ORGANIC CHEMISTRY

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Organic Chemistry

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Module 1. Hydrogen bonding and hydrophobic interactions

Lesson 1
CONCEPTS OF HYDROGEN BONDING

1.1 Introduction

- Covalent bond between two atoms - formed by sharing of electrons present in outer most valence shells of bonding atoms.
- Covalent bond is formed between two similar or dissimilar atoms.
  - The shared electron pair is attracted equally by both the atoms
  - Therefore, the pair of electrons lies exactly midway between the two bonded atoms.
  - The bond is not polarized – non-polar covalent bond between the identical atoms.
  - Example: molecules are H₂, F₂, O₂, N₂, etc.

![Figure 1.1 Homonuclear diatomic molecules](image)

- In case of covalent bond formed between two dissimilar atoms
  - Generally, one of the atoms has a higher affinity for the shared/bonded electrons.
  - Therefore, the shared electron pair is pulled closer to that atom (atom of high electronegativity elements → F, O, N). It is a polarized bond.
  - Examples → HF, H₂O, NH₃

![Figure 1.2 Heteronuclear molecules](image)

- Such asymmetric distribution of bonded electrons leads development of partial charge on atoms of the molecule called polar covalent bond represented in Fig 1.3.
  - The atoms of electronegative element (F, O, N) acquire partial negative charge.
  - The hydrogen atom acquires partial positive charge denoted as δ⁺.

![Figure 1.3 Polar covalent bond](image)
• The partial positive charge on hydrogen atom in such molecules will be attracted electrostatically by the partial negative charge on atom of the electronegative element in other molecule of such compound.

(Figure 1.4 Intermolecular hydrogen bonding)

• The electrostatic attraction between hydrogen atom of one molecule and electronegative atom of another molecule (generally of the same substance) is known as hydrogen bond/bonding.
• Hydrogen bond is represented by a dotted line (.....).
• It is a weak secondary bond with low bond energy and purely electrostatic in nature.
• It acts as a bridge between two electronegative atoms of the molecules via hydrogen atom.
• Organic compounds such as given below also form hydrogen bond

Alcohols : R-OH
Phenols : Ar-OH
Carboxylic acids : R-COOH
Amines : R-NH₂ (Primary) and R₂-NH (Secondary)
Amides : R-CNH₂

(Figure 1.5 Hydrogen bonding in organic molecules)
• Molecules of water and primary amines have two hydrogen atoms; therefore involves three hydrogen bonding per molecule.
• Molecule of other compounds have only one hydrogen atom; therefore involves two hydrogen bonding per molecule.
• Amongst the examples given above
  • In carboxylic acids, the hydrogen bonding is limited to the association of two molecules only.
  • In other compounds, the hydrogen bonding may extend to several molecules—association of several molecules.
• Hydrogen bonding may occur between molecules of different substances also—a common example is formation of hydrogen bond between molecule of water and molecule of compounds referred above (alcohol, amine, phenol etc.).

(Figure 1.6 Solubility on the basis of hydrogen bonding)

• Even compounds like lower aldehydes and ketones, which do not form hydrogen bond between their own molecules, they do form this bond with molecule of water.
• Therefore low molecular weight aldehydes (formaldehyde and acetaldehyde) and ketone (acetone) are soluble in water because of hydrogen bonding.

(Figure 1.7 Solubility of lower aldehydes and ketones on the basis of hydrogen bonding)

The extent of hydrogen bonding in alcohols increases as R/OH ratio increases so higher alcohol are insoluble in water and waxy solids.

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Lesson 2
INTER AND INTRA MOLECULAR HYDROGEN BONDING IN ALCOHOLS, CARBOXYLIC ACIDS AND OTHER MOLECULES AND THEIR SIGNIFICANCE

2.1 Introduction

- When hydrogen bonding is formed between atoms of different molecules, it is called intermolecular hydrogen bonding.
- All the examples given so far in Lesson 1 are of intermolecular hydrogen bonding (HF, H₂O etc).
- Intermolecular- between different molecules.
- When hydrogen bonding is formed between atoms of same molecule, it is called intramolecular hydrogen bonding.
- Intramolecular- within the molecule
- Examples are given below

![Figure 2.1 Intramolecular hydrogen bonding](image)

- Intramolecular hydrogen bonding forms when –O-H group and electronegative element atom in the same molecule are present close together in such a position that a ring can form without disturbing the normal bond angles.
- The new five- or six- membered rings formed above as a result of intramolecular hydrogen bonding are known as chelate rings and such compounds are often referred to as chelate compounds (phenomenon as chelation).

2.2 Conditions For Hydrogen Bonding

- An effective hydrogen bond will form only when hydrogen atom is covalently attached to an atom which is
  - Strongly electronegative and
  - Small in size
- When the electronegativity is not high or if that atom has a large atomic radius
  - The electrostatic forces of attraction will be weak and
  - As a result hydrogen bond will not be very effective
Fluorine, oxygen and nitrogen are the only three elements
• Which have sufficiently high electronegativity and
• Are small enough to form effective hydrogen bonds

Chlorine has electronegativity comparable to that of nitrogen, still it does not form effective hydrogen bonding due to its relatively larger size
Bromine and iodine are not as highly electronegative as required for hydrogen bonding.

2.3 Strength of Hydrogen Bonding

• Being electrostatic in nature, they are much weaker than the covalent bonds
• The strength of a hydrogen bonding is of the order of
  • 2 to 10 kcal/mole or
  • 10 to 40 kJ/mole (i.e. per $6.022 \times 10^{23}$ bonds)
• The strength of a normal covalent bond is of the order of
  • 50-100 kcal/mole
• Greater the electronegativity and smaller the size of the electronegative atom, stronger is the hydrogen bond
• Therefore, hydrogen bonding involving F, O or N atoms have strengths of 10, 7 and 2 kcal/mole respectively.

2.4 Effects of Hydrogen Bonding on Physical Properties

• The existence of hydrogen bonding in molecules has a marked effect on their physical properties such as melting and boiling points, solubility, spectral characteristics, density etc.

2.4.1 Effect on melting and boiling points

• Ordinarily, compounds with similar molecular weights have similar melting/boiling points and there is regular increase in these physical constants with rising molecular weights.
• Compounds containing intermolecular hydrogen bonding show unusually high melting/boiling points.
• This is evident from boiling points of hydrogen compounds with elements of group V, VI, VII-shown in the Table below

| Table 2.1 Boiling point of hydrogen bonded compounds |
In the series of hydrogen compounds given below, all the compounds have almost similar mol. wt., but there is a wide difference in their boiling points.

Table 2.2

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<th>Structure</th>
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<th>B.P. (°C)</th>
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<td>n-Butane</td>
<td>CH₃-CH₂-CCH₃</td>
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<td>0</td>
</tr>
<tr>
<td>2</td>
<td>n-Propylamine</td>
<td>CH₃-C₂H₄-NH₂</td>
<td>59</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>n-Propylalcohol</td>
<td>CH₃-C₂H₅-C₂H₅</td>
<td>60</td>
<td>98</td>
</tr>
<tr>
<td>4</td>
<td>Acetic acid</td>
<td>CH₂-C(OH)</td>
<td>60</td>
<td>118</td>
</tr>
<tr>
<td>5</td>
<td>Ethylene glycol</td>
<td>CH₂-C₂H₅-C₂H₅-OH</td>
<td>62</td>
<td>197</td>
</tr>
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</table>

- n-Butane contains only hydrogen and carbon lacks protonic hydrogen therefore non-polar covalent bond- no hydrogen bond formation- no molecular association- lower boiling point.
- Other compounds contain strongly polar covalent bond (N-H or O-H).
- Leads formation of intermolecular hydrogen bonding causes large molecular association - therefore much more energy would be needed to break them apart - therefore higher boiling points.
- The effect of intramolecular hydrogen bonding on melting/boiling points is opposite to that observed in the case of intermolecular hydrogen bonding
- Example = o-Nitrophenol (m.p. 440°C) (steam volatile) and p-Nitrophenol (m.p. 1140°C) (non-volatile in steam)
Figure 2.2 Intra- and Inter- molecular hydrogen bonding

- In ortho derivatives presence of intramolecular hydrogen bonding prevents formation of intermolecular hydrogen bonding- therefore intermolecular association does not takes place- therefore they have lower melting point and higher volatility.
- In para derivatives intermolecular hydrogen bonding formation takes places- leads to intermolecular association resulting into an increase in melting point and lower volatility.
- Another example: cis- and trans isomers of HOOC-CH=CH-COOH

Figure 2.3 Melting points of Maleic acid and Fumaric acid on the basis of hydrogen bonding

2.4.2 Effect on solubility

- Compounds which can form intermolecular hydrogen bonds with a solvent (water) would be generally soluble in that solvent
- Compounds in which hydrogen bonding with solvent (water) molecules is restricted (prevented) due to intramolecular hydrogen bonding would be less soluble or insoluble in that solvent
- Examples
  - Hydroxy compounds like methyl alcohol, ethyl alcohol, sugars, etc are highly soluble in water due to formation of hydrogen bonds between molecules of these compounds and molecules of water
  - In o-nitrophenol, - OH group is not available for hydrogen bonding with water- therefore it is less soluble in water. Whereas, in p-nitrophenol – OH group is available for hydrogen bonding with water - therefore it is more soluble in water

2.4.3 Stability of crystal structure

- Intramolecular hydrogen bonding in maleic acid halves its ability to from intermolecular bonds
- In fumaric acid all hydrogen bonds are intermolecular = therefore it gives stronger, interlinked crystal structure

2.4.4 Effect on spectral characteristics

- Spectral characteristic of compounds are significantly affected if these compounds contain hydrogen bonds- examples.
• An Infra-red (IR) spectral study of free –OH group at 3500 cm$^{-1}$ is shifted to lower frequencies if the –OH group involves hydrogen bonding -around 3200 cm$^{-1}$ in alcohol and around 3000 cm$^{-1}$ in carboxylic acids
• Much higher shift in the acids compared to alcohols- suggests that H bonds in the acids are stronger than that in alcohols.

2.4.5 Unusual behaviors of water

• Two unusual behaviors of water

  1. Lower density in solid state (ice) than that in liquid state - therefore ice floats over water – unusual – because density of solid is lower
  2. Water contract when heated between 0°C and 4°C - volume is minimum at 4°C - therefore its density is maximum at 4°C - unusual= substances expand when heated in all temperature ranges

• Both these peculiar features of water are attributed to formation of hydrogen bonding by water molecules

![Figure 2.4 The tetrahedral hydrogen bonded cage-like structure of ice](image)

• Hydrogen atoms
• Central oxygen atom
• Four oxygen atoms surrounding the central hydrogen atom
• Hydrogen bonding between water molecules is more extensive in ice than in liquid water.
• Because a substance in solid state has a definite structure and the molecules are more rigidly fixed relative to one another than in liquid state.
• In ice, the water molecules are tetrahedrally oriented with respect to one another
• Each oxygen atom is surrounded tetrahedrally by four hydrogen atoms, two of these are bonded covalently and the other two by hydrogen bonds
• The hydrogen bonds are weaker- therefore longer than the covalent bonds- this arrangement gives rise to an open cage-like structure – as indicated in the figure given above.
• There are number of holes or open spaces – because the hydrogen bonds holding the water molecules in ice are directed in definite angles
• In liquid water such hydrogen bonds are fewer in number
• As a result, when ice melts a large number of hydrogen bonds are broken and molecules move into the ‘holes’ or ‘open space’ - therefore come closer to one another than they were in solid state.
As liquid water is heated from 0 to 4\(^{0}\)C, hydrogen bonds continue to be broken and the molecules come closer and closer together.

- This leads to contraction – decrease in volume - increase in density.
- On rise of temperature from 0 to 4\(^{0}\)C some expansion takes place, but contraction effect predominates - therefore there is net contraction.
- Above 4\(^{0}\)C - expansion effect predominates – rise in volume (d=m/v). So water has maximum density at 4\(^{0}\)C since v (volume) is minimum at 4\(^{0}\)C.

### 2.5 Importance of Hydrogen Bonding

- Phenomenon of hydrogen bonding formation bears a great significance in various aspects
  - Retention of water on the earth in large amount
    - Without hydrogen bonding, water would have existed as a gas like hydrogen sulphide
    - In that case, no life would have been possible on this globe
  - Determines and maintains structure of various proteins which are so essential for life
  - Makes wood fibers more rigid and thus makes it an article of great utility to meet requirements of housing, furniture etc
  - Provides rigidity and tensile strength to cotton and silk fibers which are of vital importance for our clothing
  - Forms linkage with water – cotton cutting takes more time for drying
  - Affects properties of food constituent – changes viscosity, solubility and stability- desirable or undesirable
  - Involves in –chemotherapeutic action of drugs, binding of dyes to textiles, adhesive action of paints, lacquers etc
  - Collects water in animal and vegetable cells – required for various activities of the cells.
  - Exists a part in biomolecules of living cells – DNA, RNA etc
  - Formation retains moisture in each crust – i.e. with clay
  - Survival of marine life in aquatic regions.

- Water can form hydrogen bond with very large number of compound – because it can form hydrogen bond by
  1. Providing proton
     e.g. its hydrogen bond formation with oxygen atom of carbonyl group
    ![Figure 2.5 Hydrogen bonding with carbonyl group](image)

2. Accepting proton
   e.g. its hydrogen bond formation with hydrogen atom of amine groups
Figure 2.6 Hydrogen bonding with amino/imino group
Lesson 3
ELEMENTARY IDEA OF HYDROPHOBICITY, HYDROPHOBIC INTERACTIONS AND ITS IMPORTANCE IN THE STRUCTURE OF PROTEINS

3.1 Introduction

When you add some drops of oil to water, the drops combine to form a larger drop. This comes about because water molecules are attracted to each other and are cohesive because they are polar molecules. Oil molecules are non polar and thus have no charged regions on them. This means that they are neither repelled nor attracted to each other. The attractiveness of the water molecules for each other then has the effect of squeezing the oil drops together to form a larger drop. Since it looks like the oil molecules are avoiding the water, this type of interaction is called a hydrophobic interaction. Hydrophobic interaction is an effective interaction between two nonpolar molecules that tend to avoid water and, as a result, prefer to cluster around each other.

Hydrophobic interactions describe the relations between water and hydrophobes (low water-soluble molecules). Hydrophobes are nonpolar molecules and usually have a long chain of carbons that do not interact with water molecules. The mixing of fat and water is a good example of this particular interaction. The common misconception is that water and fat doesn’t mix because the Van der Waals forces that are acting upon both water and fat molecules are too weak. However, this is not the case. The behavior of a fat droplet in water has more to do with the enthalpy and entropy of the reaction than its intermolecular forces.

Hydrophobic interactions along with hydrophilic interactions help to determine the three dimensional shape of biologically important molecules and structures such as proteins and cell membranes.

Nonpolar molecules are not good acceptors of the hydrogen bond. When a nonpolar molecule is placed in water, the hydrogen bonding network of water is disrupted. The water molecules therefore reorganize around the solute and make a sort of cage, similar to the structure of water in ice, in order to gain back the broken hydrogen bonds. This reorganization results in a considerable loss in the configurational entropy of water and therefore an increase in the free energy.

If there are two or more such nonpolar molecules, the configuration in which they are spatially together (clustered together) is preferred because now the hydrogen bonding network of water is disrupted in one (albeit bigger) pocket, rather than in several small pockets. Therefore, the entropy of water is larger when the nonpolar molecules are clustered together, leading to a decrease in the free energy.

The hydrophobic interaction is entropy-driven and thus intrinsically temperature sensitive. For instance, the solubility of methane in water decreases with increasing temperature at low temperatures (after reaching a minimum at about 350 K, the solubility increases with higher temperature). In liquid water, a
single water molecule can form four hydrogen bonds with nearby water molecules. However, around an apolar solute, surrounding waters can not form hydrogen bonds with it. Therefore the orientation of waters near the hydrophobic solute is more ordered and the entropy of the system is reduced. For this reason, apolar solutes tend to be lumped together to minimize the number of waters affected by them.

3.2 Causes of Hydrophobic Interactions

American chemist Walter Kauzmann discovered that nonpolar substances like fat molecules tend to clump up together rather than distributing itself in a water medium, because this allow the fat molecules to have minimal contact with water.

When a hydrophobe is dropped in an aqueous medium, hydrogen bonds between water molecules will be broken to make room for the hydrophobe; however, water molecules do not react with hydrophobe. This is considered an endothermic reaction, because when bonds are broken heat is put into the system. Water molecules that are distorted by the presence of the hydrophobe will make new hydrogen bonds and form an ice-like cage structure called a clathrate cage around the hydrophobe. This orientation makes the system (hydrophobe) more ordered. With a decrease in disorder, the entropy of the system decreases.

The change in enthalpy of the system can be negative, zero, or positive because the new hydrogen bonds can partially, completely, or over compensate for the hydrogen bonds broken by the entrance of the hydrophobe. The change in enthalpy, however, is insignificant in determining the spontaneity of the reaction (mixing of hydrophobic molecules and water) because the change in entropy is very large.

3.3 Strength of Hydrophobic Interactions

Hydrophobic interactions are relatively stronger than other weak intermolecular forces (i.e. Van der Waals interactions or Hydrogen bonds). The strength of hydrophobic interactions depends on following factors

1. Temperature

As temperature increases, the strength of hydrophobic interactions increases also. However, at an extreme temperature, hydrophobic interactions will denature.

2. Number of carbons on the hydrophobes

Molecules with the greatest number of carbons will have the strongest hydrophobic interactions.

3. The shape of the hydrophobes

Aliphatic organic molecules have stronger interactions than aromatic compounds. Branches on a carbon chain will reduce the hydrophobic effect of that molecule. This is so because carbon branches produce steric hindrance, so it is harder for two hydrophobes to have very close interactions with each other to minimize their contact to water. The linear carbon chain can produce the largest hydrophobic interaction.
3.4 Biological Importance of Hydrophobic Interactions

Hydrophobic Interactions are important for the folding of proteins. This is important in keeping a protein alive and biologically active, because it allow to the protein to decrease in surface area and reduce the undesirable interactions with water. Apart from proteins, there are many other biological substances that rely on hydrophobic interactions for its survival and functions, like the phospholipid bilayer membranes in every cell.

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Module 2. Alcohols, aldehydes and ketones

Lesson 4

IMPORTANT PROPERTIES OF MONO, DI AND TRIHYDRIC ALCOHOLS (GLYCOL AND GLYCEROL)

4.1 Introduction

- Organic hydroxyl compounds of the general formula R-OH, where ‘R’ is an alkyl or substituted alkyl group
- For example

(Figure 4.1 Examples of alcohols)

Contain hydroxyl (-OH) group as the functional group - determines characteristics and properties of this family.

4.2. Classification

Classified on different basis

- Based on number of hydroxyl groups

1. According to number of hydroxyl groups content
2. As mono-, di-, tri- and polyhydroxy

(Figure 4.2 Classification on the basis of no. of –OH groups)
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- Based on kind of carbon atom/degree of carbon atom to which –OH is attached.

1. According to the kind of carbon atom that bears hydroxyl group
2. A carbon atom may be classified as primary, secondary and tertiary
3. According to number of carbon atoms attached it

(Figure 4.3 Classification of alcohols on the basis of nature of carbon atom)

- Aliphatic alcohols and Aromatic alcohols

1. Alcohols of different classes differ in rate and/or mechanism of reaction
2. Substitution may also affect the reactivity and physical constants.

4.2.1 Physical properties

- Alcohols are considerably different from hydrocarbons due to presence of polar
–OH group – polar compounds

- Form intermolecular hydrogen bonds – as shown already

Therefore;

  o Their boiling points are much higher
  o Lower members (methanol, ethanol and propanol-1) are miscible with water
  o Ethylene glycol is used as an antifreeze in automobiles.

4.2.2 Chemical properties

- Determined by its functional group- hydroxyl group → -OH
- Reactions of an alcohol can involve the breaking of either of two bonds =

1. C-OH bond - leads to removal of -OH group or
2. O-H bond - leads to removal of -H

- In either kind substitution or elimination takes place.
- The substitution replaces –OH or –H
- The elimination forms double bond
4.2.3 Industrial sources

- There are two principal ways to get the simple alcohols = by hydration of alkenes and by fermentation of carbohydrates

4.2.3.1 Hydration of alkenes

- Alkene - obtained by cracking of petroleum
- converted into alcohol by addition of water

\[ \text{H}_2\text{C} = \text{CH}_2 + \text{H}_2\text{O} \xrightarrow{\text{H}_2\text{SO}_4} \text{H}_3\text{C} - \text{CH}_2 + \text{OH} \]

(Figure 4.4 Addition of water to alkenes)

4.2.3.2 Fermentation of carbohydrates

- Fermentation of sugars by yeast is used for manufacture of ethyl alcohol

\[ \text{C}_{12}\text{H}_{22}\text{O}_{11} + \text{H}_2\text{O} \xrightarrow{\text{Fermentation}} \text{C}_5\text{H}_12\text{O}_6 + \text{C}_6\text{H}_{12}\text{O}_6 \]

Sugar → Glucose + Fructose

\[ \text{C}_6\text{H}_{12}\text{O}_6 \xrightarrow{\text{yeast}, 25-36^\circ \text{C}} 2\text{C}_2\text{H}_5\text{OH} + 2\text{CO}_2 \]

Glucose → Ethanol

(Figure 4.5 Fermentation reactions

- The sugars come from a variety of sources - mostly from molasses from sugar cane or starch from grains (maize, peas, potatoes etc).

a) Ethyl alcohol

Widely used

- As a solvent for lacquers, varnishes, perfumes and flavoring
- As a medium for chemical reactions and in recrystallizations
- As an important raw material for synthesis of aliphatic compounds – alkenes, alkyl halides, ethers, aldehydes, ketones, acids, esters etc

If nothing is mentioned about type of alcohol
• To be considered as ethyl alcohol

\textit{b) 95\% ethyl alcohol (an azeotrope = constant boiling point mixture)}

• Generally ethyl alcohol used is a mixture of 95\% alcohol and 5\% water
• After production alcohol is purified and concentrated by fractional distillation
• In distillation first material to distill is the one which has the highest volatility i.e. lowest boiling point
• In a mixture of ethyl alcohol and water the lowest boiling component is not water (boiling point 100^\circ C) or ethyl alcohol (boiling point 72.30^\circ C) but a mixture of alcohol and water (95:5) since its boiling point is 78.15^\circ C therefore ethyl alcohol is concentrated upto 95\%
• A liquid mixture that has the peculiar property of giving a vapour of the same composition is called azeotrope or constant-boiling mixture

c) \textit{Absolute alcohol}

• 100\% ethyl alcohol is called absolute alcohol
• Obtained by taking advantage of the another azeotrope
• When a mixture containing 150 g of 95\% alcohol and 74 g benzene is distilled – a ternary azeotrope consisting of 7.5\% water, 18.5\% alcohol and 74\% benzene distilled off first due to its lower boiling point (64.90^\circ C), leaving pure (anhydrous) alcohol behind
• Traces of water from the absolute alcohol can be removed by treatment with metallic magnesium
• Water is converted into Mg(OH)\textsubscript{2} from which alcohol is distilled.
Lesson 5
REACTIONS OF ALDEHYDES AND KETONES

5.1 Introduction
• Aldehydes and ketones are compounds of the following general formula

\[
\begin{array}{c}
\text{Aldehyde} \\
\text{R} – \text{CHO} \\
\text{Ketones} \\
\text{R} – \text{CO} – \text{R'}
\end{array}
\]

Where R & R’ are alkyl or aryl groups
If R& R’ are same: Simple ketones
Different: mixed ketones

- Both aldehydes and ketones contain the carbonyl (>C=O) group – therefore collectively termed as carbonyl compounds.
- This group largely determines the chemistry of aldehydes and ketones
- Aldehydes and ketones resemble each other closely in most of their properties
- However, in aldehydes a hydrogen atom is attached to carbonyl carbon, whereas, in ketones the two alkyl groups are attached to the carbonyl carbon.
- This difference in structure have two effects on their properties

1. Aldehydes are more susceptible to oxidation by mild oxidizing agents such as dil. H\textsubscript{2}SO\textsubscript{4}/KMnO\textsubscript{4} or K\textsubscript{2}Cr\textsubscript{2}O\textsubscript{7} whereas ketones are oxidized only by drastic oxidizing agents such as conc. HNO\textsubscript{3} or H\textsubscript{2}CrO\textsubscript{4}.
2. Aldehydes are usually more reactive than ketones toward nucleophilic* addition, the characteristic reaction of carbonyl compounds

[*Nucleophile/Nucleophilic reagent (Nucleo = Nucleus, philes = loving/seeking/attaching).

An atomic or molecular species which can afford a pair of electrons for covalent-coordinate bond formation is called nucleophile. e.g. OH\textsuperscript{-}, halide ions, NH\textsubscript{3}].
5.2 Structure of Carbonyl Group

(Figure 5.2 Structure of carbonyl group)

- Carbonyl carbon is joined to three other atoms by σ bonds - since formation of these bonds utilizes sp² orbital, these lie in a plane and 120° apart.
- The remaining p orbital of the carbon forms π bond with oxygen. Carbon and oxygen are joined by a double bond.
- The part of the molecule immediately surrounding carbonyl carbon is flat - i.e. oxygen, carbonyl carbon and other two atoms directly attached to the carbonyl carbon lie in the same plane.
- The electronegativity of carbonyl carbon and the associated oxygen is quite different - hence electrons are strongly pulled towards more electronegative oxygen atom - therefore bond becomes polar.

5.2.1 Physical properties

1. Formaldehyde is gas at ordinary temperature, however, other aldehyde and ketones up to about C_{11} are colorless mobile liquids and the higher members are solids.
2. Aldehydes possess an unpleasant pungent odour while ketones have a pleasant smell. However, the higher aldehydes possess fruity odour.
3. The first few members of both series are fairly soluble in water – because of hydrogen bonding with water.
4. Their boiling points are higher than those of alkanes of comparable molecular weight - because of polar nature.

However, their boiling points are lower than those of alcohols from which they are made - because lack of hydrogen bond formation between their molecules = because they contain hydrogen bonded only to carbon ( and not with F, O or N).

5.2.2 Chemical reactions

>\text{C}=\text{O} = >\text{C}^{+}\text{O}^{-}

- Carbonyl (>\text{C}=\text{O}) group governs the chemistry of aldehydes and ketones.
- As discussed earlier carbonyl group is polar and flat.
• Therefore, it is susceptible to unhindered attack from the above and below in a direction perpendicular to the carbonyl group
• It is highly reactive

• The reactions of carbonyl group generally involve formation of bond with the electron-deficient carbonyl carbon
• The carbonyl group is most susceptible to attack by electron-rich (nucleophilic) reagents e.g. CN⁻, SO₃H⁺ etc.
• The reactions are mostly addition reactions - therefore called

• Nucleophilic addition reactions
• Can be represented as shown below (Z= nucleophilic reagent or nucleophile)

(Figure 5.3 Nucleophilic addition reaction)

• The nucleophilic reaction of carbonyl group is catalysed by acids
• Because they accentuate the electron-deficiency of the carbonyl carbon by combining with the electron-rich oxygen
• This prior protonation of carbonyl oxygen helps the movement of π electrons towards oxygen and lower the activation energy of >C=O group without having the oxygen atom to develop negative charge.
5.2.3 Relative reactivity of aldehydes and ketones

Aliphatic aldehydes and ketones are characterized by the following:

- Nucleophilic addition reactions in carbonyl compounds are controlled by two factors: steric and electronic.
- The carbonyl group is trigonal and in transition state it starts acquiring tetrahedral configuration in the reactions.
  - Thus the attached groups are brought closer together in transition state during nucleophilic addition reaction.
  - The quantum of steric hindrance in nucleophilic addition is in the following order: Methanal > Ethanal > Propanone-2 > Butanone-2 > Pentanone-3 etc.
  - The larger the ‘R’ and ‘R’’ groups, the greater would be the resistance to accommodate them in the transition state of the reaction.
  - Ketones contain two alkyl or aryl groups, whereas, in aldehyde there is one alkyl or aryl group and one hydrogen atom.
- The second alkyl of aryl group of ketone is larger than the hydrogen of an aldehyde - therefore creates more steric hindrance.
- Alkyl group attached to carbonyl carbon releases electrons (+I effect i.e. inductive effect) and thereby destabilize the transition state by intensifying the negative charge developing on carbonyl oxygen.
- Aldehydes contain only one alkyl group, whereas ketones contain two alkyl groups – therefore the destabilizing effect resulting from a ketone would be greater than that of an aldehyde (steric factor and polarity of >C=O group).
- Aromatic aldehydes or ketones are characterized by the following:
  - The electron-withdrawing effect (-I effect) of the aryl group (benzene ring) is expected to increase electron-deficiency at carbonyl carbon, thereby facilitating nucleophilic attack at the carbonyl carbon.
  - However, electron releasing through resonance by the benzene ring (+R effect) decreases the electron-deficiency at carbonyl carbon.
  - Consequently deactivate the carbonyl carbon towards nucleophilic attack.
  - Resonance effect (+R) outweighs the inductive effect (-I); this causes net deactivation of carbonyl group in aromatic aldehyde/ketone towards nucleophilic attack.

1. Addition of sodium bisulphite
• Most aldehydes and many ketones (especially methyl ketones) form crystalline adduct with sodium bisulphite

![Diagram of adduct formation]

Figure 5.5 Adduct formation

• Addition of acid or alkali can destroy the bisulphite ion in equilibrium with the adduct and regenerate the carbonyl compound

![Diagram of regeneration of carbonyl compounds]

Figure 5.6 Regeneration of carbonyl compounds

• This reaction furnishes a method for purification and separation of suitable carbonyl compounds from non-carbonyl compounds

2. Addition of hydrogen cyanide

• Forms α-hydroxynitriles (cyanohydrins)
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**Figure 5.7 Addition of HCN and hydrolysis of cyanohydrins**

- Cyanohydrins can be easily hydrolysed into $\alpha$-hydroxy acid.

**Figure 5.8 Hydrolysis of acetaldehyde cyanohydrins**

3. **Addition of Grignard reagents**

- Ketones form tertiary alcohols upon hydrolysis of intermediate products

(Figure 5.9 Formation of 30 alcohol)

- The reaction is useful
- In the synthesizing of series of organic compounds - because of formation of C-C bond
- For preparation of 10, 20 and 30 alcohols

4. **Addition of alcohol**

- Aldehyde (but not ketone) forms acetal when treated with alcohol in presence of anhydrous acid
• Dilute mineral acid decomposes acetal into the parent anhydride and alcohol even at ordinary temperature
• Therefore acetal formation is often used to protect aldehyde group, especially when to be treated with alkali- to avoid alkali induced condensation (polymerization)

5. Reaction with derivatives of ammonia (at pH = 3.5)

• Forms product containing carbon-nitrogen double bond – resulting from elimination of water molecule from an intermediate product

<table>
<thead>
<tr>
<th>Derivative of ammonia used</th>
<th>Product formed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Hydroxylamine</td>
<td>H$_2$N – OH</td>
</tr>
<tr>
<td>2 Hydrazine</td>
<td>H$_2$N – NH$_2$</td>
</tr>
<tr>
<td>3 Phenylhydrazine</td>
<td>H$_2$N – N&lt;sup&gt;H&lt;/sup&gt;</td>
</tr>
<tr>
<td>4 Semicarbazide</td>
<td>H$_2$N – N&lt;sup&gt;H&lt;/sup&gt; – NH$_2$</td>
</tr>
<tr>
<td>5 Oxime</td>
<td>C = N – OH</td>
</tr>
<tr>
<td>6 Hydrazide</td>
<td>C = N – NH$_2$</td>
</tr>
<tr>
<td>7 Phenylhydrazide</td>
<td>C = N – N&lt;sup&gt;H&lt;/sup&gt;</td>
</tr>
<tr>
<td>8 Semicarbazide</td>
<td>C = N – N&lt;sup&gt;H&lt;/sup&gt; – NH$_2$</td>
</tr>
</tbody>
</table>

(Figure 5.12 Amino compounds)

• The products are usually crystalline compounds with well-defined melting points- therefore very useful for characterization and identification of aldehydes and ketones
• The derivatives can be decomposed to regenerate the parent carbonyl compounds by boiling with dilute mineral acid- therefore used for purification of carbonyl compounds.

6. Aldol condensation

• Under influence of a dilute alkali two molecules of an aldehyde or ketone combine to form a β-hydroxy aldehyde or ketone
• This reaction is called aldol condensation
In every case addition of one molecule or aldehyde (or ketone) to a second molecule occurs in such a way that the α-carbon of the first molecule gets attached to the carbonyl carbon of the second molecule.

Aldol condensation is possible only when the carbonyl compound contains at least one α-hydrogen.

The compounds like formaldehyde, trimethylacetadehyde, aromatic aldehydes and diaryl ketones do not undergo aldol condensation.

Aldols still have acidic hydrogen - therefore aldol condensation may be repeated leading to formation of complex polymeric resinous product especially when strong alkali is used to induce aldol condensation.

Aldol condensation has a great synthetic potential.

β-hydroxycarbonyl compounds and α, β-unsaturated carbonyl compounds are usually synthesized via aldol condensation reactions.

From these unsaturated carbonyl compounds α, β-unsaturated alcohol is produced by reduction.

On catalytic hydrogenation saturated alcohol is obtained from the unsaturated alcohol - i.e. yields long chain saturated alcohol.

7. Cannizarro reaction

In presence of concentrated alkali, aldehydes having no α-hydrogen undergo self oxidation-reduction to yield a mixture of an alcohol and a salt of a carboxylic acid (Fig. 5.14).

Tertiary butyl aldehyde [(CH₃)₃CCHO] also undergoes cannizarro reaction.
A crossed Cannizzaro reaction between an aromatic aldehyde and formaldehyde is an important synthetic reaction due to greater tendency of formaldehyde to undergo oxidation and gives almost exclusively the sodium formate and the alcohol corresponding to the aromatic aldehyde (Fig. 5.15).

8. Perkin condensation

- Acid anhydrides added to aromatic aldehydes in the presence of base to yield α,β-unsaturated acids
- Reaction is called Perkin condensation and resembles aldol condensation.
- The base most commonly used is the sodium salt of carboxylic acid from which the anhydride is derived
• By varying the substituent in the aromatic aldehyde, it is possible to make a wide variety of substituted cinnamic acids (C6H5CH=CHCOOH)- from which corresponding saturated acids may be obtained by hydrogenation.

9. Reduction to alcohol

• Aldehydes can be reduced to primary alcohols and ketones to secondary alcohols, either by catalytic hydrogenation or by use of chemical reducing agents like lithium aluminium hydride (LiAlH₄).

(Figure 5.17 Catalytic reduction)

(Figure 5.18 Reduction reaction)

• Such reduction is useful for preparation of certain alcohols that are less available than the corresponding carbonyl compounds

10. Reduction to hydrocarbon

• Aldehydes and ketones can be reduced to hydrocarbon by

  1. Clemmensen Reduction – by action of amalgamated zinc and concentrated hydrochloric acid – for compounds sensitive to base
  2. Wolff-Kishner Reduction – by action of hydrazine (H₂N-NH₂) and a strong base like potassium hydroxide (KOH)- for compounds sensitive to acid
Figure 5.19 Clemmensen and Wolff Kishner reductions

11. Oxidation

- Aldehydes are oxidized to acids containing same numbers of carbon atoms - even by a weak oxidizing agent e.g. KMnO₄ or K₂Cr₂O₇ / dil. H₂SO₄.

\[
\text{R} \text{-- CHO} \xrightarrow{(O)} \text{R} \text{-- COOH}
\]

Figure 5.20

- Oxidation of ketones requires the breaking of carbon-carbon bonds - therefore it occurs only under drastic conditions

\[
\text{R} - \text{C} - \text{CH}_2 - \text{R'} \xrightarrow{\text{K}_2\text{Cr}_2\text{O}_7, \text{conc. H}_2\text{SO}_4} \text{R} - \text{COOH} + \text{R'} - \text{COOH}
\]

Figure 5.21 Oxidation of unsymmetrical ketones: Popoff’s Rule

- The extent with which aldehydes undergo oxidation is useful mainly for detecting these compounds and in particular for differentiating them from ketones
- Mild oxidizing agents used for this purpose are
  1. Tollén’s Regent : ammonical solution of silver nitrate - Ag(NH₃)₂⁺ ion
  2. Fehling Solution : alkaline solution of cupric ion in presence of sodium potassium tartrate (Rochelle salt) - Cu(OH)₂
  3. Benedict Solution : alkaline solution of cupric ion in presence of citrate - Cu(OH)₂

\[
\text{CH}_3\text{--CHO} + 2\text{Ag(NH}_3\text{)}^{+} + 3\text{OH}^- \rightarrow \text{CH}_3\text{COO}^- + 2\text{NH}_3 + 2\text{H}_2\text{O} + 2\text{Ag} \quad \text{(Silver mirror)}
\]

\[
\text{CH}_3\text{--CHO} + 2\text{Cu(OH)}_2 \xrightarrow{\text{Tartarate or Citrate}} \text{CH}_3\text{COOH} + 2\text{H}_2\text{O} + \text{Cu}_2\text{O}
\]

Figure 5.22 Test for –CHO group

- The reaction is of value in synthesis of unsaturated acids from unsaturated aldehydes obtained from aldol condensation
Where advantage is taken of the fact that the mild oxidizing agents (reagents) does not attack carbon-carbon double bonds

\[ \begin{array}{c}
R - C = C - \overset{\beta}{\overset{\alpha}{\text{O}}} \quad \text{Oxidizing agent} \\
\text{(milk)}
\end{array} \quad \begin{array}{c}
R - C = C - \overset{\beta}{\overset{\alpha}{\text{COOH}}} \\
\text{α, β - Unsaturated aldehyde} \\
\text{α, β - Unsaturated acid}
\end{array} \]

(Figure 5.23 Oxidation of unsaturated aldehyde)

12. Haloform reaction

The oxidation reaction consists of treating a methyl ketone with sodium hypochlorite (hypohalite) = oxidises the ketones to acids containing one carbon atom less that the parent methyl ketone and haloform forms simultaneously.

\[ \begin{array}{c}
\overset{\text{O}}{R - C - CH_3} + 3\text{NaOX} \\
\text{Haloform}
\end{array} \rightarrow \begin{array}{c}
R - \overset{\text{COONa}}{\text{COONa}} + \overset{\text{CHX}_3}{\text{CHX}_3} + 2\text{NaOH}
\end{array} \]

(Figure 5.24 Haloform Test/Reaction)

Iodoform test: used as a diagnostic test for methyl ketone is based on this reaction

\[ \begin{array}{c}
\overset{\text{O}}{R - C - CH_3} + 3\text{NaOIl} \\
\text{Iodoform}
\end{array} \rightarrow \begin{array}{c}
R - \overset{\text{COONa}}{\text{COONa}} + \overset{\text{CHI}_3}{\text{CHI}_3} + 2\text{NaOH}
\end{array} \]

(Figure 5.25 Iodoform Test)

*****😊*****
Lesson 6
IMPORTANCE OF CARBONYL COMPOUNDS IN FOOD FLAVORS

6.1 Introduction

Carbonyl compounds are molecules containing the carbonyl group, C=O. Flavor compounds interact with olfactory and lingual receptors. Volatile compounds are generally responsible for odor perception and nonvolatile compounds for taste. The possibility of the interaction of flavor compounds at subthreshold concentrations giving rise to a detectable flavor has many implications in flavor research. Carbonyls have categorized into various fractions such as painty, oily, mushroom, metallic, tallowy, and cucumber.

In some foods, natural flavors may result from a number of compounds, none of which exist at their flavor threshold. The same phenomenon could account for off-flavor development. However, in the case of off-flavors it is conceivable that when any objectionable compound reaches its flavor threshold, the defect would become evident. The carbonyls may arise due to carbohydrate or citrate metabolism, lipid oxidation or amino acid degradation in various dairy and food products.

The Maillard reaction is one pathway falling under nonenzymatic browning (caramelization, Maillard reaction, and ascorbic acid browning). Of these pathways, the Maillard reaction plays the major key role in flavor development. This reaction is responsible for some of the most pleasant flavors enjoyed by man. There is no question that freshly baked bread, a steak, a freshly brewed cup of coffee, or a piece of chocolate is appreciated by the consumer. Yet none of the characterizing flavors existed in the product until the food processor (or cook) heated the product to develop the flavor.

Generally speaking, the Maillard reaction is a reaction between carbonyls and amines. The carbonyls in foods most often are reducing sugars, while the amines come from either amino acids or proteins. In the flavor industry, the carbonyl may be a pure compound (e.g., diacetyl) and the amine, simply ammonia, or an amine. The major end products of the Maillard reaction are melanoidins and other nonvolatile compounds. The major pathway leading to the formation of carbonyls is the Strecker degradation. This reaction occurs between dicarbonyls and free amino acids. The dicarbonyls involved have vicinal carbonyls (carbonyl groups separated by one double bond) or conjugated double bonds. While these carbonyls typically are intermediates in the Maillard reaction, they may also be normal constituents of the food (e.g., ascorbic acid), be end products of enzymatic browning (e.g., quinones), or be products of lipid oxidation.

Carbonyl compounds make a particularly significant contribution to the flavor of fermented dairy products. Diacetyl, characterized as having a buttery, nut-like aroma, is one of the most important carbonyls to the flavor of these products. Diacetyl is produced via the fermentation of citrate. The most important citric acid fermenters are Leuconostoc citrovorum, L. creamoris, L. dextranicum, Streptococcus lactis subspecies diacetylactis, S. Thermophilus, and certain strains of Propionibacterium shermani. The metabolic pathway leading to the synthesis of diacetyl involves the degradation of citrate to acetate and oxaloacetate, and the oxaloacetate is then decarboxylated to form pyruvate. Pyruvate plus acetaldehyde forms α-acetolactate, and ultimately, diacetyl. Diacetyl is relatively nontoxic to the bacteria cell so excess pyruvate is channeled into diacetyl. Unfortunately diacetyl is not stable in most
Organic Chemistry

cultured food products. The microorganisms that synthesize diacetyl also contain diacetyl reductase that reduce diacetyl to acetoin and 2, 3-butanediol.

A second dairy product where carbonyls are considered as flavor impact compounds is yogurt. Carbonyl compounds comprise the main aromatic substances in yoghurt, among which acetaldehyde is the compound that contributes most to the typical flavor of yogurt. Acetaldehyde is a metabolic end product of L. bulgaricus and/or S. thermophilus during the fermentation of milk to yogurt. Pure acetaldehyde possesses a pungent irritating odor but at dilute concentrations it gives a pleasant fruity aroma. Acetaldehyde imparts yogurt its characteristic green apple or nutty flavor. Acetaldehyde is an indispensable aroma compound in yogurt; good flavored yogurt results when proper levels (23–40 mg/kg and at least 8–10 mg/kg) of acetaldehyde are produced. Diacetyl is an important aroma compound that gives the buttery flavor and it may improve yogurt flavor quality at elevated concentrations. Diacetyl reportedly contributes to the delicate, full flavor and aroma of yogurt and is especially important for products that contain low acetaldehyde concentrations. Diacetyl is a diketone, derived by the fermentation of citrate present in milk and dairy mixes. Small quantities of diacetyl, ranging from traces to 0.90 mg/kg or more contribute to the pleasant and delicate flavor and aroma of yogurt. The typical concentrations of diacetyl in yogurt ranged from 0.2 mg/kg to 3 mg/kg. Acetoin is a common flavor substance in many cultured dairy products. Acetoin has a mild creamy, slightly sweet, butter-like flavor that is similar to that of diacetyl. Meanwhile, the flavor of acetoin is considerably weaker than that of diacetyl and it tends to reduce the harshness of diacetyl. Typical acetoin concentrations in yogurt ranged from 1.2 to 28.2 mg/kg. Acetoin combined with acetaldehyde imparts the mild, pleasant, buttery taste, and they are critical to the rich perception of yogurt. Acetone has a sweet, fruity aroma and is known to influence the aroma and flavor qualities of yoghurt. 2-butanone is significant for eliciting yogurt odor and contributes to the “fruity” flavor. Although each of these carbonyl compounds constitutes a recognizable aroma alone, yogurt flavor is determined by a balanced mixture of the important volatile compounds. For example, a 1:1 acetaldehyde and diacetyl ratio would give a preferred typical yogurt flavor, while too much acetaldehyde compared to diacetyl would lead to a “green” off-flavor. Also, the ratio of acetaldehyde to acetone plays a significant role in the development of yogurt flavor, and a ratio of 2.8:1 results in the desired “fullness” flavor.

Carbonyls are quite important to the flavor of cheeses. The 29 carbonyls have been identified in Cheddar cheese. Carbonyls (methyl ketones) may arise in fermented products initially via lipase activity of the starter culture. Dairy products contain a significant quantity of α-keto acids which are readily hydrolyzed from the triglyceride by microbial lipases and then decarboxylated to form odd carbon number methyl ketones. Methyl ketones and aldehydes may also be formed via microbially induced lipid oxidations. The oxidation may be initiated by microbial lipases, hydrogen peroxide produced by microorganisms and/or lipoxidase-like activity. Carbonyl compounds typically are significant to the flavor of most fermented food products.

Some of examples of carbonyl compounds found in dairy/food products are mentioned at below.

Table 2.1 Examples of carbonyl compounds
**Carbonyl compounds**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaldehyde</td>
<td>Ethereal, fresh, green, pungent</td>
</tr>
<tr>
<td>Acetone</td>
<td>Sweet, fruity</td>
</tr>
<tr>
<td>2-Propanone</td>
<td>Sweet, fruity</td>
</tr>
<tr>
<td>2-Butanone</td>
<td>Varnish-like, sweet, fruity</td>
</tr>
<tr>
<td>Diacetyl</td>
<td>Buttery, creamy, vanilla</td>
</tr>
<tr>
<td>Acetoin</td>
<td>Buttery</td>
</tr>
<tr>
<td>3-Methyl-2-butenal</td>
<td>Metallic, aldehydic, herbaceous</td>
</tr>
<tr>
<td>2-Pentanone</td>
<td>Fruity, acetone</td>
</tr>
<tr>
<td>3-Methylbutanal</td>
<td>Green, malty, unripe, cocoa, malty</td>
</tr>
<tr>
<td>2,3-pentanedione</td>
<td>Butter, vanilla, mild</td>
</tr>
<tr>
<td>Hexanal</td>
<td>Green, cut-grass</td>
</tr>
<tr>
<td>2-Hexanone</td>
<td>Floral, fruity</td>
</tr>
<tr>
<td>Heptanal</td>
<td>Green, sweet</td>
</tr>
<tr>
<td>Nonanal</td>
<td>Sweet, floral, citrus, grass-like</td>
</tr>
<tr>
<td>2-Nonanone</td>
<td>Fruity, musty</td>
</tr>
<tr>
<td>2-Pentanone</td>
<td>Sweet, fruity, cheesy</td>
</tr>
<tr>
<td>Heptanone</td>
<td>Fruity, spicy, cinnamon</td>
</tr>
<tr>
<td>3-Octanone</td>
<td>Mushroom, fruity</td>
</tr>
<tr>
<td>1-Octen-3-one</td>
<td>Mushroom-like, earthy, fruity</td>
</tr>
<tr>
<td>Nonanone</td>
<td>Grassy-herbal, green-fruity, floral</td>
</tr>
<tr>
<td>2-Undecanone</td>
<td>Floral, rose-like, herbaceous</td>
</tr>
<tr>
<td>g-Dodecalactone</td>
<td>Fresh fruit, peach, par, plum, coconut, buttery</td>
</tr>
<tr>
<td>d-Dodecalactone</td>
<td>Peach, coconut, buttery, musky</td>
</tr>
<tr>
<td>2-Phenylacetaldelyde</td>
<td>Flowery</td>
</tr>
</tbody>
</table>
Lesson 7
IONIZATION CONSTANT AND STRENGTH OF CARBOXYLIC ACIDS

7.1 Introduction

- Organic compounds containing carboxylic (-COOH) group (s) are called carboxylic acids
- They are represented by general formula R-COOH, where R may be
  - Aliphatic, alicyclic or aromatic
  - Saturated or unsaturated
  - Substituted or unsubstituted

- The common names of aliphatic acids are generally related to their source rather than to their structure e.g.
  - Formic acid = Latin: formica means red ants
  - Acetic acid = Latin : acetum means vinegar (Acetobacter acetii)
  - Caprylic, caproic and capric acids = Latin: caper means goat

- Branch-chain acids and substituted acids = named as derivatives of straight-chain acids
- The position of the branch/substituent are indicated by Greek prefixes α-,β-,γ-, δ-, etc

(Figure 7.1 Trivial names)

7.2 Structure Of Carboxylate Ion And Carboxylic Acid

- A carboxylic acid is a resonance hybrid of structure I & II
Figure 7.2 Resonance structure

Due to positive charge on the oxygen atom of the hydroxyl group there would be a displacement of the electron pair of the O-H bond towards the oxygen atom.

This would facilitate the release of hydrogen as a proton.

Figure 7.3 Acidic nature

Since similar resonance is not possible in alcohols, the proton in alcohol would be released with greater difficulty.

Therefore alcohols are much weaker acids than carboxylic acids.

The carboxylate anion is also resonance-stabilized.

Figure 7.4 Resonance stability of carboxylate ion

Since the resonance hybrid of the carboxylate anion involves two equivalent contributing structures, the negative charge is evenly distributed over both the oxygen atoms.

The carbon in carboxylate anion is literally joined to each oxygen atom “by one and a half” bond. Neither it is a pure C-O single bond nor it is >C=O double bond.

Figure 7.5 Resonance hybrid structure

This view is supported by the evidence of bond length.

Both the carbon-oxygen bonds in the carboxylate ion has same bond length (1.27 Å) rather than 1.21 Å for C=O and 1.41 Å for C-OH.

As the double bond character of the C=O linkage in carboxylic acid and carboxylate is reduced considerably, they do not show characteristic of the carbonyl group.

Do not form oximes, phenylhydrazones, semicarbazones, etc.
7.3 Acidity Of Carboxylic Acids

![Resonating structures](image)

- Carboxylate ion is resonance stabilized
- Both the structure are equivalent, the resonance energy of the anion is much greater than that of the acid
- Loss of a proton from the carboxylic acid produces a more stable structure
- Thus accounts for an easy release of the protein from the acid
- Ionization of carboxylic acid

\[
\text{R-COOH} + \text{H}_2\text{O} \rightleftharpoons \text{R-COO}^- + \text{H}_3\text{O}^+
\]

According to law of mass action, the equilibrium constant, K is expressed as

\[
K = \frac{[\text{RCOO}^-][\text{H}_2\text{O}]}{[\text{RCOOH}][\text{H}_2\text{O}]}\]

As the concentration of water remains constant, so \(K \times [\text{H}_2\text{O}] = K_a\)

\[
K \times [\text{H}_2\text{O}] = \frac{[\text{RCOO}^-][\text{H}_3\text{O}^+]}{[\text{RCOOH}]}\quad \therefore \quad K_{a,\text{aq}} = \frac{[\text{RCOO}^-][\text{H}^+]}{[\text{RCOOH}]}\quad \{[\text{H}_3\text{O}^+]=[\text{H}^+] \text{ For Conveniency}\}
\]

- The value of \(K_a\) – ionization constant of the acid
- Directly proportional to ionized entity - \([\text{RCOO}^-]\) and \([\text{H}^+]\)
- Inversely proportional to unionized acid - \([\text{RCOOH}]\)
- Greater the concentration of \(\text{H}^+\) - large the value of \(K_a\) stronger will be the acid
- \(K_a\) would be a true index of the acidity – acid strength
- Now a days \(K_a\) values are replaced by \(pK_a\) values ; \(pK_a = -\log K_a\)
- Each carboxylic acid has a characteristic value of \(K_a\) or \(pK_a\)
- Simple unsubstituted aliphatic and aromatic have \(K_a = \text{about 10-5}\)

These acids, compared with mineral acids (\(K_a = 108\)) are weak

★★★★ ★★★★★
Lesson 8
IMPORTANT REACTIONS OF CARBOXYLIC ACID

8.1 Introduction

8.2 Reactions of carboxylic acids

1. Reactions with alkali (Neutralization)
   - Carboxylic acids are recognized by their acidity
   - Like conventional acids (HCl, H₂SO₄, HNO₃ etc), carboxylic acids (R-COOH) also react with aqueous hydroxides (e.g. NaOH) to form salt and water - used for determination of the acid

   \[ R\text{-}\text{COOH} + \text{NaOH} = R\text{-}\text{COONa} + \text{H}_2\text{O} \]
   salt

   - Reaction with aqueous sodium bicarbonate and carbonate forms salt, water and evolve carbon dioxide - effervescence - forms the basis for detection of –COOH group in an organic compound

   \[ 2R\text{-}\text{COOH} + \text{Na}_2\text{CO}_3 = 2R\text{-}\text{COONa} + \text{H}_2\text{O} + \text{CO}_2 \uparrow \]
   \[ R\text{-}\text{COOH} + \text{NaHCO}_3 = R\text{-}\text{COONa} + \text{H}_2\text{O} + \text{CO}_2 \uparrow \]

   - When the salt is treated with mineral acid= converted back into carboxylic acid

   \[ R\text{-}\text{COONa} + \text{HCl} = R\text{COOH} + \text{NaCl} \]
   Salt mineral acid carboxylic acid

2. Reduction to alcohol
   - Reducing agent - Lithium aluminum hydride - LiAlH₄ -gives excellent yield, but somewhat expensive

   \[ R\text{-}\text{COOH} \xrightarrow{\text{LiAlH}_4} R\text{-}\text{CH}_2\text{OH} \]

   Figure 8.1 Reduction

3. Conversion into aldehyde and ketone
   - By heating calcium salt of the acid at high temperature (decarboxylation)
   - When a mixture of Ca format and Ca salt of higher acid is heated - aldehyde will form
Organic Chemistry

4. Substitution of side chain e.g. Chlorination

8.3 Dicarboxylic Acids

8.4 Tricarboxylic acid

8.4.1 Physical constants of citric acid

- Melting Point: 153 °C
- Solubility: 240 g/100 g water at 25 °C
- Ionization constant (K_a × 10^5): K_a = 74
- Important chelating agent – chelate Ca^{+2}
  - Improve heat stability of milk
• Processed cheese manufacturing
• Reduces hardness of water
• Improves resistance of human body.
Module 3. Carboxylic acids

Lesson 9
DERIVATIVES: ESTERS, AMIDES, LACTONES AND THEIR PREPARATION AND REACTIONS

9.1 Introduction

- Class of organic compounds which are derived from carboxylic acids by replacing –OH of the –COOH group with other atom or group
- include acid chlorides, acid anhydrides, acid amides and esters
- Called functional derivatives of carboxylic acids
- In acyl compounds group is common.

**Derivatives of carboxylic acids**

(Figure 9.1 Derivates of carboxylic acid)

9.1.1 Nomenclature

- Names are taken from corresponding carboxylic acid
9.2 Acid Chlorides

- Prepared by interaction of carboxylic acids with thionyl chloride (SOCl₂), phosphorus trichloride (PCl₃) or phosphorus pentachloride (PCl₅)

\[
2\text{R-COOH} + \text{Na}_2\text{CCl}_3 = 2\text{R-COONa} + \text{H}_2\text{O} + \text{CO}_2 \\
\text{R-COOH} + \text{NaHCO}_3 = \text{R-COONa} + \text{H}_2\text{O} + \text{CO}_2
\]

9.3 Reactions

- Typically undergo nucleophilic substitution
- Chlorine is expelled as chloride and its place is taken by other basic group
- Because of carbonyl group these reactions take place much more rapidly than the corresponding alkyl halide
- Acid chloride are the most reactive of the derivatives of carboxylic acids = therefore used as acylating agents
## 9.4 Acid Anhydride

- Acetic anhydride is immensely important
- Prepared by

(Figure 9.4 Preparation of acid anhydrides)
9.4.1 Reactions

- Undergo the same reactions as acid chlorides, but
- A little more slowly
- Yield a molecule of carboxylic acid (instead of HCl)
- Anhydrides of acids more convenient acylating agents
- Acetic anhydride is cheap, less volatile and more easily handled than acetyl chloride and does not form corrosive HCl

(Figure 9.5 Reactions involved)

9.5 Acid Amides
Figure 9.6 Preparations of acid amides

9.5.1 Reactions

- The electron-deficiency of the carbonyl carbon is somewhat made up by the electron donor action of the nitrogen atom

\[
\begin{align*}
\text{R} - \text{C} - \text{Cl} + 2\text{NH}_3 & \quad \rightarrow \quad \text{R} - \text{C} - \text{NH}_2 + \text{NH}_4\text{Cl} \\
\text{R} - \text{C} - \text{O} - \text{C} - \text{R} + 2\text{NH}_3 & \quad \rightarrow \quad \text{R} - \text{C} - \text{NH}_2 + \text{R} - \text{C} - \text{ONH}_4 \\
\text{R} - \text{C} - \text{O} - \text{R}' + \text{NH}_3 & \quad \rightarrow \quad \text{R} - \text{C} - \text{NH}_2 + \text{R}' - \text{OH}
\end{align*}
\]

**Ammonium salt by pyrolysis**

\[
\begin{align*}
\text{R} - \text{C} - \text{ONH}_2 & \quad \xrightarrow{\Delta} \quad \text{R} - \text{C} - \text{NH}_2 + \text{H}_2\text{O}
\end{align*}
\]

(Presence of acid suppress the dissociation of salt)

**Cyanides (Nitriles) by partial hydrolysis**

\[
\begin{align*}
\text{R} - \text{C} = \text{N} + \text{H}_2\text{O} & \quad \rightarrow \quad \text{R} - \text{C} - \text{NH}_2
\end{align*}
\]

Figure 9.7 Resonating structures of acid amides

Nucleophilic attack on acyl carbon is difficult than the acid chloride - therefore they are not used as acylating agents

- They are not even hydrolysed by water
- However, they are hydrolysed by heating in presence of dilute acid or alkali

\[
\begin{align*}
\text{R} - \text{C} - \text{O} + \text{NH}_3 & \quad \xleftarrow{\Delta} \quad \text{R} - \text{C} - \text{NH}_2 + \text{H}_2\text{O} & \quad \xrightarrow{\Delta} \quad \text{R} - \text{C} - \text{OH} + \text{NH}_4^+
\end{align*}
\]

Figure 9.8 Hydrolysis

- Amides may be expected to show basic character like amines, but they are almost neutral compounds
- Because the electron pair on N in \(\text{CONH}_2\) group is not normally available for protonation
9.6 Esters

- Widely distributed in nature
- The fragrance of fruits, flowers and essential oils largely due to esters
- Fats and oils are esters of higher fatty acids with glycerol
- Waxes are also high molecule weight esters

9.6.1 Preparation

1. By action of carboxylic acids on alcohol

   - Boiling an acid with an alcohol in presence of acid catalyst (e.g. H₂SO₄)

\[
\begin{align*}
\text{By action of carboxylic acids on alcohol} & : \quad R-C-\overset{\text{OH}}{\text{O}} + H^+ & \overset{\text{H}^+}{\rightleftharpoons} & \quad R-C-O-R' + H_2O \\
\end{align*}
\]

   - Relative reactivity of carboxylic acids and alcohols – markedly dependent on their structure
   - Greater the bulk of the substituent near –OH and /or –COOH group, the slower the reaction rate – because steric hindrance.
   - Relative reactivity order in esterification

   Alcohols – CH₃OH > CH₃CH₂OH > (CH₃)₂CHOH > (CH₃)₃COH (1° > 2° > 3°)
   Acids - HCOOH > CH₃COOH > (CH₃)₂CHCOOH > (CH₃)₃COOH

2. From acid chlorides by alcoholysis

\[
\begin{align*}
\text{From acid chlorides by alcoholysis} & : \quad R-C-\overset{\text{Cl}}{\text{O}} + HO-\overset{\text{R'}}{\text{R}} & \rightarrow & \quad R-C-O-R' + HCl \\
\end{align*}
\]

   - This reaction is not reversible - therefore faster than that of the direct esterification reaction – therefore better yield of the product i.e. ester
   - Phenyl esters and esters of sterically hindered acids and/or alcohols are generally prepared by this method
3. From acid anhydride by alcoholysis

$$\begin{align*}
\text{Anhydride} & \quad \text{alcohol} \\
\ce{R-C-O-C-R + HO-R'} & \rightarrow \ce{R-C-O-R' + R-COOH}
\end{align*}$$

Figure 9.11 Reaction of acid anhydride with alcohol

4. From esters by trans-esterification

- In esterification of an acid, an alcohol act as a nucleophilic reagent
- In hydrolysis of an esters, an alcohol is displaced by a nucleophilic reagent
- These observation gave an idea – that the alcohol present in an ester can be displaced by another alcohol to form new ester
- This alcoholysis (cleavage by an alcohol) of an ester is called transesterification

$$\begin{align*}
\text{Esters} & \quad \text{Substituting alcohol} \\
\ce{R-C-O-R' + HO-R''} & \xrightleftharpoons[\text{H^+ or OH^-}]{\text{Modified ester}} \ce{R-C-O-R'' + HO-R'}
\end{align*}$$

Figure 9.12 Transesterification

9.6.2 Reactions

1. **Hydrolysis**
   - Conversion into acid & alcohol
   - $$\ce{R-C-O-R' + H_2O} \xrightarrow{\text{H}^+} \ce{R-COOH + R'-OH}$$

2. **Ammonolysis**
   - Conversion into amides
   - $$\ce{R-C-O-R' + NH_3} \rightarrow \ce{R-C-NH_2 + R'-OH}$$

3. **Alcoholysis** (Transesterification)
   - Conversion into esters
   - $$\ce{R-C-O-R' + R''-OH} \rightarrow \ce{R-C-O-R'' + R'-OH}$$

4. **Hydrogenation** (Hydrogenolysis)
   - $$\ce{R-C-O-R' + 2H_2} \xrightarrow{\text{CuO} +_{\text{Cu}_2} \text{CO}_3, 250^\circ C, 3000 \text{ psi}} \ce{RCH_2 - OH + R'-OH}$$

Figure 9.13 Summary of main reactions
9.7 Lactones

- Lactones are cyclic esters formed by the intramolecular interaction of ‘-OH’ and ‘-COOH’ groups present on the same molecule - intramolecular ester formation – formation of ester within the molecule
- In hydroxyl carboxylic acids ‘-OH’ and ‘-COOH’ groups required for formation of ester, are present in the same molecule

(Figure 9.14 Lactones)

- Due to steric hindrance lactone smaller than γ-lactone are unstable and not formed
- Lactones are produced during thermal oxidation of saturated and unsaturated fatty acids
- High level of lactones in fresh milk is foreign to its natural flavor and considered as flavor defect
- In beverage milk trace (1-2 ppm) of these odoriferous compounds is optimal
- In butter 5 to 10 ppm are desirable
- The development of lactones is stored whole milk powder imparts coconut flavor
- In confectioneries and candies it is a major source of their unique flavor.

***** ☺ *****
Lesson 10
SUBSTITUTED CARBOXYLIC ACIDS: HALOGEN, KETO AND HYDROXY ACIDS AND THEIR IMPORTANT REACTIONS

10.1 Effect of Substitution on Acidity of Carboxylic Acids

- Any factor that stabilizes the conjugate anion should increase the acidity
- Any factor that destabilizes the conjugate anion should decrease the acidity
- Electron-withdrawing substituent - F, Cl etc - because inductive effect (-I effect)- disperse the negative charge, stabilized the conjugate anion - therefore increase acidity
- Electron-releasing substituent – alkyl group (e.g. CH₃-) - intensify the negative charge on the anion, destabilize the anion - therefore decrease acidity

![Figure 10.1 Effect of substituent](image)

Table 10.1 Dissociation constants of carboxylic acid

<table>
<thead>
<tr>
<th>Acid</th>
<th>(K_a \times 10^{-5})</th>
<th>Acid</th>
<th>(K_a \times 10^{-5})</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH₃COOH</td>
<td>1.85</td>
<td>HCOOH</td>
<td>24</td>
</tr>
<tr>
<td>ClCH₂COOH</td>
<td>155</td>
<td>CH₂COOH</td>
<td>1.85</td>
</tr>
<tr>
<td>Cl₂CHCOOH</td>
<td>5140</td>
<td>CH₃CH₂COOH</td>
<td>1.52</td>
</tr>
<tr>
<td>Cl₃COOH</td>
<td>24000</td>
<td>CH₃CH₂CH₂COOH</td>
<td>1.50</td>
</tr>
</tbody>
</table>

> Electron-withdrawing group (EWG) | Electron-donating group (EDG)

| NO₂ >F>Cl>Br>I>OCH₃>C₆H₅ | (CH₃)₃-C-CH₂-C=O > C₆H₅ > C₂H₅ > CH₃ |

Make the substituted carboxylic acid stronger | Make the substituted carboxylic acid weaker

- The inductive effect decreases with distance - i.e. increase in chain length causes inductive elective/group to away from the carboxylic group (position of substituent).
Aromatic acids (benzoic acid) are similarly affected by substituent

- Cl, NO\textsubscript{2}, etc. make the acid stronger
- CH\textsubscript{3}, C\textsubscript{2}H\textsubscript{5}, etc. display both kind of effect depending upon their position (para or meta) of substitution

  o Meta position: electron-withdrawing - makes the acid stronger
  o Para position: electron-releasing - makes the acid weaker

Ortho substitution shows unusual behavior

  o Unusually large effect
  o Makes the acid stronger irrespective of the type of substituent - electron releasing or electron-withdrawing
  o Known as ortho effect - reason not completely understood (may be electronic or kinetic reasons).

10.2 Note

* Inductive effect
The process of electron shift along a chain of atoms due to the presence in it of a polar covalent bond is called inductive effect. When the hetero atom is such that it attracts the electron pair to itself, (e.g. chlorine), it is said to exert a –I effect or electron – withdrawing inductive effect. On the other hand when the hetero atom (or group of atoms) pushes the electrons away from itself, it exerts a +I effect, or electron-releasing inductive effect.
Table 10.2 Effect of substituent position on the ionization constant

<table>
<thead>
<tr>
<th>Substituent</th>
<th>Ionization constant $K_a \times 10^7$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Para</td>
</tr>
<tr>
<td>-(None)</td>
<td>6.3</td>
</tr>
<tr>
<td>Cl</td>
<td>10.4</td>
</tr>
<tr>
<td>NO₂</td>
<td>35</td>
</tr>
<tr>
<td>CH₃</td>
<td>4.3</td>
</tr>
<tr>
<td>OCH₃</td>
<td>3.2</td>
</tr>
<tr>
<td>OH</td>
<td>2.5</td>
</tr>
</tbody>
</table>

10.B.1 Reactions of Carboxylic Acids

1. Reactions with alkali (Neutralization)

- Carboxylic acids are recognized by their acidity
- Like conventional acids (HCl, H₂SO₄, HNO₃ etc), carboxylic acids (R-COOH) also react with aqueous hydroxides (e.g. NaOH) to form salt and water = used for determination of the acid

$$\text{R-COOH} + \text{NaOH} \rightarrow \text{R-COONa} + \text{H}_2\text{O}$$

- Reaction with aqueous sodium bicarbonate and carbonate forms salt, water and evolve carbon dioxide - effervescence -forms the basis for detection of –COOH group in an organic compound

$$2\text{R-COOH} + \text{Na}_2\text{CO}_3 = 2\text{R-COONa} + \text{H}_2\text{O} + \text{CO}_2$$
$$\text{R-COOH} + \text{NaHCO}_3 = \text{R-COONa} + \text{H}_2\text{O} + \text{CO}_2$$

- When the salt is treated with mineral acid- it is converted back into carboxylic acid

$$\text{R-COONa} + \text{HCl} = \text{RCOOH} + \text{NaCl}$$
Salt mineral acid carboxylic acid

2. Reduction to alcohol

- Reducing agent - Lithium aluminum hydride - LiAlH₄ -gives excellent yield, but somewhat expensive

$$\text{R-COOH} \rightarrow \text{R-CH}_2\text{OH}$$

3. Conversion into aldehyde and ketone

- By heating calcium salt of the acid at high temperature
- When a mixture of Caformate and Ca salt of higher acid is heated - aldehyde will form
\[(\text{HCOO})_2\text{Ca} + (\text{R-COO})_2\text{Ca} = 2\text{RCHO} + 2\text{CaCO}_3\]

aldehyde

- When Ca salt of an acid other than formic acid is heated – ketone will form

\[
(R-\text{COO})\text{Ca} + (R'-\text{COO})\text{Ca} = R\quad C\quad R + 2\text{CaCO}_3
\]

4. Substitution of side chain

- Chlorine reacts with carboxylic acids to form chloro substituted carboxylic acids

\[
\text{R-CH}_2\text{COCH} + \text{Cl}_2 \rightarrow \text{R-CH-COOH} + \text{HCl}
\]

* **** ☹ **** *
Lesson 11
BASIC CHARACTER OF AMINES AND THEIR IMPORTANT REACTIONS

11.1 Introduction

• Amines are Alkyl/Aryl derivatives of ammonia
• One or more hydrogen atoms of ammonia are replaced by alkyl or aryl groups

(Figure 11.1 Amines)

11.2 Basic Character of Amine

• Like ammonia, amines are basic - because they contain a pair of unshared electrons on nitrogen
• Compound which accept the proton - base

$$R\text{-NH}_2 + H_2O = R\text{-NH}_3^+ + OH^-$$

Equilibrium constant K for the reaction
(Figure 11.2 Basic strength of amines)

- $K_b$
  - ionization constant of the amine
  - measure of the extent to which the amine accepts the hydrogen ion ($H^+$) from water
  - indicates basicity of the amine

- each amine has a characteristic values of $K_b$
- In aliphatic amines $K_b$ varies from $10^{-3}$ to $10^{-4}$
- Factors which increase the ability of nitrogen in an amine to share its electron pair increase the basicity of the amine

(Figure 11.3 Protonation)

- The electron-releasing alkyl group (R)
  - Stabilises the substituted ammonium ion by dispersing its positive charge
Organic Chemistry

- Pushes electrons towards nitrogen in the amine (R-NH₂), which makes the unshared electron pair more available for sharing with the proton
- Therefore alkyl amines are stronger bases than ammonia

- Simple aryl group (Ar) has an electron-withdrawing effect NH₂ → Ar : tends to make the unshared electron pair on nitrogen less available for sharing with the proton
- Therefore arylamines are weaker bases than ammonia

\[
\text{Aniline} \quad \begin{array}{c}
\text{C} \\
\text{H} \\
\text{N} \\
\text{H}_2
\end{array}
\]

\[K_b = 4.2 \times 10^{-10}\]

**Figure 11.4 Aniline**

- Basicities of primary, secondary and tertiary alkylamines

Theoretically Tertiary > Secondary > Primary - because of electronic factors
But practically 2° Amine > 1° Amine >3° Amine > NH₃

- Effect of nuclear substitution in aryl amines

**Table 11.1 Dissociation constants \(K_b\) of substituted anilines.**

<table>
<thead>
<tr>
<th>Substituent</th>
<th>(K_b \times 10^{-10})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ortho</td>
</tr>
<tr>
<td>-CH₃</td>
<td>2.5</td>
</tr>
<tr>
<td>-NH₂</td>
<td>3.0</td>
</tr>
<tr>
<td>-OCH₃</td>
<td>3.0</td>
</tr>
<tr>
<td>-NC₂</td>
<td>0.0005</td>
</tr>
<tr>
<td>-Cl</td>
<td>0.05</td>
</tr>
</tbody>
</table>

- Electron-releasing substituents : -CH₃, -NH₂ - Increase basicities of aniline
- Electron –withdrawing substituents : NO₂, Cl - decrease basicities of aniline
- Ortho-substituted amines have lower \(K_b\) than that of aniline, irrespective of the nature of substituent (e-releasing or withdrawing)
- Base-weakening effect of e- withdrawing substituent is most marked when they are present on ortho position.

**11.3 Reactions**

1. **Salt formation**

- Amines being base react with acids to form salts
Figure 11.5 Formation of salt

- When the amine salts are treated with strong base like NaOH, the present amines are regenerated.

\[
\text{R} - \text{NH}_2 + \text{HX} \rightarrow \text{R} - \text{NH}_2\text{X}^{-}
\]

1° Amine

\[
\text{R}_2 - \text{NH} + \text{HX} \rightarrow \text{R}_2 - \text{NH}_2\text{X}^{-}
\]

2° Amine

\[
\text{R}_3 - \text{N} + \text{HX} \rightarrow \text{R}_3 - \text{NHX}^{-}
\]

3° Amine

Figure 11.6 Decomposition of amino salts

\[
\text{R} - \text{NH}_3\text{X}^{-} + \text{NaOH} \rightarrow \text{R} - \text{NH}_2 + \text{H}_2\text{O} + \text{NaCl}
\]

Salt (Soluble in water)

Amine (Insoluble in water)

2. Alkylation

- 10, 20, 30 amines react with alkyl halides to form 40 ammonium salt as an ultimate product.

\[
\begin{align*}
\text{R} - \text{NH}_3 & \quad \xrightarrow{\text{R-x}} \quad \text{R} - \text{NH} \\
\text{R}_2 - \text{NH} & \quad \xrightarrow{\text{R-x}} \quad \text{R}_3 - \text{N} \\
\text{R}_4 - \text{N} & \quad \xrightarrow{\text{R-x}} \quad \text{R}_4 - \text{NH}
\end{align*}
\]

1° Amine \quad 2° Amine \quad 3° Amine \quad 4° Ammonium salt

Figure 11.7 Alkylation

3. Acylation

- 1°, 2° (but not 3°) amines react with acid chloride of carboxylic acids to form substituted amides.
**Figure 11.8 Acylation**

4. Reaction with nitrous acid

$1^0$, $2^0$, $3^0$ amines behave differently in this reaction (Distinction).

### 11.4 Aliphatic Amines

1. Primary amines

\[
R - \text{NH}_2 + \text{NaNO}_2 + \text{HCl} \rightarrow R\text{N}_2\text{Cl} \rightarrow \uparrow \text{N}_2 + \text{Complex mixture of products}
\]

*Figure 11.9 Decomposition of aliphatic diazo salt*

The mixture includes, alcohols, alkenes, alkyl chloride etc.

2. Secondary amines reacts slowly

\[
R_2\text{-NH} + \text{HNO}_2 \rightarrow R_2\text{NN}=\text{O} + \text{H}_2\text{O}
\]

- Nitrous acid
- Nitrosoamine
  (characterized by Libermann’s test)

3. Tertiary amine : dissolves in cold HNO$_2$ and reacts readily

\[
R_3\text{-N} + \text{HNO}_2 \rightarrow R_3\text{N\hspace{1pt} NO}_2 \rightarrow R_2\text{N - NO} + \text{Aldehydes / Ketones}
\]

*Figure 11.10 Action of nitrous acid on tertiary amines*
11.5 Aromatic Amines

11.5.1 Primary amine

• Reacts readily at low temperature and in presence of mineral acid

\[ \text{Ar} - \text{NH}_2 + \text{HNO}_2 \xrightarrow{\text{Cold (0°C)}} \text{Ar} - \text{N}^+ \text{NX} + 2\text{H}_2\text{O} \]

Diazonium salt

**Figure 11.11 Diazotization (Peter Grieze Reaction)**

11.5.2 Secondary amine

\[ \text{Ar} - \text{N} - \text{R} + \text{HNO}_2 \xrightarrow{\text{H}^+} \text{Ar} - \text{N} - \text{N} = \text{O} + \text{H}_2\text{O} \]

N-nitrosoamine (yellow)

**Figure 11.12 Action of nitrous acid on secondary amine**

11.5.3 Tertiary amine

• Undergo nitrosation in aromatic ring and form green coloured p-nitrosoamine

\[ (\text{CH}_3)_2\text{N} - \xrightarrow{\text{Cold}} \xrightarrow{\text{H}^+} (\text{CH}_3)_2\text{N} - \text{N} = \text{O} + \text{H}_2\text{O} \]

N,N-Dimethylaniline       p-Nitroso-N,N-Dimethylaniline (green)

**Figure 11.13 Action of nitrous acid on aromatic tertiary amine**

11.5.4 Reactions with aldehydes and ketones

• Amines undergo condensation with aldehydes and ketones to give products - known as Schiff’s base (or anil)

• The product can be hydrolysed to regenerate parent amines

• Therefore this reaction is used for protecting -NH\textsubscript{2} in certain reaction

**Figure 11.14 Condensation of aniline with aldehydes**
11.5.5 Oxidation

- Arylamines (unlike aliphatic amines) – very susceptible to oxidation - darken in color on standing even at room temperature
- Characteristic colors are obtained by the action of oxidizing agents e.g. controlled oxidation of aniline with chromic acid gives a yellow crystallization substance : p-benzoquinone

\[
\text{NH}_2 \quad \overset{\text{Na}_2\text{Cr}_2\text{O}_7 + \text{H}_2\text{SO}_4}{\longrightarrow} \quad \text{O} = \text{C} = \text{O} \\
\text{p-Benzquinone (yellow)}
\]

Figure 11.15 Oxidation
Lesson 12
ACIDIC CHARACTER OF PHENOLS AND EFFECT OF NUCLEAR SUBSTITUTION ON IT.
IMPORTANT REACTIONS OF PHENOLS

12.1 Introduction

- Phenols are benzene ring hydroxy compounds represented by the general formula Ar-OH
- Where Ar- is phenyl (C₆H₅-) group, substituted phenyl group or aryl group derived from polynuclear aromatic hydrocarbon
- May monohydric, dihydric, trihydric, etc- depending on number of –OH groups attached to the aromatic nucleus
- The simplest member of this family – hydroxybenzene – phenol
- Other phenols are generally named as derivatives of phenol

![Figure 12.1]

- Phenols containing more than one –OH groups in benzene ring are better known their by special names

![Figure 12.2]

- Phenols differ from alcohols in having –OH group attached directly to the aromatic nucleus benzene ring.
- Being hydroxyl compounds, they resemble alcohols in certain respects – e.g. can be converted into ethers and esters
- They differ substantially in most of their properties – therefore they deserve to be classified as different family
12.2 Physical Properties

- Pure phenols are generally colourless solids or liquids, but they turn reddish due to atmospheric oxidation
- Phenol itself is somewhat soluble in water (9 g/100g) – because of H bonding with water
- Most other phenols are insoluble in water
- Its boiling point is very high due to intermolecular H bonding

12.3 Chemical reaction

12.3.1 Salt formation (Acidic character)

- Phenols are fairly acidic compounds which form salts –
- Phenoxide – on reaction with alkali metal hydroxide

\[
\text{R-OH} + \text{NaOH} \rightarrow \text{R-ONa} + \text{H}_2\text{O} \\
\text{Na-phenoxide}
\]

Figure 12.3

- The salt decomposes by mineral acids, carboxylic acids or even by carbonic acid- gives back the free phenol

\[
\text{R-ONa} + \text{H}_2\text{CO}_3 \rightarrow \text{R-OH} + \text{NaHCO}_3 \\
\text{Na-phenoxide} \quad \text{Carbonic acid} \quad \text{Phenol} \quad \text{Na-bicarbonate}
\]

Figure 12.4

R-COONa + H₂CO₃ - No reaction
Therefore phenols are less acidic than carboxylic acids or even H₂CO₃

- Acid strength

<table>
<thead>
<tr>
<th></th>
<th>(K_a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple phenols</td>
<td>(10^{-9})</td>
</tr>
<tr>
<td>Carboxylic acids</td>
<td>(10^{-5})</td>
</tr>
<tr>
<td>Alcohols</td>
<td>(10^{-16}) to (10^{-19})</td>
</tr>
<tr>
<td>Carbonic acid</td>
<td>(10^{-7})</td>
</tr>
</tbody>
</table>
• Phenols are much more acidic than alcohols but less than carboxylic acids even carbonic acid
• Electron-withdrawing substituents -NO₂, -Cl, etc. enhance the acidity of phenols, but electron releasing substitutes -CH₃ decreases it.

12.3.2 Esterfication

• Phenols react with acids, in presence of polyphosphoric acid or toluene sulphonlic acid to give phenyl esters
• The yields are poorer than those obtained in case of alcohols

![Figure 12.5](image)

\[
\text{R-C-OH} + \text{HO-Ar} \rightleftharpoons \text{R-C-O-Ar} + \text{H₂O}
\]

Carboxylic acid
phenol
Phenylester

Figure 12.5

• Phenyl esters are better prepared by the action of acid chlorides or acid anhydrides

![Figure 12.6](image)

\[
\text{Phenyl benzoate}
\]

\[
\text{O₂N} + \text{H₃C-C-O-C-CH₃} + \text{OH⁻} \rightarrow \text{O₂N-C-O-C-CH₃} + \text{CH₃COOH}
\]

\[
\text{N - Nitrophenol} + \text{Acetic anhydride} \rightarrow \text{N - Nitrophenyl acetate} + \text{Acetic acid}
\]

Figure 12.6

12.3.3 Etherification

• Phenols can be converted into ethers by treatment with alkyl halides or alkylsulphates =

![Figure 12.7](image)

\[
\text{Ethoxy benzene (Phenetole)}
\]

\[
\text{Anisol}
\]

(Figure 12.7)
12.3.4 Reaction with ferric chloride

- Phenols (unlike alcohols) give characteristic colours with neutral ferric chloride – green, blue, violet, red etc.
- Due to formation of complexes, but their precise nature is unknown.

12.3.5 Libermann Nitroso reaction

- When phenol is treated with NaNO₂ and concentrated H₂SO₄, it develops a deep green or blue colour which turns red on careful dilution with water. Addition of NaOH solution brings back the green or blue color.

(Figure 12.8 Libermann nitroso reaction)

12.3.6 Phthalein reaction

- When phenols are heated with phthalic anhydride in presence of concentrated H₂SO₄ or anhydrous ZnCl₂, phthaleins are formed – give characteristic colour depending on reaction medium pH.

(Figure 12.9 Phthalein reaction)
12.3.7 Reaction with formaldehyde

- Results into formation of polymer – phenol-formaldehyde resins - also known as Bakelite
- It is among the oldest of the synthetic polymers and still extremely important
- When phenol is treated with formaldehyde in the presence of acid or alkali – polymer is formed

(Figure 12.10 Condensation of phenol with formaldehyde)

- Stages involved in the formation of the polymer
- Phenol reacts with formaldehyde to form o- or p- hydroxymethyl-phenol
- Hydroxymethyl phenol than reacts with another molecule of phenol with loss of water to form compound in which two rings are joined by a \(-\text{CH}_2-\) link
- This process than continues to yield a product of high molecular weight- a polymer (Thermosetting polymer)
- Since three positions in each phenol molecule are susceptible to attack, the final product contains many cross-links
- Hence has a rigid linear or cross-linked structure.

★★★★★
Module 5. Amino acids and peptides

Lesson 13
SYNTHETIC AND NATURAL AMINO ACIDS

13.1 Introduction

Amino acids which are obtained from natural sources, proteins are called natural amino acids. Amino acids which are synthesized by various methods are called synthetic amino acids. Because of the importance of amino acids in organic chemistry, bio-chemistry, medicine, food etc, a great deal of research has been devoted to develop methods for the synthesis of specific amino acids with the result most of the commercially available amino acids are prepared now-a-days synthetically.

Generally, a synthesis is a more convenient way of preparing an amino acid than preparing it from natural sources.

Several important methods for synthesizing α-amino acids are discussed below.

13.2 Amination of α-Halogenated Acids

An α-halogenated acid is treated with a large excess of ammonia. α-halogenated acids are more easily obtained by treating aliphatic carboxylic acids with chlorine or bromine in the presence of a small amount of phosphorous. Remember that in this reaction only α-hydrogen atom is replaced by halogen.

Glycine, alanine, serine, threonine, valine, and leucine, have been prepared by this method

13.3 Gabriel - Phthalimide Synthesis

Based on the "R" group present in α-halogenated acid ester the different kinds of amino acids will be formed.
If R= H then it is Glycine.
R = CH₃- then it is Alanine.
R = (CH₃)₂CH- group (Isopropyl) then it is Valine.

and so on. As we change the "R" group attached to α-halogenated acid ester we get different kinds of amino acids.

13.4 Malonic Ester Synthesis

This method is really an extension of method no:1 as it offers a means of preparing α-halogenated acids. This method offers a means for preparing the following acids: phenylalanine, proline, leucine, isoleucine, and methionine.

13.5 Phthalimidomalonic Ester Synthesis

This method is the combination of the previous two methods, malonic ester synthesis and Gabriel pthalimide synthesis.
In this method pthalimidomalonic ester (prepared from potassium phthalimide and monobromomalonic ester) is treated with the appropriate alkyl chloride in presence of sodium ethoxide and the product is hydrolysed to yield the amino acid.

Phenylalanine, cystine, tyrosine, proline, serine, aspartic acid, lysine and methionine have been synthesized by this method.

13.6 Streecker Synthesis
In this method an aldehyde or ketone is treated with a mixture of ammonium chloride and sodium cyanide (ammonium cyanide) to form cyanohydrin which on hydrolysis gives the corresponding amino acid.

This method is useful for preparing glycine, alanine, serine, valine, methionine, glutamic acid, leucine, and phenylalanine.

★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★
Lesson 14
GENERAL PROPERTIES OF AMINO ACIDS

14.1 Introduction

Figure 14.1 General structure of amino acid

\[
\begin{align*}
\text{H} \\
R - \text{C} - \text{NH}_2 \\
\text{COOH}
\end{align*}
\]

R- Side chain-aliphatic, aromatic or heterocyclic
Have both an amino and a carboxylic acid groups attached to the same carbon atom

- Amino group is on the carbon atom adjacent to carboxyl group (α-carbon atom) - therefore known as α-amino acids
- All naturally occurring amino acids have ‘L’ configuration therefore α-L-amino acid- obtained by acid or enzymatic hydrolysis of proteins
- On hydrolysis with alkali - L form converted into mixture of ‘D’ and ‘L’ form (Racemization).
- D-amino acids occurs in cells and some peptides (e.g. antibiotics containing peptide group) but not in proteins
- About 20 amino acids are usually found as constituents in most of the naturally occurring proteins
- More than 80 amino acids exist in small concentration - specific for proteins derived from specific source-not usual and not always found

e.g. Citrulline= in watermelon
Lanthionine= in sheepwool
Hydroxylysine= in collagen of body tissue

- Different amino acids have different taste -bitter (arginine), sweet (glycine), tasteless (tyrosine), aspartame (substitute for sugar, known as sugar free gold).
- Classified according to chemical nature of side chain ‘R’
- Amino acids (naturally occurring)
**Figure 14.2**

**Different Types Of Amino Acids**

<table>
<thead>
<tr>
<th>Type</th>
<th>Name</th>
<th>Abbreviation</th>
<th>Structural Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple</td>
<td>Glycine</td>
<td>Gly</td>
<td>H - C - COOH</td>
</tr>
<tr>
<td></td>
<td>Alanine</td>
<td>Ala</td>
<td>H - C - COOH</td>
</tr>
<tr>
<td></td>
<td>Valine</td>
<td>Val</td>
<td>H2C - CH - CH - NH2</td>
</tr>
<tr>
<td></td>
<td>Leucine</td>
<td>Leu</td>
<td>H2C - CH - CH - NH2</td>
</tr>
<tr>
<td></td>
<td>Isoleucine</td>
<td>Ileu</td>
<td>H2C - CH - CH - NH2</td>
</tr>
<tr>
<td>Hydroxy</td>
<td>Serine</td>
<td>Ser</td>
<td>HO - CH - C - COOH</td>
</tr>
<tr>
<td></td>
<td>Threonine</td>
<td>Thr</td>
<td>H2C - CH - C - COOH</td>
</tr>
<tr>
<td>Basic</td>
<td>Lysine</td>
<td>Lys</td>
<td>H2N - CH - CH - CH - CH - C - COOH</td>
</tr>
<tr>
<td></td>
<td>Arginine</td>
<td>Arg</td>
<td>H2N - C - N - C - C - COOH</td>
</tr>
<tr>
<td>Acidic</td>
<td>Aspartic Acid</td>
<td>Asp</td>
<td>HOOC - CH - C - COOH</td>
</tr>
<tr>
<td></td>
<td>Glutamic Acid</td>
<td>Glu</td>
<td>HOOC - CH - CH - C - COOH</td>
</tr>
<tr>
<td>Amide</td>
<td>Asparagine</td>
<td>Asp(NH2)</td>
<td>H2N - C - CH - C - COOH</td>
</tr>
<tr>
<td></td>
<td>Glutamine</td>
<td>Glu(NH2)</td>
<td>H2N - C - CH - CH - C - COOH</td>
</tr>
<tr>
<td>Sulphur Containing</td>
<td>Cysteine</td>
<td>Cys</td>
<td>HS - CH - C - COOH</td>
</tr>
<tr>
<td></td>
<td>Cystine</td>
<td>Cys - Cys</td>
<td>HOOC - CH - S - S - CH - C - COOH</td>
</tr>
<tr>
<td></td>
<td>Methionine</td>
<td>Met</td>
<td>H2C - S - CH - C - COOH</td>
</tr>
</tbody>
</table>
Note on the given Table

- Most of the amino acids contain C, H, O and N elements, but a few contain ‘S’ also
- All amino acids, except glycine, contain an asymmetric carbon atom = therefore they are optically active
- Most of the α-amino acids are Laevo (-) rotator, but some are Dextro (+) rotator as well.
- Except proline and hydroxy proline, all other amino acids have common denominations and free carboxylic group and a free unsubstituted amino group on the ‘α’ carbon atom
- Proline and hydroxyproline are immino acids
- Some amino acids contain free or potentially free second carboxyl group, such amino acids - called acidic amino acids
- Some amino acids contain second basic group like amine, guanidine, amidazole, etc - known as basic amino acids.

14.2 Essential Amino Acids

- “Amino acids which are not synthesized in human body and these must be supplied in diet from exogenous sources for normal physiological functions of the body”
- Indispensable (essential) amino acids — include —


“A HILL MP TTV”

- Ten amino acids which the human body can synthesize are called non-essential or dispensable amino acids.
Lesson 15

ZWITTER ION FORM AND ITS PROPERTIES VIZ. MELTING POINT AND VOLATILITY

15.1 Introduction

- Molecular formula - free carboxylic and amino groups
- Several behaviors (properties) - not in accordance with the structure - abnormal behavior/properties - e.g.

1. Relatively non-volatile, crystalline solids - melt with decomposition at fairly high temperature (~200 °C) (if amine and carboxylic acid)
2. Insoluble in non-polar solvents (benzene) but soluble in polar solvents (water)
3. Neutral substances have $K_a 1.6 \times 10^{-12}$ and $K_b 2.5 \times 10^{-12}$ - aliphatic amines have $k_b 10^{-14}$ and carboxylic acids have $k_a 10^{-5}$
4. Aqueous solutions - have a high dielectric constant - dipole movement - reflection occurrence of both positive and negative charges on the same molecule

- These abnormal properties can be explained by - existence of dipolar (salt like) structure of amino acids

\[
\begin{align*}
\text{H} \\
\text{R} \quad \text{C} \quad \text{COO}^- \\
\downarrow & & \downarrow \\
\text{NH}_3 & & \text{COO}^-
\end{align*}
\]

**Figure 15.1**

The inner salt structure - results due to salt forming character of acids and amines

- Inner salt - Zwitter ion or ampholyte or dipolar ion.

\[
\begin{align*}
\text{H} \\
\text{R} \quad \text{C} \quad \text{COO}^- \\
\downarrow & & \downarrow \\
\text{NH}_3 & & \text{COO}^-
\end{align*}
\]

**Figure 15.2**

Electrostatic attraction (--------) between oppositely charged groups - stabilizes crystalline state - therefore high melting point.
Figure 15.3

Weak electrostatic interaction (------) between amino acid molecules and water molecules - increases solubility (decrease in potential energy=P.E.).

- Zwitter (dipolar) form of amino acid remains in equilibrium with non-zwitter (apolar) form of amino acid- therefore amino acid can undergo the typical reactions of amines and carboxylic acid

Figure 15.4

15.2 Amphoteric Nature of Amino Acids

- Compound - reacts both with acid and base - amphoteric compound
- This characteristic - amphoteric nature
- Zwitter ion of amino acids in water - acts as an acid as well as base - proton donor as well as proton acceptor- i.e. have amphoteric nature

Amphoteric Nature Of Amino Acids

- Acidic group of amino acid: -NH\(^+\_3\) - donates proton
- Basic group of amino acid: -COO\(^-\) - accept proton
15.3 Isoelectric Point of Amino Acids

- Amino acid in solutions - action of electric field - moves towards anode or cathode - depending on pH of the solution
- In alkaline solution - amino group predominates - therefore migrate towards anode
- In acidic solution - cation predominates - therefore migrate towards cathode
- At particular pH - Zwitter ion may be present (or cation and anion present in equal amount) - no net migration of amino acid - isoelectric point pH - pI
- “A particular pH at which a particular amino acid does not have net charge and does not migrate under the influence of an electric field is called isoelectric point of that amino acid”.
- Each amino acid has a characteristic isoelectric point - e.g. 6.1 for gly
- In general,
  - Monoamino monocarboxylic acid - pI - 6.0
  - Diamino monocarboxylic acid - pI = > 6.0
  - Monoamino dicarboxylic acid - pI = < 6.0

- At pI amino acid has minimum solubility - used for separation of amino acids in mix.

**Table 15.1 Solubility of different amino acids in water at 25 °C (g/l)**
When Zwitterions is titrated with acid, the $\text{–COO}^-$ group becomes protonated ($\text{–COOH}$)

pH at which concentration of $\text{–COO}^-$ and $\text{–COOH}$ are equal - known as $\text{pK}_{a1} (-\log K_{a1})$

When Zwitterions is titrated with base, the $\text{–NH}_3^+$ group becomes deprotonated ($\text{–NH}_2$)

pH at which concentration of $\text{–NH}_3^+$ and $\text{–NH}_2$ are equal - known as $\text{pK}_{a2} (-\log K_{a2})$

In addition to $\alpha$-$\text{NH}_2$ and $\alpha$-$\text{COOH}$ groups, side chains of some amino acids also contain ionizable groups.
Arg., Asp., Cys., Glu., His., Lys, and Tyr

- pH at which concentration of their respective ionized and unionized form are equal - known as $pK_{a3}$ (-log$K_{a3}$)

- $pI$ of amino acids can be estimated from their $pK_{a1}$, $pK_{a2}$ and $pK_{a3}$, using the following expressions

  - For neutral amino acids $pI = (pK_{a1} + pK_{a2})/2$ (no charged side chain)
  - For acidic amino acids $pI = (pK_{a1} + pK_{a3})/2$
  - For basic amino acids $pI = (pK_{a2} + pK_{a3})/2$

- In proteins, the $\alpha$-COOH of one amino acid is coupled to $\alpha$-NH$_2$ group of the next amino acid through peptide (amide) bond - these groups are not free to ionize
- The only ionisable groups in protein are
  - N-terminal –NH$_2$
  - C-terminal –COOH group and
  - Ionizable groups present in side chain of amino acids

- The $pK_a$ of ionisable groups in side chain of amino acids are different from those of free amino acids

- In protein, the $pK_{a3}$ values of
  - Acidic amino acids (Glu and Aps) are larger and
  - Basic amino acids (Arg and Lys) are smaller than those of the corresponding free amino acids

  - Degree of ionization of a group at a given pH of solution can be calculated using **Henderson-Hasselbach equation.**

\[
\text{pH} = pK_a + \log \frac{[\text{Conjugated base}]}{[\text{Conjugated acid}]}
\]

### 15.4 Hydrophobicity

- One of the major factors affecting physicochemical properties of peptides and protein is Hydrophobicity of the constituent amino acid residues.

- Structure, solubility, taste, interaction with other elements and/or constituents

- Defined as the excess free energy of a solute dissolved in water compared to that in an organic solvent (e.g. ethanol) under similar conditions
### Table 15.2 Hydrophobicity of amino acid side chain at 25°C (kJ/mol)

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>Hydrophobicity at 25°C (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>2.09</td>
</tr>
<tr>
<td>Arginine</td>
<td>-</td>
</tr>
<tr>
<td>Asparagine</td>
<td>0.00</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>2.09</td>
</tr>
<tr>
<td>Cysteine</td>
<td>4.18</td>
</tr>
<tr>
<td>Glutamine</td>
<td>-0.42</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>2.09</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.00</td>
</tr>
<tr>
<td>Histidine</td>
<td>2.09</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>12.54</td>
</tr>
<tr>
<td>Leucine</td>
<td>9.61</td>
</tr>
<tr>
<td>Lysine</td>
<td>-</td>
</tr>
<tr>
<td>Methionine</td>
<td>5.43</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>10.45</td>
</tr>
<tr>
<td>Proline</td>
<td>10.87</td>
</tr>
<tr>
<td>Serine</td>
<td>-1.25</td>
</tr>
<tr>
<td>Threonine</td>
<td>1.67</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>14.21</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>9.61</td>
</tr>
<tr>
<td>Valine</td>
<td>6.27</td>
</tr>
</tbody>
</table>

*****😊*****
Lesson 16
IMPORTANT REACTIONS OF AMINO ACIDS

16.1 Introduction

1. Reaction with formaldehyde

- Zwitter ion structure is eliminated - carboxylic acid group sets free - can be quantitatively titrated against standard alkali solution using phenolphthalein as an indicator - used for quantitative determination

2. Peptide bond formation

- COOH group of α-carbon of one amino acid reacts with –NH₂ group of α-carbon of another amino acid - formation of peptide bond

- One more –NH₂ group of amino acid react with free –COOH group of dipeptide to give tripeptide - the reaction can go on indefinitely hundreds of amino acids
- When 100 or more amino acids are condensed through peptide linkage resulting a polypeptide having molecular weight/mass more than 10,000 is called protein. So α-amino acids are the building blocks of proteins.
3. Reaction with ninhydrin

- Often used for qualitative and quantitative analysis of free amino acids
- When an excess amount of ninhydrin reacts with amino acid
  - For each mole of amino acid reacted with ninhydrin, one mole each of ammonia, aldehyde, carbon dioxide and hydrindantin are formed as intermediates.

**Figure 16.4 Reaction with ninhydrin**

- The liberated ammonia subsequently reacts with one mole of ninhydrin and one mole of hydrindantin, forming a purple product
  - Known as Ruhemann’s purple
  - Has maximum absorbance at 570 nm
- Proline and hydroxyproline give a yellow product that has a maximum absorbance at 440 nm
- These colour reactions are the basis for colorimetric determination of amino acids

**Figure 16.5 Estimation of amino acids**
Module 6. Proteins

Lesson 17
DEFINITION AND CLASSIFICATION OF PROTEINS

17.1 Introduction

- The terms ‘protein’ - coined by Dutch physiological chemist G.J. Mulder in 1838.
- The term - derived from Greek word “πρώτα” - meaning- the first or the top most position or the eminent of the significant
- Protein- a common constituent of all biological materials, without which life is not possible - an essential constituent of all living cells.
- Average 2/3rd of total dry of the cell - composed of protein
- A complex nitrogenous organic compound – a polymer of amino acids - therefore defined as

“Protein may be defined as high molecular weight polymers of low molecular weight monomers known as amino acids, which are linked to gather by peptide bonds”

17.2 Elementary Composition

- Important elements – C, H, O and N
- Sometimes - S, P and I

Table 17.1

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Element</th>
<th>Symbol</th>
<th>Range (%)</th>
<th>Average (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Carbon</td>
<td>C</td>
<td>50-55</td>
<td>52</td>
</tr>
<tr>
<td>2</td>
<td>Hydrogen</td>
<td>H</td>
<td>6-8</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>Oxygen</td>
<td>O</td>
<td>20-23</td>
<td>22</td>
</tr>
<tr>
<td>4</td>
<td>Nitrogen</td>
<td>N</td>
<td>15-18</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>Sulphur</td>
<td>S</td>
<td>0-4</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>Phosphorus</td>
<td>P</td>
<td>0-1</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

- Some time Fe, Zn, Cu, Mn, Mg, etc found present
  - Not directly attached with amino acids of proteins
  - Attached to non-protein substances to form complex which inturn attach to amino acid
17.3 Classification

- Classified on the basis of composition, shape of molecules and solubility

17.3.1 On the basis of composition

Three groups- simple, conjugated and derived proteins
1. Simple proteins
   - Consist of only amino acids- do not contain other class of compounds

2. Conjugated proteins
   - Consist of amino acids as well as other class of compounds
   - Further classified into six subgroups

Table 17.2

<table>
<thead>
<tr>
<th>Sr No.</th>
<th>Class</th>
<th>Other compound present</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chromoprotein</td>
<td>Coloured pigment</td>
<td>Haemoglobin</td>
</tr>
<tr>
<td>2</td>
<td>Glycoprotein</td>
<td>Carbohydrate</td>
<td>Mucin, Ig</td>
</tr>
<tr>
<td>3</td>
<td>Phosphoprotein</td>
<td>Phosphoric acid</td>
<td>Casein (in milk)</td>
</tr>
<tr>
<td>4</td>
<td>Lipoprotein</td>
<td>Liquid</td>
<td>Lipovitelin (in egg yolk)</td>
</tr>
<tr>
<td>5</td>
<td>Nucleoprotein</td>
<td>Nucleic acid</td>
<td>Viruses</td>
</tr>
<tr>
<td>6</td>
<td>Metalloprotein</td>
<td>Metal</td>
<td>Circulating (Cu)</td>
</tr>
</tbody>
</table>

- Obtained from simple or conjugated protein as derivative - generally hydrolysis products - proteose, peptone, peptide and ultimately α-amino acids.

The sequence is characterized by decreasing order of molecular weight with increasing order of solubility in water

17.3.2 On the basis of shape of molecules

Two main groups : fibrous proteins and globular proteins
1. Fibrous proteins
   - Long and thread or ribbon like, lie side by side to form fibers
   - Generally insoluble in water - because of strong intermolecular attraction
   - Serve as chief structural material of animal tissues
   - e.g. keratin, myosin, collagen etc
2. Globular proteins

- Spherical in shape
- Generally soluble in water of aqueous solution of acid, base or salt
- Involved in physiological processes of animal body
- e.g. enzymes, some hormones, haemoglobin, etc.

17.3.3 On the basis of solubility

1. Albumin – soluble in distilled water, dilute salt, acid and base solutions
2. Globulin- insoluble in distilled water, but soluble in dilute salt, acid and base solutions
3. Protamine and Histones- highly soluble in distilled water - because small molecules, stable to heat i.e. not coagulated. Protamine soluble in NH₄OH, whereas Histones insoluble NH₄OH.
4. Glutelins- insoluble in distilled water and alcohol but soluble in dilute acid and base solution.
5. Prolamine- insoluble in distilled water, but soluble in dilute acid, dilute base and 70-80% alcohol
6. Scleroproteins- insoluble in most of the solvents- water, dilute acid, dilute base, dilute salt etc.
   They are fibrous proteins

17.4 Functions of Proteins

- Nutritional and physiological

17.4.1 Nutritional functions

1. Primary and important function - role as a source of essential amino acids - for synthesis of new cells and maintenance of wear and tear
2. Supply energy - 4.4 kcal/g

17.4.2 Physiological functions

1. Structural - fibrous protein- keratin (hair), myosin (muscles), etc
2. Catalyst- enzymes – in digestion, absorption etc
3. Transport- haemoglobin (O₂) , Albumin (fatty acids, amino acids)
4. Regulation - hormones- insulin- digestion
5. Buffering- albumin maintain pH and osmotic pressure
6. Protection - immunoglobulins (antibodies)

***** ☺ *****
Lesson 18
A PRIMARY, SECONDARY, TERTIARY AND QUATERNARY STRUCTURE OF PROTEINS

18.A.1 Introduction

Four basic structural levels of organization in proteins – primary, secondary, tertiary and quaternary.

1. Primary structure: \( \text{H}_2\text{N}---------\text{COOH} \)
   - Refers to combination of amino acids in a proper sequence via peptide bonds (a covalent bond)
   - A linear sequence of amino acid residues making a polypeptide chain

2. Secondary structure
   - Refers to the regular reoccurring arrangement of polypeptide chain in one direction
   - A helical spiral of polypeptide is formed, which is stabilized by hydrogen bonding

3. Tertiary structure
   - Refers to bending or folding of polypeptide chain in three dimensional space.
   - Stabilize by disulphide linkage, salt linkage, hydrophobic bond, dipole-dipole interaction, phosphodiester linkage etc.

4. Quaternary structure
   - Refers to how the individual polypeptide chains in the protein are arranged relative to each other-
     arrangement and interrelationship of the polypeptide chains
   - Most of the high mol.wt. proteins consist of two or more polypeptide chains- known as oligomer proteins
   - Stabilized by any one or all types of forces which can exist between amino acids side chain, except covalent bond.

18. A. 2 Primary Structure

- Refers to the linear sequence in which the constituent amino acids are covalently linked through peptide (amide) bonds - results from condensation of \( \alpha\)-COOH group of one amino acid and \( \alpha\)-NH\(_2\) group of another amino acid
- All the amino acids are in L-configuration
- Protein with \( n \) amino acid residues contains \( n-1 \) peptide linkages
- In a given protein

- Type of amino acids
- Number of amino acids and
- Sequence in which the amino acids are linked
• Determine the physical, chemical, biological, functional and structural properties of the protein

• The peptide bond is represented as a single covalent bond. However in reality it has a partial double bond character- because of resonance structure caused by delocalization of electrons

![Figure 18.1 Resonating structure](image_url)

• This has several important structural implications in proteins

1. The resonance structure includes protonation of peptide group
2. The partial double bond character, the rotation of peptide bond is restricted to a max. of 60°, known as ‘w’ angle - restricted rotational freedom drastically reduces backbone flexibility. Only N-Cα and Cα-C bonds have rotational freedom – termed as φ (phi) and ψ (psi) dihedral angles respectively.

3. Delocalization of electrons impart partial : positive charge to hydrogen atom of the group and negative charge to oxygen atom of the group. - because this hydrogen bonding between and groups of the peptide backbone is possible under appropriate conditions
4. Four atoms attached to the peptide bond can exist either in cis or trans form

![Figure 18.2 Geometrical isomerism in peptide linkage](image_url)

• However, almost all protein peptide bonds exists in trans configuration - because grater thermodynamic stability
• Since trans-cis transformation increases free energy of the peptide bond by 34.8 kJ/mol, such isomerization does not occur in protein.
18. A.3 Secondary Structure

- Refers to periodic spatial arrangement of amino acid residues
- The periodic (regular) structures – two forms
  - Helical structure
  - Extended sheet-like structure

18.A.3.1 Helical structure

- Three types – α-helix, 310-helix and π-helix
  - However, α-helix is the major form found in proteins and it is the most stable form among the three forms
  - The α-helixes are stabilized by hydrogen bonding
  - Each backbone group is hydrogen bonded to the >C=O group of the fourth preceding residue
  - The pitch of the helix (axial length occupied per rotation) – 5.40 Å
  - Each amino acid residue extends the axial length by 1.50 Å
  - Each helical rotation involves 3.6 amino acid residues
  - Thirteen backbone atoms are in the hydrogen bonded loop- therefore α-helix is sometimes also called 3.613 helix
  - H bonds are oriented parallel to the helix axis
  - N, H, and O atoms at the H bond lie almost in a straight line - therefore H bond angle is almost zero
  - H bond length is about 2.90 Å
  - Strength of H bond is about 18.8 kJ/mole
  - The α-helix can exist in either a right or left handed orientation
  - However, the right handed orientation is more stable
  - In proline and hydroxy proline residues

![Figure 18.3 Proline and hydroxyproline](image)

1. N of the N-H group is involved in ring structure- rigid - therefore rotation of the N-Cα bond is not possible, therefore φ angle has a fixed value of 70°
2. H of the N-H involves in peptide bond formation- therefore no free H atom remains available to form hydrogen, therefore it can not form H bond

- Because of these two attributes segment containing high amount of proline or hydroxyproline residues and their uniform distribution can not form α-helices- therefore these amino acid residues are considered to be an α-helix breakage.
- e.g. In β-casein proline residues constitute 17% of the total amino acids and 8.5% in αs1 casein - therefore α-helices are not present in these proteins, therefore these proteins have random structure
18.A.3.2 β-sheet structure: (Extended structure)

- The \(\text{C}^\text{\scriptsize{O}}-\text{N}^\text{\scriptsize{H}}\) groups are oriented perpendicular to direction of the chain - therefore hydrogen bonding is possible only between segments (i.e. intersegments) and not within a segment (i.e. intersegment)
- The β-strands are usually about 5-15 amino acid residues long
- In proteins, two β-strands of the same molecule interact via hydrogen bonds, forming a sheet-like structure - known as β-pleated sheet
- In this structure, the side chains of amino acid are oriented perpendicular (above and below) to the plane of the sheet
- Depending on the N→C directional orientation of the strands - two types of β-sheet structures can form: parallel sheet and antiparallel sheet
- In parallel β-sheet the direction of the β-strands run parallel to each other
- In antiparallel β-sheet the direction of the β-strands run antiparallel (opposite) to each other

\[ \beta - \text{Sheet Structure} \]

(Figure 18.4 β-sheet structure)

- These differences in chain directions affect the geometry of H bonds.
- In antiparallel β-sheets N-H----O-C atoms lie in a straight line- zero H-bond angle, which enhances the stability of this bond
- In parallel β-sheets the atoms lie at an angle, which reduces stability of the H bonds

Therefore antiparallel β-sheets are more stable than parallel β-sheets

- Polypeptide segments containing alternating polar and nonpolar residues have a high propensity to form β-sheet structure
- Segment rich in bulky hydrophobic side chains (e.g. Val and Ile) also have tendency to form β-sheet structure
18.A.4 Forces Involved In Structural Organization of Proteins

1. Peptide bond: (Amide linkage)
   - Back bone of protein structure and covalent in nature
   - Formed by reaction of $\alpha$-NH$_2$ group of one amino acid with $\alpha$-COOH of another amino acid.

   ![Amide Linkage](image)

   (Figure 18.5 Formation of Peptide linkage)

2. Hydrogen Bond
   - A weak electrostatic interaction between two strongly electronegative atoms (e.g. O and N) via hydrogen atom in Fig. 18.6.
   - In protein hydrogen bond is formed between oxygen of carbonyl group (-C=O-) and nitrogen of imino group (-N-H-)

   ![Figure 18.6 Formation of hydrogen bond](image)

3. Disulfide linkage
   - Formed by reaction of two sulphydryl groups of cystein

   ![Figure 18.7 Disulfide Linkage](image)
4. Hydrophobic bond

- Formed when two long chains of hydrocarbon or aromatic rings come nearer to each other
- They have tendency to repel the water

![Figure 18.8 Hydrophobic Bond](image)

5. Ionic bond

- Formed by interaction between two opposity charged ions or groups—e.g. positively charged group of basic amino acid (lysine) and negatively charged group of acidic amino acid (glutamic or aspartic acid)

![Figure 18.9 Ionic bond](image)

6. Salt bridge

- Formed by interaction between two negatively charged groups of acidic amino acids with divalent cation.

![Figure 18.10 Salt bridge](image)

7. Dipole-dipole interaction

- Formed by interaction between two hydroxyl groups of hydroxy amino acids (serine and threonine)
8. Phosphodiester linkage

- Formed by interaction of two hydroxyl groups of hydroxy amino acids with a phosphoric acid molecule

![Phosphodiester Linkage](image)

**18.5 Schematic Representation of Different Types of Forces Or Interactions In Protein Molecule**

(Figure 18.13 Schematic representations of different types of forces or interactions in protein molecule)

**18.5.1 Terminals of proteins**

H₂N--------------------------COOH

N-terminal C-terminal
• Information of peptide chain
• At one end –NH$_2$ group remains free – N- terminal- L.H.S.
• At other end –COOH group remains free- C- terminal- R.H.S.

**18.1 Introduction**

• Three important conformations of proteins- helical structure (α-helix), extended chain structure (β-structure) and folded (globular) structure

  1. Helical structure (α-helix)

  • Obtained by winding the peptide chain around an imaginary cylinder to form one turn to the next
  • The winding - in such a way that –CONH- groups faces each other at a suitable distance
  • First proposed by Poulung and Corey.

![Helical Structure](image)

(Figure 18.14 Helical Structure)

**18.B.1.1 Important characteristics**

a) Peptide bonds are planner
b) Right handed helix – most stable conformation of polypeptide chain
c) ‘R’ groups- protruded outward- therefore tight helix formation
d) Number of amino acids per turn of the helix- 3.6
e) Distance travelled per turn – 5.4 $\text{Å}$ (0.54 nm)
f) The helix – stabilized by hydrogen bonds H atom attached to peptide N and O attached to peptide C-bond length 2.8$\text{Å}$
g) Generally present in globular proteins
h) Certain amino acids disrupt the α-helix formation e.g.

- proline and hydroxyproline –α-N is in rigid ring structure—therefore no free rotation (turning)- therefore causes sharp bending of polypeptide.
• if charge groups (\(-\text{COO}^-, \text{NH}_3^+\), etc) in R-are cultured – cause electrostatic repulsion – disrupt the \(\alpha\)-helix formation.

2. Extended chain: (\(\beta\)-structure)

• In this structure- peptide chains are essentially linear and tend to lie side by side
• The structure is maintained by multiple hydrogen bonding between the peptide chains

(Figure 18.15 Extended Chain)

• Two adjacent polypeptide chains run in antiparallel direction- one chain run from N-terminal to C-terminal while the other run from C- terminal to N-terminal
• Each of the chain also form hydrogen bond with still another polypeptide chain and so on – therefore large protein aggregate
• The structure- formed only if R groups are relatively small- so that the chains held sufficiently near to form hydrogen bonds – e.g. amino acids glycine , alanine, serine etc
• Found in structural proteins – i.e. fibrous proteins

18.B.2 Properties of Proteins

1. Each protein has its own isoelectric point pH (casein 4.6)
2. Amphoteric in nature- reacts both with acid and alkali
3. Can bind both anion and cation- some ions form insoluble salt – causes precipitation of protein – used for isolation
4. Solubility depends on pH and ionic composition
5. May interact with aqueous solution and swell – gel structure
6. Have optical activity - because activity of amino acids
7. Absorb in UV light at 280 nm because of aromatic ring of aromatic amino acids – used for estimation of proteins
8. Many proteins can be obtained in crystalline form.
18.B.3 Native State of Proteins

• Highly ordered conformation in which biological activity is manifested
• Have three dimensional configuration
• Native – as such or unchanged- as occurring in nature

18.B.4 Denaturation Of Proteins

• Any change caused in native state of protein
• Fundamentally a disorganization of molecular configuration
• Does not include cleavage of peptide bond- primary structure of protein is not affected.
• Secondary, tertiary and quaternary structure of proteins are affected- forces responsible for these
structures are destroyed partially or completely

18.B.5 Effect Of Denaturation On Proteins

1. Decreased solubility and loss of biological value e.g. enzymes
2. Soluble globular protein changed to insoluble – fibrous
3. Increased reactivity – reactive groups- exposed- because unfolding
4. Loss of crystallizability
5. Marked increase in viscosity of solution
6. Increased susceptibility to enzymatic hydrolysis- therefore better digestibility- therefore food is
cooked
7. Change in sedimentation pattern

18.B.5.1 Denaturing agents

Certain reagents and conditions

1. Physical treatments- heat, UV-light, violent agitation
2. Chemical reagents

• Organic solvents- alcohol, acetone etc – breaks H bonds
• Acids and alkalies- break ionic bonds and slat bridges
• Salts of heavy metals- AgNO₃, HgNO₃ – cation binds with –COO⁻ - precipitation
• Alkloidal reagents- tannic acid, picric acid, phosphotungestic acid
• Urea- used for fractionation of casein (α, β, κ, γ)
• Detergent- Lauryl sulphate- ionic bonds
• Oxidising and reducing agents= -S-S- bond – for hair style change
• Dyes- microscopic examination of micro organisms after staining, determination of protein

18.B.6 Colloidal Behaviour of Proteins

• Most of the proteins form colloidal solution – of emulsion type- solid dispersed in liquid medium
• Both hydrophobic and hydrophilic interaction present in protein – therefore colloidal state forms.
• Stability of colloidal particle rests on – charge and hydration
18.B.6.1 Charges

- Because ionized groups on the surface = -COO\(^{-}\), PO\(_4\)^{3-}, NH\(_3^+\), etc in case of proteins
- Equal and similar charge on all the particles
- Similar charge – electrostatic repulsion- keeps the particle away from each other

18.B.6.2 Hydration

- Water binding capacity= because hydrophilic groups such as –OH, side chain R of amino acids, serine and threonine
- Forms a covering layer around the particle and prevents intimate contact between the particles
- Charge and water of hydration- removed= coagulation of protein

(Figure 18.16 Colloidal behaviour of proteins)

- Charge and water of hydration- vary from protein to protein= because variation in amino acids make-up = qualitative and quantitative

18.B.7 Note

* Isoelectric point

Like amino acids, there exists for each protein a certain pH, known as the isoelectric point, at which its ionization is minimum and it is least soluble. For example casein, the major milk protein, has an isoelectric point of 4.6. This character of protein is often made use of in their isolation.
Lesson 19
QUALITATIVE TEST FOR PROTEINS

19.1 Introduction

As the name suggests amino acids are organic compounds that contain amino and carboxyl groups. The R- in the above formula stands for different chemical groups (may be aliphatic, aromatic or heterocyclic) and this determines the characteristics of the amino acids. The color tests have frequently been used for qualitative detection of amino acids. Not all amino acids contain the same reactive groups. For this reason the various color tests yield reactions varying in intensity and type of color according to the nature of groups contained in the particular amino acid under examination.

19.2 The Detection of Proteins

19.2.1 Millon’s reaction

19.2.1.1 Principle

The reaction is due to the presence of the hydroxyphenyl group, C₆H₅OH in the amino acid molecule; and any phenolic compound which is unsubstituted in the 3,5 positions such as tyrosine, phenol and thymol will give the reaction. Solutions of nitric acid containing mercuric nitrate reacts with phenols, producing red colors or yellow precipitates which react with nitric acid to form red solution. The reaction probably depends on the formation of a nitro compound; which then reacts with phenol.

19.2.1.2 Materials

1. Millon’s reagent

19.2.1.3 Method

Add 3 to 4 drops of Millon’s reagent to 5 ml of test solution. Mix and bring the mixture gradually to a boiling point by heating over a small flame. Development of red color is due to the presence of protein. Excess of reagent should however be avoided since it may produce a yellow color which is not a positive reaction.

19.2.2 Millon-Nasse reaction

19.2.2.1 Materials

1. Millon-Nasse reagent
2. 1% NaNO₂

19.2.2.2 Method

Add 1 ml of Millon-Nasse reagent to 5 ml of test solution. Place the tube in a boiling water bath for 10 mins. and cool the contents in water bath for 5 to 10 mins. and add 1 ml of 1% NaNO₂. A deep red color indicates tyrosine or other 3,5 unsubstituted phenol.
19.2.3 Xanthoproteic reaction

19.2.3.1 Principle

This reaction is due to the presence in the amino acid molecule of the phenyl group –C₆H₅, with which the nitric acid forms certain nitro modifications. The particular amino acids which are of especial importance in this connection are those of tyrosine and tryptophan. Phenylalanine does not respond to this test as it is ordinarily preformed.

19.2.3.2 Materials

1. Conc. HNO₃
2. Ammonium hydroxide
3. Sodium hydroxide

19.2.3.3 Method

Add 1 ml of conc. Nitric acid to 2 to 3 ml of test solution in a test tube. A white precipitate forms, which upon heating turns yellow and finally dissolves, imparting to the solution a yellow color, cool the solution and carefully add ammonium hydroxide or sodium hydroxide in excess. Note that the yellow color deepens into an orange.

19.2.4 Hopkins-Cole reaction

19.2.4.1 Principle

The formation in this test color is due to the presence of indoyl group. Gelatin does not respond to this test due to lack of amino acid tryptophan. Violet to blue colors develop when a mixture of protein and an aldehyde is layered over conc. sulphuric acid. A number of tests based on this principle have been suggested; all depends on the presence of the indoly group of tryptophan which reacts as follows (using glyoxylic acid as an example of an aldehyde).

This is called Hopkin-Cole test- A similar test was at one time recommended for detection of formaldehyde that had been as a preservative to milk, the formaldehyde reacting with indolyl groups of milk proteins to give a color.

19.2.4.2 Materials

1. Hopkin-Cole reagent
2. Conc. H₂SO₄

19.2.4.3 Method

Place 2 to 3 ml of test solution and an equal volume of Hopkins-Cole reagent in a test tube and mix thoroughly. Incline the tube and permit 5 to 6 ml of conc. sulphuric acid to flow slowly down the side of the tube, thus forming a sharp layer of acid beneath the amino acid solution. When stratified in this manner a reddish-violet color forms at the zone of contact of the two fluids. If the color does not appear after starting for a few minutes, the tube may be rocked gently to cause a slight mixing of the liquids are mixed by gentle stirring the precipitate of protein dissolves and the violet color spread throughout the
19.2.5 Biuret test

19.2.5.1 Principle

The Biuret test is given by those substances whose molecules contain two cabamyl (-CONH₂) groups joined either directly or through a single atom of nitrogen or carbon. Similar substances which contain -CSNH₂, -C(NH)NH₂, or – CH₂NH₂ in place of the –CONH₂ group also respond to the test. It follows from this fact that substance which are non-protein in character but which contain the necessary groups will respond to the biuret test.

Protein responds positively since there are pairs of CONH groups in the molecule. A copper coordination complex with the ring structure is probably produced. Short chain polypeptides give a pinkish violet color, longer one including proteins a more purple blue. The amino acid histidine gives a pink color, which depends on the peptide linkage, does not vary greatly in intensity from protein to protein. Several procedure based on this method have been suggested for quantitative determination of milk proteins, but it is not in general use in dairy research.

19.2.5.2 Materials

1. 10% NaOH
2. 0.5% CuSO₄

19.2.5.3 Method

To 2 to 3 ml of test solution in a test tube add an equal volume of 10% sodium hydroxide solution, mix thoroughly, and add a 0.5% copper sulphate solution drop by drop, mixing between drops, until a purplish-violet or pinkish-violet color is produced. The color depends upon the nature of the protein, proteoses and peptones give a decided pink; the color produced with gelatin is not far removed from a blue.

19.2.6. Ninhydrin reaction

19.2.6.1 Principle

This test gives positive results with proteins, peptones, peptides, amino acids and other primary amines, including ammonia. Proline and hydroxyproline give yellow color with ninhydrin, while other acids give blue to purple color.

19.2.6.2 Materials

1. 0.1% ninhydrin
2. pH paper

19.2.6.3 Method

To 5 ml of dilute test solution, which must be approximately between pH 5 and pH 7 (a few drops of
Organic Chemistry

pyridine or a few crystals of sodium acetate may be used to adjust the pH), add 0.5 ml of 0.1 \% ninhydrin, heat to boiling for one to two minutes, and allow to cool. A blue color develops if the test is positive.

19.2.7 Folin test

A phosphomolybdatungstic acid reagent designed by Folin for phenol has been widely used for detection and analysis of indolyl and phenol groups in amino acids. A characteristic blue color is formed when amino acid solution is warmed with this reagent. The color so formed is due to the reaction of alkaline copper with the amino acid and the reduction of phosphomolybdate by tyrosine and tryptophan present.

19.2.7.1 Materials

1. Alkaline Na$_2$CO$_3$ solution (2\% in 0.1 N NaOH)
2. CuSO$_4$-Na; K tartarate solution (0.5 \% CuSO$_4$) in 1 \% Na, K tartarate) prepared fresh by mixing stock solutions.
3. “Alkaline solution” (prepared by mixing 50 ml of the reagent (1) and 1 ml of the reagent (2)).
4. Folin-Ciocalteau reagent

19.2.7.2 Method

Add 5ml of the alkaline solution to 1 ml of the test solution. Mix thoroughly and allow to stand at room temperature for 10 mins. Add 0.5 ml diluted Folin-Ciocalteau reagent rapidly with immediate mixing. Observe for development of color after 30 mins. Development of characteristic blue color indicates presence of indolyl or phenol group.

19.2.8 Sakaguchi test

19.2.8.1 Principle

Arginine and other guanidyl derivatives (glycocamine, methylgyanidine etc) react with hypo bromide and alpha napthol to give a red colored product.

19.2.8.2 Materials

1. Sodium hydroxide solution (40\%)
2. Alpha napthol solution (1\% in alcohol)
3. Bromine water (a few drops of bromine in 100 ml distilled water)

19.2.8.3 Method

Mix 1 ml of sodium hydroxide with 3 ml of test solution and add 2 drops of alpha napthol. Mix thoroughly and add 4 to 5 drops of bromine water. Note the color formed. Formation of a red color indicates presence of guanidine group. This is a very sensitive and specific test.
19.2.9 Nitroprusside test

19.2.9.1 Principle

Sodium nitroprusside reacts with compounds containing sulphahydryl groups produce an intensely red but somewhat unstable color.

19.2.9.2 Materials

1. Sulphur amino acids (1.0% cystine, cysteine and methionine)
2. Sodium nitroprusside (2% prepared fresh)
3. Ammonium hydroxide

19.2.9.3 Method

Mix 0.5 ml of a fresh solution of sodium nitroprusside with 2 ml of the test solution and add 0.5 ml of ammonium hydroxide.

19.2.10 Spectrophometric method

The use of infrared and ultraviolet spectra offers a mean of identification of amino acids and their derivatives. Spectrophometric method of detecting and determining amino acids in the intact protein offer advantages over chemical methods in that they do not involve hydrolysis; which often leads to partial decomposition of some amino acids.

The absorption of UV radiation at wave lengths of 280 nm, can be used as a method for detecting and determining some amino acids content. The light is absorbed by the amino acids tyrosine, tryptophan and phenylalanine.

*****😊*****
Lesson 20
DEFINITION, CLASSIFICATION AND ISOMERISM

20.1 Introduction

• Major class of organic compounds occurring in nature - important to maintain life
• Include - glucose, sucrose, starch, cellulose etc

20.2 Definition

• The French term ‘hydrate de carbone’ meaning – hydrates of carbon – therefore defined as hydrates of carbon having an empirical formula \( C_nH_{2n}O_n \) or \( (CH_2O)_n \)
• Some non-carbohydrate compounds also fit into the empirical formula—e.g. formaldehyde, acetic acid, lactic acid etc
• Some carbohydrates do not have H to O ratio of 2:1 – e.g. deoxy ribose: \( C_5H_{10}O_4 \)
• Carbohydrates containing N and S, in addition to C, H and O do not fit into the empirical formula so the word carbohydrate has only historical importance.
• Carbohydrates now defined as —“ polyhydroxy aldehyde or ketone compounds or compounds which yield these on hydrolysis and contain at least one asymmetric/ chiral carbon atom ”

20.3 Nomenclature

Two types – nature of source and trivial names

1. According to nature of source

Beet sugar, cane sugar, grape sugar, malt sugar, milk sugar

2. Trivial names

Prefix - related to source, number of carbon atom content, aldehyde or ketone group content of rotation of plane polarized light

Suffix - ‘ose’ – indicates compound to be carbohydrate (sugar)

20.3.1 Source

Fruit – fructose
Lactis – Lactose (Lactis- Milk)
Malt- Maltose
Xylum - Xylose (Xylum- wood)
Cell – Cellulose

20.3.2 Number of carbon atom content

3- Tri- Triose
4- Tetra- Tetrose
5-Penta- Pentose
6-Hexa- Hexose
7- Hepta- Heptose

20.3.3 Type of functional group content

Aldehyde- Aldose
Ketone- Ketose

20.3.4 Rotation of plane polarized light

Dextro rotatory- Dextrose
Laevor rotator- Levulose

20.3.5 Classification

(Figure 20.1 Carbohydrates)
Organic Chemistry

Three major classes: sugars, non-sugars and derived carbohydrates

1. Sugars - sweet, crystalline and water-soluble
2. Non-sugars - Tasteless, Amorphous and Water-insoluble
3. Derived carbohydrates - Derivatives of carbohydrates

- Oxidation products - ascorbic acid, saccharic acid etc
- Reduction products - glycerol, inositol, sorbitol etc
- Deoxy products - 2-deoxy ribose, methyl pentose etc
- Amino products - glucose amine, galactosamine

*****😊*****
Lesson 21
STRUCTURE OF GLUCOSE - OPEN CHAIN AND RING STRUCTURE AND EVIDENCES FOR THE RING STRUCTURE

21.A.1 Introduction

The structure of carbohydrates (taking glucose as an example) is given below

21.A.1.1 Open chain structure (Bayer’s formula)

- Arrangement of six carbon atoms- a straight chain
- Aldehyde group – in terminal position
- One of the five –OH groups as –CH₂OH and in terminal position

```
    CHO
   |   
  CHOH   
 |   
  CHOH
 |   
  CHOH
 |   
  CHOH
 |   
CH₂OH
```

Figure 21.1 Open chain structure of glucose

21.A.1.2 Fisher’s projection formula

- Emil Fisher- German organic chemist
- In glucose- for asymmetric carbon atoms- therefore 2⁴ = 16 stereoisomeric structures possible
- Therefore essential to define the configuration about each of the four asymmetric carbon atoms
- Glucose- assigned the configuration as shown here
- Horizontal lines represent bonds coming out of the plane- i.e. towards us.
- Vertical lines represent bonds going behind the plane – i.e. away from us.
21.A.1.3 Ring structure

- Chemistry of glucose- anomalies in behavior
- Lacks some characteristic reactions of aldehyde
  - Does not give schiff’s test
  - Does not form a bisulphate product

- Kiliani cyanohydrins synthesis is not rapid (difficult)
- Exists in two isomeric forms – ‘α’ and ‘β’ – both undergo mutarotation
- These anomalies explained on the basis of cyclic (ring) structure – resulting from intramolecular hemiacetal formation
- A hemiacetal – a compound formed by reaction between aldehyde and alcohol

Hemiacetals – quite reactive compounds – give many, but not all the reactions of aldehyde
- Hemiketals and ketals- formed from ketones
- “An intramolecular hemiacetal is formed in glucose by reaction between aldehyde group and one of the hydroxyl group of the molecule – resulting into cyclic (ring) structure”
- The angles of tetrahedral carbon atoms tend to bend the glucose molecule in such a way that the -OH group on carbon number five forms the hemiacetal by reaction with –CHO group of the molecule in two stereoisomeric forms known as Anomers.
Figure 21.4 Anamors, Glucopyranose structures

- The carbon atoms which held aldehyde or ketone group - anomeric carbon atom/ The glycosidic carbon.
- The hydroxyl group formed from the carbonyl oxygen due to hemiacetal (or hemiketal) formation – anomeric hydroxyl group.
- The hemiacetal linkage, formed between first and fifth carbon atoms- amylene oxide ring.
- The first carbon atom becomes asymmetric/chiral due to hemiacetal formation- therefore two modifications of this form (structure) exist = namely ‘α’ and ‘β’ as shown.

21.B.1 Introduction

The confirmation indicates arrangement of groups or element around anomeric (reference) carbon atom - ‘α’ and ‘β’ denotes conformation.
In solution these forms are in equilibrium with each other via aldehyde form and have \([\alpha]^{D_{20^\circ} C} = 52.5^0\)

\[
\begin{align*}
\text{H} & \text{C} \text{OH} \quad \Leftrightarrow \quad \text{H} & \text{C} \quad \Leftrightarrow \quad \text{H} \text{C} \text{OH} \\
\alpha & - \text{Glucose} & \text{Aldehyde} & \beta & - \text{Glucose}
\end{align*}
\]

(Figure 21.6 Mutarotation)

One form (\(\alpha\) or \(\beta\)) dissolved- changes to another till equilibrium established between the two – process- Mutarotation.

Configuration indicates arrangement of groups or atoms around number carbon atom – denotes by ‘D’ and ‘L’

\[
\begin{align*}
\text{CHO} & \quad \text{O} = \text{C} \\
\text{H} & \quad \text{HO} - \text{C} \quad \text{H} \\
\text{HO} & \quad \text{H} - \text{C} \quad \text{OH} \\
\text{H} & \quad \text{H} - \text{C} \quad \text{OH} \\
\text{H} & \quad \text{H} - \text{C} \quad \text{OH} \\
\text{CH}_2 & \quad \text{OH}
\end{align*}
\]

\(\text{D} - \text{Glucose} \quad \text{L} - \text{Ascorbic acid}

(Figure 21.7 D- and L- configuration)

- Position of –OH group on asymmetric carbon atom farthest from the functional group (C-5 this case)
  - on right hand side – ‘D’ configuration
  - on left hand side – ‘L’ configuration

- Optical rotation: rotation of direction of plane polarized light
  - towards right- clock-wise direction- dextrorotatory – denoted by (+) sign
  - towards left- anticlock-wise direction- denoted by sign (-) = laevorotatory.
  - Previously dextro and levo rotations were denoted by (d) and (l) signs but they create confusion with ‘D’ and ‘L’ configuration
    - Therefore (d) and (l) signs are now replaced by (+) and (-) respectively for representing dextro and levo rotations
    - e.g. D-(+)-glucose, D(-)-fructose etc.

1. Haworth formula: (cyclic form)
W.H. Haworth (1929) – an English chemist
Emphasized structural relationship of carbohydrates with furan and pyran

(Figure 21.8 Haworth formula)

- Three dimensional representation of molecule
- Atoms of the ring lie in a single plane and perpendicular to the plane of the paper
- Groups or atoms (H, OH etc) attached to the carbon atoms are projected at right angles to the plane of the ring – above or below the plane
- Position of OH group- indicated by drawing only bond position of single H atom- not indicated (not to draw bonds)
Lesson 22

STEREOCHEMISTRY AND STABILITY OF ANOMERS

22.1 Introduction

• Branch of chemistry which deals with the structures of molecules

22.2 Isomerism

Distinctive feature of organic compounds

• Isomers: compounds which have same molecular formula but different structural formula
• Isomerism: phenomenon that deals with isomers
• Types: two: structural isomerism and stereoisomerism

22.3 Structural Isomerism

• Due to the difference in the arrangement of atoms within a molecule- structural isomers
• Divided into four kinds
• Chain isomerism, position isomerism, functional group isomerism and metamerism

22.4 Sterioisomerism (Space isomerism)

• Due to the difference in spatial position of atoms in a molecule
• Isomers differ from each other only in the way in which atoms in a molecule are oriented in space
• Types- three- optical isomerism, geometric isomerism and conformational isomerism

1. Optical isomerism (enantiomerism)

• Light - ordinary, monochromatic, plane polarized
  o Ordinary light - rays of different wavelength, vibrating in all directions (unpolarized light)
  o Monochromatic light - rays of single wavelength, vibrating in all directions
  o Plane polarized light - vibrating only in one direction

• Substance which rotates plane of polarized light- optically active
  o This property -phenomenon- optical activity –Dextro (+) or Laevo (-)

• For the compound to be optically active
  o Must have at least one asymmetric (chiral) carbon atom

• Asymmetric carbon atom
  o The carbon atom which has four different atoms or groups or atoms and groups
• Enantiomer - pair of compounds whose structures differ only in being mirror image of each other
  
  o Phenomenon- enantiomerism

• Enantiomeric pairs have identical gross structure, but differ from each other in having different spatial arrangement of atoms and/or groups constituting them
• Enantiomers differ from each other in direction of rotating the plane of polarized light
  
  o One - dextrorotatory - rotates clockwise
  o The other - laevorotatory- rotates anti-clockwise

• Racemic modification (mixture) - denoted by (±)
• A mixture of equal parts of enantiomers (+ and – forms)
• Optically in active - because rotation caused by one isomer of the mixture is cancelled out by equal and opposite rotation caused by its enantiomer.
• Compounds with two asymmetric carbon atoms:
  • These two asymmetric carbon atoms may be
    
    o Dissimilar - atoms/ groups attached to one of the asymmetric carbon atom are different from those attached to the other
    o Similar - atoms/groups attached to both the asymmetric carbon atoms are similar (identical)

• Stereochemistry of compound containing two dissimilar asymmetric carbon atoms - e.g. 3-chlorobutanol-2
  • An optically inactive compound whose molecules are superimposed on their mirror images despite the presence of asymmetric (chiral) carbon atoms is known as mesocompound (having plane of symmetry).

2. Geometric isomerism

• The double bonded carbon atoms and the four atoms attached to them should be flat
  • e.g. ethylene molecule of the type abc=cab
  • The flatness is due to geometric arrangement of the bonding orbitals and their overlapping that leads to formation of π bond so hindered rotation
• In cis- arrangement similar groups (atoms) lie on the same side of the ethylenic carbons e.g.
• In trans- arrangement similar groups (atoms) lie on opposite sides of the ethylenic carbons e.g.
• Interconversion of these two structures (cis- and trans-) requires breaking of the π bond
• The breaking of π bond requires very high amount of energy - 70 kcal/mol
• Therefore, the two structures (cis- and trans-) have independent existence
• Such cis- and trans- structures are isomeric
  
  o Referred to as geometric ( or cis-trans) isomers
  o The phenomenon is referred as geometric isomerism or cis-trans isomerism

• The cis- and trans- isomers differ from each other only in the manner of the substituent atoms/group of atoms oriented in space.
  
  o Therefore cis- and trans- isomers are a kind of stereoisomers

• Geometric isomers have different physical properties
Melting points, boiling points, solubilities, densities, refractive index, dipole movements, heat of hydrogenation, selectivity etc.

Therefore can be separated easily

3. Conformational isomerism

- The electron distribution of the sigma molecular orbital is symmetrical around the inter nuclear axis of a sigma bond
  - Therefore sigma bond (single covalent bond) permits free rotation about its axis e.g. H₃C-CH₃.
  - As a result, it is possible for such a compound to have different relative arrangements of their atoms in space which can change into one another usually denoted as Eclipsed and Staggered in case of C₂H₆

- Such arrangements of atoms which can be converted into one another by rotation around single bonds are called conformations or rotational isomers.

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Lesson 23
REACTIONS OF MONOSACCHARIDES

23. A.1 Introduction

Carbohydrates which cannot be hydrolyzed into simpler compounds - therefore also known as simple sugars
Contain 3 to 10 carbon atoms - triose, tetrose, pentose, hexose etc
Contain –CHO or >C=O group - aldose or ketose
Hexoses - the most important class of monosaccharides – widely distributed in nature
The most important hexoses - glucose, galactose, mannose and fructose
Some aldopentoses - biologically important - Ribose and Deoxy ribose – component of DNA, RNA , ATP etc.

Table 23.A.1 Example of monosaccharides

<table>
<thead>
<tr>
<th>Type of sugar</th>
<th>Aldose</th>
<th>Ketose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triose(C₃H₆O₃)</td>
<td>Glyceraldehyde</td>
<td>Dihydroxyacetone</td>
</tr>
<tr>
<td>Tetrose(C₄H₈O₄)</td>
<td>Erythrose</td>
<td>Erythulose</td>
</tr>
<tr>
<td>Pentose(C₅H₁₀O₅)</td>
<td>Xylose, Ribose</td>
<td>Xylose, Ribulose</td>
</tr>
<tr>
<td>Hexose(C₆H₁₂O₆)</td>
<td>Glucose, Galactose</td>
<td>Fructose, Sorbose</td>
</tr>
<tr>
<td>Heptose(C₇H₁₄O₇)</td>
<td>-</td>
<td>Sedo hephulose</td>
</tr>
</tbody>
</table>

23.A.2 Reactions of Monosaccharides

• Differences in structures - frequently affect rate of reaction and sometimes ability to react at all – the differences – useful in detection and determination of sugar.
• Reactive groups

1. Hydroxyl groups
   - Anomeric hydroxyl group - more reactive
   - primary alcohol group

2. Carbonyl group - -CHO and >C=O

1. Glycoside formation

• Acetal formed from carbohydrate - glycoside
• Glycoside - general name - specific name given – based on particular sugar involved - e.g. glucoside,
galactoside, fructoside etc

- The hydroxyl group on anomeric carbon involved in glycoside formation.

(Figure 23.1 Glycoside formation)

- Disaccharides are glycoside in which ‘alcohol’ is another monosaccharide (i.e. its primary hydroxyl group)
- Glycoside formation fixes the ring structure of monosaccharide – no shift from α to β or β to α form – therefore glycosides do not mutarotate and prevents many reactions of –CHO or >C=O

2. Ester formation

- Esterification of hydroxyl group – useful reaction – helps in identification of structure
- Esterified by treatment with acid anhydride or acid halide – to acetate, stearate etc.

(Figure 23.2 Ester formation)
• Formation of esters with inorganic acids - e.g. phosphoric acid = phosphoesters (phosphates) - great biological significance = phosphates of ribose and deoxyribose - component of vital natural compounds – Nucleic acids

\[
\begin{align*}
\text{Ribose} & \quad \text{Phosphoric Acid} \\
\text{CHO} & \quad \text{HO–PO–OH} & \quad \text{CHO} \\
\text{H–C–OH} & \quad \text{H–C–OH} & \quad \text{H–C–OH} \\
\text{H–C–OH} & \quad \text{H–C–OH} \\
\text{CH₂OH} & \quad \text{CH₂–O–PO–OH} & \quad \text{CH₂OH}
\end{align*}
\]

\[\text{Ribose -5- Phosphate}\]

(Figure 23.3 Formation of phosphoester)

3. **Copper reduction: (Reducing properties)**

• Possessing free of potentially free aldehyde or ketone group – therefore have ability to reduce certain metal ions – copper
• Cupric hydroxide (blue) in alkaline medium (Fehling solution) – heated in presence of reducing agent (sugar) - reduced to insoluble cuprous oxide (brick red) - used as qualitative and quantitative tests for the reducing sugars.

23. **B.1 Introduction**

The reactions of monosaccharides are mentioned below

1. **Osazone formation**
   
   • With calculated amount of phenylhydrazine reducing sugars form phenylhydrazones.
With excess of phenyldrazine – a more complex reaction takes place - forming osazone

Time required for osazone formation- vary with sugar and typical for a given sugar.

Osazones- yellow solid- each sugar has its own characteristic crystalline osazone- therefore useful in identification of sugars-which are difficult to crystallize.

2. Oxidation

- Product- depends on type of oxidizing agent (mild or strong)
- Mild oxidizing agent- only aldehydeic group is oxidized to –COOH group.
- Strong agent- terminal hydroxyl group oxidized- dicarboxylic acid (terminal).
- Very strong agent- both aldehyde and terminal hydroxyl group oxidized- saccharic acid (mucic acid - very old name)
3. Reduction

- Carbonyl group (-CHO or >C=O) of sugar- reduced to an alcohol group- product known as alditol
- By variety of agent = H₂, Pt, Sodium amalgam

- Sorbitol- obtained by reduction of glucose- has commercial importance- used in manufacture of emulsifying agents and ascorbic acid.
4. Action of alkali (Alkaline interconversion)

- Lobry de Bruyn and Alberda Van Ekensiein rearrangement – reversible isomerization of monosaccharides- bring about the dilute alkalies

5. Dehydrocyclization (Action of acid and heat)

- Monosaccharides- relatively stable in dilute acid
- Treatment with hot strong acid- complex reaction – dehydrocyclization- loss of water and formation of furfural or furfural derivative
- Aldo pentose yields furfural

(Figure 23.9 Action of alkali)
• Aldohexose yields hydroxymethyl furfural (HMF)

• Furfurals- very reactive- give characteristic color with polyhydric phenols (resorcinol, orcinol etc) = used in qualitative and quantitative analysis of sugars
• Appearance of color – identify the sugar- pentose or hexose, aldose or ketose etc
• Intensity of color- used to determine concentration
• HMF- an treatment of hot acid= transferred into levulinic acid and large amount of dark, insoluble condensation products (humins)- leads to browning – caramalization
• HMF determination- serves as an index of caramalization

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Lesson 24  
DETECTION OF CARBOHYDRATES

Carbohydrates are polyhydroxy aldehydic or ketonic compounds or anhydrides of such compounds or their derivatives.

Carbohydrates have reactive groups which are responsible for their chemical behavior. There are
1. The glycosidic hydroxyl group
2. The alcoholic hydroxyl group and,
3. The free aldehyde or ketone group

Most of the reactions of Carbohydrates are carried out in aqueous solution, which means that all three types of groups will be present and available for reaction in mutarotating mono- and di- saccharide. When chemical reagents react specifically with 1 or 3 in mono and di saccharides, the equilibrium, alpha form $\leftarrow = \Rightarrow$ aldehyde form $\leftarrow = \Rightarrow$ beta form (referred to previous structures mention)
or alpha form $\leftarrow = \Rightarrow$ ketone form $\leftarrow = \Rightarrow$ beta form (referred to previous structures mention) is shifted in favour of the group which is being used up. Some reagents react with more than one group. Such, reactions are the basis for detection of different types or classes of carbohydrates.

24.1 Alpha-Napthol Reaction (Molisch test)

24.1.1 Principle

Concentrated sulphuric acid hydrolyses glycosidic bonds to give the mono- saccharides which are then dehydrated to furfural and its derivatives such as hydroxyl methyl furfural. These products then combine with sulphonated alpha-napthol to give a purple complex (conc. solution of organic compounds may give a red or violet color due to the charring action of the sulphuric acid).

This reaction is a general one for the presence of carbohydrate and other organic compound that give furfural with conc. sulphuric acid.

24.1.2 Materials

1. Conc. $\text{H}_2\text{SO}_4$
2. Alpha-napthol (5% in ethanol)
3. 1% sugar solution

24.1.3 Method

Add two drops of the alpha napthol solution to 2 ml of test solution, then carefully pour about 1 ml conc. $\text{H}_2\text{SO}_4$ down the side of the tube so as to form two layers. A redish-violet zone appears at the junction between the two liquids. Repeat the test using water.
24.2 The Anthrone Reaction

24.2.1 Principle

The anthrone reaction is generally test for carbohydrates. The principle is the same as outlined above except that the furfural reacts with anthrone to give blue-green complex.

24.2.2 Materials

1. Conc. H$_2$SO$_4$
2. Anthrone solution (0.2% in conc. H$_2$SO$_4$)
3. 1% sugar solution

24.2.3 Method

Add 5 drops of the test solution to about 2 ml of the anthrone reagent, mix thoroughly and observe the color change. Blue green color indicates presence of carbohydrate.

24.3 Fehling’s Test (for reducing sugar)

24.3.1 Principle

Carbohydrates with a free or pontentially free carbonyl group have the ability to reduce solution of various metallic ions such as Fehling’s solution in which case rust-brown cuprous oxide is precipitated. Fehling’s test is too sensitive for the routine detection of glucose in urine since it can give a false positive test due to the action of urates in urine. Excess ammonia and ammonium salts interfere with the test.

24.3.2 Materials

1. Fehling’s solution A
2. Fehling’s solution B
3. Sugar solution

24.3.2 Method

Mix equal volumes of Fehling’s solution A and B and a few drops of the test solution to 1 ml of the mixed Fehling’s solution and boil. Brick red precipitates show the presence of reducing sugars. Test the Fehling’s solution with water.

24.4 Benedict’s Test (for reducing sugar)

24.4.1 Principle

Benedict modified the Fehling’s test to product single solution which is more convenient for tests as well as being more stable than Fehling’s reagents. This is also a copper reduction test in which sugars are oxidized to their corresponding acids and cupric hydroxide is reduced to cuprous oxide.
24.4.2 Materials

Add 5 drops of the solution to 2 ml of Benedict’s reagent and place in a boiling water bath for 5 mins. Compare the sensitivity of Benedict’s and Fehling’s test, using increasing dilutions of 1% glucose.

24.5 Picric Acid Test (for reducing sugar)

24.5.1 Principle

Reduction of picric acid to picramic acid by the reducing sugar produces mahogany-red color (dark brown-red).

24.5.2 Materials

1. Sugar solution
2. Saturated picric acid
3. 10% Na₂CO₃

24.5.3 Method

To 5 ml of the sugar solution add 2 to 3 ml of saturated picric acid solution, add about 1 ml of 10% Na₂CO₃. Warm. Note the development of mahogany-red color is the presence of reducing sugar due to the reduction of picramic acid.

24.6 Barfoed’s Test (for monosaccharide)

24.6.1 Principle

Barfoed’s reagent is weakly acidic and is only reduced by monosaccharides. Prolonged boiling may hydrolyse di-saccharide to give a false positive reaction. The precipitate of cuprous oxide is less dense than with the previous two tests and it is best to leave the tube to stand to allow the precipitate to settle. The color of the cuprous oxide is also different, being more a brick-red rather than orange-brown obtained in the previous tests.

24.6.2 Materials

1. Sugar solution
2. Barfoed’s reagent

24.6.3 Method

Add 1 ml of the test solution to 2 ml of Barfoed’s reagent, boil for one minute and allow to stand. Reduction indicated glucose, fructose, mannose, galactose, pentose, possibly along with non-reacting carbohydrates. No reduction indicates lactose, or maltose or both.

24.7 Bial’s Test (for pentose)

24.7.1 Principle

When pentoses are heated with conc. HCl, furfural is formed which condenses with orcinol in the presence of ferric ions to give a blue-green color. The reaction is not absolutely specific for pentose since prolonged heating of some hexoses yields hydroxymethyl furfural which also reacts with orcinol to give colored complexes.
24.7.2 Materials

1. Sugar solution
2. Amyl alcohol
3. Bial’s orcinol reagent

24.7.3 Method

Add about 2 ml of the test solution to 5 ml of reagent in a test tube and heat until boiling commences. A blue green color indicates positive result. Cool the tube, then add 2-3 ml of amyl alcohol and shake.

24.8 Selwanoff’s Test (for ketose)

24.8.1 Principle

Ketoses are dehydrated more rapidly than aldoses to give furfural derivatives which then condenses with resorcinol to form a red complex, prolonged heating of the solution under investigation must, therefore, be avoided. Furfural-hydrochloric acid reacts with resorcinol with formation of orange to red color.

24.8.2 Materials

1. Sugar solution
2. Selwanoff’s reagent

24.8.3 Method

Add two drops of carbohydrate solution to 2 ml of Selwanoff’s reagent and warm in a boiling water bath for 1 min. Note the appearance of a red color.

24.9 Iodine Test (for polysaccharide)

24.9.1 Principle

Iodine forms colored adsorption complexes with polysaccharides, starch gives a blue color with iodine, while glycogen and partially hydrolyzed starch react to form red-brown colors and no color with sugars (only color of iodine).

24.9.2 Materials

1. Cellulose, glycogen, starch, inulin and sugars (1% solution)
2. Iodine solution

24.9.3 Method

Acidify the test solution with dilute HCl, then add 2 drops of iodine and compare the colors obtained with that of water and iodine.

24.10 Osazone Formation Test

The ‘osazones’ are yellow solids with characteristic crystalline forms and rates of formations. Osazone
formation is, therefore, very useful in the identification of carbohydrates which are usually difficult to crystallize.

24.10.1 Materials

1. Phenylhydrazine mixture
2. 5% sugar solution
3. Alcohol

24.10.2 Principle

When a solution of reducing sugar is heated with phenylhydrazine yellow crystalline compound called osazone are formed. In general, each individual sugar will give rise to an osazone of a definite crystalline form which is typical for that sugar.

The reaction of phenylhydrazine with free aldehyde and ketone groups of sugars may be considered to be an example of the formation of a special types of Schiff’s base, a hydrazone, followed by the formation of a double Schiff’s base, the osazone.

24.10.3 Procedure

To 300 mg phenylhydrazine mixture add 5 ml of the sugar solution, shake well and heat on a boiling water bath for 30 to 45 mins. Allow the tube to cool slowly. Note the time required for crystal formation. Also examine crystals for size, shape, melting point etc. The crystals can be purified by recrystalization from alcohol.

24.10.4 Note

Determination of the time required for formation of the insoluble yellow osazone is a valuable means of identifying the various sugars. The time required for osazone formation (mins.) is given in table below:

Table 24.1 Time required for osazone formation

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mannose</td>
<td>1-5</td>
</tr>
<tr>
<td>Fructose</td>
<td>2</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.5</td>
</tr>
<tr>
<td>Xylose</td>
<td>7</td>
</tr>
<tr>
<td>Arabinose</td>
<td>10</td>
</tr>
<tr>
<td>Galactose</td>
<td>20</td>
</tr>
<tr>
<td>Sucrose</td>
<td>30 (Glucose formed after hydrolysis)</td>
</tr>
<tr>
<td>Lactose</td>
<td>soluble in hot water</td>
</tr>
<tr>
<td>Maltose</td>
<td>soluble in hot water</td>
</tr>
</tbody>
</table>
Lesson 25
DEFINITION AND CLASSIFICATION

25.1 Introduction

- Various terms - used interchangeably for lipids.
- Lipids - Lipins - Lipoids- Fats - oils i.e. used synonymously.
  - Term ‘lipid’ - coined by Bloor
  - Denotes the class of compounds which are insoluble in water but soluble in usual organic solvents like alcohol, ether, chloroform, benzene etc.

25.2 Importance (functions) of Lipids

1. Rich source of energy - 9 kcal/g
2. Serves as a carrier of fat soluble vitamins - A,D, E and K
3. Carrier of essential fatty acids
4. Structural component of cell wall- play important role in cell permeability
5. Structural component of nerve tissues - protect them

25.3 Chemically What is Lipid, Fat and Oil ?

- When alcohol and acid react - ester is formed

\[
R-\overset{\text{H}}{\text{O}}H + H\overset{\text{O}}{\text{O}}\overset{\text{H}}{\text{O}} \rightarrow R-O-C-R' + H_2O
\]

- Trihydric alcohol (Glycerol) -esterified with carboxylic acids - ester - known as glyceride.
Fats or oils - chemically glycerides- an ester of fatty acids with glycerol.
The difference between them - physical state - solid or liquid - at room temperature
The difference - due to difference in their fatty acids make up
  - relatively more unsaturated fatty acids content in oil - therefore low melting point – therefore liquid
  - relatively less unsaturated fatty acids content in fat - therefore high melting point - therefore solid

Oils and fats - synthesize - plants or animals
  - Some other organic compounds also synthesize and those which are oil/fat soluble get associated with them (become soluble in oil/fat)

- Include- sterols – cholesterol (animal) and phytosterol (plant)
- Vitamins – A,D,E,K
- Hydrocarbons- squelene
- Pigments-carotenoids- carotenes- β-carotene
- Waxes, long chain alcohols and acids etc
  - Glycerides (fats/oils) with associated compounds- collectively known as ‘lipid’

Therefore Lipid = Glyceride + Glyceride soluble compounds
(fat/oil)

25.4 Classification of Lipids

- Lipids – classified on several basis- complexity, saponification, polarity etc.
25.4.1 Based on complexity

Three groups - simple, complex and derived lipids

Table 25.1 Three groups - simple, complex and derived lipids

<table>
<thead>
<tr>
<th>Simple lipids</th>
<th>Compound lipids</th>
<th>Derived lipids</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Fats</td>
<td>1. Phospholipids</td>
<td>1. Sterols</td>
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<td></td>
<td>4. Lipoproteins</td>
<td>4. Alcohols</td>
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<td>5. Pigments</td>
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<td></td>
<td></td>
<td>6. Fatty acids</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7. Hydrocarbons</td>
</tr>
</tbody>
</table>

25.4.2 Based on polarity

Two groups - polar and non-polar
1. Polar lipids
   - They are charged molecules
   - Soluble in polar solvents - alcohol, chloroform etc
   - e.g. phospholipids, fatty acids etc

2. Non-polar lipids
   - They are uncharged molecules
   - Soluble in non-polar solvents- ether, benzene, hexane, etc
   - e.g. Glycerides, cholesterol esters, vitamins

25.4.3 Based on saponification

Ability to react with alkality to give soap
Two groups - saponifiable and unsaponifiable
1. Saponifiable lipids
   - React with alkali and form soap
   - Present in large amount
   - e.g. Glycerides, phospholipids, fatty acids, cholesterol

2. Unsaponifiable lipids
   - Do not react with alkali to form soap
   - Present in relatively small amount
   - e.g. vitamins, sterols, hydrocarbons, carbonyls etc.

25.5 Traditional Classification of Edible Fats/Oils

Based on source of fat/oil and constituent fatty acids
25.5.1 Milk fat

- Fats of this group are derived from milk of mammals, particularly from cows and buffalo.
- Majority of fatty acids of milk fat are palmitic ($C_{16:0}$), stearic ($C_{18:0}$) & oleic ($C_{18:1}$).
- This fat is unique in that it contains appreciable amounts of short chain fatty acids ($C_{4:0}$, $C_{6:0}$, $C_{8:0}$, $C_{10:0}$).
- Butyric acid ($C_{4:0}$) is unique to milk fat.

25.5.2 Lauryl or lauric acid fat

- Fat of this group are derived from certain species of palm - such as coconut and babasu.
- These fats are characterized by their high amounts (content) of lauric acid - 40 to 50%.
- Contain moderate amount of $C_{6:0}$, $C_{8:0}$, $C_{10:0}$ fatty acids.
- Content of unsaturated fatty acids is low.
- Even though melting point is low due to short chain fatty acids.

25.5.3 Vegetable butters

- Fats of this groups are derived from the seeds of various tropical trees.
- They are distinguished by their narrow melting range - which is mainly due to arrangement of fatty acids in the triacylglycerol molecules.
- They are extensively used in the manufacture of confections.
- Cocoa butter being the most important member of this group.

25.5.4 Oleic–linoleic acids fats

- Fat in this group are the most abundant.
- Vegetable origin and contain large amounts of oleic and linoleic acids and less than 20% saturated fatty acids.
- The most important members of this group are cottonseed, corn, peanut, sunflower, palm olive and sesame oils.

25.5.5 Linolenic acid fats

- Fat in this group contain substantial amount of linolenic acid.
- Examples are soybean, rapeseed and flexseed, wheat germ, hempoise and perilla oils; with soybean being the most important.
- The abundance of linolenic acid in soybean oils is responsible for the development of an off-flavour problem known as flavour reversion.

25.5.6 Animal body fats

- This group consists of depot fats from domestic land animals e.g. lard and tallow.
- All contain large amounts of $C_{16}>C_{18}$ fatty acids, medium amounts of unsaturated fatty acids (mostly $C_{18:1}>C_{18:2}$) and small amount of odd-numbered acids.
- Also contain appreciable amounts of fully saturated triacylglycerol and exhibits relatively high melting points.
- Egg lipids are of particular importance because of their emulsifying properties and their high content of cholesterol.
This lipid content whole egg is approx. 12%, most exclusively present in the yolk
The yolk contains 32-36 % lipids
Yolk lipids consist of about 66 % triacylglycerol and 28% phospholipids and 5% cholesterol
The major phospholipids of egg yolk are phosphoatidylcholine (73%) and phosphatidylethanolamine (18%)

25.5.7 Marine oils

- These oils typically contain large amounts of long-chain omega-3-polyunsaturated fatty acids, with upto six double bonds
- They usually rich in vitamins A & D.

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Lesson 26

IMPORTANT REACTION OF FATTY ACIDS (SATURATED AND UNSATURATED)

26.1 Introduction

The various reactions of fatty acids are mentioned below

26.2 Saturated Fatty Acids

26.2.1 Saponification value

The process of mixing a strong alkali with glycerides and heating splits the glycerides yielding glycerol and alkaline salts of fatty acids (soaps). This is known as saponification. The saponification value is defined as the number of milligrams of KOH required to saponify 1 gm of fat/oil. The saponification value is inversely proportional to the mean molecular weight of fatty acids. This is because a unit weight of fat containing lower mol. weight fatty acids would contain larger number of molecules than equal weight of fats containing higher mol. weight of fatty acids. Each molecule of glyceride irrespective of mol. weight requires 3 molecules of alkali for saponification. Thus glycerides containing higher mol. weight fatty acids would require less KOH.

Determination of saponification value gives idea about the proportion of fatty acids in respect to molecular weight and chain length. The mineral oil e.g. liquid paraffin is not acted upon by alkali and so there is no uniform solution upon saponification; thus its presence is detected in butterfat. From the knowledge of RM value and Saponification value, as both are higher in for butter fat, the adulteration of animal body fat or vegetable fat/oil can be detected. The Saponification value is also important in speculation about certain physical properties like melting point, solidification point, refractive index etc. of fat.

26.2.1.1 Principle

Heating with alcoholic KOH saponifies the fats/oils. Fats are water insoluble and hence the rate of hydrolysis by aqueous KOH is slow so alcoholic KOH is used for the reaction. The amount of alkali consumed for saponification of fat is estimated by back titration with an acid. Refluxing is the collection of distillate back in the original content from which it has been vaporized.
26.2.2 Free Fatty Acids

Milk fat contains little concentration of free fatty acids in their composition. Upon passage of time due to splitting of glycerides by the action of lipase the concentration of free fatty acids increase resulting into hydrolytic rancidity.

26.2.2.1 Principle

The free fatty acids present in ghee are estimated by titration with weak alkali using phenolphthalein as indicator.

In milk and cream, lactic acid is important, contributing major part of acidity, whereas, in butter and ghee wide range of fatty acids occur which vary widely in molecular weight. It is therefore preferable to present the free fatty acids in terms of acid value.

26.2.3 Reichert-Meissle and Polenske Values

The method does not determine the total quantities of volatile fatty acids, soluble and insoluble in water,
present in combination in fat. The amount of these acids actually determined by this process is
dependent on strict adherence to the dimensions of the apparatus and the details of the procedure.

The Reichert-Meissl value (R.M. value) is the number of ml of 0.1 N aqueous alkali solution required to
neutralize the water-soluble steam volatile fatty acids distilled from 5g of ghee under the precise
conditions specified in the method.

The Polenske value is the number of ml of 0.1 N aqueous alkali solution required to neutralize the
water-insoluble steam volatile fatty acids distilled from 5g of ghee under the precise conditions
specified in the method.

The Kirschner value is the number of ml of 0.1 N aqueous alkali solution required to neutralize the
water-soluble steam volatile fatty acids which form water-soluble silver salts distilled from 5g of ghee
under the precise conditions specified in the method.

26.2.3.1 Principle

Upon saponification of fats with strong alkali under vigorous heat, the acylglycerols split into glycerol
and alkaline salt of fatty acids. The fatty acid salts thus obtained are treated with dilute sulphuric acid to
set free the fatty acids during steam distillation. A portion of the fatty acids, being volatile under the
given condition, pass into the distillate along with excess of water.

The volatile portion again consists of two types of fatty acids (i) water-soluble and (ii) water-insoluble.
The water-insoluble fatty acids are harvested by washing the condenser, still head, receiver and filter
paper with alcohol/rectified spirit where as water-soluble fatty acids remain in the filtrate of the
distillates. Amount of the water-soluble fatty acids is estimated by titrating the filtrate with standard
alkali. That of water-insoluble fatty acids is estimated by titrating the alcohol extract with standard
alkali.

The distillation time should be between 19 and 21min. Overheating results into decarboxylation of fatty
acids and migration of double bonds. Hence, prolonged heating or distillation brings decomposition of
nonvolatile fatty acids and yield volatile products which makes the determination nonquantitative.
26.3 Unsaturated Fatty Acids

26.3.1 Iodine value

Iodine value is the number of grams of iodine absorbed by 100 grams of fat or oil under the specified conditions. It ranges from 26 to 33 for normal fresh ghee. It is determined as % by weight of halogen calculated as iodine. This value is a measure of degree or unsaturation of fat. The extent of hydrogenation of fat and adulteration of ghee with vegetable oil or animal body fats can also be detected from the iodine value. Iodine value is affected by factors like season, feed, oxidative rancidity, extent of hydrogenation etc.

The volumetric analysis in which the use of standard iodine solution is made to estimate the amount of a substance dissolved in a given solution is known as iodimetry. Iodometry is a volumetric titration in which the iodine liberated during a chemical reaction from the substance containing iodine is determined.

26.3.1.1 Principle

The unsaturated fatty acids absorb halogen and form addition products. The addition of halogen iodide or monochloride to the double bond is quantitative. Iodine is absorbed very slowly by the fat/oil and hence to bring about the reaction carried out, iodine monochloride is used. The method is based on the treatment of known weight of fat/oil with a known volume of standard solution of iodine monochloride and estimation of free ICl.

Iodine has low solubility in water (only 0.335g in 1 lit. water at 25° C). Also an aqueous solution of iodine has an appreciable vapour pressure of iodine and therefore decreases slightly in concentration on account of volatilization. These difficulties are overcome by dissolving iodine in aqueous potassium iodide solution. The increased solubility is due to formation of tri iodate ion (I_2+I^-→I_3^-). The resulting solution has much less vapour pressure. When an iodide solution of iodine is treated with the reducing agent, this displaces the equilibrium to the left and eventually all the tri iodate is decomposed. The solution is therefore behaves as a solution of free iodine. This free iodine is titrated with 0.1 N sodium thiosulphate solution using starch as an indicator.
26.3.2 Peroxide values

The oxidation of unsaturated lipids is initiated by two oxygen addition mechanisms: (a) addition of molecular oxygen on methylene group adjacent to double bond, i.e. autocatalytic radical chain reaction mechanism, or (b) concreted addition “ene” reaction: addition of singlet oxygen directly at double bond in unsaturated lipids. Autoxidation may be considered as a two step process: (i) Primary autoxidation reaction which may lead to the formation of hydroperoxides. (ii) Further reaction of decomposition of hydroperoxides by cleavage of C-C bond to form volatile compounds.

The peroxide value is a measure of the oxidative rancidity in ghee and is expressed as milliliters of 0.002 N sodium thiosulphate per gram of sample, or as milliequivalents of peroxide oxygen per kg of sample. Two methods are recommended by BIS (a) Iodometric Method and (b) Oxygen Absorption Method. Here the determinations will be performed by Iodometric Method. The other chemical methods to measure oxidative deterioration in lipid and lipid containing food products include: (i) Determination of peroxide by modified Stamm method, (ii) Loftus-Hill method, (iii) Thio-Barbutyric Acid (TBA) value, (iv) Determination of carbonyl content, etc.

Autoxidation of unsaturated fatty acids involve their reaction with molecular oxygen and as a result several peroxide structure compounds are formed as intermediate products. Amount of the peroxides is estimated iodometrically. In acidic conditions, these peroxides liberate free iodine from potassium iodide. Amount of the liberated iodine, which is in proportion to the amount of peroxides present in the sample, is determined by titrating with standard sodium thiosulphate.
\[
\begin{align*}
\text{-CH}_2\text{-CH=CH-CH}_2^- & \quad \text{(original radical)} \\
\text{-C}^\cdot \text{H-CH=CH-CH}_2^- & \quad \text{(free radical)} \\
\text{-CH(OO)-CH=CH-CH}_2^- & \quad \text{(peroxide radical)} \\
\text{-CH(OOH)-CH=CH-CH}_2^- & \quad \text{(original radical)} \\
\text{-CH}_2\text{-CH=CH-CH}_2^- & \quad \text{(hydroperoxide radical)} \\
\text{-H-CH=CH-CH}_2^- & \quad \text{(free radical)}
\end{align*}
\]

\[
\begin{align*}
\text{ROOH} & \rightarrow \text{ROH} + \text{O} \\
\text{Iodometric titration/estimation:} \\
2\text{KI} + 2\text{CH}_3\text{COOH} + \text{O} & \rightarrow \text{I}_2 + 2\text{CH}_3\text{COOK} + \text{H}_2\text{O}
\end{align*}
\]

Iodometric titration/estimation:
\[
\begin{align*}
2\text{Na}_2\text{S}_2\text{O}_3 & + \text{I}_2 + \text{Starch} \\
\text{blue} & \\
2\text{NaI} & + \text{Na}_2\text{S}_2\text{O}_6 + \text{Starch} \\
\text{Sodium tetrathiosulphate} & \text{ (colourless)}
\end{align*}
\]

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Lesson 27
STRUCTURE AND PROPERTIES OF NEUTRAL LIPIDS, PHOSPHOLIPIDS AND CHOLESTEROL

27.A.1 Introduction

- Include –fats, oils and waxes
- Uncharged- therefore termed as non-polar or neutral lipids
- On hydrolysis yield only alcohol (glycerol) and fatty acids
- Chemically -fat and oil - glycerides

27.A.2 Glycerides

- Esters of fatty acids with glycerol
- Classified - mono-, di- and triglycerides - depending upon number of –OH of glycerol esterified.

<table>
<thead>
<tr>
<th>Simple Lipids</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Monoglycerides</strong></td>
</tr>
<tr>
<td>H₂C—O—C—R</td>
</tr>
<tr>
<td>H₂C—OH</td>
</tr>
<tr>
<td>H₂C—OH</td>
</tr>
<tr>
<td>H₂C—OH</td>
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<tr>
<td>H₂C—OH</td>
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27.A.3 Phospholipids

- Heterogeneous group of compounds belonging to class lipid and contain ester phosphate-therefore complex lipid
- White waxy solids- turns dark on exposure to air or light - because polymerization- because contained excessive USFAs.
- Not readily soluble in acetone, but soluble in other fat solvents-used for precipitation from extract
- Have net negative charge at phosphate group at around pH 7.0- therefore known as polar lipids
- Hygroscopic and mix well with water and form cloudy colloidal solution.

27.A.4 Importance In Dairy And Food Industry

1. Used as emulsifier in product formulation

   - Purified preparation (Lecithin)- moderately effective in lowering surface tension of aqueous solutions, but when combined or adsorbed to carbohydrates or proteins – become remarkably active and constitute valuable agent for emulsification of fats and oils
   - Large quantities of soyabean lecithin- used as emulsifying and smoothing agents in food industry
   - Used as a wetting agent in manufacturing of instant milk powder

2. Component of milk fat globule membrane

   - Play vital role in stability of fat globule emulsion form- provides net negative charge on the globule – therefore causes electrostatic repulsion between the globules

3. Used as synergistic antioxidant – specifically cephalin – prevents or delay oxidative deterioration of lipids.

27.A.5 Classification of Phospholipids

Classified into a variety of groups and sub-groups - based on structural components present in the molecule

I. Phosphoglycerides
   1. Phosphatidyl choline (Lecithin)
   2. Phosphatidyl ethanolamine (Cephalin)
   3. Phosphatidyl serine
   4. Plasmalogens
   5. Diphosphatidyl glycerol (cardiolipin)

II. Phosphoinositides

Inositol or Lipositol

III. Phosphosphingosides

Sphingomyelins
27.A.6 Phosphoglycerides

- The most common phospholipids
- Derived from a phosphatidic acid

**Figure 27.4 Formation of phosphatidic acid**

- One of the primary –OH group esterified with phosphoric acid and remaining two –OH groups by fatty acids (or in some causes forms ether linkage with a long unsaturated aliphatic chain)

**Figure 27.5 Formation of phosphoglycerides**

- Second –OH group of phosphate gets esterified with amino alcohol

**Figure 27.6 Structure of serine**

27.A.7 Lecithin

- On hydrolysis – yields – fatty acids, phosphoric acid and choline

**Figure 27.7 Structure of Lecithin**
• Variation in fatty acids composition – give rise to different lecithin
• Fatty acid at C₂ is generally unsaturated – susceptible to oxidation
• Phospholipase D- hydrolyses choline (base)
• Choline- heated – decomposes into trimethylamine and ethylene glycol

![Chemical structure of choline and its products](image)

**Figure 27.8 Thermal decomposition of Choline**

• Trimethylamine has fishy flavour therefore lecithin of fat globule membrane creates fishy flavor defect in butter

**27.A.8 Cephalin**

• On hydrolysis yields fatty acids, phosphoric acid and ethanolamine or serine

![Chemical structures of phosphatidyl ethanolamine and phosphatidyl serine](image)

**Figure 27.9 Hydrolytic products of Cephalin**

• Physical and chemical properties are similar to lecithin

**27.A.9 Plasmalogens**

• Structure- similar to lecithin or cephalin- the only difference – one of the fatty acid is replaced by enol form of long chain aldehyde, linked by ether linkage
Plasmalogens

Vinyl ether linkage - generally on position 1
Aldehyde – aliphatic and long chain (C₁₂ to C₁₈)

27.A.10 Cardiolipin

Two primary hydroxyl group of glycerol molecule - linked to two molecules of diglyceride through phosphodiester bridges

Phosphoinositides

Contains carbohydrate like inositol
27.A.12 Sphingomyelins

- In place of glycerol contain sphingosine – a complex unsaturated amino alcohol
REFERENCES


