

Pioneer Junior College
Higher 2 Chemistry (9647)
Organic Chemistry - Nitrogen Compounds

References

- 1) A Level Chemistry (4th Edition) by E.N. Ramsden
- 2) Organic Chemistry (8th Edition) by John McMurry
- 3) Chemistry in Context (5th Edition) by Hill and Holman

Content

- Amines (exemplified by ethylamine and phenylamine)
 - (i) Their formation
 - (ii) Salt formation
 - (ii) Other reactions of phenylamine
- Amides (exemplified by ethanamide)
 - (i) Their formation from acyl chlorides
 - (ii) Their hydrolysis
- Amino acids (exemplified by aminoethanoic acid)
 - (i) Their acid and base properties
 - (ii) Zwitterion formation
- Proteins
 - (i) Protein structure: primary; secondary; tertiary; quaternary structures
 - (ii) The hydrolysis of proteins
 - (iii) Denaturation of proteins

Learning Outcomes

Candidates should be able to:

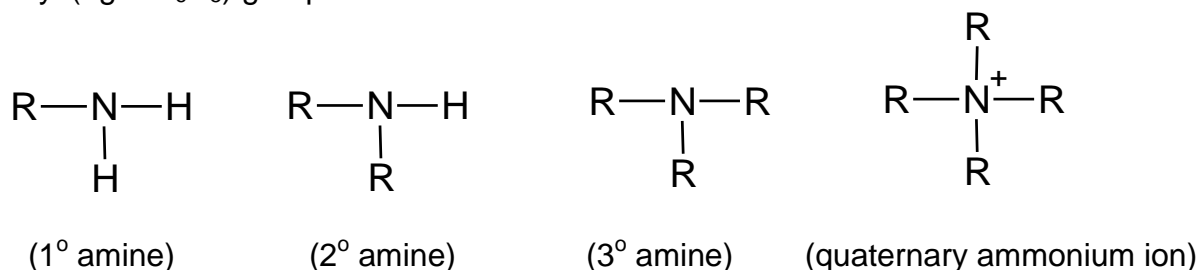
- (a) describe the formation of ethylamine (by nitrile reduction) and of phenylamine (by the reduction of nitrobenzene)
- (b) explain the basicity of amines
- (c) explain the relative basicities of ammonia, ethylamine and phenylamine in terms of their structures
- (d) describe the reaction of phenylamine with aqueous bromine
- (e) describe the formation of amides from the reaction between RNH_2 and $\text{R}'\text{COCl}$
- (f) describe amide hydrolysis on treatment with aqueous alkali or acid
- (g) describe the acid/base properties of amino acids and the formation of zwitterions
- (h) describe the formation of peptide (amide) bonds between amino acids and, hence, explain protein formation
- (i) list the major functions of proteins in the body
- (j) describe the hydrolysis of proteins
- (k) explain the term primary structure of proteins
- (l) recognise that the twenty amino acids that make up all the proteins in the body are α -amino acids with the general formula $\text{RCH}(\text{NH}_2)\text{CO}_2\text{H}$, and be able to interpret the properties of α -amino acids in terms of the nature of the R group
- (m) describe the secondary structure of proteins: α -helix and β -pleated sheet and the stabilisation of these structures by hydrogen bonding

- (n) state the importance of the tertiary protein structure and explain the stabilisation of the tertiary structure with regard to the R groups in the amino acid residues (ionic linkages, disulfide bridges, hydrogen bonds and van der Waals' forces)
- (o) describe
 - (i) the quaternary structure of proteins
 - (ii) the protein components of haemoglobin
- (p) explain denaturation of proteins by heavy metal ions, extremes of temperature and pH changes
- (q) apply the knowledge of the loss and formation of secondary and tertiary structures to interpret common everyday phenomena

AMINES

1 INTRODUCTION

Amines are derivatives of ammonia. They are formed when at least one of the hydrogen atoms in the NH_3 molecule has been replaced by either alkyl (eg. $-\text{CH}_3$) or aryl (eg. $-\text{C}_6\text{H}_5$) groups.

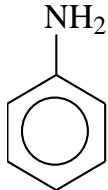
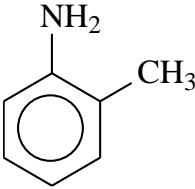
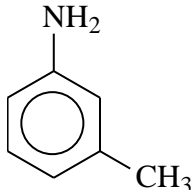
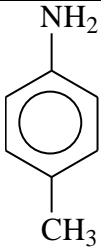
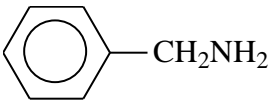
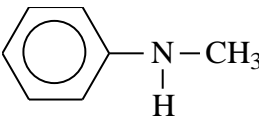


where *R* represents either an alkyl or aryl group.

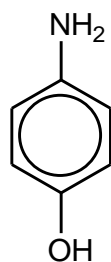
2 NOMENCLATURE

Amines are named by the alkyl or aryl group(s) attached to nitrogen, followed by the suffix -amine.

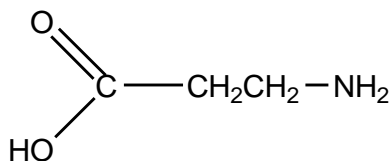
Molecular Formula	Structural Formula	IUPAC Name	Classification
CH_5N	CH_3NH_2	methylamine	primary
$\text{C}_2\text{H}_7\text{N}$	$\text{CH}_3\text{CH}_2\text{NH}_2$	ethylamine	primary
	$\begin{array}{c} \text{H}-\text{N}-\text{CH}_3 \\ \\ \text{CH}_3 \end{array}$	dimethylamine	secondary
$\text{C}_3\text{H}_9\text{N}$	$\text{CH}_3\text{CH}_2\text{CH}_2\text{NH}_2$	propylamine	primary
	$\begin{array}{c} \text{CH}_3\text{CHNH}_2 \\ \\ \text{CH}_3 \end{array}$	1-methylethylamine	primary

Molecular Formula	Structural Formula	IUPAC Name	Classification
	$\begin{array}{c} \text{CH}_3\text{CH}_2\text{N}-\text{H} \\ \\ \text{CH}_3 \end{array}$	N-methylethylamine	secondary
	$\begin{array}{c} \text{CH}_3 \\ \\ \text{CH}_3-\text{N}-\text{CH}_3 \end{array}$	trimethylamine	tertiary
C ₆ H ₇ N		phenylamine	primary
C ₇ H ₉ N		2-methylphenylamine	primary
		3-methylphenylamine	primary
		4-methylphenylamine	primary
		phenylmethanamine	primary
		N-methylphenylamine	secondary

In the presence of other functional groups with higher priorities (*refer to the lecture notes on "Introduction to Organic Chemistry"*), the amine functional group will be considered as a substituent and is given the prefix -amino.

Examples:

4-aminophenol



3-aminopropanoic acid

3 PHYSICAL PROPERTIES**3.1 Boiling Points**3.1.1 Comparison between Isomeric Amines

The table below shows the boiling points of three isomeric amines.

Structural Formula	$\text{CH}_3\text{CH}_2\text{CH}_2\text{NH}_2$	$\begin{array}{c} \text{CH}_3\text{CH}_2\text{N}-\text{CH}_3 \\ \\ \text{H} \end{array}$	$\begin{array}{c} \text{CH}_3-\text{N}-\text{CH}_3 \\ \\ \text{CH}_3 \end{array}$
Classification	Primary	Secondary	Tertiary
Boiling point	49 °C	37 °C	3.5 °C

From the table, it is observed that for isomeric amines,

Boiling point of primary amine \geq secondary amine \geq tertiary amine

Reason?

- primary and secondary amines can form intermolecular hydrogen bonds between its molecules (since there is at least 1 hydrogen atom attached directly to the electronegative nitrogen atom).
- however, the presence of two alkyl groups in the 2° amine molecule hinders the formation of hydrogen bonds (steric hindrance), which results in less extensive hydrogen bonding, and hence a lower boiling point than 1° amine.
- tertiary amines, however, **cannot** form intermolecular hydrogen bonds between its own molecules as there is no hydrogen atom bonded to the nitrogen atom directly.
- lesser energy is required to overcome the weak permanent dipole – dipole interactions between 3° molecules. Hence, 3° has the lowest boiling point.

3.1.2 Comparison with Alcohols

The table below shows the boiling points of an amine and alcohol of comparable M_r .

Structural Formula	$\text{CH}_3\text{CH}_2\text{CH}_2\text{NH}_2$	$\text{CH}_3\text{CH}_2\text{CH}_2\text{OH}$
M_r	59.0	60.0
Boiling point	49 °C	65 °C

From the table, it is observed that the boiling point of an amine is lower than that of an alcohol with comparable M_r .

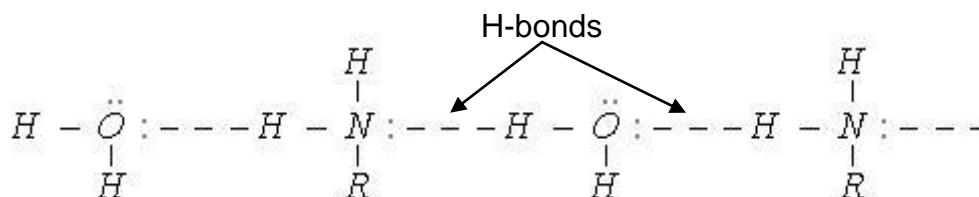
Reason?

- The N atom in the amine is less electronegative than the O atom in the alcohol.
- Hence, the H-N bond is less polar than the H-O bond.
- As a result, the hydrogen bonds that are formed between molecules of the amine are not as strong as those that are formed in the alcohol.

3.2 Solubility

Primary, secondary and tertiary amines are soluble in water because they can form hydrogen bonds (i.e. favourable solute-solvent interactions which release sufficient heat to overcome the solute-solute and solvent-solvent interactions) with water molecules.

eg. CH_3NH_2 is very soluble in water.



However, with increasing number of carbon atoms in the hydrocarbon chain, the solubility of the amines in water will decrease.

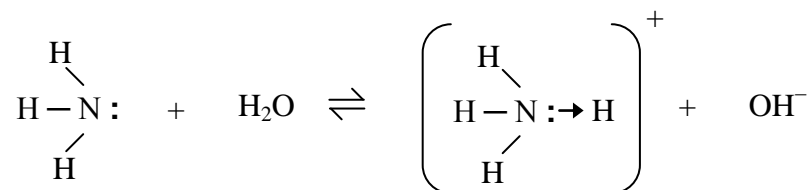
eg. $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2$ is only sparingly soluble in water.

Phenylamine is only sparingly soluble in water. This is due to two reasons:

- delocalisation of the lone pair of electrons on N atom into the benzene ring decreases the tendency to form hydrogen bonds with water;
- presence of non-polar benzene ring that does not form favourable solute-solvent interactions with water molecules.

3.3 Basicity

(b) *explain the basicity of amines*



The ammonia molecule contains a nitrogen atom with a lone pair of electrons. This enables it to act as a base by accepting a proton (or H^+) from water.

Ammonia is a weak base which only ionises partially in water. A solution of ammonia is alkaline (pH > 7).

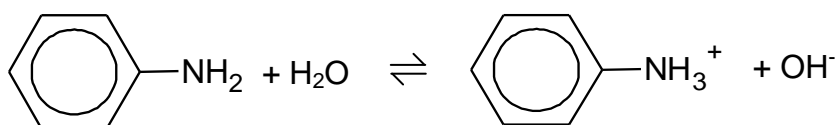
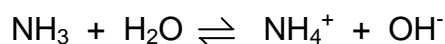
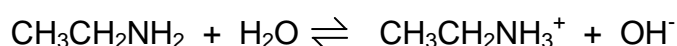
In the same way, amines are weak bases which partially ionise in water.

The important factor that affects basicity is given below:

***** Availability of lone pair of electrons on N atom for protonation / to accept a proton**

3.3.1 Relative Basicities

(c) *explain the relative basicities of ammonia, ethylamine and phenylamine in terms of their structures*

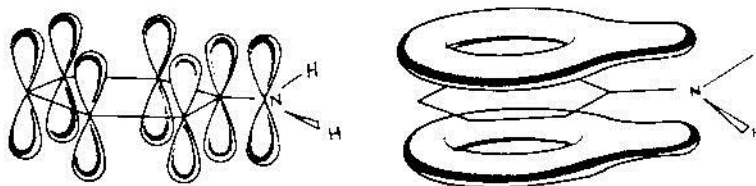


Basic strength: ethylamine > ammonia > phenylamine
 K_b : 5.01×10^{-4} 1.58×10^{-5} 3.98×10^{-10}

Base dissociation constant, K_b , is a measure of the strength of a base.

The larger the value of K_b , the stronger the base.
(will be covered in depth in the chapter of "Ionic Equilibria")

- Ethylamine is a stronger base than ammonia because:
 - ethyl group (CH_3CH_2-) is electron-donating;
 - increases electron density around N atom;
 - makes the lone pair on N atom more available to accept a proton.
- Phenylamine is a weaker base than ammonia because:



- lone pair of electrons on the N atom can be partially delocalised into the benzene ring
- making it less available to accept a proton.

Note:

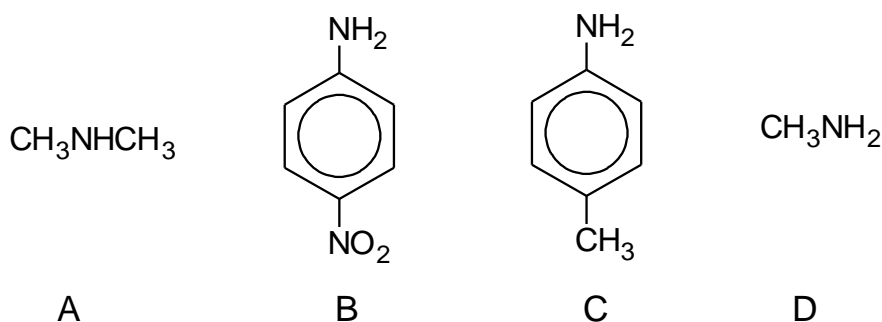
Substituents in the benzene ring can affect the basicity of phenylamine.

Electron-donating groups (eg. $-\text{CH}_3$, $-\text{C}_2\text{H}_5$) make the lone pair of electrons on N atom **more available** to accept a proton → **increase basicity**.

Electron-withdrawing groups (eg. $-\text{NO}_2$, $-\text{Cl}$) make the lone pair of electrons on N atom **less available** to accept a proton → **decrease basicity**.

Example:

Arrange the following compounds in the order of increasing basic strength. Briefly explain your answer.



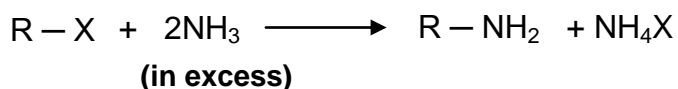
Answer: (least basic) B, C, D, A (most basic)

- phenylamines are generally less basic than aliphatic amines as the lone pair of electrons on the N atom can be partially delocalised into the ring, making it less available for protonation.
- For compounds B and C, $-\text{NO}_2$ is an electron-withdrawing group, while $-\text{CH}_3$ is an electron-donating group. Hence, the lone pair of electrons on N atom in compound C is more available for protonation than that in compound B. Therefore, compound C is more basic than B.
- For compounds A and D, $-\text{CH}_3$ group is electron-donating. There are 2 $-\text{CH}_3$ groups attached directly to the N atom in compound A, making the lone pair of electrons more available for protonation. Hence, compound A is more basic than compound D.

4 PREPARATION OF AMINES

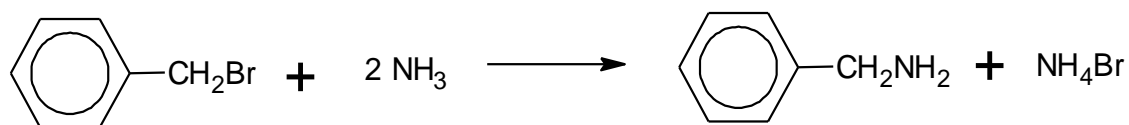
4.1 Nucleophilic Substitution of Alkyl Halide

Reagent & Conditions: excess NH_3 in ethanol, heat in a sealed tube
Type of Reaction: Nucleophilic Substitution

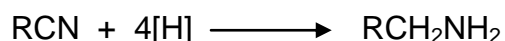
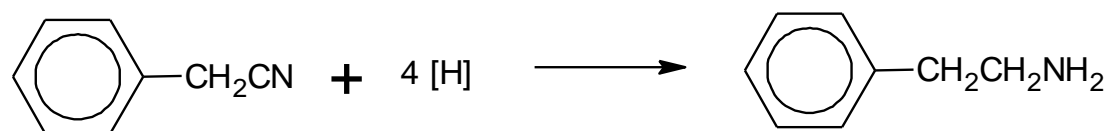
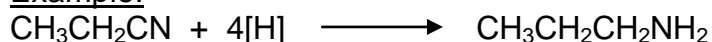


Note:

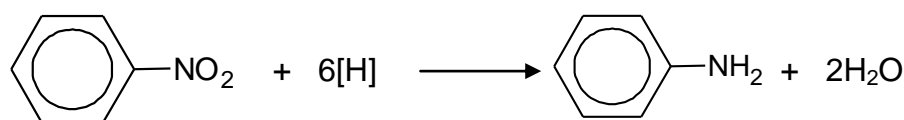
This method is **not** feasible for aryl halides. Hence phenylamine cannot be prepared this way.

Example:**4.2 Reduction of a Nitrile**

Reagent & Conditions: LiAlH₄ in dry ether **OR** H₂, Ni, 200°C
Type of Reaction: Reduction

Example:**4.3 Reduction of Nitrobenzene (for Aromatic Amines only)**

Reagent & Conditions: Step 1: Sn, concentrated HCl, heat
 Step 2: NaOH(aq)
Type of Reaction: Step 1: Reduction; Step 2: Acid-base

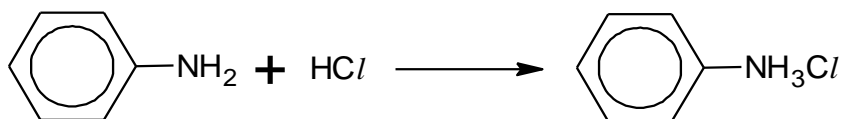
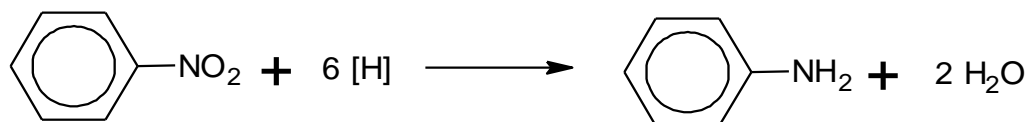
Question:

Why is the 2nd step necessary for the preparation of phenylamine?

Answer:

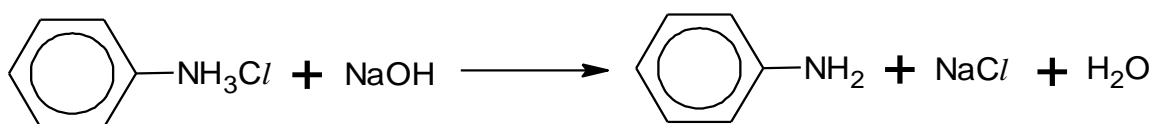
In step 1, the phenylamine produced will react with the excess HCl to form a salt.

Step 1:



Hence in step 2, the basic NaOH(aq) is added to liberate the free amine.

Step 2:



5 REACTIONS OF AMINES

(a) *describe the formation of ethylamine (by nitrile reduction) and of phenylamine (by the reduction of nitrobenzene)*

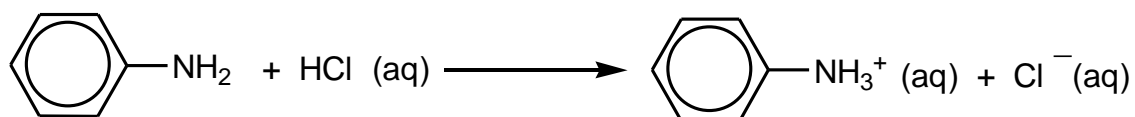
5.1 Salt Formation with Acids

Aliphatic and aryl amines form soluble salts with mineral acids.

Reagent & Conditions: HCl(aq) or H₂SO₄(aq), room temperature

Type of Reaction: Acid-base

Examples:



Note:

Phenylamine is insoluble in water (two separate immiscible layers are formed when they are mixed), but dissolve readily in HCl (aq) (only one clear colourless miscible layer is formed) due to the formation of a soluble salt. White crystalline solid of phenylammonium chloride is obtained on evaporation.

5.2 Reaction with Aqueous Bromine

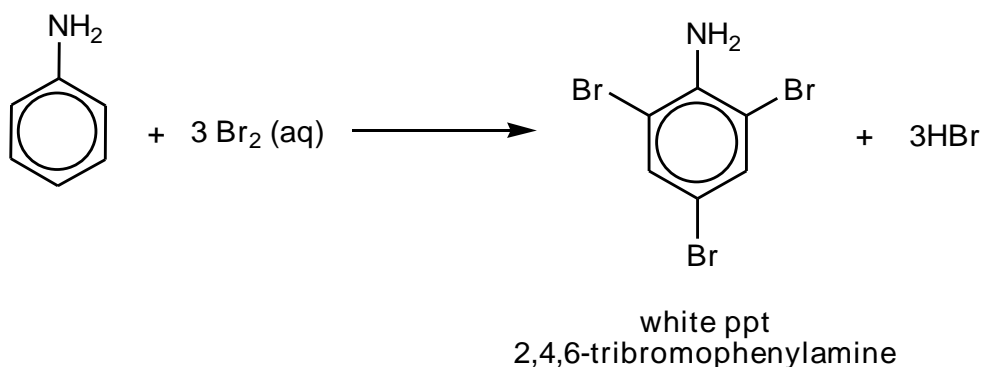
(d) describe the reaction of phenylamine with aqueous bromine

Aliphatic amines **do not** react with aqueous bromine.

Phenylamine undergoes electrophilic substitution with aqueous bromine at room temperature (similar to reaction of phenol with aqueous bromine).

Reagent & Conditions: $\text{Br}_2(\text{aq})$, room temperature

Type of Reaction: Electrophilic Substitution



Observations:

- Orange bromine water decolourises.
- White precipitate is formed.

5.3 Formation of Amides (Acylation)

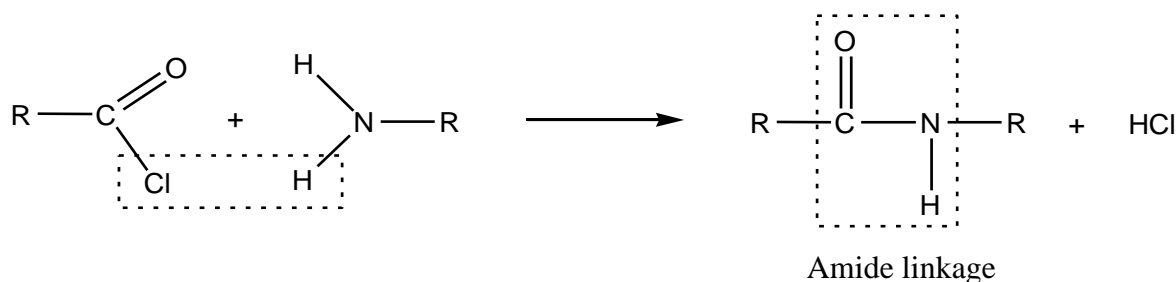
(e) describe the formation of amides from the reaction between RNH_2 and $\text{R}'\text{COCl}$

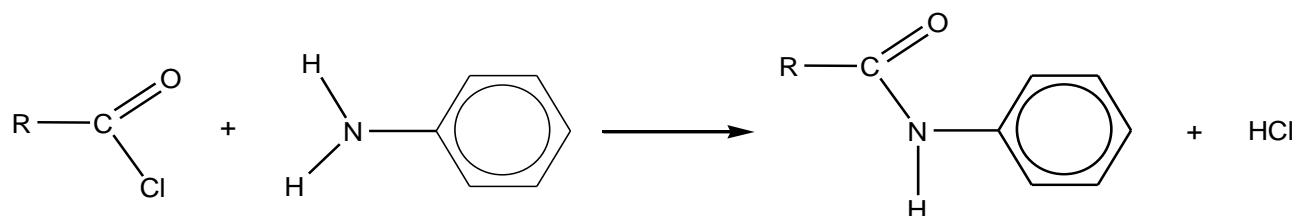
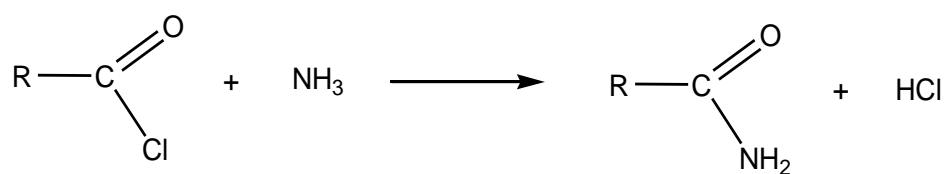
Amides are prepared by the reaction of an acyl chloride RCOCl with either NH_3 , 1° or 2° amines.

3° amine **does not** undergo acylation because it does not have any replaceable H atoms.

Reagent & Conditions: RCOCl reacting with either NH_3 , 1° or 2° amines
room temperature

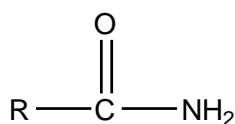
Type of Reaction: Nucleophilic Substitution



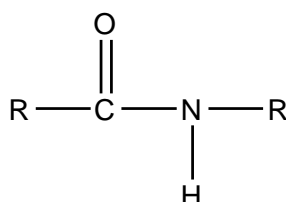
Examples:**AMIDES****1 INTRODUCTION**

Amides are derivatives of carboxylic acids.

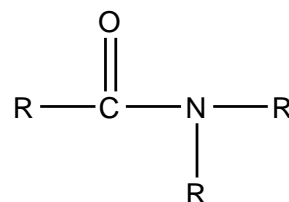
There are three different classes of amides, namely primary, secondary and tertiary.



Primary (1°)



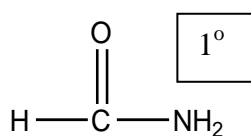
Secondary (2°)



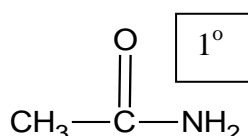
Tertiary (3°)

2 NOMENCLATURE

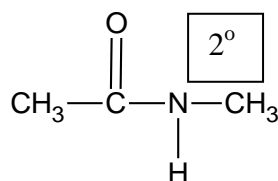
To name an amide, replace -oic acid of the parent carboxylic acid with -amide.



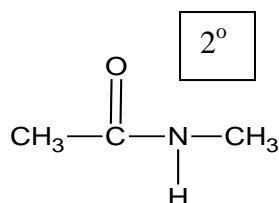
methanamide



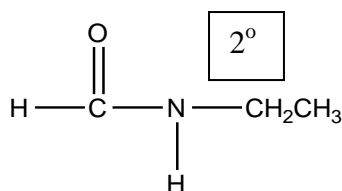
ethanamide



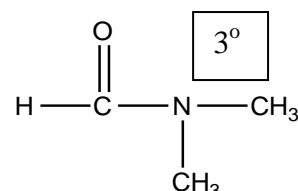
N-methylethanamide



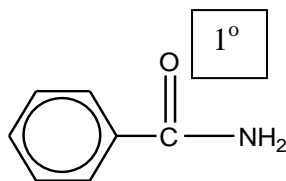
N-methylethanamide



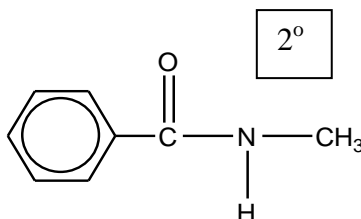
N-ethylmethanamide



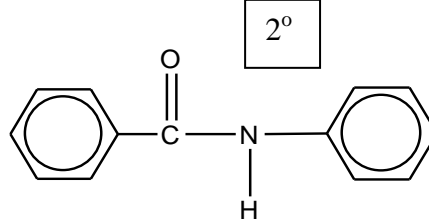
N,N-dimethylmethanamide



benzamide



N-methylbenzamide



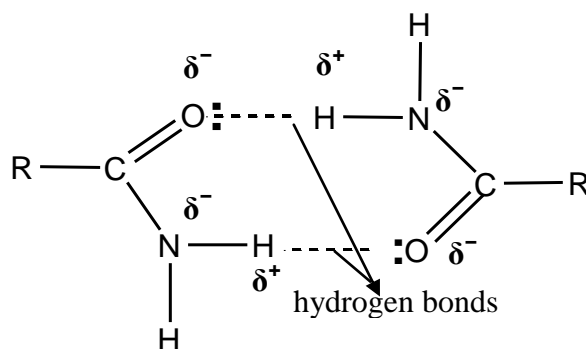
N-phenylbenzamide

Exercise:

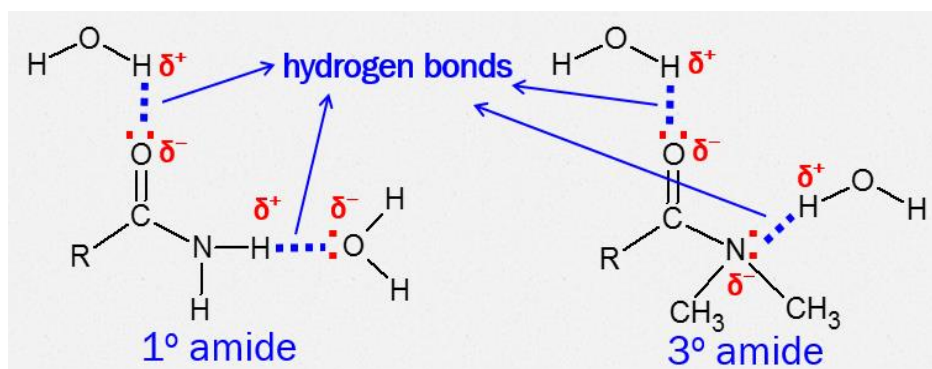
Classify the amides above.

3 PHYSICAL PROPERTIES

- Primary and secondary amides can form intermolecular hydrogen bonding.
- Tertiary amides **cannot** form such H-bonding because there is no H atom directly attached to the N atom. Weaker permanent dipole – dipole (pd-d) interactions exist between 3° amide molecules.



- Amides have relatively high melting and boiling points due to intermolecular hydrogen bonding. Hence, all amides (except methanamide) are crystalline solids at room temperature.
- Smaller amides are soluble in water.

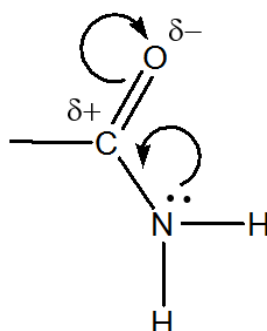


As the number of carbon atoms increases, the amides become less soluble as the increasing hydrocarbon chain prevents the formation of favourable solute-solvent interactions with water molecules, and it hinders the formation of hydrogen bonds.

- Solutions of amides are **neutral**, unlike amines.

Reason?

- *** The powerful **electron-withdrawing effect of the carbonyl group C=O** reduces the electron density on the N atom, making it less effective as a proton acceptor.
- The lone pair of electrons on the N atom can be delocalised with the π electrons of the C=O double bond.



4 REACTIONS OF AMIDES

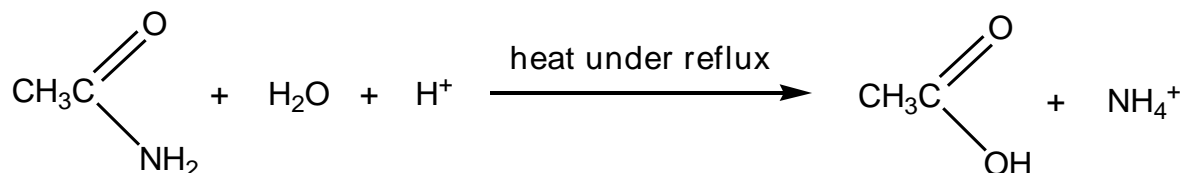
(f) *describe amide hydrolysis on treatment with aqueous alkali or acid*

4.1 Hydrolysis of Amides

4.1.1 Acidic Hydrolysis

Amides are hydrolysed to form carboxylic acids and ammonium salts.

Reagent & Conditions: $\text{H}_2\text{SO}_4(\text{aq})$ **OR** $\text{HCl}(\text{aq})$, heat under reflux
 Type of Reaction: acid hydrolysis

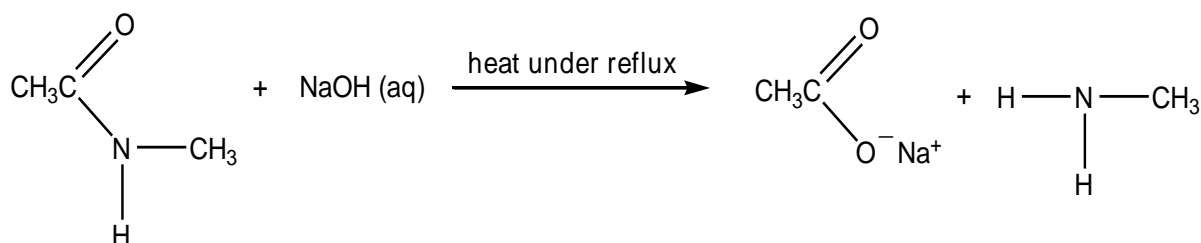
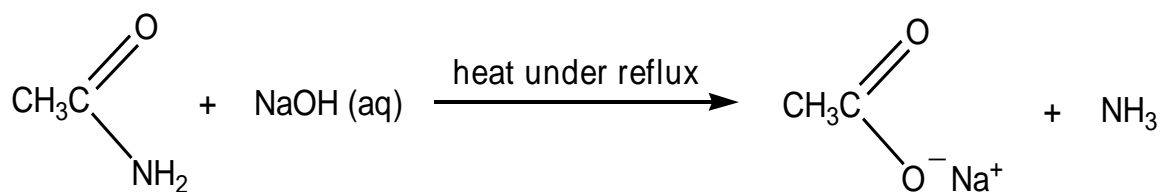


4.1.2 Alkaline Hydrolysis

Amides are hydrolysed to produce carboxylate salts.

Reagent & Conditions: NaOH(aq), heat under reflux

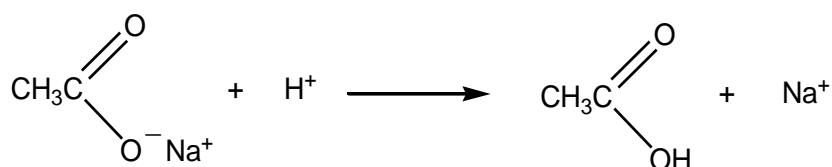
Type of Reaction: alkaline hydrolysis



The salt can then be acidified to give the carboxylic acid.

Reagent & Conditions: H₂SO₄(aq) **OR** HCl(aq), room temperature

Type of Reaction: acidification



Note:

This reaction can be used to distinguish an amide with the formula RCONH₂ (primary amide) from an ammonium salt (eg CH₃COO⁻NH₄⁺).

- The primary amide liberates NH₃ when **heated** with aqueous NaOH.



- The ammonium salt liberates NH₃ with aqueous NaOH **without heating**.

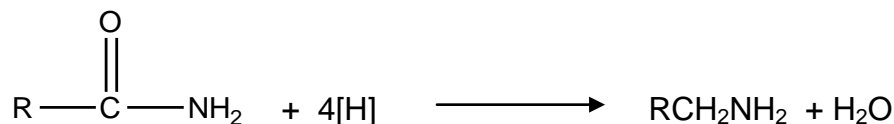


4.2 Reduction of Amide

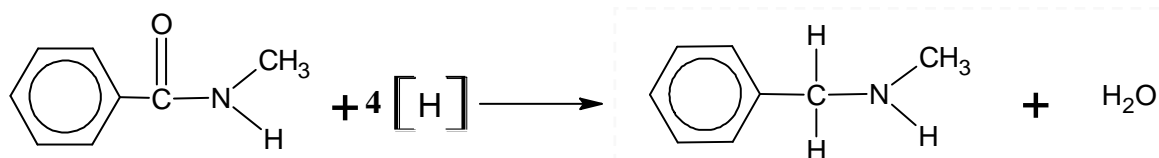
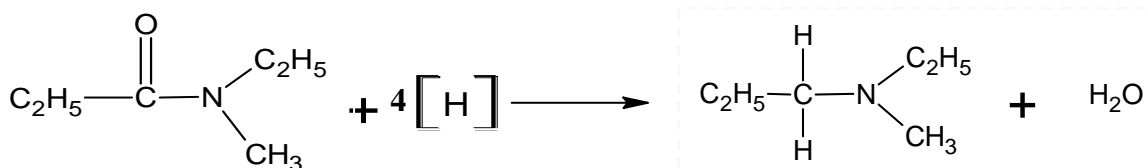
1°, 2° and 3° amides can be reduced to produce 1°, 2° and 3° amines respectively.

Reagent & Conditions: LiAlH₄ in dry ether

Type of Reaction: reduction



Exercise:



AMINO ACIDS

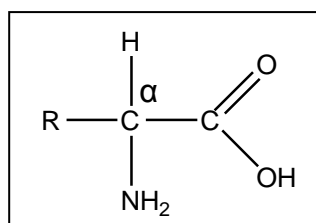
- (I) recognise that the twenty amino acids that make up all the proteins in the body are α -amino acids with the general formula $\text{RCH}(\text{NH}_2)\text{CO}_2\text{H}$, and be able to interpret the properties of α -amino acids in terms of the nature of the R group

1 INTRODUCTION

An amino acid is an organic molecule which contains the **amino ($-\text{NH}_2$) group** and the **carboxyl ($-\text{COOH}$) group** on the same C. They are important compounds in nature and are the basic building blocks of proteins.

While there are many forms of amino acids, the important ones that function as building blocks for protein syntheses are called the **α -amino acids**.

All α -amino acids have the following general structure:





There are about 20 amino acids found in proteins in the body and all of them are α -amino acids.

All α -amino acids (except glycine) have at least one chiral carbon atom. Hence they exhibit optical isomerism. Most of the α -amino acids are L-stereoisomers (left-handed isomers), although a few D-amino acids (right-handed isomers) occur in bacterial envelopes and some antibiotics.

Amino acids can be categorised into:

- (i) Non-essential amino acids: Can be synthesised by the human body for normal metabolic processes.
- (ii) Essential amino acids: Cannot be synthesised by the human body and hence the necessary raw materials must be obtained from the diet. There are eight essential amino acids required by adults and are indicated in the table below by an 'E'. Small children also need histidine during rapid growth in addition to the other eight essential amino acids.

List of common α -amino acids in proteins:

Name	Abbreviated name	R	Essential amino acid
Glycine	Gly	— H	
Alanine	Ala	— CH ₃	
Valine	Val	— CH(CH ₃) ₂	E
Leucine	Leu	— CH ₂ CH(CH ₃) ₂	E
Isoleucine	Ileu	— CH(CH ₃)(CH ₂ CH ₃)	E
Serine	Ser	— CH ₂ OH	
Threonine	Thr	— CH(OH)CH ₃	E
Aspartic acid	Asp	— CH ₂ COOH	
Asparagine	Asn	— CH ₂ CONH ₂	
Glutamic acid	Glu	— CH ₂ CH ₂ CO ₂ H	
Glutamine	Gln	— CH ₂ CH ₂ CONH ₂	
Lysine	Lys	— (CH ₂) ₄ NH ₂	E
5-Hydroxylysine		— (CH ₂) ₂ CH(OH)CH ₂ NH ₂	
Arginine	Arg	— (CH ₂) ₃ NHC(NH ₂)NH ₂	
Cysteine	CysH	— CH ₂ SH	
Cystine	Cys – Cys	— (CH ₂) ₂ SSCH ₂ CH(NH ₂)CO ₂ H	
Methionine	Met	— (CH ₂) ₂ SCH ₃	E
Phenylalanine	Phe	— CH ₂ — 	E
Tyrosine	Tyr		

		$-\text{CH}_2- \quad -\text{OH}$	
Tryptophan	Try	$ \begin{array}{c} -\text{CH}_2-\text{C}-\text{C}_6\text{H}_5 \\ \parallel \\ \text{HC} \\ \backslash \\ \text{NH} \end{array} $	E
Histidine	His	$ \begin{array}{c} -\text{CH}_2-\text{C}=\text{CH} \\ \backslash \quad \quad / \\ \text{HN} \quad \quad \text{N} \\ \quad \quad \backslash \quad / \\ \quad \quad \text{CH} \end{array} $	E (For infants)

Classifications of amino acids according to the nature of the R group:

Structure	Name	Remarks
$ \begin{array}{c} \text{H} \\ \\ \text{H}_2\text{N}-\text{C}-\text{COOH} \\ \\ \text{H} \end{array} $	2-aminoethanoic acid (glycine)	Simplest amino acid. No optical activity
$ \begin{array}{c} \text{H} \\ \\ \text{H}_2\text{N}-\text{C}-\text{COOH} \\ \\ \text{CH}_3 \end{array} $	2-aminopropanoic acid (alanine)	Contains a chiral carbon and exists in optically active forms (enantiomers).
$ \begin{array}{c} \text{H} \\ \\ \text{H}_2\text{N}-\text{C}-\text{COOH} \\ \\ \text{CH}_2\text{SH} \end{array} $	2-amino-3-thiopropionic acid (cysteine)	Example of sulfur-containing amino acid. Able to form strong <u>disulfide linkages</u> in <u>3°</u> and some of the <u>4° structures</u> of proteins.
$ \begin{array}{c} \text{H} \\ \\ \text{H}_2\text{N}-\text{C}-\text{COOH} \\ \\ \text{CH}_2 \\ \\ \text{COOH} \end{array} $	2-aminobutane-1,4-dioic acid (aspartic acid)	Examples of acidic amino acids. Each molecule contains one basic amino group and two acidic carboxyl groups.

Structure	Name	Remarks
$ \begin{array}{c} \text{H} \\ \\ \text{H}_2\text{N}-\text{C}-\text{COOH} \\ \\ \text{CH}_2 \\ \\ \text{CH}_2 \\ \\ \text{COOH} \end{array} $	2-aminopentane-1,5-dioic acid (glutamic acid)	
$ \begin{array}{c} \text{H} \\ \\ \text{H}_2\text{N}-\text{C}-\text{COOH} \\ \\ (\text{CH}_2)_4 \\ \\ \text{NH}_2 \end{array} $	2,6-diaminohexanoic acid (lysine)	Example of basic amino acid. The molecule contains two basic amino groups and one acidic carboxyl group.
$ \begin{array}{c} \text{H} \\ \\ \text{H}_2\text{N}-\text{C}-\text{COOH} \\ \\ \text{CH}_2 \\ \\ \text{H}-\text{C}-\text{CH}_3 \\ \\ \text{CH}_3 \end{array} $	2-amino-4-methylpentanoic acid (leucine)	Example of neutral amino acids. (same number of carboxyl and amino groups)
$ \begin{array}{c} \text{H} \\ \\ \text{H}_2\text{N}-\text{C}-\text{COOH} \\ \\ \text{H}-\text{C}-\text{CH}_3 \\ \\ \text{CH}_2 \\ \\ \text{CH}_3 \end{array} $	2-amino-3-methylpentanoic acid (isoleucine)	
$ \begin{array}{c} \text{H} \\ \\ \text{H}_2\text{N}-\text{C}-\text{COOH} \\ \\ \text{H}-\text{C}-\text{OH} \\ \\ \text{CH}_3 \end{array} $	2-amino-3-hydroxybutanoic acid (threonine)	Examples of amino acids containing a hydroxy group. Able to form <u>hydrogen bonds</u> in <u>3^o structure</u> of proteins

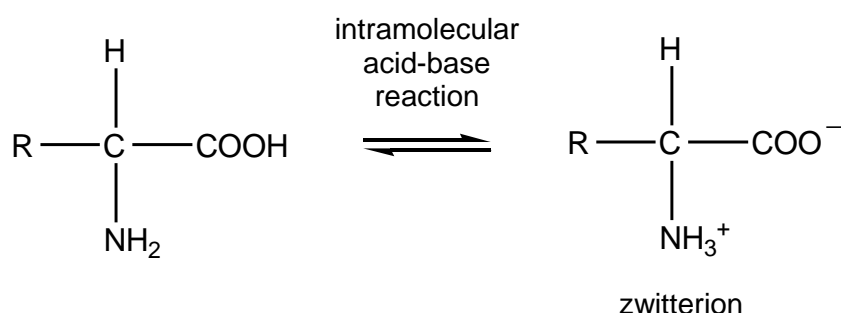
Structure	Name	Remarks
$ \begin{array}{c} \text{H} \\ \\ \text{H}_2\text{N} - \text{C} - \text{COOH} \\ \\ \text{CH}_2\text{OH} \end{array} $	2-amino-3-hydroxypropanoic acid (serine)	

2 PROPERTIES OF AMINO ACIDS

(g) describe the acid/base properties of amino acids and the formation of zwitterions

In aqueous solution and in the solid state, amino acids exist as **dipolar ions** or **zwitterions**, which are **electrically neutral**.

A zwitterion is formed through an acid-base reaction between the carboxyl group and the amino group of the same amino acid molecule.



2.1 Physical Properties

Amino acids are crystalline solids with **high melting points**.

Why?

- In the solid state, amino acids exist in the zwitterionic form.
- Strong electrostatic forces of attraction / ionic bonds exist between the zwitterions.
- Large amount of energy is required to overcome these forces of attraction.

Amino acids are **soluble in water**, but **insoluble in non-polar solvents**.

Why?

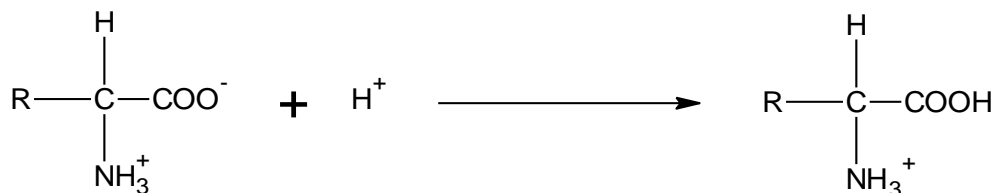
- Formation of favourable ion-dipole interactions between water and the zwitterions.
- Energy released is sufficient in overcome the ionic bonds between the zwitterions.

2.2 Acid-Base Properties

Aqueous solutions of most amino acids are **neutral** as the acidic nature of the carboxyl group is counterbalanced by the basic nature of the amino group.

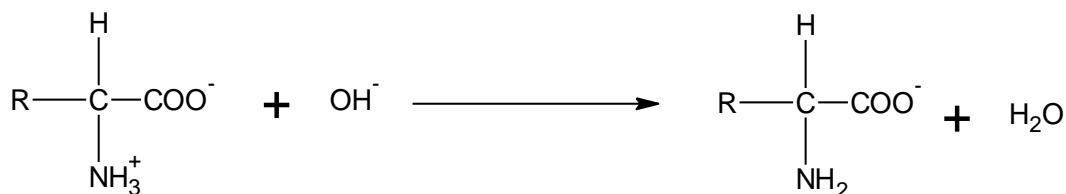
Amino acids are **amphoteric** in nature i.e. they show properties of both a base (due to presence of -NH_2 group) and an acid (presence of -COOH group).

In **acidic** medium (low pH), the amino acid acts as a base and accepts a proton, thus forming a **cation**.



- Since the resulting species is positively charged, it will migrate towards the negative electrode (cathode) when a potential difference is applied.

In **basic** medium (high pH), the amino acid acts as an acid and donates a proton to OH^- , thus forming **anion**.



- Since the resulting species is negatively charged, it will migrate towards the positive electrode (anode) when a potential difference is applied.

2.3 Isoelectric Point

The **pH** at which an amino acid has a **net charge of zero** is known as the **isoelectric point (pI)**.

At this pH, the amino acid will **NOT** migrate under the influence of an electric field as it has no **net** charge.

Different amino acids have different isoelectric points. The isoelectric point is a characteristic of each amino acid.

3 SEPARATION OF AMINO ACIDS BY ELECTROPHORESIS

Mixtures of amino acids (usually obtained from the hydrolysis of proteins) can be **separated** and **identified** using electrophoresis.

Electrophoresis refers to the separation of charged particles in solution by the difference in their tendencies to migrate towards the oppositely charged electrodes.

Two factors that affect the rate at which the charged particles move during electrophoresis are:

- 1) **Charge** on the particle
- 2) **Mass** of the particle

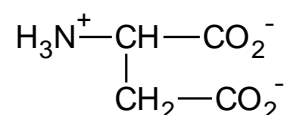
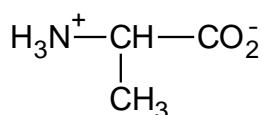
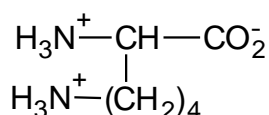
Procedures involved in electrophoresis:

- A strip of filter paper or gel is soaked in a buffer solution of a specific pH.
- A streak of the amino acid mixture is placed in the centre of each strip.
- Two electrodes are placed in contact with the edges of the paper, and a potential difference is applied across the electrodes.
- Positively charged (cationic) amino acids migrate towards the negative electrode.
- Negatively charged (anionic) amino acids migrate towards the positive electrode.
- An amino acid at its isoelectric point has no net charge. Hence it does not move.

After a period of time, the separated amino acids are recovered by cutting the paper. If electrophoresis is being used as an analytical technique (to determine the amino acids present in the mixture), the paper is treated with a reagent such as ninhydrin to make the bands visible. The amino acids are then identified by comparing their positions with those of standards.

Example:

Consider a mixture of lysine (pI = 9.7), alanine (pI = 6.0), and aspartic acid (pI = 2.8). In a buffer solution at pH of 6, their structures are as follows:



Lysine (net charge= +1)

- pH 6 is more acidic than pH 9.7, so lysine is in the cationic form.
- migrates towards cathode.

Alanine (net charge= 0)

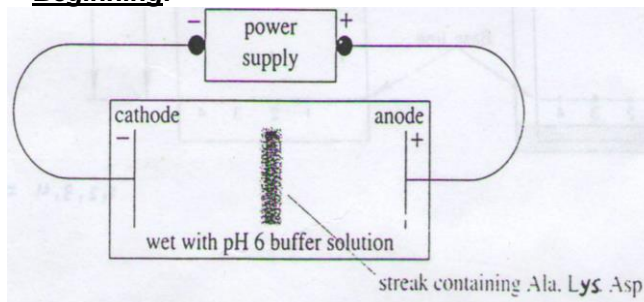
- Exists as zwitterion.
- Does not move.

Aspartic acid (net charge= -1)

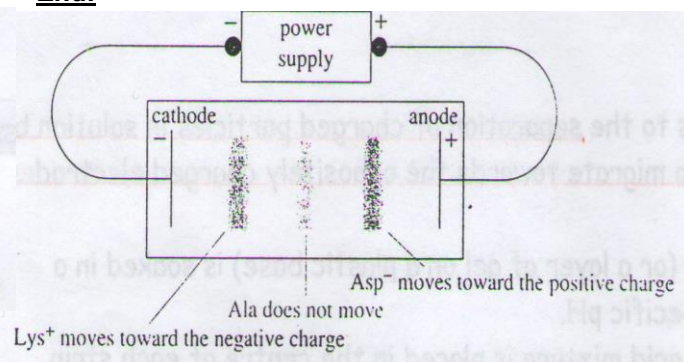
- pH 6 is more alkaline than pH 2.8, so aspartic acid is in the anionic form.
- migrates towards anode.

Their separation would be as shown in the diagrams below:

Beginning:



End:



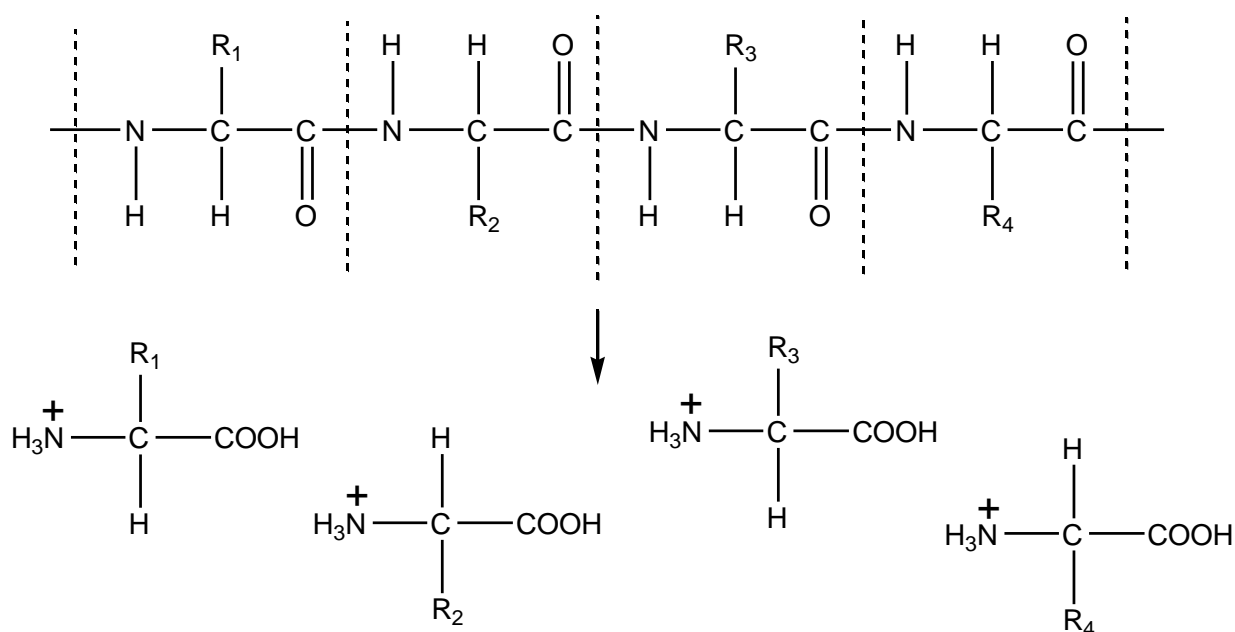
5 HYDROLYSIS OF PROTEIN

(j) *describe the hydrolysis of proteins*

Proteins can be broken down into its constituent amino acids by heating them under reflux in acidic or alkaline conditions.

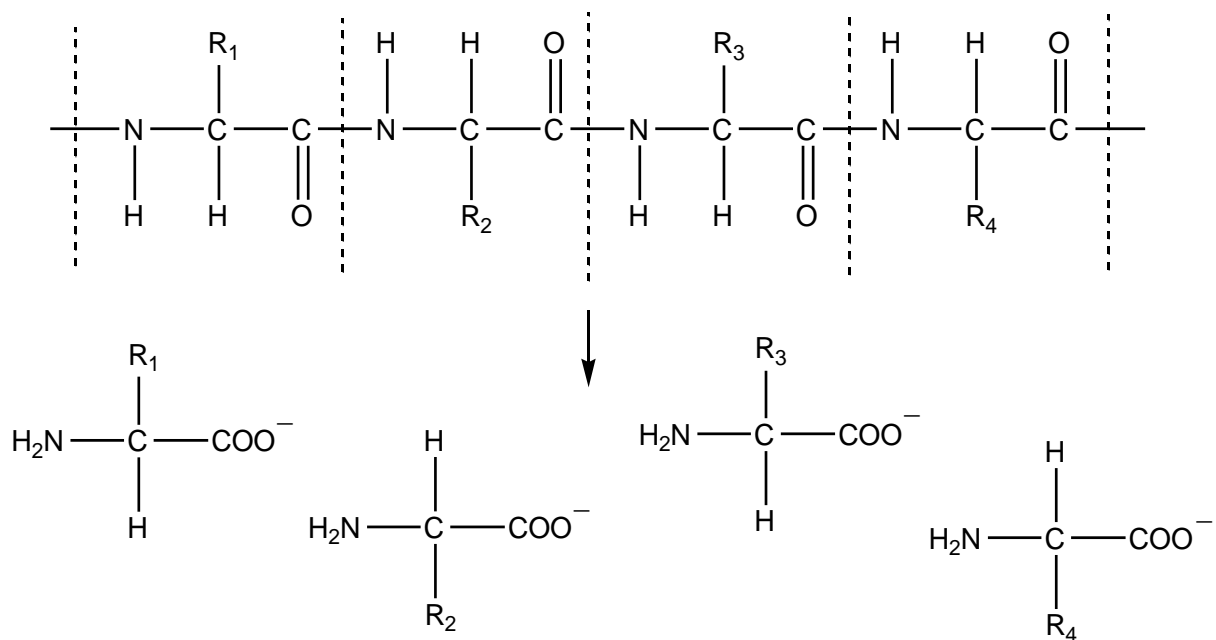
5.1 Acid Hydrolysis

Reagent & Conditions: $\text{H}_2\text{SO}_4(\text{aq})$, heat under reflux for a long time



5.2 Alkaline Hydrolysis

Reagent & Conditions: $\text{NaOH}(\text{aq})$, heat under reflux for a long time



Summary (exemplified by phenyl)

