimate of is usually referred to as the population.

Sample. Only a fraction of the population is usually selected for analysis, which is referred to as the sample. The sample may be comprised of one or more sub-samples selected from different regions within the population.

Laboratory Sample. The sample may be too large to conveniently analyze using a laboratory procedure and so only a fraction of it is actually used in the final laboratory analysis. This fraction is usually referred to as the laboratory sample.

The primary objective of sample selection is to ensure that the properties of the laboratory sample are representative of the properties of the population, otherwise erroneous results will be obtained. one must always be aware that analysis of a limited number of samples can only give an estimate of the true value of the whole population.

Sampling Plans. To ensure that the estimated value obtained from the laboratory sample is a good representation of the true value of the population it is necessary to develop a sampling plan. A sampling plan is a clearly written document that contains precise details that an analyst uses to decide the sample size, the locations from which the sample should be selected, the method used to collect the sample, and the method used to preserve them prior to analysis. It should also stipulate the required documentation of procedures carried out during the sampling process.. For certain products and types of populations sampling plans have already been developed and documented by various organizations which authorize official methods, e.g., the Association of Official Analytical Chemists (AOAC).

The choice of a particular sampling plan depends on

- the purpose of the analysis,
- the property to be measured,
- the nature of the total population and of the individual samples, and
- the type of analytical technique used to characterize the samples.

For certain products and types of populations sampling plans have already been developed and documented by various organizations which authorize official methods, e.g., the Association of Official Analytical Chemists (AOAC).

Explain diff methods of sampling
Explain procedure for preparation of standard solution.
solution ?

GENERAL CONSIDERATIONS IN TAKING THE LABORATORY SAMPLES;

Developing a Sampling Plan

After considering the above factors one should be able to select or develop a sampling plan which is most suitable for a particular application. Different sampling plans have been designed to take into account differences in the types of samples and populations encountered, the information required and the analytical techniques used. Some of the features that are commonly specified in official sampling plans are listed below.

To ensure that the estimated value obtained from the laboratory sample is a good representation of the true value of the population, it is necessary to develop a sampling plan. A sampling plan should be a clearly written document that contains precise details that an analyst uses

- to decide the sample size,
- locations from which the sample should be selected,
- method used to collect the sample,
- the method used to preserve them prior to analysis
- It should also stipulate the required documentation . of procedures carried out during the sampling process.

Sample size. The size of the sample selected for analysis largely depends on

- the expected variations in properties within a population,
- the seriousness of the outcome if a bad sample is not detected,
- the cost of analysis, and
- the type of analytical technique used.

Given this information it is often possible to use statistical techniques to design a sampling plan that specifies the minimum number of sub-samples that need to be analyzed to obtain an accurate representation of the population. Often the size of the sample is impractically large, and so a process known as sequential sampling is used. Here sub-samples selected from the population are examined sequentially until the results are sufficiently definite from a statistical viewpoint. **For example, sub-samples are analyzed until the ratio of good ones to bad ones falls within some statistically predefined value that enables one to confidently reject or accept the population.**

Sampling ;Consist of obtaining a larger group (referred to as the population). From this sample, one hopes to obtain an estimate of the true value of the parameters of interest with sufficient accuracy for the intended purposes. Sampling permits a reduction in cost and personnel while allowing information to be obtained quickly and comprehensively. **A laboratory sample is anything that is sent to a laboratory for analysis. It can be of any size or quantity.**

SAMPLING PROCEDURE-The anticipated use of the results from the sample will determine the sampling procedure.

Populations may be finite or infinite

For finite populations, sampling provides an estimate of lot. For infinite populations, sampling determines concern at many points.

.TYPES OF SAMPLING

1.Non-probability sampling;Judgments sampling; Convenience sampling;Haphazard sampling;

2.Probability sampling: Simple random sampling;Systematic sampling;Stratified random sampling;Clustered sampling;Composite sampling;Mixed sampling

1. **NON-PROBABILITY SAMPLING**;Probability of inclusion of any portion of the whole in the sample is not equal.In these sampling plans the investigator determines which sample will be selected.

Accurate estimates of the entire population are not possible because sampling error cannot be determined.

a) Judgments sampling;Depends on the person choosing the sample.Frequently this method is the only practical and feasible way to obtain a sample. If the investigator is experienced in sample selection and the limitations in extrapolation of the results are understood, this method may better represent the true state of the population than the random sampling.

b) Convenience sampling;It is often referred to as 'chunk sampling' or 'grab sampling'The first pallet or easiest to get the box is selected. Such a sample is not representative of the whole.
Restricted sampling may be required when the entire population is not accessible. Results are not representatives of the entire population.

c) Haphazard sampling;Selection of any portion of the sample. It should be avoided

d) Quota sampling; It is the division of the lot into groups representing various categories. A specific number of samples are selected from each group by judgment. The sampling plan is less costly than random sampling but is also less reliable.

PROBABILITY SAMPLING;It provides a scientific method for selection of samples according to a statistical plan.The chance of including each item is known and sampling error may be calculated.

a) **Simple random sampling;**It requires that the number of units in the population to the sample is known.Each unit is assigned (in order) a number. A specific quantity of random numbers is selected between 1 and the total number of units in the lot. Random number tables may be used.
Units are chosen corresponding to the random numbers and evaluated for the characteristics of interest.

b)Systematic sampling;It is applied when a complete list of sample units is not available.
The first sample unit is selected at random and every nth unit after that is selected. This plan is used when materials are continuously distributed over time or space.It is most frequently used in production line sample.

c)**Stratified random sampling** Population to be sampled is divided into subgroups such that units within each group are as homogenous as possible. Group means are as widely different as possible.Samples are taken randomly from each subgroup.This procedure provides the most representative cross section of the entire population because no part is excluded. It is less expensive than simple random sampling.

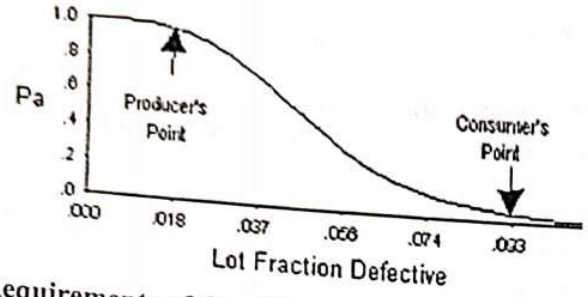
d) **Clustered sampling** Population is divided into subgroups termed 'clusters' such that each subgroup is as similar to all others as possible. Heterogeneity is within the cluster. This process is more efficient and less costly than simple random sampling for populations that can be easily divided into homogenous groups. Clusters should be small and number of sample units from each cluster is about the same.

e) **Composite sampling;**It is commonly used for flour, seeds and other items in bags.It is also useful for solid samples in bulk.Two or more random samples are combined to give one sample for analysis. This procedure averages differences within the population.

3) **Mixed sampling;**Mixed sampling combines random and purposeful sampling.The lot is subdivided based on purposeful sampling methods and items from within the group are selected randomly.

PROBLEMS IN SAMPLING
Sample bias-Bias may be introduced by non-probability sampling plans such as purposeful or convenience sampling. Bias may also result from substitution of a more conveniently acquired sample by the sampler.Lack of understanding of the population distribution.Storage of sample: Sample should be placed in an appropriate container that protects it from moisture loss or absorption during transport and storage.

4 **Operating Characteristic (OC) Curves**; Operating Characteristic (OC) curves are used extensively in acceptance sampling. The Operating Characteristic (OC) curve shows the probability of acceptance, Pa, for any level of lot quality. On the horizontal axis is the quality characteristic. This OC curve enables you to evaluate the probability of acceptance for any true lot quality level-on a what-if basis. This way, you can design sampling plans that perform the way you want.



1) If the lot quality is 0.093 fraction defective, then the probability of acceptance, Pa, is 0.05.

2) If the lot quality is 0.018 fraction defective, then the probability of acceptance, Pa, is 0.95.

Requirements of Good Sampling Methods ;

- good sampling techniques and good sampling practices.
- Inspection of the lot before sampling.
- Use of suitable sampling devices for the particular commodity and type of sample desired.
- Use of suitable containers to hold the sample.
- Maintenance of the integrity of the sample and associated records.
- Use of adequate precautions in preserving, packing and delivery of the sample to the lab in a timely manner.
- Provision of appropriate storage conditions for the sample both prior to and following analysis.
- cost versus benefits analysis, and
- A review of program objectives and regularity requirements,

are to be assessed and brought together in a sampling plan that serves as a guide to management, as well as to operating personnel as a firm plan to achieve quality in sampling.

SAMPLE LOCATION

1. Homogeneous versus heterogeneous population

The ideal population would be exactly the same at every location. Such a population would be **homogeneous Sampling** from a homogeneous population is simple .Unfortunately in the real world such populations are rare Most populations are **heterogeneous**. Results obtained with samples taken from heterogeneous population will depend on the location of sampling.The food material within the sample selected from the population is usually heterogeneous, i.e., its properties vary from one location to another. Sample heterogeneity may either be caused by in the properties of different units within the sample **(inter-unit variation)** eg.a box of oranges, some of good quality and some of bad quality. Variations within the individual units in the sample **(intra-unit variation)**. Eg. individual orange, whose skin has different properties than its flesh.

2. Manual versus continuous sampling

Manual sampling is done by humans. Regardless of the process, it is imperative that the unit being sample be as homogenous as possible prior to sampling. For liquids in small containers, this is achieved by shaking prior to sampling. For liquids in silos, aeration maintains a homogenous unit.
For grains in rail cars samples are probed from several points at random and a composite sample prepared to represent the whole. Granular or powdered samples may be taken with the aid of triers or probes that are inserted into the material. The solid products may be sampled by cutting representative portions from specific areas
.Continuous sampling is performed by mechanical sampling devices

Sample collection. Sample selection may either be carried out manually by a human being or by specialized mechanical sampling devices. Manual sampling may involve simply picking a sample from a conveyor belt or a truck, or using special cups or containers to collect samples from a tank or sack. The manner in which samples are selected is usually specified in sampling plans.

Preparation of Laboratory Samples

Once we have selected a sample that represents the properties of the whole population, we must prepare it for analysis in the laboratory. The preparation of a sample for analysis must be done very carefully in order to make accurate and precise measurements.

Making Samples Homogeneous

The food material within the sample selected from the population is usually heterogeneous, i.e., its properties vary from one location to another. Sample heterogeneity may either be caused by variations in the properties of different units within the sample (inter-unit variation) and/or it may be caused by variations within the individual units in the sample (intra-unit variation). The units in the sample could be apples, potatoes, bottles of ketchup, containers of milk etc. An example of inter-unit variation would be a box of oranges, some of good quality and some of bad quality. An example of intra-unit variation would be an individual orange, whose skin has different properties than its flesh. For this reason it is usually necessary to make samples homogeneous before they are analyzed, otherwise it would be difficult to select a representative laboratory sample from the sample. A number of mechanical devices have been developed for homogenizing foods, and the type used depends on the properties of the food being analyzed (e.g., solid, semi-solid, liquid). Homogenization can be achieved using mechanical devices (e.g., grinders, mixers, slicers, blenders), enzymatic methods (e.g., proteases, cellulases, lipases) or chemical methods (e.g., strong acids, strong bases, detergents).

Reducing Sample Size

Once the sample has been made homogeneous, a small more manageable portion is selected for analysis. This is usually referred to as a laboratory sample, and ideally it will have properties which are representative of the population from which it was originally selected. Sampling plans often define the method for reducing the size of a sample in order to obtain reliable and repeatable results.

Preventing Changes in Sample

Once we have selected our sample we have to ensure that it does not undergo any significant changes in its properties from the moment of sampling to the time when the actual analysis is carried out, e.g., enzymatic, chemical, microbial or physical changes. There are a number of ways these changes can be prevented.

Enzymatic Inactivation. Many foods contain active enzymes they can cause changes in the properties of the food prior to analysis, e.g., proteases, cellulases, lipases, etc. If the action of one of these enzymes alters the characteristics of the compound being analyzed then it will lead to erroneous data and it should therefore be inactivated or eliminated. Freezing, drying, heat treatment and chemical preservatives (or a combination) are often used to control enzyme activity, with the method used depending on the type of food being analyzed and the purpose of the analysis.

Lipid Protection. Unsaturated lipids may be altered by various oxidation reactions. Exposure to light, elevated temperatures, oxygen or pro-oxidants can increase the rate at which these reactions proceed. Consequently, it is usually necessary to store samples that have high unsaturated lipid contents under nitrogen or some other inert gas, in dark rooms or covered bottles and in refrigerated temperatures. Providing that they do not interfere with the analysis antioxidants may be added to retard oxidation.

Microbial Growth and Contamination. Microorganisms are present naturally in many foods and if they are not controlled they can alter the composition of the sample to be analyzed. Freezing, drying, heat treatment and chemical preservatives (or a combination) are often used to control the growth of microbes in foods.

Physical Changes. A number of physical changes may occur in a sample, e.g., water may be lost due to evaporation or gained due to condensation; fat or ice may melt or crystallize; structural properties may be disturbed. Physical changes can be minimized by controlling the temperature of the sample, and the forces that it experiences.

SAMPLE IDENTIFICATION

Laboratory samples should always be labelled carefully so that if any problem develops its origin can easily be identified. The information used to identify a sample includes: a) Sample description, b) Time sample

was taken, c) Location sample was taken from, d) Person who took the sample, and, e) Method used to select the sample. The analyst should always keep a detailed notebook clearly documenting the sample selection and preparation procedures performed and recording the results of any analytical procedures carried out on each sample. Each sample should be marked with a code on its label that can be correlated to the notebook. Thus if any problem arises, it can easily be identified.

Data Analysis and Reporting

Food analysis usually involves making a number of repeated measurements on the same sample to provide confidence that the analysis was carried out correctly and to obtain a best estimate of the value being measured and a statistical indication of the reliability of the value.A variety of statistical techniques are available that enable us to obtain this information about the laboratory sample from multiple measurements.

2 Measure of Central Tendency of Data

The most commonly used parameter for representing the overall properties of a number of measurements is the mean: Here n is the total number of measurements, xi is the individually measured values and is the mean value. The mean is the best experimental estimate of the value that can be obtained from the measurements. It does not necessarily have to correspond to the true value of the parameter one is trying to measure. There may be some form of systematic error in our analytical method that means that the measured value is not the same as the true value. Accuracy refers to how closely the measured value agrees with the true value. The problem with determining the accuracy is that the true value of the parameter being measured is often not known. Nevertheless, it is sometimes possible to purchase or prepare standards that have known properties and analyze these standards using the same analytical technique as used for the unknown food samples. The absolute error Eabs, which is the difference between the true value (xtrue) and the measured value (xi), can then be determined: Eabs = (xi - xtrue). For these reasons, analytical instruments should be carefully maintained and frequently calibrated to ensure that they are operating correctly.

Measure of Spread of Data

The spread of the data is a measurement of how closely together repeated measurements are to each other. The standard deviation is the most commonly used measure of the spread of experimental measurements. This is determined by assuming that the experimental measurements vary randomly about the mean, so that they can be represented by a normal distribution.◆ The standard deviation SD of a set of experimental measurements is given by the following equation:

Measured values within the specified range:

1 SD means 68% values within range (x - SD) to (x + SD)
2SD means 95% values within range (x - 2SD) to (x + 2SD)
3SD means >99% values within range (x - 3SD) to (x + 3SD)

Another parameter that is commonly used to provide an indication of the relative spread of the data around the mean is the coefficient of variation, CV = [SD /] ◆ 100%.

SOURCES OF ERROR

There are three common sources of error in any analytical technique:

Personal Errors (Blunders). These occur when the analytical test is not carried out correctly: the wrong chemical reagent or equipment might have been used; some of the sample may have been spilt; a volume or mass may have been recorded incorrectly; etc. It is partly for this reason that analytical measurements should be repeated a number of times using freshly prepared laboratory samples.◆ Blunders are usually easy to identify and can be eliminated by carrying out the analytical method again more carefully.

Random Errors. These produce data that vary in a non-reproducible fashion from one measurement to the next e.g., instrumental noise.

Systematic Errors. A systematic error produces results that consistently deviate from the true answer in some systematic way, e.g., measurements may always be 10% too high.. For example, a nominally 100 cm3 pipette may always deliver 101 cm3 instead of the correct value.

To make accurate and precise measurements it is important when designing and setting up an analytical procedure to identify the various sources of error and to minimize their effects. Often, one particular step will be the largest source of error, and the best improvement in accuracy or precision can be achieved by minimizing the error in this step.

PROPAGATION OF ERRORS

Most analytical procedures involve a number of steps (e.g., weighing, volume measurement, reading dials), and there will be an error associated with each step. These individual errors accumulate to determine the overall error in the final result. For random errors there are a number of simple rules that can be followed to calculate the error in the final result:

Addition ($Z = X+Y$) and Subtraction ($Z = X-Y$): (3)

Multiplication ($Z = XY$) and Division ($Z = X/Y$): (4)

Here, $\pm X$ is the standard deviation of the mean value X, $\pm Y$ is the standard deviation of the mean value Y, and $\pm Z$ is the standard deviation of the mean value Z. These simple rules should be learnt and used when calculating the overall error in a final result.

As an example, let us assume that we want to determine the fat content of a food and that we have previously measured the mass of extracted fat extracted from the food (ME) and the initial mass of the food (MI):..

ME = 3.1 ± 0.3 g

MI = 10. 1 ±- 0.7 g

% Fat Content = 100 x ME / MI

To calculate the mean and standard deviation of the fat content we need to use the multiplication rule (Z=X/Y) given by Equation 4. Initially, we assign values to the various parameters in the appropriate propagation of error equation:

X = 3.1; ±x = 0.3

Y = 10.5; ±y= 0.7

% Fat Content = Z = 100X X/Y = 100 x 3.1/10.5 = 29.5%

±Z = Z [(X±x)2+(±y/Y)2] = 29.5% [(0.3/3.1)2+(0.7/10.5)2] = 3.5%

Hence, the fat content of the food is 29.5± 3.5%. In reality, it may be necessary to carry out a number of different steps in a calculation, some that involve addition/subtraction and some that involve multiplication/division. When carrying out multiplication/division calculations it is necessary to ensure that all appropriate addition/subtraction calculations have been completed first.

SIGNIFICANT FIGURES AND ROUNDING

The number of significant figures used in reporting a final result is determined by the standard deviation of the measurements. A final result is reported to the correct number of significant figures when it contains all the digits that are known to be correct, plus a final one that is known to be uncertain. For example, a reported value of 12.13, means that the 12.1 is known to be correct but the 3 at the end is uncertain, it could be either a 2 or a 4 instead.

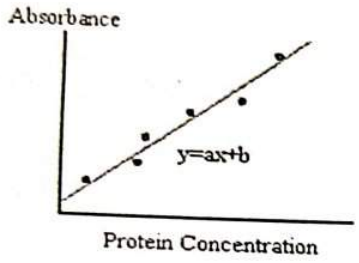
For multiplication ($Z = Xx Y$) and division ($Z = X/Y$), the significant figures in the final result (Z) should be equal to the significant figures in the number from which it was calculated (X or Y) that has the lowest significant figures. For example, 12.312 (5 significant figures) x 31.1 (3 significant figures) = 383 (3 significant figures). **For addition** ($Z = X + Y$) and **subtraction** ($Z = X - Y$), the significant figures in the final result (Z) are determined by the number from which it was calculated (X or Y) that has the last significant figure in the highest decimal column. For example, 123.4567 (last significant figure in the "0.0001" decimal column) + 0.31 (last significant figure in the "0.01" decimal column) = 123.77 (last significant figure in the "0.01" decimal column). Or, 1310 (last significant figure in the "10" decimal column) + 12.1 (last significant figure in the "0.1" decimal column) = 1320 (last significant figure in the "10" decimal column).

ROUNDING NUMBERS: always round any number with a final digit less than 5 downwards, and 5 or more upwards, e.g. 23.453 becomes 23.45; 23.455 becomes 23.46; 23.458 becomes 23.46. It is usually desirable to carry extra digits throughout the calculations and then round off the final result.

STANDARD CURVES: REGRESSION ANALYSIS

When carrying out certain analytical procedures it is necessary to prepare standard curves that are used to determine some property of an unknown material. A series of calibration experiments is carried out using samples with known properties and a standard curve is plotted from this data. For example, a series of protein solutions with known concentration of protein could be prepared and their absorbance of electromagnetic radiation at 280 nm could be measured using a UV-visible spectrophotometer. For dilute protein solutions there is a linear relationship between absorbance and protein concentration:

A best-fit line is drawn through the date using regression analysis, which has a gradient of a and a y-intercept of b. The concentration of protein in an unknown sample can then be determined by measuring its absorbance: x = (y-b)/a, where in this example x is the protein concentration and y is the absorbance. How well the straight-line fits the experimental data is expressed by the correlation coefficient r2, which has a value between 0 and 1. The closer the value is to 1 the better the fit between the straight line and the experimental values: r2 = 1 is a perfect fit. Most modern calculators and spreadsheet programs have routines that can be used to automatically determine the regression coefficient, the slope and the intercept of a set of data.



2.4.7. Rejecting Data

When carrying out an experimental analytical procedure it will sometimes be observed that one of the measured values is very different from all of the other values, e.g., as the result of a blunder in the analytical procedure. Occasionally, this value may be treated as being incorrect, and it can be rejected. There are certain rules based on statistics that allow us to decide whether a particular point can be rejected or not. A test called the Q-test is commonly used to decide whether an experimental value can be rejected or not.

Here XBAD is the questionable value, XNEXT is the next closet value to XBAD, XHIGH is the highest value of the data set and XLOW is the lowest value of the data set. If the Q-value is higher than the value given in a Q-test table for the number of samples being analyzed then it can be rejected:

Number of Observations (90% confidence level)	Q-value for Data Rejection
3	0.94
4	0.76
5	0.64
6	0.56
7	0.51
8	0.47
9	0.44
10	0.41

For example, if five measurements were carried out and one measurement was very different from the rest (e.g., 20,22,25,50,21), having a Q-value of 0.84, then it could be safely rejected (because it is higher than the value of 0.64 given in the Q-test table for five observations).

CHAPTER-5 PROXIMATE ANALYSIS

The proximate analysis comprises:

1. Moisture
2. Crude Fat
3. Crude Protein
4. Ash
5. Carbohydrate and
6. Crude Fibre

Total carbohydrate = 100-[moisture + crude fat + crude protein + ash].

Determination of Moisture and Total Solids

1. MOISTURE

.Moisture content is one of the most commonly measured properties of food materials. It is important to food scientists for a number of different reasons:

- Legal and Labeling Requirements.
- Economic.
- Microbial Stability.
- Food Quality.
- Food Processing Operations.

It is therefore important for food scientists to be able to reliably measure moisture contents

Despite having the same chemical formula (H_2O) the water molecules in a food may be present in a variety of different molecular environments depending on their interaction with the surrounding molecules.

The water molecules in these different environments normally have different physiochemical properties: , melting point, boiling point, density, compressibility, heat of vaporization, electromagnetic absorption spectra.

- **Bulk water.** Bulk water is free from any other constituents, so that each water molecule is surrounded only by other water molecules. It therefore has physicochemical properties that are the same as those of pure water,.
- **Capillary or trapped water.** Capillary water is held in narrow channels between certain food components because of capillary forces. Trapped water is held within spaces within a food that are surrounded by a physical barrier that prevents the water molecules from easily escaping, e.g., an emulsion droplet or a biological cell.
- **Physically bound water.** A significant fraction of the water molecules in many foods are not completely surrounded by other water molecules, but are in molecular contact with other food constituents, e.g. proteins, carbohydrates or minerals.This type of water has different physicochemical properties than bulk water
- **Chemically bound water.** Some of the water molecules present in a food may be chemically bonded to other molecules as water of crystallization or as hydrates, e.g. NaSO4.10H20. This has very different physicochemical properties to bulk water

Foods are heterogeneous materials that contain different proportions of **chemically bound, physically bound, capillary, trapped or bulk water.** In addition, foods **may contain water that is present in different physical states: gas, liquid or solid.).**

EVAPORATION METHOD

Sample preparation

Selection of a representative sample, and prevention of changes in the properties of the sample prior to analysis, are two major potential sources of error in any food analysis procedure. When determining the moisture content of a food it is important to prevent any loss or gain of water. For this reason, exposure of a sample to the atmosphere, and excessive temperature fluctuations, should be minimized. When samples are

POLYMERASE CHAIN REACTION (PCR)

Introduction

polymerase chain reaction (PCR) is a widely used enzymatic process that rapidly and exponentially amplifies a specific region of DNA, producing millions to billions of copies of a particular DNA sequence.
PCR was invented in 1983 by American biochemist Kary Mullis. It is used in a wide range of applications, including the analysis of ancient DNA samples, identification of infectious agents, gene cloning and manipulation, DNA sequencing etc. The PCR process involves several steps. The PCR method typically relies on thermal cycling which involves exposing the reaction to repeated cycles of heating and cooling. These temp^r changes facilitate. specific reactions, such as DNA melting and enzyme-driven DNA replication.

Principle

The principle of PCR involves the amplification of a specific segment of DNA through a series of temperature cycles. The process begins with denaturation, where the target DNA is heated to separate the two strands, resulting in single-stranded DNA. Next, specific primers, designed to bind to each target DNA strand, are added. These primers serve as starting points for DNA synthesis.

Mechanism of PCR

=) For PCR, nucleic acid is first extracted from the organism or a clinical sample potentially containing the target organism by heat, chemical, or enzymatic methods.

=) Once extracted, target nucleic acid is added to the reaction mix containing all the necessary components of PCR (primers, nucleotides, covalent ions, buffer and enzyme) and placed into a thermal cycler to undergo amplification.

PCR involves 25 - 50 repetitive cycles, with each cycle comprising three sequential reactions.

1. Initialization : This step is required for DNA polymerases that need heat activation. The reaction chamber is heated to a temp^{r} of 94-96°C (or 98°C for extremely thermostable polymerases) and held for 1-10 min.

2. Denaturation : The reaction chamber is heated to 94-98°C for 20-30 sec. This causes the double stranded DNA template to denature, separating it into two single-stranded DNA molecules.

3. Annealing : The temperature is lowered to 50-65°C for 20-40 sec. During this step, short DNA sequences called primers anneal to each of the single-stranded DNA templates. primers are designed to bind to

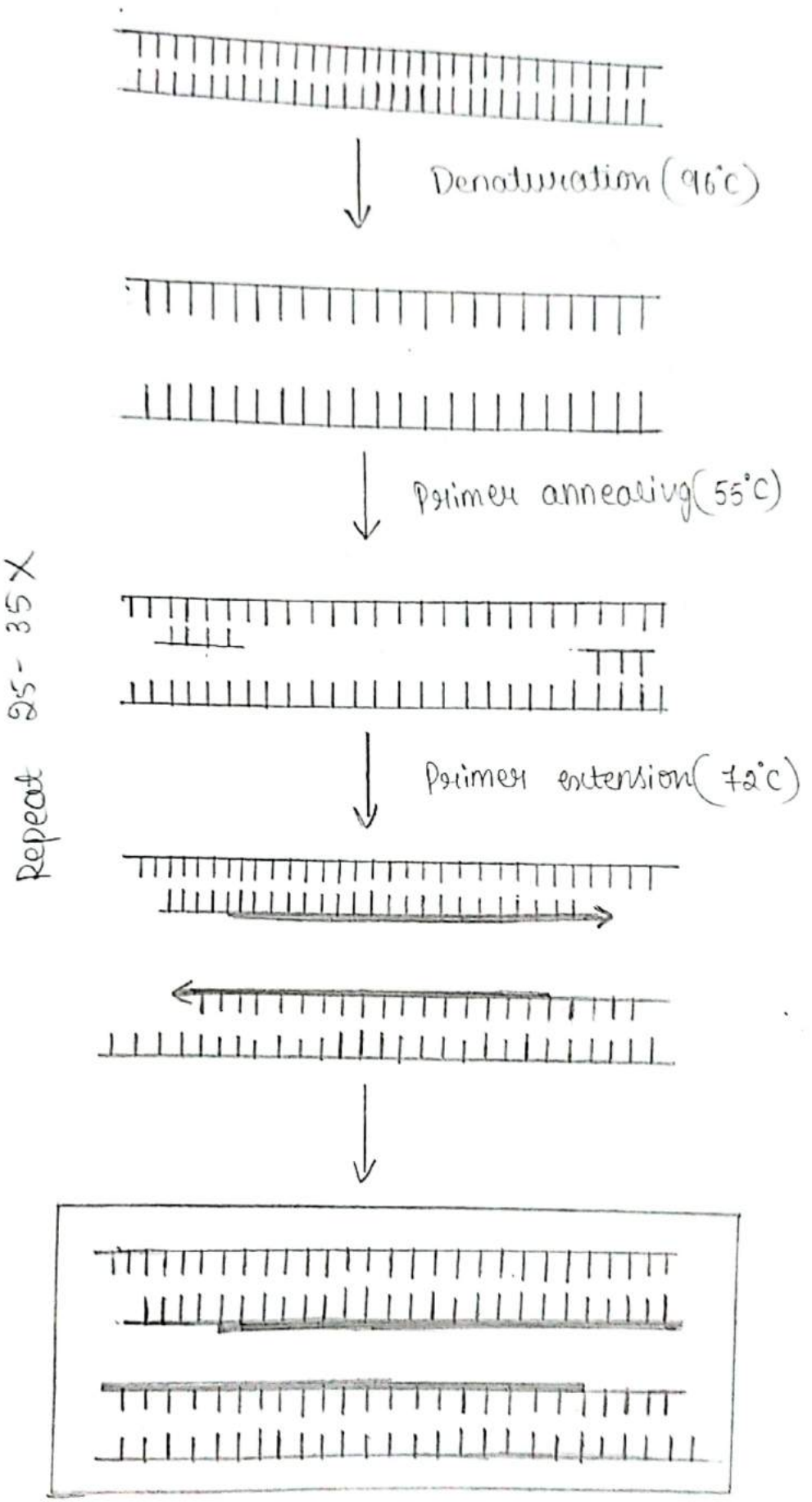
specific regions flanking the target DNA sequence.

4. Extension / Elongation : The temp^{r} is raised to the optimal activity temp^{r} of the DNA polymerase used, typically around 72 - 80°C. The DNA polymerase synthesizes a new DNA strand complementary to the template strand by adding nucleotides (dNTPs) in the 5'-3' direction. This step extends the primers and synthesizes new DNA strands.

5. cycling : Steps 2-4 are repeated for 20-40 cycles. with each cycle, the target DNA is doubled, resulting in exponential amplification of the specific DNA sequence.

6. Final elongation : After the last PCR cycle, a final elongation step is performed at a temp^{r} of 70-74°C for 5-15 min to ensure complete elongation of any remaining single-stranded DNA.

7. Final hold : The reaction chamber is cooled to 4-15°C for an indefinite time. This step allows for short-term storage of the PCR products.



Result after 1 cycle of DNA molecules doubled

Applications of PCR

* Diagnosis of infections : PCR approaches are widely used in clinical laboratories to diagnose infections caused by bacteria, viruses, protozoa, and fungi. These techniques offer specific and sensitive detection and quantification of infectious agents.

* Diagnosis of genetic defects : PCR based detection systems are used to accurately identify genetic disorders before the onset of disease and confirm their presence after the onset. PCR allows for the detection of inherited genetic changes and spontaneous genetic mutations.

* Diagnosis and prognosis of Cancers : PCR-based approaches can identify cancer-related genes and analyze their expression patterns. This helps in determining genetic predisposition to certain types of cancer, confirming the cancer type, predicting prognosis and guiding treatment decisions.

* personalized medicine : PCR technologies play a crucial role in pharmacogenomics and pharmacogenetics. Genetic markers tracked by PCR help determine individual responses to treatments, design tailored drugs, and prescribe effective drug doses.

* Forensic Sciences : PCR is utilized in forensic sciences to amplify DNA samples obtained from crime scenes. Even poor-quality and low-quantity DNA samples can be reliably analyzed using PCR, aiding in criminal identification and investigations.

* Meat traceability: PCR methods are used to identify and quantify adulteration of meat in raw and processed food products. This helps ensure the accuracy and integrity of meat labeling and traceability systems.

* Identifying medicinal plants: PCR-based DNA barcoding is a rapid and accurate tool for identifying medicinal plant species. This approach is used in various fields, including medicine, ecology and conservation biology, to identify endangered and new species.

* Recombinant DNA technology: PCR is used in recombinant DNA technology to generate hybrid DNA molecules with precision. It is also employed to clone DNA into specific vectors for protein expression and production.

Advantages of PCR.

1. Simplicity and Speed : PCR is a relatively simple technique to understand and perform. It involves a few basic steps and can be carried out in a relatively short period of time, typically a few hours. This rapid turnaround time allows for quick diagnosis and identification of target sequences.

2. High Sensitivity : PCR is highly sensitive and has the potential to amplify even trace amounts of target. DNA. It can produce millions to billions of copies of a specific DNA fragment

3. Versatility and flexibility : PCR is a versatile technique that can be used in various applications. It allows for the amplification of DNA from a wide range of sources, including genomic DNA, cDNA, and even degraded or old DNA samples. It can be adapted for different purposes, such as sequencing, cloning, mutation analysis, and genotyping.

4. Specificity : PCR offers high specificity when designed properly. By using specific primers that target and desired DNA sequence, PCR can selectively amplify the target region while minimizing amplification of non-target DNA. This specificity ensures reliable and accurate results.

Limitations of PCR

1. Target sequence requirement : PCR requires prior knowledge of the target DNA sequence to design the primers for selective amplification. This means researchers need to know the specific sequence upstream of the target region on each single-stranded template. without this information, PCR amplification cannot be performed.

2. potential for errors and mutations: DNA polymerases used. in PCR are prone to errors during DNA synthesis, leading to mutations in the amplified fragments. These errors can introduce inaccuracies in the results and affect downstream analysis. or interpretation.

3. Size limitations; Large DNA fragments may not be efficiently amplified or may require modified PCR protocols.

4. Inhibition by environmental samples : Environmental samples that contain humic acids, such as. soil or water samples, can inhibit PCR amplification and result in inaccurate or failed results. specialized purification methods or alternative PCR protocols. may be required to overcome this limitation.

5. Contamination risks : Even a small amount of contamina-ting DNA, such as from previous PCR reactions or environmental sources, can be amplified and lead to false or ambiguous results.

AAS

Atomic Absorption Spectrometer

- Another name of AAS is AES (Atomic emission spectroscopy)

Introduction:

- AAS was developed in 1950s by Alan Walsh and rapid -ly became a widely used analytical tool
- AAS is an elemental analysis technique capable of provi -ding quantitative information of about 70 elements.
- AAS is used for analysis of metallic elements and trace elements such as Al, Cd, Pb, Cu, Ni, Mo, Mg, Zn etc
- It is a very sensitive technique - it can detect the elements upto ppm (parts per billion) level and for some elements it can detect upto ppb or ppt level also.
- Another advantages is that a given element can be determined in the presence of other elements, which do not interfere by absorption of the analyte wavelength.
- Food, soil, water samples are generally analyzed by AAS for presence of trace elements. (Cu, Fe, Zn, Cr, Co, Ni, Mo, Se)

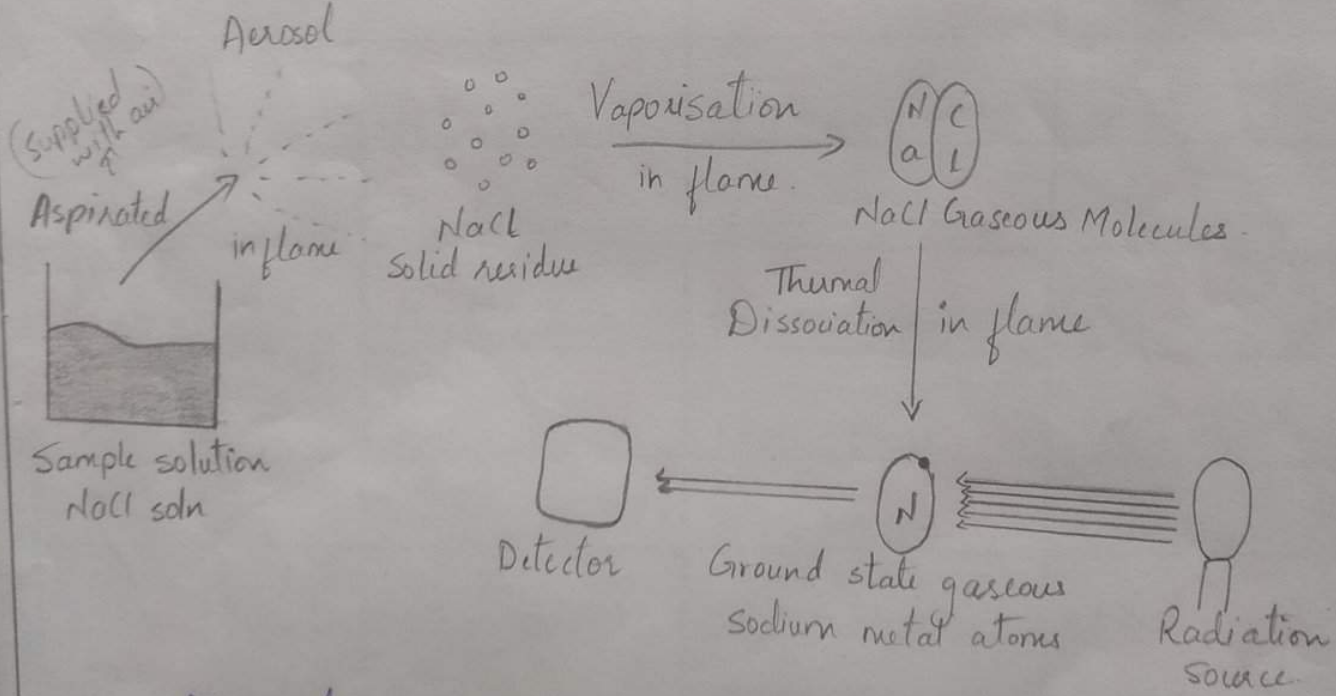
Principle of AAS

- Sample solution is atomized in a atomizer (flame atomizer)
- Droplets of sample solution evaporates in flame giving solid residue of sample.
- This solid gets evaporated and the solid sample molecules converts into gaseous sample molecule due to

flame temperature.

- Further gaseous molecule dissociated to give gaseous neutral metal atoms (ground state).
- Due to flame temperature some of the ground state gaseous metal atoms will be exited but most of atoms remains in ground state / unexited.
- Electromagnetic radiations of particular wavelengths are passed through these ground state (unexcited) gaseous metal atoms which will absorb those radiations.

and this absorption of radiation by the gaseous metal atoms is the basis of AAS. The absorbed radiation are measured by the detector



Absorption of radiations by ground state (unexcited) gaseous metal atoms is the basis of AAS.

The absorption of radiations follows Beer-Lambert's law

$$\text{Log } I_0/I_t = KLN_0$$

I_0 - intensity of radiations incident
I_t - intensity of radiations transmitted from metal atoms
K - characteristic constant
L - path length of flame in cm.
N_0 - No. of atoms in the ground state

Oxidants and Fuels mixtures used in AAS.

Oxidant gas	Fuel gas	Flame Temperature in °C	Max Burning Velocity (cm/s)
Oxygen	Natural gas	2700 - 2800	370 - 390
Oxygen	Hydrogen	2550 - 2700	900 - 1400
Oxygen	Acetylene	3050 - 3150	1100 - 2480
Air	Natural gas	1700 - 1900	39 - 34
Air	Hydrogen	2000 - 2100	300 - 440
Air	Acetylene	2100 - 2400	158 - 266
Nitrous Oxide	Acetylene	2600 - 2800	285

Comparison of Flame Photometry and AAS.

Similarities.

Both are atomic techniques.

Both are used to for analysis of soil, food, water samples.

Differences.

Flame Photometry	AAS
Measurement of emitted radiations is basis of Flame Photometry	Measurement of absorbed radiations is the basis of AAS
Emission of radiation depends upon number of gaseous excited metal atoms	Absorption of radiation depends upon number of gaseous unexcited metal atoms.
Emission phenomenon depends on flame temperature	Absorption phenomenon is independent of flame temperature.
Limited to only alkali and alkaline earth metals.	Approximately 70 elements can be analyzed by AAS.

* Working nature:

AAS is an analytical technique used to determine how much of certain elements are in a sample.

Basic Components of AAS: the sample introduction area, the light (radiation) source, the monochromator or polychromator and the detector.

Application

1. Analysis of water, soil and air samples for metallic pollutants. (river, industrial, waste and same as)
2. Analysis of food samples for metallic content.
3. Determination of trace elements in various samples. (such as the sample of paints, capsz, rubber sample)
4. Analysis of biological samples such as blood, plasma, serum for Ca, Mg, Li, Na, Fe etc.
5. Determination of Lead in petrol is done by AAS
6. Hair analysis for heavy metal poisoning.
7. AAS can be used for both Qualitative and Quantitative analysis.

Qualitative → detection of elements i.e which element are present in the sample

Quantitative → the element are present in how much quantity

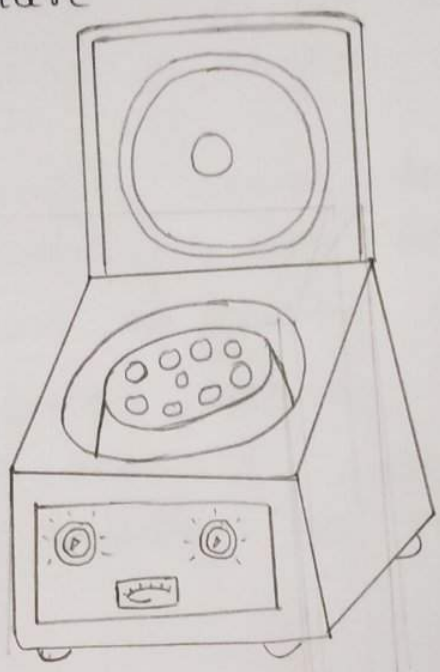
CENTRIFUGATION

Definition:- It is a unit operation working for separation separating the consequent present in a dispersion with the help of centrifugal force. for example centrifugal force includes the earth revolves around the sun. It is a technique which involves the application of centrifugal force to separate particles from a solution according to their size, shape, density, the viscosity of the medium and rotor speed.

Principle of centrifugation

1. The centrifuge involve the principle of sedimentation
2. The principle of the centrifugation technique is to separate the particles suspended in liquid media under the influence of a centrifugal field. These are placed either in tubes or bottles in a rotor in the centrifuge
3. Sedimentation is a phenomenon where suspended materials settles out of the fluids by gravity. The suspended material can be particles such as clay or powder. Example tea leaves falling to the bottom in a teacup
4. The particles having size more than 5 micrometres are separated by simple filtration process while the particles having size 5 micrometre or less do not sediment under gravity. The central force is useful to separate those particles

Working Nature



1) Preparation :- Gather the mixture you want to separate, and ensure it is properly homogenized to distribute the components evenly
2) Selection of centrifuge :- Choose the appropriate centrifuge based on the volume and type of sample Different centrifuges have varying speed and capacity capabilities
3) Loading the sample : Carefully pour the mixture into centrifuge tubes or bottles ensuring they are balanced with equal masses
4) Centrifugation : Place the loaded samples into the centrifuge rotor, which spins rapidly to create a centrifugal force. This force pulls the denser components outward, causing them to settle at the bottom of the tube
5) Formation of layers : As the centrifuge spins, the mixture's components separate into distinct layers based on their densities. With the denser particles forming a pellet at the bottom and the lighter components remaining as supernatant at the top

6) Stopping the centrifuge: Once the desired separation is achieved. turn off the centrifuge and allow it to come to a complete stop before opening the lid

7) Collection: Carefully remove the separated components from the centrifuge tubes using appropriate techniques such as pipetting, decanting or pouring

8) Analysis or further processing: The separated components can now be analyzed, quantified or subjected to additional procedures as required.

Applications

1. Production of bulk drugs
2. Production of biological products
3. Evaluation of suspensions and emulsion
4. Determination of molecular weight of collides
5. Separating chalk powder from water
6. Removing fat from milk to produce skimmed milk
7. The Clarification and Stabilization of the wine
8. Biopharmaceutical analysis of drugs
9. Use in water treatment
10. Separating particles from an airflow using cyclonic separation.

Advantages

1. Rapid separation: They can quickly separate different components of a mixture based on their density. allowing for efficient processing
2. High capacity: Centrifuges can handle large volumes of sample at once, increasing productivity in laboratories and industrial settings
3. Versatility: They are adaptable for various applications such as cell separation. DNA/RNA extraction and protein purification
4. Precise results: Centrifugation provides reliable and reproducible results, ensuring accurate analysis and experimentation
5. Easy operation: Modern centrifuges are user-friendly. making them accessible to both experienced scientist and newcomers
6. Low maintenance: They typically have simple maintenance requirement. reducing downtime and operational costs
7. Compact size: Many models are compact and don't require extensive space, making them suitable for laboratories with limited room.

Disadvantage

1. Heat generation: Centrifugation can generate heat during operation. potentially affecting sensitive samples or causing denaturation of biomolecules.
2. Sample damage: High centrifugal forces may cause damage to delicate samples leading to altered results or reduced sample integrity

3 Time-consuming: Certain centrifugation procedures can be time-consuming especially when dealing with large volumes or high density samples

4 Cost: Centrifuges can be expensive to purchase and maintain, making them less accessible for some laboratories with budget constraints

5 Noise and vibration: Centrifuges can produce noise and vibration during operation

6 Energy consumption: Centrifugation machines consume significant amounts of energy, contributing to operational costs and environmental impact.

COLORIMETER

Defn:— A colorimeter is a device that used in colorimetry It helps specific solution to absorb a particular wavelength of light. The colorimeter is usually used to measure the concentration of known solute in given solution / with the help of Beer-Lambert law.

The colorimeter was invented in the year 1870 by Louis J Duboscq.

Principle of Colorimeter

It is photometric technique which states that when a beam of incident light of Intensity I_o passes through a solution, Then following occur.

- A part of it is reflected which is denoted as I_r
- A part of it is absorbed which is denoted as I_a
- Rest of light transmitted & denoted as I_t

$$\therefore I_o = I_r + I_a + I_t$$

Colorimeter is based on 2 fundamental laws of photometry. They are

1) Beer's law
2) Lambert's law.

Beer's law :—

According to this law amount of light absorbed is Proportional to the solute concentration present in Solution.

$$A = \varepsilon bC$$

A - Absorbance

ε - Molar absorptivity

b - Length of ~~sample~~ light path

C - Solution of concentration in mole per unit

Lambert's law :—

According to this law the amount of light absorbed is Proportional to the length as well as thickness of solution taken for analysis

$$A = \varepsilon bc$$

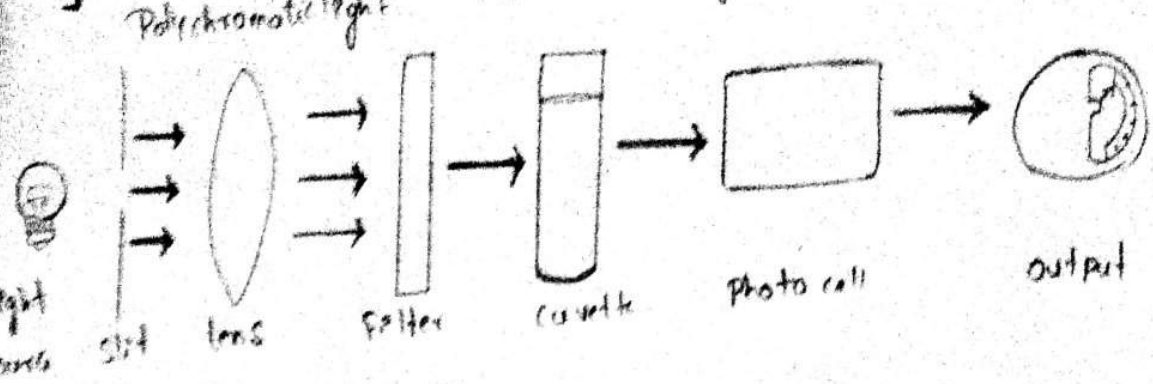
A - Absorbance

ε - Molar absorptivity

b - length of light path

c - concentration of solution

Diagram of colorimeter



Working of colorimeter

Step 1:— Before starting the experiment it is important to calibrate the colorimeter. It is done by using standard solution of the known solute concentration that has to be determined. Fill the standard solution in cuvette & place it in cuvette holder of colorimeter.

Step 2:— A light ray of certain wavelength, which is specific for assay in the direction of solution. The light passes through a series of different lenses & filters. The coloured light navigates with help of lenses, & filters. The coloured light navigates with helps of lens, & Filter helps to split a beam of light in to different wavelength allowing only required wavelength to passe through it & reach cuvette of std test solution

Step 3:— When the beam of light reaches' cuvette, it is transmitted, reflected & absorbed by solution.
The transmitted rays falls on photocell system where it measures the intensity of transmited light. It converts into electrical signals & sends it to galvanometer.

Step 4:— The electrical signals measured by the galvanometer are displayed in the digital form.

Uses of colorimeter

It is used in laboratories & hospitals to estimate biochemical sample such as urine, Plasma. Serum, etc

* It is used in manufacturing of paints.
* It is used in textile & food industry.
* It is used in quantitative analysis of protein glucose, & other biochemical compounds.
* It is used to test water quality
* It is used to determination concentration of haemoglobin in the blood.

Advantages & disadvantages of colorimeter

→ It is an inexpensive method
→ Widely used in quantitative analysis of coloured samples, easy to carry & transport.

→ Analysis of colourless compounds is not possible, does not work in IR & UV regions

-: LIQUID CHROMATOGRAPHY :-

LIQUID CHROMATOGRAPHY:

Liquid chromatography (LC) is a powerful and widely used analytical technique that plays a crucial role in separa-ting, identifying, and quantifying components in a mixture. It is a type of chromatographic method and the stationary phase can be either solid or liquid. LC is employed in various fields, including chemistry, biology, pharmaceuticals, environmental sciences, food analysis and many others.

PRINCIPLE OF LIQUID CHROMATOGRAPHY:

The principle behind liquid chromatography is based on the differential partitioning of analytes (the substances being separated) between a stationary phase and a mobile phase. The stationary phase is a solid material packed into a column or coated on a surface, while mobile phase is a liquid solvent that carries the sample through the column.

When the sample is injected into the chromatographic system and the mobile phase is allowed to flow, the components of the sample interact differently with the stationary phase. Some components will have stronger interactions and will be retained longer on the stationary phase, while others will have weaker interactions and will have weaker interactions and will move faster with the mobile phase.

The differential reaction causes the components to separate as they elute (come out) from the column at different times. The time it takes for a component to elute is known as the retention time, and it is used to identify and quantify the substances present in the sample.

WORKING NATURE OF LIQUID CHROMATOGRAPHY:

Liquid chromatography works by passing a liquid sample through a column containing, work the stationary phase. As the sample flows through the column, the components of the sample interact with the stationary phase to varying degrees, leading to their separation. The mobile phase, often a solvent, continuously carries the sample through the column at a controlled flow rate

1. COMPONENTS OF CHROMATOGRAPHY:

* STATIONARY PHASE:

It is a solid or liquid material fixed in a column through which the sample will pass. The stationary phase interacts differently with different components of the sample.

* MOBILE PHASE:

It is a liquid solvent that carries the sample through the stationary phase. The choice of the

mobile phase depends on the type of liquid chromato-graphy being used.

2. SAMPLE INJECTION:

A small volume of the sample is injected into the liquid chromatography system.

3. SAMPLE SEPARATION:

As the mobile phase flows through the stationary phase, the components of the sample interact differen-tly with it.

Components that have stronger interactions with the stationary phase will move more slowly through the column, while those with weaker interactions will move faster.

4. DETECTION:

As each component elutes (comes out) of the column, it passes through a dectector that measures its concentration.

The detector generates a signal that corresponds to the concentration of each component.

5. DATA ANALYSIS:

The data obtained from the detector is analyzed to identify and quantify the components in the sample.

The retention times (time taken for a component to elute from the column) and peak areas are used for identification and quantification.

APPLICATIONS AND USES OF LIQUID CHROMATOGRAPHY:

1. PHARMACEUTICALS:

For analyzing drug purity, identifying impurities, and testing drug formulations.

2. ENVIRONMENTAL ANALYSIS:

Detecting and quantifying pollutants in water, soil and air samples.

3. FOOD AND BEVERAGE INDUSTRY:

Ensuring food safety by detecting contaminants and additives in food products.

4. FORENSIC SCIENCE:

Analyzing biological samples like blood and urine for drug testing and identifying unknown compounds.

5. BIOCHEMISTRY AND BIOTECHNOLOGY:

Separating and analyzing proteins, nucleic acids, amino acids and other biomolecules.

6. QUALITY CONTROL:

Used in industries to monitor and ensure the quality of products during manufacturing processes.

7. RESEARCH AND DEVELOPMENT:

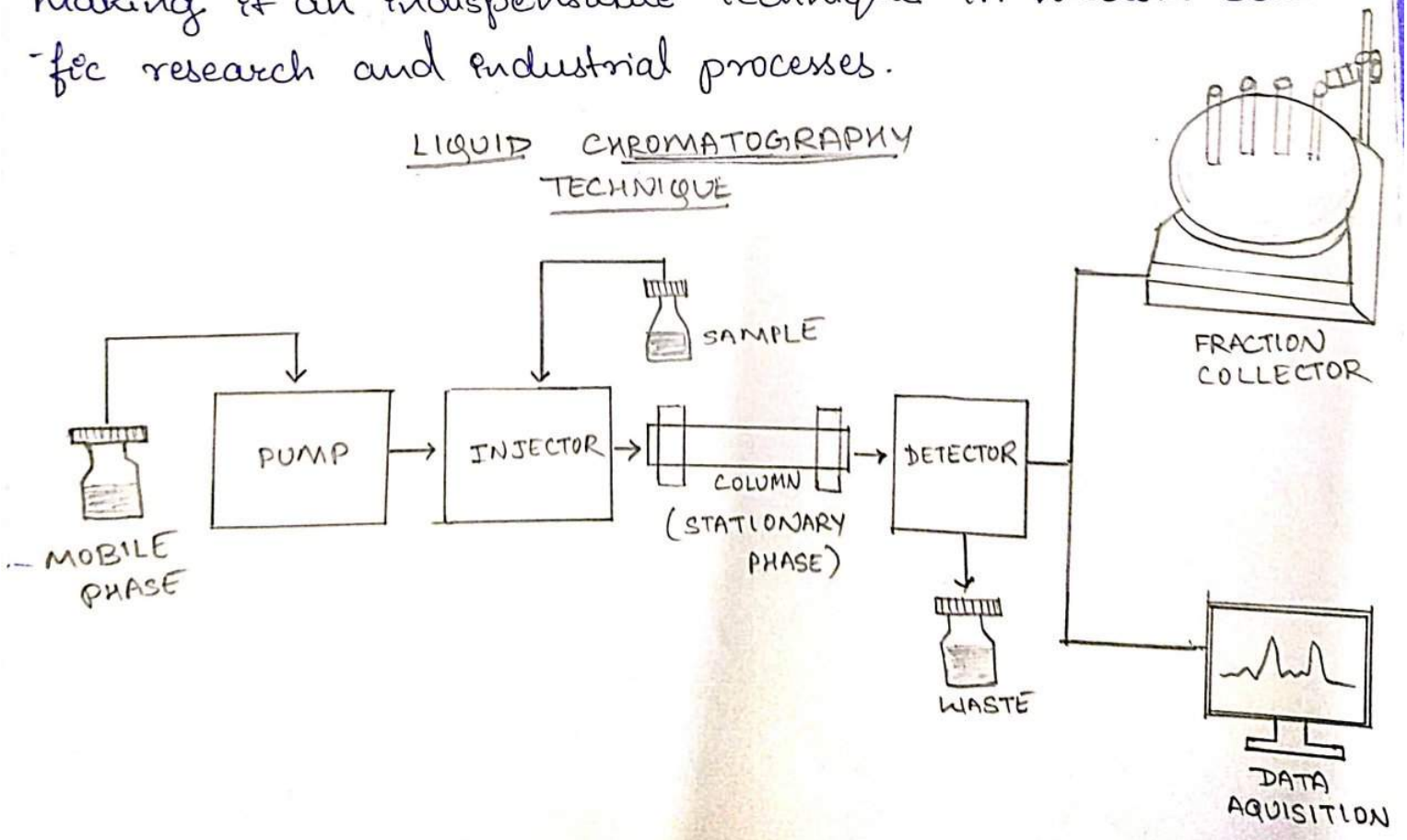
For characterizing and purifying compounds in the labaratories.

8. CLINICAL DIAGNOSIS:

Used in medical labaratories for testing patient samples to diagnose diseases and monitor health conditions.

Overall, liquid chromatography is a powerful analytical toal with a wide range of applications, making it an indispensable technique in modern scientific research and industrial processes.

LIQUID CHROMATOGRAPHY TECHNIQUE



SOXHLET APPARATUS

INTRODUCTION.

A Soxhlet Extractor is a piece of laboratory apparatus invented in 1879 by Franz Von Soxhlet, It is originally designed for the extraction of a lipid from a solid material. Soxhlet extraction is used for when the desired compound has a limited solubility in a solvent and the impurity insoluble in that solvent.

PRINCIPLE.

Soxhlet extractor extracts the components using the condensed vapours of the solvent, The condensed vapours come in contact with the sample powder and the soluble part in the powder gets mixed with the solvent.

WORKING NATURE.

First we need to understand that the desired component from the sample powder must be soluble in the solvent at high temperatures, then only we would be able to seperate it from other components, It's okay if other components are soluble too because we can separate them later.

- First we turn on the heat and the hot iron plate gets heated. The Round bottom flask (RBF) which contains our solvent starts boiling.
- The vapours from the RBF travel from RBF to Condenser via the distillation tube.
- The Condenser Condenses the vapour of solvent and those Condensed vapour fall down to thimble.
- We put our sample powder inside the thimble. The powder has to be covered from the bottom with the filter paper To avoid power directly falling into the thimble. And also cover the powder from the top.
- So, when the Condensed Vapors fall into the thimble, the powder gets wet with the solvent and the components which are soluble in the solvent get mix along with it.
- Siphon tube connects the thimble to RBF. The solvent mixture starts boiling filling thimble and siphon starts overflowing Under the influence of gravity in the RBF it falls back to RBF.
- Since the siphon directly connects RBF. the overflowed liquid falls back to RBF.

This marks the first cycle, as I mentioned earlier. we can perform as many cycles as we want, but only 1 sample at a time

- One thing to mention is we don't change the solvent for every cycle, that when the solvent vaporizes, the components from the sample do not get vaporized. So. each time we get 100% pure solvent vapour.

- When we think that we have exhausted the sample sufficiently, we stop the cycles. Now we have the mixture of solvent & the components from the sample which are soluble in that solvent
- After that filteration we separate the oil from the solvent by filter paper to get According to Soxhlet Extractor it takes maximum 72 cycles for about 8 hours for complete Efficient oil Extration

CONSTRUCTION ASSEMBLY

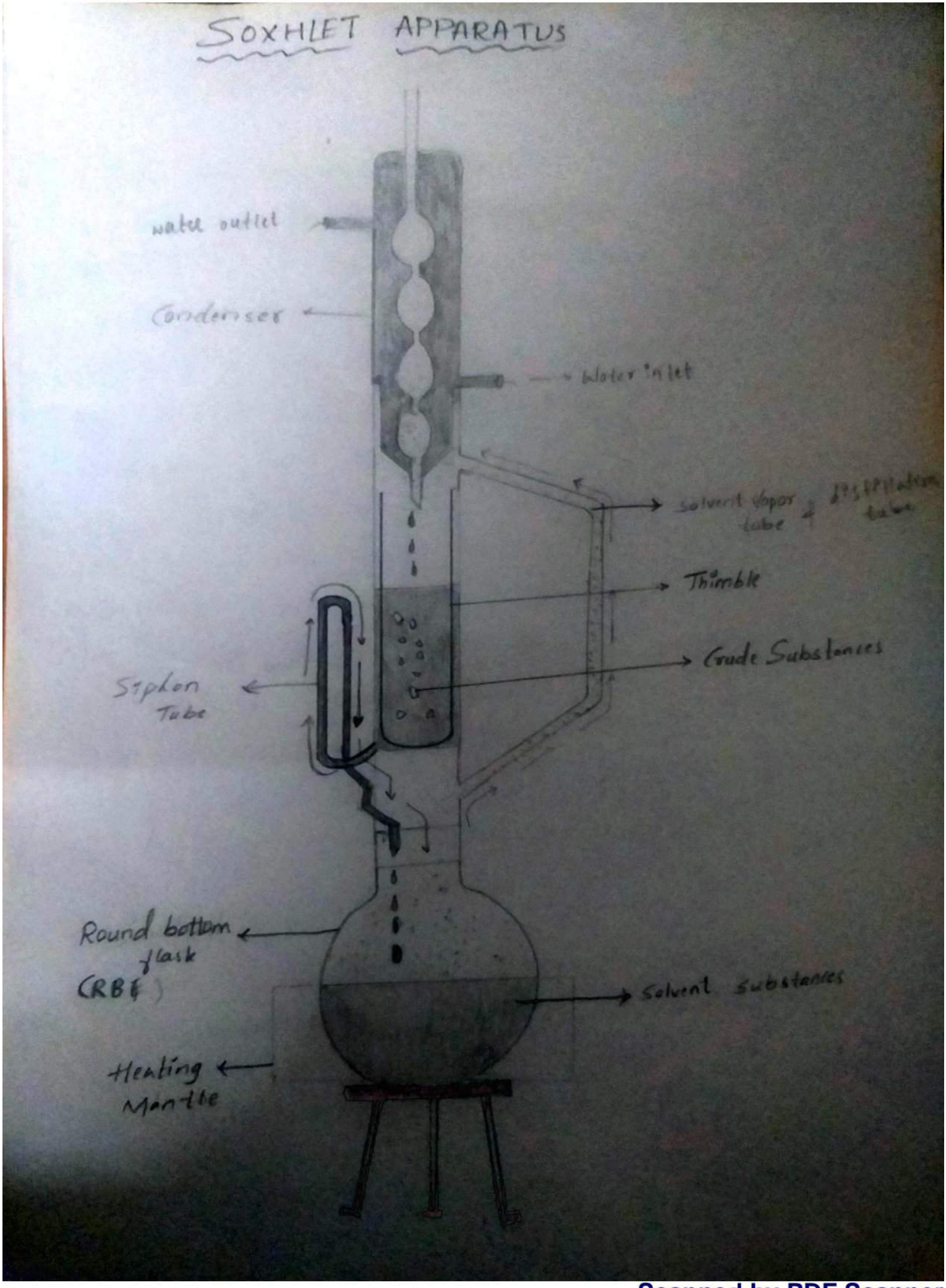
- As shown in the figure, it has a hot plate of iron, to heat the round bottom flask containing solvent
- We place RBF on the top of that hot plate. The distillation tube connects RBF & Condenser.

For better understanding before looking at the middle part, See the ~~your~~ Upper part first. It has a condensation assembly It has a way to allow cool water in and out

Now, the middle part 'Thimble' connects to the condensation assembly

One more arm connects the thimble and RBF we call this arm Siphon tube.

SOXHLET APPARATUS



ADVANTAGES

- Efficient and Continuous Extraction.
- It needs less solvent to yield Concentrated extract.
- It needs less we can Continue the process until the powder gets Completely exhausted. Due to which extration Efficiency is much greater than the traditional extractor.
- We can use modified Soxhlet extractors to meet different needs and increase efficiency further.
- By modifying certain things, we can use the soxhlet extractor on the industry level.
- It is semple & clear design.
- The production process is continuity.
- Ease of visual monitoring the process.
- A low flow of solvent & the possibility of its we use after stripping & distillation.
- It need less solvent to yield concentrated extract.

DISADVANTAGES

- Extraction by Soxhlet is only possible with boiling solvents.
- we Cannot extract from more that one sample at a time long operation time required.
- Evaporation and Concentration is needed at the end of the extraction.
- The desired Components must be soluble in the solvent at a high temperature.

Applications

- In food Industry for Analysing food components and food testing
- Helps in Extracting lipids in foods.
- Biofuel Extraction from coffee Beans.
- Extraction of Caffience from Beverages Tea plants using Soxhlet method.
- Peperine Extraction from black papper
- Enviromental analysis of soils, sludge & wastes the Soxhlet method is used

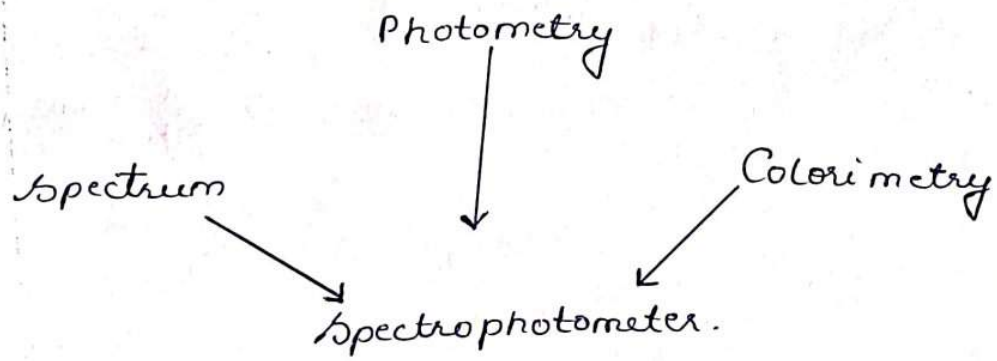
SPECTOPHOTOMETER

DEFINITION:-

A spectrophotometer is an instrument that measures the amount of light absorbed by a sample.

→ A scientist named Arnold J. Beckman and his colleagues at the National Technologics Laboratory (NTL) invented the Beckman DU spectrophotometer in 1940.
→ Results come through simple process within few minutes
→ Results were 99.99% accurate.

MEANING OF SPECTROPHOTOMETER



→ Uses a type of light to detect molecules in a solution.
→ Light is a type of energy and the energy is reported as wavelengths in nanometers (nm).

TYPES OF SPECTROPHOTOMETER.

1) Ultraviolet (UV) Spectrophotometers : Uses ultraviolet light of wavelength from 200nm - 350nm.
2) Visible (VLS) Light spectrum Spectrophotometers : Uses visible light (white light) of wave lengths from 350 nm - 700 nm.

WORKING PRINCIPLE OF SPECTROPHOTOMETER.

→ When a beam of monochromatic light passed through a solution it may transmitted as a such or some of may be absorbed.

→ Proportional the transmitted light can be respresen-ted by intensity of the incident radiation.

$$T = I / IO$$

Absorbance (A) of light through a solution in inverse-ly proportional to log 10 of % T.

$$A = \log (I/T)$$
$$= \log (I/IO)$$

where, I = Intensity of Transmitted light
IO = Intensity of incident light.

* The quantitative determination of compounds by spectrometeric techniqie is based on two law.

1) Lamberts Law

2) Beers Law.

1) Lambert's Law.

It state that light absorbed by solution is direct-ly proportional to length of the light through the solution.

$$A = Log\ (I_0/I) = E.C.I$$

where,

A = Absorbance

E = Molar absorptivity co-efficient

I = Path length of the sample

C = Concentration of solution

* Calculating the absorbance of a sample using the equation depends on the absorbance is directly proportional to the path length of the sample.

2) Beer's Law.

It state the amount of light absorbed is directly proportional to concentration of absorbing solute in the solution.

$$A = \log (I_0/I) = E.C.I.$$

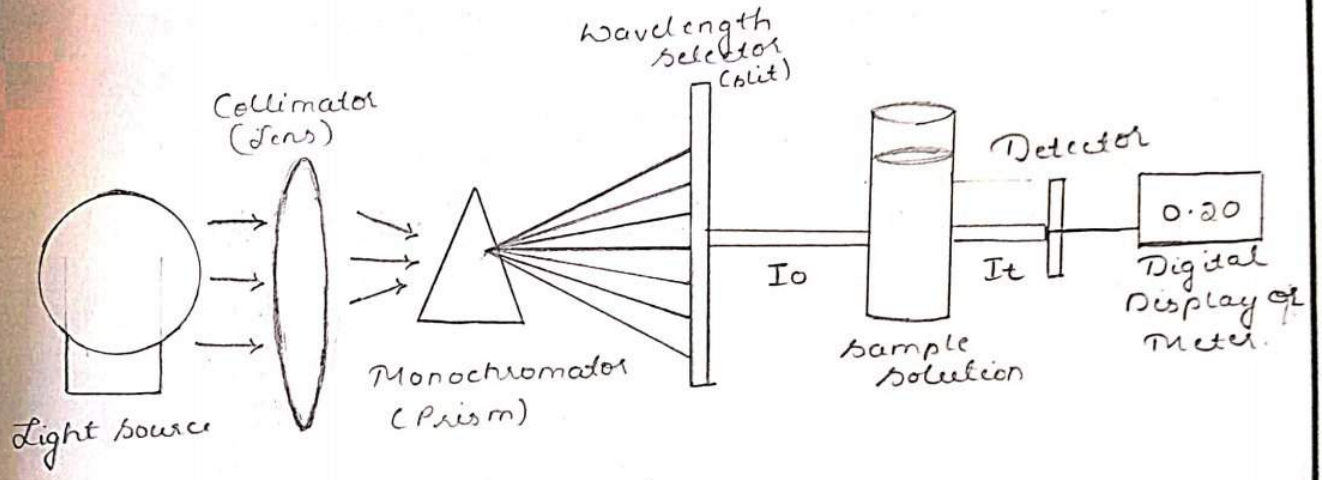
where, C = Concentration of solution mole per liter.

E = Molar absorptivity co-efficient

I = Path length of the sample.

* Calculating the absorbance of a sample using the equation depends on the absorbance is directly proportional to the concentration of the sample.

HOW A SPECTROPHOTOMETER WORKS



1) White light hits the prism or grating, it is split into the colors of the rainbow.

2) The wavelength knob rotates the prism / grating directing different color of light toward the sample.

3) The wavelength of light produced by the tungsten lamp range from about 350 nm - 700 nm.

4) The molecules in the sample either absorb or Transmit the light energy of one wavelength or another.

5) The detector measures the amount of light being transmitted by the sample and reports that value directly or converts it to the amount of light absorbed in absorbance units using Beer's law.

APPLICATIONS:

→ Determines the presence and concentration of samples.
→ Determines the purity of a sample.
→ Look at the change of sample over time.
→ Molecular weight determination of compounds.
→ Respiratory gas analysis in hospitals.
→ The visible and UV spectrophotometer may be used to identify classes of compounds in both the pure state and in biological preparations.

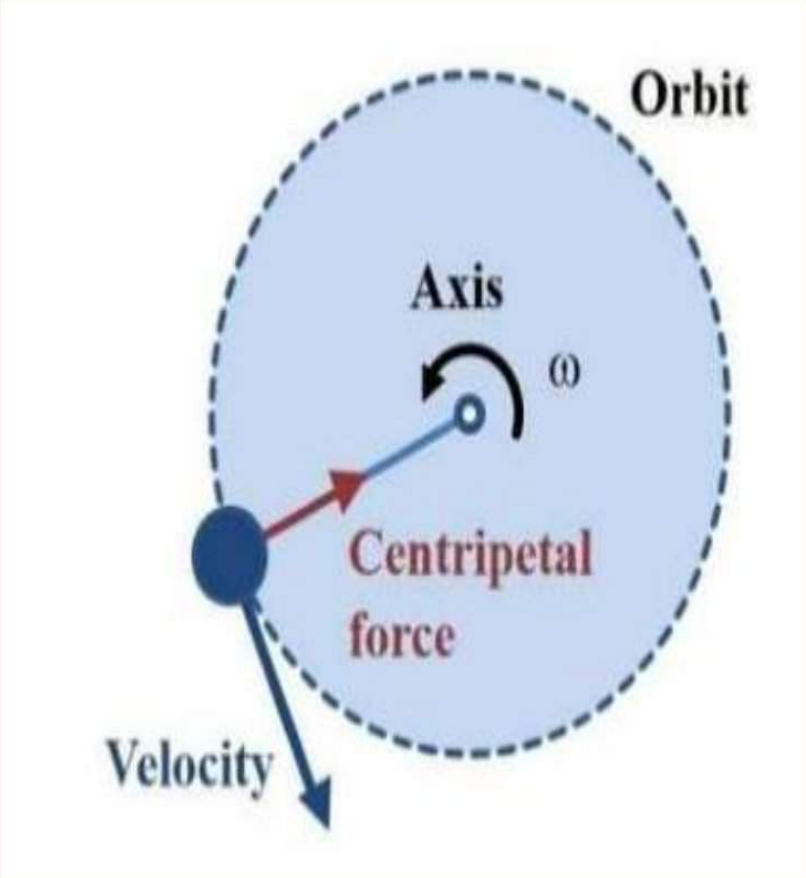
ADVANTAGES - Incredibly powerful & can offer more in depth measurements than a colorimeter.

DISADVANTAGES - High cost, low sensitivity due to poor light throughput, low reliability due to more complexity.

Centrifugation- Principle, Types and Applications

PG Sem II
Paper CC6
Unit IIA

Definition of Centrifugation



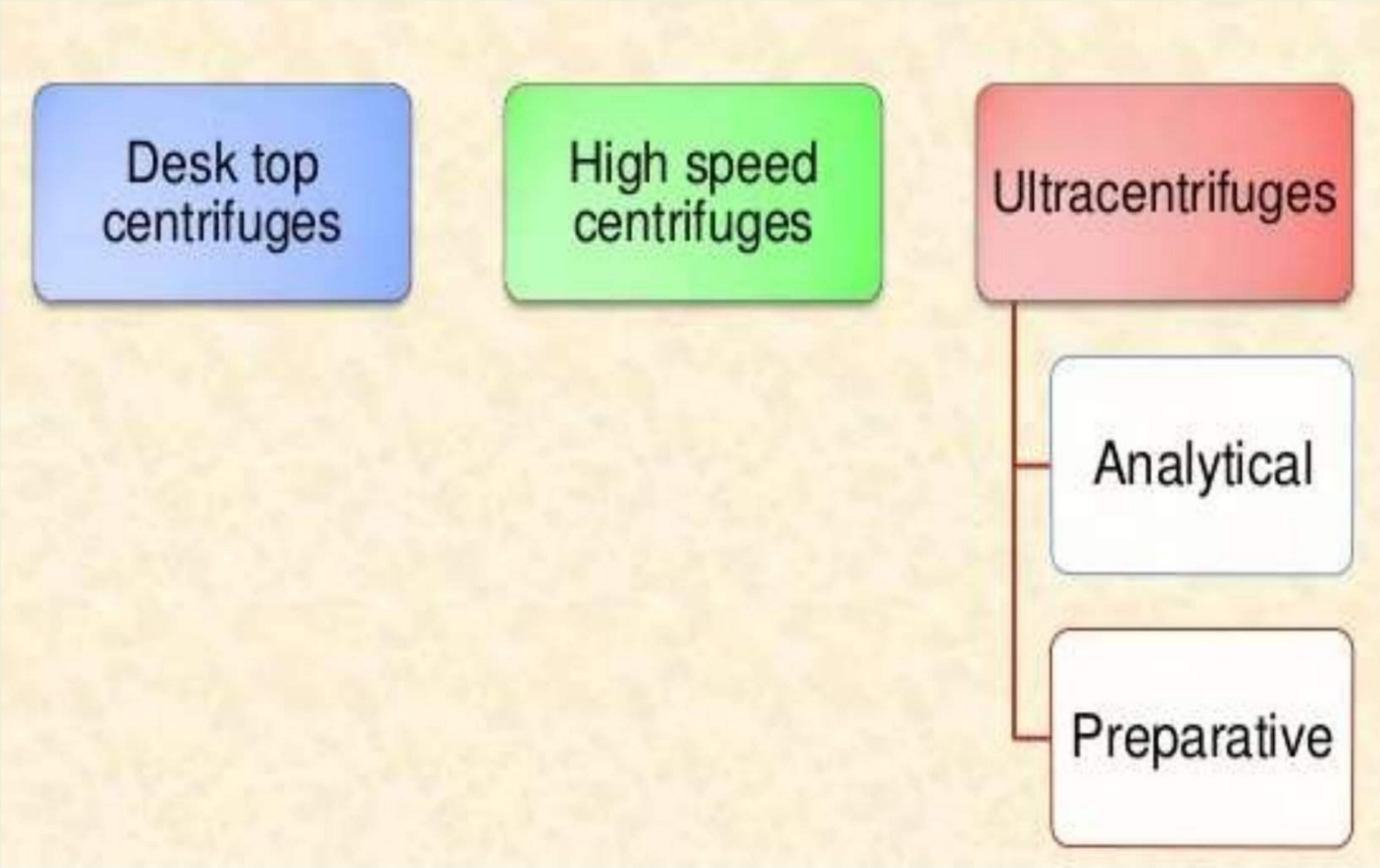
- Centrifugation is a technique of separating substances which involves the application of centrifugal force.The particles are separated from a solution according to their size, shape, density, the viscosity of the medium and rotor speed.

Principle of Centrifugation

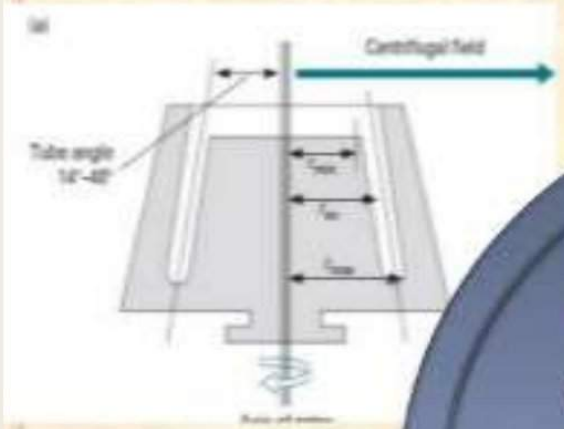
- In a solution, particles whose density is higher than that of the solvent sink (sediment), and particles that are lighter than it floats to the top.
- The greater the difference in density, the faster they move. If there is no difference in density (isopycnic conditions), the particles stay steady.
- To take advantage of even tiny differences in density to separate various particles in a solution, gravity can be replaced with the much more powerful "centrifugal force" provided by a centrifuge.
- A centrifuge is a piece of equipment that puts an object in rotation around a fixed axis (spins it in a circle), applying a potentially strong force perpendicular to the axis of spin (outward).

- The centrifuge works using the sedimentation principle, where the centripetal acceleration causes denser substances and particles to move outward in the radial direction.
- At the same time, objects that are less dense are displaced and move to the center.
- In a laboratory centrifuge that uses sample tubes, the radial acceleration causes denser particles to settle to the bottom of the tube, while low- density substances rise to the top.

Types of Centrifuge

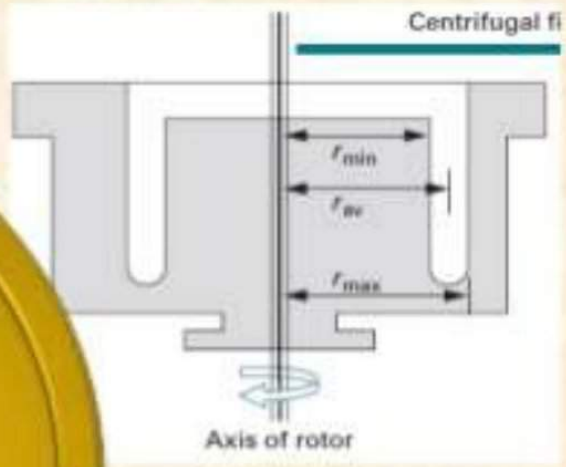


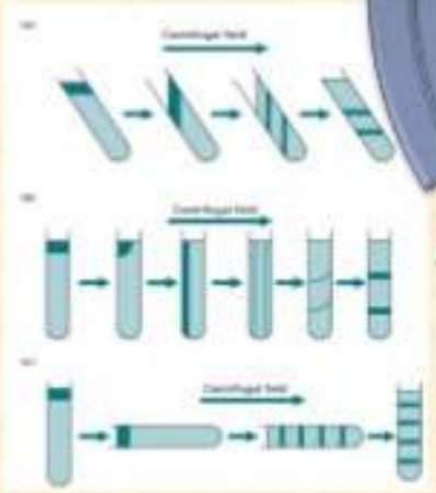
Types of rotor



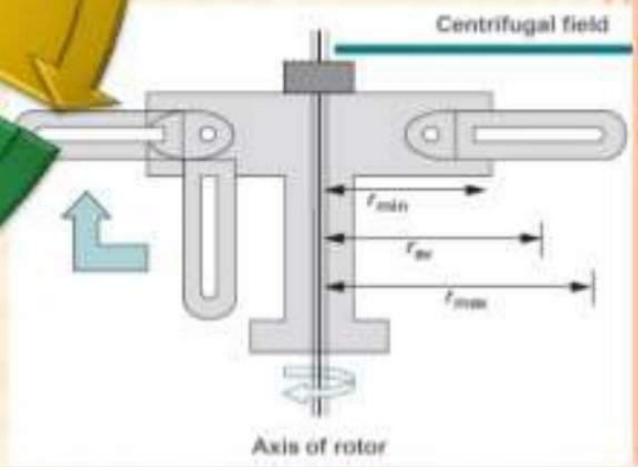
Fixed angle rotors

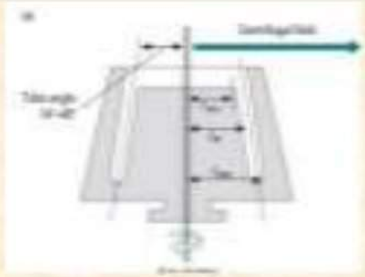
Vertical tube rotors



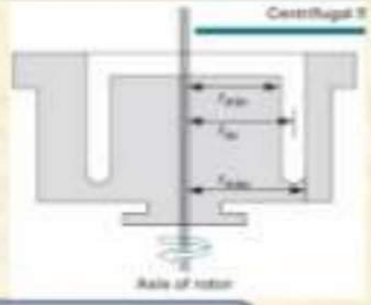


Swinging-bucket rotors





Types of rotor



Fixed angle rotors

- Tubes are held at angle of 14 to 40^0 to the vertical.
- Particles move radially outwards, travel a short distance.
- Useful for differential centrifugation
- Reorientation of the tube occurs during acceleration and deceleration of the rotor.

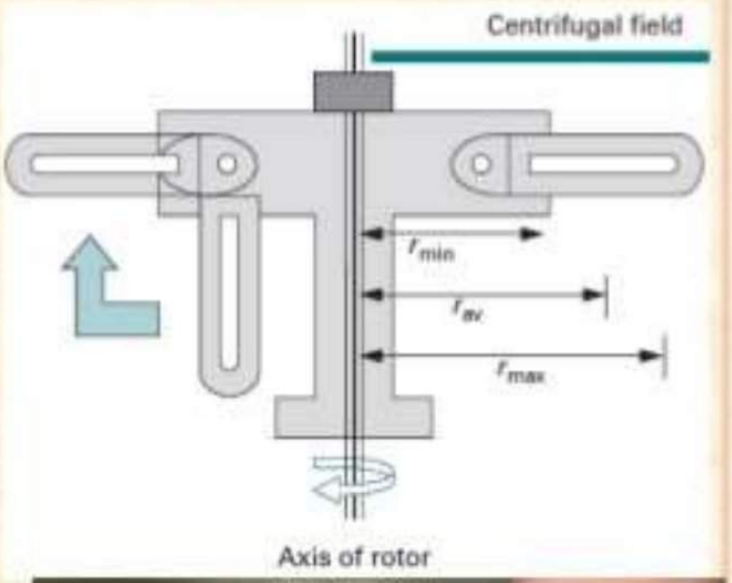
Vertical tube rotors

- Held vertical parallel to rotor axis.
- Particles move short distance.
- Time of separation is shorter.
- Disadvantage: pellet may fall back into solution at end of centrifugation.

Types of rotor

Swinging-bucket rotors

- Sing out to horizontal position when rotor accelerates.
- Longer distance of travel may allow better separation, such as in density gradient centrifugation.
- Easier to withdraw supernatant without disturbing pellet.
- Normally used for density-gradient centrifugation.



LOW-SPEED CENTRIFUGE

1) Most laboratories have a standard low-speed centrifuge used for routine sedimentation of heavy particles
2) The low-speed centrifuge has a maximum speed of 4000-5000rpm
3) These instruments usually operate at room temperatures with no means of temperature control.
4) Two types of rotors are used in it,

- Fixed angle
- Swinging bucket.

5) It is used for sedimentation of red blood cells until the particles are tightly packed into a pellet and supernatant is separated by decantation.

HIGH-SPEED CENTRIFUGES

- High-speed centrifuges are used in more sophisticated biochemical applications, higher speeds and temperature control of the rotor chamber are essential.
- The high-speed centrifuge has a maximum speed of 15,000 – 20,000 RPM
- The operator of this instrument can carefully control speed and temperature which is required for sensitive biological samples.
- Three types of rotors are available for high-speed centrifugation-

- Fixed angle
- Swinging bucket
- Vertical rotor

High speed centrifuges

- Maximum speed of 25000rpm, providing 90000g centrifugal forces.
- Equipped with refrigeration to remove heat generated.
- Temperature maintained at 0-4^{0}C by means of thermocouple.
- Used to collect microorganism, cell debris, cells, large cellular organelles, precipitates of chemical reactions.
- Also useful in isolating the sub-cellular organelles(nuclei, mitochondria, lysosomes)

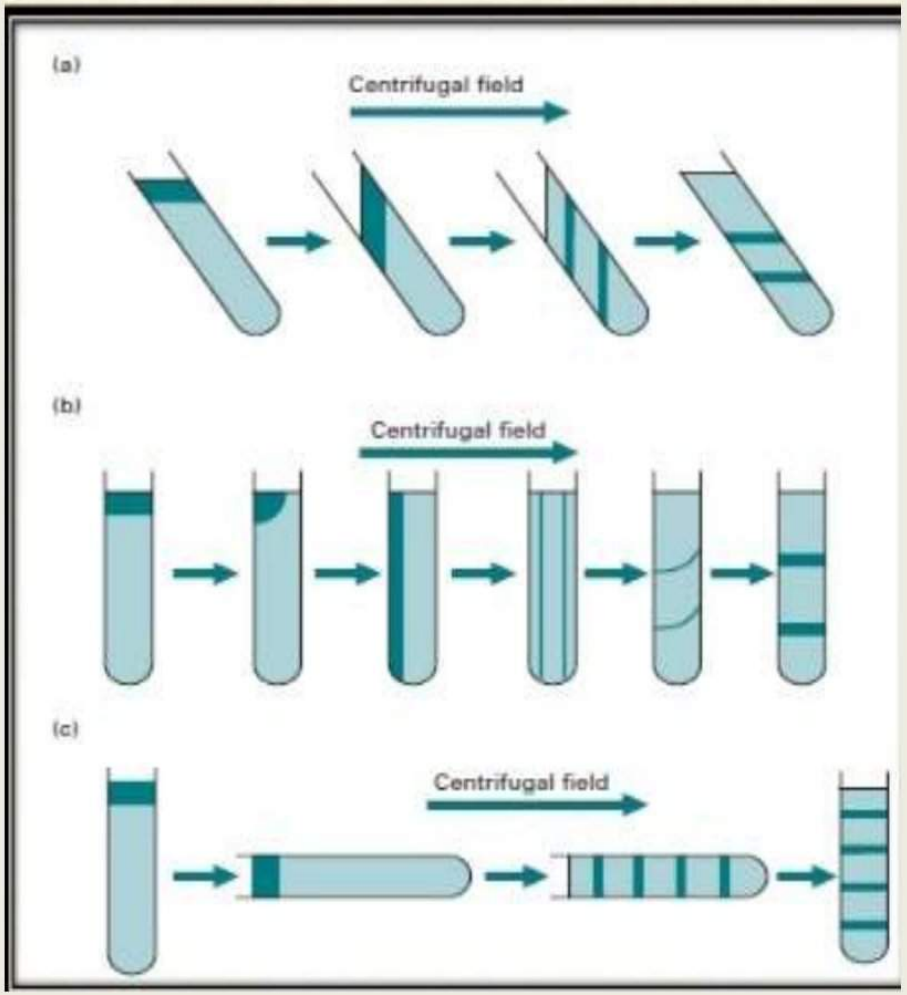
ULTRACENTRIFUGES

- It is the most sophisticated instrument.
- Ultracentrifuge has a maximum speed of 65,000 RPM (100,000's x g).
- Intense heat is generated due to high speed thus the spinning chambers must be refrigerated and kept at a high vacuum.
- It is used for both preparative work and analytical work

Ultracentrifuges

- Operate at speed of 75,000rpm, providing the centrifugal force of 500,000g.
- Rotor chamber is sealed and evacuated by pump to attain vacuum.
- Refrigeration system (temp 0-4^{0}C).
- Rotor chamber is always enclosed in a heavy armor plate.
- Centrifugation for isolation and purification of components is known as preparatory centrifugation, while that carried out with a desire for characterization is known as analytical centrifugation.

Types of Centrifugation



Preparative centrifugation

- Is concerned with the actual isolation of biological material for subsequent biochemical investigations.
- Divided into two main techniques depending on suspension medium in which separation occur.
  - Homogenous medium – differential centrifugation
  - Density gradient medium – density gradient centrifugation

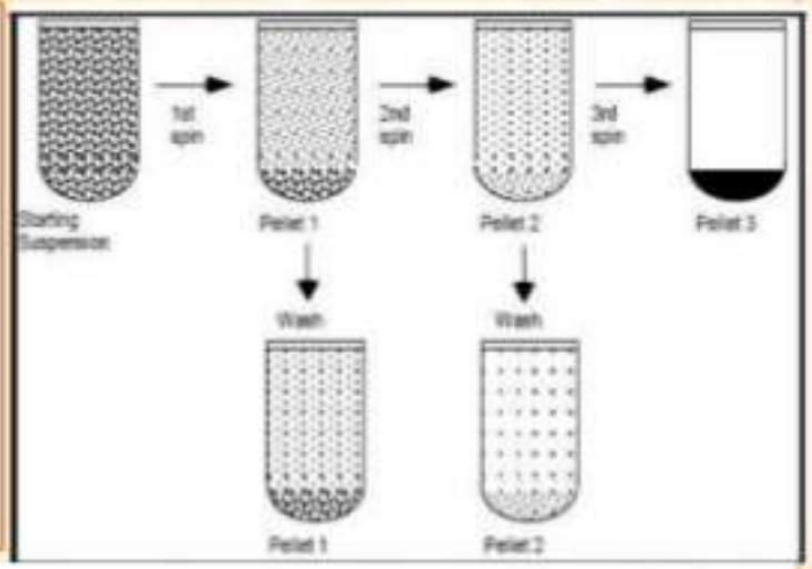
1. Differential centrifugation

- Separation is achieved based in the size of particles in differential centrifugation.
- Commonly used in simple pelleting and obtaining the partially pure separation of subcellular organelles and macromolecules.
- Used for study of subcellular organelle, tissues or cells (first disrupted to study internal content)

It is the most common type of centrifugation employed. Tissue such as the liver is homogenized at 32 degrees in a sucrose solution that contains buffer.

The homogenate is then placed in a centrifuge and spun at constant centrifugal force at a constant temperature. After some time a sediment forms at the bottom of a centrifuge called pellet and an overlying solution called supernatant. The overlying solution is then placed in another centrifuge tube which is then rotated at higher speeds in progressing steps.

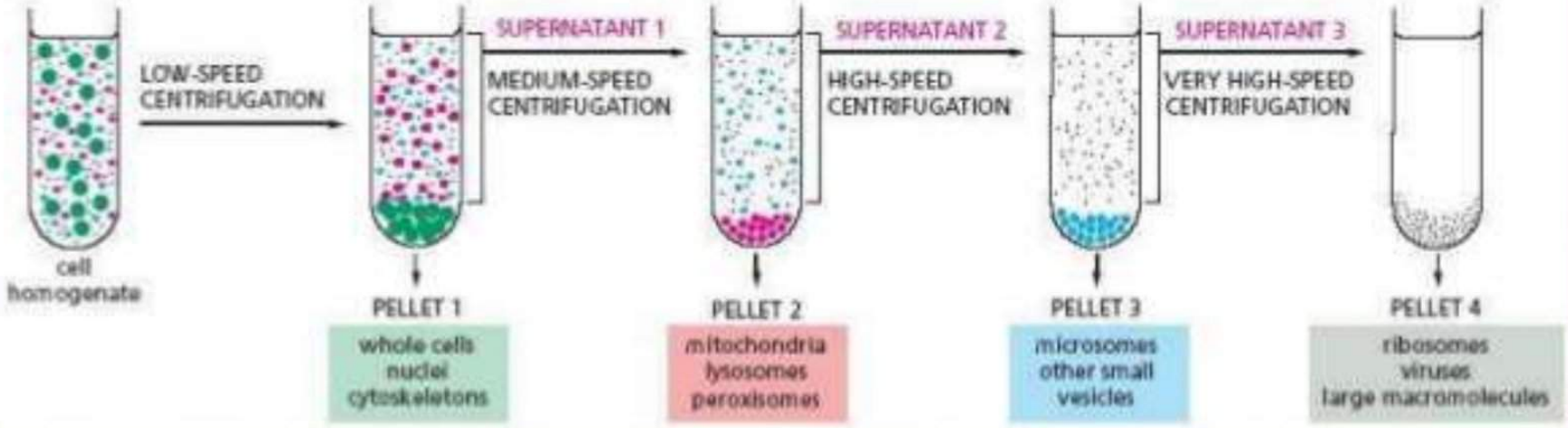
- During centrifugation, larger particles sediment faster than the smaller ones.
- At a series of progressive higher g-force generate partially purified organelles.



DIFFERENTIAL CENTRIFUGATION

Repeated centrifugation at progressively higher speeds will fractionate cell homogenates into their components.

Centrifugation separates cell components on the basis of size and density. The larger and denser components experience the greatest centrifugal force and move most rapidly. They sediment to form a pellet at the bottom of the tube, while smaller, less dense components remain in suspension above, a portion called the supernatant.



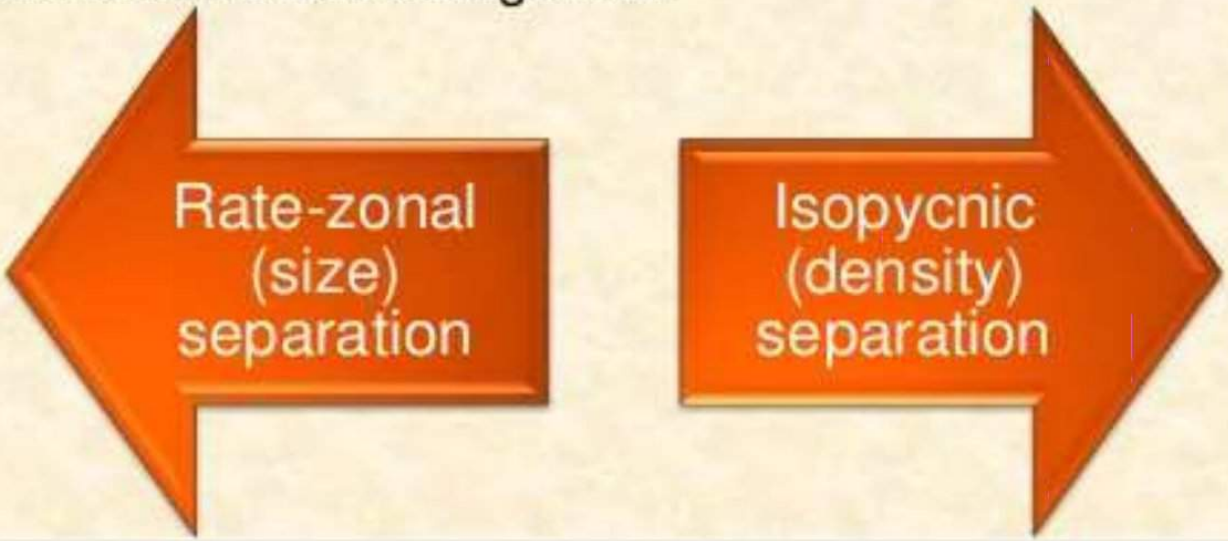
- Inspite of its reduced yield differential centrifugation remains probably the most commonly used method for isolation of intracellular organelle from tissue homogenates because of its;
  - relative ease
  - Convenience
  - Time economy
- Drawback is its poor yield and fact that preparation obtained never pure.

Density Gradient Centrifugation

- This type of centrifugation is mainly used to purify viruses, ribosomes, membranes, etc.
- A sucrose density gradient is created by gently overlaying lower concentrations of sucrose on higher concentrations in centrifuge tubes
- The particles of interest are placed on top of the gradient and centrifuge in ultracentrifuges.
- The particles travel through the gradient until they reach a point at which their density matches the density of surrounding sucrose.
- The fraction is removed and analyzed.

2. Density gradient centrifugation

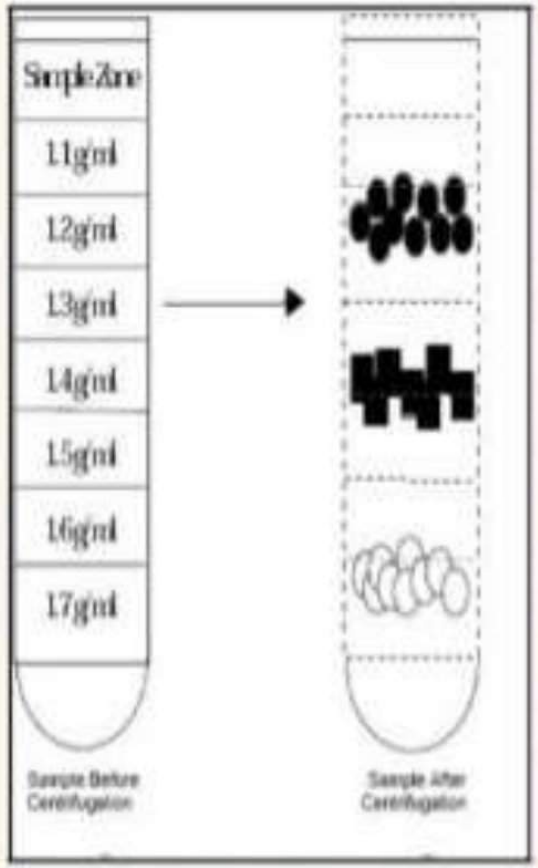
- It is the preferred method to purify subcellular organelles and macromolecules.
- Density gradient can be generated by placing layer after layer of gradient media such as sucrose in tube, with heaviest layer at the bottom and lightest at the top in either.
- Classified into two categories:



- Gradient material used are:
  - Sucrose (66%, 5^0C)
  - Silica sols
  - Glycerol
  - CsCl
  - Cs Acetate
  - Ficol (high molecular wgt sucrose polymer & epichlorhydrin)
  - Sorbitol
  - Polyvinylpyrrolidone

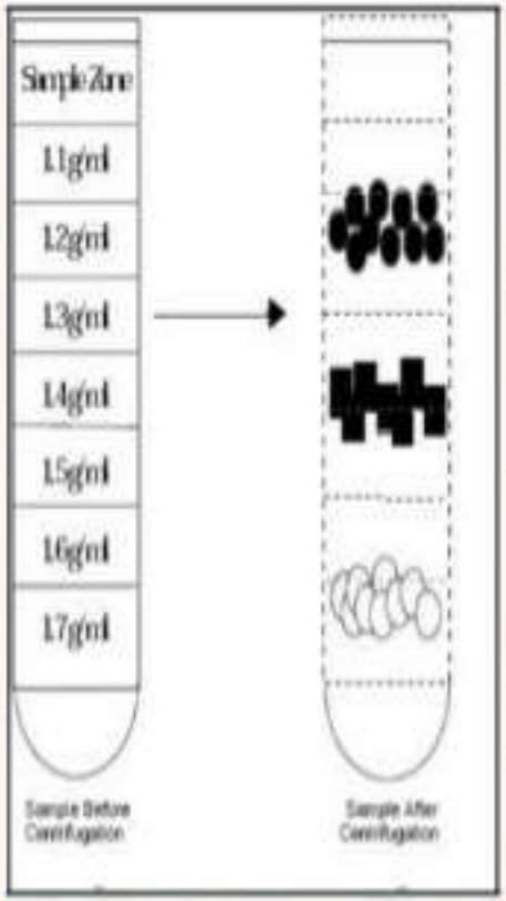
2.1 Rate zonal centrifugation

- Gradient centrifugation.
- Take advantage of particle size and mass instead of particle density for sedimentation.
- Ex: for common application include separation of cellular organelle such as endosomes or proteins (such as antibodies)



2.1 Rate zonal centrifugation

- Criteria for successful rate-zonal centrifugation:
  - Density of sample solution must be less than that of the lowest density portion of the gradient.
  - Density of sample particle must be greater than that of highest density portion of the gradient.
  - Path length of gradient must be sufficient for the separation to occur.
  - Time is important, if you perform too long runs, particles may all pellet at the bottom of the tube.



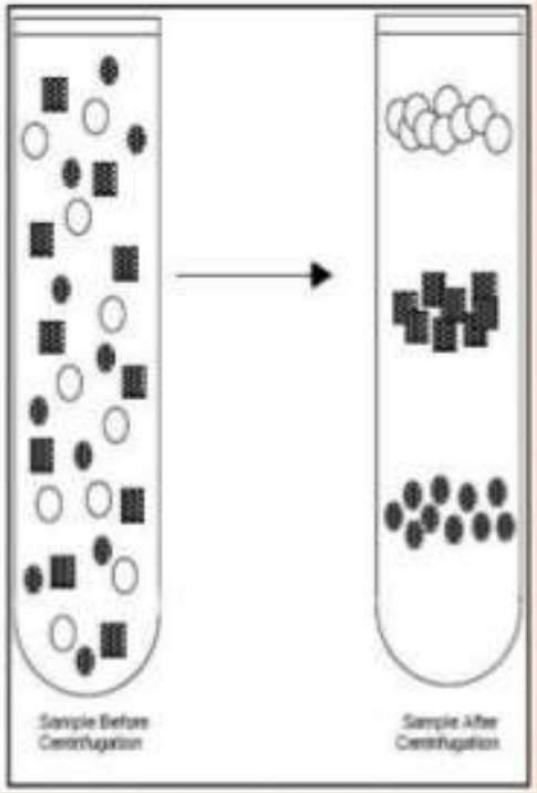
Rate-Zonal Density-Gradient Centrifugation

- Zonal centrifugation is also known as band or gradient centrifugation
- It relies on the concept of sedimentation coefficient (i.e. movement of sediment through the liquid medium)
- In this technique, a density gradient is created in a test tube with sucrose and high density at the bottom.
- The sample of protein is placed on the top of the gradient and then centrifuged.
- With centrifugation, faster-sedimenting particles in sample move ahead of slower ones i.e. sample separated as zones in the gradient.
- The protein sediment according to their sedimentation coefficient and the fractions are collected by creating a hole at the bottom of the tube.

2.2 Isopycnic centrifugation

- Particle of a particular density will sink during centrifugation until a position is reaches where the density of the surrounding solution is exactly the same as the density of the particle.
- Once quasi-equilibrium is reached, the length of centrifugation doesnot have any influence on the migration of particle.
- Ex: separation of Nucleic acid in CsCl (Caseium chloride) gradient.

Figure 3. ISOPYCNIC (DENSITY) SEPARATION



Isopycnic Centrifugation

- The sample is loaded into the tube with the gradient-forming solution (on top of or below pre-formed gradient, or mixed in with self-forming gradient)
- The solution of the biological sample and cesium salt is uniformly distributed in a centrifuge tube and rotated in an ultracentrifuge.
- Under the influence of centrifugal force, the cesium salts redistribute to form a density gradient from top to bottom.
- Particles move to point where their buoyant density equals that part of gradient and form bands. This is to say the sample molecules move to the region where their density equals the density of gradient.
- It is a "true" equilibrium procedure since depends on bouyant densities, not velocities
- Eg: CsCl, NaI gradients for macromolecules and nucleotides – "self-forming" gradients under centrifugal force.

	Rate-Zonal	Isopycnic
Synonym	S-zonal, sedimentation velocity	Density equilibrium, sedimentation equilibrium
Gradient	•Shallow, •Maximum gradient density less than the least dense sedimenting specie, •Gradient continuous.	•Steep, •Maximum gradient density greater than that of the most dense sedimenting specie, •Continuous or discontinuous gradients.
Centrifuga-tion	•Incomplete sedimentation, •Low speed, •Short time	•Complete sedimentation till equilibrium is achieved, •High speed, • Long time.
Separation	RNA- DNA hybrids, ribosomal subunits, etc.,	DNA, plasma lipoproteins, lysosomes, mitochondria, peroxisomes, etc.,

Applications of Centrifugation

- To separate two miscible substances
- To analyze the hydrodynamic properties of macromolecules
- Purification of mammalian cells
- Fractionation of subcellular organelles (including membranes/membrane fractions) Fractionation of membrane vesicles
- Separating chalk powder from water
- Removing fat from milk to produce skimmed milk
- Separating particles from an air-flow using cyclonic separation
- The clarification and stabilization of wine
- Separation of urine components and blood components in forensic and research laboratories
- Aids in the separation of proteins using purification techniques such as salting out, e.g. ammonium sulfate precipitation

Definition of Refrigeration

It is defined as:

"The method of reducing temperature of a system below surrounding temperature and maintains it by continuosly abstracting heat from it.

Definition of Refrigerator:

The device used to maintain the low temperature within required space is called refrigerator.

Principle of Refrigeration:

→ In refrigeration heat is removed continuously from a system at a lower temperature.

→ And transfer to the surrounding at a higher temperature.

→ According to second low of thermodynamics, external work is required to transfer heat from cold body to hot body.

→ Therefore in refrigeration, power is consumed to cool the space below the atmospheric temperature.

Aplication of Referigeration

We can keep food in this referigerator

1) storage & transportation of food like fruits, Vegetables, Fish, dairy products etc.

ex we can keep medicine & syrups also in the refer

2) Preservation of medicine & syrups.

3) Manufacturing of ice & photographic films, cooling of water etc....

4) comfirt air conditioning of auditorium, hospitals, offices, Vehicles etc....

5) Processing of petroleum & other chemical products

6) Liquification of gases like N_2, O_2, H_2 etc ....

Referigeration Effect

- The amount of heat absorbed by the referigeration system from the space to be cooled in a given time is called referigeration effect.
- Its unit is KJ/min.

(Refrigerant): what is heat? heat is a form of energy as energy is measured in joules similarly heat would be measured in

• The refrigerant is heat carrying medium which absorbs heat from space by evaporating at low temperature & rejects heat to atmosphere by condensing at a high temperature.

Refrigerants commonly used:

Ammonia, carbon dioxide, air, R11, R12, R22 etc....

Trichloro fluoromethane

Dichlorodi fluoromethane

Chloro difluoro methane

(Properties of good Refrigerant) what are the properties required for a good refrigerant

Thermodynamic properties:

1) It should have low boiling point.

2) It should have low freezing point.

3) It should have high latent heat of vaporization.

Physical properties:

1) It should have low specific volume. (is volume of a unit mass of substance)

2) It should have low liquid specific heat & high vapour specific heat, (The quantity of heat required to rise the temp of 1g of sub by 1°C)

3) It should have high thermal conductivity. (The ability of material to conduct heat from its one side to the other.)

4) It should have low viscosity.

viscosity is the tendency of a fluid to resist any change in its shape or motion.

ex:- Honey is thicker & has much higher viscosity than water.

Chemical Properties:

1) It should be non-toxic
2) It should be non-flammable
3) It should be non-corrosive

Other properties:

1) It should be easily available
2) It should not have any bad impact on ozone layer
3) Leakage should be easily detected.

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HPLC

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

★ **Introduction:**

HPLC, formerly referred to as high-pressure liquid chromatography, is a technique in analytical chemistry used to seperate, identify, and quantify each component in a mixture

It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material.

PRINCIPLE :—

The specific intermolecular interaction btwn the molecules of a sample and the packing material define their time "on-column" Hence, different constituents of a sample are eluted at diff times. Thereby, the seperation of the sample ingredients is achieved.

WORKING NATURE :—

- HPLC consists of a variety of components including a solvent delivery pump, a degassing unit, a sample injector, a column oven, a detector and a data processor

- As for HPLC, the pump delivers the mobile phase at controlled flow rate(s). Air can easily dissolve in the mobile phase under the standard atmospheric pressure in which we live

- If the mobile phase contains air bubbles and enters the delivery pump, troubles such as flow rate fluctuations and baseline noise/drift may occers.
- The degassing unit helps prevent this issue by removing air bubbles in the mobile phrase. (b)
- After the dissolved air has been removed the mobile phase is diliverd to the column.
- The sample injector then introduces a standard solution or sample solution into the mobile phase (c).
- Tempt fluctuations can affect the seporation of compounds in the column.
- The column is placed in a column oven to keep the temperature constant (d)
- Compounds eluted from the column are detected by a detector which is placed downstreams of the column (e).
A workstation processes the signal from the detector to obtain a chromatogram to identify and quantify the compound (f)

Seperation process

To perform a HPLC seperation, the user injects a sample dissolved in a solvent into a packed column. The column is lined with a solid seperating material. As the solvent sample matrix is injected into the column and passes through it, the sample molecules are effectively washed out and seperated by molecular affinity.

USES OF HPLC

- Purification of water
- Impurity detection in the pharmaceutical Industry that
- Trace components, are pre-concentrated
- Chromatography based on ligand exchange
- protein chromatography via ion exchange
- Carbohydrate and oligosaccharide anion-exchange chromatography at high pH.

Applications

- Drug evaluation
- Synthetic polymer analysis
- pollution analysis in environmental analytics
- Drug determination in biological matrices
- Isolation of high-value goods.

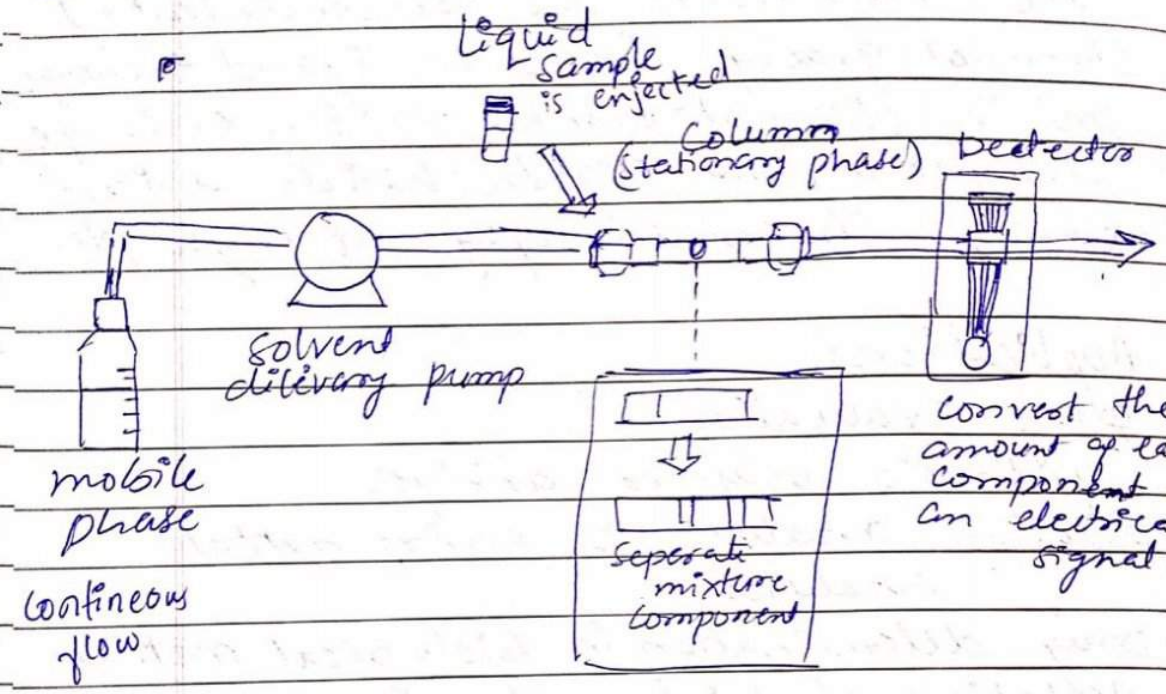
Advantages :—

- HPLC has high resolution & speed of analysis
- High surface area
- It has high pressure gradient.
- It has wide range of stationary phases
- Precise flow rate control.
- Senstive detection methods
- Low sample method requirment.
- Accurate peak identification using HPLC.

Disadvantages :—

- HPLC has high cost
- High quality components are needed
- The solvents and columns used in HPLC are expensive.
- Regular maintainance & calibration is

is needed which add extra cost
- Sophisticated software is required for data analysis
- Research and development cost.



HPLC Diagram.

Gas Chromatography

Introduction :-

It is the an analytical technique used to separate the chemical components of sample mixture & then detect them to determine their presence or absence. It is also used to figure out - how much is present in sample.

Principle :

The sample solution injected into the instrument enters a gas stream which transports the sample into a separation tube known as the column.

Uses :-

⟹ Used in food beverage, flavour & fragrance analysis

⟹ In forensics. It is used in detection of body fluid, for testing of fibers, blood alchol detection of poison.

⟹ Skin sample analysis

⟹ Geochemical search.

* List of components

⟹ Carrier gas

⟹ Flow regulator

⟹ Injector

⟹ Column

⟹ Stationary phase

⟹ Oven

⟹ Detectors

⟹ Display device.

* Carrier gas :-

Carrier gas is an inert gas used to carry Samples. Helium (He), nitrogen (N_2), Hydrogen (H_2) and argon (Ar) are often used. Helium & nitrogen are most commonly used and the use of helium is desirable when using a capillary column.

* Flow regulator :=

Regulates the carrier gas flow in gas chromatography. Constant flow of carrier gas → Column efficiency & reproducible elution time. Magnitude of carrier gas flow rate depends → Types of column.

* Injector :

The Injector contains a heated chamber containing a glass Liner into which the sample is injected through the Septum. The carrier gas enters the chamber & can leave by three routes (when the injector is in split mode). The sample vapourises to form a mixture of carrier gas, vapourised Solvent and vapourised Solutes.

* Columns :

There are 2 types of Columns

1) Packed Columns 2) Capillary Column.

* Packed Phase is coated directly on the column
* Applicable for both GSC and GLC
* Liquid phase is adsorbed onto the surface of the beads in a thin layer or onto the solid inert Packing.

2) Capillary column.

* Stationary phase is coated with the inner wall of the column
* Applicable only for GLC
* Liquid stationary phase is immobilized on the capillary tubing walls.

* Stationary phase:

The stationary phase that are used in GC are primarily silicone based oils with high temperature stability. These stationary phase are available in different types as totally non-polar, mid polar and polar like methyl silicone, methyl Phenyl silicone, methyl cyano propyl silicone, methyl trifluro propyl silicone etc.

* Oven:

Oven is to heat the sample and the stationary Phase in the column to facilitate the separation of the sample components.

* Detectors:

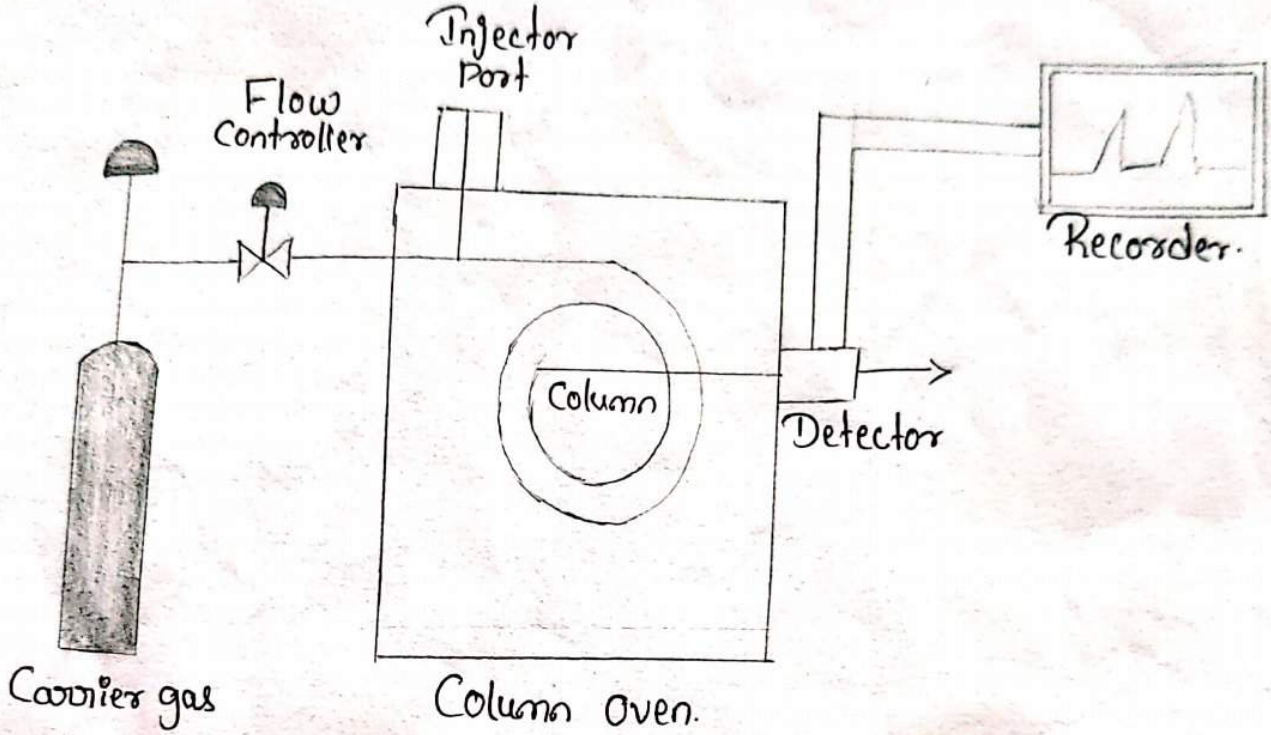
Flame ionization detectors. The FID is the most common detector used in gas chromato-graphy. The FID is sensitive to and capable of detecting, compound that contain carbon atoms (C), which accounts for almost all organic compounds.

Working nature:

Gas chromatography is a novel technique for separating and quantitating vapourized compounds using an inert carries gas. It operates on similar principles to column permeation chromatography. Where a sample is dissolved in a mobile phase and passed through a porous stationary structure.

* Gas Chromatography Procedure.

- Sample is injected in column. Gas chromatography
- Oven heats the system to vaporize the sample and speed its passage through the column.
- The different components of the sample will be separated by the column because each of the components "sticks" to the liquid coating that on the column packing differently.
- When a substance leaves the column, it is sensed by a detector.
- The detector generates the voltages that is proportional to the amount of the substance.



Gas Chromatography.

ELECTROPHORESIS

INTRODUCTION -

Electrophoresis is the motion of dispersed particles relative to all a fluid under the influence of a spatially uniform electric field.

Electrophoresis was introduced by the scientist Arne Tiselius in 1931.

Electrophoresis of positively charged particles is sometimes called cataphoresis, while negatively charged particles sometimes called anaphoresis.

PRINCIPLE -

The migration of and separation of charged particles under the influence of an electric field.

TYPES -

1. Gel electrophoresis.
2. Immuno electrophoresis.
3. Paper electrophoresis.

GEL ELECTROPHORESIS -

Gel electrophoresis is a technique, used to separate DNA fragments based on their size and charge.

PRINCIPLE -

Charged molecules move through a gel when an electric current is passed across it.

WORKING -

5 steps involved are;

Step - 1 → Negatively charged DNA fragments move towards the anode under an electric field.

step - 2 → DNA fragments separate according to their size through sieving effect provided by agarose gel.

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Step-3 → Stained with ethodium bromide and exposed to UV light to visualize.

Step-4 → Elution - Cutting and extracting separated bands of DNA from agarose gel.

Step-5 → The DNA ~~separated~~ fragments purified this way is used for recombinations.

USES OF ELECTROPHORESIS -

- Electrophoresis is used in DNA sequencing.
- Medical research.
- Protein research purification.
- Separation of organic acids, DNA and RNA fragments.

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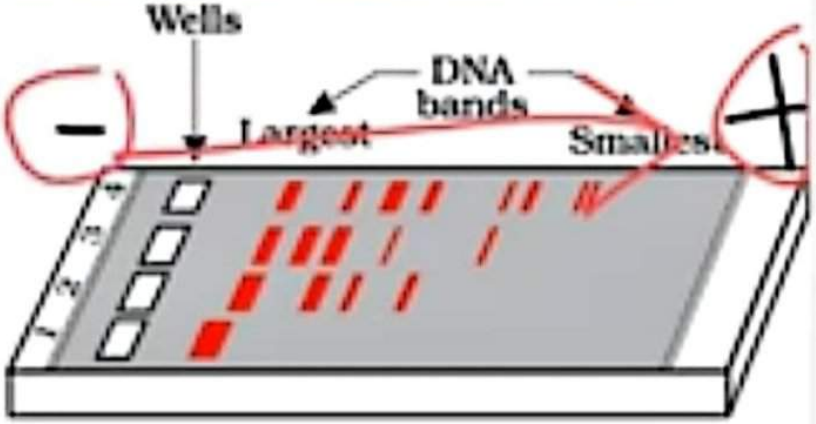
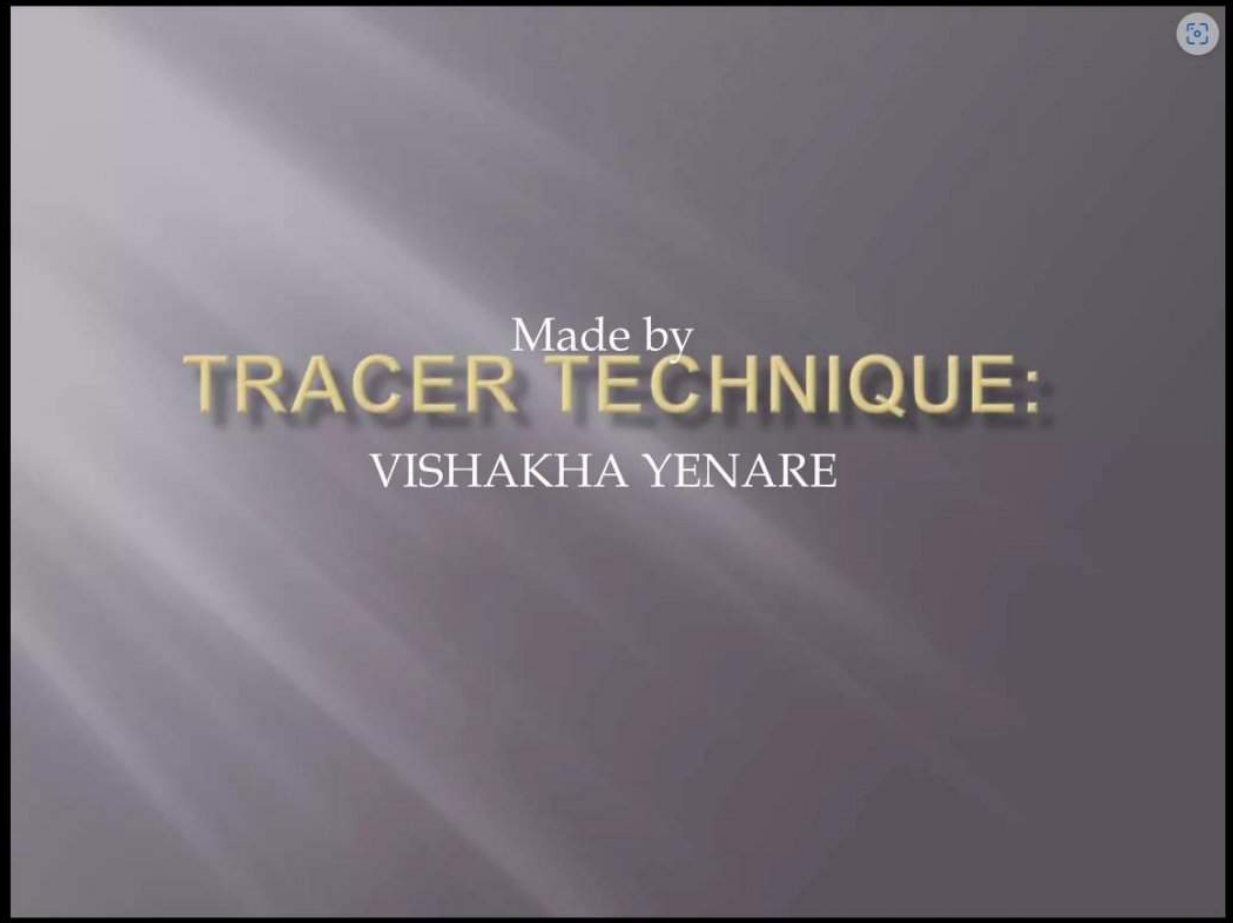
Technique of Separation and isolation of DNA fragments

Steps:-

1. Negatively charged DNA fragments move towards the anode under an electric field.
2. DNA fragments separate according to their size through sieving effect provided by agarose gel.
3. stained With ethodium bromide & exposed to UV light to visualize
4. Elution-"cutting and extracting Separated bands of DNA from agarose gel"
5. The DNA fragment purified this way is used for recombination.





TRACER TECHNIQUE:

Tracer technique is an effective tool to study these biosynthetic pathways. This technique makes use of different isotopes, mainly the radioactive isotopes, which are incorporated into the presumed precursors of plant metabolites and are used as markers in biogenetic experiments such as

- Shikkmic acid pathway
- Mevalonic pathway
- Acetate pathway

TRACER TECHNIQUE:

Tracer technique is an effective tool to study these biosynthetic pathways. This technique makes use of different isotopes, mainly the radioactive isotopes, which are incorporated into the presumed precursors of plant metabolites and are used as markers in biogenetic experiments such as

- Shikkmic acid pathway
- Mevalonic pathway
- Acetate pathway

SIGNIFICANCE OF TRACER TECHNIQUE:

- High sensitivity.
- Applicable to living system.
- Wide ranges of isotopes are available.
- More reliable, easy administration & isolation procedure. Gives accurate result, if proper metabolic time & technique applied.
- Location & Quantity of compound containing tracer 14 C labelled glucose is used for determination of glucose in biological system.
- Different tracers can be used for different studies. Ex. For studies on nitrogen and amino acid, Labelled nitrogen give specific information than carbon.

CRITERIA FOR TRACER / ISOTOPE SELECTION:

Two types of isotopes are generally used for labelling

1. Radioactive isotopes-
   - Unstable nucleus
   - Decomposes spontaneously by emission of nuclear electron or helium nucleus.

CRITERIA FOR TRACER / ISOTOPE SELECTION:

2. Stable isotopes.

- No tendency to undergo tendency to undergo radioactive breakdown
- Mass and NMR are Used in detection process
- Eg- heavy water
  cholesterol

STEPS INVOLVED IN TRACER TECHNIQUE:

- Preparation of labelled compound.
- Introduction of labelled compound into a biological system.
- Separation & determination of labelled compound in various biochemical fractions.
- Methods for tracer technique

Preparation of Labelled Compound :

- The labelled compound produce by growing them in atmosphere of $^{14}CO_2$.
- All carbon compounds get 14 C labelled. The 3 H (tritium) labelled compound are commercially available .
- Tritium labelling is effected by catalytic exchange in aqueous media by hydrogenation of unsaturated compound with tritium gas.
- Tritium is pure β – emitter of low intensity & its radiation energy is lower than 14 C.
- By the use of organic synthesis :
- $CH_3MgBr + {}^{14}CO_2 \longrightarrow CH_3{}^{14}COOHMgBr + H_2O$

$$CH_3{}^{14}COOH + Mg(OH)Br$$

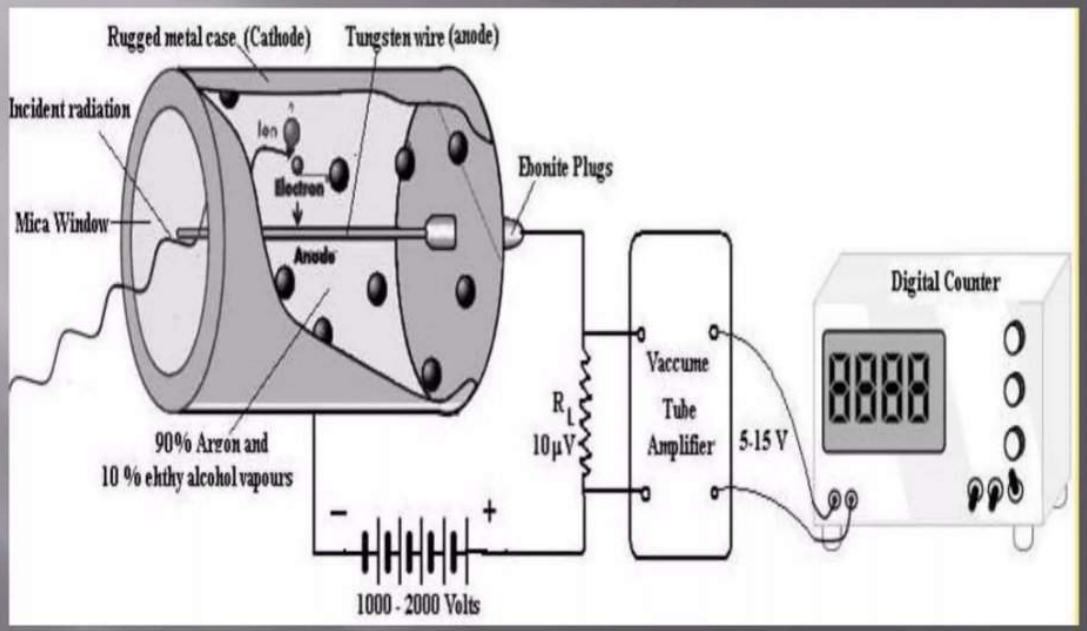
Introduction of labelled compound into biological system :

- ✓ Root feeding
- ✓ Stem feeding
- ✓ Direct injection
- ✓ Infiltration
- ✓ Floating method
- ✓ Spray technique

Separation & Detection Techniques :

- GM Counters
- Liquid Scintillation Chamber
- Gas Ionization Chamber
- Mass Spectrophotometer
- NMR Spectrophotometer
- Auto-Radiography

Geiger–Muller (GM) Counters:

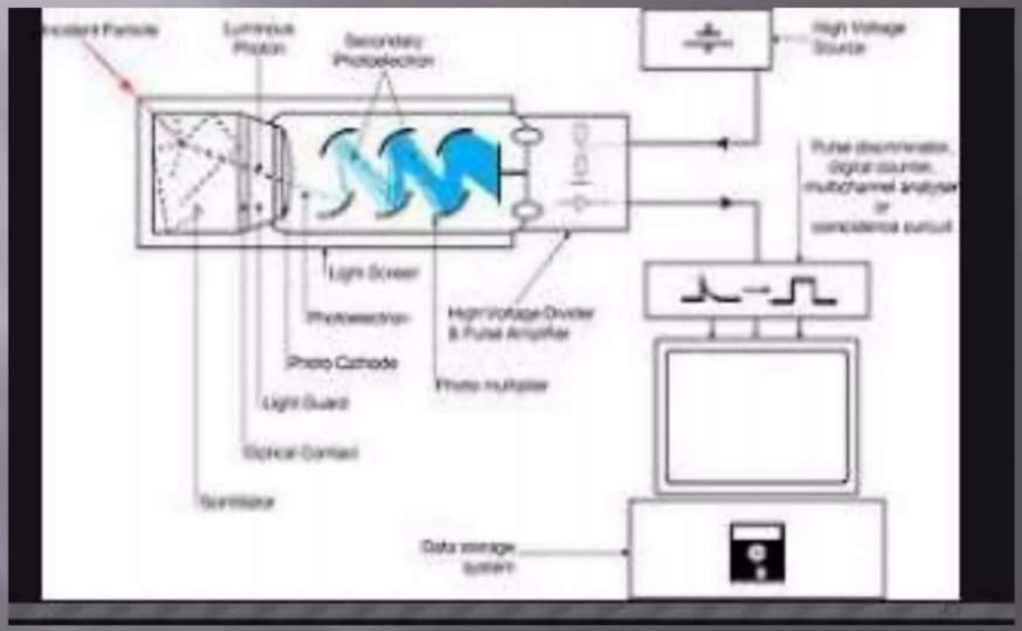


Geiger–Muller (GM) Counters:

A Geiger–Muller counter, is a type of particle detector that measures ionizing radiation

- e.g. alpha, beta particles, or gamma rays — by the ionization produced in a low-pressure gas usually helium , neon or argon with halogens added in a Geiger–Muller tube which briefly conducts electrical charge when a particle or photon of radiation makes the gas conductive by ionization.
- This charge has been detected in form of current pulse.

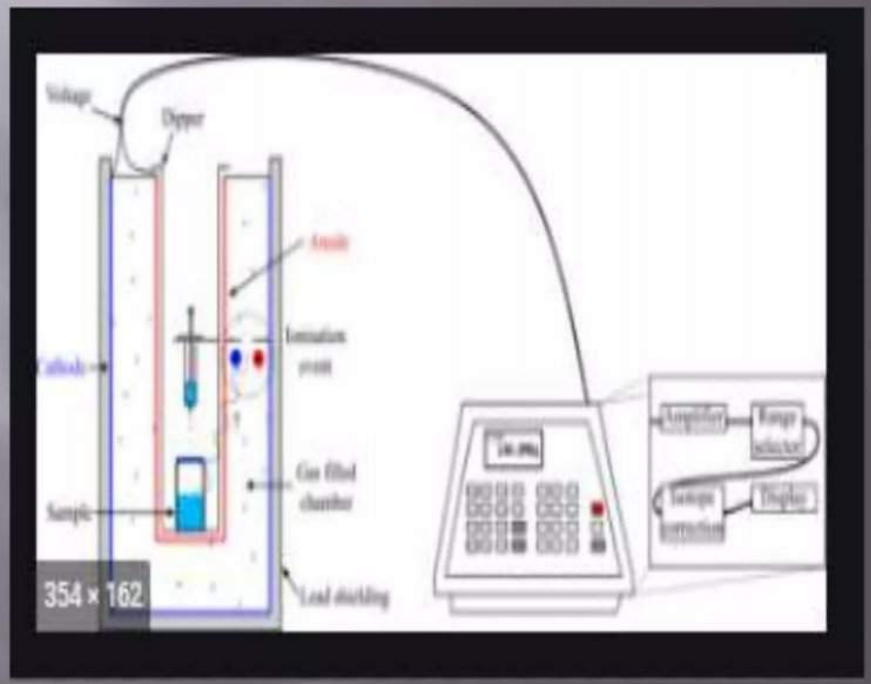
Liquid Scintillation Chamber:



Liquid Scintillation Chamber:

- A scintillation detector or scintillation counter is obtained when a scintillator is coupled to an electronic light sensor such as a photomultiplier tube (PMT) or a photodiode .
- A scintillator is a material that exhibits scintillation —
- the property of luminescence when excited by ionizing radiation . Samples are dissolved or suspended in a "cocktail" containing a solvent (aromatic organics such as benzene or toluene), typically some form of a surfactant , and small amounts of scintillators.

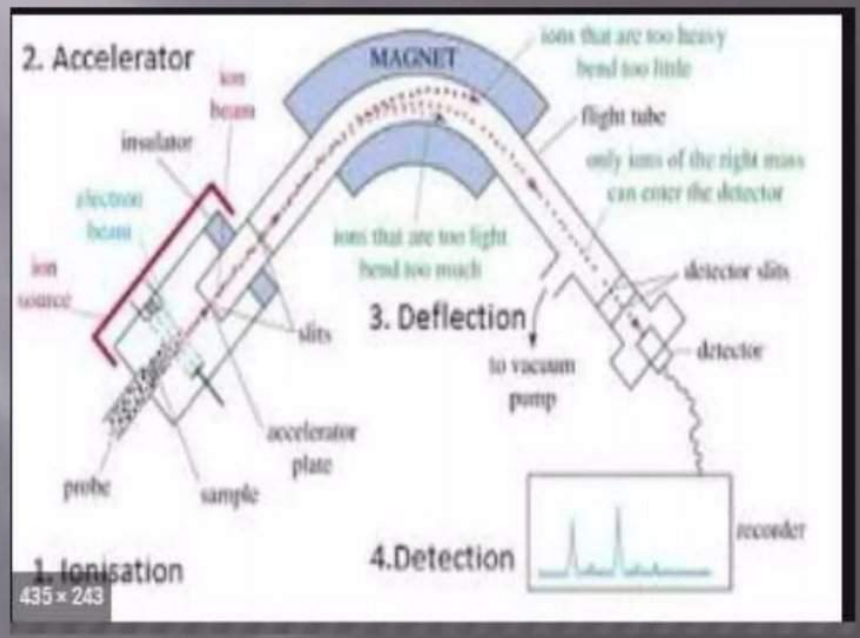
Gas Ionization Chamber:



Gas Ionization Chamber:

The ionization chamber is the simplest of all gas-filled radiation detectors, and is widely used for ionizing radiation ; X-rays , gamma rays and beta particles. Conventionally, the term "ionization chamber" is used exclusively to describe those detectors which collect all the charges created by direct ionization within the gas through the application of an electric field.

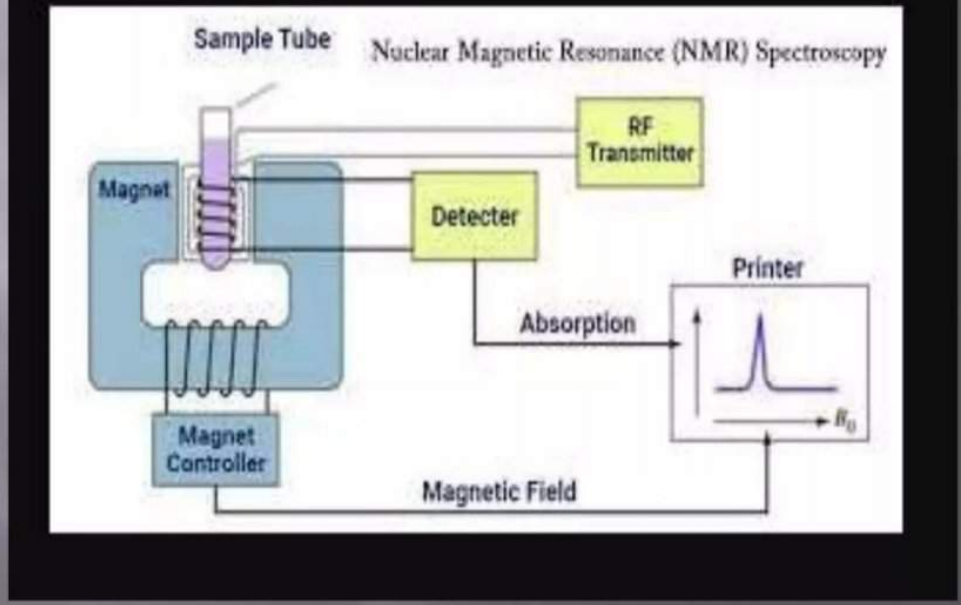
Mass Spectrophotometer:



Mass Spectrophotometer:

Mass spectrometry (MS) is an analytical technique that measures the mass-to-charge ratio of charged particles. It is used for determining masses of particles, for determining the elemental composition of a sample or molecule , and for elucidating the chemical structures of molecules, such as peptides and other chemical compounds .

NMR Spectrophotometer:



NMR Spectrophotometer:

NMR spectroscopy , is a research technique that exploits the magnetic properties of certain atomic nuclei to determine physical and chemical properties of atoms or the molecules in which they are contained. It relies on the phenomenon of nuclear magnetic resonance and can provide detailed information about the structure, dynamics, reaction state, and chemical environment of molecules.

Autoradiography:

Autoradiography is a method for investigating the distribution of radioactive material in a plant object, e.g. histological tissues, a chromatography plate. This techniques uses a photographic film or emulsion as detector of ionizing radiation. The sample is in close contact with emulsion for a certain period of time

METHODS FOR TRACER TECHNIQUE:

- Precursor Product Sequences
- Double & Multiple Labelling
- Competitive Feeding
- Isotope Incorporation
- Sequential Analysis

PRECURSOR PRODUCT SEQUENCE :

In this technique, the presumed precursor of the constituent under investigation on a labelled form is fed into the plant and after a suitable time the constituent is isolated, purified and radioactivity is determined.

Application:

- Stopping of hordenine production in barley seedling after 15 – 20 days of germination.
- Restricted synthesis of hyoscine , distinct from hyoscyamine in Datura stramonium .

DOUBLE & MULTIPLE LABELLING

This method give the evidence for nature of biochemical incorporation of precursor arises double & triple labelling. In this method specifically labelled precursor and their subsequent degradation of recover product are more employed

Application:

- This method is extensively applied to study the biogenesis of plant secondary metabolite.
- Used for study of morphine alkaloid. E.g. Leete ,
- use Doubly labelled lysine used to determine which hydrogen of lysine molecule was involved in formation of piperidine ring of anabasine in Nicotina glauca .

COMPETITIVE FEEDING :

This method provides the possible intermediates that plant normally used during biogenesis.

Application:

- This method is used for elucidation of biogenesis of propane alkaloids.
- Biosynthesis of hemlock alkaloids (conline, conhydrine etc) using 14 C labelled compounds.

COMPETITIVE FEEDING :

This method provides the possible intermediates that plant normally used during biogenesis.

Application:

- This method is used for elucidation of biogenesis of propane alkaloids.
- Biosynthesis of hemlock alkaloids (conline, conhydrine etc) using 14 C labelled compounds.

ISOTOPE INCORPORATION :

- This method provides information about the position of bond cleavage & their formation during reaction.
- E.g. Glucose – 1- phosphatase cleavage as catalyzed by alkaline phosphatase this reaction occur with cleavage of either C – O bond or P – O bond.

SEQUENTIAL ANALYSIS : -

The principle of this method of investigation is to grow plant in atmosphere of 14 CO 2 & then analyze the plant at given time interval to obtain the sequence in which various correlated compound become labelled.

Application: -

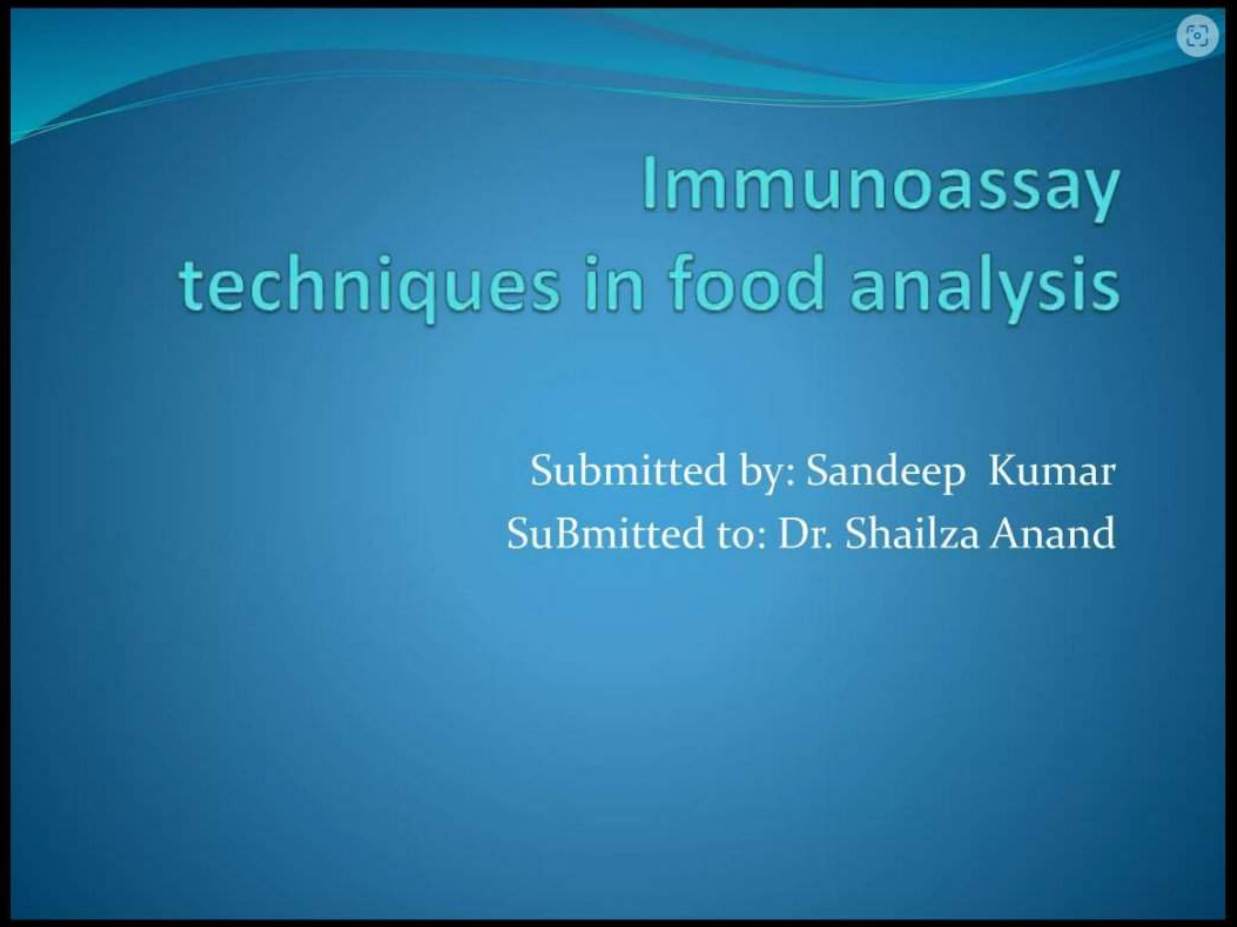
- 14 CO2 sequential analysis has been very successfully used in elucidation of carbon in photosynthesis.
- Determination of sequential formation of opium hemlock and tobacco alkaloids.

APPLICATION OF TRACER TECHNIQUE:

- Tracing of bio-synthetic pathway of cyanogenetic glycoside "prunacine"; by incorporating 14 C into phenylaniline Interrelationship among 4 – methyl sterols & 4, 4 dimethyl sterols, by use of 14 C acetate.
- Study of squalene cyclization by use of 14 C, 3 H labelled mevalonic acid. Terpenoid biosynthesis by chloroplast isolated in organic solvent, by use of 2-14 C mevalonate.
- Study the formation of cinnamic acid in pathway of coumarin from labelled coumarin. Origin of carbon & nitrogen atoms of purine ring system by use of 14 C or 15 N labelled precursor.

APPLICATION OF TRACER TECHNIQUE:

- Study of formation of scopoletin by use of labelled phenylalanine. By use of 45 Ca as tracer, - found that the uptake of calcium by plants from the soil. (CaO & CaCO2).
- By adding ammonium phosphate labelled with 32 P of known specific activity the uptake of phosphorus is followed by measuring the radioactivity as label reaches first in lower part of plant, than the upper part i.e. branches, leaves etc.



CONTENTS

- Introduction
- Method of immunoassay
- Principle
- Isotopic immunoassay
- Non isptopic immunoassay
- Application in food industry
- summary

Introduction

- An immunoassay is a test that uses antibody and antigen complexes as a means of generating measurable result.

or

- An immunoassay is an analytical method which uses antibodies as reagents to quantitate specific analytes

- An antibody:antigen complex is also known as an immuno-complex.
- The assay operates like the immune system to identify a substance (usually a protein) based on it's capacity to act like an antigen
- Highly specific "lock and key" system: antibody – antigen reaction

Principle

- The **immunoassay** is a technique which incorporates the binding reaction of a target substance (antigen) with an antibody. Antibodies are basically immunoglobins that bind to different natural and synthetic antigens in the body such as carbohydrates, lipids, proteins and nucleic acids.

Antibodies

- An antibody is a protein that is produced by the body in response to an invading (foreign) substance.
- Antibodies are produced as part of the body's immune response to protect itself.
- Antibodies (Ab) are a type of protein called immunoglobulin.
- The most common one is immunoglobulin G(IgG).
- IgG is a protein composed of two main structural and functional regions

Types of antibodies

- Polyclonal antibodies
- Monoclonal antibodies

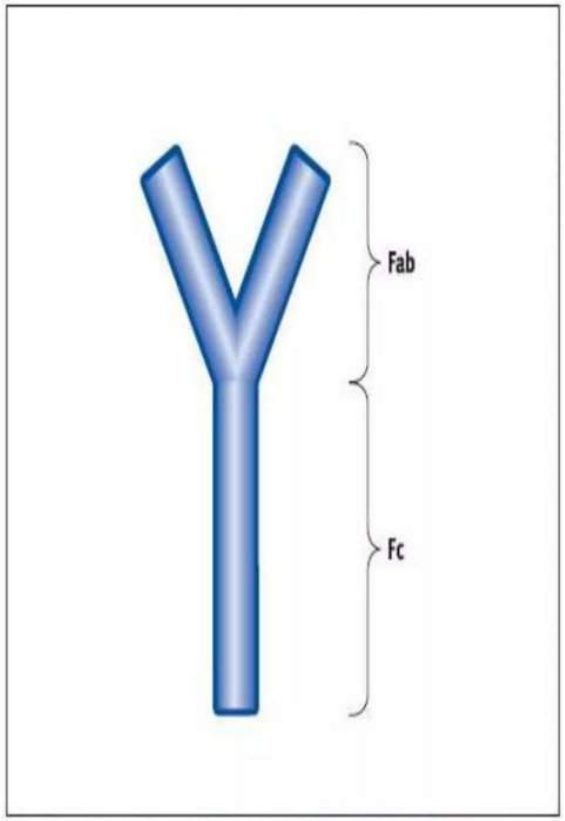
- Polyclonal antibodies recognize multiple sites on antigens, or monoclonal antibodies recognize single sites on antigens.

Antigen

- An antigen is the substance that the body is trying to fight off (eliminate or reduce) by mounting an immune response.
- In a test to measure the concentration of a drug, e.g., the drug is the antigen that binds to the antibody.

Analyte

- An analyte is anything measured by a laboratory test. In immunoassay testing, the analyte may be either an antibody, or an antigen.



Fab region: Contains the antigen (Ag) binding site that varies between different antibodies.

Fc region: Region of constant structure within an antibody class.

FIGURE 1-1 Antibody Structure and Functional Sites

Methodology for immunoassay

- Isotopic immunoassay
- Nonisotopic immunoassay

Isotopic immunoassay

- Based on competition for antibody between radioactive indicator antigen and unlabelled antigen in test sample.
- Increase in count of unlabeled antigen in test sample decrease the labeled antigen in bound.
- The concentration of the test antigen can be determined by comparison with a standard calibrated curve with known concentration of purified antigen.

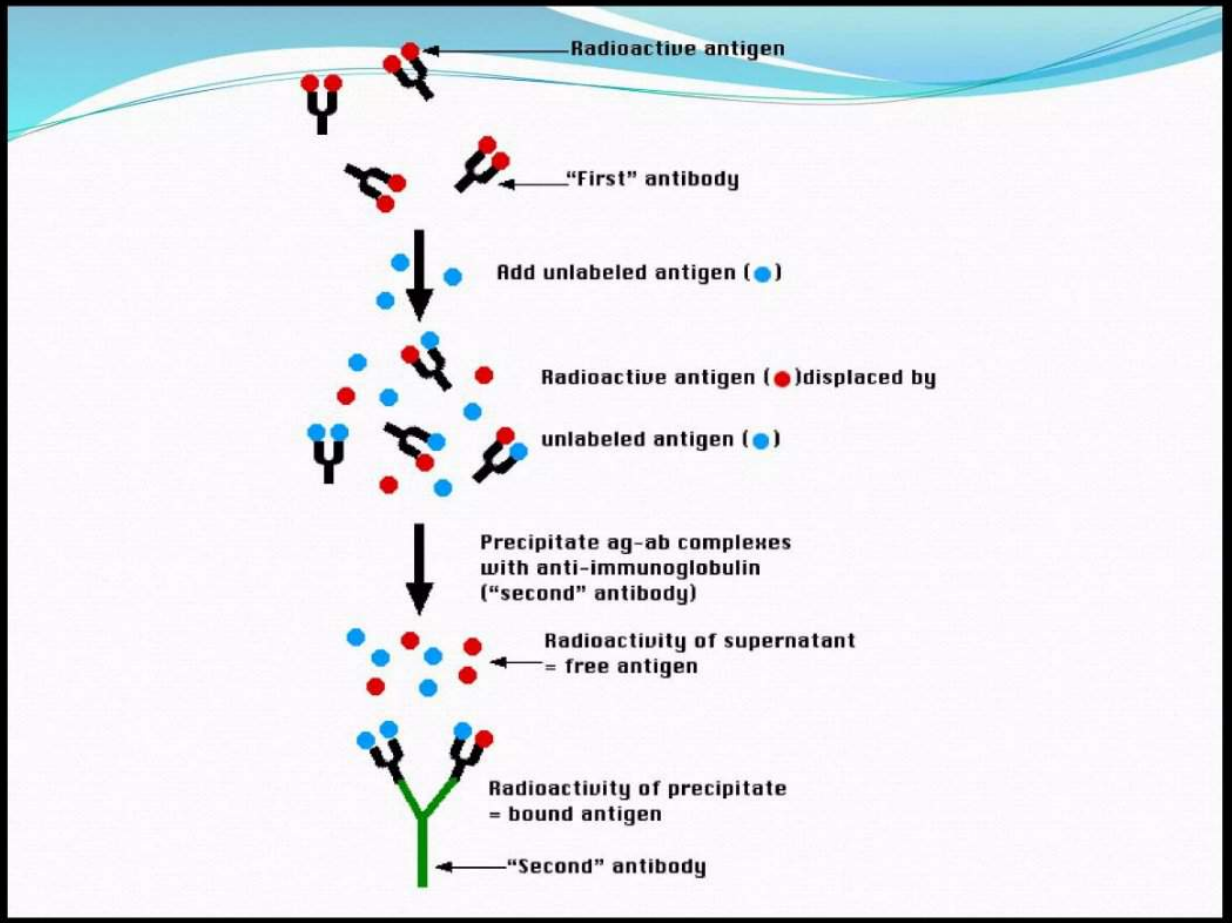
Radioimmunoassay

- Radioimmunoassay (RIA) involves the separation of a protein (from a mixture) using the specificity of antibody - antigen binding and quantitation using radioactivity

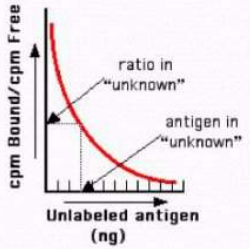
The technique

- A mixture is prepared of
  - radioactive antigen
    - Because of the ease with which iodine atoms can be introduced into tyrosine residues in a protein, the radioactive isotopes ^{125}I or ^{131}I are often used.
  - antibodies against that antigen.
- Known amounts of unlabeled ("cold") antigen are added to samples of the mixture. These compete for the binding sites of the antibodies.

- At increasing concentrations of unlabeled antigen, an increasing amount of radioactive antigen is displaced from the antibody molecules.
- The antibody-bound antigen is separated from the free antigen in the supernatant fluid, and
- the radioactivity of each is measured



- After determining the ratio of bound to free antigen in each unknown, the antigen concentrations can be read directly from the standard curve.



- The main drawbacks to radioimmunoassay are the expense and hazards if preparing and handling the radioactive antigen.
- Both ^{125}I or ^{131}I emit gamma radiation that requires special counting equipment;
- The body concentrates iodine atoms — radioactive or not — in the thyroid gland where they are incorporated in thyroxine (T_4).

Nonisotopic immunoassay

- Differ from isotopic immunoassay in:-
  - Type of label used
  - Means of end point detection
  - Possibility of eliminating a separation test
- Two types of nonisotopie immunoassay are:-
  - Fluoroimmunoassay
  - ELISA (Enzyme-Linked Immunosorbent assay)

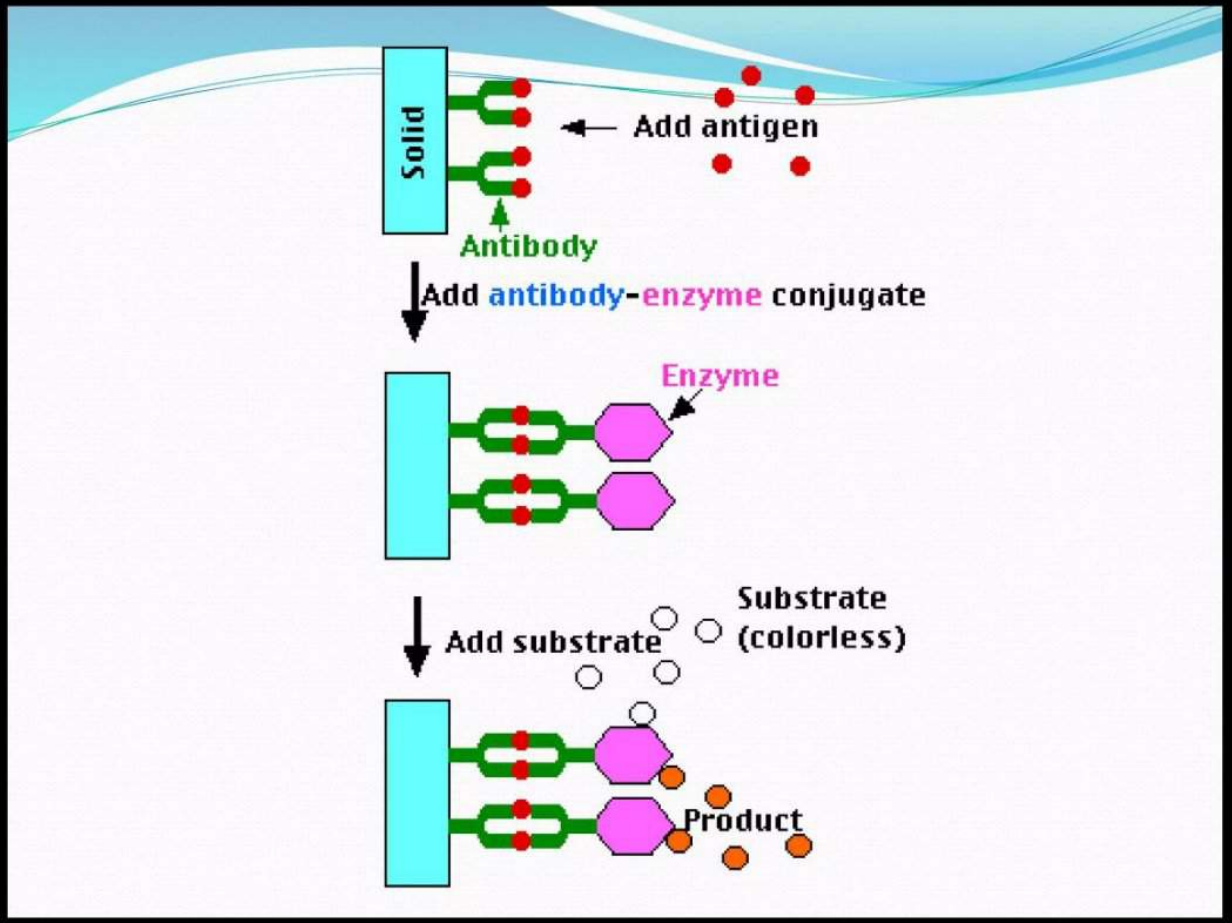
ELISA (Enzyme-Linked Immunosorbent assay)

- ELISA is a widely-used method for measuring the concentration of a particular molecule (e.g., a hormone or drug) in a fluid such as serum or urine. It is also known as enzyme immunoassay or **EIA**.
- ELISA has many of the advantages (e.g., sensitivity, ease of handling multiple samples) without the disadvantages of dealing with radioactivity (like in RIA).

- The test requires:
  - the antibodies fixed to a solid surface, such as the inner surface of a test tube;
  - a preparation of the same antibodies coupled to an enzyme. This is one (e.g., β-galactosidase) that produces a colored product from a colorless substrate.

Performing the Test

- The tubes are filled with the antigen solution (e.g., urine) to be assayed. Any antigen molecules present bind to the immobilized antibody molecules.
- The antibody-enzyme conjugate is added to the reaction mixture. The antibody part of the conjugate binds to any antigen molecules that were bound previously, creating an antibody-antigen-antibody "sandwich".
- After washing away any unbound conjugate, the substrate solution is added.
- After a set interval, the reaction is stopped (e.g., by adding 1 N NaOH) and the concentration of colored product formed is measured in a spectrophotometer. The intensity of color is proportional to the concentration of bound antigen.



- **detecting allergens** in food and house dust
- **measuring toxins** in contaminated food

Fluoroimmunoassay

- Radioisotopes and enzymes were replaced by new fluorescent compound in labeling the antibodies and antigen.
- Substances used such as chelate of Europium which fluoresces when it excite by light in the presence of a developing solution
- Emission duration is short but less than that of the background noise. can be measured by special instrument which is unfortunately very expensive.
- It is 10 times more sensitive than RIA.

- A modern fluorescent based immunoassay uses as the detection reagent a fluorescent compound which absorbs light or energy (excitation energy) at a specific wavelength and then emits light or energy at a different wavelength
- The difference between the wavelength of the excitation light and the emission light is called the Stokes shift.

Application of immunoassay in food Industry

- Many of the macromolecule that can found in food are good antigen and antibodies are capable of recognizing them and small molecule.
- Composition of raw material and final product
- Harmful and useful minor substances with biological activity (toxins and allergens)
- Enzyme detection
- Contaminants detection and determination (hormones ,drug, pesticides residue)

Summary

- Immunoassay are tests that use antibody and antigen complexes (also called immunocomplexes) to measure the presence of a specific analyte in a sample.
- Antibodies are proteins that are normally produced by the immune system in response to a foreign substance.
- Antigens are the molecules that antibodies bind to, which in the body could be an invading pathogen, or the foreign molecules injected into an animal to trigger the immune response.

Summary

- Isotopic and non isotopic are two type of immunoassay
- Isotopic immunoassay have radioimmunoassay in which radiation is used to quantified the amount of antigen.
- Nonisotopic immunoassay have no radiation process and is very much safer then isotopic immunoassay.
-

Summary

- Immunoassay techniques are use in determining the composition of raw material and final produce, to identify toxins, allergens, defects in food, enzyme detection, quantity of additives, contamination detection, and drug detection.

6Th Semester Theory

DSE4T : Research Methodology and Design
Tools techniques for research in Physiology and Health Science :

COLUMN CHROMATOGRAPHY :

➢ What Is Column Chromatography?

In chemistry, Column chromatography is a technique which is used to separate a single chemical compound from a mixture dissolved in a fluid. It separates substances based on differential adsorption of compounds to the adsorbent as the compounds move through the column at different rates which allow them to get separated in fractions. This technique can be used on small scale as well as large scale to purify materials that can be used in future experiments. This method is a type of adsorption chromatography technique.

➢ Column Chromatography Principle

When the mobile phase along with the mixture that needs to be separated is introduced from the top of the column, the movement of the individual components of the mixture is at different rates. The components with lower adsorption and affinity to stationary phase travel faster when compared to the greater adsorption and affinity with the stationary phase. The components that move fast are removed first whereas the components that move slow are eluted out last.

The adsorption of solute molecules to the column occurs in a reversible manner. The rate of the movement of the components is expressed as:

R_f = the distance travelled by solute/ the distance travelled by solvent

R_f is the retardation factor.

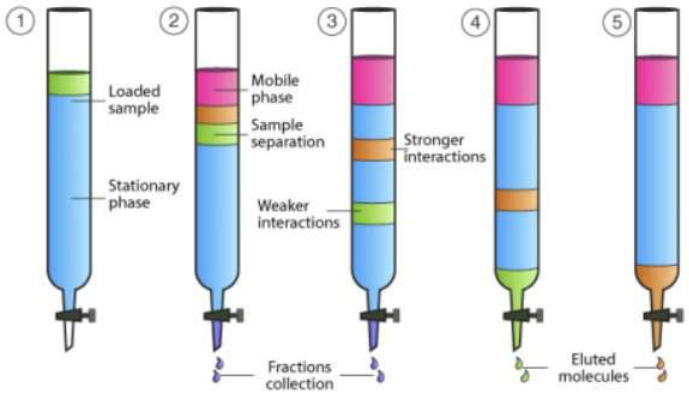


Figure : Column Chromatography

➢ Column Chromatography Procedure

Before starting with the Column Chromatography Experiment let us understand the different phases involved.

Mobile phase – This phase is made up of solvents and it performs the following functions:

1. It acts as a solvent – sample mixture can be introduced in the column.
2. It acts as a developing agent – helps in the separation of components in the sample to form bands.
3. It acts as an eluting agent – the components that are separated during the experiment are removed from the column
4. Some examples of solvents used as mobile phase based on their polarity are – ethanol, acetone, water, acetic acid, pyridine, etc.

Stationary phase – It is a solid material which should have good adsorption property and meet the conditions given below:

1. Shape and size of particle: Particles should have uniform shape and size in the range of 60 – 200μ in diameter.
2. Stability and inertness of particles: high mechanical stability and chemically inert. Also, no reaction with acids or bases or any other solvents used during the experiment.
3. It should be colourless, inexpensive and readily available.
4. Should allow free flow of mobile phase
5. It should be suitable for the separation of mixtures of various compounds.

➢ Column Chromatography Experiment

- The stationary phase is made wet with the help of solvent as the upper level of the mobile phase and the stationary phase should match. The mobile phase or eluent is either solvent or mixture of solvents. In the first step the compound mixture that needs to be separated, is added from the top of the column without disturbing the top level. The tap is turned on and the adsorption process on the surface of silica begins.
- Without disturbing the stationary phase solvent mixture is added slowly by touching the sides of the glass column. The solvent is added throughout the experiment as per the requirement.
- The tap is turned on to initiate the movement of compounds in the mixture. The movement is based on the polarity of molecules in the sample. The non-polar components move at a greater speed when compared to the polar components.
- For example, a compound mixture consists of three different compounds viz red, blue, green then their order based on polarity will be as follows blue>red>green
- As the polarity of the green compound is less, it will move first. When it arrives at the end of the column it is collected in a clean test tube. After this,

the red compound is collected and at last blue compound is collected. All these are collected in separate test tubes.

➢ Column Chromatography Applications

- Column Chromatography is used to isolate active ingredients.
- It is very helpful in Separating compound mixtures.
- It is used to determine drug estimation from drug formulations
- It is used to remove impurities.
- Used to isolation metabolites from biological fluids.

➢ Types of Column Chromatography:

1. Adsorption column chromatography – Adsorption chromatography is a technique of separation, in which the components of the mixture are adsorbed on the surface of the adsorbent.

2. Partition column chromatography – The stationary phase, as well as mobile phase, are liquid in partition chromatography.

3. Gel column chromatography – In this method of chromatography, the separation takes place through a column packed with gel. The stationary phase is a solvent held in the gap of a solvent.

4. Ion exchange column chromatography – A chromatography technique in which the stationary phase is always ion exchange resin.