GOVERNMENT COLLEGE (AUTONOMOUS) RAJAMAHENDRAVARAM

DEPARTMENT OF MICROBIOLOGY

QUESTION BANK FOR

MICROBIAL AND ANALYTICAL TECHNIQUES (C-7)

(B.Sc Honours-Microbiology)

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Course-7 (MICROBIAL AND ANALYTICAL TECHNIQUES)

Question Bank-2024-25

Essay type questions (Select any Two from each Unit for Internal Choice)

Unit -I

Q.No	Questions	Marks	BL	CO	PO
1.	Explain the principle and construction of light microscope.	8	2	1	
2.	Generalise principle and applications of Transmission Electron microscope.	8	3	1	
3.	Generalise principle and applications of Scanning Electron microscope.	8	3	1	

Unit -II

Q.No	Questions	Marks	BL	CO	PO
1.	A. State definitions and examples of Fungicide, Viricide, Bacteriostatic and Bactericidal agents B. Write a short note on sterilization by UV and Gama Rays	4 4	1 &3 2	2	
2.	Describe working principle and applications of Autoclave	8	2	2	
3.	Evaluate different methods of Chemical means of sterilization	8	4	2	

Unit -III

Q.No	Questions	Marks	BL	CO	PO
1.	Justify streak plate and spread plate methods and their Uses in obtaining pure cultures	8	5	3	
2.	Define MTCC and ATCC. Evaluate Subculture, Lyophilization and lower temperature methods for the preservation of stock cultures	2+6	1& 4	3	
3.	Explain different methods of cultivating Anaerobic Bacteria	8	3	3	

Unit -IV

Q.No	Questions	Marks	BL	CO	PO
1.	Describe the instrumentation and Applications of Spectroscopy	8	3	4	
2.	Explain principle and applications of Paper Chromatography	8	2& 3	4	
3.	A. Explain about principle of Ion Exchange Chromatography B. Explain principle of Affinity Chromatography	4+4	3	4	

Unit -V

Q.No	Questions	Marks	BL	CO	PO
1.	Differentiate types of Centrifuges and their applications	8	2	5	

2.	Give outlines of working principle and applications of Gel Electrophoresis.	8	3	5	
3.	Analyse characters and applications of Radio Isotopes	8	4	5	

ESSAY TYPE QUESTIONS

1. Explain the principle and construction of light microscope

Principles:

Magnification:

This represents the number of times the image of a specimen is amplified. 10x means the size of the image is increased by ten times. The magnifying power of the lens is limited. After a certain point the magnification results in a blurred image and is termed empty magnification. Even with the best optics, 1400x is the highest useful magnification achieved. Magnifying power of an objective is determined by the dividing the optical tube length by the focal length of the lens. Optical tube length is the length of the microscope body tube between the nosepiece opening, where the objective is mounted, and the top edge of the observation tubes where the eyepieces are inserted. In most microscopes, it is fixed at 160mm.

Low power dry objective: 160/16 = 10x
High power dry objective: 160/4 = 40x
Oil immersion objective: 160/1.7 = 94x or approximately 100x

Numerical Aperture:

The numerical aperture of the lens is an important consideration in optics as it dictates the angle at which the light enters it. The light-gathering ability of a microscope objective is quantitatively expressed in terms of the numerical aperture. Higher values of numerical aperture allow increasingly oblique rays to enter the objective front lens, producing a more highly resolved image.

It is defined by the following formula: Numerical Aperture (NA) = n × sin(θ)where n is the refractive index of the medium between the object and the objective θ is one-half the angular aperture (angle of aperture is the angle formed by the two most divergent rays of light which enter the objective, starting from the center of the object).

Refractive index of oil is 1.5 and that of air is 1.0.

NA of dry objective: 1 x sin 90° = 1, but practically the highest practical numerical aperture of a dry lens is 0.95.

NA of oil immersion objective: 1.5 x sin 90° = 1.5, however in practice only 1.4 is achieved for apochromatic objective and 1.3 for achromatic objective.

Limit of resolution or resolving power: In simple words, it is the ability to see two closely placed dots as two separate dots. If the distance between the two points is lessened, it would appear as a single point. It is expressed quantitatively as limit of resolution. The resolution of

human unaided eye is 200 µm. This means that human eye can not see objects small than 200 µm. The resolving power of compound microscope is 0.2 µm and that of electron microscope is 1-10 nm. The limit of resolution depends on the wavelength of the light used. Resolution increases with the decreasing wavelength of light. Violet colour light offers more resolution that red coloured light. Electron beams, which have very low wavelength offers maximum resolution. It is calculated by using formula:

$$LR = \frac{0.61 \text{ x wavelength of light}}{\text{Numerical aperture}}$$

Or

$$L.R = \frac{\text{wavelength of light}}{2xNA}$$

For example, if green light of wavelength 0.55 µm is used and oil immersion objective with NA 1.4 is used, the maximum resolution obtained is 0.24 µm

$$\frac{0.55}{2x1.4} = 0.24 \text{ µm}$$

Resolution

This is the capacity of the objective to render the outline of the image clear and distinct. Definition of an image is disturbed by spherical or chromatic aberrations. The central part of the image is usually well focused but the edges may suffer some aberration, which are of two types; spherical or chromatic. In spherical aberration, the periphery of the image appears out of focus. This happens because all the light passing through the lens doesn't condense at the same point. In chromatic aberration, the light is split into different colours at the peripheral part of the image since the edges of the lens act like a prism. The aberrations can be corrected by using achromatic or apochromatic lenses.

Construction of Microscope:

The most commonly used microscope for general purposes is the standard compound microscope. It magnifies the size of the object by a complex system of lens arrangement.

It has a series of two lenses; (i) the objective lens close to the object to be observed and (ii) the ocular lens or eyepiece, through which the image is viewed by eye. Light from a light source (mirror or electric lamp) passes through a thin transparent object

The objective lens produces a magnified 'real image' first image) of the object. This image is again magnified by the ocular lens (eyepiece) to obtain a magnified 'virtual image' (final image), which can be seen by eye through the eyepiece. As light passes directly from the source to the eye through the two lenses, the field of vision is brightly illuminated. That is why; it is a bright-field microscope.

The parts of a compound microscope are of two categories as given below:

(i) Mechanical Parts:

These are the parts, which support the optical parts and help in their adjustment for focusing the object.

The components of mechanical parts are as follows:

1. Base or Metal Stand:

The whole microscope rests on this base. Mirror, if present, is fitted to it.

2. Pillars:

It is a pair of elevations on the base, by which the body of the microscope is held to the base

3. Inclination joint:

It is a movable joint, through which the body of the microscope is held to the base by the pillars. The body can be bent at this joint into any inclined position, as desired by the observer, for easier observation. In new models, the body is permanently fixed to the base in an inclined position, thus needing no pillar or joint.

4. Curved Arm:

It is a curved structure held by the pillars. It holds the stage, body tube, fine adjustment and coarse adjustment.

5. Body Tube:

It is usually a vertical tube holding the eyepiece at the top and the revolving nosepiece with the objectives at the bottom. The length of the draw tube is called 'mechanical tube length' and is usually 140-180 mm (mostly 160 mm).

6. Draw Tube:

It is the upper part of the body tube, slightly narrower, into which the eyepiece is slipped during observation.

7. Coarse Adjustment:

It is a knob with rack and pinion mechanism to move the body tube up and down for focusing the object in the visible field. As rotation of the knob through a small angle moves the body tube through a long distance relative to the object, it can perform coarse adjustment. In modern microscopes, it moves the stage up and down and the body tube is fixed to the arm

8. Fine Adjustment:

It is a relatively smaller knob. Its rotation through a large angle can move the body tube only through a small vertical distance. It is used for fine adjustment to get the final clear image. In modern microscopes, fine adjustment is done by moving the stage up and down by the fine adjustment.

9. Stage:

It is a horizontal platform projecting from the curved arm. It has a hole at the center, upon which the object to be viewed is placed on a slide. Light from the light source below the stage passes through the object into the objective.

10. Mechanical Stage (Slide Mover):

Mechanical stage consists of two knobs with rack and pinion mechanism. The slide containing the object is clipped to it and moved on the stage in two dimensions by rotating the knobs, so as to focus the required portion of the object.

11. Revolving Nosepiece:

It is a rotatable disc at the bottom of the body tube with three or four objectives screwed to it. The objectives have different magnifying powers. Based on the required magnification, the nosepiece is rotated, so that only the objective specified for the required magnification remains in line with the light path.

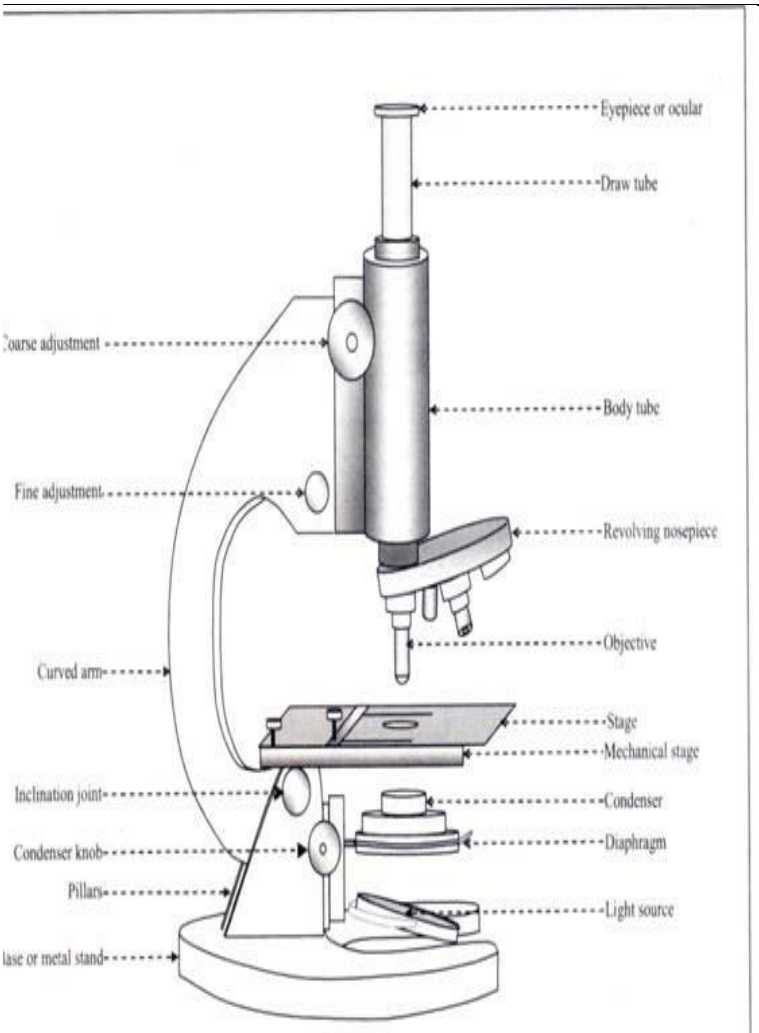
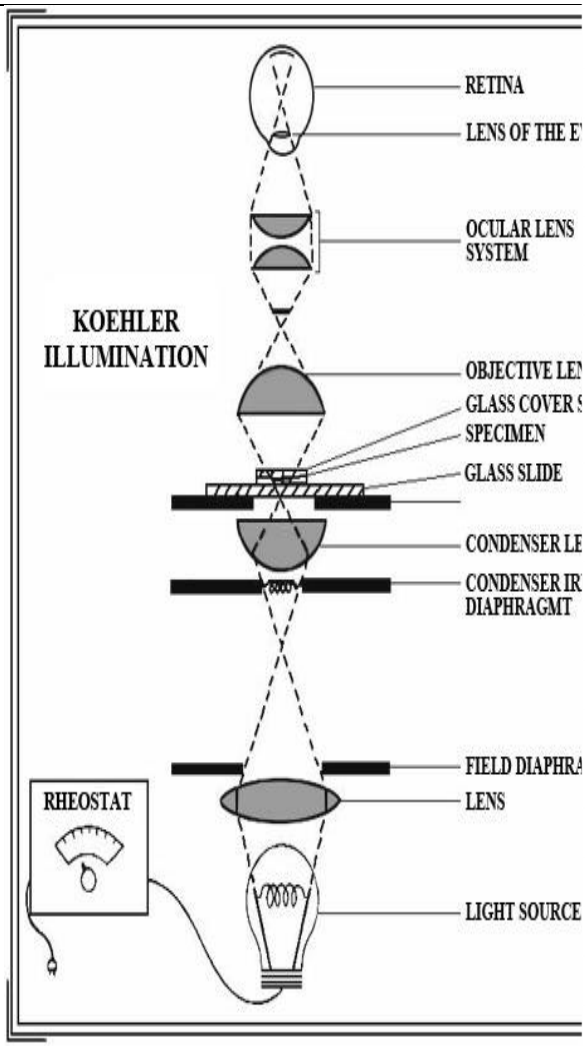


Figure 4.6: A compound microscope



PATH OF LIGHT

(ii) Optical Parts:

These parts are involved in passing the light through the object and magnifying its size.

The components of optical parts include the following:

1. Light Source:

Modern microscopes have in-built electric light source in the base. The source is connected to the mains through a regulator, which controls the brightness of the field. But in old models, a mirror is used as the light source. It is fixed to the base by a binnacle, through which it can be rotated, so as to converge light on the object. The mirror is plane on one side and concave on the other.

2. Diaphragm:
If light coming from the light source is brilliant and all the light is allowed to pass to the object through the condenser, the object gets brilliantly illuminated and cannot be visualized properly. Therefore, an iris diaphragm is fixed below the condenser to control the amount of light entering into the condenser.

3. Condenser:
The condenser or sub-stage condenser is located between the light source and the stage. It has a series of lenses to converge on the object, light rays coming from the light source. After passing through the object, the light rays enter into the objective.

The 'light condensing', 'light converging' or 'light gathering' capacity of a condenser is called 'numerical aperture of the condenser'. Similarly, the 'light gathering' capacity of an objective is called 'numerical aperture of the objective'. If the condenser converges light in a wide angle, its numerical aperture is greater and vice versa.

4. Objective:
It is the most important lens in a microscope. Usually three objectives with different magnifying powers are screwed to the revolving nosepiece.

The objectives are:
(a) Low power objective (X 10):
It produces ten times magnification of the object.

(b) High dry objective (X 40):
It gives a magnification of forty times.

(c) Oil-immersion objective (X100):
It gives a magnification of hundred times, when immersion oil fills the space between the object and the objective

5. Eyepiece:
The eyepiece is a drum, which fits loosely into the draw tube. It magnifies the magnified real image formed by the objective to a still greatly magnified virtual image to be seen by the eye

Useful magnification:
It is the magnification that makes visible the smallest resolvable particle. The useful magnification in a light microscope is between X1000 and X2000. Any magnification beyond X2000 makes the image blurred.

2. Generalise principle and applications of Transmission Electron microscope.

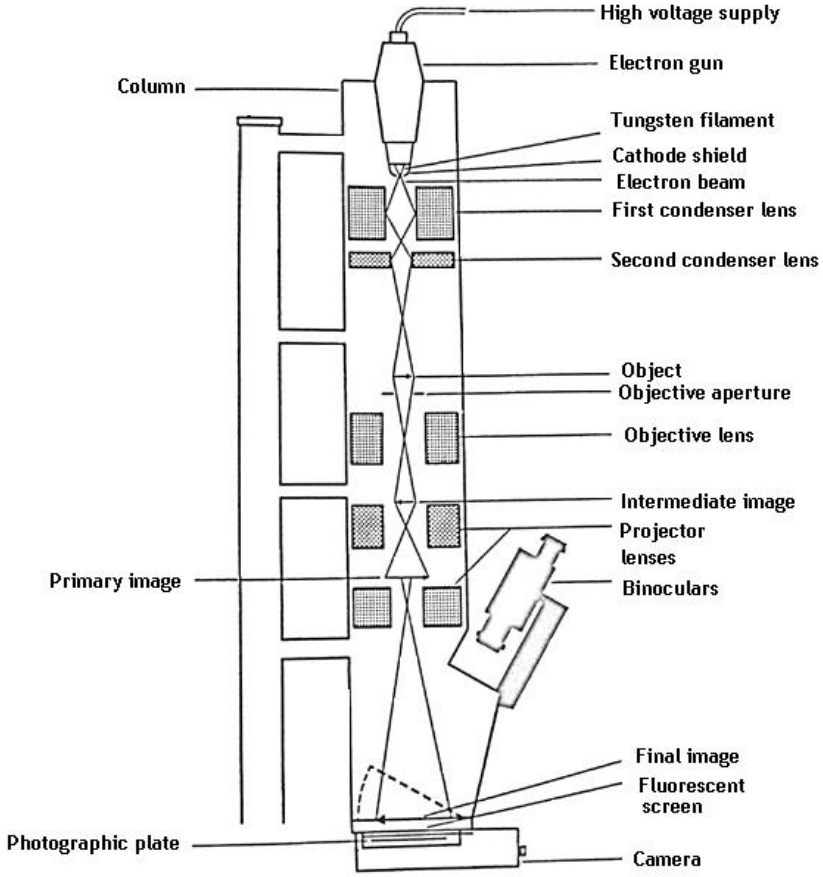
In transmission electron microscope (TEM), the source of illumination is a beam of electrons of very short wavelength, emitted from a tungsten filament at the top of a cylindrical column of about 2 m high. The whole optical system of the microscope is enclosed in vacuum. Air must be evacuated from the column to create a vacuum so that the collision of electrons with air molecules and hence the scattering of electrons are avoided. Along the column, at specific intervals magnetic coils are placed. Just as the light is focused by the glass lenses in a light microscope, these magnetic coils in the electron microscope focus the electron beam. The magnetic coils placed at specific intervals in the column acts as an electromagnetic condenser lense system. The specimen stained with an electron dense material and is placed in the vacuum. The electron beams are passes through the specimen and scattered by the internal structures.

The resolving power of a microscope is directly related to the wavelength of the irradiation, which used to form an image. The faster the electrons travel, the shorter their wavelength. As the wavelength is reduced, the resolution is increased. Therefore, the resolution of the microscope is increased if the accelerating voltage of the electron beam is increased.
Transmission electron microscopy involves a high voltage beam of electron emitted by a cathode and formed by magnetic lenses. The beam of electron that has been partially transmitted through the very thin specimen carries information about the structure of the specimen. The spatial variation in this information (the "image") is then magnified by a series of magnetic lenses until it is recorded by hitting a fluorescent screen, photographic plate, or light sensitive sensor like CCD (charge-coupled device) camera. The image detected by the CCD may be displayed in real time on a monitor or computer.
The TEM has the ability ability to determine the positions of atoms within materials which has made an indispensable tool for nano-technologies research and development in many fields, including heterogeneous catalysis and the development of semiconductor devices for photonics and electronics. In the life sciences, it is still mainly the specimen preparation which limits the resolution of what we can see in the electron microscope, rather than the microscope itself.

There are four parts for a transmission electron microscope:

- Electron source
- Electromagnetic lens system
- Sample holder
- Imaging system

The electron source is an electron gun which consists of a tungsten filament. This filament emits electrons when it is heated. The beam of electrons are the focused on the specimen by the condenser which consists of electromagnets called magnetic lenses. The sample holder consists of a mechanical arm which holds the specimen. The imaging system also consists of electromagnetic lens system and a screen which has a phosphorescent plate. The plate glows when hit by the electrons after passing through the specimen.



Advantages

A Transmission Electron Microscope is an impressive instrument with a number of advantages such as:

- TEMs offer the most powerful magnification, potentially over one million times or more
- TEMs have a wide-range of applications and can be utilized in a variety of different scientific, educational and industrial fields
- TEMs provide information on element and compound structure
- Images are high-quality and detailed
- TEMs are able to yield information of surface features, shape, size and structure
- They are easy to operate with proper training

Disadvantages

- Some cons of electron microscopes include:
- TEMs are large and very expensive
- Laborious sample preparation
- Potential artifacts from sample preparation
- Operation and analysis requires special training
- Samples are limited to those that are electron transparent, able to tolerate the vacuum chamber and small enough to fit in the chamber
- TEMs require special housing and maintenance

- Images are black and white

Electron microscopes are sensitive to vibration and electromagnetic fields and must be housed in an area that isolates them from possible exposure.

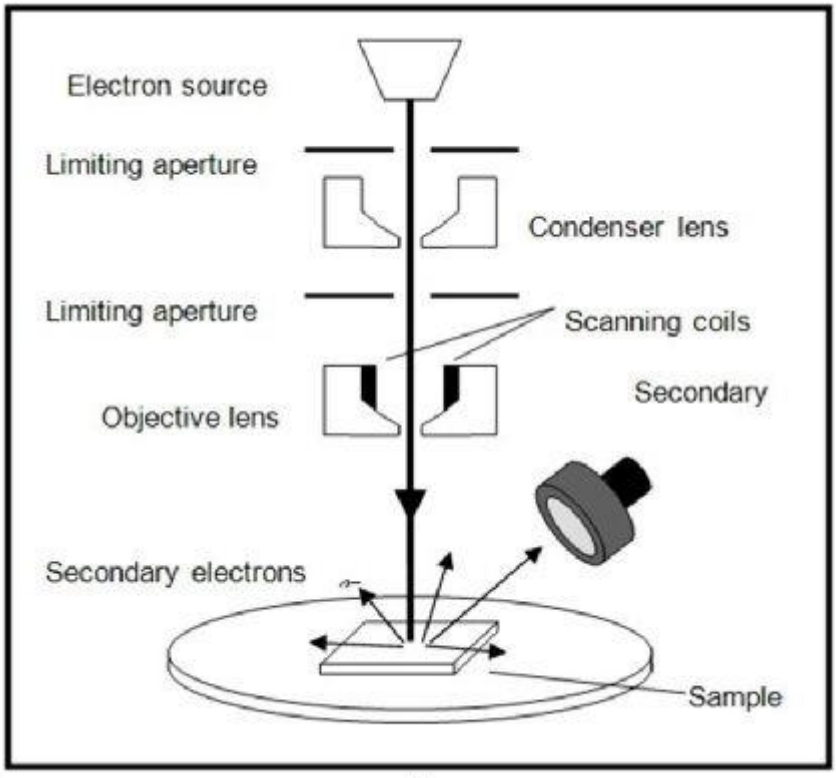
A Transmission Electron Microscope requires constant upkeep including maintaining voltage, currents to the electromagnetic coils and cooling water.

3. Generalise principle and applications of Scanning Electron microscope

Scanning Electron Microscope (SEM)

Unlike the TEM, where the electrons in the primary beam are transmitted through the sample, the Scanning Electron Microscope (SEM) produces images by detecting secondary electrons which are emitted from the surface due to excitation by the primary electron beam. In the SEM, the electron beam is scanned across the surface of the sample in a raster pattern (layer by layer), with detectors building up an image by mapping the detected signals with beam position.

The SEM image relies on electron interactions at the surface rather than transmission it is able to image bulk samples and has a much greater depth of view, and so can produce images that are a good representation of the 3D structure of the sample. SEM images are therefore considered to provide us with 3D, topographical information about the sample surface but will still always be only in black and white.



SEM uses focused Beam of high energy electron to generate a variety of signalS AT THE SURFACE of solid specimens. The signal from electron and sample interactions revels the information about the sample like ,its external morphology, chemical composition, chrystaline structure, and orientation of the materials present in the specimen. Areas ranging from 1cm to 5 microns in width(magnification in the range of 20x to 30,000x) can be imaged in a SEM.

Principle of SEM:

Accelerated electrons in a SEM carry significant amounts of kinetic energy and is converted in to a variety of signals by interaction with the specimen or solid sample. These signals include secondary electrons, Back scattered electrons diffracted back scattered electrons, photons, visible light and heat. Secondary electrons and back scattered electrons were used for imaging samples. Secondary electrons are valuable for the morphology and topography on samples and back scattered electrons are useful in illustrating contrasts in composition in multiphase samples. X-rays are generated by collisions of the electron with the atoms in the sample, thus characteristic X- rays were produced for each element in a mineral that is excited by the electron beam. the exited elements come back to their lower energy state with out any destruction. By studying the X-rays detection of elements in the sample is possible and analyze same material repeatedly

SAMPLE PREPARATION

Sputter Coating for the SEM examination: - an ultra-thin coating of electrically-conducting material, deposited by low vacuum coating of the sample. This is done to prevent charging of the specimen which would occur because of the accumulation of static electric fields due to the electron irradiation required during imaging. It also increases the amount of secondary electrons that can be detected from the surface of the sample in the SEM and therefore increases the signal to noise ratio. Such coatings include gold, gold/palladium, platinum, chromium etc.

4. a. State definitions and examples of Fungicide, Viricide, Bacteriostatic and Bactericidal gents
b. Write a short note on sterilization by UV and Gama Rays

A **fungicide** is a type of pesticide specifically designed to kill or inhibit the growth of fungi, including molds, yeasts, and other fungal pathogens that can damage plants, crops, or stored products. Fungicides are commonly used in agriculture, gardening, and in various industries to prevent fungal infections.

Example:

One well-known fungicide is **chlorothalonil**, which is used to control a variety of fungal diseases in crops like tomatoes and cucumbers. It helps prevent issues such as leaf spots, blights, and molds, promoting healthier plant growth and higher yields.

A **viricide** is a type of antiviral agent that is specifically designed to kill or inactivate viruses. These compounds can be used in various applications, including medical treatments, disinfectants, and personal care products.

Example:

An example of a viricide is **oseltamivir** (commonly known as Tamiflu), which is used to treat and prevent influenza virus infections. It works by inhibiting the viral enzyme neuraminidase, thereby preventing the virus from spreading within the body.

Bacteriocidal:

- **Definition**: Bacteriocidal agents are substances that kill bacteria outright. They lead to the death of bacterial cells.
- **Example**: **Penicillin** is a bacteriocidal antibiotic that disrupts the synthesis of bacterial cell walls, leading to cell lysis and death.

Bacteriostatic:

- **Definition**: Bacteriostatic agents inhibit the growth and reproduction of bacteria without killing them. This allows the immune system to eliminate the bacteria.
- **Example**: **Tetracycline** is a bacteriostatic antibiotic that interferes with protein synthesis in bacteria, preventing their growth and reproduction.

Sterilization by UV rays

It refers to the use of ultraviolet (UV) light to kill or inactivate microorganisms, including bacteria, viruses, and fungi. UV light, particularly UV-C (wavelengths between 100 to 280 nanometres), is effective at disrupting the DNA or RNA of pathogens, preventing them from replicating and causing infections.

Mechanism:

- **DNA Damage**: UV-C light causes the formation of thymine dimers in the DNA of microorganisms. This disrupts normal DNA replication and function, leading to cell death or inactivation.

Applications:

- **Water Treatment**: UV sterilization is commonly used to disinfect drinking water and wastewater by eliminating harmful pathogens without the use of chemicals.
- **Surface Disinfection**: UV light can be used to sterilize surfaces in laboratories, hospitals, and food processing facilities.
- **Air Purification**: UV lamps are used in HVAC systems to reduce airborne pathogens.

Advantages:

- **Chemical-Free**: UV sterilization does not involve chemicals, reducing the risk of chemical residues.

- **Effective**: It can quickly and effectively reduce microbial loads.

Limitations:

- **Shadows**: UV light is only effective on surfaces directly exposed to it; shadows can limit its effectiveness.
- **Material Degradation**: Prolonged exposure to UV light can damage certain materials, such as plastics.

Sterilization by gamma rays

It is a process that uses high-energy gamma radiation to kill or inactivate microorganisms, including bacteria, viruses, fungi, and spores. This method is widely used for sterilizing medical equipment, pharmaceuticals, and food products.

Mechanism:

- **Ionizing Radiation**: Gamma rays are a form of ionizing radiation. When they pass through materials, they ionize atoms and molecules, leading to the formation of free radicals. These free radicals can damage essential cellular components, including DNA, proteins, and membranes, ultimately resulting in cell death or inactivation.

Applications:

- **Medical Equipment**: Gamma sterilization is commonly used for items such as surgical instruments, syringes, and implants, ensuring they are free from pathogens before use.
- **Pharmaceuticals**: Certain drugs and medical supplies that are sensitive to heat or moisture can be sterilized using gamma radiation.
- **Food Preservation**: Gamma rays can be used to extend the shelf life of food by killing spoilage organisms and pathogens.

5. Describe working principle and applications of Autoclave

An autoclave is a machine that provides a physical method of sterilization by killing bacteria, viruses, and even spores present in the material put inside of the vessel using steam under pressure.
Autoclave sterilizes the materials by heating them up to a particular temperature for a specific period of time. The autoclave is also called a steam sterilizer that is commonly used in healthcare facilities and industries for various purposes. The autoclave is considered a more effective method of sterilization as it is based on moist heat sterilization.

Autoclave parts / components

The simplest form of the autoclave is the pressure cooker type or laboratory bench autoclaves. The following is the detailed description of different components/ parts of an autoclave

a. Pressure Chamber

- The pressure chamber is the main component of a steam autoclave consisting of an inner chamber and an outer jacket.

- The inner chamber is made up of stainless steel or gunmetal, which is present inside the out chamber made up of an iron case.
- The autoclaves used in healthcare laboratories have an outer jacket that is filled with steam to reduce the time taken to reach the sterilization temperature.
- The inner chamber is the case where the materials to be sterilized are put.
- The size of the pressure chamber ranges from 100 L to 3000 L.

b. Lid/ Door

- The next important component of an autoclave is the lid or door of the autoclave.
- The purpose of the lid is to seal off the outside atmosphere and create a sterilized condition on ht inside of the autoclave.
- The lid is made airtight via the screw clamps and asbestos washer.
- The lid consists of various other components like:

Pressure gauge

- A pressure gauge is present on the lid of the autoclave to indicate the pressure created in the autoclave during sterilization.
- The pressure gauge is essential as it assures the safety of the autoclave and the working condition of the operation.

Pressure releasing unit/ Whistle

- A whistle is present on the lid of the autoclave is the same as that of the pressure cooker.
- The whistle controls the pressure inside the chamber by releasing a certain amount of vapor by lifting itself.

Safety valve

- A safety valve is present on the lid of the autoclave, which is crucial in cases where the autoclave fails to perform its action or the pressure inside increases uncontrollably.
- The valve has a thin layer of rubber that bursts itself to release the pressure and to avoid the danger of explosion.

c. Steam generator/ Electrical heater

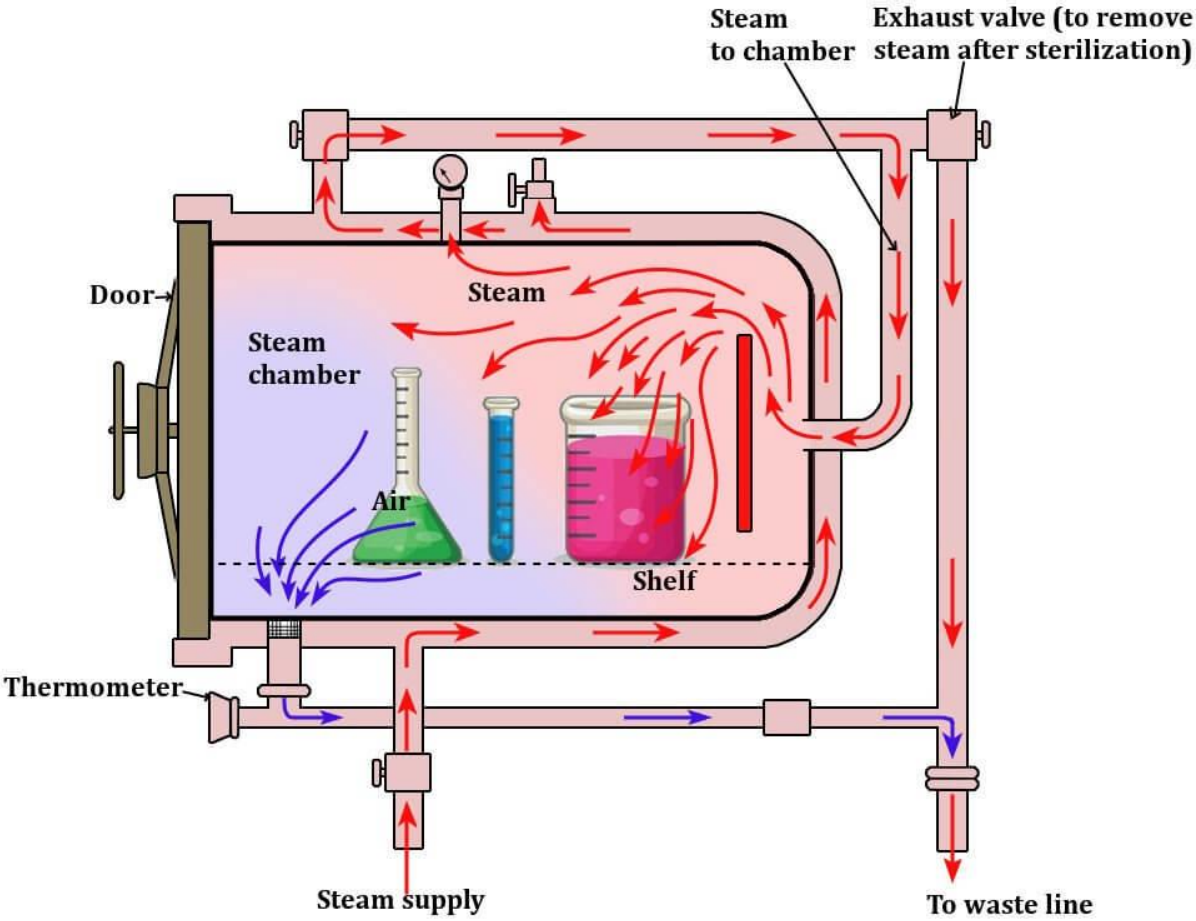
- An electrical steam generator or boiler is present underneath the chamber that uses an electric heating system to heat the water and generate steam in the inner and the outer chamber.
- The level of water present in the inner chamber is vital as if the water is not sufficient; there are chances of the burning of the heating system.
- Similarly, if the water is more than necessary, it might interfere with the trays and other components present inside the chamber.

d. Vacuum generator (if applicable)

- In some types of autoclaves, a separate vacuum generator is present which pulls out the air from the inside of the chamber to create a vacuum inside the chamber.
- The presence of some air pockets inside the chamber might support the growth of different microorganisms. This is why the vacuum chamber is an important component of an autoclave.

e. Wastewater cooler

- Many autoclaves are provided with a system to cool the effluent before it enters the draining pipes.
- This system prevents any damage to the drainage pipe due to the boiling water being sent out of the autoclave.



Principle:

- he autoclave works on the principle of moist heat sterilization where steam under pressure is used to sterilize the material present inside the chamber.
- The high pressure increases the boiling point of water and thus helps achieve a higher temperature for sterilization.
- Water usually boils at 100°C under normal atmospheric pressure (760 mm of Hg); however, the boiling point of water increases if the pressure is to be increased.
- Similarly, the high pressure also facilitates the rapid penetration of heat into deeper parts of the material, and moisture present in the steam causes the coagulation of proteins causing an irreversible loss of function and activity of microbes.
- This principle is employed in an autoclave where the water boils at 121°C at the pressure of 15 psi or 775 mm of Hg.
- When this steam comes in contact with the surface, it kills the microbes by giving off latent heat.
- The condensed liquid ensures the moist killing of the microbes.
- Once the sterilization phase is completed (which depends on the level of contamination of material inside), the pressure is released from the inside of the chamber through the whistle.
- The pressure inside the chamber is then restored back to the ambient pressure while the components inside remain hot for some time.

Uses of Autoclave:

Autoclaves are important devices to ensure the sterilization of materials containing water as they cannot be sterilized by dry heat sterilization. Besides, autoclaves are used for various other purposes.

1. They are used to decontaminate specific biological waste and sterilize media, instruments, and labware.
2. Regulated medical waste that might contain bacteria, viruses, and other biological materials is recommended to be inactivated by autoclaving before disposal.
3. In medical labs, autoclaves are used to sterilize medical equipment, glassware, surgical equipment, and medical wastes.
4. Similarly, autoclaves are used for the sterilization of culture media, autoclavable containers, plastic tubes, and pipette tips.

6.Evaluate different methods of Chemical means of sterilization

Alcohols:

Ethyl alcohol and isopropyl alcohol are frequently used as chemical agents for disinfection. Both of the chemicals facilitate the protein denaturation of bacterial proteins. 70% ethyl alcohol is the standard concentration which is used for disinfection. These are used as skin antiseptics. Apart from this methyl alcohol has activity against fungal spores and used to disinfection of inoculation cabinets.

Aldehydes:

Formaldehyde:

It is known for its bactericidal, sporicidal and virucidal activities. It can be used in both aqueous and gaseous form. A 10% formalin solution is a standard chemical disinfectant. It is used for

- Prevention of tissues for histological examinations.
- Sterilization of bacterial vaccines
- Preparation of toxoids from toxins.

Glutaraldehyde:

It has its activity against bacteria (*Mycobacterium tuberculosis)*, fungi and viruses (including HIV, hepatitis B, etc). It can also kill spores and is known for its less toxic nature. It is used as a 2% buffered solution. Glutaraldehyde is used for

- Sterilization of cystoscopes, endoscopes, and bronchoscopes
- Sterilization of plastic endotracheal tubes, face masks, metal instruments, etc.

Phenols:

Lister (father of antiseptic surgery) used phenol for the first time in the sterilization of surgical instruments. Phenols work as a disinfectant and kill microorganisms by cell membrane damage. It is toxic for the skin. Different derivatives of phenol are used as antiseptics which are following

Cresols:

An example of cresol is Lysol which is mostly used for sterilization of infected glasswares, floors, etc.

Halogens:

Chlorine and iodine are commonly used disinfectants. Chlorine is used in water supplies, swimming pools, food, and dairy industries. Chlorine compounds in the form of bleaching powder, sodium hypochlorite, and chloramines. The disinfection action of all the chlorine compounds is due to the release of free chlorine which becomes a strong oxidative agent. Iodine in alcoholic and aqueous solution is used as a skin disinfectant. It is active against *M tuberculosis* and slightly active against spores. Compounds with iodine with surface-active agents known as iodophors are claimed to be more active than aqueous or alcohol solution.

Oxidizing Agents-Hydrogen peroxide:

It is effective against most organisms in the concentration of 3-6 %. However, it kills spores at higher concentrations (10-25%). The mode of action is by the liberation of free hydroxyl

radical on the decomposition of hydrogen peroxide. These free radicals are active ingredients in the disinfection process.

Peracetic acid:

It is an oxidizing agent and is a more potent germicidal agent than hydrogen peroxide.

Salts:

Slats of heavy metals have a toxic effect on bacteria. The salts of copper, silver, and mercury are used as a disinfectant. They are protein coagulant ant act by combining with sulphydryl groups of bacterial proteins and other essential intracellular compounds. Merthiolate (sodium ethyl mercurithiosalicylate) is used in a dilution of 1:10000 for the preservation of sera.

Dyes:

Two groups of dyes, aniline and acridine dyes have been used as a skin and wound antiseptics. Both the dyes have bacteriostatic activity. Aniline dyes include crystal violet, brilliant green, and malachite green. Acridine dyes include acriflavine, cuflavin, proflavin, and aminacrine.

Ethylene Oxide:It is a colorless liquid with a boiling point of 10.7^0C. It is effective against all types of microorganisms including viruses and spores. It acts by alkylating the amnio carboxyl, hydroxyl and sulphydryl groups in protein molecules. In addition, it reacts with DNA and RNA. It is specially used for sterilizing plastic and rubber articles, respirators, heart-lung machines, dental equipment, etc.

Beta propiolactone (BPO)

This is a condensation product of ketane and formaldehyde. It has rapid action and used in0.2%. It is more efficient in fumigation than formaldehyde. BPO is used for the inactivation of vaccines.

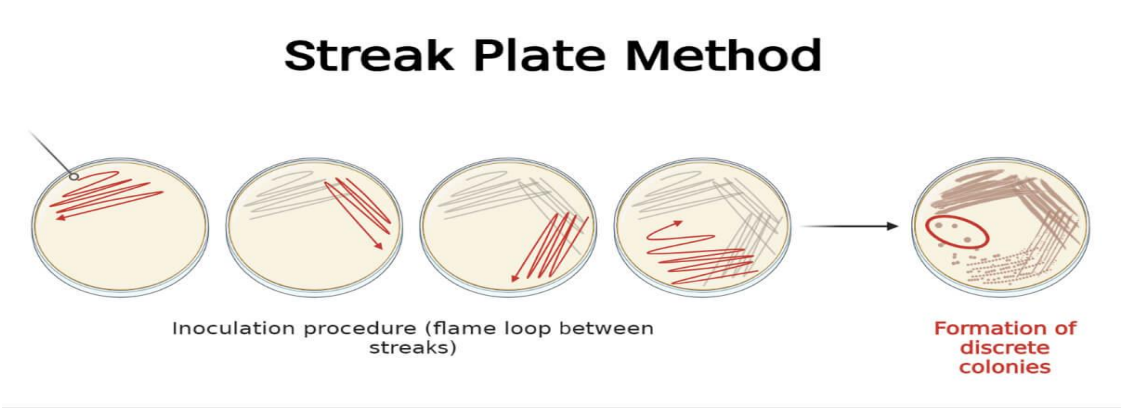
Q.7 Justify streak plate and spread plate methods and their Uses in obtaining pure cultures

Streak Plate Method

Streak literally means "*a long, thin line*": and the streak plate method is a microbiological culture technique where a sample is spread in a petri dish in the form of a long, thin line over the surface of solid media.

The streak plate method is based on dilution during the process of mechanical spreading of inoculum over the surface of solidified culture media in order to obtain well-isolated colonies of the sample at the terminal streaks. Sample can be either colony on solid media or suspension in broth. The sample is picked by using different tools, mostly using a sterile inoculating loop or swab. The sample is placed over a surface of sterile solid media at one edge of the petri dish and a smear is prepared. Using the tool, the smear is successively streaked over the agar medium on different patterns. As the streaking proceeds, the inoculum is gradually diluted to the point where bacterial cells are separated as individual cells or as a colony-forming unit (CFU) at a gap of a few millimeters. When these inoculated plates are incubated, the isolated bacterium or a CFU will give rise to a well-isolated colony. This will allow us to get a pure culture as well as describe the colony morphology of the organism.

This method dilutes the bacterial load, over the surface of agar medium, successively as streaking proceeds, and ultimately only a few bacterial cells will be inoculated at the end giving well-isolated colonies in the final streaks. Thus, this method mechanically isolated the bacteria from a mixed population of either the same or different species. After inoculation, the same types of colonies are seen in the terminal streaks if the specimen contained single species, whereas, different types of colonies may be seen if the specimen contained different species.



Application of Styreak plate Method:

1. Used to obtain a pure culture from the mixed culture in order to perform morphological, biochemical, and molecular tests to identify and for other applications.
2. Used to define the specimen as pure or mixed species.
3. Used to study colony characters of bacteria.
4. Used to produce a colony of genetically identical individuals
5. Used in inoculation of clinical specimens in diagnostic laboratories to grow isolated colonies of pathogen
6. Used in urine culture to isolate pathogens and semi-quantify the uropathogens to determine the significance of the infection. A calibrated loop is used for this purpose.

Spread plate method

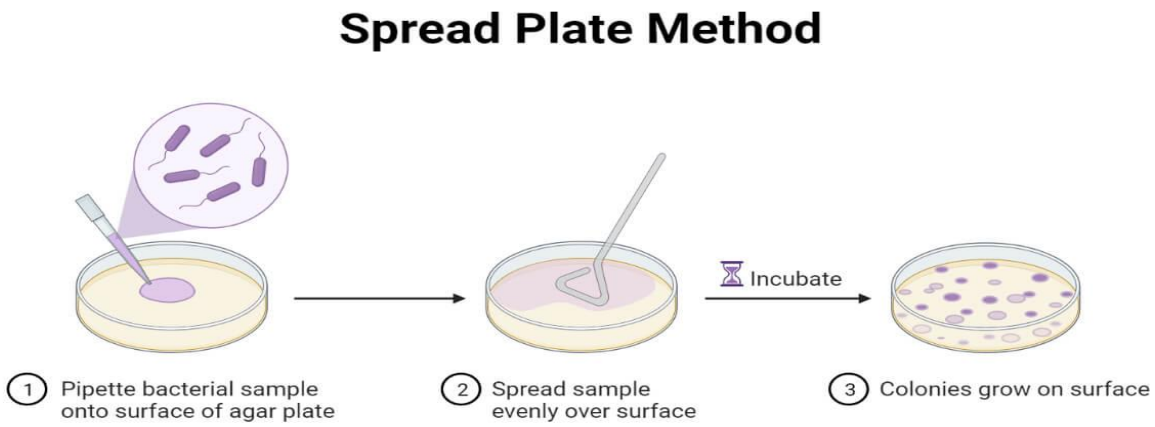
Spread Plate Method is one of the widely used culture techniques in microbiology laboratories due to its ease and simplicity. This method is suitable for aerobic and facultative aerobic microorganisms. It is an easy, simple, and economical method; however, it requires the sample to be in liquid or suspension.

Principle of Spread plate method

When a diluted liquid specimen containing one or more microorganisms, same or different species, is spread over a suitable solid agar media, each of the viable microorganisms will multiply forming a separate colony. These colonies can be counted and expressed in terms of the CFU/mL which can be used to calculate the microbial load in the sample.

A certain volume of the diluted sample, mostly 0.1 mL (0 to 1 mL can be used), is dispensed over the surface of a pre-sterilized solid medium. Usually, a bent glass rod or swab or glass beads are used to spread the sample. Following the incubation for usually 24 – 48 hours at 37°C, the viable microorganisms in the sample will grow into discrete visible colonies on the surface of the medium. The visible colonies can be counted and CFU/mL can be calculated using the following formula;

$$CFU/mL = \frac{Total\ number\ of\ colonies\ obtained \times dilution\ factor}{volume\ of\ specimen\ used\ (aliquot)}$$



Applications:

1. Used to isolate bacteria and fungi from a given sample
2. Used in antimicrobial sensitivity testing, and enrichment and screening experiments.
3. Used to calculate the number of viable microorganisms (i.e. calculate CFU/mL) in a sample
4. Used in food industries, pharmaceutical industries, soil studies, etc. in order to isolate and enumerate spoilers or contaminants to check for the quality of the products.
5. Used to mass culture the stock culture or fresh specimen
6. Used in clinical laboratories to inoculate the clinical specimens
7. Used to study growth curves, metabolic activities, and biochemical features of microorganisms, and also the effect of environmental factors on them.
8. Used in separating pure culture from a mixed culture
9. Used in generating discrete and pure colonies in order to study colony characters, genetic features, and other biochemical features of the isolates.

Q-8-Define MTCC and ATCC.
Evaluate Subculture, Lyophilization and lower temperature methods for the preservation of stock cultures

The Microbial Type Culture Collection and Gene Bank (MTCC) is a national facility in India that preserves microbial cultures for research and industry:

- **Preserves microbial cultures**
- **Provides characterization services**
- **Researches microbial diversity**
- **Funded by the Government of India**
- **Located in Chandigarh**

The American Type Culture Collection (ATCC) is a private, nonprofit organization that acquires, preserves, authenticates, and distributes biological materials. ATCC is the world's

largest general service culture collection, with collections in six areas: Bacteriology, Cell Culture, Molecular Biology, Mycology, Protistology, and Virology. ATCC offers a variety of resources, including:

- **Cell lines**

  ATCC has the world's largest catalog of human and animal cell lines for research purposes. ATCC cell lines are used in the discovery, invention, and development of new scientific advances

Methods of preservation:

Subculturing/ periodic transfer:

Periodic transfer to fresh media (Subculturing) -Strains can be maintained by periodically preparing a fresh culture from the previous stock culture. The culture medium, the storage temperature, and the time interval at which the transfers are made vary with the species and must be ascertained beforehand. The temperature and the type of medium chosen should support a slow rather than a rapid rate of growth so that the time interval between transfers can be as long as possible. Many of the more common heterotrophs remain viable for several weeks or months on a medium like Nutrient Agar.

Advantages

It is a simple method, any special apparatus are not required.

Easy to recover the culture

Disadvantages:

Since repeated subculturing is time consuming, it becomes difficult to maintain a large number of pure cultures successfully for a long time.

Risk of contamination is more

Possibility of genetic changes due to the development of variants and mutants and changes in biochemical characteristics Therefore, it is now being replaced by some modern methods that do not need frequent subculturing.

Freez dying (Lyophilization)

Freeze-drying is a process where water and other solvents are removed from a frozen product via sublimation. Sublimation occurs when a frozen liquid goes directly to a gaseous state without entering a liquid phase. It is recommended using slow rates of cooling, as this will result in the formation of vertical ice crystal structures, thus allowing for more efficient water sublimation from the frozen product. Freeze-dried products are hygroscopic and must be protected from moisture during storage. Under these conditions, the microbial cells are dehydrated and their metabolic activities are stopped; as a result, the microbes go into dormant state and retain viability for years. Lyophilized or freeze-dried pure cultures and then sealed and stored in the dark at 4°C in refrigerators. Freeze-drying method is the most frequently used technique by culture collection centers. Many species of bacteria preserved by this method have remained viable and unchanged in their characteristics for more than 30 years.

Advantage of Lyophilization

Only minimal storage space is required;

hundreds of lyophilized cultures can be stored in a small area.

Small vials can be sent conveniently through the mail to other microbiology laboratories when packaged in a special sealed mailing containers. Lyophilized cultures can be revived by opening the vials, adding liquid medium, and transferring the rehydrated culture to a suitable growth medium.

Low Temperatures:
Refrigeration- Pure cultures can be successfully stored at 0-4°C either in refrigerators or in coldrooms. This method is applied for short duration (2-3 weeks for bacteria and 3-4 months for fungi) because the metabolic activities of the microorganisms are greatly slowed down but not stopped. Thus their growth continues slowly, nutrients are utilized and waste products released in medium. This results in, finally, the death of the microbes after sometime.

Cryopreservation
Cryopreservation (i.e., freezing in liquid nitrogen at -196°C or in the gas phase above the liquid nitrogen at -150°C) helps survival of pure cultures for long storage times. In this method the microorganisms of culture are rapidly frozen in liquid nitrogen at -196°C in the presence of stabilizing agents such as glycerol or Dimethyl Sulfoxide (DMSO) that prevent the cell damage due to formation of ice crystals and promote cell survival. This liquid nitrogen method has been successful with many species that cannot be preserved by lyophilization and most species can remain viable under these conditions for 10 to 30 years without undergoing change in their characteristics, however this method is expensive.

Q-9-Explain different methods of cultivating Anaerobic Bacteria

Anaerobic Culture

Obligate anaerobes are bacteria that can live only in the absence of oxygen. These anaerobes are killed when exposed to the atmosphere for as briefly as 10 minutes. Some anaer-obes are tolerant to small amounts of oxygen. Facultative anaerobes are those anaerobes that grow with or without oxygen.

Anaerobic bacterial culture is a method used to grow anaer-obes from a clinical specimen. Culture and identification of anaerobes is essential for initiating appropriate treatment.

Culture Media

The commonly used media for anaerobic culture include Robertson cooked meat broth, thioglycollate broth, Willis and Hobbs' media, and neomycin blood agar. Robertson cooked meat (RCM) broth is the most widely used medium in an anaer-obic culture. It consists of nutrient broth and pieces of fat-free minced cooked meat of ox heart with a layer of sterile liquid paraffin over it. Unsaturated fatty acids and even glutathione and cysteine present in the meat extract utilize oxygen for auto-oxidation. The medium before inoculation is usually boiled at 80°C in a water bath to make the medium free of oxygen. The media after inoculation and incubation allows the growth of even strict anaerobes and also indicates their saccharolytic or proteolytic activities as meat is turned red or black, respectively.

Methods of Anaerobic Culture

Anaerobic cultures are carried out in an environment that is free of oxygen, followed by incubation at 95°F (35°C) for at least 48 hours before the plates are examined for growth. The cultures of anaerobic bacteria are carried out as follows:

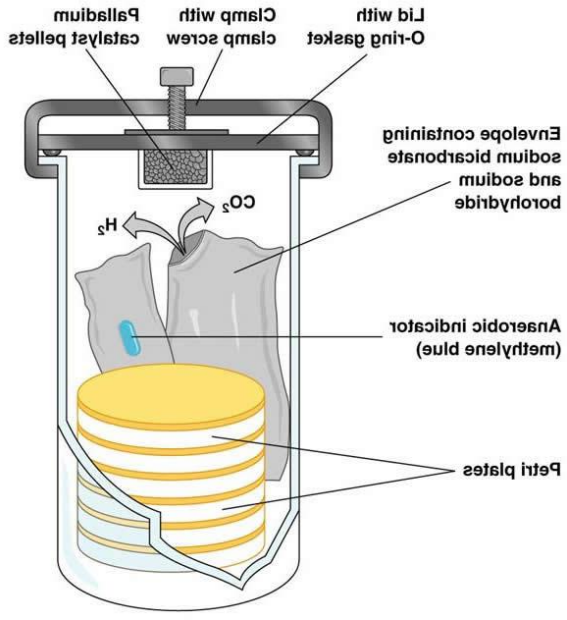
McIntosh–Fildes anaerobic jar: It is the most widely usedand dependable method of anaerobiosis. It consists of a glass or metal jar with a metal lid that can be clamped air tight with the help of a screw. The lid has one inlet tube and another outlet tube. The outlet tube is

connected to a vacuum pump by which the air is evacuated out of the jar. The inlet tube is connected to a source of hydrogen supply. The lid has two electric terminals also that can be connected to an electric supply. The underside of the lid contains a catalyst (e.g., alumina pellets coated with palladium) that catalyzes the combination of hydro-gen with residual oxygen present in the air. This method ensures complete anaerobiosis.

The culture media are inoculated with the specimens suspected to contain anaerobic bacteria. The inoculated media are then kept inside the jar, and the lid is closed air tight. The anaerobiosis in the jar is carried out by first evacuating the air from the jar through outlet tube with the help of a vacuum pump. The outlet tube is closed, then the sealed jar containing the culture plates is replaced with hydrogen gas passed through inlet tube till reduced atmospheric pressure is brought to normal atmospheric pressure, which is monitored on the vacuum gauge as zero. The electrical terminals are then switched on to heat the catalyst that catalyzes combination of hydrogen with residual oxygen and ensures complete anaerobiosis in the jar.

Reduced methylene blue is used as the indicator of anaerobiosis in the jar. If anaerobiosis is complete, it remains colorless; if anaerobiosis is not complete, it turns blue on exposure to oxygen.

Gas pack system is a simple and effective method of production of hydrogen gas for anaerobiosis. It does not require the cumbersome method of evacuation and filing up of gases after evacuation. Carbon dioxide, which is also generated, is required for growth by some anaerobes. Water activates the gas pack system, resulting in the production of hydrogen and carbon dioxide. Hydrogen combines with oxygen in the air in the presence of catalyst and maintains anaerobiosis. In this method, the inoculated plates are kept along with the gas pack envelope with water added in the air tight jar.



2. Anaerobic glove box: The anaerobic glove box is anotherinnovation developed for isolating anaerobic bacteria. It is essentially a large clear-vinyl chamber with attached gloves, containing a mixture of 80% nitrogen, 10% hydrogen, and 10% carbon dioxide. A lock at one end of the chamber is fit-ted with two hatches, one leading to outside and the other to the inside of the chamber. Specimens are placed in the lock, the outside hatch is closed, and the air in the lock

is evacu-ated and replaced with the gas mixture. The inside hatch is then opened to introduce the specimen into the chamber.

3**. Anoxomat**: This is a fully automated system that evacu-ates a portion of the jar contents and refills the jar with an anaerobic gas mixture. During this procedure, the oxygen concentration in the air is rarefied. For anaero-bic atmosphere, this procedure is repeated three times, after which the oxygen concentration is rarefied to 0.16%. A small catalyst removes this very small percentage of oxygen content. Anoxomat is capable of producing micro-aerophilic conditions also. The method is being increas-ingly used for processing clinical specimens for isolation of anaerobic bacteria.

Q-10-Explain about principle of Ion Exchange Chromatography
Explain principle of Affinity Chromatography

Spectroscopy: Introduction, Principles, Types and Applications

UV-Vis Spectroscopy or Ultraviolet-visible spectroscopy or Ultraviolet-visible spectrophotometer (UV-Vis) is also called absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region.

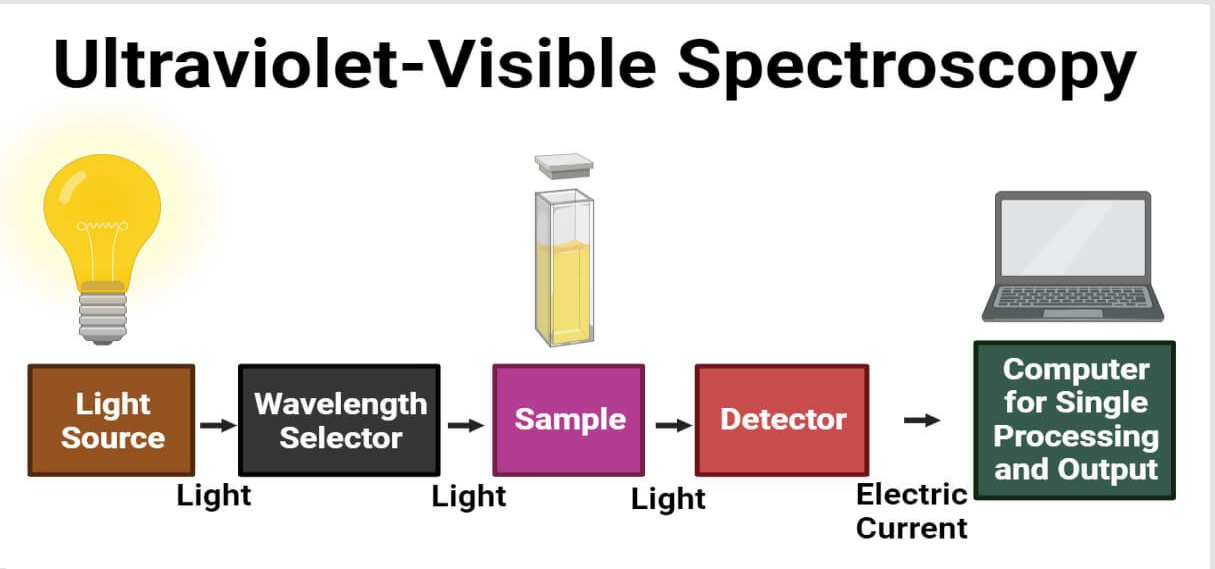
UV-VISIBLE SPECTROSCOPY

When a specific wavelength of light hits a molecule, that molecule gets excited. Once the electron excites, it excites from the ground (lower) energy state to the higher energy state. When an electron jumps off, it absorbs light energy because electrons in the orbital at a lower energy state utilize energy to move to a higher energy level.

Energy is neither created nor destroyed but can transform energy from one form to another. On passing EMR (UV- Vis range 200- 800 nm), only light possessing the precise amount of energy that can cause transitions from one level to another will absorb because matter's energy levels are quantized.

If the energy is utilized, the intensity of light received is lost. At this time, the energy absorbed by the electrons will equal the energy difference between the two energy levels.

During this stage, electron transition occurs. So, after the interaction of electromagnet radiation, the spectra received are called absorption spectra. Hence, it is called electron spectroscopy. Similarly, when electrons in the orbital at a higher energy level move to the ground energy level, the spectra received are called emissions.



INSTRUMENTATION OF UV VISIBLE SPECTROSCOPY

The main components of UV- Vis spectrophotometer are:

1. Light Source
2. Wavelength selector
3. Sample container
4. Detectors

1. Light Source

It is essential for emitting light in a wide range of wavelengths to work in a UV-Vis spectrometer. Commonly, a high-intensity light source used for both UV and Visible ranges is a xenon lamp. In contrast to tungsten and halogen lamps, it is less stable and more costly. So, the two lamps for this instrument are a deuterium lamp for UV light and a halogen or tungsten lamp for visible light as a source of light. The two lamps provide good intensity. While measuring the intensity of the light, the spectrometer ought to switch. A smoother transition is possible when the switchover occurs between 300 and 350 nm because the light emission for both visible and UV light sources is the same amount of light at that wavelength.

2. Wavelength selector

In order to allow sample examination using the wavelengths that the light source emits, wavelength selection helps to ascertain which wavelength is appropriate for the type of analyte and sample. The commonly used wavelength selector in the UV-Vis spectrometer is the monochromator. It separates light into a narrow band of wavelength.

From the entrance slit, radiation of different wavelengths will enter the monochromator. At a particular angle, the beam will collide and strike the dispersing element. A monochromator contains a prism that separates all different wavelengths of light in a single beam. It bends the monochromatic light and produces non-linear dispersion. Only single radiation or color of a specific wavelength will allow it to leave the monochromator and pass through its ultimate chain or exit slit.

3. Sample Container

In a single-beam spectrophotometer, all the radiation coming from the light source passes through the sample as one beam. Single-beam spectrophotometers can determine color by comparing the light sources' intensities before and after a sample is inserted. The wavelength range measure is 190–750 nm; however, it may go up to 1100 nm.

4. Detector

Detectors rely on photoelectric coatings or semiconductors. It converts the incoming light from the sample into an electric signal or current. The higher the current, the greater the intensity. It has the properties of low noise and high sensitivity, so it gives a linear response. Each detector has a variety of wavelength ranges and different sensitivity. Finally, The data recorder usually plots the absorbance against wavelength (nm) in the UV and visible section of the electromagnetic spectrum.

APPLICATIONS

DNA and RNA analysis

It focuses on verifying the concentration and purity of DNA and RNA, which plays a crucial role in downstream applications like sequencing. It ensures whether the DNA or RNA samples prepared for sequencing are contaminant or pure.

Since pure DNA has an absorbance ratio of 1.8 and pure RNA has a ratio of 2, the 260 nm/280 nm absorbance ratio is crucial for displaying protein contamination in nucleic acids. 260nm/230nm absorbance ratio varies for RNA and DNA (2.15 to 2.50).

Pharmaceutical analysis

It is essential in drug discovery and development, quantifying impurities in drug ingredients, dissolution testing of solid oral dosage forms like tablets, and chemical identification and quantification. It allows overlapping absorbance peaks in the original spectra using mathematical derivatives to identify pharmaceutical compounds.
Likewise, the Identification of pharmaceutical compounds, Chlortetracycline (antibiotic) and benzocaine (anesthetic) in veterinary powder formulation, by overlapping the absorbance peaks in UV spectra using mathematical derivatives.
Food and Beverage Applications
It applies to assessing the sensory attributes, nutritional components of food and its products such as beer, wine, juices, energy and soft drinks, waters, other thin liquids and thick liquids (honey, oils), fruits, vegetables, caffeine content, etc., and the chemical composition of ingredients and detect contaminants or adulterant to ensure the product is safe and healthier.
It can be used in quality control in wine by identifying anthocyanin in blueberries, raspberries, and cherries. It can evaluate food and food product color, flavor, and aroma.
Bacterial culture
It is essential in the biomass growth curve. It is used in culturing bacteria by estimating cell concentrations and growth tracking in measuring optical density at 600 nm. 600 nm is best to preserve the optical properties of culture media where bacteria grow and to avoid cell damage when there is a need for continuous experimentation.
Other Applications

1. In the cosmetic industry, it is used to evaluate photostability agents and color index, quantify dyes and antioxidants, and detect adulteration.

2. It is used in material science, like the characterization of small nanoparticles and to determine battery composition.
3. It is used to examine structural protein changes by tracking changes in peak wavelength absorbance.
4. In wastewater treatment, it is employed in kinetics and monitoring studies of dyes and dye byproducts to ensure adequate dye removal by comparing their spectra over time.
5. It is used in cancer research to estimate hemoglobin concentration.
6. It is used to measure color index to monitor transformer oil as a preventive measure to ensure electric power is delivered safely.
7. It is used in petrochemistry for characterizing crude oil, quality of crude oil gravity, formulation of indices for aromatic content, and sulfur content.
8. In the biochemistry and genetic fields, it is used to quantify DNA, protein/enzyme, and thermal denaturation of protein.

Q-11: Explain principle and applications of Paper Chromatography

PARTITION CHROMATOGRAPHY / Paper chromatography

What is Partition Chromatography?

Partition Chromatography technique is defined as the separation of components between two liquid phases viz original solvent and the film of solvent used in the column.
This separation theory was introduced in the year 1940s which was published by **Richard Laurence Millington Synge and Archer Martin**. It is also known as *Liquid-liquid chromatography (LLC)*. Or if gas is the mobile phase it is called *Gas-liquid chromatography (GLC)*.

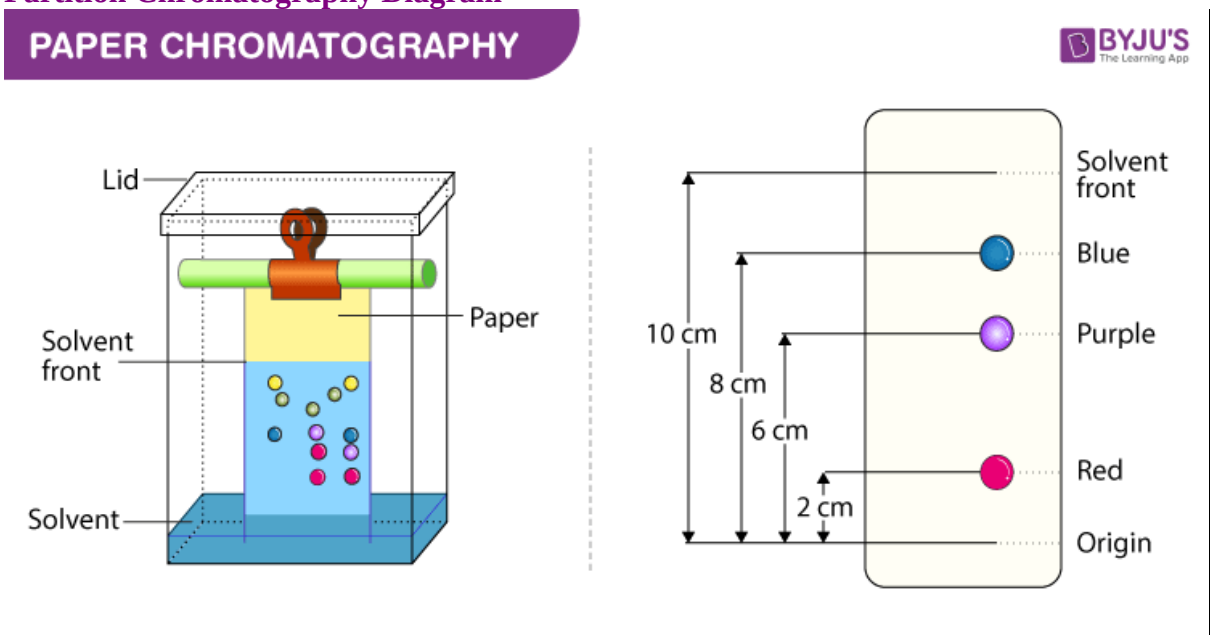
Principle of Chromatography

- Chromatography is a separation method where the analyte is contained within a liquid or gaseous mobile phase, which is pumped through a stationary phase.
- Usually, one phase is hydrophilic and the other lipophilic. The components of the analyte interact differently with these two phases.
- Depending on the polarity they spend more or less time interacting with the stationary phase and are thus retarded to a greater or lesser extent.
- This leads to the separation of the different components present in the sample.

Partition Chromatography Principle

The separation of the components from the sample mixture is carried out by the process of partition of the components between 2 phases. Both phases are in liquid form. In this process, the immiscible solid surface coated with the liquid surface on the stationary phase is in the mobile phase. The liquid surface is immobilised by a stationary phase which results in making it a stationary phase. The mobile phase moves from the stationary phase and components get separated. The separation depends on different partition coefficients.

Partition Chromatography Diagram



Partition Chromatography Procedure

Below we have explained the procedure to conduct Paper Chromatography Experiment for easy understanding

Apparatus required – chromatography jar, liquid impregnated paper (stationary phase), capillary tube (to apply sample mixture), mobile phase (example chloroform, methanol, acetone, ethanol).

1. Take a clean and dry chromatography jar.
2. To make sure that the environment of the jar is saturated with solvent vapours, a paper impregnated in the mobile phase is set to the walls.
3. Add the mobile phase to the jar. Around 0.5 cm to 1 cm from the bottom of the jar.
4. Close the jar.
5. Allow attaining equilibrium.
6. Mark the baseline on the adsorbent.

7. Apply sample to the paper with the help of a capillary tube.
8. Air-dry the sample spot.
9. Place the paper in the jar and close it.
10. Allow the system to stand till the solvent moves to some distance from the baseline.
11. Take out the paper and dry it.
12. If the sample components are separated, showing colours, then dry them in ordinary light. If it is a colourless component, then dry it in a UV lamp.Store the chromatogram and Calculate the Rf value.

Partition Chromatography Applications

There are various applications of Paper Chromatography. Some of the applications are mentioned below:

1. To separate and identify amino acids.To separate and identify tannins.
2. To separate and identify alkaloids.
3. To separate and identify carbohydrates.
4. To separate and identify glycosides.

Types of Partition Chromatography

1. Liquid-liquid Chromatography – It is a chromatography technique where a sheet of blotting paper, is used instead of adsorption column. The components are separated based on their differential migratory velocities. On separating, they are stained to make the chromatogram visible.
2. Gas-liquid Chromatography – A chromatography technique in which the separation of the mixture is done by an inert gas along a tube. The tube is filled with finely divided inert solid. The solid is coated with a nonvolatile oil. The migration of each component occurs at a rate determined by its solubility in oil as well as its vapour pressure.

Application of partition chromatography

- Remove the detergent from the protein solution.
- Steroids, bile acids, and mycotoxins are separated.
- Pesticides, phenols, and insecticides are all being removed.
- Concentration of trace metals in aqueous solution.

Q-12. Explain about principle of Ion Exchange Chromatography

Explain principle of Affinity Chromatography

Ion Exchange Chromatography

Ion exchange chromatography (or ion chromatography) is a process that allows the separation of ions and polar molecules based on their affinity to ion exchangers.

The principle of separation is thus by reversible exchange of ions between the target ions present in the sample solution to the ions present on ion exchangers.

In this process, two types of exchangers i.e., cationic and anionic exchangers can be used.

1. Cationic exchangers possess negatively charged group, and these will attract positively charged cations. These exchangers are also called “Acidic ion exchange” materials, because their negative charges result from the ionization of acidic group.
2. Anionic exchangers have positively charged groups that will attract negatively charged anions. These are also called “Basic ion exchange” materials.

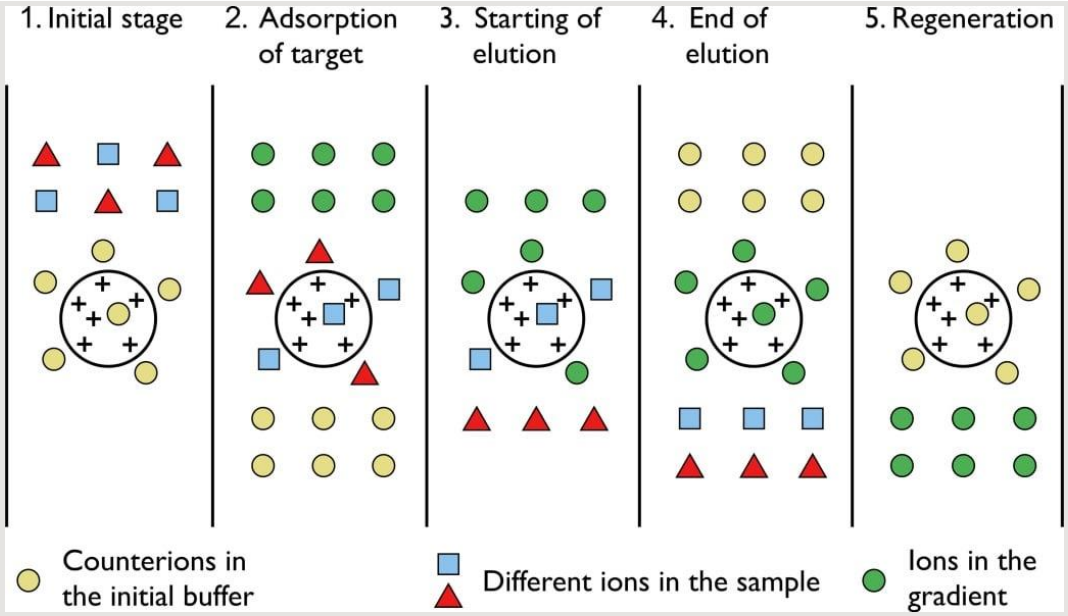
- Ion exchange chromatography is most often performed in the form of column chromatography. However, there are also thin-layer chromatographic methods that work basically based on the principle of ion exchange.

Principle:

This form of chromatography relies on the attraction between oppositely charged stationary phase, known as an ion exchanger, and analyte.

- The ion exchangers basically contain charged groups covalently linked to the surface of an insoluble matrix.
- The charged groups of the matrix can be positively or negatively charged.
- When suspended in an aqueous solution, the charged groups of the matrix will be surrounded by ions of the opposite charge.
- In this “ion cloud”, ions can be reversibly exchanged without changing the nature and the properties of the matrix.

Procedure of Ion exchange chromatography:



- Ion exchange separations are carried out mainly in columns packed with an ion-exchanger.
- These ionic exchangers are commercially available. They are made up of styrene and divinyl benzene. Example. DEAE-cellulose is an anionic exchanger, CM-cellulose is a cationic exchanger.
- The choice of the exchanger depends upon the charge of particle to be separated. To separate anions “Anionic exchanger” is used, to separate cations “Cationic exchanger” is used.

- First the column is filled with ion exchanger then the sample is applied followed by the buffer. The tris-buffer, pyridine buffer, acetate buffer, citrate and phosphate buffers are widely used.
- The particles which have high affinity for ion exchanger will come down the column along with buffers.
- In next step using corresponding buffer separates the tightly bound particles.
- Then these particles are analyzed spectroscopically.

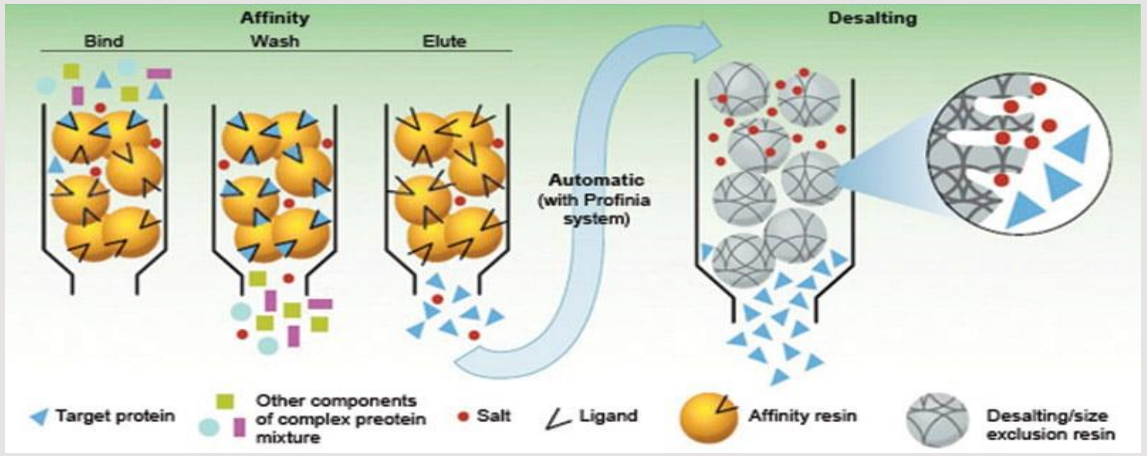
Applications:

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

AFFINITY CHROMATOGRAPHY

- Chromatography is an important biophysical technique that enables the separation, identification, and purification of the components of a mixture for qualitative and quantitative analysis.
- It is a separation technique in which a mobile phase carrying a mixture is caused to move in contact with a selectively absorbent stationary phase.
- Affinity chromatography is a type of liquid **chromatography** for the separation, purification or specific analysis of sample components.
- It utilizes the reversible biological interaction or molecular recognition called affinity which refers to the attracting forced exerted in different degrees between atoms which cause them to remain in combination.

Example: Enzyme with an inhibitor, antigen with an antibody, etc.



PRINCIPLE

- he stationary phase consists of a support medium, on which the substrate (ligand) is bound covalently, in such a way that the reactive groups that are essential for binding of the target molecule are exposed.
- As the crude mixture of the substances is passed through the chromatography column, substances with binding site for the immobilized substrate bind to the stationary phase, while all other substances is eluted in the void volume of the column.
- Once the other substances are eluted, the bound target molecules can be eluted by methods such as including a competing ligand in the mobile phase or changing the pH, ionic strength or polarity conditions.

APPLICATIONS

Affinity chromatography is one of the most useful methods for the separation and purification of specific products.

- It is essentially a sample purification technique, used primarily for biological molecules such as proteins.

Its major application includes:

- Separation of mixture of compounds.
- Removal of impurities or in purification process.
- In enzyme assays
- Detection of substrates
- Investigation of binding sites of enzymes
- In in vitro antigen-antibody reactions
- Detection of Single Nuceotide polymorphisms and mutations in nucleic acids

Q-13- Differentiate types of Centrifuges and their applications

Centrifugation is a term used to describe a method of separating mixtures using spinning and centrifugal force. Several characteristics can separate particles during centrifugation, including size, shape, density, and viscosity.

Principle of a Centrifuge:

The centrifuge utilizes the sedimentation principle due to gravitational force. The centrifugation technique uses a centrifugal field to separate particles suspended in a liquid medium. These are put in the centrifuge's rotor either in bottles or tubes. Sedimentation is a process whereby gravity causes suspended particles to separate from fluids. The suspended substance may consist of powder or clay-like particles.

Simple filtration filters particles larger than 5 micrometres from those less than 5 micrometres, which start Brownian motion and do not sediment under gravity. These particles can be separated with the help of the central force.

Types of Centrifugation techniques

There are two types of centrifugation techniques, namely, **preparatory centrifugation** and **analytical centrifugation**. Preparatory centrifugation deals with the isolation and purification of components such as tissue, cells, subcellular structure, membrane vesicles, and other particles of biochemical interest. In contrast, analytical centrifugation is carried out to characterize purified biomolecules.

Preparative Centrifugation

Based on suspension, preparative centrifugation is divided into two different types. They are:

i) Differential centrifugation

It separates particles based on shape, size, and density. A suspension of particles with varying densities or sizes will sediment at varying speeds, with the larger and denser particles sedimenting more quickly. Following a series of rising centrifugal force cycles on a suspension of cells, a series of pellets containing cells with a decreasing sedimentation rate will result.

ii) Density gradient centrifugation

It separates particles based on their buoyant density or sedimentation rate. A sample mixture is placed on the top of a preformed liquid density gradients such as CsCl for DNA banding and isolation of plasmids, nucleoproteins, and viruses; NaBr and NaI for fractionation of lipoprotein; Per coll, Ficoll, Metrizamide, Dextran for separation of whole cells and sucrose solution for the separation of DNase, RNase and Protease.

The two subtypes of density gradient centrifugation are rate-zonal and isopycnic centrifugation.

Rate-zonal centrifugation

On top of a density gradient, the sample is overlaid as a small zone. Depending on their mass, particles travel under centrifugal force at various speeds. Size and mass are the main determinants of how quickly particles settle. As the band of particles descends through the density medium, zones with particles of comparable size develop as the faster sedimenting particles pass the slower ones.

Isopycnic centrifugation

Particles are separated exclusively based on their density in an isopycnic separation, also known as buoyant or equilibrium separation. It is necessary for the gradient medium to have a higher density than the particles that need to be separated.

Particles migrate under the influence of centrifugal force from a uniformly mixed sample and density gradient until their densities are equal to those of the surrounding medium. After centrifugation, particles of a certain density settle until their density equals that of the gradient media (i.e., the equilibrium position).

Analytical Centrifugation

It aims to collect information to characterize the spun sample (sedimentation velocity, viscosity, concentration, etc.), determine the relative molecular weight of the solutes, purity of biomolecules, detect conformational changes of protein structure, etc.

Types of Centrifuges:

Benchtop or Tabletop centrifuges

- They can be handy for tiny labs with limited space because of their diminutive size.
- These are compact and are frequently employed in research and clinical laboratories.
- A tabletop centrifuge is furnished with a lid that covers the apparatus used to run the centrifuge and a rotor with racks for the test tubes.

Gas centrifuges

- These are used to separate molecules based on their masses and to separate gases based on their isotopes.
- Specifically, they are employed in the extraction and separation of uranium-235 and uranium-238.

Haematocrit centrifuge

- Haematocrit centrifuges operate between 7000 and 15000 rpm.

- The main purpose of hematocrit centrifuges is to calculate the volume-based erythrocyte percentage in blood. It is used to produce plasma for photometric analysis of the bilirubin concentration of neonatal blood.

Microcentrifuge

- They have a very small footprint and take up minimal room on the workstation because of their highly compact form.
- These work well with small tubes (up to 2.0 ml) and are frequently employed in biological applications.
- They are used to microfilter small amounts of aqueous samples and hold pelleted nucleic acids, proteins from solutions, and other substances.

Refrigerated Centrifuges

- These centrifuges run at their top speeds while keeping a constant temperature.
- It is used to analyze DNA, RNA, PCR, and antibodies because its temperature range is between -20 and -40 degrees Celsius.
- They are frequently used to collect sedimenting materials quickly, including yeast cells, chloroplasts, and more.

High-Speed Centrifuges

- A high-speed centrifuge is a type of centrifuge that can work at somewhat faster rates ranging between 15,000 and 30,000 revolutions per minute.
- High-speed centrifuges contain a device for regulating both the temperature and speed of the operation for the critical analysis of delicate biological molecules.
- These centrifuges employ three rotors: fixed angle, swinging bucket, and vertical.

Low-speed centrifuges

- These are frequently used in laboratories for routine particle sorting operated at a maximum speed of 4000-5000 rpm.
- There are few instances of temperature regulation, and they are often operated at room temperature.
- These centrifuges employ swinging bucket and fixed-angle rotor types.

Continuous flow centrifuges

- It enables the centrifugation of large quantities of samples without influencing sedimentation rates.
- They also have greater capacities, which saves time by eliminating the need to load and unload the sample repeatedly as is necessary with standard centrifuges.

Ultracentrifuges

- The ultracentrifuge is a highly developed and sophisticated centrifuge that can separate tiny molecules that conventional centrifuges can’t separate at a fast rate.
- Ultracentrifuge rotor speeds can range from 60,000 to 150,000 rpm.
- They run samples in groups or as continuous flow systems and are larger.

Types of Ultracentrifuges

1. **Preparative Ultracentrifuges**

- Preparative ultracentrifuges are centrifuges that are used to isolate and separate particles inside an experiment using centrifugation.

- When a run is prepared for an ultracentrifuge, the contents of the tubes are examined after the centrifugation procedure, as opposed to the centrifuge for analysis, which examines during the centrifugation.

2. **Analytical Ultracentrifuges**

- Analytical centrifuges are ultracentrifuges used to examine different particles inside the specimen.
- It is utilized to perform a qualitative examination of macromolecules in solution.
- These are fitted with sensing devices that track the spin and movement of the constituents in real time to calculate the sedimentation coefficient

Applications of Centrifuge

- Centrifuges are employed in chemistry, biology, biochemical, and clinical laboratories, such as testing the sedimentation rates of various blood cells.
- These are utilized in dairy industries to separate cream (fat) from milk, and this process is known as churning.
- Giant centrifuging machines are used in water treatment, where it spins the mud and sludge out of the water to produce cleaner water. Likewise, solid matter is separated from freshly drilled-out petroleum in oil rigs.
- Moreover, big spinning wheels are used to simulate a high-gravity environment to practice for the pilots. Hence, these are important in aeronautics and space.
- Centrifugation is used to produce biological products and bulk drugs and perform biopharmaceutical analysis of drugs.
- It is applied in removing water from lettuce after washing it in a salad spinner and separating chalk powder from water.
- It is useful for separating the isotopes for nuclear weapon programming.

Advantages of Centrifuge

- Enclosed operation and consequently clean appearance
- Quick start-up and shutdown
- Easy automation and continuous operation if necessary
- Low capital cost-to-capacity ratio
- Quick adjustment of operating parameters
- High flexibility and outstanding performance
- Simple operation and easy installation

Q-14_ working principle and applications of Gel Electrophoresis

Agarose gel electrophoresis is a method of gel electrophoresis used in biochemistry, molecular biology, genetics, and clinical chemistry to separate a mixed population of macromolecules such as DNA , RNA or proteins in a matrix of agarose.

- Agarose is a natural linear polymer extracted from seaweed that forms a gel matrix by hydrogen-bonding when heated in a buffer and allowed to cool.
- They are the most popular medium for the separation of moderate and large-sized nucleic acids and have a wide range of separation.

Principle:

Gel electrophoresis separates DNA fragments by size in a solid support medium such as an agarose gel. Sample (DNA) are pipetted into the sample wells, followed by the application of an electric current which causes the negatively-charged DNA to migrate (electrophorese)

towards the anodal, positive (+ve) end. The rate of migration is proportional to size: smaller fragments move more quickly and wind up at the bottom of the gel.
DNA is visualized by including in the gel an intercalating dye, ethidium bromide. DNA fragments take up the dye as they migrate through the gel. Illumination with ultraviolet light causes the intercalated dye to fluoresce.
The larger fragments fluoresce more intensely. Although each of the fragments of a single class of molecule is present in equimolar proportions, the smaller fragments include less mass of DNA, take up less dye, and therefore fluoresce less intensely. A "ladder" set of DNA fragments of known size can be run simultaneously and used to estimate the sizes of the other unknown fragments.

Requirements:

The equipment and supplies necessary for conducting agarose gel electrophoresis are relatively simple and include:

1. An **electrophoresis chamber** and **power supply**
2. **Gel casting trays**, which are available in a variety of sizes and composed of UVtransparent plastic. The open ends of the trays are closed with tape while the gel is being cast, then removed prior to electrophoresis.
3. **Sample combs**, around which molten medium is poured to form sample wells in the gel.
4. **Electrophoresis buffer**, usually Tris-acetate-EDTA (TAE) or Tris-borate-EDTA (TBE).
5. **Loading buffer**, which contains something dense (e.g. glycerol) to allow the sample to "fall" into the sample wells, and one or two tracking dyes, which migrate in the gel and allow visual monitoring or how far the electrophoresis has proceeded.
6. **Staining**: DNA molecules are easily visualized under an ultraviolet lamp when electrphoresed in the presence of the extrinsic fluor ethidium bromide. Alternatively, nucleic acids can be stained after electrophoretic separation by soaking the gel in a solution of ethidium bromide. When intercalated into doublestranded DNA, fluorescence of this molecule increases greatly. It is also possible to detect DNA with the extrinsic fluor 1-anilino 8-naphthalene sulphonate.
7. **Transilluminator** (an ultraviolet light box), which is used to visualize stained DNA in gels.

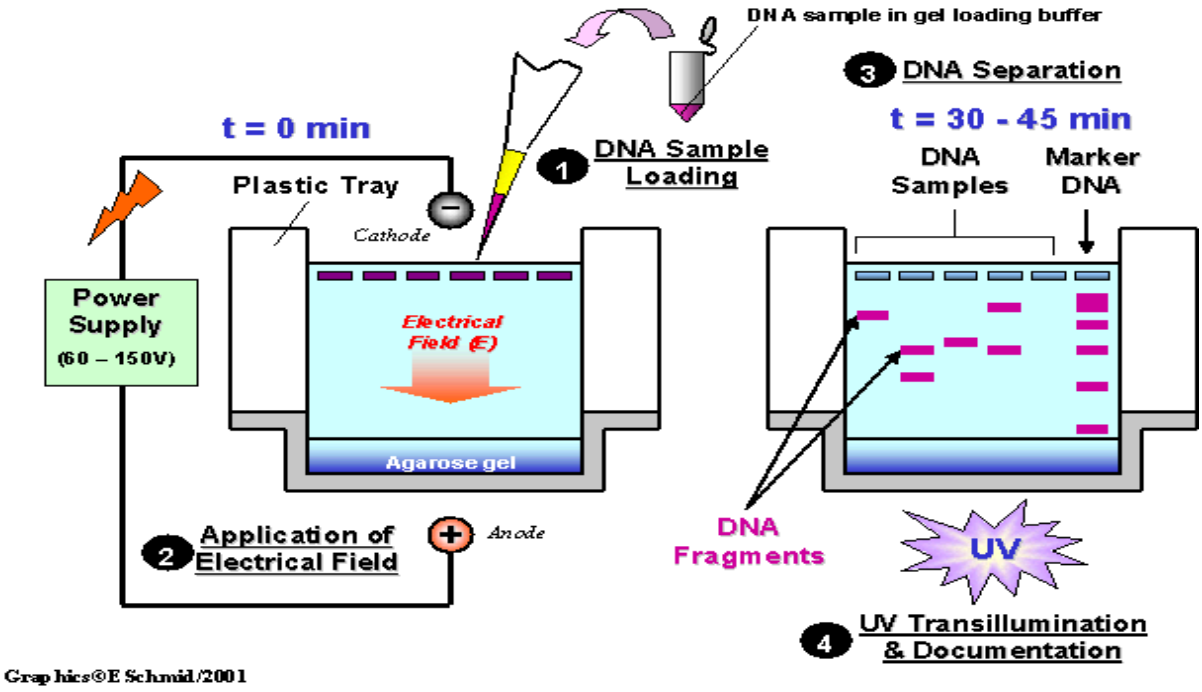
PREPARATION OF GEL:

1. To prepare gel, agarose powder is mixed with electrophoresis buffer to the desired concentration, and heated in a microwave oven to melt it.

The concentration of Agarose Gel

- The percentage of agarose used depends on the size of fragments to be resolved.
- The concentration of agarose is referred to as a percentage of agarose to volume of buffer (w/v), and agarose gels are normally in the range of 0.2% to 3%.
- The lower the concentration of agarose, the faster the DNA fragments migrate.
- In general, if the aim is to separate large DNA fragments, a low concentration of agarose should be used, and if the aim is to separate small DNA fragments, a high concentration of agarose is recommended.

2. Ethidium bromide is added to the gel (final concentration 0.5 ug/ml) to facilitate visualization of DNA after electrophoresis.



3. After cooling the solution to about 60oC, it is poured into a casting tray containing a sample comb and allowed to solidify at room temperature.
4. After the gel has solidified, the comb is removed, taking care not to rip the bottom of the wells.
5. The gel, still in plastic tray, is inserted horizontally into the electrophoresis chamber and is covered with buffer.
6. Samples containing DNA mixed with loading buffer are then pipetted into the sample wells, the lid and power leads are placed on the apparatus, and a current is applied.
7. The current flow can be confirmed by observing bubbles coming off the electrodes.
8. DNA will migrate towards the positive electrode, which is usually colored red, in view of its negative charge.
9. The distance DNA has migrated in the gel can be judged by visually monitoring migration of the tracking dyes like bromophenol blue and xylene cyanol dyes.

APPLICATIONS OF AGAROSE GEL ELECTROPHORESIS

Agarose gel electrophoresis is a routinely used method for separating proteins, DNA or RNA.

- Estimation of the size of DNA molecules
- Analysis of PCR products, e.g. in molecular genetic diagnosis or genetic fingerprinting
- Separation of restricted genomic DNA prior to Southern analysis, or of RNA prior to Northern analysis.
- The agarose gel electrophoresis is widely employed to estimate the size of DNA fragments after digesting with restriction enzymes, e.g. in restriction mapping of cloned DNA.
- Agarose gel electrophoresis is commonly used to resolve circular DNA with different supercoiling topology, and to resolve fragments that differ due to DNA synthesis.
- In addition to providing an excellent medium for fragment size analyses, agarose gels allow purification of DNA fragments. Since purification of DNA fragments size separated in an agarose gel is necessary for a number molecular techniques such as cloning, it is vital to be able to purify fragments of interest from the gel.

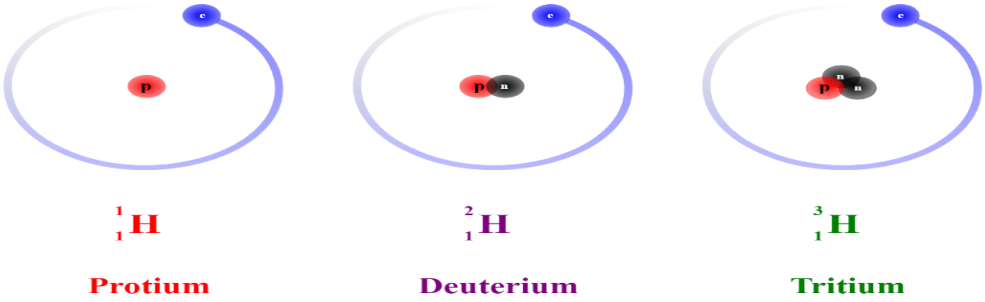
ADVANTAGES:

- For most applications, only a single-component agarose is needed and no polymerization catalysts are required. Therefore, agarose gels are simple and rapid to prepare.
- The gel is easily poured, does not denature the samples.
- The samples can also be recovered.

Q-15- Analyse characters and applications of Radioisotopes

Isotopes Definition:

Isotopes are atoms with the same atomic number but different mass numbers. They are the subspecies of the same chemical element & occupy the same position in periodic table, but have different properties.



Two classes of isotopes: 1- Stable IsotopesThese do not have distinguishing characteristics other than their masses. These are obtained from natural resources by fractional procedure. 2- Unstable – Isotopes that continuously and spontaneously break down/decay in other lower atomic weight isotopes. These are called Radio active isotopes.

Radioactivity:

Radioactivity is the spontaneous degradation of nucleus & transmission of one element to another with consequent emission of rays (or) particles. In other wordsRadioactivity is the process whereby unstable atomic nuclei release energetic subatomic particles. ❖ First discovered in 1896 by the French scientist Henri Becquerel, after whom the SI unit for radiation, the Becquerel, is named.

Radioisotopes:

Radioisotopes/radioactive isotopes of an element can be defined as atoms that contain an unstable nucleus and dissipate excess energy by spontaneously emitting radiation in the form of alpha, beta and gamma rays. How do radioisotopes occur? NaturalOccur in nature in traces, as in radium-226, Carbon-12 ArtificialThey are prepared artificially by altering the atoms,using a nuclear reactor or a cyclotron.

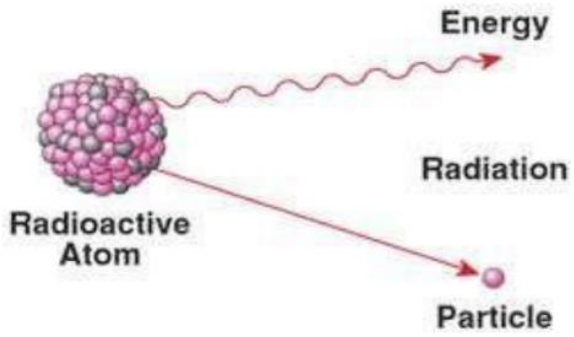
Properties of Radioactive Isotopes

1. Emits radiation
2. Half life(t ½)
3. Penetration property
4. .Same chemical properties
5. Different physical properties

1. Emits radiation

Radioactive isotopes are unstable so they undergo radioactive decay emitting radiations, till they become stable.

Three types of radiations • Alpha particles(α) • Beta particles(β) • Gamma rays(Y)



Alpha Decay

An alpha particle is identical to a helium nucleus. It contains two protons and two neutrons.

Hence, it can be written as He2+. Alpha particles are a highly ionising form of particle radiation

As its ionising power is so high it does not penetrate very deeply into matter. Thus it has very low penetrating power (absorbed by 10 cm of air, 0.01 mm lead or a sheet of paper).

Beta Decay

Beta decay occurs when a neutron changes into a proton (+) and an electron (-). A beta particle is identical to electron. It is Emitted from the nucleus of an atom undergoing radioactive decay. Beta particles are high-energy, high-speed electrons emitted by certain types of radioactive nuclei such as potassium-40. Form of ionising radiation also known as beta rays. The high energy electrons have greater range of penetration than alpha particles, but still much less than gamma rays.

GammaDecay

Gamma rays are not charged particles like α and β particles. They are released with these particles. Gamma rays are electromagnetic radiation with high frequency. When atoms decay by emitting α or β particles to form a new atom, the nuclei of the new atom formed may still have too much energy to be completely stable. This excess energy is emitted as gamma rays (gamma ray photons have energies of ~ 1 x 10-12 J). These have Low ionising power. These have Very high penetrating power.

Same chemical properties

Isotopes of the same elements have the same chemical properties.

Due to the same number of electrons in the outermost shell. Different physical properties

Differ from isotopes to isotopes. Based on the number of neutrons.

Applications of Radioactive Isotopes

a) Scientific research

b) Analytical

c) Diagnostic

d) Therapeutic

A. Applications of Radioisotopes in Biological Sciences/ Research

Radioisotopes are frequently used for tracing metabolic path ways.

Mixing radiolabelled substrates & samples of the experimental material & collecting samples at various times , extract & separate the products by chromatography. It is possible to predict the fate of individual carbon atoms of (14C) acetate through TCA cycle.

Radio labelled drugs are useful in pharmacokinetic studies

B.Analytical application of Radioisotopes

Virtually any enzyme reaction can be assayed using radioactive tracer methods.

Radioisotopes have been used in study of The mechanism of enzyme action& In studies of ligand binding to membrane receptors.

By isotope dilution analysis plasma volume , total body water, E.C.F volume , RBC cell volume , total exchangeable sodium can be measured.

Radio immunoassays are useful in analysis of hormones , growth factors , tumour markers, cytokines , bacterial antigens, vitamin D & various biological molecules.

In RIA either antigen or antibody is radiolabelled. Radiolabelling must not interfere in the binding of antigen & antibody , has to be compared with unlabelled ones .

C.Applications of Radioisotopes in Diagnostic purposes

The branch of medicine that deals with the diagnostic applications of radioactivity is referred to as Nuclear Medicine. A quick and accurate diagnosis can be made by radio imaging of organs like thyroid, liver, bone etc.

Radio active iodine uptake & imaging reveals the functional status of thyroid tissue , including nodules , the whole thyroid gland & metastatic foci .

131 -Iodine is used for thyroid cancer imaging & management.

123- Iodine is used for thyroid scan.

D. Applications of Radioisotopes in Diagnostic purposes

Ex: Radio immunoassay

E. Therapeutic applications of Radioisotopes

Radioisotopes have role in management of malignancies. Tumor tissues are attacked by beam of radiation.

Two routes

1-From outside the patient's body ((External sources)

2-From within the body (Internal sources)

Therapeutic applications of Radioisotopes

a)Tele therapy: 60 Co is the source of radiation , radiation occurs from a distant source.

Treatment of various malignant disorders. Advantage: penetrate deep into tissues; does not cause skin reactions.

b) Beads, needles and applicators:

Radioactive material is impregnated into body in form of beeds or needles or as surface applicants.

e.g: **60Co** for CA Cervix, encapsulated in gold or silver needles, wires, rods orcylinders.**32P** applied to paper or polythene sheets for SCC, superficial angiomas, mycosis fungoides senile keratosis.**90Sr** applicators used for lesions of cornea, conjunctiva sclera.

c)Extracorporeal irradiation of blood:

E.g: C/c leukaemia-blood is taken out of patient via forearm artery, circulated around 137Cs source which emits powerful rays, and then irradiated blood is returned to the same patient via forearm vein.

Advantage: avoid bone marrow depression by 'radiomimetic alkylating agents'.

Multiple Choice Questions (MCQ)

UNIT-1

Which of the following is true about the principle of a Bright-field microscope?
a) It uses phase-shifted light to visualize specimens.
b) It illuminates the specimen directly, producing a dark image against a bright background.
c) It only works for live organisms.
d) It requires a vacuum for imaging.
Answer: b) It illuminates the specimen directly, producing a dark image against a bright background.

1. In Micrometry, the unit most commonly used for measuring microscopic objects is:
   a) Millimeter
   b) Centimeter
   c) Micrometer
   d) Nanometer
   Answer: c) Micrometer
2. Which staining technique is used to differentiate bacteria into Gram-positive and Gram-negative?
   a) Simple staining

b) Gram staining
c) Negative staining
d) Acid-fast staining
Answer: b) Gram staining

3. In Spore staining, what color do the spores appear after staining with malachite green?
a) Red
b) Blue
c) Green
d) Pink
Answer: c) Green

Fill in the Blanks

5. In Bright-field microscopy, the image is formed by light that is _____ by the specimen.
Answer: transmitted

6. Electron microscopes use a beam of _____ instead of visible light to achieve higher resolution.
Answer: electrons

7. Acid-fast staining is primarily used to identify organisms like _____ that have waxy cell walls.
Answer: Mycobacterium

8. Negative staining is particularly useful for observing _____, which may not be easily stained with other methods.
Answer: capsules

True or False

9. True or False: Simple staining uses only one dye, making it effective for differentiating between multiple types of microorganisms.
Answer: False

10. True or False: Scanning Electron Microscopy (SEM) is ideal for observing the surface details of a specimen.
Answer: True

UNIT-2

Multiple Choice Questions (MCQ)

1. Which method of sterilization uses dry heat to destroy microorganisms?
a) Autoclave
b) Incineration
c) Filtration
d) UV radiation
Answer: b) Incineration

2. Which of the following is not a physical method of microbial control?
a) Radiation
b) Autoclave
c) Fumigants

d) Hot air oven
Answer: c) Fumigants

3. Alcohols act as disinfectants by:
   a) Denaturing proteins and disrupting cell membranes
   b) Binding to DNA and causing mutations
   c) Preventing protein synthesis
   d) Inhibiting cell wall formation
   Answer: a) Denaturing proteins and disrupting cell membranes
4. What is the main mode of action of UV radiation in controlling microbial growth?
   a) Disrupting cell membranes
   b) Causing thymine dimers in DNA
   c) Denaturing enzymes
   d) Destroying bacterial spores
   Answer: b) Causing thymine dimers in DNA

Fill in the Blanks

5. Autoclaving sterilizes by using _____ heat under pressure to kill microorganisms, including spores.
Answer: moist
6. Gamma rays are a type of ionizing radiation that sterilizes by breaking _____ bonds within microbial DNA.
Answer: chemical
7. Fungicides are chemical agents specifically designed to kill _____.
Answer: fungi

True or False

8. True or False: Moist heat sterilization, like autoclaving, is more effective than dry heat sterilization because water conducts heat more effectively than air.
Answer: True
9. True or False: Phenols disrupt cell walls and membranes, making them effective disinfectants, especially in healthcare settings.
Answer: True
10. True or False: Filtration is a method of sterilization that removes microorganisms from solutions by trapping them in filters, and it is especially useful for heat-sensitive materials.
Answer: True

UNIT-3

Multiple Choice Questions (MCQ)

1. Which method is commonly used for isolating pure bacterial cultures?
   a) Streak plate method
   b) Filtration
   c) Spore staining
   d) Gram staining
   Answer: a) Streak plate method

2. Which of the following is used to maintain bacterial cultures over long periods of time by lowering metabolic activity?
   a) Lyophilization
   b) Serial dilution
   c) Streaking
   d) Gram staining
   Answer: a) Lyophilization
3. Which device is used in isolating single cells during the pure culture technique?
   a) Centrifuge
   b) Autoclave
   c) Micromanipulator
   d) Spectrophotometer
   Answer: c) Micromanipulator
4. What is the primary goal of serial dilution in microbiology?
   a) To decrease the number of bacteria
   b) To isolate individual colonies from a mixed culture
   c) To increase the concentration of a sample
   d) To measure the bacterial motility
   Answer: b) To isolate individual colonies from a mixed culture
5. Which of the following culture collection centers is located in India?
   a) MTCC
   b) ATCC
   c) DSMZ
   d) NCCS
   Answer: a) MTCC

Fill in the Blanks

6. Subculturing is the process of transferring microorganisms from one _____ to another to maintain pure cultures.
Answer: medium

7. Anaerobic bacteria require specialized conditions for cultivation because they cannot tolerate _____ in their environment.
   Answer: oxygen

True or False

8. True or False: Sand cultures are a method used to preserve bacteria in sterile, dry sand at room temperature.
Answer: True

9. True or False: ATCC is an international culture collection center that provides cultures of bacteria, fungi, and other microorganisms.
Answer: True

10. True or False: The cultivation of fungi typically requires an acidic medium, such as Sabouraud's agar, to inhibit bacterial growth.
Answer: True

UNIT-4

Multiple Choice Questions (MCQ)

1. Which law explains the relationship between absorbance and concentration in UV-Visible spectrophotometry?
   a) Beer-Lambert Law
   b) Newton's Law
   c) Dalton's Law
   d) Charles' Law
   Answer: a) Beer-Lambert Law
2. The absorbance of a solution is directly proportional to:
   a) Concentration of the solution
   b) Wavelength of light
   c) pH of the solution
   d) Temperature
   Answer: a) Concentration of the solution
3. In thin layer chromatography (TLC), the stationary phase is typically composed of:
   a) Paper
   b) Silica gel
   c) Agarose
   d) Cellulose
   Answer: b) Silica gel
4. Which type of chromatography is most suitable for separating proteins based on their charge?
   a) Paper chromatography
   b) Adsorption chromatography
   c) Ion-exchange chromatography
   d) Gel-filtration chromatography
   Answer: c) Ion-exchange chromatography

Fill in the Blanks

5. Beer-Lambert law states that the absorbance of a solution is directly proportional to the _____ of the solution and the path length.
Answer: concentration
6. In colorimetry, a solution's concentration is determined by measuring the intensity of the _____ produced by a specific color.
Answer: light
7. In affinity chromatography, specific interactions between a target molecule and a ligand attached to the stationary phase are used to achieve _____.
Answer: separation

True or False

8. True or False: In column chromatography, partition chromatography separates components based on their different solubilities in two immiscible liquids.
Answer: True
9. True or False: In adsorption chromatography, the mobile phase is a solid, and the stationary phase is a liquid.
Answer: False
10. True or False: Gel filtration chromatography separates molecules based on their size, with smaller molecules eluting first.
Answer: False

UNIT-5

Multiple Choice Questions (MCQ)

1. The principle of centrifugation is based on which of the following?
   a) Gravitational force
   b) Electromagnetic force
   c) Centripetal force
   d) Centrifugal force
   Answer: d) Centrifugal force
2. Which type of centrifugation is commonly used to separate cellular organelles based on their size and density?
   a) Ultracentrifugation
   b) Density gradient centrifugation
   c) Differential centrifugation
   d) Microcentrifugation
   Answer: c) Differential centrifugation
3. What is the purpose of SDS in SDS-PAGE electrophoresis?
   a) To denature proteins and give them a uniform negative charge
   b) To stain proteins for visualization
   c) To act as a buffer solution
   d) To separate proteins by charge
   Answer: a) To denature proteins and give them a uniform negative charge
4. In autoradiography, which of the following is used to detect radioactive materials?
   a) X-ray film
   b) UV light
   c) Fluorescence
   d) Mass spectrometry
   Answer: a) X-ray film

Fill in the Blanks

5. In ultracentrifugation, particles are separated based on their _____ and buoyant density, using extremely high rotational speeds.
Answer: size
6. Agarose gel electrophoresis is commonly used to separate _____ based on size.
Answer: nucleic acids (DNA/RNA)
7. SDS-PAGE electrophoresis uses polyacrylamide gels to separate proteins based on their _____.
Answer: molecular weight

True or False

9. True or False: In density gradient centrifugation, the sample forms layers based on the density of the particles, with heavier particles moving to the top.
Answer: False
10. True or False: Autoradiography allows visualization of radioactive molecules on a gel after electrophoresis.
Answer: True