1. a) How would you differentiate between Gly-Ala and Ala-Gly by Edman's degradation method? Write down the reactions involved.

    - **Differentiation using Edman's Degradation Method:** Edman's degradation method is used to determine the N-terminal amino acid of a peptide and sequence a peptide from its N-terminus. It works by selectively cleaving the N-terminal amino acid without hydrolyzing the rest of the peptide chain.

        - **For Gly-Ala (Glycylalanine):**

            - **Step 1: Reaction with Phenylisothiocyanate (PITC):** Phenylisothiocyanate reacts with the free amino group of Glycine (Gly), the N-terminal amino acid, to form a phenylthiocarbamoyl (PTC) derivative of Gly-Ala.
            $C_6H_5-N=C=Stext(PITC)+H_2N-CH_2-CO-NH-CH(CH_3)-COOHtext(Gly-Ala) longrightarrowC_6H_5-NH-CS-NH-CH_2-CO-NH-CH(CH_3)-COOHtext(PTC-Gly-Ala)$

            - **Step 2: Cyclization under Mild Acidic Conditions:** The PTC derivative cyclizes to form a 2-anilino-5-thiazolinone derivative, simultaneously cleaving the N-terminal amino acid from the peptide.
            $PTC-Gly-AlaxrightarrowH+text2-anilino-5-thiazolinoneofGlycine+H_2N-CH(CH_3)-COOHtext(Alanine,Ala)$

            - **Step 3: Rearrangement to Phenylthiohydantoin (PTH) Derivative:** The unstable 2-anilino-5-thiazolinone derivative rearranges under stronger acidic conditions (e.g., anhydrous HF or aqueous HCl) to a more stable phenylthiohydantoin (PTH) derivative of the N-terminal amino acid.
            $text2-anilino-5-thiazolinoneofGlycinexrightarrowH+textPTH-Glycine$

- **Identification:** The PTH-Glycine derivative can be identified by chromatography (e.g., HPLC) by comparing its retention time with known PTH-amino acid standards. In this case, the identification of PTH-Glycine confirms that Glycine was the N-terminal amino acid of Gly-Ala. The remaining peptide is Alanine.

- **For Ala-Gly (Alanylglycine):**
  - **Step 1: Reaction with PITC:** Phenylisothiocyanate reacts with the free amino group of Alanine (Ala), the N-terminal amino acid, to form a phenylthiocarbamoyl (PTC) derivative of Ala-Gly. $C_6H_5-N=C=S\text{(PITC)}+H_2N-CH(CH_3)-CO-NH-CH_2-COOH\text{(Ala-Gly)} \longrightarrow C_6H_5-NH-CS-NH-CH(CH_3)-CO-NH-CH_2-COOH\text{(PTC-Ala-Gly)}$
  - **Step 2: Cyclization:** $\text{PTC-Ala-Gly} \xrightarrow{} H+\text{2-anilino-5-thiazolinone of Alanine}+H_2N-CH_2-COOH\text{(Glycine, Gly)}$
  - **Step 3: Rearrangement to PTH Derivative:** $\text{2-anilino-5-thiazolinone of Alanine} \xrightarrow{} H+\text{PTH-Alanine}$
  - **Identification:** The PTH-Alanine derivative is identified chromatographically. This confirms Alanine as the N-terminal amino acid of Ala-Gly. The remaining peptide is Glycine.

Differentiation: By carrying out one cycle of Edman's degradation, Gly-Ala will yield PTH-Glycine, while Ala-Gly will yield PTH-Alanine. These two PTH-amino acids are distinct and can be easily identified, thus differentiating the two dipeptides.

b) How will you synthesize valine by Gabriel's phthalimide method?

- **Gabriel's Phthalimide Synthesis of Amino Acids (Valine):** Gabriel's phthalimide synthesis is a classic method for synthesizing primary amines, and it can be adapted for amino acid synthesis, particularly alpha-amino acids. The key idea is to use phthalimide to protect the nitrogen, allow for alkylation at the alpha-carbon, and then hydrolyze to liberate the amino acid.
  - **Reactions Involved:**
  1. **Preparation of Potassium Phthalimide:**
     - Phthalimide reacts with a strong base like potassium hydroxide (KOH) to form potassium phthalimide. This makes the nitrogen nucleophilic.
     - Phthalimide + KOH longrightarrow Potassium Phthalimide + H_2O
  2. **Alkylation of Potassium Phthalimide with Ethyl alpha-Bromo-isovalerate:**
     - In Gabriel's synthesis, we need to introduce the specific side chain of valine at the alpha-carbon. This is typically done by reacting potassium phthalimide with an alpha-haloester. For valine, the precursor is ethyl alpha-bromo-isovalerate.
     - The nitrogen of potassium phthalimide acts as a nucleophile and attacks the alpha-carbon of the ethyl alpha-bromo-isovalerate, displacing the bromine (S$_N$2 reaction).
     - Potassium Phthalimide + Br−CH(CH(CH_3)_2)−COOEttext(Ethylalphatext−bromo−isovalerate) longrightarrow N-(1-(ethoxycarbonyl)-2-methylpropyl)phthalimide + KBr

3. **Hydrolysis (or Hydrazinolysis):**
    - The phthalimide group (which is protecting the amine) is removed, and the ester is hydrolyzed to a carboxylic acid. This can be achieved by:
        - **Acidic Hydrolysis:** Heating with strong aqueous acid (e.g., concentrated HCl). This can be harsh and may lead to side reactions or racemization.
        - **Alkaline Hydrolysis:** Heating with strong aqueous base (e.g., NaOH). This can also be harsh.
        - **Hydrazinolysis (Preferred Method for Mildness):** Reaction with hydrazine (N_2H_4). This is generally milder and prevents racemization.

    - **Using Hydrazine:**
        - N-(1-(ethoxycarbonyl)-2-methylpropyl)phthalimide + $N_2H_4 \longrightarrow$ Phthalhydrazide + $H_2N-CH(CH(CH_3)_2)-COOH$ text(Valine)

- **Summary of Steps for Valine Synthesis:**
    1. Start with diethyl malonate (or an equivalent precursor to the alpha-carbon).
    2. Alkylate diethyl malonate with isopropyl bromide (to introduce the valine side chain) using a base like sodium ethoxide. This forms diethyl isopropylmalonate.

3. Hydrolyze one ester group of diethyl isopropylmalonate and decarboxylate to form isovaleric acid.
4. Brominate the alpha-carbon of isovaleric acid to get alpha-bromoisovaleric acid. Then esterify it to ethyl alpha-bromo-isovalerate.
5. React ethyl alpha-bromo-isovalerate with potassium phthalimide.
6. Hydrolyze (or hydrazinolyze) the resulting N-substituted phthalimide derivative to yield valine.

(Note: The most direct adaptation of Gabriel synthesis for alpha-amino acids usually involves reacting potassium phthalimide with an alpha-haloester, where the halogen is on the carbon that will become the alpha-carbon of the amino acid. The side chain is already present in the alpha-haloester. The ethyl alpha-bromo-isovalerate already has the isopropyl side chain. The explanation above focuses on the key Gabriel steps after obtaining this bromoester.)

c) How will you differentiate between RNA and DNA by alkaline hydrolysis. Give mechanism of the reaction involved.

- **Differentiation by Alkaline Hydrolysis:**
  - **RNA (Ribonucleic Acid):** RNA is susceptible to alkaline hydrolysis and will be completely hydrolyzed into its constituent ribonucleotides (and further to nucleosides and phosphates) when treated with a strong base (like NaOH or KOH).
  - **DNA (Deoxyribonucleic Acid):** DNA is stable to alkaline hydrolysis and will not be cleaved or degraded under similar conditions.
- **Mechanism of Alkaline Hydrolysis of RNA:** The difference in stability arises from the presence of a **2'-hydroxyl (-OH) group**

on the ribose sugar in RNA, which is absent in the deoxyribose sugar of DNA. This 2'-hydroxyl group acts as an internal nucleophile, facilitating the cleavage of the phosphodiester bonds.

1. **Deprotonation of 2'-OH:** In alkaline conditions, the 2'-hydroxyl group of the ribose sugar is deprotonated by a hydroxide ion (OH^-) to form a highly reactive 2'-alkoxide ion.
    - $2'-\text{textOH}+OH^- \rightleftharpoons 2'-\text{textO}^-+H_2O$
2. **Intramolecular Nucleophilic Attack:** The negatively charged 2'-alkoxide ion acts as an intramolecular nucleophile. It attacks the electrophilic phosphorus atom of the adjacent phosphodiester bond (specifically, the phosphate group linking the 2' carbon to the 3' carbon of the upstream nucleotide, or the 3' carbon to the 5' carbon of the downstream nucleotide). This attack forms a **cyclic 2',3'-phosphodiester intermediate**.
    - The attack leads to a pentavalent phosphorus intermediate, followed by the breaking of the P-O bond with the 5'-carbon of the next nucleotide in the chain.
3. **Cleavage of Phosphodiester Bond:** The formation of the cyclic intermediate results in the cleavage of the phosphodiester bond, breaking the RNA backbone.
4. **Hydrolysis of Cyclic Intermediate:** The cyclic 2',3'-phosphodiester intermediate is then rapidly hydrolyzed (by water, often catalyzed by the base) to yield a mixture of 2'-monophosphate and 3'-monophosphate ribonucleotides.

        - Cyclic 2',3'-phosphodiester xrightarrowH_2O/OH− 2'-monophosphate + 3'-monophosphate ribonucleotides

- **Why DNA is stable:**
    - DNA lacks the 2'-hydroxyl group on its deoxyribose sugar. Without this crucial nucleophilic group, the intramolecular attack on the phosphorus atom cannot occur. Therefore, DNA's phosphodiester backbone remains intact under alkaline conditions, making it stable to alkaline hydrolysis.
- **Differentiation:**
    - To differentiate, you would treat samples of RNA and DNA with a strong alkaline solution (e.g., 0.1 M NaOH) at room temperature for a period (e.g., 24 hours).
    - After treatment, analyze the products. RNA will show a complete breakdown into smaller nucleotide fragments, while DNA will remain largely intact. This can be observed by techniques like gel electrophoresis (RNA fragments will migrate much faster), spectrophotometry (change in absorbance profile), or chromatographic methods.

d) Write structure of NAD^+. Explain its role in an enzyme catalyzed reaction.

- **Structure of NAD^+ (Nicotinamide Adenine Dinucleotide):** NAD^+ is a coenzyme consisting of two nucleotides joined through their phosphate groups by a pyrophosphate bond.
    - One nucleotide contains **adenine** linked to **ribose** and a **phosphate** group (Adenosine Monophosphate or AMP).
    - The other nucleotide contains **nicotinamide** (a derivative of Vitamin B3, Niacin) linked to **ribose** and a **phosphate** group (Nicotinamide Monophosphate).

The structure can be visualized as: **Nicotinamide ring - Ribose - Phosphate - Phosphate - Ribose - Adenine**

- **Key features:**
  - The **nicotinamide ring** is the active part, specifically the nitrogen atom in the ring. It carries a positive charge in NAD^+ (hence the $^+$ superscript).
  - The **ribose sugar** is connected to the nicotinamide and to the phosphate group.
  - The **phosphate groups** link the two nucleotides via a pyrophosphate bond.
  - The **adenine base** is part of the adenosine monophosphate moiety, which serves as a recognition and binding site for enzymes but is not directly involved in redox reactions.

(Imagine drawing a flat nicotinamide ring with the nitrogen being quaternary and positively charged. Then link it to a ribose sugar, then a phosphate, then another phosphate, then another ribose sugar, and finally an adenine base.)

- **Role in Enzyme-Catalyzed Reactions:** NAD^+ primarily functions as a **redox coenzyme** in many metabolic pathways. Its main role is to act as an **electron carrier** in oxidation-reduction (redox) reactions catalyzed by enzymes known as **dehydrogenases**.
  - **Electron Acceptor (Oxidizing Agent) - NAD^+:**
    - In catabolic (breakdown) reactions, where substrates are oxidized (lose electrons), NAD^+ acts as an **electron acceptor**.

- The nicotinamide ring of NAD^+ accepts two electrons and one proton (a hydride ion, H^-) from the substrate.
- This reduces NAD^+ to **NADH**. The positive charge on the nicotinamide nitrogen is neutralized, and a new double bond is formed in the ring.
- **Reaction:**
  Substrate_reduced+NAD+xrightarrowDehydrogenaseSubstrate_oxidized+NADH+H+
- Example: Oxidation of glucose in glycolysis and the citric acid cycle.

- **Electron Donor (Reducing Agent) - NADH:**
  - The NADH (the reduced form) then carries these high-energy electrons to other enzyme systems, such as the electron transport chain in mitochondria.
  - In anabolic (synthesis) reactions or in the electron transport chain, NADH acts as an **electron donor**, donating its electrons (and a proton) to another molecule, thereby regenerating NAD^+.
  - **Reaction:**
    NADH+H++Acceptor_oxidizedxrightarrowDehydrogenaseNAD++Acceptor_reduced
  - Example: Oxidative phosphorylation, where NADH donates electrons to the electron transport chain to generate ATP.
- **Key Functions:**

- **Interconversion:** NAD^+ and NADH are interconverted during redox reactions, allowing for the continuous transfer of electrons.
- **Energy Production:** NADH is a crucial molecule in cellular respiration, as it carries electrons to the electron transport chain, which ultimately drives ATP synthesis.
- **Metabolic Regulation:** The ratio of NAD^+ to NADH within a cell is an important indicator of its metabolic state and helps regulate various biochemical pathways.

e) Discuss the effect of urea and heat on secondary structure of protein.

- **Secondary Structure of Proteins:** Secondary structure refers to the local folding patterns of the polypeptide chain, primarily stabilized by hydrogen bonds between the backbone amide groups. The two most common types are the alpha-helix and the beta-pleated sheet.
- **Effect of Heat on Secondary Structure:**
  - **Denaturation:** Heat is a potent denaturing agent. When a protein is heated above a certain temperature (its melting temperature), the increased kinetic energy of the atoms and molecules causes them to vibrate more vigorously.
  - **Disruption of Hydrogen Bonds:** This increased vibration directly disrupts the relatively weak hydrogen bonds that stabilize the alpha-helices and beta-sheets.
  - **Unfolding:** As these hydrogen bonds break, the organized secondary structures unravel and unfold into a more random coil conformation.

- **Irreversibility:** If heating is prolonged or intense, the denaturation can become irreversible, leading to aggregation and precipitation of the protein, as the polypeptide chains lose their native conformation and interact non-specifically. This is why a boiled egg cannot be "unboiled" - the proteins have irreversibly denatured.

- **Effect of Urea on Secondary Structure:**
  - **Mechanism:** Urea is a chaotropic agent, meaning it disrupts the structure of water and weakens non-covalent interactions within proteins. It is a powerful denaturant often used in biochemistry laboratories.
  - **Hydrogen Bond Disruption:** Urea directly interferes with the hydrogen bonding network that stabilizes alpha-helices and beta-sheets. It can form its own hydrogen bonds with the backbone amide and carbonyl groups of the polypeptide chain, effectively competing with and breaking the intramolecular hydrogen bonds that maintain the secondary structure.
  - **Hydrophobic Interactions:** Urea also disrupts hydrophobic interactions, which play a role in tertiary and quaternary structures, but their disruption can indirectly affect secondary structure stability by altering the overall protein environment.
  - **Unfolding:** Similar to heat, the disruption of hydrogen bonds by urea leads to the unfolding of the alpha-helices and beta-sheets, causing the protein to adopt a more disordered random coil state.
  - **Reversibility:** Unlike heat, denaturation by urea is often **reversible**. If the urea is gradually removed (e.g., by dialysis), the protein can sometimes refold back into its native secondary (and tertiary) structures, assuming no

irreversible aggregation occurred. This reversibility is due to the non-covalent nature of the interactions broken by urea.

2. a) The reaction of nonapeptide "A" with dansyl chloride gives dansyl derivative of Cysteine. Peptide "A" on reaction with cyanogen-bromide gives tripeptide containing Cys, Met, Lys and hexapeptide containing Try, Gly, Ala, Phe, Leu and Asp. Partial hydrolysis of "A" yields Lys-Met-Leu, Ala-Gly-Try, Cys-Lys, Leu-Phe-Ala, Gly-Try-Asp and Met-Leu- Phe. Deduce the structure of "A". Give all the reactions involved. Write down the products obtained when B is treated with Carboxypeptidase and Chymotrypsin.

- **Deducing the Structure of Nonapeptide "A":**

    1. **N-terminal analysis with Dansyl Chloride:**

        - "Reaction of nonapeptide 'A' with dansyl chloride gives dansyl derivative of Cysteine."
        - This immediately tells us that **Cysteine (Cys)** is the N-terminal amino acid of peptide "A".
        - So, peptide A starts with: **Cys-...**

    2. **Cyanogen Bromide (CNBr) Cleavage:**

        - "Peptide 'A' on reaction with cyanogen-bromide gives tripeptide containing Cys, Met, Lys and hexapeptide containing Try, Gly, Ala, Phe, Leu and Asp."
        - Cyanogen bromide specifically cleaves peptide bonds on the C-terminal side of **Methionine (Met)** residues.
        - This means there is only one Met residue in peptide "A", and it is located such that it separates a tripeptide from a hexapeptide.
        - Since Cys is the N-terminus, the tripeptide must be **Cys-X-Met** (where X is another amino acid).

- The tripeptide contains Cys, Met, Lys. So, the tripeptide sequence must be **Cys-Lys-Met**.
- The hexapeptide contains Try, Gly, Ala, Phe, Leu, Asp. This means the Met must be followed by these six amino acids.
- So, peptide A looks like: **Cys-Lys-Met - (Try, Gly, Ala, Phe, Leu, Asp) (hexapeptide)**

3. **Partial Hydrolysis Fragments:**
   - "Partial hydrolysis of 'A' yields Lys-Met-Leu, Ala-Gly-Try, Cys-Lys, Leu-Phe-Ala, Gly-Try-Asp and Met-Leu- Phe."
   - Let's use these fragments to connect the hexapeptide sequence and confirm the tripeptide.
   - We have:
     - Fragment 1: **Lys-Met-Leu**
     - Fragment 2: **Ala-Gly-Try**
     - Fragment 3: **Cys-Lys**
     - Fragment 4: **Leu-Phe-Ala**
     - Fragment 5: **Gly-Try-Asp**
     - Fragment 6: **Met-Leu-Phe**
   - **Connecting Fragments:**
     - From CNBr, we know the tripeptide is Cys-Lys-Met. Fragment 3 (Cys-Lys) confirms the first two amino acids. Fragment 1 (Lys-Met-Leu) confirms Lys-Met and extends it to Leu. Fragment 6 (Met-Leu-Phe) confirms Met-Leu and extends it to Phe.
     - So, the beginning sequence is **Cys-Lys-Met-Leu-Phe**. This uses Cys, Lys, Met, Leu, Phe.

        - Now let's look at the hexapeptide's remaining amino acids: Try, Gly, Ala, Asp.
        - We have Leu-Phe-Ala (Fragment 4). This fits after Leu-Phe.
        - So, we have **Cys-Lys-Met-Leu-Phe-Ala**. This uses Cys, Lys, Met, Leu, Phe, Ala.
        - Remaining amino acids are Gly, Try, Asp.
        - We have Gly-Try-Asp (Fragment 5). This fits perfectly at the end.
        - We also have Ala-Gly-Try (Fragment 2) which further confirms the sequence after Ala.
    - **Final Sequence Deduction for "A":** Combining all the information:
        - N-terminus: Cys
        - CNBr cleavage after Met: Cys-Lys-Met (tripeptide) and remaining hexapeptide.
        - Fragments:
            - Cys-Lys (from Cys-Lys-Met)
            - Lys-Met-Leu (confirms Lys-Met and adds Leu)
            - Met-Leu-Phe (confirms Met-Leu and adds Phe)
            - Leu-Phe-Ala (confirms Leu-Phe and adds Ala)
            - Ala-Gly-Try (confirms Ala and adds Gly-Try)
            - Gly-Try-Asp (confirms Gly-Try and adds Asp)
        - Therefore, the full sequence of nonapeptide "A" is: **Cys-Lys-Met-Leu-Phe-Ala-Gly-Try-Asp**

- **Reactions Involved (in brief):**

1. **Dansyl Chloride Reaction:**
    - **Reactant:** Dansyl chloride (5−(dimethylamino)naphthalene−1−sulfonylchloride)
    - **Mechanism:** It reacts with the free N-terminal amino group of the peptide in a slightly alkaline medium to form a stable N-dansyl derivative.
    - Peptide−NH_2+Dansyl−CllongrightarrowDansyl−NH−Peptide+HCl
    - Subsequent hydrolysis of the entire peptide (e.g., with 6M HCl) releases the N-dansyl amino acid, which is fluorescent and can be identified.
    - For peptide A: Cys−Lys−Met−...+Dansyl−CllongrightarrowDansyl−Cys−(peptide_remaining)xrightarrow6MHClDansyl−Cys+textfreeaminoacids

2. **Cyanogen Bromide (CNBr) Cleavage:**
    - **Reactant:** Cyanogen bromide (BrCN)
    - **Mechanism:** Cleaves peptide bonds specifically at the C-terminal side of methionine residues. It involves the formation of a homoserine lactone at the C-terminus of the cleaved fragment.
    - For peptide A: Cys−Lys−Met−Leu−Phe−Ala−Gly−Try−AspxrightarrowBrCN
        - Cys−Lys−HomoserineLactone (Tripeptide)
        - H_2N−Leu−Phe−Ala−Gly−Try−Asp (Hexapeptide)

- **Products obtained when "B" is treated with Carboxypeptidase and Chymotrypsin:**
    - The question mentions "Peptide 'A' on reaction with cyanogen-bromide gives tripeptide containing Cys, Met, Lys and hexapeptide containing Try, Gly, Ala, Phe, Leu and Asp." It then asks about products when "B" is treated. Assuming "B" refers to the **hexapeptide product** from CNBr cleavage.
    - Hexapeptide (B) = **Leu-Phe-Ala-Gly-Try-Asp** (N-terminal Leu, C-terminal Asp)

    1. **Treatment with Carboxypeptidase:**
        - **Specificity:** Carboxypeptidases are exopeptidases that cleave amino acids from the C-terminal end of a polypeptide chain.
        - **Carboxypeptidase A:** Cleaves all C-terminal amino acids except Pro, Arg, Lys. It is most efficient with C-terminal hydrophobic amino acids.
        - **Carboxypeptidase B:** Cleaves C-terminal Arg and Lys.
        - **For Hexapeptide B (Leu-Phe-Ala-Gly-Try-Asp):**
            - The C-terminal amino acid is **Aspartic Acid (Asp)**.
            - Carboxypeptidase A (or general carboxypeptidase activity) would cleave Asp.
            - **Product:** The hexapeptide would be progressively degraded from the C-terminus:
                - Leu-Phe-Ala-Gly-Try-Asp xrightarrowCarboxypeptidase Leu-Phe-Ala-Gly-Try + Asp

- Leu-Phe-Ala-Gly-Try xrightarrowCarboxypeptidase Leu-Phe-Ala-Gly + Try
- ...and so on, until the enzyme reaches a resistant residue or the peptide is too short.

2. **Treatment with Chymotrypsin:**
   - **Specificity:** Chymotrypsin is a serine protease that specifically cleaves peptide bonds on the C-terminal side of large **hydrophobic amino acids**, particularly **Tyrosine (Tyr), Tryptophan (Trp), and Phenylalanine (Phe)**. It can also cleave after Leucine (Leu) and Methionine (Met) but less efficiently.
   - **For Hexapeptide B (Leu-Phe-Ala-Gly-Try-Asp):**
     - We have **Phenylalanine (Phe)** at position 2 and **Tryptophan (Try)** at position 5.
     - Chymotrypsin will cleave after Phe and Try.
     - **Cleavage after Phe:** The bond between Phe and Ala will be cleaved.
       - Leu-Phe downarrow Ala-Gly-Try-Asp
       - Products: **Dipeptide (Leu-Phe)** and **Tetrapeptide (Ala-Gly-Try-Asp)**.
     - **Cleavage after Try:** The bond between Try and Asp will be cleaved.
       - Ala-Gly-Try downarrow Asp
       - Products: **Tripeptide (Ala-Gly-Try)** and **Amino acid (Asp)**.
     - **Overall Products from Chymotrypsin digestion of Hexapeptide B:**

1. **Leu-Phe**
2. **Ala-Gly-Try**
3. **Asp**

b) What are the structures of lysine at pH = 1.5, 3.2, 9.74 and 12? To which electrode does lysine migrate at each pH? Which of the structure will be present at isoelectric point?

- **Lysine (Lys):** Lysine is a basic amino acid with three ionizable groups:
    1. alpha-carboxyl group (pKa approx 2.2)
    2. alpha-amino group (pKa approx 9.0)
    3. epsilon-amino group on the side chain (pKa approx 10.53)
- **Isoelectric Point (pI) of Lysine:** Since it's a basic amino acid, the pI will be calculated using the two highest pKa values (the alpha-amino and epsilon-amino groups). pI=fracpKa(alpha−NH_3+)+pKa(epsilon−NH_3+)2=frac9.0+10.532=frac19.532=9.765
    - The question states 9.74 as a pH value, which is very close to the pI. For practical purposes, we can consider pH 9.74 as the pI for this problem.
- **Structures and Migration at Different pH Values:**
    - **At pH = 1.5 (Strongly Acidic):**
        - pH < all pKa values. All ionizable groups will be protonated.
        - alpha-COOH: Protonated (COOH)
        - alpha-NH_2: Protonated (NH_3+)
        - epsilon-NH_2 (side chain): Protonated (NH_3+)

- **Structure:**
  H_3N+−CH_2−CH_2−CH_2−CH_2−CH(NH_3+)−COOH
  (Net charge: +2)
- **Migration:** Migrates towards the **cathode** (negative electrode) due to its overall positive charge.

- **At pH = 3.2 (Acidic, between pKa1 and pKa2):**
  - pH > pKa(alpha-COOH) but < pKa(alpha-NH_2), pKa(epsilon-NH_2).
  - alpha-COOH: Deprotonated (COO−)
  - alpha-NH_2: Protonated (NH_3+)
  - epsilon-NH_2 (side chain): Protonated (NH_3+)
  - **Structure:**
    H_3N+−CH_2−CH_2−CH_2−CH_2−CH(NH_3+)−COO−
    (Net charge: +1)
  - **Migration:** Migrates towards the **cathode** (negative electrode) due to its overall positive charge.
- **At pH = 9.74 (Near Isoelectric Point):**
  - This pH is very close to the pI of Lysine (9.765). At the pI, the molecule exists predominantly as a zwitterion with a net zero charge.
  - alpha-COOH: Deprotonated (COO−)
  - alpha-NH_2: Deprotonated (NH_2)
  - epsilon-NH_2 (side chain): Protonated (NH_3+) (Since 9.74 is still below 10.53)
  - **Structure:**
    H_3N+−CH_2−CH_2−CH_2−CH_2−CH(NH_2)−COO−
    (Net charge: 0)

        - **Migration:** At the isoelectric point, lysine will **not migrate** significantly towards either electrode in an electric field, as its net charge is zero.

    - **At pH = 12 (Strongly Alkaline):**

        - pH > all pKa values. All ionizable groups will be deprotonated.
        - alpha-COOH: Deprotonated (COO−)
        - alpha-NH_2: Deprotonated (NH_2)
        - epsilon-NH_2 (side chain): Deprotonated (NH_2)
        - **Structure:** H_2N−CH_2−CH_2−CH_2−CH_2−CH(NH_2)−COO− (Net charge: -1)
        - **Migration:** Migrates towards the **anode** (positive electrode) due to its overall negative charge.

- **Structure present at Isoelectric Point:**
    - The structure present at the isoelectric point (pH approx 9.74) is the **zwitterionic form** with a net charge of zero: H_3N+−CH_2−CH_2−CH_2−CH_2−CH(NH_2)−COO−

3. a) Name the monomers used in preparation of resin used in Solid Phase Merrifield method. How would you synthesize a tripeptide Leu-Ala-Lys by this method? Give its advantages over general method of synthesis.

- **Monomers for Merrifield Resin:** The resin commonly used in the Solid Phase Merrifield method is a cross-linked polymer. The primary monomers are:
    1. **Styrene:** Forms the main polymer backbone.

2. **Divinylbenzene (DVB):** Acts as a cross-linking agent, providing rigidity and insolubility to the resin beads, which is crucial for solid-phase synthesis.
    - The resin is typically functionalized with chloromethyl groups ($-CH_2Cl$) (Merrifield resin) or other linkers like Rink amide resin, Wang resin, etc., to allow for the attachment of the first amino acid.

- **Synthesis of Tripeptide Leu-Ala-Lys by Merrifield Method:** The Merrifield method involves synthesizing a peptide by adding amino acids one by one to a growing chain that is covalently attached to an insoluble polymeric resin. The synthesis proceeds from the C-terminus to the N-terminus.
    - **Target Peptide:** Leu-Ala-Lys (N-terminus to C-terminus)
    - **Amino Acid Sequence for Synthesis:** Lys-Ala-Leu (C-terminus to N-terminus, starting with Lys)
    - **Steps:**

1. **Preparation of Protected Amino Acids:**
    - All amino acids must be protected at their alpha-amino group (e.g., with Boc or Fmoc group) and their side chain functional groups (if present).
    - For Lysine, the epsilon-amino group on the side chain needs protection.
    - Boc-Lys(epsilon-protectant)-OH (e.g., Boc-Lys(Cbz)-OH or Boc-Lys(Fmoc)-OH)
    - Boc-Ala-OH
    - Boc-Leu-OH
2. **Loading the First Amino Acid (Lysine) onto the Resin:**

- The C-terminal amino acid (Lysine, with alpha-amino and side chain protected) is covalently attached to the chloromethylated Merrifield resin via an ester bond (or amide bond if Rink amide resin is used). This is often done by heating in the presence of an organic base.
- Boc−Lys(protectant)−OH+Resin−CH_2ClongrightarrowBoc−Lys(protectant)−O−CH_2−Resin+HCl

3. **Deprotection of alpha-Amino Group (e.g., Boc removal):**
    - The Boc protecting group is removed from the alpha-amino group using a reagent like trifluoroacetic acid (TFA) in a non-aqueous solvent. This regenerates the free alpha-amino group, which is now ready for the next amino acid coupling. The side chain protecting group remains intact.
    - Boc−Lys(protectant)−O−CH_2−ResinxrightarrowTFAH_2N−Lys(protectant)−O−CH_2−Resin+CO_2+(CH_3)_3C+
4. **Coupling of the Second Amino Acid (Alanine):**
    - The next protected amino acid (Boc-Ala-OH) is coupled to the deprotected amino group of Lysine on the resin. A coupling reagent (e.g., DCC/DIC or HBTU/HOBt) is used to activate the carboxyl group of the incoming amino acid, forming a peptide bond.
    - Boc−Ala−OH+H_2N−Lys(protectant)−O−CH_2−ResinxrightarrowCouplingAgentBoc−Ala−Lys(protectant)−O−CH_2−Resin+textbyproducts
5. **Repeat Steps 3 and 4:**
    - Deprotect the alpha-amino group of Alanine (Boc removal).

- Couple the third protected amino acid (Boc-Leu-OH) to the deprotected Alanine.
- $H_2N-Ala-Lys(protectant)-O-CH_2-Resin \xrightarrow{Boc-Leu-OH + CouplingAgent} Boc-Leu-Ala-Lys(protectant)-O-CH_2-Resin$

6. **Cleavage from Resin and Side Chain Deprotection:**
   - Once the full peptide chain is synthesized, the peptide is cleaved from the resin and simultaneously deprotected (all side chain protecting groups removed). This is typically achieved using a strong acid like anhydrous HF or TFA.
   - $Boc-Leu-Ala-Lys(protectant)-O-CH_2-Resin \xrightarrow{HF \text{ or } TFA} Leu-Ala-Lys + \text{spent resin} + \text{deprotection products}$
   - The cleaved peptide (Leu-Ala-Lys) is then purified.

- **Advantages over General Method (Solution Phase Synthesis):**
  1. **Ease of Purification:** After each step (deprotection, coupling), the excess reagents and byproducts are simply washed away, as the growing peptide chain remains attached to the insoluble resin. This eliminates tedious purification steps (crystallization, chromatography) after each coupling, which is a major advantage over solution-phase synthesis.
  2. **Higher Yields:** The reactions can be driven to completion by using excess reagents, and the unreacted reagents are easily removed. This leads to higher yields for each coupling step, which is crucial for synthesizing longer peptides.
  3. **Automation:** The repetitive nature of the steps (washing, deprotection, coupling) makes the Merrifield method highly amenable to automation. Automated peptide synthesizers can synthesize peptides rapidly and efficiently.

4. **Reduced Loss of Product:** Because the product is always tethered to the solid support, there are minimal losses during transfers and workup procedures.
5. **Synthesis of Longer Peptides:** The ability to perform rapid and efficient couplings with easy purification at each step makes it feasible to synthesize much longer peptides and even small proteins, which would be exceedingly difficult by solution-phase methods.

3. b) Discuss the following about the Trypsin:
    - i. Specificity
        - **Specificity of Trypsin:** Trypsin is a highly specific **serine protease** found in the digestive system. Its primary role is to break down proteins into smaller peptides in the small intestine.
        - **Recognition Site:** Trypsin specifically cleaves peptide bonds on the **C-terminal side of basic amino acids**, namely **Lysine (Lys) and Arginine (Arg)**.
        - **Mechanism of Specificity:** This high specificity is due to a negatively charged aspartate residue (Asp189) located at the bottom of the S1 specificity pocket of the trypsin active site.
            - When a positively charged Lysine or Arginine side chain enters this pocket, it forms an ionic bond with the negatively charged Asp189. This electrostatic interaction correctly positions the scissile peptide bond (the bond to be cleaved) near the catalytic triad for hydrolysis.
        - **Exceptions/Considerations:**
            - Trypsin will not cleave if the basic residue is followed by Proline (Pro), as the cyclic nature of

proline's imino group prevents the proper fit into the active site.

- Sometimes, limited cleavage can occur at other sites if the environment or substrate conditions are unusual, but its primary specificity remains Lys and Arg.

- ii. Catalytic Triad
  - **Definition:** The catalytic triad is a characteristic arrangement of three amino acid residues (a serine, a histidine, and an aspartate) found in the active site of many hydrolytic enzymes, especially serine proteases like trypsin, chymotrypsin, and elastase. These three residues work cooperatively to carry out the enzymatic reaction.
  - **Composition in Trypsin:** For Trypsin, the catalytic triad consists of:
    - **Serine (Ser195):** The primary nucleophile. Its hydroxyl group (O−H) acts as the attacking species.
    - **Histidine (His57):** Acts as a general acid-base catalyst. It helps to activate the serine hydroxyl by abstracting a proton.
    - **Aspartate (Asp102):** Provides an electrostatic environment that stabilizes the protonated histidine, enhancing its basicity and facilitating proton transfer.
  - **Mechanism of Action:**
    1. **Activation of Serine:** The Aspartate residue polarizes the Histidine imidazole ring, making it a stronger base. This Histidine then abstracts a proton from the Serine hydroxyl group, making the

Serine oxygen atom a much more powerful nucleophile (a highly reactive alkoxide ion).

2. **Nucleophilic Attack:** This activated Serine oxygen then launches a nucleophilic attack on the carbonyl carbon of the peptide bond to be cleaved.
3. **Tetrahedral Intermediate:** This attack leads to the formation of a short-lived **tetrahedral intermediate**, with the carbonyl oxygen becoming an oxyanion.
4. **Proton Transfer & Cleavage:** The Histidine then donates a proton back to the leaving group (the N-terminus of the second peptide fragment), facilitating the cleavage of the peptide bond.
5. **Acyl-Enzyme Intermediate:** This results in the formation of an **acyl-enzyme intermediate**, where the C-terminal portion of the original substrate is covalently attached to the Serine residue.
6. **Deacylation:** A water molecule then attacks the acyl-enzyme intermediate, facilitated by Histidine as a general base, leading to the release of the remaining peptide fragment and regeneration of the free enzyme.

- iii. Pocket at the active site
  - **S1 Specificity Pocket:** The "pocket" at the active site of trypsin (and other proteases) is known as the **S1 specificity pocket**. This pocket is a crucial determinant of the enzyme's substrate specificity. It is a cleft or cavity in the enzyme's active site that accommodates the side chain of the amino acid residue immediately preceding the scissile peptide bond (i.e., the P1 residue).
  - **Structure of Trypsin's S1 Pocket:**

        - In trypsin, the S1 pocket is deep and relatively narrow.
        - Crucially, at the bottom of this pocket, there is a **negatively charged aspartate residue (Asp189)**.
    - **Role in Specificity:**
        - The negative charge of Asp189 in the S1 pocket specifically attracts and binds positively charged amino acid side chains.
        - Therefore, only **Lysine (Lys)** and **Arginine (Arg)**, with their long, positively charged side chains, can fit into and be specifically accommodated by this pocket.
        - This precise fit correctly positions the peptide bond adjacent to the Lys or Arg residue for cleavage by the catalytic triad. Without this specific interaction, the peptide bond cannot be optimally presented for hydrolysis, ensuring trypsin's high specificity.

c) Explain the various types of forces that are responsible for the stabilization of tertiary structures of proteins.

- **Tertiary Structure of Proteins:** Tertiary structure refers to the overall three-dimensional folding of a single polypeptide chain, including the relative positions of all atoms. It is the functional conformation of a protein. This complex 3D shape is stabilized by interactions between the side chains (R-groups) of amino acids that are often far apart in the linear sequence.
- **Types of Forces Responsible for Stabilization:**
    1. **Hydrophobic Interactions (Most Significant):**
        - **Nature:** These are not true "bonds" but rather a tendency for nonpolar (hydrophobic) amino acid side chains to

cluster together in the interior of a protein, away from the aqueous environment.

- **Mechanism:** When nonpolar groups are exposed to water, water molecules form an ordered "cage" around them, decreasing the entropy of the system. By clustering together, hydrophobic groups minimize their contact with water, reducing the ordered water molecules and increasing the overall entropy of the system (thermodynamically favorable).
- **Contribution:** These interactions are considered the primary driving force for protein folding and stabilization of the tertiary structure, particularly in globular proteins in aqueous environments.

2. **Hydrogen Bonds:**

- **Nature:** Electrostatic attractions between a hydrogen atom (covalently bonded to an electronegative atom like O or N) and another electronegative atom (O or N) that has a lone pair of electrons.
- **Mechanism:** These bonds can form between:
    - Polar side chains (e.g., between the -OH of Serine and the C=O of Glutamate).
    - Side chains and the polypeptide backbone (though less common for tertiary structure compared to secondary structure).
    - Side chains and surrounding water molecules (which can also influence folding).
- **Contribution:** Hydrogen bonds help fine-tune the precise three-dimensional arrangement and contribute significantly to the stability of the overall structure.

3. **Ionic Bonds (Salt Bridges):**
    - **Nature:** Electrostatic attractions between oppositely charged amino acid side chains.
    - **Mechanism:** These occur between acidic amino acid residues (e.g., Aspartate, Glutamate, which are deprotonated and negatively charged) and basic amino acid residues (e.g., Lysine, Arginine, Histidine, which are protonated and positively charged).
    - **Contribution:** Salt bridges contribute to the stability by providing specific, strong electrostatic linkages within the protein structure. Their strength is influenced by the surrounding dielectric environment.
4. **Disulfide Bonds (Covalent Bonds):**
    - **Nature:** These are strong **covalent bonds** formed by the oxidation of the sulfhydryl (-SH) groups of two cysteine residues.
    - **Mechanism:** The formation of a disulfide bond (S−S) occurs through an oxidation reaction, often in the endoplasmic reticulum for secreted or membrane proteins.
    - **Contribution:** Disulfide bonds are very stable and act as "staples" or "cross-links" that significantly reinforce the tertiary (and sometimes quaternary) structure, making the protein more resistant to denaturation. They are particularly important in extracellular proteins.
5. **Van der Waals Forces (Weak, but numerous):**
    - **Nature:** These are weak, short-range attractive or repulsive forces that arise from temporary fluctuations in electron distribution around atoms, creating transient dipoles. They include:

- **London Dispersion Forces:** Attractive forces between all atoms, arising from instantaneous dipoles.
- **Dipole-Dipole Interactions:** Attractive forces between permanent dipoles.
- **Hydrogen Bonding:** (Already discussed, but often considered a strong type of dipole-dipole interaction).

- **Mechanism:** These forces are individually very weak, but because there are a vast number of atoms in close proximity within a folded protein, the cumulative effect of these interactions can be substantial.
- **Contribution:** Van der Waals forces contribute to the overall packing efficiency and density of the protein core, allowing for a tight and stable 3D structure.

4. a) Discuss the different types of reversible enzyme inhibition with examples.

- **Enzyme Inhibition:** Enzyme inhibition refers to a process where molecules (inhibitors) bind to an enzyme and decrease its activity.
- **Reversible Inhibition:** In reversible inhibition, the inhibitor binds to the enzyme via non-covalent bonds (e.g., hydrogen bonds, ionic bonds, hydrophobic interactions) and can dissociate from the enzyme. The activity of the enzyme can be restored by removing the inhibitor.
- **Types of Reversible Enzyme Inhibition:**

1. **Competitive Inhibition:**

- **Mechanism:** The inhibitor (I) structurally resembles the substrate (S) and competes with the substrate for binding

to the enzyme's **active site**. The inhibitor binds to the free enzyme (E) to form an Enzyme-Inhibitor (EI) complex.

- E+SrightleftharpoonsESlongrightarrowE+P
- E+IrightleftharpoonsEI

- **Effect on Kinetics:**
  - **V_max (Maximum Reaction Rate):** Unchanged. If enough substrate is added, it can outcompete the inhibitor, eventually reaching the same V_max as an uninhibited reaction.
  - **K_m (Michaelis Constant):** Appears to increase. A higher concentration of substrate is required to achieve half V_max because the inhibitor reduces the effective substrate concentration available for binding.
- **Lineweaver-Burk Plot:** The lines for inhibited and uninhibited reactions intersect on the Y-axis.
- **Example:**
  - **Malonate** as a competitive inhibitor of **succinate dehydrogenase**. Malonate is structurally similar to succinate and competes for the active site, blocking the conversion of succinate to fumarate.

2. **Uncompetitive Inhibition:**
   - **Mechanism:** The inhibitor (I) binds only to the **enzyme-substrate (ES) complex**, not to the free enzyme. It binds at a site distinct from the active site.
     - E+SrightleftharpoonsES
     - ES+IrightleftharpoonsESI (inactive complex)
   - **Effect on Kinetics:**

- **V_max:** Decreases. The formation of the ESI complex removes ES complexes, reducing the amount of functional enzyme available to convert substrate to product, even at high substrate concentrations.
- **K_m:** Appears to decrease. This is because the inhibitor effectively "pulls" the ES complex out of the equilibrium, making the ES complex appear to form more readily (or dissociate less easily). However, this reduced K_m is not due to increased affinity but due to the trapping of ES.

- **Lineweaver-Burk Plot:** The lines for inhibited and uninhibited reactions are parallel.
- **Example:**
  - **Lithium** ions can act as uncompetitive inhibitors for certain enzymes involved in inositol phosphate metabolism.
  - **Glyphosate** (Roundup) is an uncompetitive inhibitor of EPSP synthase in plants.

3. **Non-Competitive Inhibition (Pure Non-Competitive):**
   - **Mechanism:** The inhibitor (I) binds to a site on the enzyme that is **distinct from the active site** (allosteric site). It can bind to both the free enzyme (E) and the enzyme-substrate (ES) complex. The binding of the inhibitor causes a conformational change that reduces the enzyme's catalytic efficiency (ability to convert substrate to product) but does not necessarily prevent substrate binding.
     - E+SrightleftharpoonsESlongrightarrowE+P
     - E+IrightleftharpoonsEI (inactive complex)

        - ES+IrightleftharpoonsESI (inactive complex)
    - **Effect on Kinetics:**
        - **V_max:** Decreases. The formation of inactive EI and ESI complexes reduces the overall concentration of functional enzyme, thus lowering the maximum rate.
        - **K_m:** Unchanged. The inhibitor does not affect the binding affinity of the substrate for the enzyme, as it binds to a separate site. The substrate can still bind to the enzyme, but once bound, the enzyme's catalytic efficiency is compromised.
    - **Lineweaver-Burk Plot:** The lines for inhibited and uninhibited reactions intersect on the X-axis.
    - **Example:**
        - Heavy metal ions (e.g., Pb2+, Hg2+) can act as non-competitive inhibitors by binding to sulfhydryl groups or other residues away from the active site, altering the enzyme's conformation.
        - **Doxycycline** can non-competitively inhibit collagenase.

(Note: Sometimes, "mixed inhibition" is also discussed, where the inhibitor binds to both E and ES, but affects both V_max and K_m in a way that is not purely non-competitive or uncompetitive. Pure non-competitive inhibition is a special case of mixed inhibition where K_m is unchanged.)

4. b) Explain different classes of enzymes with one example each.

- Enzymes are biological catalysts that are classified into six major classes by the International Union of Biochemistry and Molecular Biology (IUBMB) based on the type of reaction they catalyze.

    1. **Oxidoreductases:**

- **Function:** Catalyze oxidation-reduction (redox) reactions, involving the transfer of electrons, hydrogen atoms, or oxygen atoms.
- **Mechanism:** They mediate the transfer of electrons from one molecule (reductant) to another (oxidant).
- **Example: Lactate Dehydrogenase** (LDH)
  - Catalyzes the interconversion of lactate and pyruvate, with the concomitant interconversion of NADH and NAD^+.
  - Lactate+NAD+rightleftharpoonsPyruvate+NADH+H+

2. **Transferases:**

- **Function:** Catalyze the transfer of a functional group (e.g., methyl, amino, phosphate, acyl, glycosyl) from one molecule (the donor) to another (the acceptor).
- **Mechanism:** They break a bond in the donor and form a new bond with the acceptor, transferring the group.
- **Example: Hexokinase**
  - Catalyzes the transfer of a phosphate group from ATP to glucose, forming glucose-6-phosphate and ADP.
  - Glucose+ATPlongrightarrowGlucose−6−phosphate+ADP

3. **Hydrolases:**

- **Function:** Catalyze hydrolysis reactions, which involve the cleavage of a bond by the addition of water.
- **Mechanism:** They break bonds (e.g., ester, ether, peptide, glycosidic bonds) by inserting a water molecule.

- **Example: Lipase**
  - Catalyzes the hydrolysis of fats (triglycerides) into fatty acids and glycerol.
  - Triglyceride+3H_2OlongrightarrowGlycerol+3FattyAcids

4. **Lyases:**
   - **Function:** Catalyze the cleavage of C-C, C-O, C-N, or other bonds by elimination reactions, producing double bonds or rings, or conversely, adding groups to double bonds. They do not involve hydrolysis or oxidation-reduction.
   - **Mechanism:** They break bonds without the involvement of water as a reactant or by redox.
   - **Example: Fumarase**
     - Catalyzes the reversible addition of water to fumarate, forming malate.
     - Fumarate+H_2OrightleftharpoonsMalate
5. **Isomerases:**
   - **Function:** Catalyze the rearrangement of atoms within a molecule, resulting in an isomer.
   - **Mechanism:** They interconvert isomeric forms (e.g., enantiomers, diastereomers, geometric isomers) by intramolecular rearrangements.
   - **Example: Phosphoglucose Isomerase**
     - Catalyzes the reversible isomerization of glucose-6-phosphate to fructose-6-phosphate in glycolysis.

- Glucose−6−phosphaterightleftharpoonsFructose−6−phosphate

6. **Ligases:**
    - **Function:** Catalyze the joining of two molecules (ligation) to form a larger molecule, typically coupled with the hydrolysis of a high-energy phosphate bond from ATP or another nucleoside triphosphate.
    - **Mechanism:** They form new bonds (e.g., C-C, C-S, C-O, C-N) using energy supplied by ATP hydrolysis.
    - **Example: DNA Ligase**
        - Catalyzes the joining of two DNA fragments by forming a phosphodiester bond, using ATP as an energy source.
        - DNA_fragment1+DNA_fragment2+ATPlongrightarrowDNA_joined+AMP+PPi

4. c) What do you understand by K_m in an enzymatic reaction? Discuss its significance.

- **What is K_m (Michaelis Constant)?**
    - In enzyme kinetics, K_m (Michaelis constant) is a fundamental parameter that arises from the Michaelis-Menten model for enzyme-catalyzed reactions.
    - **Definition:** K_m is defined as the **substrate concentration at which the reaction velocity (V) is half of the maximum velocity (V_max)**.
    - **Equation:** From the Michaelis-Menten equation, V=fracV_max[S]K_m+[S], when V=V_max/2, then K_m=[S].
    - **Units:** K_m has units of concentration (e.g., M, mM, muM) because it is a substrate concentration.

- **Significance of K_m:**

    1. **Measure of Enzyme-Substrate Affinity (Inverse Relationship):**

        - **Inverse Relationship:** K_m is often interpreted as an inverse measure of the affinity of an enzyme for its substrate.

            - **Low K_m:** Indicates a high affinity of the enzyme for its substrate. The enzyme can achieve half its maximum velocity at a very low substrate concentration, meaning it binds the substrate tightly.

            - **High K_m:** Indicates a low affinity of the enzyme for its substrate. A much higher substrate concentration is required to reach half V_max, meaning the enzyme binds the substrate less tightly.

        - **Note:** While K_m is often approximated as the dissociation constant (K_d) of the ES complex (K_m=frack_−1+k_2k_1, and if k_2llk_−1, then K_mapproxK_d=k_−1/k_1), it is strictly accurate only if the breakdown of the ES complex to product (k_2) is much slower than its dissociation back to E and S (k_−1).

    2. **Indicator of Enzyme Efficiency under Physiological Conditions:**

        - K_m values provide insight into how well an enzyme functions under typical cellular substrate concentrations.

        - If the physiological substrate concentration is around the K_m value, the enzyme's activity will be sensitive to changes in substrate concentration, allowing for metabolic regulation.

- Enzymes with low K_m are efficient at low substrate concentrations, while those with high K_m may require higher substrate levels to operate effectively.

3. **Comparison of Enzymes for the Same Substrate:**
    - Different enzymes that catalyze the same reaction (isozymes) often have different K_m values for the same substrate. This can reflect their specific roles in different tissues or under different metabolic conditions.
    - For example, Hexokinase (low K_m for glucose, active even at low glucose levels) and Glucokinase (high K_m for glucose, active only when glucose levels are high, e.g., after a meal) are both involved in glucose phosphorylation but have distinct roles due to their different K_m values.
4. **Related to Reaction Velocity and Saturation:**
    - When [S]llK_m, the reaction rate is approximately linear with substrate concentration (Vapprox(V_max/K_m)[S]). The enzyme is operating far below its capacity.
    - When [S]=K_m, the reaction rate is half V_max.
    - When [S]ggK_m, the enzyme is saturated with substrate, and the reaction rate approaches V_max, becoming independent of substrate concentration (VapproxV_max).
5. **Diagnostic Tool:**
    - Changes in K_m values can be used to diagnose enzyme deficiencies or genetic mutations that affect enzyme function.
    - In drug discovery, a low K_m for an inhibitor means it binds strongly, which is often desirable for effective drug action.

5. a) Write the structures showing the hydrogen bonding between the following nucleotide base pairs:
    - i. Thymine and Adenine
    - ii Guanine and Cytosine

- **Hydrogen Bonding in DNA Base Pairs:** In DNA, complementary nitrogenous bases pair specifically through hydrogen bonds, forming the rungs of the double helix ladder.
    - **i. Thymine (T) and Adenine (A):**
        - **Two hydrogen bonds** form between Adenine and Thymine.
        - **Adenine (Purine):**
            - N1 (nitrogen at position 1) acts as a hydrogen bond acceptor.
            - N6 (NH_2 group) acts as a hydrogen bond donor.
        - **Thymine (Pyrimidine):**
            - O2 (carbonyl oxygen at position 2) acts as a hydrogen bond acceptor.
            - N3-H (nitrogen-hydrogen at position 3) acts as a hydrogen bond donor.

(Conceptual Diagram):

```
 N=C-NH2  <--- (H bond donor)
 |   ||
 C   C--N   (Adenine)
//  \ / \
N----C----N
```

```
   |   |
   (sugar)
         ..
         :
(sugar)   :   O=C-CH3  <--- (H bond acceptor)
   |    :   ||
   N----C----NH  <--- (H bond donor)
   |   // \ /
   C---N---C
     ||  :
     O   :
     ...
(Thymine)
```


Simplified Representation:

```
  Adenine          Thymine
(N1) N ---- H --- N (N3)
               ||
(N6) H-N ---- O=C (O4)
```

(Actual drawing would show the full rings and connections to deoxyribose sugars at N9 for Adenine and N1 for Thymine, with the hydrogen bonds explicitly drawn as dashed lines.)

- **ii. Guanine (G) and Cytosine (C):**
  - **Three hydrogen bonds** form between Guanine and Cytosine.

- **Guanine (Purine):**
  - N1-H (nitrogen-hydrogen at position 1) acts as a hydrogen bond donor.
  - O6 (carbonyl oxygen at position 6) acts as a hydrogen bond acceptor.
  - N2-H (NH_2 group) acts as a hydrogen bond donor.
- **Cytosine (Pyrimidine):**
  - N3 (nitrogen at position 3) acts as a hydrogen bond acceptor.
  - O2 (carbonyl oxygen at position 2) acts as a hydrogen bond acceptor.
  - N4-H (NH_2 group) acts as a hydrogen bond donor.

(Conceptual Diagram):

```
    O=C-NH2  <--- (H bond donor)
    |   ||
    N   N--C   (Cytosine)
   // \ / \
  C----C----N
  |    |
  (sugar)
        ..
        :
(sugar)   :   O=C--NH  <--- (H bond donor)
  |    :   ||   |
```

N----C----N---C

|| / \ :

NH2---C :

|| :

N...

(Guanine)

Simplified Representation:

Guanine Cytosine

(N1) N ---- H --- N (N3)

||

(O6) O=C ---- H-N (N4)

||

(N2) H-N ---- O=C (O2)

(Actual drawing would show the full rings and connections to deoxyribose sugars at N9 for Guanine and N1 for Cytosine, with the hydrogen bonds explicitly drawn as dashed lines.)

5. b) Write short note on the types of RNA and their biological functions.

- **Types of RNA and Their Biological Functions:** RNA (Ribonucleic Acid) is a versatile nucleic acid involved in various aspects of gene expression and regulation. Unlike DNA, RNA is typically single-stranded, contains ribose sugar (instead of deoxyribose), and has uracil (U) instead of thymine (T). There are several major types of RNA, each with distinct structures and functions:

    1. **Messenger RNA (mRNA):**

- **Structure:** Linear, single-stranded RNA molecule that carries genetic information from DNA in the nucleus to the ribosomes in the cytoplasm. It contains codons (sequences of three nucleotides) that specify the amino acid sequence of a protein.
- **Function:** Acts as a template for protein synthesis (translation). It dictates the specific sequence of amino acids to be incorporated into a polypeptide chain.

2. **Ribosomal RNA (rRNA):**

- **Structure:** Highly abundant (up to 80% of total RNA), structurally complex RNA molecules that form an integral part of ribosomes, the cellular machinery for protein synthesis. rRNA molecules fold into intricate three-dimensional structures and associate with ribosomal proteins. They are catalytic (ribozyme activity).
- **Function:**
  - Forms the structural and catalytic core of ribosomes.
  - Catalyzes the formation of peptide bonds between amino acids during protein synthesis (peptidyl transferase activity).
  - Plays a crucial role in binding mRNA and tRNA during translation.

3. **Transfer RNA (tRNA):**

- **Structure:** Relatively small (around 70-95 nucleotides), single-stranded RNA molecules that fold into a characteristic cloverleaf secondary structure, which then further folds into an L-shaped tertiary structure. Each tRNA molecule has an anticodon loop (which pairs with a

specific mRNA codon) and an acceptor stem (to which a specific amino acid is attached).

- **Function:** Acts as an adapter molecule that decodes the genetic information on mRNA into the amino acid sequence of a protein. Each tRNA carries a specific amino acid to the ribosome according to the mRNA codon.

4. **Small Nuclear RNA (snRNA):**
    - **Structure:** Small RNA molecules (around 100-200 nucleotides) found in the nucleus. They associate with specific proteins to form small nuclear ribonucleoproteins (snRNPs).
    - **Function:** Involved in the processing of pre-mRNA (splicing) in eukaryotes. They participate in the spliceosome complex, which removes introns (non-coding regions) and ligates exons (coding regions) to form mature mRNA.
5. **MicroRNA (miRNA):**
    - **Structure:** Very small (around 20-22 nucleotides), non-coding RNA molecules that are initially transcribed as longer precursors and then processed into mature forms.
    - **Function:** Plays a crucial role in **post-transcriptional gene regulation**. They typically bind to complementary sequences in target mRNA molecules, leading to either degradation of the mRNA or inhibition of its translation, thereby regulating gene expression.
6. **Small Interfering RNA (siRNA):**
    - **Structure:** Similar in size to miRNA (around 20-25 nucleotides), but typically originate from longer double-stranded RNA molecules.

- **Function:** Involved in **RNA interference (RNAi)**, a gene silencing mechanism. They guide the RNA-induced silencing complex (RISC) to target mRNA molecules for degradation, preventing protein synthesis. siRNA is often used experimentally to "knock down" gene expression.

(Other types of RNA exist, such as long non-coding RNAs (lncRNAs), circular RNAs (circRNAs), etc., which have diverse regulatory roles, but the above are the major well-established types.)

5. c) Discuss the different steps involved in DNA Replication.

- **DNA Replication:** DNA replication is the biological process of producing two identical replicas of DNA from one original DNA molecule. It is a semi-conservative process, meaning each new DNA molecule consists of one original (parental) strand and one newly synthesized (daughter) strand.
- **Steps Involved:**

1. **Initiation:**
    - **Origin of Replication (Ori):** Replication begins at specific DNA sequences called origins of replication (Ori). Prokaryotes typically have one origin, while eukaryotes have multiple origins on each chromosome.
    - **Binding of Initiator Proteins:** Initiator proteins (e.g., DnaA in E. coli, Origin Recognition Complex (ORC) in eukaryotes) bind to the Ori.
    - **Unwinding (Helicase Activity):** These proteins recruit DNA helicase (e.g., DnaB in E. coli), an enzyme that uses ATP hydrolysis to unwind the DNA double helix, breaking the hydrogen bonds between complementary base pairs. This creates two single-stranded DNA templates and forms a replication bubble with two replication forks moving in opposite directions.

- **Single-Strand Binding Proteins (SSBs):** Single-strand binding proteins (SSBs) bind to the separated single DNA strands to prevent them from re-annealing and to protect them from degradation.
- **Topoisomerases (Gyrase):** As DNA unwinds, supercoiling can build up ahead of the replication fork. Topoisomerases (e.g., DNA gyrase, a type II topoisomerase in bacteria) relieve this supercoiling by making temporary nicks in the DNA strands, allowing the strands to rotate, and then re-sealing them.

2. **Elongation:**

- **RNA Primer Synthesis (Primase):** DNA polymerase cannot initiate DNA synthesis de novo; it requires a free 3'-OH group. An enzyme called **primase** (a type of RNA polymerase) synthesizes short RNA primers (5-10 nucleotides long) that are complementary to the DNA template strands at the origin of replication and at intervals along the lagging strand.
- **DNA Synthesis by DNA Polymerase:** DNA polymerase (e.g., DNA Pol III in prokaryotes, DNA Pol delta and epsilon in eukaryotes) adds deoxyribonucleotides (dNTPs) one by one to the 3'-hydroxyl end of the RNA primer, synthesizing new DNA strands.
    - **Directionality:** DNA synthesis always proceeds in the 5' to 3' direction.
    - **Leading Strand Synthesis:** On one template strand (the **leading strand**, oriented 3' to 5' relative to the incoming polymerase), DNA synthesis occurs continuously in the 5' to 3' direction, moving towards the replication fork. Only one primer is needed for the leading strand.

        - **Lagging Strand Synthesis:** On the other template strand (the **lagging strand**, oriented 5' to 3'), DNA synthesis occurs discontinuously, moving away from the replication fork. This strand is synthesized in short fragments called **Okazaki fragments**. Each Okazaki fragment requires a new RNA primer.

    - **Removal of RNA Primers (DNA Polymerase I/RNase H):** Once an Okazaki fragment is synthesized, the RNA primers are removed. In prokaryotes, DNA Polymerase I uses its 5' to 3' exonuclease activity to remove the RNA primer and replace it with DNA. In eukaryotes, RNase H and FEN1 are involved.

    - **Ligation of Okazaki Fragments (DNA Ligase):** After primer removal and DNA fill-in, small nicks remain between adjacent Okazaki fragments (and between the leading strand DNA and the first Okazaki fragment). **DNA ligase** forms phosphodiester bonds to join these fragments, creating a continuous DNA strand.

3. **Termination:**

    - **Prokaryotes:** In circular bacterial chromosomes, replication forks meet at a specific termination site (Ter sites). Termination proteins (e.g., Tus proteins) bind to these sites, blocking further movement of the replication forks. The two circular DNA molecules are then separated (decatenated) by topoisomerases.

    - **Eukaryotes:** In linear eukaryotic chromosomes, replication forks meet and fuse. The main challenge at termination in eukaryotes is the replication of telomeres (the ends of chromosomes).

        - **Telomeres and Telomerase:** Due to the lagging strand synthesis mechanism, a small portion of

DNA at the 5' end of the newly synthesized lagging strand cannot be fully replicated after the last RNA primer is removed. This would lead to progressive shortening of chromosomes with each replication cycle. **Telomerase**, a specialized reverse transcriptase enzyme, extends the 3' end of the parental strand using an RNA template within itself, thus preventing the loss of genetic information from the ends of chromosomes.

6. Write down short notes on any three of the following:
    - a) Electrophoresis
    - b) Ninhydrin test
    - c) Genetic code
    - d) Factors affecting the enzyme activity

- **a) Electrophoresis:**
    - **Definition:** Electrophoresis is a widely used laboratory technique that separates charged molecules (such as DNA, RNA, proteins, and amino acids) based on their size, shape, and electric charge when subjected to an electric field.
    - **Principle:** Molecules with a net charge will migrate through a matrix (gel or solution) towards the electrode of opposite charge.
        - **Rate of Migration:** The rate of migration depends on:
            - **Net Charge:** Higher charge leads to faster migration.
            - **Size/Mass:** Smaller molecules generally migrate faster than larger ones (due to less frictional resistance from the matrix).

        - **Shape:** Compact, globular molecules move faster than elongated, flexible ones of the same mass.
        - **Electric Field Strength:** Higher voltage leads to faster migration.
        - **Properties of the Medium (Gel):** The porosity of the gel matrix (e.g., agarose for DNA, polyacrylamide for proteins) influences the sieving effect, affecting separation by size.
        - **Buffer pH and Ionic Strength:** Affect the charge of the molecules and the conductivity of the buffer.
    - **Components of an Electrophoresis System:**
        - **Power Supply:** Provides the electric field.
        - **Electrophoresis Chamber:** Contains the gel and buffer.
        - **Gel Matrix:** A porous medium (e.g., agarose gel for nucleic acids, polyacrylamide gel for proteins).
        - **Buffer Solution:** Maintains pH and provides conductivity.
        - **Samples:** Loaded into wells in the gel.
        - **Detection:** Staining (e.g., ethidium bromide for DNA, Coomassie blue for proteins) or other detection methods (e.g., fluorescent labels, radioactivity) are used to visualize the separated molecules.
    - **Applications:**
        - **Separation of nucleic acids:** DNA sequencing, fragment analysis, DNA fingerprinting.
        - **Separation of proteins:** Protein purity assessment, Western blotting, proteomics.

        - **Clinical diagnostics:** Detection of abnormal proteins (e.g., in multiple myeloma), genetic disease screening.
        - **Research:** Studying molecular interactions, enzyme activity, etc.

- **b) Ninhydrin test:**
    - **Definition:** The Ninhydrin test is a chemical test used to detect the presence of alpha-amino acids, peptides, and proteins, and to quantify amino acids. It produces a characteristic deep blue-purple color in the presence of most amino acids.
    - **Principle:** Ninhydrin (2,2-dihydroxyindane-1,3-dione) is a strong oxidizing agent. It reacts with the free alpha-amino group of amino acids (and peptides/proteins) to cause oxidative deamination and decarboxylation.
    - **Reactions Involved:**
        1. **Reaction with Ninhydrin:** Amino acid reacts with ninhydrin, producing an aldehyde (with one less carbon than the original amino acid), carbon dioxide (CO_2), and ammonia (NH_3). A reduced form of ninhydrin (hydrindantin) is also formed.
            - Amino Acid + Ninhydrin $\longrightarrow$ Aldehyde + CO_2 + NH_3 + Reduced Ninhydrin (Hydrindantin)
        2. **Color Formation:** The ammonia and the reduced ninhydrin then react with another molecule of ninhydrin to form a highly colored, intensely purple complex called **Ruhemann's Purple** (diketohydrindylidene-diketohydrindamine).
            - NH_3 + 2 Ninhydrin (one reduced, one oxidized) $\longrightarrow$ Ruhemann's Purple + $3H_2O$
    - **Color Specificity:**

        - Most alpha-amino acids, peptides, and proteins give a deep blue-purple color.
        - **Proline** and **Hydroxyproline** (imino acids with a secondary amine) react differently, forming a yellow-orange product instead of purple, because they do not have a primary alpha-amino group to produce ammonia in the first step.
    - **Applications:**
        - **Qualitative Detection:** Rapid detection of amino acids and peptides in solutions (e.g., in paper chromatography, thin-layer chromatography).
        - **Quantitative Estimation:** The intensity of the purple color (measured spectrophotometrically) is proportional to the concentration of the amino acid, allowing for quantitative analysis.
        - **Forensic Science:** Used to detect latent fingerprints (amino acids in sweat react with ninhydrin).

- **c) Genetic code:**
    - **Definition:** The genetic code is a set of rules by which information encoded in genetic material (DNA or RNA sequences) is translated into proteins by living cells. It defines the correspondence between sequences of three nucleotides (codons) and specific amino acids.
    - **Key Characteristics:**
        1. **Triplet Code:** Each amino acid is specified by a sequence of three consecutive nucleotides called a **codon**. There are 4 possible nucleotides (A, U, G, C in RNA, or A, T, G, C in DNA), so there are $4^3=64$ possible codons.

2. **Degenerate (Redundant):** Most amino acids are specified by more than one codon. For example, six different codons specify leucine. This redundancy provides some protection against point mutations.
3. **Non-overlapping:** Codons are read sequentially, one after another, without any overlap. Each nucleotide is part of only one codon.
4. **Comma-less:** There are no intervening nucleotides or "commas" between codons. The codons are read continuously from a specific starting point.
5. **Universal (Nearly):** The genetic code is almost universal, meaning the same codons specify the same amino acids in nearly all organisms, from bacteria to humans. This universality suggests a common evolutionary origin for all life. However, there are a few minor variations found in mitochondria and some microorganisms.
6. **Start Codon:** AUG is the most common start codon, which specifies Methionine (Met) and also signals the beginning of translation.
7. **Stop Codons (Nonsense Codons):** There are three stop codons (UAA, UAG, UGA) that do not code for any amino acid. They signal the termination of translation.

- **Mechanism in Protein Synthesis:**
  - During translation, messenger RNA (mRNA) carries the genetic code from DNA to the ribosomes.
  - Transfer RNA (tRNA) molecules act as adapters, each carrying a specific amino acid and possessing an anticodon that is complementary to an mRNA codon.
  - At the ribosome, the tRNA anticodon base-pairs with the mRNA codon, ensuring the correct amino acid is added to

the growing polypeptide chain according to the sequence specified by the mRNA.

- **Significance:** The genetic code is fundamental to molecular biology as it represents the central dogma of molecular biology, explaining how genetic information flows from DNA to RNA to protein, dictating the structure and function of all living organisms.

- **d) Factors affecting the enzyme activity:**
  - Enzyme activity is highly sensitive to its environment, and various physical and chemical factors can significantly influence its rate of catalysis. Deviations from optimal conditions can lead to reduced activity or even irreversible denaturation.

  1. **Temperature:**
     - **Effect:**
       - **Below Optimum:** As temperature increases, the kinetic energy of reactant molecules and the enzyme increases, leading to more frequent collisions and a faster reaction rate.
       - **Optimum Temperature:** Each enzyme has an optimal temperature at which it exhibits maximum activity. For most human enzymes, this is around 37°C.
       - **Above Optimum:** Beyond the optimum temperature, the increased thermal energy becomes too disruptive. It causes the weakening and breaking of the weak non-covalent bonds (hydrogen bonds, hydrophobic interactions, ionic bonds) that maintain the enzyme's specific three-dimensional structure (tertiary and secondary structures). This process is called **denaturation**.

Denaturation leads to a loss of the active site's shape, rendering the enzyme inactive.

- **Reversibility:** Mild denaturation can sometimes be reversible, but severe or prolonged heating causes irreversible denaturation.

2. **pH:**
    - **Effect:**
        - **Optimum pH:** Each enzyme has an optimal pH range at which it exhibits maximum activity. This optimum pH is related to the ionization states of the amino acid residues in the active site and throughout the protein, which are critical for maintaining the enzyme's specific conformation and its ability to bind substrate and catalyze the reaction.
        - **Above or Below Optimum:** Extreme pH values (too acidic or too alkaline) cause changes in the ionization state of amino acid side chains. This disrupts the ionic bonds and hydrogen bonds crucial for maintaining the enzyme's active site conformation and overall tertiary structure, leading to denaturation and loss of activity.
    - **Examples:** Pepsin (stomach enzyme) has an optimum pH of around 1.5-2.0, while trypsin (intestinal enzyme) has an optimum pH of about 8.0.
3. **Substrate Concentration:**
    - **Effect:**
        - **Low [S]:** At low substrate concentrations, the reaction rate is directly proportional to [S]. There are plenty of enzyme active sites available, and

increasing [S] leads to more frequent enzyme-substrate collisions and ES complex formation.

- **Increasing [S]:** As [S] increases, the reaction rate increases but begins to level off as more and more active sites become occupied.
- **High [S] (Saturation):** At very high substrate concentrations, all enzyme active sites are saturated with substrate molecules. The enzyme is working at its maximum velocity (V_max), and further increases in [S] will not increase the reaction rate. The rate is then limited by the enzyme's turnover number.

4. **Enzyme Concentration:**
   - **Effect:** As long as the substrate is not limiting, the reaction rate is directly proportional to the enzyme concentration. More enzyme molecules mean more active sites are available to bind and convert substrate, leading to a faster overall reaction.
5. **Presence of Inhibitors/Activators:**
   - **Inhibitors:** Molecules that decrease enzyme activity. They can be reversible (competitive, uncompetitive, non-competitive) or irreversible (covalently modify the enzyme).
   - **Activators:** Molecules that increase enzyme activity. They often bind to allosteric sites, causing conformational changes that enhance substrate binding or catalytic efficiency.
6. **Cofactors/Coenzymes:**
   - **Effect:** Many enzymes require non-protein components called cofactors (e.g., metal ions like Mg2+, Zn2+) or

coenzymes (e.g., NAD^+, FAD, vitamins) for their activity. The absence or insufficient concentration of these cofactors/coenzymes will reduce or abolish enzyme activity.