

Question 1: What is RNA polymerase? Explain its function and uses.

Answer: RNA polymerase

RNA polymerase is an enzyme that is responsible for copying a DNA sequence into an RNA sequence, during the process of transcription

Function

RNA polymerase I transcribe ribosomal RNA (rRNA), which is used in the construction of ribosomes. RNA polymerase II transcribes mRNA, which is then translated into proteins. RNA polymerase III transcribes small nuclear RNAs (snRNAs), which are involved in splicing.

Uses

RNA polymerase is essential to life, and is found in all living organisms and many viruses. Depending on the organism, a RNA polymerase can be a protein complex (multi-subunit RNAP) or only consist of one subunit (single-subunit RNAP, ssRNAP), each representing an independent lineage.

Question2: Define transcription factors?

Answer: Transcription factors

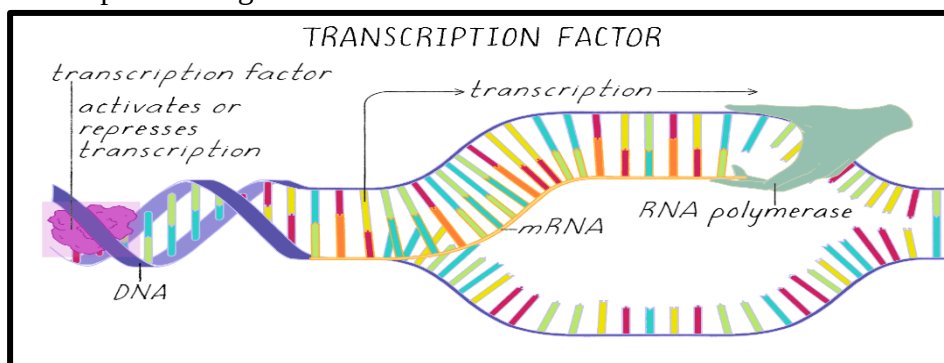
Transcription factors are specialized proteins that bind to specific DNA sequences to control the rate at longer or shorter intervals—turning genes "on" or "off" by helping or blocking RNA polymer.

Structural Domains

- **DNA-Binding Domain (DBD):** Recognizes and attaches to specific DNA sequences like promoters or enhancers.
- **Transactivation/Regulatory Domain:** Interacts with other proteins, co activators, or co repressors to modulate activity.
- **Dimerization Domain:** Allows different transcription factor proteins to pair up.

Functions

1. **Activators:** Boost gene transcription by recruiting RNA polymerase.
2. **Repressors:** Decrease or block transcription to silence specific genes.
3. **General Factors:** Form a pre initiation complex at promoter regions necessary for basic transcription to begin.



Question3: What is a gene? Explain its structure and functions.

Answer: Gene

A gene is a basic physical and functional unit of heredity made up of DNA that provides instructions to make proteins or functional RNA.

Composition: Made of DNA nucleotide base pairs (Adenine, Thymine, Cytosine, Guanine).

Location: Found on chromosomes inside the nucleus of eukaryotic cells.

Structure of a Gene

Promoter Sequence: A DNA region at the start of a gene where enzymes bind to start expression.

Coding Region: The stretch of DNA containing exons (coding sequences) and introns (non-coding sequences).

Terminator Sequence: The DNA sequence that marks the end of gene expression.

Functions of Genes

Protein Synthesis: Genes carry codes that direct cells to assemble amino acids into specific proteins. These proteins form body structures like muscles, bones, and organs.

Determining Traits: Genes influence physical characteristics such as eye color, hair color, and height.

Regulating Cellular Activity: Genes control chemical signals and processes, including immune responses, cell growth, and division.

Heredity: Genes pass biological information from one generation to the next.

Question 4: Differentiate Promotor and Operator.

Answer: Difference between Promotor and Operator

PROMOTER	OPERATOR
• DNA sequence where RNA polymerase binds to initiate transcription • Required for the initiation of transcription • Binding site for RNA polymerase with sigma factor • Located upstream 5' end of structural genes • Essential for transcription to start • Not a binding site for repressor proteins • Contains -35 and -10 regions • Example -35 TTGACA and -10 TATAAT	• Regulatory DNA sequence where repressor protein binds to control transcription • Not required for initiation — regulates whether transcription proceeds • Binding site for regulatory proteins — repressor or activator • Usually overlaps with or is adjacent to the promoter • Functions as an ON/OFF switch • Binds repressor — inhibitor prevents RNA polymerase from transcribing • Function depends on regulatory mechanism e.g. <i>lac operon</i> and <i>trp operon</i> • Example — <i>lac</i> operator <i>lacO</i> binds <i>Lac</i> repressor

Question5: Briefly describe the central dogma of molecular Biology.

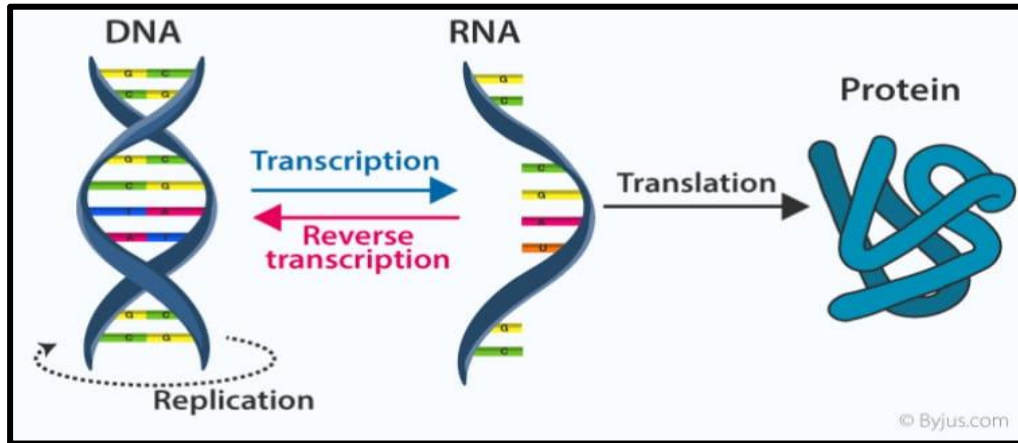
Answer: Central Dogma

“Central dogma is the process in which the genetic information flows from DNA to RNA, to make a functional product protein.”

Steps

1. **Replication:** DNA makes an exact copy of itself so cells can divide.

2. **Transcription:** An enzyme reads a DNA strand to create a messenger RNA (mRNA) molecule.
3. **Translation:** Ribosomes read the mRNA code to link amino acids together and form a functional protein.



Question6: What is reverse central dogma? Explain with example.

Answer: Reverse Central Dogma

The reverse central dogma describes the flow of genetic information from RNA → DNA, which is reverse of the usual DNA → RNA → Protein pathway.

Process

RNA → DNA

This process is called reverse transcription and is carried out by the enzyme reverse transcriptase.

Example

Retroviruses such as HIV: The viral RNA is converted into DNA by reverse transcriptase. The newly formed viral DNA can then integrate into the host cell's genomes.

Flow:

RNA--- Reverse transcriptase---DNA

Significance

It allows RNA genomes of retrovirus to produce a DNA copy inside the host cell.

Question7: What do you understand by 'Genetic code'?

Answer: Genetic Code

It is a set of rules that specifies how the information in DNA and RNA translates into the sequence of amino acids to form proteins. It's the language the cell uses to read the instructions in the genetic material and produce the proteins that carry out various bodily functions. The genetic code is universal and standard to almost all living organisms, from bacteria to humans.

The genetic code is read in groups of three nucleotides (triplets) on the mRNA molecule. Each triplet of nucleotides is called a codon. There are 64 possible codons (4 nucleotide options for each of the three positions in a codon), and each codon relates to a specific amino acid to start or stop protein synthesis.

The codon AUG serves as the start codon, indicating the beginning of protein synthesis. It also codes for methionine amino acids. There are three stop codons (UAA, UAG, and UGA), which signal the termination of protein synthesis.

Question.8 Write short note on Anticodon.

Answer: Anticodon

Anticodon is a trinucleotide sequence present on one arm of the tRNA (transfer RNA) which is complementary to the sequence or codon present on mRNA (messenger RNA). The anticodon sequence is present on the anticodon loop of the tRNA and is involved in the process of translation. The amino acid binds to the 3' adenosine end of the tRNA which yields an aminoacyl tRNA.

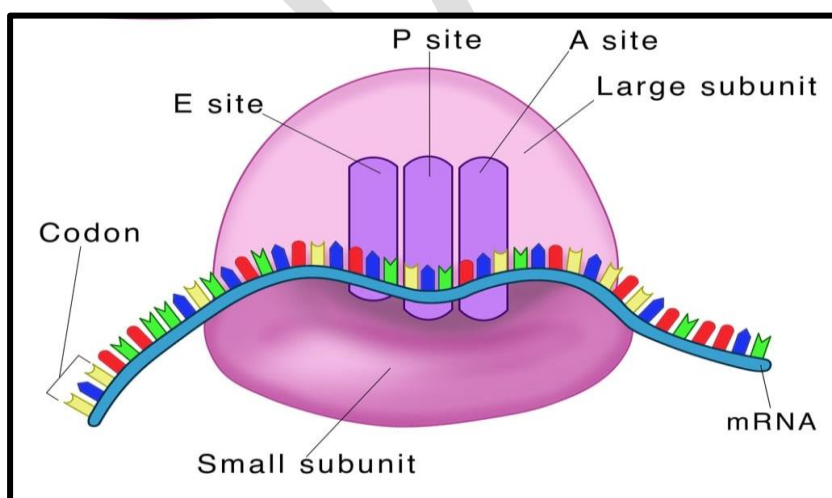
Functions

1. Responsible for the process of translation in which a protein polypeptide is formed. Determines the amino acid specificity.
2. Anticodon is responsible for the initiation and termination of the translation process.
3. The first anticodon in prokaryotes and eukaryotes is UAC which binds to the sequence AUG on the mRNA.
4. The initiator anticodon of the tRNA binds with a complementary codon on mRNA which adds the first amino acid of the protein polypeptide. The first amino acid in prokaryotes is N-formyl-methionine, whereas the one in eukaryotes is unmodified methionine.
5. Anticodon is responsible for the initiation of the translation process, it is also responsible for the termination of translation.
6. Anticodon on tRNA is – AUU, AUC, and ACU, one of these three sequences termination is achieved.

Question.9: What are ribosomes? Describe their functions.

Answer: Ribosomes

The ribosome word is derived – 'ribo' from ribonucleic acid and 'somes' from the Greek word 'soma' which means 'body'. Ribosomes are tiny spheroidal dense particles (of 150 to 200 Å diameters) that are primarily found in most prokaryotic and eukaryotic.



Functions

1. It assembles amino acids to form proteins that are essential to carry out cellular functions.
2. The DNA produces mRNA by the process of DNA transcription.
3. The mRNA is synthesized in the nucleus and transported to the cytoplasm for the process of protein synthesis.
4. The ribosomal subunits in the cytoplasm are bound around mRNA polymers. The tRNA then synthesizes proteins.
5. Ribosomes are the site of protein synthesis.
6. The proteins synthesized in the cytoplasm are utilized in the cytoplasm itself, the proteins synthesized by bound ribosomes are transported outside the cell.

Question10: Differentiate between Transcription and Translation.

Answer: Differences between Transcription and Translation

1. The purpose of transcription is to make RNA copies of genes. The purpose of translation is to synthesize proteins for cellular functions.
2. Translation produces proteins, while transcription produces mRNA, tRNA, and non-coding RNA.
3. In prokaryotes, translation and transcription occurs in the cytoplasm. While in eukaryotes, translation happens on the ribosomes and transcription occurs in the nucleus.
4. Transcription begins when an RNA polymerase protein binds to a promoter in DNA and creates a transcription initiation complex. Translation begins when a ribosome, initiation factor, and tRNA bind mRNA near a starting codon.
5. The molecules (RNA) that result from transcription contributes to what is known as the cell's transcriptome, while translation generates proteins which contribute to the proteome.

Question 11: What is DNA sequencing? Explain the principle and applications of DNA sequencing.

Answer: DNA Sequencing

DNA sequencing is the process of determining the exact sequence of nucleotides bases adenine, guanine, cytosine, and thymine in a piece of DNA.

Principle:

DNA synthesis is stopped when a dideoxynucleotide (ddNTP) is incorporated into the growing DNA strand because ddNTPs lack a 3'-OH group.

Requirements

1. Template DNA
2. DNA primer
3. DNA polymerase
4. Normal nucleotides (dNTPs)
5. Chain-terminating nucleotides (ddNTPs)

Steps

1. Denaturation: Double-stranded DNA is separated into single strands.
2. Primer annealing: A primer attaches to the template.
3. DNA synthesis: DNA polymerase adds nucleotides.
4. Chain termination: DNA fragments are separated according to size by electrophoresis.

Applications

1. Genome sequencing
2. Cancer mutation detection
3. Genetic disease studies
4. DNA fingerprinting
5. Molecular biology research
6. Personalized medicine

Question12: Define pribnow box?

Answer: Pribnow box

The Pribnow box (also known as the Pribnow - Schaller box) is a sequence of TATAAT of six nucleotides that is an essential part of a promoter site on DNA for transcription to occur in bacteria. It is also known as the "-10 box".

Functions

Role in transcription: It is a crucial binding site for RNA polymerase, which is necessary to start the process of creating an RNA copy from a DNA template.

Sequence: The consensus sequence is TATAAT, though variations can exist.

Location: It is positioned in the promoter region of genes, about 10 base pairs before the start site of transcription.

Origin: It was named after **David Pribnow**, who studied it in bacterial and viral DNA.

Question13: What is TATA box?

Answer: TATA box

The TATA box is a conserved DNA sequence in the promoter region of many eukaryotic and archaeal genes. It has the core consensus sequence 5'-TATAAA-3' (or TATAWAAR, where W is A or T, and R is A or G). Found about 25 to 35 base pairs upstream the transcription start site (+1). Rich in thymine and adenine bases, making it easier to unwind the DNA strand.

Function

Acts as a binding site for the TATA-Binding Protein (TBP) to help position RNA polymerase II and start gene transcription.

Question14: Write a short note on Capping.

Answer: Capping

RNA 5' capping is a crucial post-transcriptional modification that adds a 7-methyl guanosine structure to the start of eukaryotic messenger RNA.

Step

RNA Triphosphatase: Removes a terminal phosphate group from the 5' end of the pre-mRNA.

Guanyl transferase: Attaches a GMP molecule (from GTP) to form the 5'-5' triphosphate bridge.

Methyl transferase: Transfers a methyl group to the N7 position of the added **guanine**, creating the final 7-methylguanosine (m⁷G) cap.

Functions

1. **Protection:** Shields the mRNA from 5' exonuclease degradation.
2. **Translation:** Helps ribosomes recognize the mRNA and initiate protein synthesis.
3. **Transport:** Assists in moving the mature mRNA out of the nucleus and into the cytoplasm.

Question15: Give a short account of RNA Editing.

Answer: RNA Editing

RNA editing is a molecular process that changes specific nucleotide sequences in an RNA molecule after transcription, altering the coded message without modifying the underlying DNA. It is a well-documented biological phenomenon across eukaryotes and is detailed further on RNA editing.

Mechanisms of RNA Editing

RNA editing generally occurs through two primary pathways:

- **Substitution (Base Modification):** Chemical alteration of individual nucleotides, such as converting cytidine (C) to uridine (U) via cytidine deaminases, or adenosine (A) to inosine (I) via ADAR enzymes. Inosine is read as guanosine (G) by the cell's translational machinery.
- Insertion and Deletion:** The non-templated addition or removal of specific bases (frequently uridine) guided by small guide RNA (gRNA) molecules, which can shift the protein's reading frame.

Question16: Describe Ribozymes.

Answer: Ribozymes

Ribozymes (ribonucleic acid enzymes) are RNA molecules that have the ability to catalyze specific biochemical reactions, including RNA splicing in gene expression, similar to the action of protein enzymes. **Thomas Cech** and Sidney **Altman** discovered them independently in the early 1980s.

Types

- **Small Ribozymes:** These are short strands that usually cut themselves. Examples include the hammerhead and hairpin ribozymes.
- **Large Ribozymes:** These are long strands that act on other molecules. Examples include RNase P and the ribosome.

Biological Roles

- **Protein Building:** The ribosome uses a ribozyme core to link amino acids into long protein chains.
- **RNA Processing:** They cut raw RNA into correct sizes during gene expression.
- **Evolution:** They support the RNA World Hypothesis, which suggests ancient life used RNA for everything before DNA and proteins evolved.

Question 17: Describe Split genes.

Answer: Split genes

A split gene is a gene that contains coding regions called exons interrupted by non-coding sequences called introns. Found mostly in eukaryotes and discovered in **1977** by **Richard J. Roberts** and **Phillip A. Sharp**.

Parts of Split Genes

Exons: Segments of DNA that code for proteins and remain in the final messenger RNA (mRNA).

Introns: Intervening sequences that do not code for proteins and are cut out during processing.

Splicing: The cellular process that removes introns and joins exons together before mRNA leaves the nucleus.

Function

Alternative Splicing: Cells can mix and match different exons, letting one single split gene build many distinct proteins for different cell tasks.

Gene Regulation: Introns often contain regulatory elements that help control when and how much protein a cell makes.

Evolutionary Flexibility: Split genes let new functional protein domains evolve faster through the shuffling of exons.

Question18: What is Western blotting? Explain its principle and applications.

Answer: Western blotting

Western blotting (also called protein immunoblot) is a key laboratory technique used to detect and identify specific target proteins within a complex biological sample using antibodies.

Principle

The method relies on specific antigen-antibody interactions. Proteins are separated by size via gel electrophoresis, transferred to a solid membrane, and targeted using labeled antibodies for visualization.

Steps

1. Sample Preparation
2. Gel Electrophoresis (SDS-PAGE)
3. Transfer (Blotting)
4. Blocking:
5. Probing (Antibody Incubation)
6. Detection

Applications

1. Confirming specific proteins and analyzing post-translational modifications.
2. Diagnosing infectious diseases (e.g., confirmatory tests for HIV and Lyme disease).
3. Detecting protein expression levels in various research fields.
4. Biomarker Discovery

Question19: Describe the steps involved Northern blotting technique.

Answer: Northern Blotting

Northern blotting is the method used to detect a specific RNA sequence in a sample of mixed RNAs. Below are the basic steps in a Northern Blot.

Electrophoresis – It segregates RNA samples based on their size into different bands.

Transfer – RNA bands in the gel are moved to the membrane by capillary action.

Identification of specific sequences – The target RNA sequence is identified by hybridisation with a tagged oligonucleotide probe made of DNA.

Northern blotting can be utilised in gene expression research because it can identify a specific RNA sequence in a sample. It can also assist in the diagnosis of diseases.

Question20: What is Protein degradation? Explain its Functions.**Answer: Protein degradation**

Protein degradation is the process of breaking down proteins into smaller peptides and amino acids, which is essential for cellular regulation, protein quality control, and recycling building blocks.

Functions

1. **Cellular regulation:** It removes regulatory proteins like cyclins that are no longer needed, which helps control cell division and other processes.
2. **Quality control:** Cells break down damaged proteins to prevent them from accumulating and potentially causing disease.
3. **Recycling:** The amino acids released from broken-down proteins can be reused to synthesize new proteins.
4. **Starvation response:** Cells can degrade certain proteins to provide amino acids for energy.

Question21: Define Molecular Chaperones.**Answer: Molecular Chaperones**

Molecular chaperones are proteins that assist the conformational folding or unfolding of proteins or macromolecular protein complexes. Molecular chaperones are specialized proteins that help other proteins fold correctly, maintain their proper structure, and prevent misfolding and aggregation.

Functions

1. **Assist protein folding** – Help newly synthesized proteins fold into their correct 3D structure.
2. **Prevent protein aggregation** – Stop improperly folded proteins from sticking together.
3. **Refold damaged proteins** – Help partially unfolded proteins regain their normal structure.
4. **Protein transport** – Assist proteins in crossing cellular membranes.
5. **Protein quality control** – Help identify and remove severely misfolded proteins.
6. **Stress protection** – Protect cells from protein damage during heat, oxidative, or other stresses.
7. **Protein assembly** – Assist in the proper assembly of protein complexes

Examples

Hsp70 – binds to newly synthesized or unfolded proteins and assists their folding.

Hsp60 (Chaperonin) – provides a protected environment for protein folding inside a barrel-like structure.

Hsp90 – helps in the maturation and stabilization of several regulatory proteins.

Hsp104 – helps disassemble protein aggregates in some organisms.

Question22: What is Ubiquitination?**Answer: Ubiquitination**

Ubiquitination is a cellular process in which a small protein called ubiquitin is attached to another protein. This attachment acts like a molecular tag that changes the protein's fate or function.

Roles of Ubiquitination

1. Marking proteins for degradation by the proteasome (the cell's protein recycling system).
2. Regulating protein activity by turning proteins on or off.
3. Changing protein location within the cell.
4. Controlling DNA repair, cell signaling, immune responses, and the cell cycle.

Ubiquitination is essential for normal cell function, and defects in this pathway are linked to diseases such as cancer, neurodegenerative disorders (like Parkinson's disease), and immune disorders.

Question23: Explain Wobble hypothesis.

Answer: Wobble hypothesis

Proposed by **Francis Crick in 1966**, the wobble hypothesis explains that the first two bases in an mRNA codon form strict, strong Watson-Crick pairs with a tRNA anticodon, while the third base has flexible, looser (“wobbly”) non-canonical pairing. This flexibility lets a single tRNA recognize multiple codons.

Significance:

1. Explains the degeneracy of the genetic code.
2. Reduces the number of different tRNAs required.
3. Helps explain efficient and accurate protein synthesis.

Question 24: Write short notes on Resolving Power of Microscope.

Answer: The resolving power of an objective lens is measured by its ability to differentiate two lines or points in an object. The greater the resolving power, the smaller the minimum distance between two lines or points that can still be distinguished. The larger the N.A., the higher the resolving power.

Resolving Power Formula The following formula is generally used for determining resolution.

Factors Affecting Resolving Power

- **Wavelength (λ):** Shorter wavelengths increase resolving power.
- **Numerical Aperture (NA):** A larger NA increases resolving power.
- **Refractive Index (n):** Using immersion oil instead of air increases n, which improves resolution.

$$\epsilon = 0.61 \times \frac{\lambda}{N.A.} \quad (\text{Reyleigh formula})$$

λ : Wavelength
 $\lambda = 0.55\mu\text{m}$ is used for visible light
 N.A.: Objective lens N.A.

Example MPLFLN100× (N.A. 0.90), $\lambda = 0.55\mu\text{m}$

$$\epsilon = 0.61 \times \frac{\lambda}{N.A.} = \frac{0.3355}{N.A.} = \frac{0.3355}{0.90} = 0.37\mu\text{m}$$

Question25: Write down the Working Principle of Electron Microscope.

Answer: Working Principle of Electron Microscope

Electron microscopes use signals arising from the interaction of an electron beam with the sample to obtain information about structure, morphology, and composition.

1. The electron gun generates electrons.
2. Two sets of condenser lenses focus the electron beam on the specimen and then into a thin tight beam.
3. To move electrons down the column, an accelerating voltage (mostly between 100 kV-1000 kV) is applied between the tungsten filament and anode.
4. The specimen to be examined is made extremely thin, at least 200 times thinner than those used in the optical microscope. Ultra-thin sections of 20-100 nm are cut which is already placed on the specimen holder.
5. The electronic beam passes through the specimen and electrons are scattered depending upon the thickness or refractive index of different parts of the specimen.
6. The denser regions in the specimen scatter more electrons and therefore appear darker in the image since fewer electrons strike that area of the screen. In contrast, transparent regions are brighter.
7. The electron beam coming out of the specimen passes to the objective lens, which has high power and forms the intermediate magnified image.
8. The ocular lenses then produce the final further magnified image.

Question 26: Describe the principle of working of Transmission Electron Microscope.

Answer: The working principle of a Transmission Electron Microscope (TEM) is passing a high-energy beam of electrons through an ultra-thin specimen to form a magnified internal image.

Electron Illumination: An electron gun emits a beam of electrons accelerated by a high voltage inside a vacuum chamber.

Short Wavelength: Electrons act like waves with a wavelength much shorter than visible light, which gives the microscope a very high resolution.

Electromagnetic Lenses: Magnetic lenses focus the electron beam into a thin, sharp path instead of using glass lenses.

Transmission and Interaction: As the beam passes through the ultra-thin sample, absorb more electrons.

Image Formation: The transmitted electrons are magnified by projector lenses and hit a fluorescent screen to create a detailed black-and-white image of the

Question 27: What is Magnification? Give its types.

Answer: Magnification

It is the optical process of enlarging the apparent size of an object, enabling detailed examination of small or distant subjects. It is quantified by the ratio of the image size to the object size. An eyepiece having a magnification 10x is used although eyepiece of higher or lower magnifications are also available. The magnification of ocular lens 10x and 45x.

Types of Magnification

1. **Linear Magnification:** This describes how much bigger or smaller an image is compared to the actual object, based on their sizes measured straight across
2. **Angular Magnification:** This measures the angle subtended by the image at the observer's eye compared to the angle subtended by the object when viewed without magnification.

Question 28: Explain the principles and applications of fluorescence microscope.

Answer: Fluorescence microscope

When a substance absorbs light, the electrons present at the outermost orbit absorb energy and get excited; on the way back to the ground state, it emits a part of the energy absorbed. This phenomenon is termed as fluorescence. Fluorescent microscope staining of specimens with special fluorescent dyes. When a fluorescent dye is applied to a substance, it absorbs a wavelength and emits light of different wavelength.

Principle

Illumination is provided by a bright mercury vapour lamp, produces light range of 200-400nm. The specimen is illuminated with light of a specific wavelength. Which is absorbed by the fluorophores, causing them to emit light of longer wavelengths. The illumination light is separated from the much weaker emitted fluorescence through the use of a spectral emission filter.

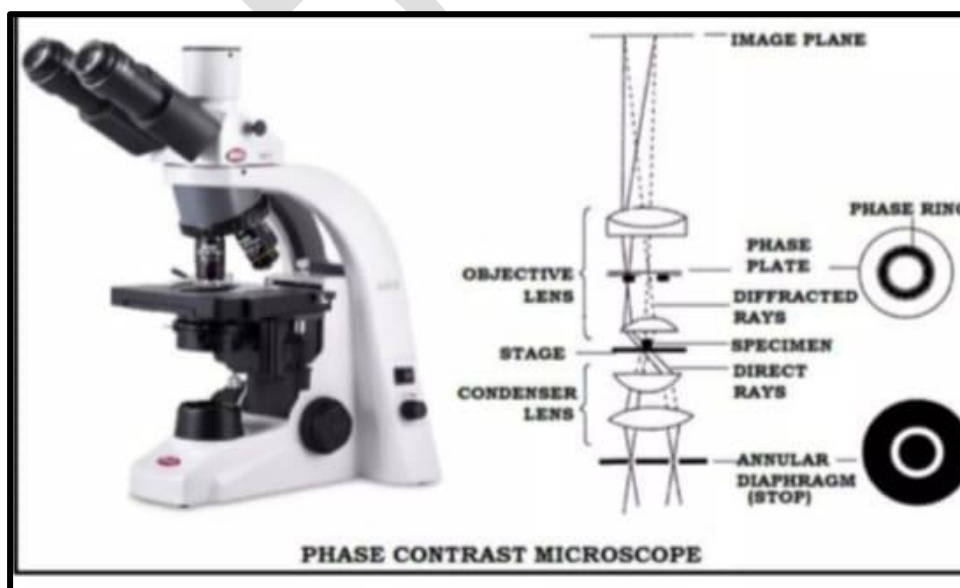
Applications

1. Imaging the genetic material (DNA & RNA) and other structural components.
2. Monitoring the environment for microbial contamination.
3. Certain micro-organism can be detected and identified only by this microscopy.
4. Neurotransmitters such as dopamine, serotonin, norepinephrine, and epinephrine can be detected using fluorescence microscopes.
5. It is employed in textile industries for the analysis of fibres and obtaining clear 3D images of fibres and yarns.

Question 29: What is Phase Contrast Microscope? Explain advantages of phase contrast microscope.

Answer: Phase Contrast Microscope

The first phase contrast microscope shifts occur, which are invisible to the human eye. In a phase-contrast microscope, these phase shifts are converted into changes in amplitude, which can be observed as differences in image contrast.



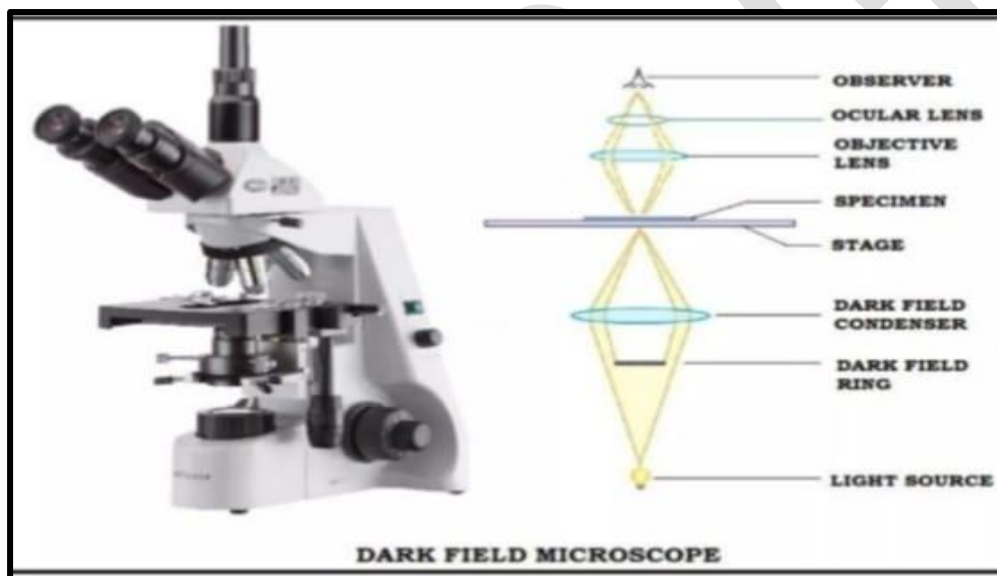
Advantages

1. Specimens which have a refractive index similar to their surroundings can be invisible in Bright field, but are well defined in Phase Contrast.
2. Phase Contrast is normally used to examine unstained biological specimens.
3. Living cells can be observed in their natural state without previous fixation.
4. Examining intracellular components of living cells at relatively high resolution. eg: The dynamic motility of mitochondria, mitotic chromosomes & vacuoles.
5. It made it possible for biologists to study living cells and how they proliferate through cell division.

Question 30: What is a Dark Field Microscope? Explain the principle and uses of Dark Field Microscope.

Answer: Dark Field Microscope

Dark field microscope is an optical microscope that illuminates unstained, transparent specimens so they appear bright against a dark background.



Principle

Light from the illumination source from the base of the Microscope stand is aimed at dark-field ring. Dark-field ring is an opaque disk blocks the central rays of the light. The marginal/peripheral light rays are directed to sub-stage dark-field condenser lens. The specimen on the stage is illuminated only with the peripheral oblique rays. As a result of this, the field appears dark. The scattered ray from bright specimen is collected by objective lens and is magnified according to its capacity, creating a primary image. The primary image is magnified by ocular lens (eye piece), which also act as magnifying glass by allowing the observer to view virtual and magnified image of the sample.

Uses of Dark Field Microscope

1. Used for examination of live sample.
2. Unstained or lightly stained specimen or fluids could also be observed.
3. Useful for diagnosis of disease.

4. Precious stones are viewed.
5. It is useful for the demonstration of very thin bacteria not visible under ordinary illumination since the reflection of the light makes them appear larger.
6. It is also useful for the demonstration of the motility of flagellated bacteria and protozoa.
7. Dark field is used to study mounted cells and tissues.

Question 31: What is Ultracentrifuge? Explain its principle, types and Applications.

Answer: Ultracentrifuge

An ultracentrifuge is a high-performance laboratory instrument that spins samples at high speeds—up to 150,000 rpm—to separate microscopic particles based on size, shape, and density.

Principle

Sedimentation: Denser and larger particles move outward faster than smaller, lighter ones under a heavy centrifugal force.

Force Balance: Rotation creates a massive centrifugal field (1,000,000 x g) counteracted by fluid friction and buoyancy. ,

Environment: Operates inside a refrigerated vacuum chamber to eliminate air friction and prevent heat damage to sensitive biological samples.

The Svedberg Unit (S): Sedimentation speed is measured in Svedberg units (10^{-13} seconds)

Types

1. Preparative Ultracentrifuge
2. Analytical Ultracentrifuge

Applications

Macromolecules: Purifying and studying proteins, DNA, and RNA.

Subcellular Structures: Isolating cell organelles, ribosomes, and membranes.

Virology: Concentrating and purifying viruses and viral vectors.

Nanotechnology: Characterizing synthetic nanoparticles and polymers.

Question 32: What is Chromatography? Write down the principle.

Answer: CHROMATOGRAPHY

Chromatography is a technique in which molecules of different substances, present in solution are separated as they move through some type of porous matrix.

The term 'chromatography' (Gr. Chromatos colour, graphos = written), was coined by

Mikhail Tswett in **1906**. Chromatography is an analytic process. It is used to separate and purify organic and inorganic substances and macromolecules. It is equally important for the fractionation of complex mixtures, separation of closely related compounds, isomers and unstable compounds.

Principle of Chromatography

Chromatography is based on partition or distribution coefficient (K_d). For two immiscible phases A and B, the value of this coefficient is constant at a given temperature and is given as

$$K_d = \frac{\text{Concentration in phase A}}{\text{Concentration in phase B}}$$

Concentration in phase B

The term effective distribution coefficient is defined as the total amount of substance in one phase divided by the total amount present in the other phase.

Effective distribution coefficient =

$\frac{\text{Total amount in phase A}}{\text{Total amount in phase B}}$

Basically all chromatographic systems consist of the stationary phase which may solid, gel, liquid or solid/liquid mixture that is immobilised, and the mobile phase which may be liquid or gas. The mobile phase flows over or through the stationary phase. The choice of stationary and mobile phase is made that the compounds to be separated. different distribution coefficient.

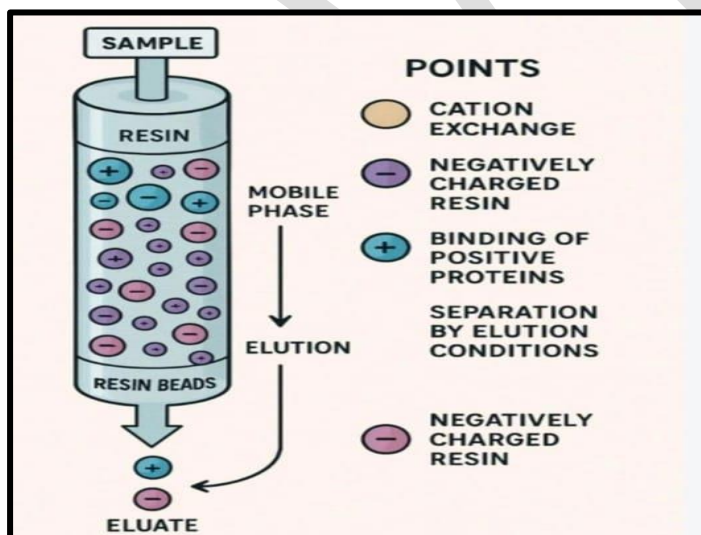
Question33: Write short note on Ion- Exchange Chromatography.

Answer: Ion Exchange Chromatography

Ion exchange 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.

Working 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.



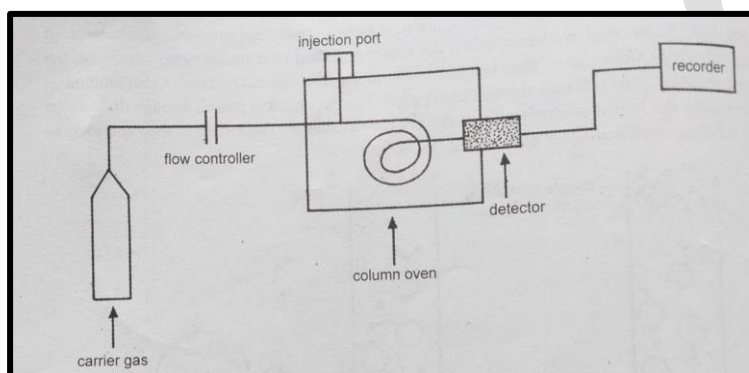
Applications

1. Routine analysis of amino acid mixtures.
2. The 20 principal amino acids from blood serum or from the hydrolysis of proteins are separated and used in clinical diagnosis.
3. This is most effective method for water purification.
4. Chelating resins are used to collect trace metals from seawater.
5. To analyze lunar rocks and rare trace elements on Earth

Question 34: Write short note on Gas Chromatography.

Answer: Gas Chromatography

This technique is used to separate volatile organic compounds. Gas chromatography is an efficient technique for identification of gases, pollutants, drugs, vitamins, alkaloids, etc. This technique was introduced by **Martin and Synge (1952)**. This type of chromatography involves injecting the volatile materials into a column containing a liquid or solid stationary phase. A gas chromatograph consists of a flowing mobile phase, an injection port, a separation column containing the stationary phase and a detector. The stationary phase may be either a liquid or solid. In gas-liquid chromatography (GLC), the mobile phase is a gas and the stationary phase is a thin layer of non-volatile liquid bound to a solid support. In gas-solid chromatography a solid adsorbent acts as the stationary phase and an adsorption process occurs. The organic compounds are separated due to differences in their partitioning behaviour between the



mobile gas phase and stationary phase in the column. In gas chromatography, the sample is injected into the injection port from where it is carried into the column. The eluate released from the column pass through a detector which is linked via an amplifier to a chart recorder which records a peak as a particular compound passes through a detector.

Question35: Write short note on Thin Layer Chromatography.

Answer: Thin Layer Chromatography (TLC)

This type of chromatography is extensively used in quantitative determination of high molecular weight compounds. Carbohydrates, dyes, pigments, vitamins, steroids, etc., can be separated and characterized by this method. In TLC, a thin layer of stationary phase is formed on a suitable flat surface such as glass, plastic or a metal foil. The movement of the mobile phase across the layer takes place rapidly by simple capillary action. As the mobile phase moves across the layer from one edge to the opposite, it transfers analytes placed on the thin layer. The movement of the analyte is expressed by its retention factor (R_f) which is expressed as follows:

$$R_f = \frac{\text{Distance moved by the analyte from origin}}{\text{Distance moved by solvent from origin}}$$

For detection of the analyte, several methods are available. A general method is to spray the plate with 50% (v/v) sulphuric acid or 25% (v/v) sulphuric acid in ethanol and heating at 110°C. This results in most of the compounds showing up as brown spots.

Question36: What is Paper Chromatography? Explain applications of Paper Chromatography.

Answer: Paper Chromatography

Chromatography technique that uses paper sheets or strips as the adsorbent being the stationary phase through which a solution is made to pass is called paper chromatography. It is a powerful analytical tool that uses very small quantities of material. Paper chromatography was discovered by Synge and Martin in the year 1943.

Paper Chromatography Applications

1. **Separating Colored Substances:** Paper chromatography is often used to separate and identify colored components, such as in the separation of plant pigments.
2. **Analyzing Mixtures:** It's valuable for analyzing mixtures of amino acids and determining the number of components present.
3. **Identifying Unknowns:** By comparing the R_f values of the separated components to those of known standards, one can identify unknown substances.
4. **Pharmaceutical Analysis:** It is used to assay pharmaceutical compounds.
- Inorganic Separation. it is used for the separation of inorganic cations.
5. Clinical and biological analysis of various biological compounds.
6. Analysis of inks and dyes to identify their components.

Question 37: What is Ion Exchange Chromatography? Explain applications of Ion exchange chromatography.

Answer: Ion Exchange Chromatography

Ion exchange chromatography is a process that allows the separation of ions and polar molecules based on their affinity to ion exchangers. In this process two types of exchangers.

1. Cationic exchangers possess negatively charged group, and these will attract positively charged cations. These exchangers are also called “Acidic ion exchange” materials,
2. Anionic exchangers have positively charged groups that will attract negatively charged anions. These are also called “Basic ion exchange” materials.

Applications -

1. An important use of ion-exchange chromatography is in the routine analysis of amino acid mixtures.
2. The 20 principal amino acids from blood serum or from the hydrolysis of proteins are separated and used in clinical diagnosis.
3. 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.
4. 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.
5. Chelating resins are used to collect trace metals from seawater.
6. To analyze lunar rocks and rare trace elements on Earth.

Question 38: Write application of gas chromatography.

Answer: Applications of gas chromatography

1. **Pharmaceuticals:** Tests drug purity, checks for trace toxins.
2. **Environmental Monitoring:** Detects harmful air pollutants, water contaminants, and soil pesticides like benzene or toluene.
3. **Food and Beverage:** Measures the exact oils and compounds that create specific food flavours, aromas, and spoilage indicators.
4. **Forensic Science:** Identifies illegal drugs, poisons, explosives, and blood alcohol levels in crime or accident investigations.
5. **Petrochemicals:** Splits complex fuel and gas mixtures to check product quality and control refinery steps.
6. **Perfume and cosmetics industry** – identification of fragrance components.
7. **Research laboratories** – separation and identification of volatile organic compounds.
8. **Clinical/medical studies** – analysis of volatile compounds in biological samples

Question 39: What is HPLC? What are the major applications of HPLC..

Answer: HPLC (High-Performance Liquid Chromatography)

A technique used to separate, identify, and quantify components in a mixture.

Components of HPLC

1. Column -A tube packed with stationary phase material.
2. Mobile Phase,-A solvent or mixture of solvents that flows through the column.
3. Detector- Measures the absorbance or other properties of the separated components.

Applications of HPLC-

1. Analysis of drugs
2. Analysis of synthetic polymers
3. Analysis of pollutants in environmental analytics
4. Determination of drugs in biological matrices
5. Isolation of valuable products
6. Product purity and quality control of industrial products and fine chemicals
7. Separation and purification of biopolymers such as enzymes or nucleic acids
8. Water purification
9. Impurity detection in the pharmaceutical industry.
10. Carbohydrate and oligosaccharide anion-exchange chromatography at high pH.

Question 40: What is Gel Filtration Chromatography? Write its applications .

Answer: Gel Filtration Chromatography

Gel filtration is a technique in which the separation of components is based on the difference in molecular weight or size .Gel Filtration Chromatography also known as size-exclusion chromatography.

Applications

1. Proteins fractionation
2. Purification
3. Molecular weight determination.
4. Separation of sugar, proteins, peptides, rubbers, and others on the basis of their size.

5. Can be used to determine the quaternary structure of purified proteins.
6. Replacing the buffer of a protein solution by removing small molecules and ions.
7. Quality control to differentiate between dimers, aggregates, and the desired product.

Question41: Write Applications of pH meter.

Answer: Applications of pH meter

1. To measure the quality of municipal drinking water, swimming pools, and rainwater.
2. To measure the pH of biological fluid such as urine, blood, etc., to know the type of biological conditions.
3. To know the pH of the soil to increase productivity.
4. It is used in the chemical industry, neutralizing effluent in the paper, steel and pulp, pharmaceutical manufacturing, biotechnology, and petrochemical industries.
5. It is used to determine the pH value of food products to maintain a high level of safety and quality.
6. It is also used to prepare the reagents of exact pH values in microbiology laboratories.

Question42: What is pH ? Describe structure of pH meter.

Answer: pH

The pH is logarithm to the base 10 of reciprocal of hydrogen ion concentration.

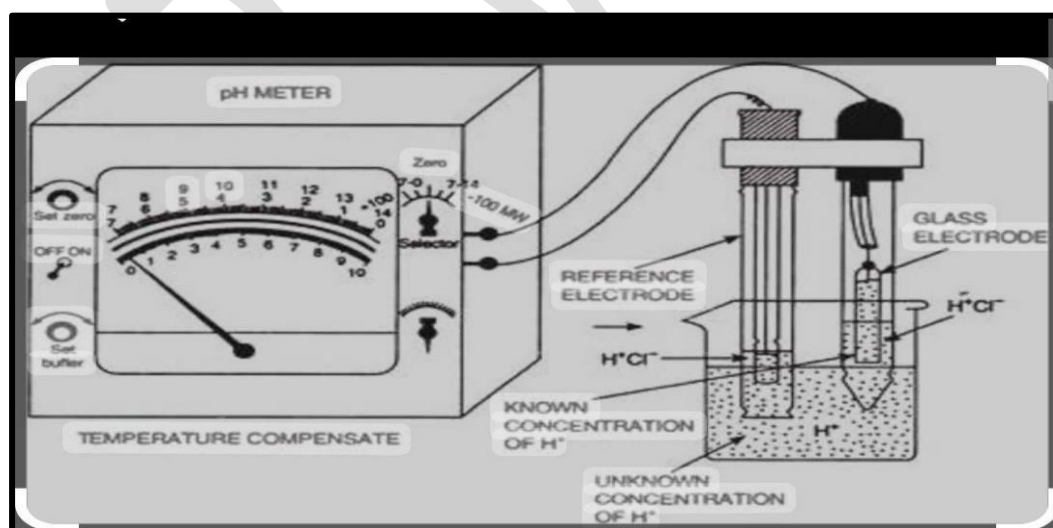
pH meter

A pH meter is an electronic tool used to measure how acidic or basic a liquid is on a scale from 0 to 14.

pH less than 7 (lemon juice, vinegar)

Neutral: pH equals 7 (pure water)

Alkaline/Basic: pH greater than 7 (soap, bleach)



Structure of pH meter

- **Glass Electrode:** Features a thin, H⁺-sensitive glass bulb tip enclosing a reference solution (like 0.1M HCl) and an internal wire (silver/silver chloride). It builds an electrical potential based on hydrogen ions in the outer test liquid.

- ▶ **Reference Electrode:** Contains a stable internal solution (like saturated KCl) and a secondary silver/silver chloride wire. It provides a steady, unchanging baseline voltage for comparison.
- ▶ **Porous Junction (Plug):** A tiny ceramic or fiber capillary leak that allows slow, controlled contact between the reference solution and the test sample to complete the electrical circuit.
- ▶ **Temperature Sensor (ATC):** Measures sample heat levels so the device can automatically correct voltage shifts caused by temperature changes.

Question 43: Write applications of Colorimeter.

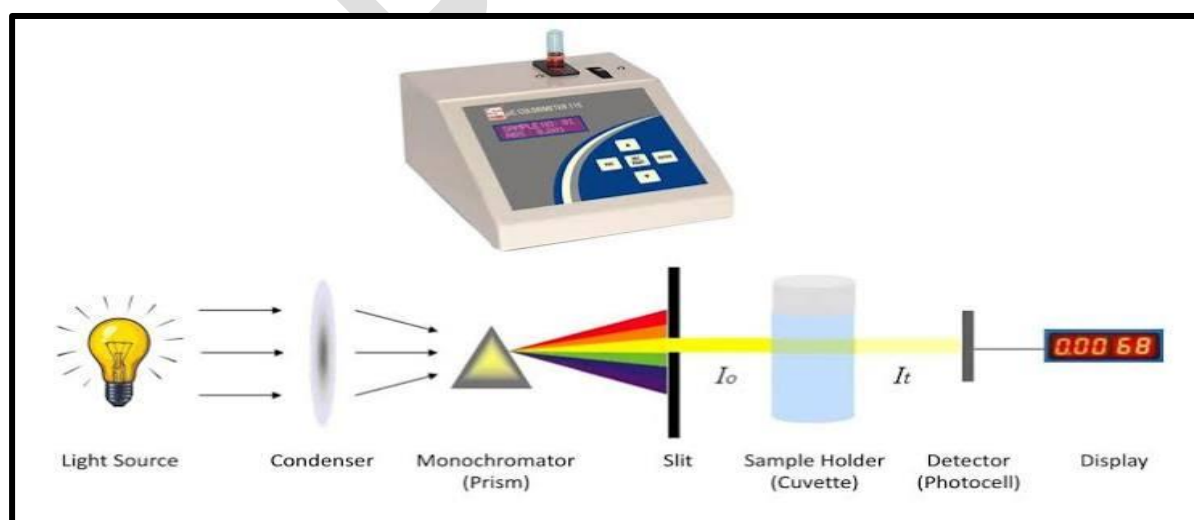
Answer: Applications of Colorimeter

- 1. Education:** Schools use colorimeter to help students with learning disabilities by placing a color overlay over their reading material or allowing them to use tinted glasses.
- 2. Engineering:** Colorimeter is vital in optical engineering, specifically for research and development. Most of the newest photography and camera technologies use colorimeter principles to produce real-life color matching.
- 3. Medical research:** Chemists and biologists use colorimeter for blood testing and analysis, laboratory quality control, calculating reaction rates, and determining bacteria growth. Doctors use colorimeter to evaluate skin tones and teeth to screen for potential diseases.
- 4. Agriculture:** Besides recognizing impurities in a water supply, spectrophotometers can identify chlorophyll, glucose, nitrate, and ammonium concentrations in plants. Spectrophotometers can also evaluate potassium, nitrogen, and phosphorus levels in soil — all vital components of growth and high crop yields.
- 5. Food and beverage processing:** Colorimeter is vital in the food and beverage industry because achieving the right color is critical for establishing quality, process control, and brand consistency.
- 6. Commercial printing:** In the printing industry, spectrophotometers are the color management system's primary measuring tools. They can also help evaluate paper and ink quality through substance analysis.

Question44: Describe Structure of Colorimeter.

Answer: Structure of Colorimeter

Colorimeter is an instrument used to measure the intensity of color in a solution.



Structure of calorimeter-

1. **Light Source** - Usually LED or tungsten lamp.
2. **Color Filters**- Selects specific wavelength of light
3. **Cuvette**- Holds the colored solution
4. **Detector/Photoelectric cell** - Measures light that passes through
5. **Display**- Shows absorbance or % transmittance.

Question45: What is Beer-Lambert law?

Answer: Beer-Lambert law

According to Beer-Lambert law, the absorption capacity of light is equal to the product of its molar absorption coefficient, molar concentration, and optical path length.

Since, $A \propto Lc$

$$A = \epsilon Lc$$

$$\epsilon = A/LC$$

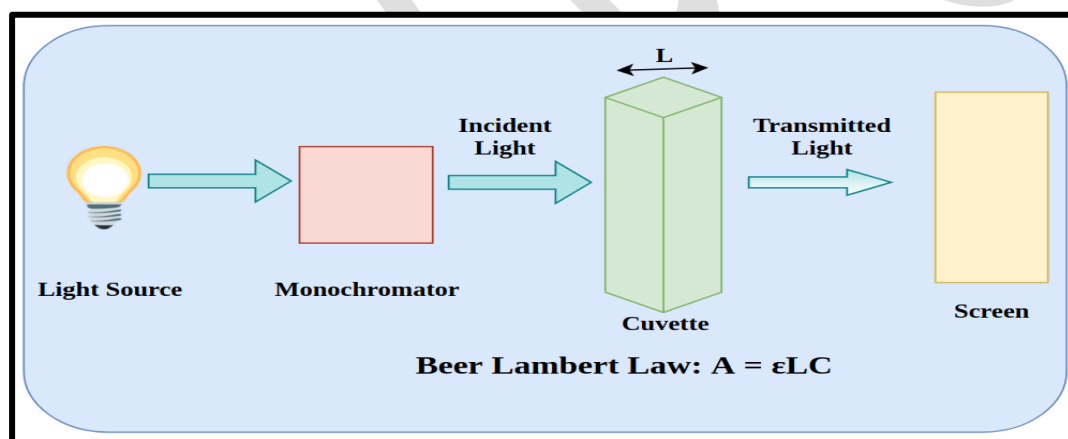
where,

A is the absorbed light for a wavelength by the solution,

ϵ is the molar absorption coefficient,

L is the distance travelled by light through the solution,

c is the solution concentration



Question 46: Summarise applications of Spectrophotometry.

Answer: Applications of Spectrophotometry

Quantitative Analysis: Measures the exact concentration of a known solute in a solution using the Beer-Lambert law.

Life Sciences & Molecular Biology: Quantifies nucleic acids (DNA/RNA) and proteins using tiny sample volumes, and measures bacterial cell density to track microbial growth curves.

Agriculture: Analysis of soil nutrients and fertilizers. Determination of chlorophyll content in plants.

Biochemistry: Studies enzyme kinetics and monitors the rates of chemical reactions over time.

Forensic Science: Detection and identification of drugs, poisons, and toxic substances. Analysis of biological samples in criminal investigations.

Pharmaceutical Industry: Assesses drug concentration, purity, and stability during quality control and drug development.

Environmental Testing: Detects and measures heavy metals, organic pollutants, and nutrients in water, as well as toxic gases like ozone and nitrogen dioxide in air quality monitoring.

Chemical & Petroleum Industry: Identifies impurities and checks fuel quality parameters, including octane numbers.

Material Science: Characterizes thin films, performs colorimetry, and evaluates optical properties in semiconductor and nano-fabrication processes.

Question 47: Write applications of radio tracer technique.

Answer: Applications of radio tracer technique

1. **Medical Diagnosis and Imaging:** Doctors use radiotracers like Fluorine-18 in FDG-PET scans to detect cancer, monitor brain activity, and evaluate heart function.
2. **Industrial Process Troubleshooting:** Engineers apply radiotracers to measure fluid flow rates, locate pipeline leaks, test mixing efficiency, and calculate residence times in industrial reactors.
3. **Biological and Metabolic Studies:** Researchers use isotopes like Carbon-14 and Tritium (Hydrogen-3) to map complex metabolic and biosynthetic pathways in plants and animals.
4. **Environmental and Hydrological Tracking:** Scientists trace the movement of pollutants in groundwater, monitor dispersion in rivers and oceans, and measure toxic elements like arsenic in drinking water.
5. **Agricultural Research:** Radioisotopes help study how plants absorb fertilizers and are used to sterilize male insects to manage pest populations safely.
6. **Protein synthesis:** Tracing amino acid during Protein synthesis.
7. **Ecology:** study of nutrients cycles and movement of substances in ecosystem.

Question 48: What is ELISA? Explain its principle, types and applications of ELISA.

Answer: ELISA (Enzyme-Linked Immunosorbent Assay)

ELISA is a biochemical technique used to detect and quantify antigens or antibodies in a sample using enzyme-linked antibodies and a color change reaction.

Principle:

ELISA is based on the antigen–antibody reaction. An enzyme is attached (linked) to an antibody or antigen. When a specific substrate is added, the enzyme catalyzes a reaction that produces a color change, indicating the presence (and often quantity) of the target molecule.

Types of ELISA:

1. Direct ELISA:
2. Indirect ELISA
3. Sandwich ELISA:
4. Competitive ELISA

Applications:

1. Detection of HIV, Hepatitis, COVID-19, pregnancy tests
2. Measurement of hormones, cytokines, and antibodies
3. Food industry: allergen detection

4. Research: protein quantification and immune response studies.

Question 49: Explain DNA Fingerprinting and its Applications.

Answer: DNA Fingerprinting

DNA fingerprinting is a laboratory technique used to determine an individual's unique genetic profile by analyzing repetitive non-coding DNA sequences. DNA fingerprinting, also called DNA profiling. It compares selected regions of DNA that vary from person to person rather than sequencing the entire genome. This method became a landmark development in biology and forensic science after it was introduced by **Sir Alec Jeffreys** in **1984**. In India, major contributions to the development and use of DNA fingerprinting were made by **Dr V. K. Kashyap** and **Dr Lalji Singh**.

Applications

1. Identifying criminals from biological evidence.
2. Resolving paternity and family disputes.
3. Solving immigration-related identity issues.
4. Identifying dead bodies in accidents or disasters.
5. Studying genetic diversity among populations.
6. Traces the inheritance of mutated genes to diagnose inherited diseases like sickle cell anaemia, thalassemia, or Huntington's disease.
7. Assists in matching tissue and donor compatibility.

Question50: Write short notes on RIA and its applications.

Answer: RIA

RIA stands for Radioimmunoassay. It is a highly sensitive immunological technique used to measure very small amounts of substances such as hormones, drugs, and antigens. It is based on the competition between radioactive-labelled and unlabelled antigen for specific antibodies. A known amount of radioactive antigen is mixed with antibody and the sample containing the unlabelled antigen. The amount of radioactivity measured is inversely proportional to the concentration of the antigen in the sample.

Applications:

1. Detection of hormones such as insulin and thyroid hormones.
2. Diagnosis of certain diseases.
3. Measurement of drugs and vitamins.
4. Research in endocrinology and immunology.

Question51: Summarise Applications of PCR.

Answer: Applications of PCR

1. **Infectious Disease Detection:** Identifies viral and bacterial genetic material quickly, allowing diagnosis of illnesses like COVID-19, HIV, and tuberculosis.
2. **Genetic Disorder Screening:** Detects mutations linked to inherited conditions such as cystic fibrosis, sickle cell anaemia, and thalassemia.
3. **Cancer Research:** Identifies oncogenes (cancer-causing genes) and monitors minimal residual disease during treatment.

4. **In research:** in addition DNA fingerprinting of new microorganisms isolated from various extreme environment is also carried out to confirm their identify by comparing with the DNA sequence of known microorganism.
5. **DNA fingerprinting:** Amplifies tiny, degraded, or mixed DNA traces from hair, blood, or skin left at crime scenes.
6. **Human Identification:** Helps match biological evidence to suspects or identify victims in mass disasters and missing person cases.
7. **Paternity Testing:** Compares child and parent DNA profiles to confirm biological relationships.
8. **GMO Detection:** Screens crops and food products for genetically modified organism markers.
9. **Pathogen and Pest Control:** Identifies agricultural plant pathogens, bacteria, or viruses affecting crop yields.
10. **Gene Cloning:** Isolates and amplifies specific genes to insert them into vectors for recombinant DNA technology.