Qualitative Tests of Amino Acids

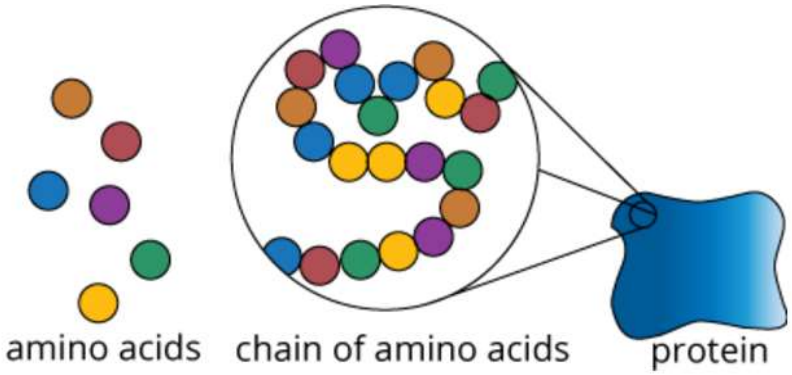
BCH303 [Practical]

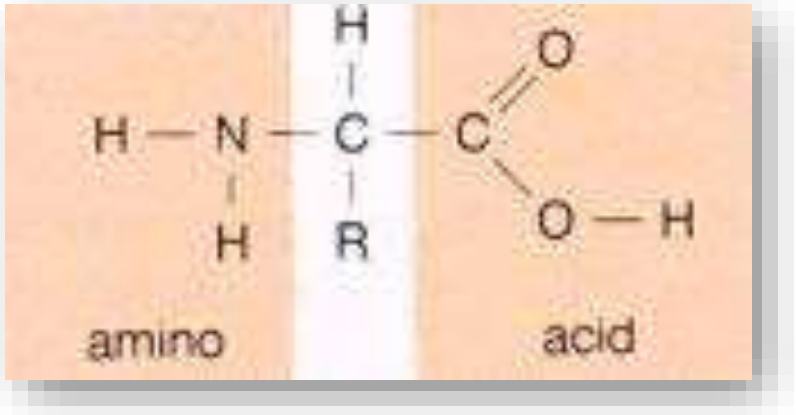
Amino Acids:

- Amino acid's role.

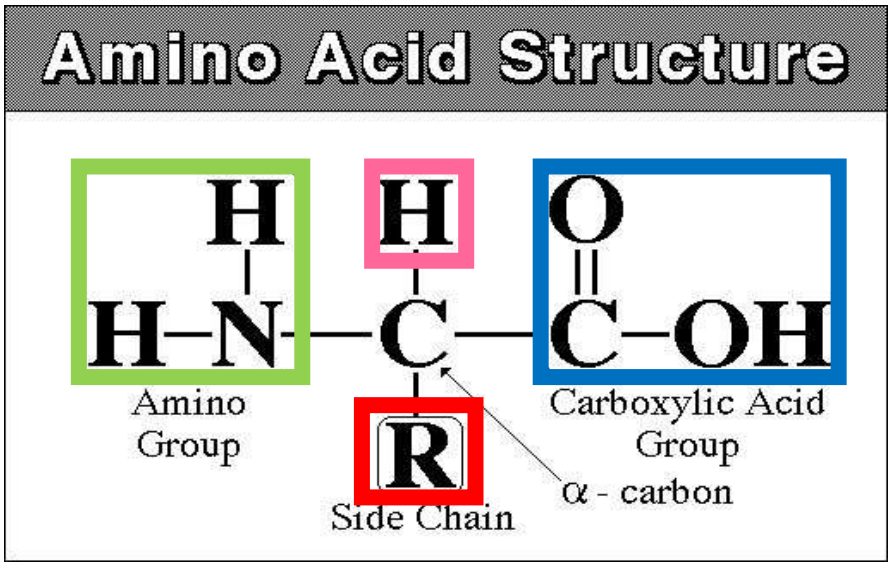
- There are 20 natural amino acids that are found within proteins.

➔ In our bodies **All of them are L-α amino acids.**

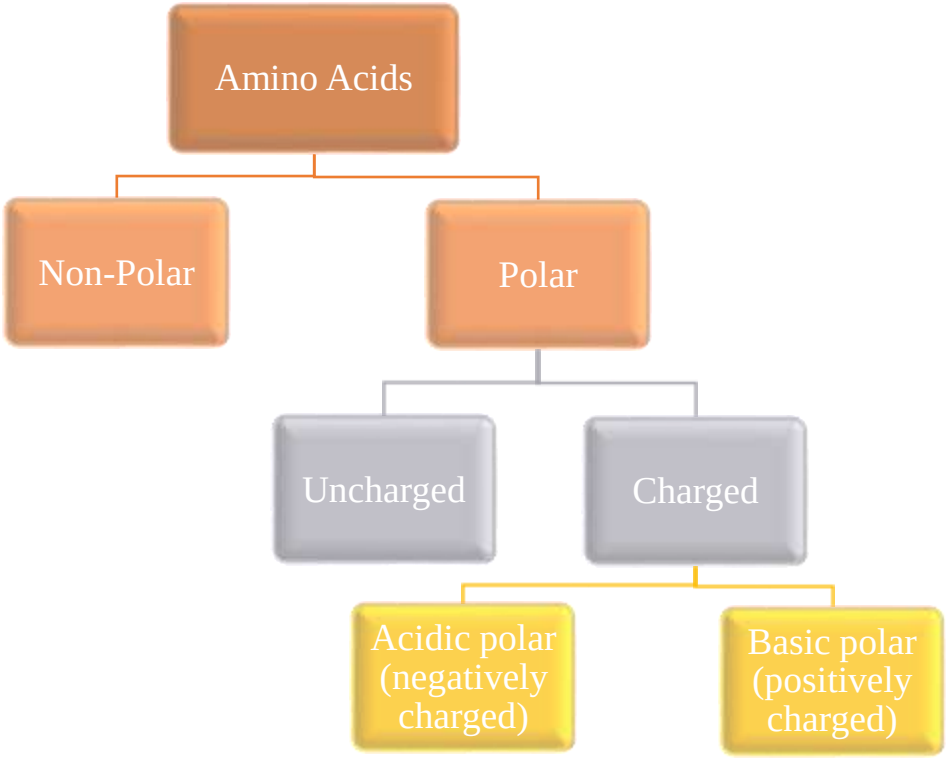




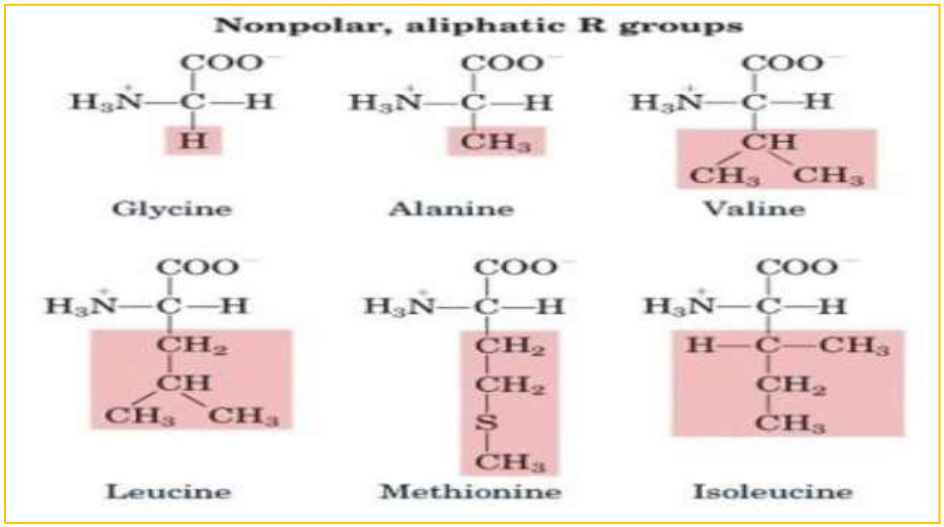
General structure of amino acids:

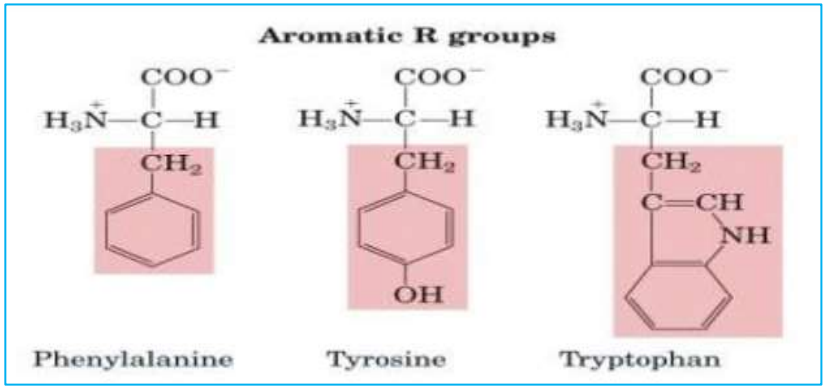


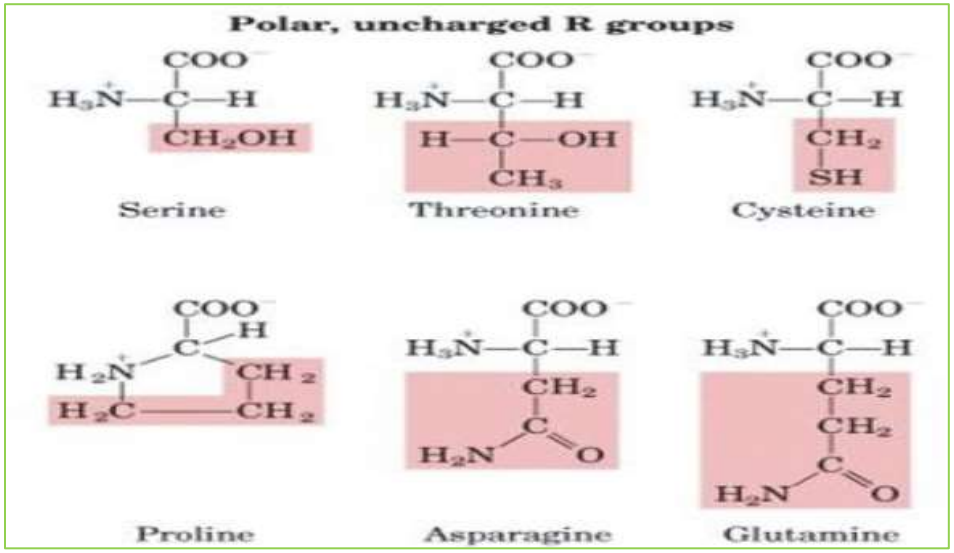
Classification of amino acids:

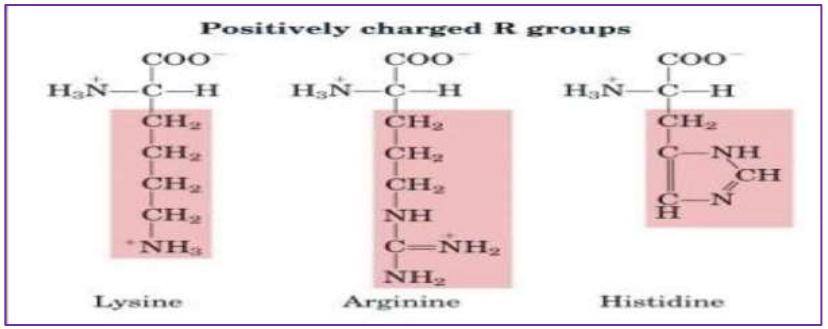


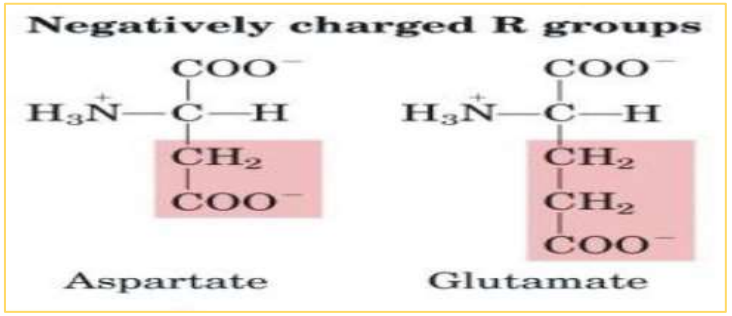
Twenty standard amino acids











Some properties of Amino Acids:

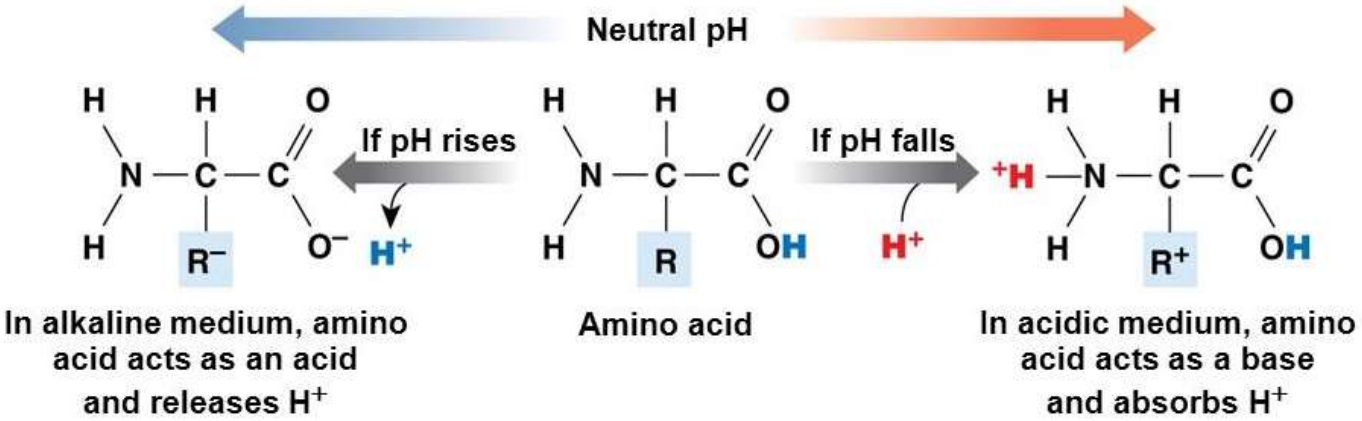
1. Amphoteric Compounds.
2. Isoelectric point (pI).
3. Optical Activity.
4. Light Absorption.

Some properties of Amino Acids:

1. Amphoteric Compounds:

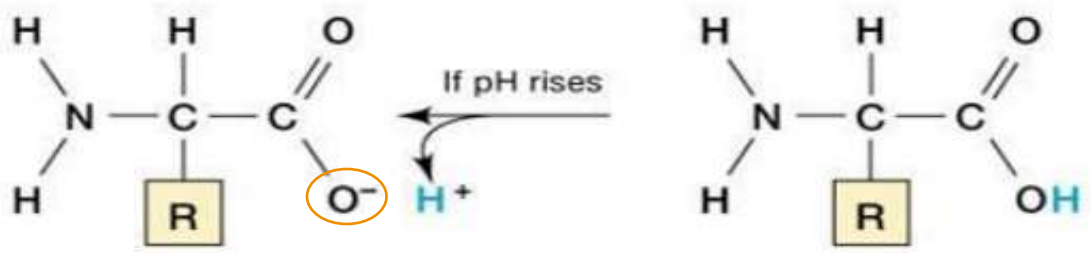
- What is **Amphoteric** compounds ?

- Amphoteric properties of amino acids due to the presence of their ionizable α-amino and α-carboxylic group can act sometimes as acids and sometimes as bases **depending on the pH of their media .**



A. Presence of **carboxyl group COOH** that able to donate proton (H^+) "acidic behavior", and converted to COO^- :

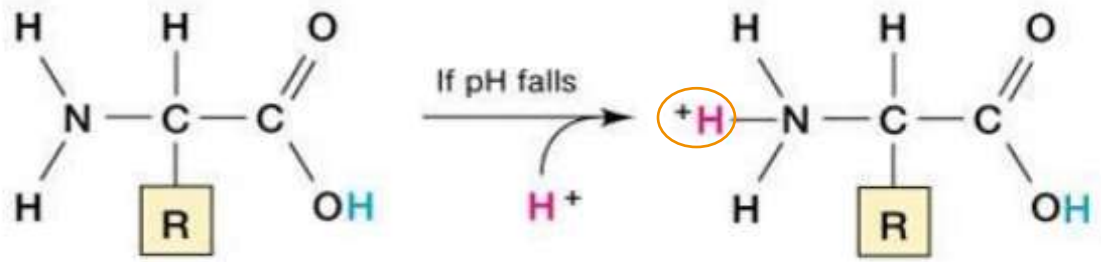
$COOH \rightarrow COO^-$



High pH➔ Act as **acids**

B. Presence of **amino group NH_2** that able to accept proton (H^+) "basic behavior", and converted to NH_3^+ :

$NH_2 \rightarrow NH_3^+$

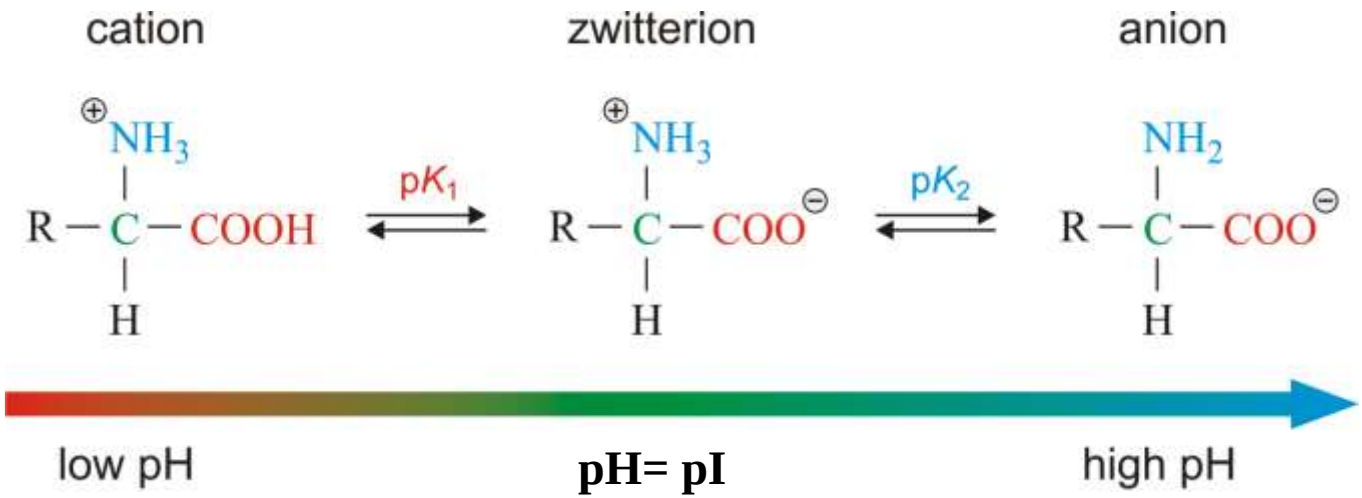


Low pH ➔ Act as **Base**

Some properties of Amino Acids cont':

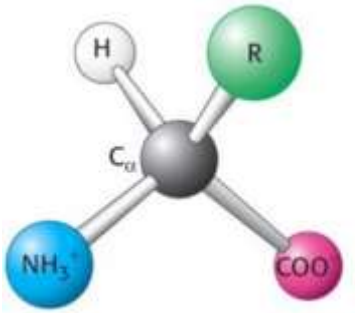
2. Isoelectric point (pI):

- It is the pH value at which the **positive charge** equals the **negative charge** (i.e. the net charge of this molecule equals **zero**) ➔ Zwitter ion
- Electric field?
- Solubility?
- Different pI.



Some properties of Amino Acids cont':

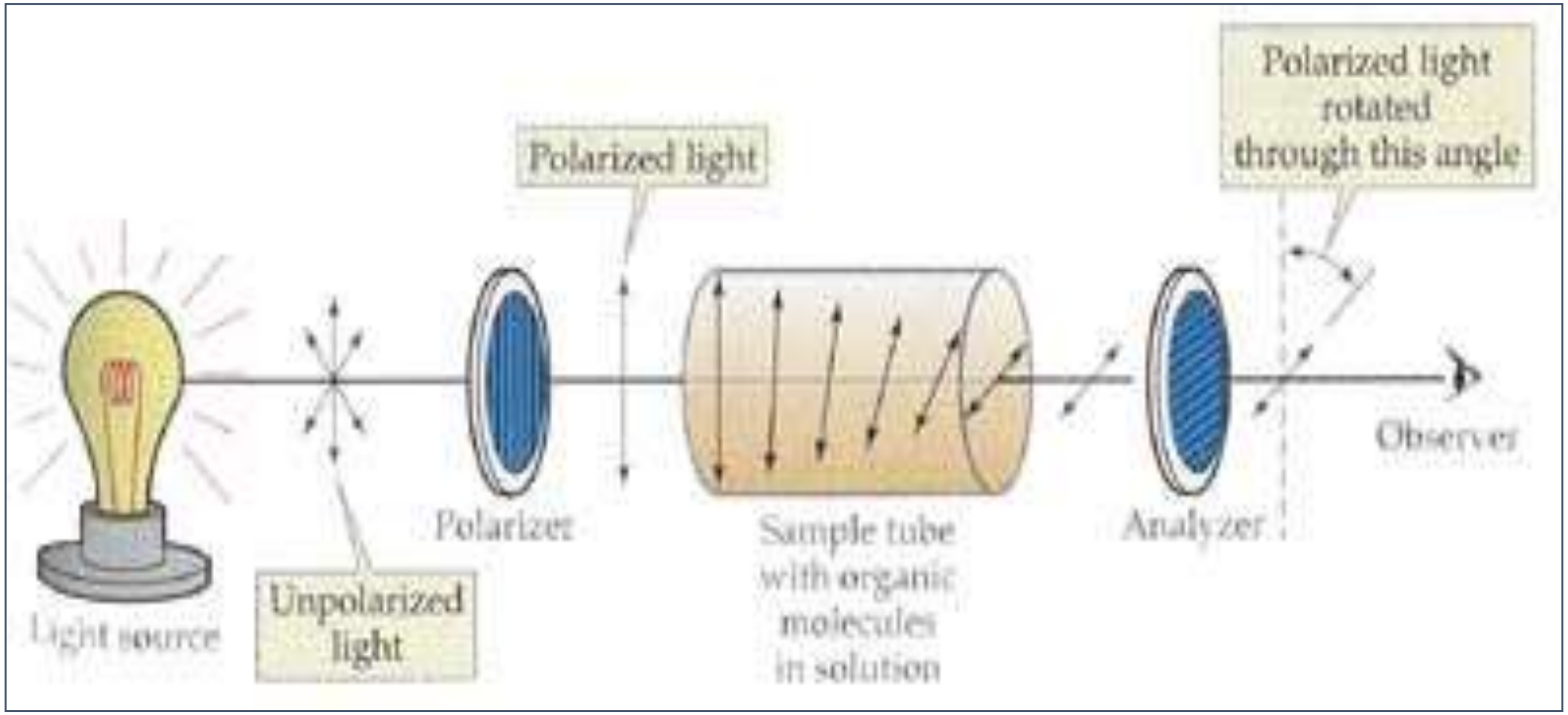
3. Optical Activity :



- Amino acids are able to <u>rotate</u> polarized light either to:
  - The left (Levorotatory) ➔ (-) – Amino acid
  - The right (Dextrorotatory) ➔ (+) – Amino acid

- **Asymmetric C atom.**

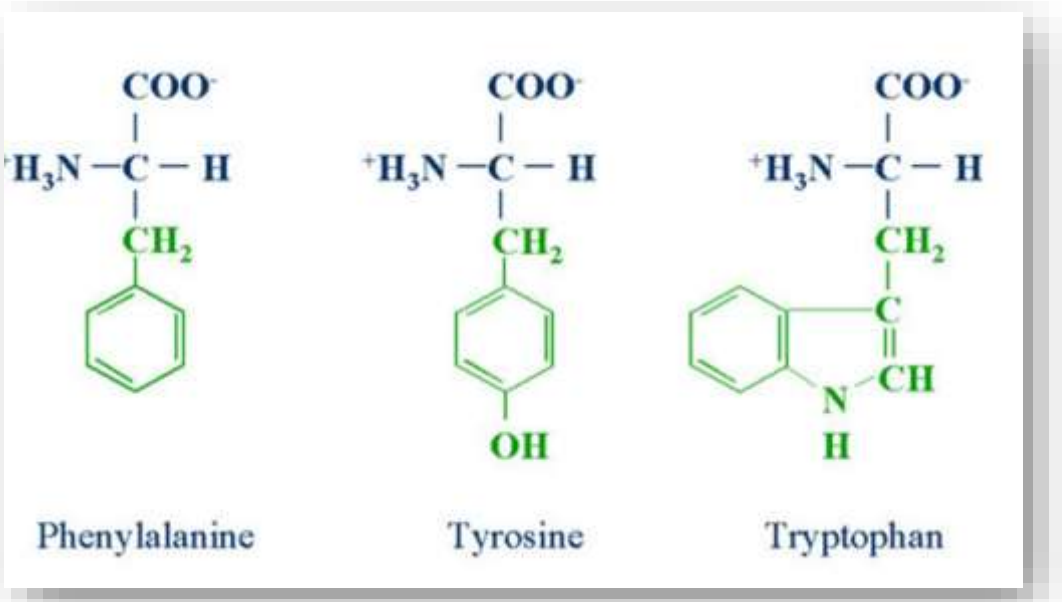
- **Glycine** ?


Polarized light
rotated
through this angle
Polarized light
Observer
Polarizer
Analyzer
Sample tube
with organic
molecules
in solution
Unpolarized
light
Light source

Some properties of Amino Acids cont':

4. Light Absorption:

- The aromatic amino acids absorb ultraviolet light **at 280nm.**
- proteins ?



Practical part

Qualitative tests of amino acids

1. Solubility Test.
2. Ninhydrin test: for α-L amino acids.
3. Xanthoproteic test: for Aromatic amino acids.
4. Sakaguchi Test: for arginine.
5. Millon's test: for amino acids containing hydroxy phenyl group (Tyrosine)
6. Lead sulfite test: for of amino acids containing sulfhydral group (- SH) (Cysteine)

Experiment 1 : Solubility Test

Objective:

- Investigate the solubility of selected amino acid in various solutions.

Principle:

- Amino acids are generally soluble in water. This is because the presence of **amino and carboxyl group.**

- Insoluble in non-polar organic solvents such as hydrocarbons (e.g. chloroform, ethanol….).

Experiment 1 : Solubility Test

Method:

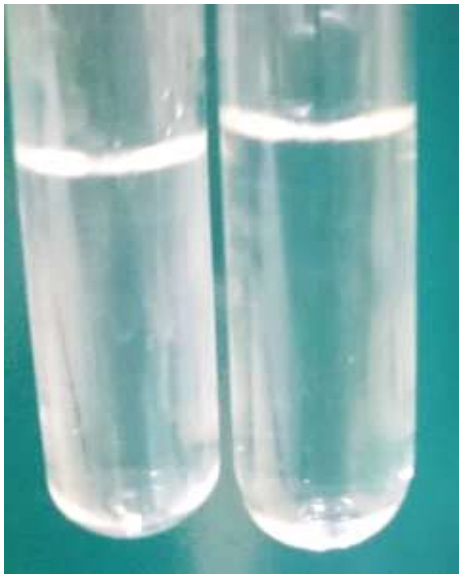
1. Add 2ml of different solvents in 4 clean test tubes then place 0.5 ml of each amino acid.
2. Shake the tubes thoroughly, then leave the solution for about one minute.
3. Notice what happened to the solution .
4. Record your result .

Results:

Amino acid	Solvent	Degree of solubility
Glycine	Water	
	NaOH	
	HCl	
	Chloroform	
Arginine	Water	
	NaOH	
	HCl	
	Chloroform	
Glutamine	Water	
	NaOH	
	HCl	
	Chloroform	

soluble

insoluble



Soluble

Insoluble

Experiment 2: Ninhydrin test

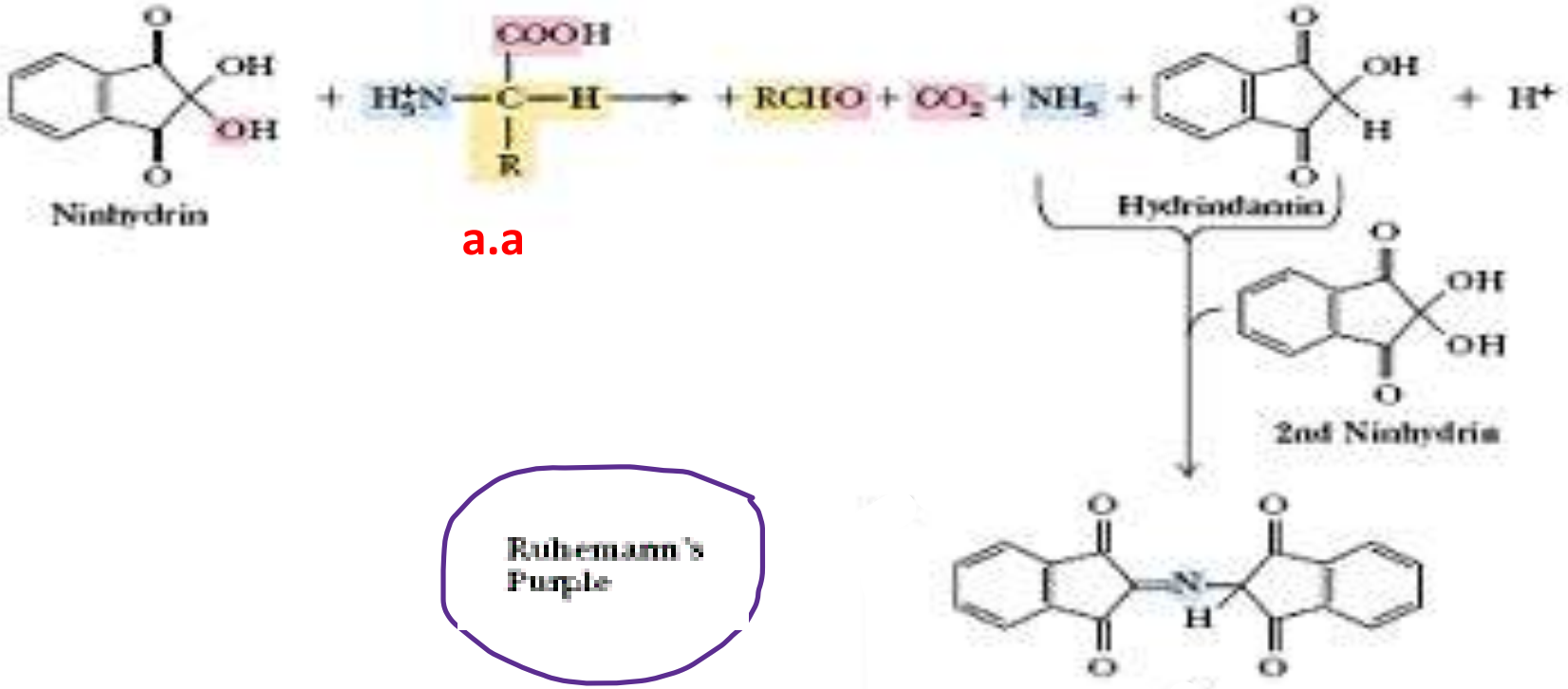
Objective:

- To detect α-amino acids.

Principle:

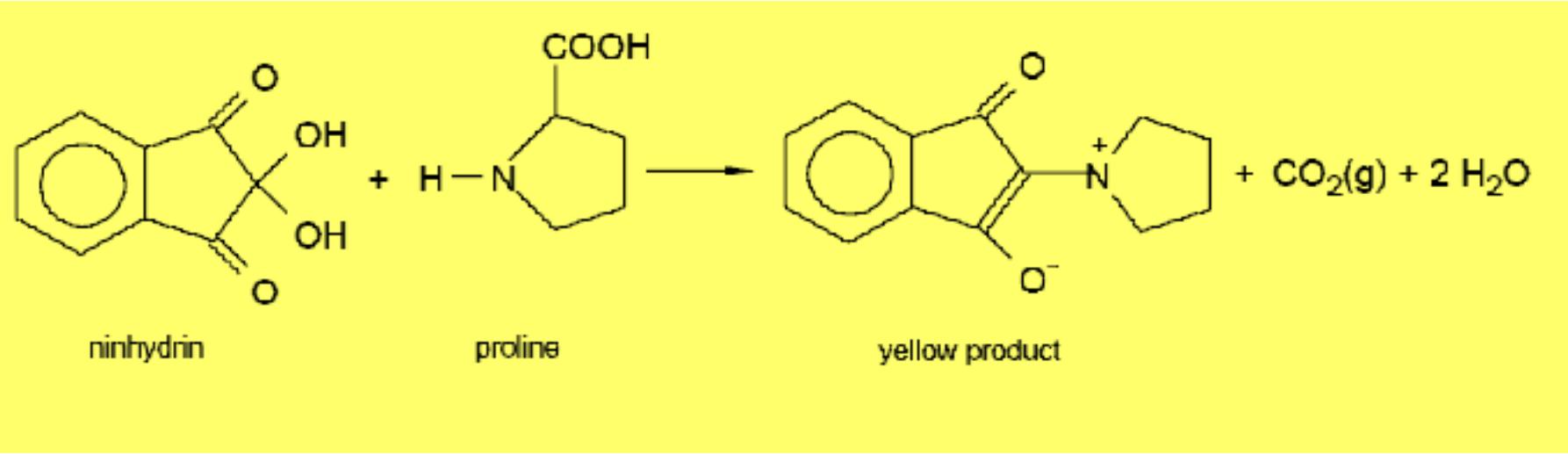
1.Ninhydrin (triketohydrindene hydrate) degrades amino acids into aldehydes (on pH range 4-8), ammonia and CO2 though a series of reactions.

2.Ninhydrin then condenses with ammonia and hydrindantin to produce an intensely blue or purple pigment, sometimes called ruhemann's purple



All amino acids that have a free amino group will give positive result (purple color) .

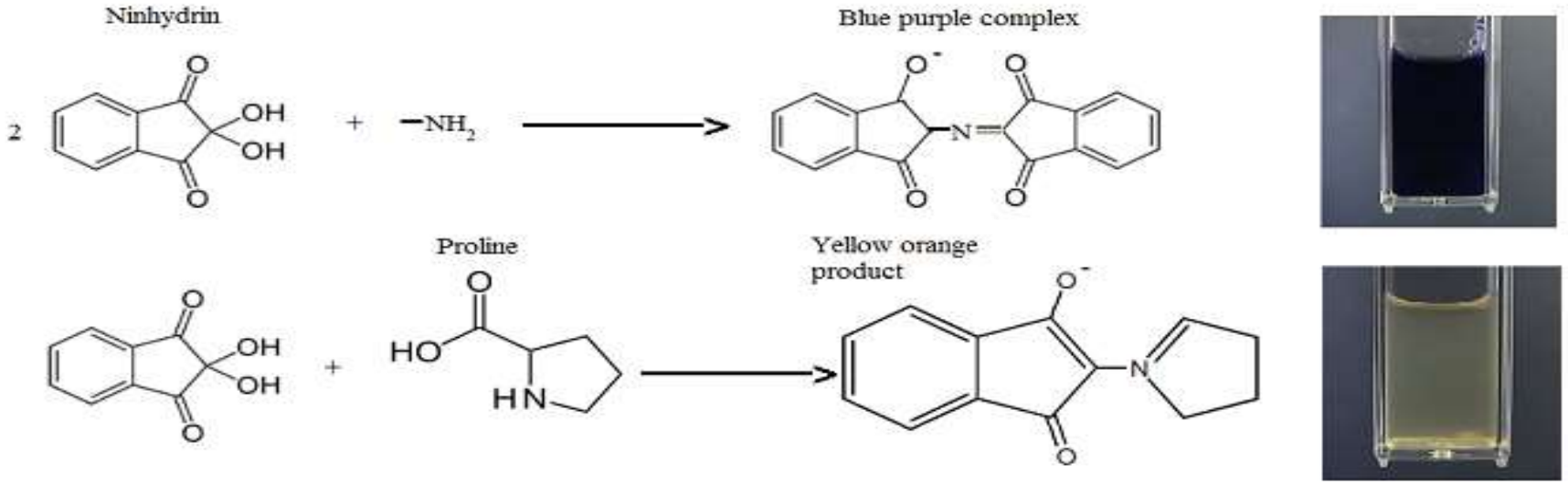
Except



While not free amino group-proline and **hydroxy-proline** (amino acids) will give a (yellow color).

Note: Many substances other than amino acids, such as amines will yield a blue color with ninhydrin, particularly if reaction is carried out on filter paper.

Experiment 2: Ninhydrin test



Experiment 2: Ninhydrin test

Method:

1-Place 1 ml of each of the a.a solutions in a test tube and add 1 ml of ninhydrin solution.
2- Boil the mixture over a water bath for 2 min.
3- Allow to cool and observe the blue-purple color formed.
4- Record your results.

Ninhydrin is a strong oxidizing agent, it should be handled with care.

Results:

Tube	Observation
Glycine	
Tryptophan	
Proline	

Experiment 3 : Xanthoproteic test

Objective:

- To differentiate between aromatic amino acids which give **positive** results and other amino acids.

Principle:

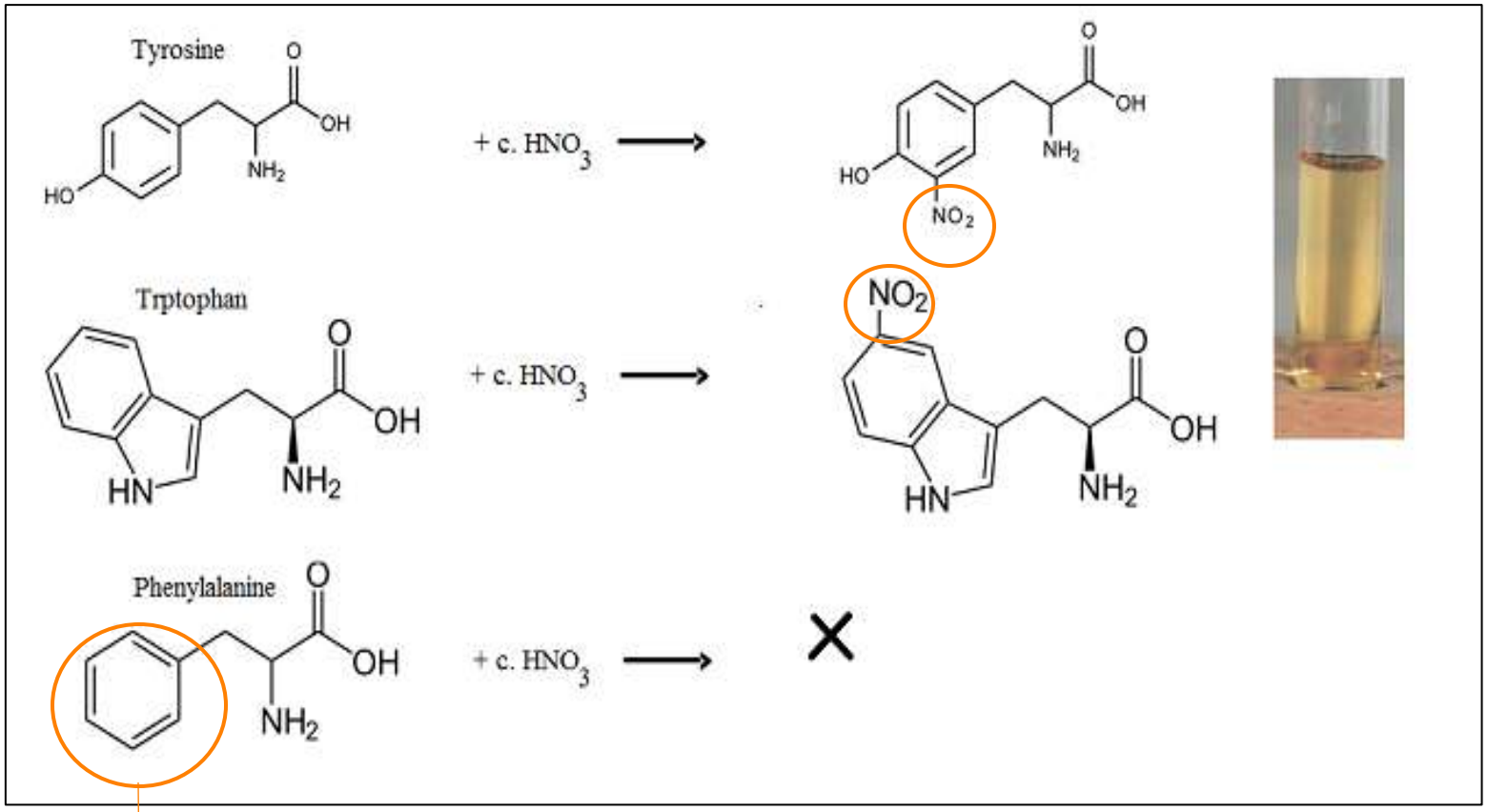
Concentrated nitric acid react with aromatic ring present in the amino acid side chain [nitration reaction] →giving the solution yellow color.

Note:

Amino acids tyrosine and tryptophan →contain activated benzene rings [aromatic nucleus] which are easily nitrated to yellow-colored compounds.

The aromatic ring of phenyl alanine dose not react with nitric acid despite it contains a benzene ring, but it is not activated, therefore it will not react

Nitration



benzene ring is not activate

Experiment 3 : Xanthoproteic test

Method:

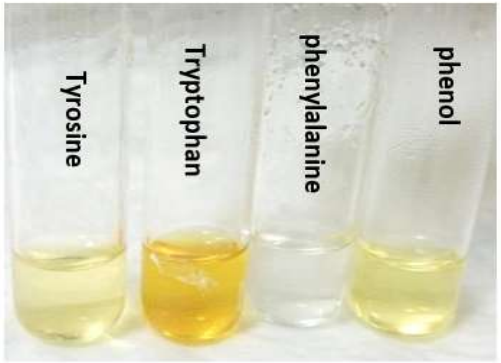
CAUTION

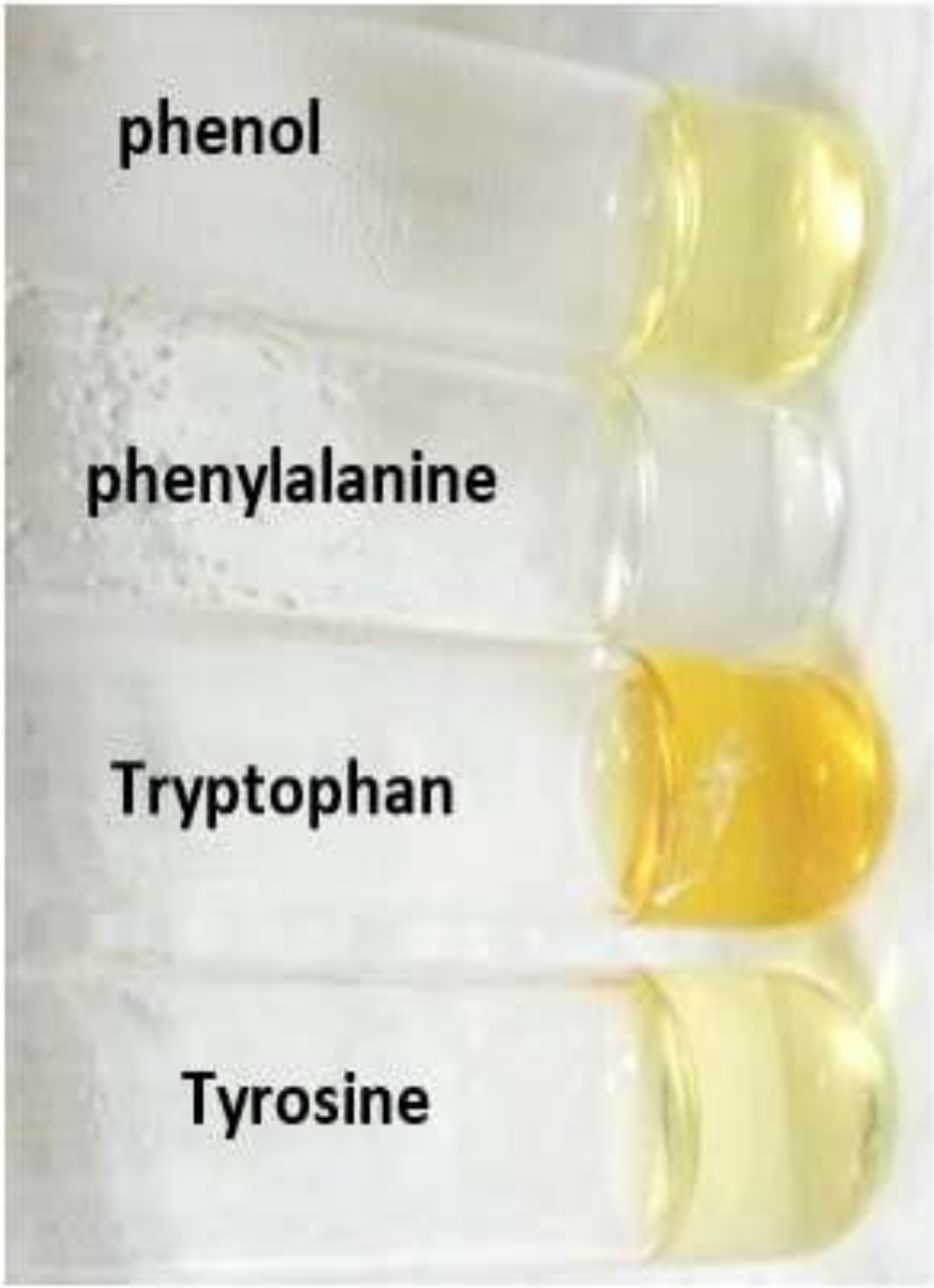
Concentrated HNO_3 is a toxic, , it should be handled with care.

1. Label four tubes (1 - 4), then add 1 ml of each amino acid solutions and phenol solution to those test tubes each alone.
2. Add 1 ml of concentrated HNO_3. then record your result
3. Now COOL THOROUGHLY under the tap and CAUTIOSLY add 5 drops of 10M NaOH to make the solution strongly alkaline (the alkaline is added to be sure about the nitration).

Results:

Tube	Observation	
	+ HNO_3	+NaOH
Tyrosine		
Tryptophan		
Phenylalanine		
Phenol		



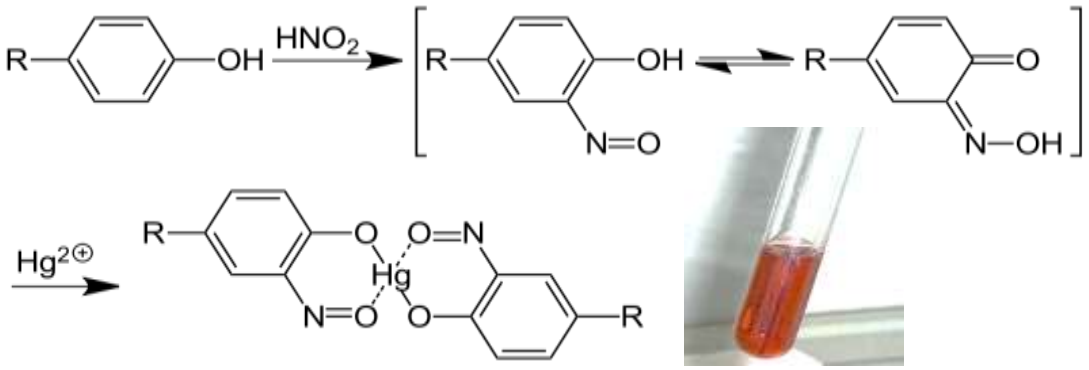

phenol
phenylalanine
Tryptophan
Tyrosine

Experiment 4 : Millon's test

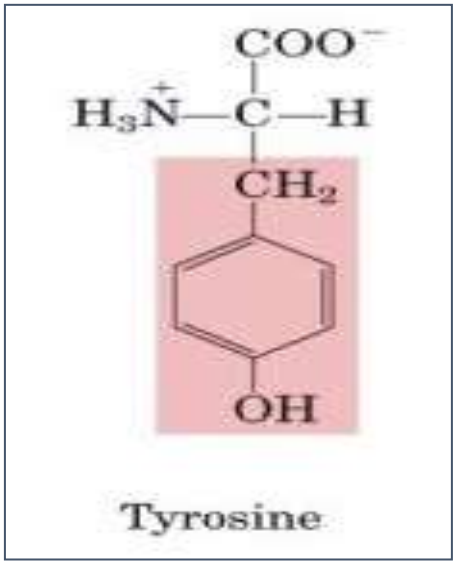
Objective:

- This test is specific for **Tyrosine** ➔ because it is the only amino acid containing a phenol group.

Principle:



Note:
All phenols (compound having benzene ring and OH attached to it) give positive results in Millon's test.



Tyrosine

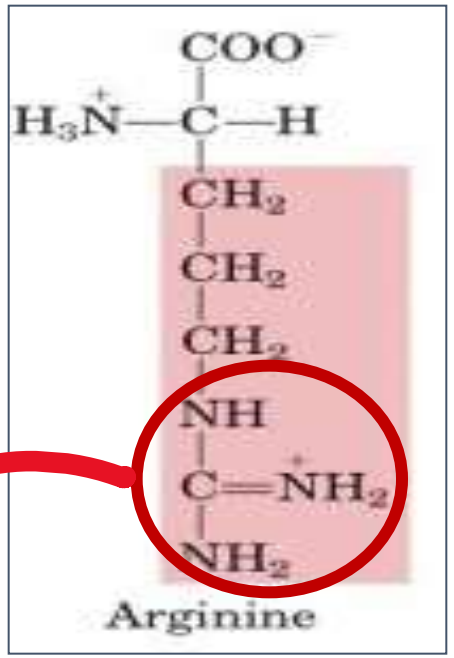
Experiment 5 : Sakaguchi Test

Objective:

- Detection of amino acid containing gauanidium group
  ➔test for **Arginine.**

Principle:

- In **alkaline** solution, arginine react with α-naphthol and sodium hypobromite /chlorite as an oxidize agent, to form red complexes as a positive result.



gauanidium group

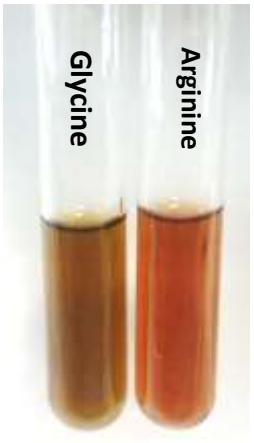
Experiment 5 : Sakaguchi Test

Method:

1. Label 2 test tube and place in each one 2 ml of the amino acid solution .
2. Add to each tube 2ml of NaOH solution. Mix well
3. Add to each tube 5 drops of α-naphthol solution. Mix well
4. Add to each tube 5 drops of sodium hypobromite solution, and record your result .

Results:

Tube	Observation
Glycine	
Arginine	



Experiment 6 : Lead Sulfite Test

Objective:

- This test specific for–SH [sulfhydral group]containing amino acid ➔ **Cysteine.**

Principle:

- Sulphur in cysteine, is converted to **sodium sulfide** by boiling with 40% NaOH.
- The Na_2S (sodium sulfide)can be detected by the precipitation of PbS (lead sulfide) from an alkaline solution when adding lead acetate $(CH_3COO)_2$ Pb .