Department of Microbiology

II B.Sc Microbiology Honours -III Semester

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	

Analyse characters and applications of Radio Isotopes

C. Explain about principle of Ion Exchange Chromatography
D. 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.
Electron transition takes place, so it is also called electron spectroscopy. It is a cost-effective, simple, versatile, and non-destructive technique that allows the sample to be used again for further analysis. It is a qualitative, quantitative, and analytical technique that compares a sample with a blank or reference sample to measure the amount of discrete ultraviolet and visible light absorbed or transmitted through a particular sample using Beer-Lambert law. It studies under vacuum conditions.

The wavelength of UV-vis spectroscopy ranges from 190 nm to 800 nm. The UV region ranges from 190 to 400 nm, and the visible region from 400 to 800 nm. Near UV region is 190 nm to 400 nm, and far UV region is below 200 nm. The shorter the wavelength, the higher will be the frequency and energy. It occurs in UV region. Similarly, the higher the wavelength, the lower the frequency and energy in the visible region.

Its properties depend on sample composition and concentration. It helps to identify, assess purity, and quantify the components of the sample by analyzing the pattern of absorption and transmission of light. It may apply in several sample types, such as monolithic solids, liquids, glass, powders, and thin films.

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.

Beer-Lambert Law equation is the principle behind absorbance spectroscopy.

The concentration of the sample can be determined directly from the absorption of spectra produced by these samples at specific wavelengths using the Beer-Lambert law.

What is Beer-Lambert Law?

When a beam of light allows it to pass through a transparent medium, the rate at which an intensity decreases with medium thickness is directly proportional to the light beam's intensity.

According to the Beer-Lambert Law, the absorbance is directly proportional to the concentration of the substance in the solution. Therefore, a sample's concentration can also be determined using UV-visible spectroscopy.

The Beer-Lambert Law can be expressed in the form of the following equation:

$A = -\log T = -\log (I/Io) = \log(Io/I) = ecl$

$A = ecl$

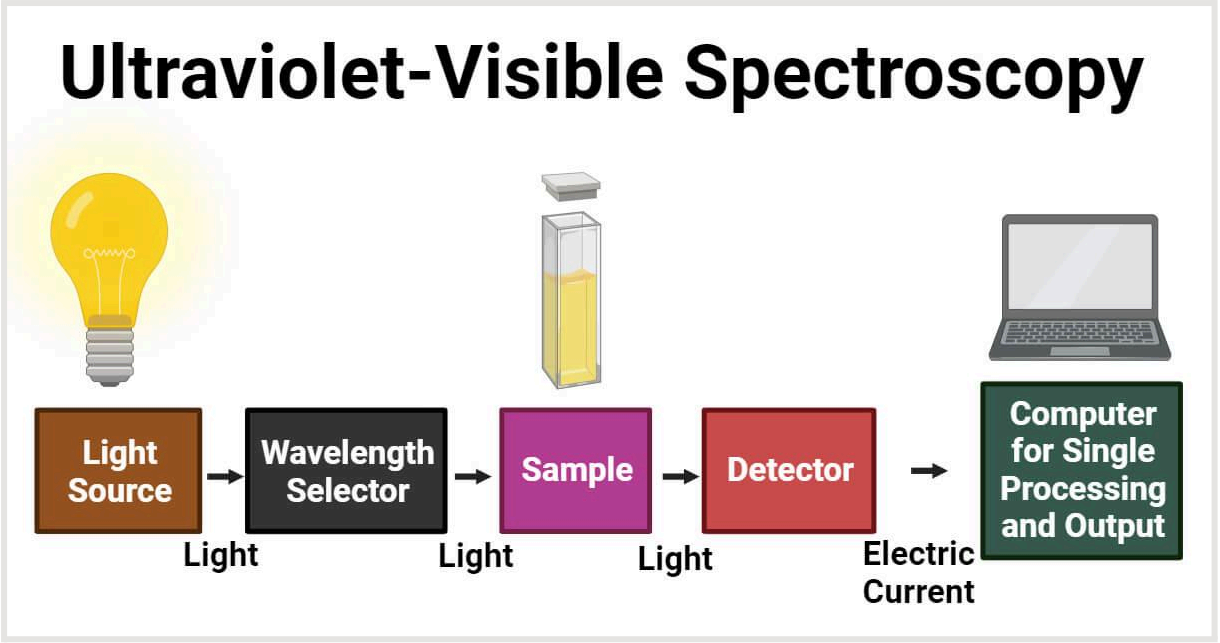
Where A = absorbance

l = optical path length of the cell or cuvette or sample holder(cm)

c = concentration of the solution (mol dm-3)

e = molar absorptivity of the compound or molecule in solution, which is constant for a particular substance at a particular wavelength (dm3 mol-1 cm-1)

Following the Beer-Lambert Law, the plot of absorbance versus concentration should be linear if the absorbance of a series of sample solutions with known concentrations is measured and plotted against equivalent concentrations. This graph is known as a calibration graph.



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.

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)*.

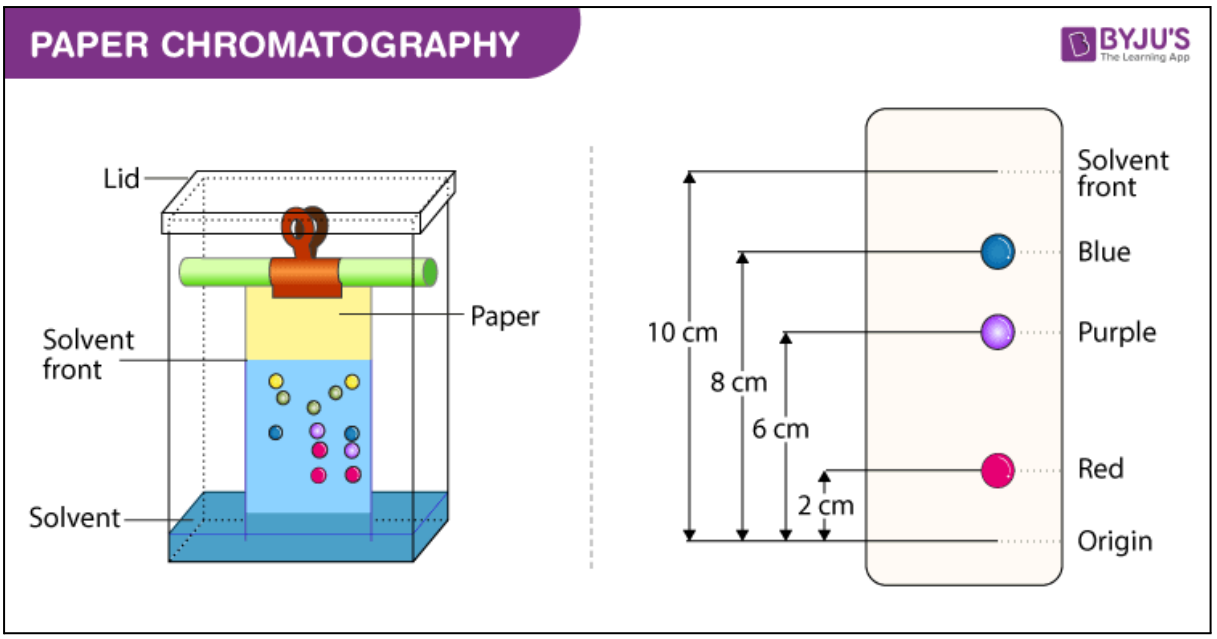
Principle of Chromatography

- 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.

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.

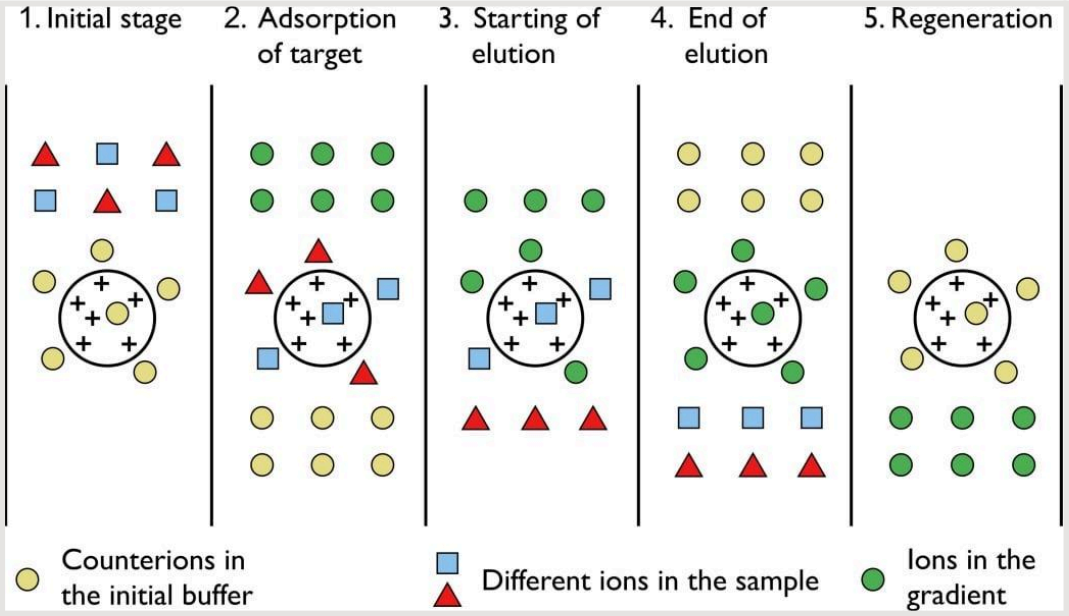
- Ion exchange chromatography is most often performed in the form of column chromatography. However, there are also thin-layer chromatographic methods that work basically based on the principle of ion exchange.

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:

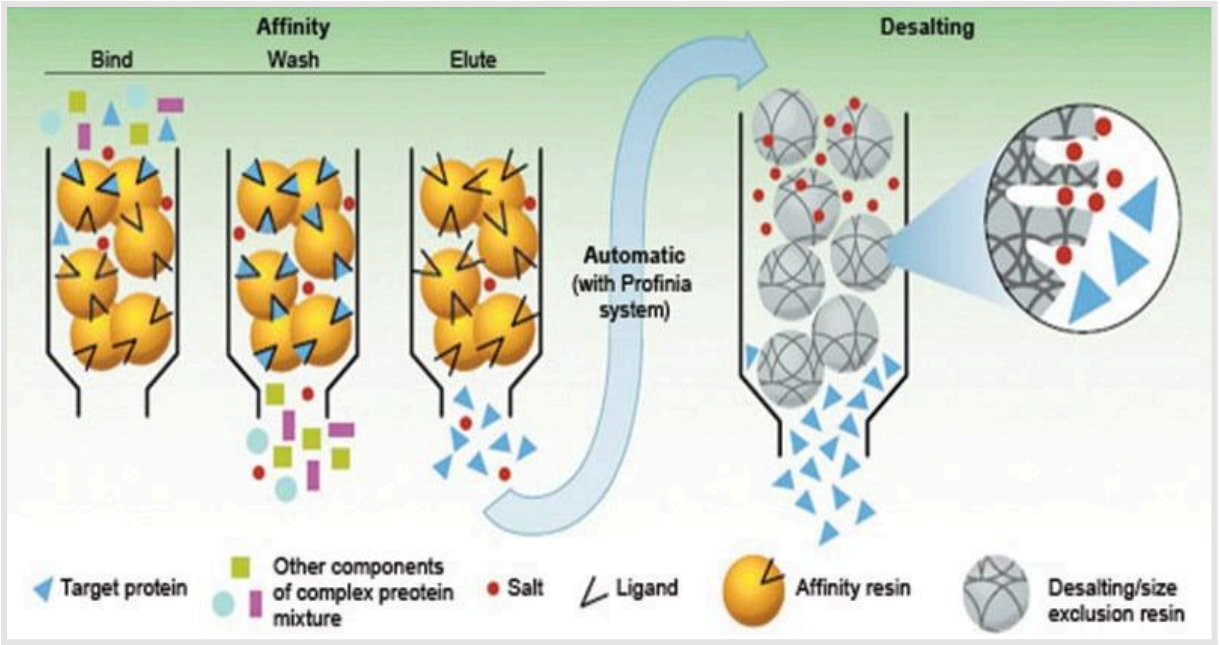
- An important use of ion-exchange chromatography is in the routine analysis of amino acid mixtures.
- The 20 principal amino acids from blood serum or from the hydrolysis of proteins are separated and used in clinical diagnosis.
- This is most effective method for water purification. Complete deionization 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.

- It was piscovered by Pedro Cuatrecasas and Meir Wilcheck.



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

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

Parts of a Centrifuge:

Some of the common parts of the centrifuge are described below:

1. **Motor:** The motor is the powerful central component of the centrifuge that creates the spin.
2. **Rotor assembly:** A drive shaft and a rotor comprise the rotor assembly. The drive shaft provides support for the rotor components. The rotor head is attached to the

motor, which bears the containers to house the tubes containing the sample to be centrifuged. It converts electrical energy to mechanical energy. Two rotors with different diameters can have the same rotational speed. Varying radii and angular momentum results in a difference in the acceleration of such rotors. Thus, relative centrifugal force (rcf) is regarded as the accepted standard unit for the rotation speed. There are mainly three types of rotors:

a) Fixed angle rotors: These rotors hold the tubes at an angle of 14 to 40° to the vertical such that particles travel a short distance while moving radially outwards and are used in differential centrifugation. The sedimentation takes place at the walls of the tubes at an angle since the sedimentation direction is the same as the direction of centrifugal force. The pellets (cluster of sediments) later settle at the corner of the base and the wall surface after colliding with the wall surface.

b) Swinging bucket/ Horizontal rotors: These rotors, along with the centrifuge tubes, swing out to a horizontal position during the time of acceleration such that particles travel a longer distance, thereby facilitating easier separation of supernatant from the pellet. These types of motors are employed in density gradient centrifugation.

c) Vertical rotors: These hold the tubes vertically, i.e., parallel to the motor axis, and the particles move shorter distances with shorter periods for separation. It is used for isopycnic and density gradient separation; however, it is not considered useful for pelleting because the pellets are spread out along the entire outer wall of the tube by centrifugal force.

3. **Containers:** Several types of containers, such as test tubes, blood bags, cuvettes, centrifuge tubes, etc., are held in the rotors such that the sample rotates along as the rotor rotates.
4. **Control Panel:** It serves the purpose of controlling different parameters such as temperature, rotational speed (rcf or rpm), etc.
5. **Latch:** When a tube breaks, or there are other issues with the centrifuge while running, the latch keeps the lid closed.
6. **Lid:** The centrifuge will only spin if the lid is closed and locked to prevent mishaps.

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

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.

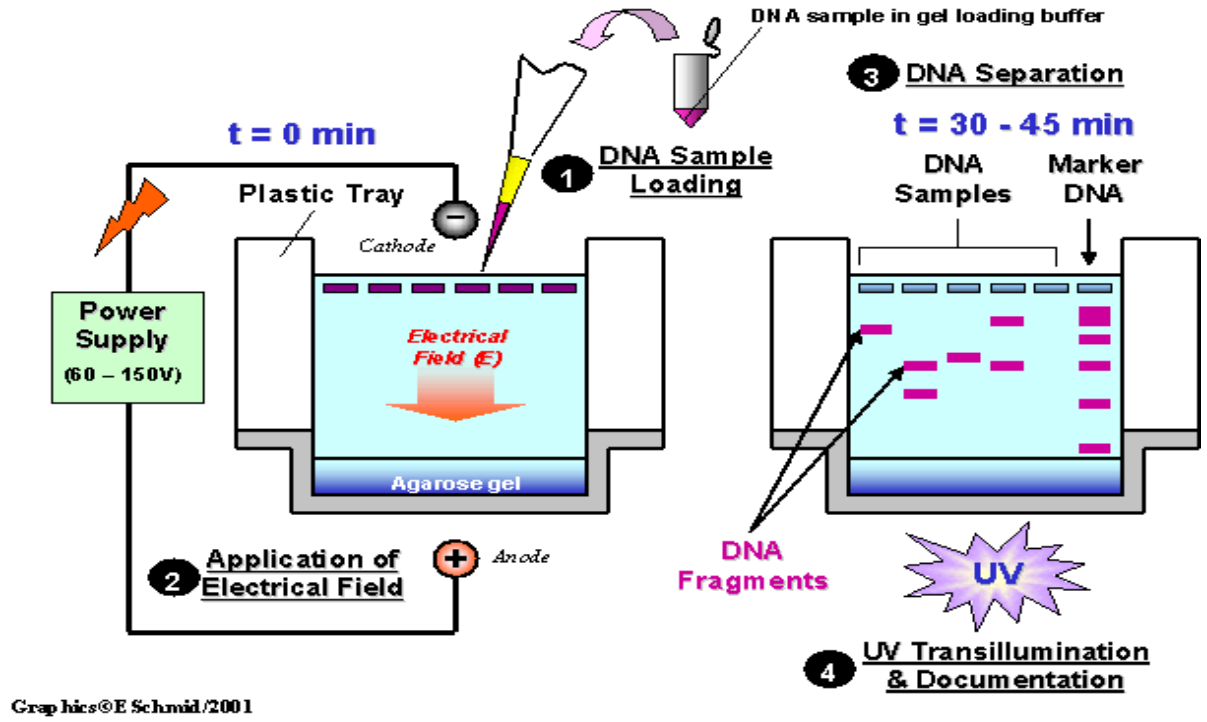
DNA is visualized by including in the gel an intercalating dye, ethidium bromide. DNA fragments take up the dye as they migrate through the gel. Illumination with ultraviolet light causes the intercalated dye to fluoresce.

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.

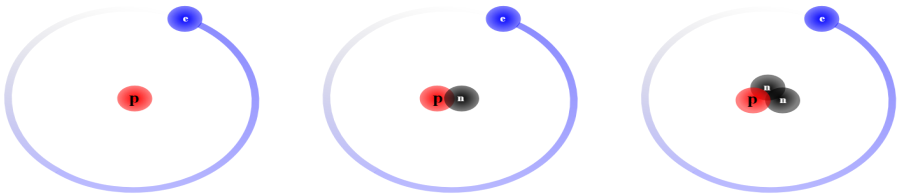
ADVANTAGES:

- 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.

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.



$^{1}_{1}H$ $^{2}_{1}H$ $^{3}_{1}H$

Protium **Deuterium** **Tritium**

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.

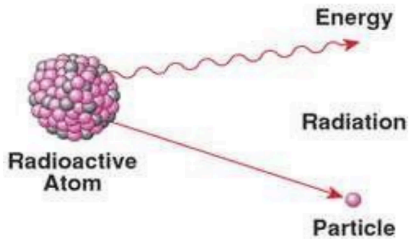
Properties of Radioactive Isotopes

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 He^{2+}.

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 haveVery 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

Applications of Radioisotopes in Biological Sciences/ Research

Radioisotopes are frequently used for tracing metabolic path ways.

Mixingradiolabelledsubstrates & 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 carbonatoms of (14C)acetatethroughTCAcycle.

Methods have been developed to isolate intermediates of the cycle & to ascertain the distribution of carbon atoms within each intermediateRadioisotopes are used in ascertaining the turnover times for particular compounds.

The rats are killed at suitable time intervals & radioactivity in organs or tissue of interest is determined.

Radioisotopes are widely used in study of the mechanism & rate of absorption, accumulation & translocation of inorganic & organic compounds in the animal.

Radiolabeled drugs are useful in pharmacokinetic studies

Analytical application of Radioisotopes

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

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

Isotope dilution analysis : when a known amount of radioactive tracer is introduced into an unknown volume , after thorough mixing , the concentration of radio tracer is estimated.

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 radiolabeled.Radiolabelling must not interfere in the binding of antigen & antibody , has to be compared with unlabeled ones .

Applications of Radioisotopes in Diagnostic purposes

vThe 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 radioimagingof 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.

Applications of RadioisotopesinDiagnostic purposes

51 Cr –EDTA , 99m Tc-DTPA(diethylene-triamine-pentaacetate)&125I –iothalamate have clearance closest to inulin (useful in measurement of GFR).

Therapeutic applications of Radioisotopes

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

Tworoutes

1-From outside the patient's body ((Externalsources)

2-From within the body(Internalsources)

Therapeutic applications of Radioisotopes

1-External Sources

a)Teletherapy:

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)Heavy Particles:

Produce dense ionisation intissuese.g: Heavy particle proton irradiation used in diabetic retinopathy to improve vision.

d)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'.

1. Explain about principle and construction of light microscope.

Microscopy;

Introduction

Microorganisms are so small that they cannot be ordinarily seen using unaided eye. The optical instrument that magnifies the image of these organisms that enables us to view their morphological features is a microscope.

Principles of microscopy and concepts

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 2

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) = $n \times \sin(\theta)$
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 = 0.61 x wavelength of light Numerical aperture
Or
L.R= 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
0.55 = 0.24 μm
2x1.4
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 3

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: 4

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 **PATH OF LIGHT**

III SEMESTER

COURSE 7: MICROBIAL AND ANALYTICAL TECHNIQUES

UNIT-1

Multiple Choice Questions (MCQ)

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.
2. In Micrometry, the unit most commonly used for measuring microscopic objects is:
   a) Millimeter
   b) Centimeter
   c) Micrometer
   d) Nanometer
   Answer: c) Micrometer
3. 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
4. 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

Government College Autonomous (Rajahmundry)-Department of Microbiology

II B.Sc Microbiology Honours -III Semester

Course-8 (CELL BIOLOGY AND GENETICS)

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.	State Cell theory. Explain detailed structure of Chloroplast	2+6	1&2	1	
2.	Discuss detailed structure and functions of Mitochondria	8	2	1	
3.	Illastrate cell cycle and it s regulation	8	3	1	

Unit -II

Q.No	Questions	Marks	BL	CO	PO
1.	Review structure and functions of Cell membrane	8	6	2	
2.	Discuss about structure and functions of nuclear membrane	8	3	2	
3.	Define and describe about Oncogenes and suppressor genes.	8	1	2	

Unit -III

Q.No	Questions	Marks	BL	CO	PO
1.	Generalise different Intracellular signal transduction pathways.	8	3	3	
2.	Explain about Programmed cell death	8	2	3	
3.	State structure and importance of Lampbrash and polytene chromosomes	8	2	3	

Unit -IV

Q.No	Questions	Marks	BL	CO	PO
1.	Compare and evaluate Mendal's Law of segregation and independent assortment	8	4&5	4	
2.	Define and distinguish between Incomplete dominance and co-dominance.	8	1&5	4	
3.	Define Multiple alleles, Lethal alleles, Epistasis and Pleiotropy.	4x2=8	1	4	

Unit -V

Q.No	Questions	Marks	BL	CO	PO

1.	Define crossing over. Interpret molecular mechanism of Crossing over	8	1&3	5	
2.	Evaluate the role of Natural selection and its role in Genetic drift and genetic shift	8	4		
3.	Write a short note on A. Sex linked inheritance B. extra chromosomal Inheritance	4+4	2	5	

COURSE 8: - CELL BIOLOGY AND GENETICS

UNIT-1

Multiple Choice Questions (MCQ)

1. Which statement is part of the cell theory?
   a) All organisms are made up of multiple cells.
   b) Cells arise only from pre-existing cells.
   c) Cells do not contain hereditary material.
   d) Energy flows outside of cells.
   Answer: b) Cells arise only from pre-existing cells.
2. Which organelle is responsible for packaging and modifying proteins in eukaryotic cells?
   a) Mitochondria
   b) Lysosomes
   c) Golgi apparatus
   d) Endoplasmic reticulum
   Answer: c) Golgi apparatus
3. What is the main function of peroxisomes?
   a) Protein synthesis
   b) Lipid synthesis
   c) Detoxification of harmful substances
   d) ATP production
   Answer: c) Detoxification of harmful substances
4. Which phase of the cell cycle involves the duplication of DNA?
   a) G1 phase
   b) S phase
   c) G2 phase
   d) M phase
   Answer: b) S phase
5. Which cytoskeletal structure is primarily involved in muscle contraction?
   a) Microtubules
   b) Intermediate filaments
   c) Actin filaments
   d) Golgi network
   Answer: c) Actin filaments

Fill in the Blanks

6. Mitochondria are often referred to as the _____ of the cell because they produce ATP through cellular respiration.
   Answer: powerhouse

7. The cytoskeleton is composed of actin filaments, intermediate filaments, and _____, each contributing to the cell's shape and internal organization.
   Answer: microtubules
8. The G1 checkpoint in the cell cycle ensures that the cell is ready to enter the S phase and begin _____ replication.
   Answer: DNA

True or False

9. True or False: Lysosomes are involved in the breakdown of cellular waste and damaged organelles.
   Answer: True
10. True or False: Intermediate filaments are primarily involved in the transport of vesicles throughout the cell.
    Answer: False

UNIT-2

Multiple Choice Questions (MCQ)

1. The primary function of the Na+/K+ pump in the cell membrane is to:
   a) Transport glucose into the cell
   b) Maintain the electrochemical gradient by moving 3 Na+ ions out and 2 K+ ions in
   c) Facilitate passive diffusion of ions
   d) Pump calcium ions into the endoplasmic reticulum
   Answer: b) Maintain the electrochemical gradient by moving 3 Na+ ions out and 2 K+ ions in
2. Which process involves the cell engulfing large particles or debris from the extracellular environment?
   a) Pinocytosis
   b) Phagocytosis
   c) Exocytosis
   d) Diffusion
   Answer: b) Phagocytosis
3. The nuclear pore complex primarily functions to:
   a) Regulate the movement of molecules between the nucleus and the cytoplasm
   b) Produce ribosomal RNA
   c) Anchor the nuclear envelope to the cytoskeleton
   d) Synthesize DNA
   Answer: a) Regulate the movement of molecules between the nucleus and the cytoplasm
4. Which gene, when mutated, is most commonly associated with promoting the development of cancer?
   a) p53
   b) BRCA1
   c) Ras
   d) Calmodulin
   Answer: c) Ras
5. Which of the following is a function of tumor suppressor genes?
   a) Promote cell growth and division

b) Initiate DNA repair mechanisms
c) Inhibit apoptosis
d) Increase the rate of cellular metabolism
Answer: b) Initiate DNA repair mechanisms

Fill in the Blanks

6. The calmodulin protein binds to _____ ions to regulate various cellular processes, including signal transduction pathways.
   Answer: calcium (Ca^{2+})
7. Exocytosis is a process where vesicles fuse with the plasma membrane to release their contents into the _____ space.
   Answer: extracellular
8. The nucleolus is the site within the nucleus responsible for synthesizing _____.
   Answer: ribosomal RNA (rRNA)

True or False

9. True or False: The nuclear lamina is a mesh-like structure that provides mechanical support to the nuclear envelope and regulates nuclear events such as DNA replication.
   Answer: True
10. True or False: Oncogenes normally function to suppress tumor growth, but when mutated, they can lead to uncontrolled cell division.
    Answer: False

UNIT-3

Multiple Choice Questions (MCQ)

1. Which organelle is primarily involved in the sorting and modification of proteins before they are transported to their destination?
   a) Mitochondria
   b) Golgi apparatus
   c) Lysosomes
   d) Nucleus
   Answer: b) Golgi apparatus
2. G protein-coupled receptors (GPCRs) activate which molecule after binding to a ligand?
   a) mTOR
   b) G proteins
   c) ERK
   d) DNA
   Answer: b) G proteins
3. Which signal transduction pathway is activated by growth factors and leads to cell proliferation?
   a) GPCR Pathway
   b) ERK/MAPK Pathway
   c) mTOR Pathway
   d) Apoptotic Pathway
   Answer: b) ERK/MAPK Pathway

4. Programmed cell death, or apoptosis, is characterized by all of the following EXCEPT:
   a) Cell shrinkage
   b) DNA fragmentation
   c) Inflammation
   d) Caspase activation
   Answer: c) Inflammation
5. Polytene chromosomes are primarily found in:
   a) Human liver cells
   b) Drosophila salivary glands
   c) Frog oocytes
   d) Mammalian neurons
   Answer: b) Drosophila salivary glands

Fill in the Blanks

6. The mTOR signaling pathway is crucial for regulating cell growth and _____, responding to nutrients, growth factors, and energy status.
   Answer: metabolism
7. Lampbrush chromosomes are large, extended chromosomes found in the oocytes of _____ and amphibians, with loops of chromatin active in transcription.
   Answer: birds
8. Stem cells have the ability to differentiate into specialized cell types and are also capable of _____ division, giving rise to identical stem cells.
   Answer: self-renewing

True or False

9. True or False: In the GPCR signaling pathway, GTP-bound G proteins activate downstream effectors such as adenylyl cyclase or phospholipase C.
   Answer: True
10. True or False: Apoptosis is an uncontrolled cell death process that results in damage to neighboring cells and tissue.
    Answer: False

UNIT-4

Multiple Choice Questions (MCQ)

1. In a monohybrid cross, which of the following is the phenotypic ratio observed in the F2 generation according to Mendel’s law of dominance?
   a) 1:2:1
   b) 3:1
   c) 9:3:3:1
   d) 1:1
   Answer: b) 3:1
2. Which of Mendel's laws states that alleles for different traits are inherited independently of one another?
   a) Law of Segregation
   b) Law of Dominance
   c) Law of Independent Assortment

d) Law of Linkage
Answer: c) Law of Independent Assortment

3. What type of inheritance is shown when both alleles in a heterozygote are fully expressed, as in AB blood type?
   a) Incomplete dominance
   b) Co-dominance
   c) Pleiotropy
   d) Epistasis
   Answer: b) Co-dominance
4. In pedigree analysis, a filled-in square represents a(n):
   a) Male without the trait
   b) Female without the trait
   c) Male with the trait
   d) Female with the trait
   Answer: c) Male with the trait
5. In a dihybrid cross, the phenotypic ratio of the F2 generation is typically:
   a) 1:2:1
   b) 9:3:3:1
   c) 3:1
   d) 1:1
   Answer: b) 9:3:3:1

Fill in the Blanks

6. Multiple alleles refer to a gene having more than two _____, such as the ABO blood group system.
   Answer: alleles
7. Lethal alleles can cause death when present in the _____ state, often resulting in altered Mendelian ratios in offspring.
   Answer: homozygous
8. Pleiotropy occurs when a single gene affects _____ traits, as seen in conditions like Marfan syndrome.
   Answer: multiple

True or False

9. True or False: In incomplete dominance, the heterozygote exhibits a phenotype that is an intermediate between the two homozygous phenotypes.
   Answer: True
10. True or False: Epistasis occurs when one gene masks or alters the expression of another gene at a different locus.
    Answer: True

UNIT-5

Multiple Choice Questions (MCQ)

1. Linkage refers to:
   a) Genes located on different chromosomes
   b) Genes that are inherited together because they are located on the same chromosome

c) Genes that do not assort independently
d) Both b and c
Answer: d) Both b and c

2. During crossing over, genetic material is exchanged between:
   a) Non-homologous chromosomes
   b) Homologous chromosomes
   c) Sister chromatids
   d) None of the above
   Answer: b) Homologous chromosomes
3. Recombination frequency is used to measure:
   a) The mutation rate in a population
   b) The intensity of linkage between genes
   c) The rate of natural selection
   d) The rate of genetic drift
   Answer: b) The intensity of linkage between genes
4. Genetic drift is most pronounced in:
   a) Large populations
   b) Small populations
   c) Stable environments
   d) Diverse ecosystems
   Answer: b) Small populations

Fill in the Blanks

5. Speciation occurs when populations of the same species become _____ and evolve into different species over time.
   Answer: reproductively isolated
6. Sex-linked inheritance typically involves genes located on the _____ chromosomes.
   Answer: sex
7. Extra-chromosomal inheritance refers to the transmission of genetic material found outside the _____.
   Answer: nucleus
8. Crossing over results in new combinations of alleles, which contributes to genetic _____ in a population.
   Answer: variation

True or False

9. True or False: The process of natural selection acts only on phenotypes, not genotypes.
   Answer: True
10. True or False: In linkage, genes that are located far apart on the same chromosome assort independently.
    Answer: False