Qualitative tests of Amino Acids

BCH302 [Practical]

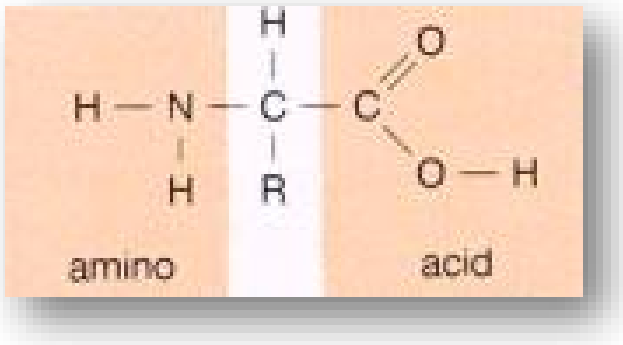
Amino Acids:

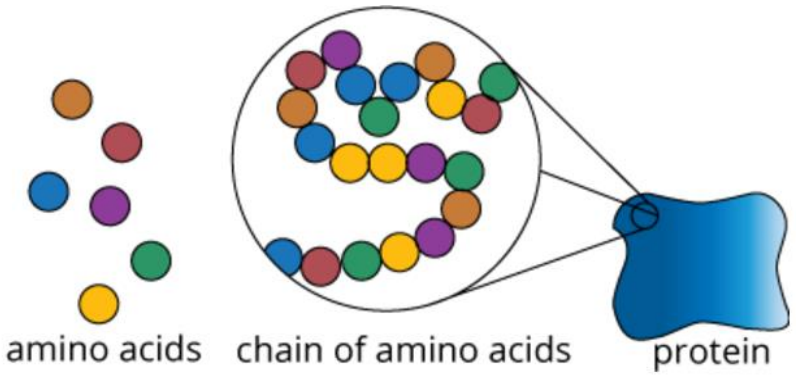
- **Amino acids play a central role:**

  i. As building blocks of **proteins.**
  ii. As intermediates in metabolism, converted to specialized products.

- There are <u>20 natural amino acids</u> that are found within proteins.

➔**All of them are L-α amino acids.**

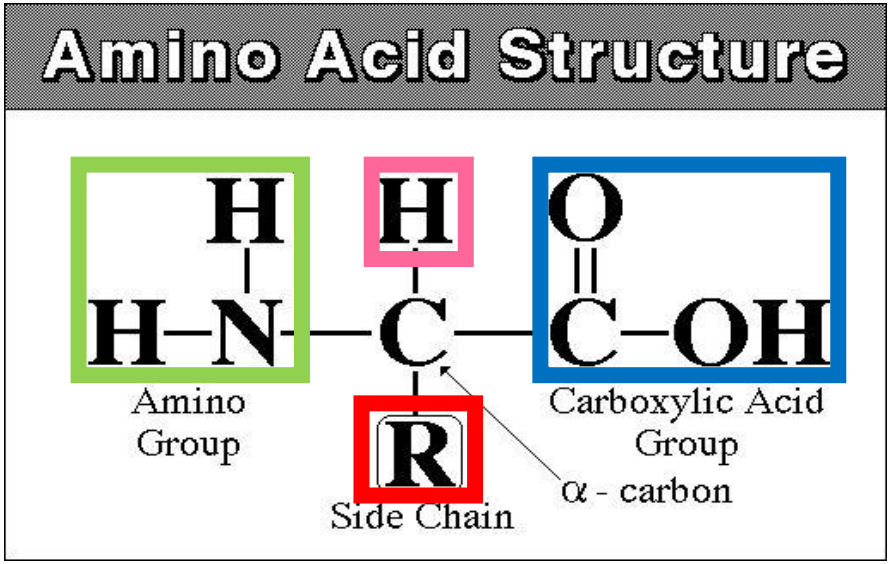




General structure of amino acids:

- **All amino acids found in proteins consist of:**

➢ A basic amino group (—NH_2)
➢ An acidic carboxyl group (—COOH)
➢ A hydrogen atom (—H)
➢ A distinctive side chain (—R).



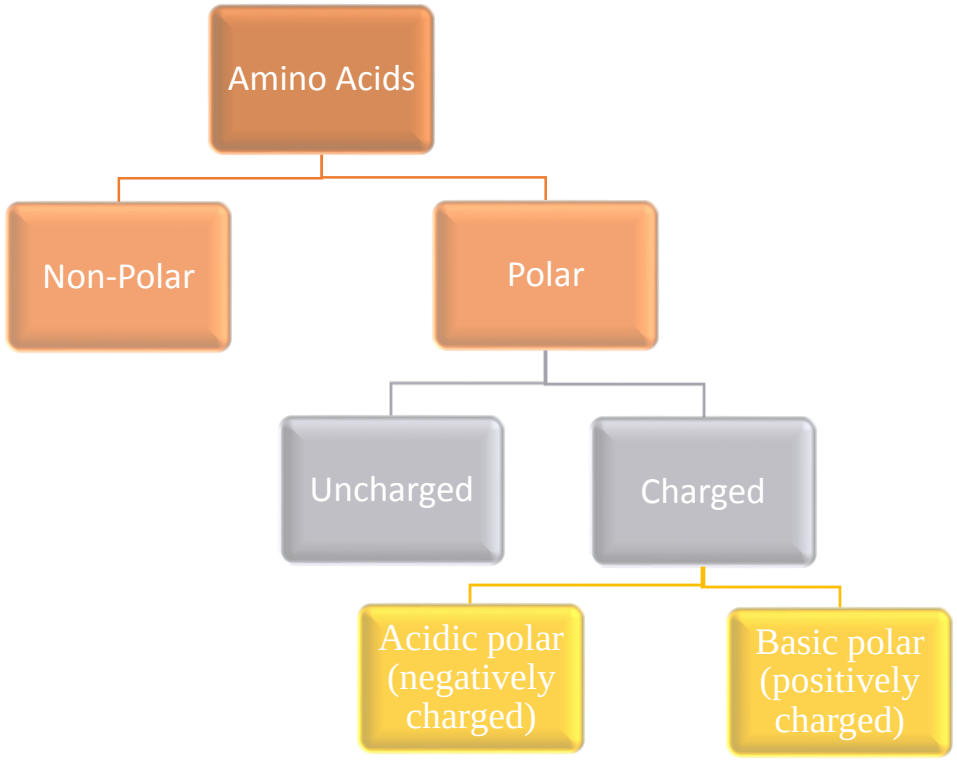
- Amino acids differing only in the structure of the R-group or the side chain.

- The simplest, and smallest, amino acid found in proteins is **glycine** for which the R-group is **hydrogen (H).**

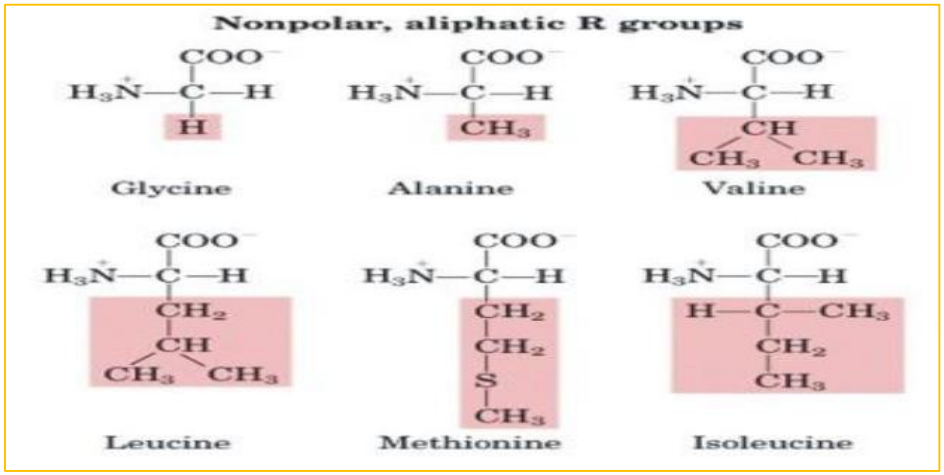
Classification of amino acids:

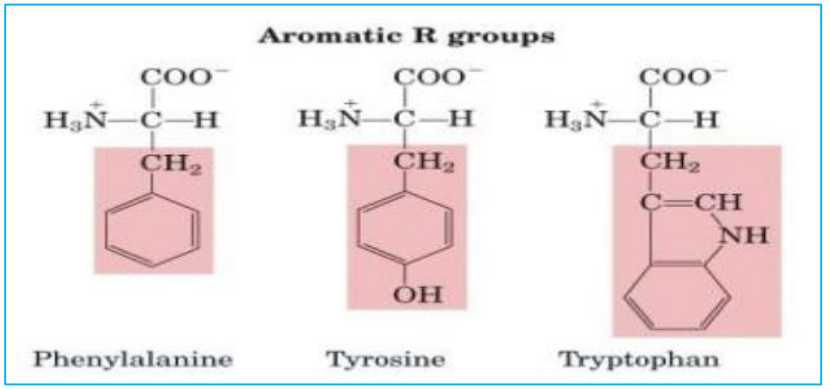
- Classification of amino acid depending on the R-group ionization (polarity) in water:

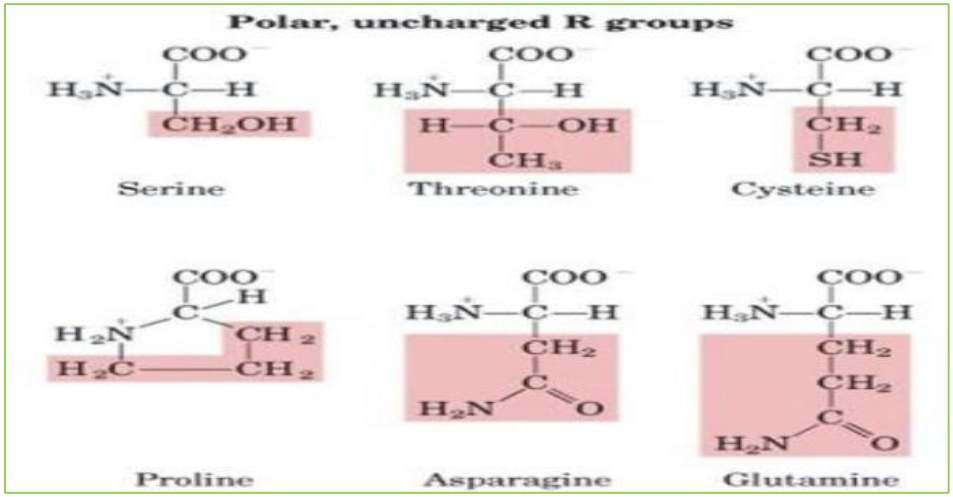
1. Non-polar.
2. Uncharged polar.
3. Charged polar amino acids :
   i. Basic polar (positively charged).
   ii. Acidic polar (negatively charged).

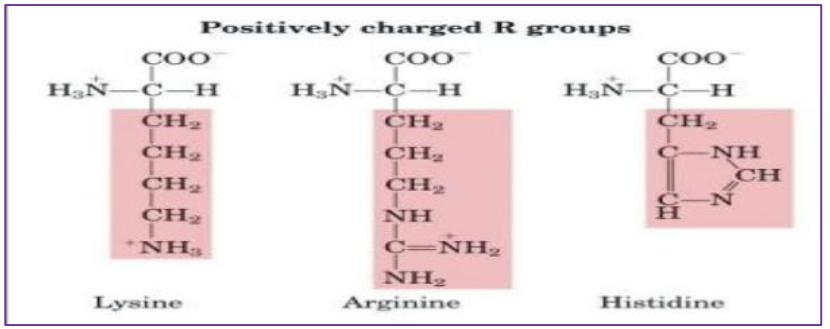


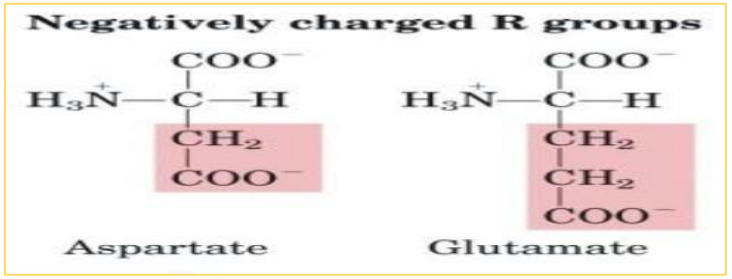
Twenty standard amino acids











Some properties of Amino Acids:

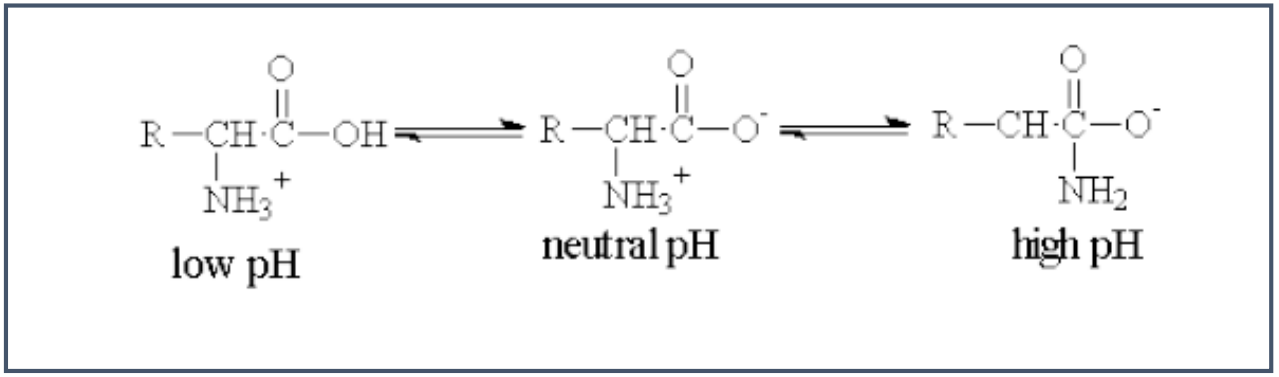
1. Amphoteric Compounds.
2. Isoelectric point (pI).
3. Optical Activity.
4. Light Absorption.

Some properties of Amino Acids:

1. Amphoteric Compounds:

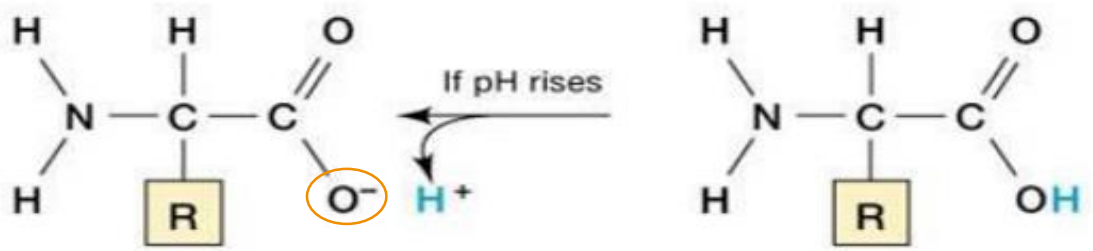
- **Amphoteric** compounds is a molecule that can can act as **acids** (donate a proton) and **bases** (accept a proton).

- 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^- :

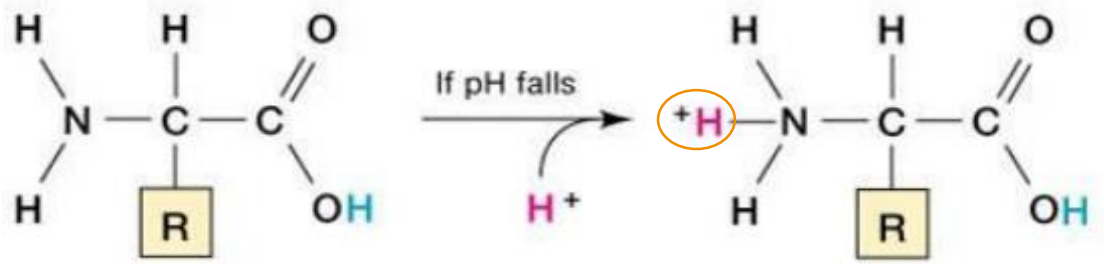
$COOH \rightarrow COO^-$



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**

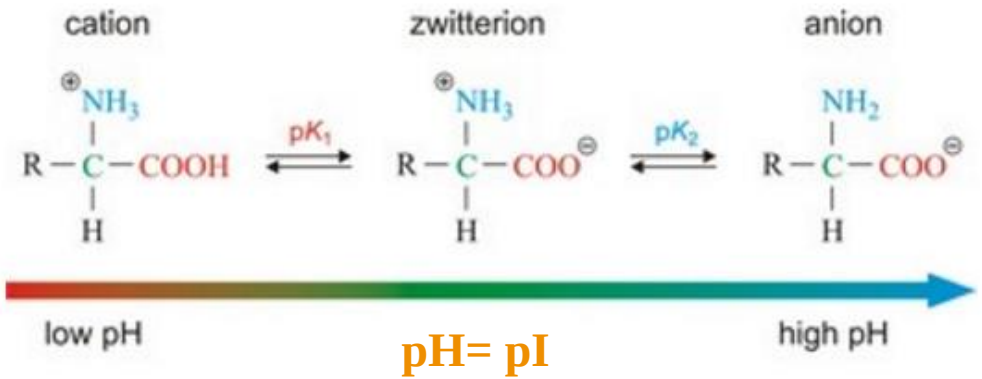
Some properties of Amino Acids cont':

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

- It is known as a point at which the molecule does not move to either cathode or anode if it is put in electric field and its solubility is minimum so it is possible to precipitate at this point.

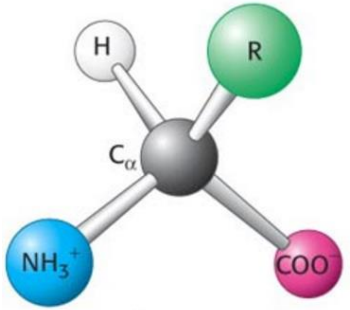
- Each amino acid have a different pI.



pH= pI

Some properties of Amino Acids cont':

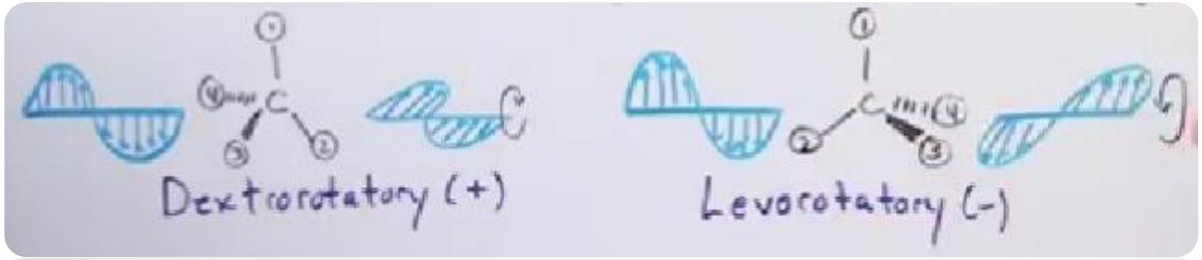
3. Optical Activity :



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

- The ability to rotate the plane of polarized light because they have an **asymmetric C atom** (a carbon atom linked to 4 different groups).

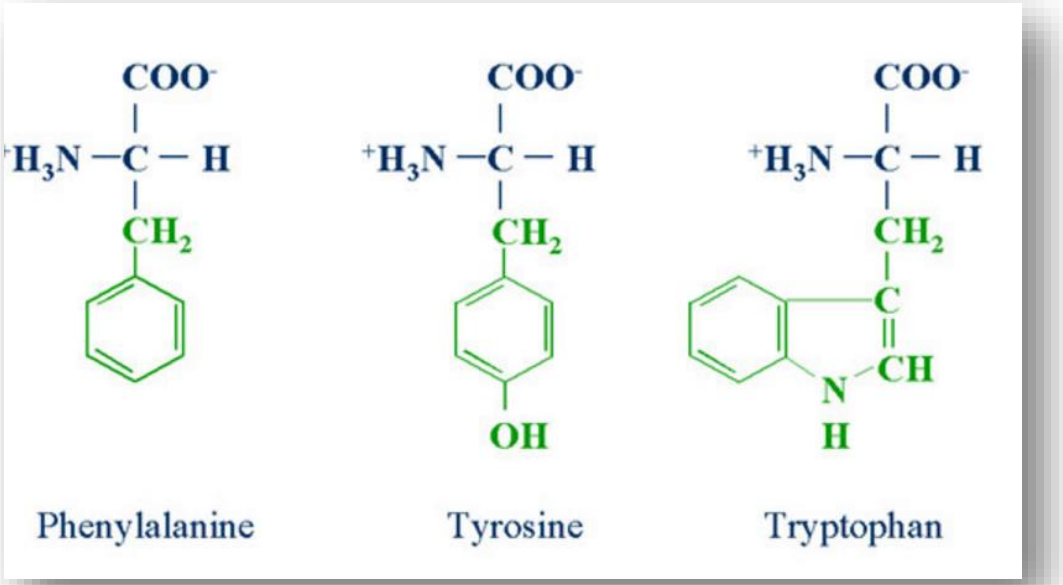
- **Glycine** is the only amino acid which **lacks** asymmetric C atom (has 2 H^+ on α-C) .



Some properties of Amino Acids cont':

4. Light Absorption:

- The aromatic amino acids tryptophan , tyrosine , and phenylalanine absorb ultraviolet light **at 280nm** ,which explains the absorption of <u>proteins at 280nm.</u>



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 and insoluble in non-polar organic solvents such as hydrocarbons.

- This is because the presence of **amino and carboxyl group** which enables amino acids to accept and donate protons to aqueous solution, and therefore, to act as acids and bases.

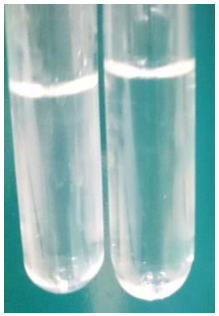
Experiment 1 : Solubility Test

Method:

1. Add 4ml of different solvents in 3 clean test tubes then place 1 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:

	Glycine	Arginine
HCl (acid)		
NaOH (base)		
Chloroform (organic solvent)		



soluble

insoluble

Experiment 2: Ninhydrin test

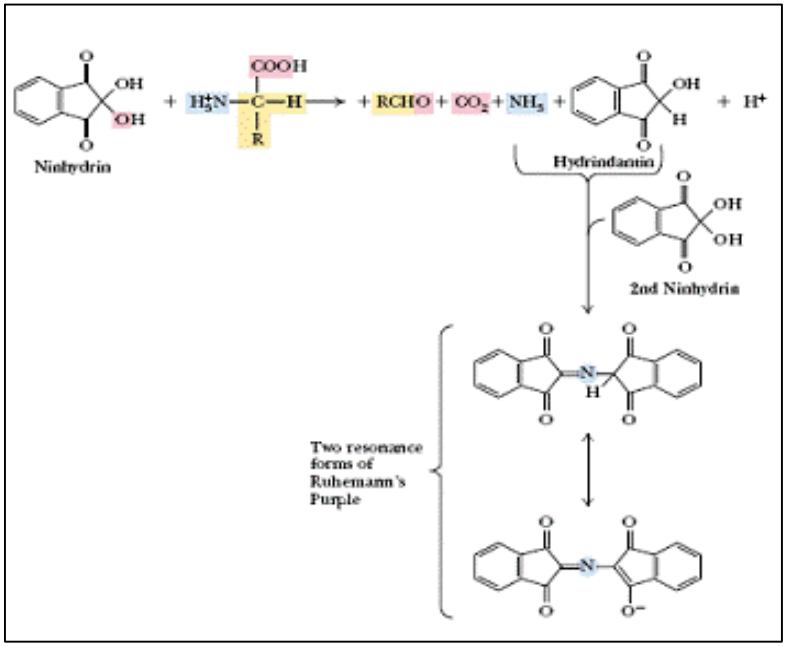
Objective:

- To detect α-L-amino acids.

Principle:

1. Ninhydrin (triketohydrindene hydrate) degrades amino acids into **aldehydes** (on pH range 4-8), **ammonia** and **CO_2** though a series of reactions.
➔ The net result is ninhydrin in a partially reduced from hydrindantin.

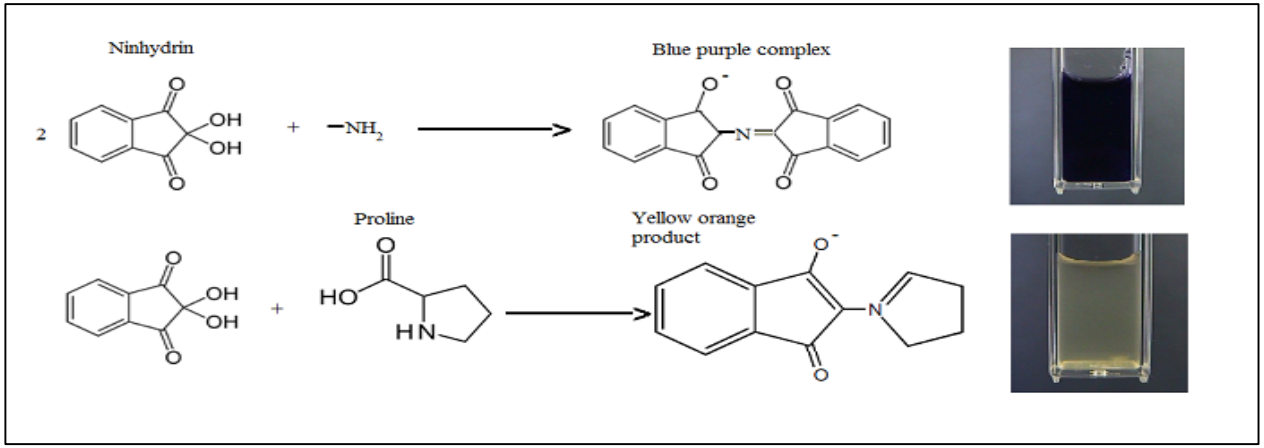
2. Ninhydrin then **condenses** with **ammonia and hydrindantin** to produce an intensely blue or purple pigment, sometimes called **ruhemann's purple.**



Experiment 2: Ninhydrin test

Principle cont':

- All amino acids that have a free amino group will give **positive result** (purple color).
- While not free amino group-proline and hydroxy-proline (amino acids) will give a (yellow color) ➔ In proline the N is not available for reaction as it is locked in the ring structure, therefore no ammonia is produced, so no blue color is presented.



Note:

Many substances other than amino acids, such as amines will yield a blue color with ninhydrin.

Experiment 2: Ninhydrin test

Method:

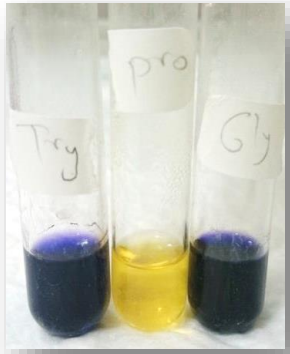
1-Place 1 ml of each of the solutions in a test tube and add 1 ml of ninhydrin solution.
2- Boil the mixture over a water bath for 2 min.
3- Allow to cool and observe the blue-purple color formed.
4- Record your results.

⚠ CAUTION

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

Results:

Tube	Observation
Glycine	
Tryptophan	
Proline	



Experiment 3 : Xanthoproteic test

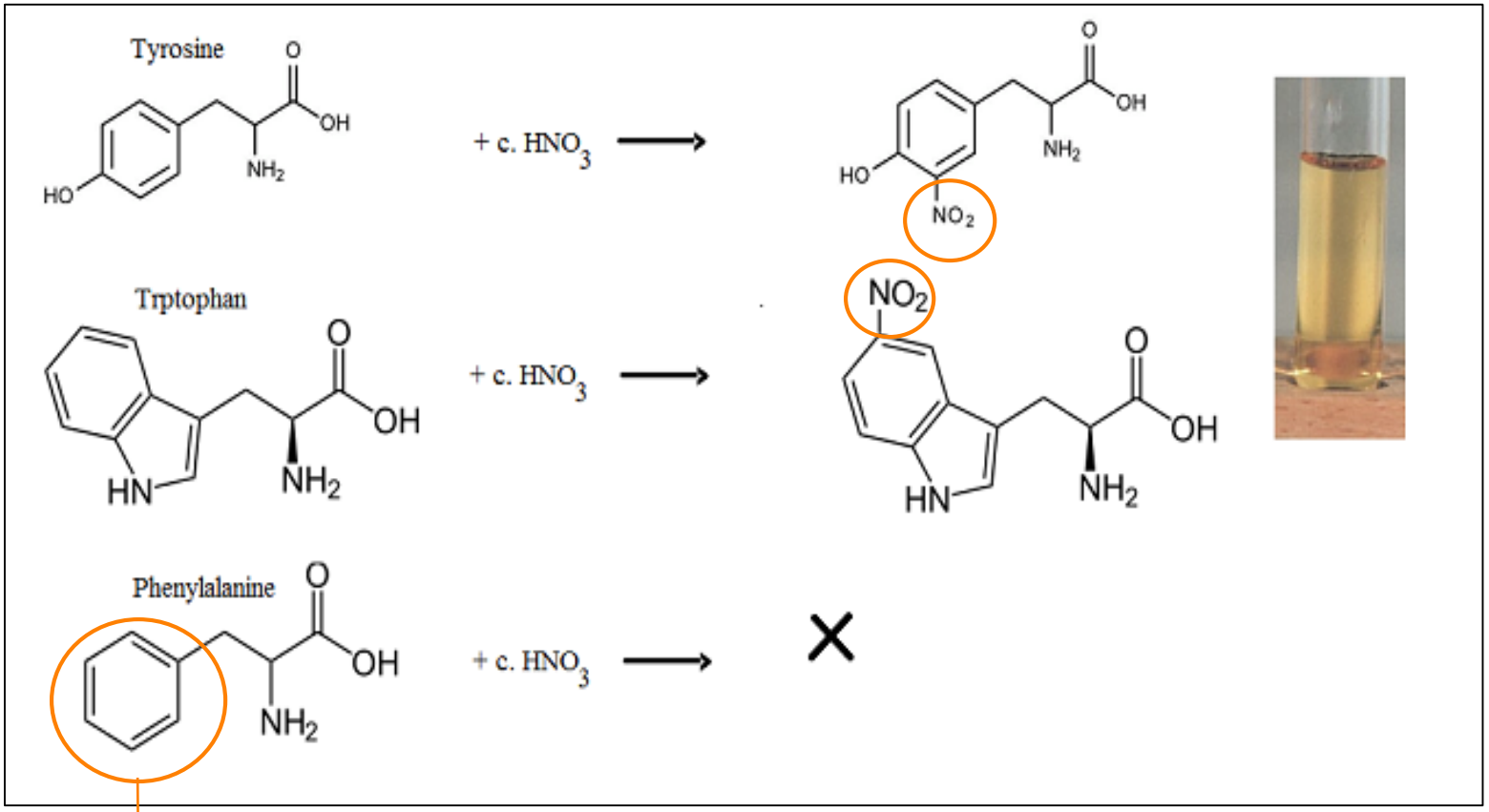
Objective:

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

Principle:

- Concentrated nitric acid (HNO_3) react with aromatic nucleus present in the amino acid side chain [**nitration reaction**] ➔ giving the solution yellow color.
- Amino acids tyrosine and tryptophan → contain activated benzene rings [aromatic nucleus] which are easily nitrated to yellow colored compounds.
- The aromatic ring of phenylalanine 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:

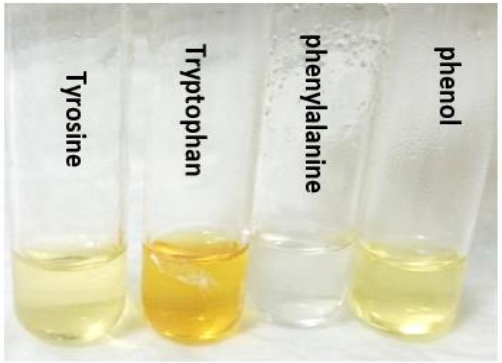
CAUTION

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).

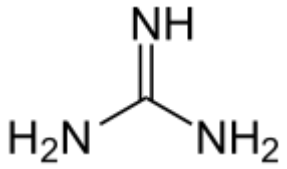
Results:

Tube	Observation	
	+ HNO_3	**+ NaOH**
Tyrosine		
Tryptophan		
Phenylalanine		
Phenol		



Experiment 4 : Sakaguchi Test

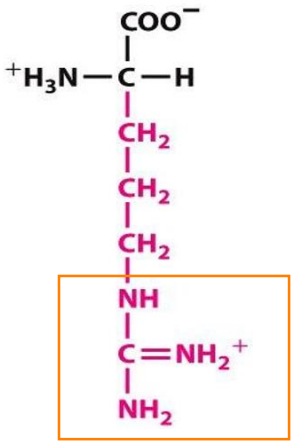
Objective:



- 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

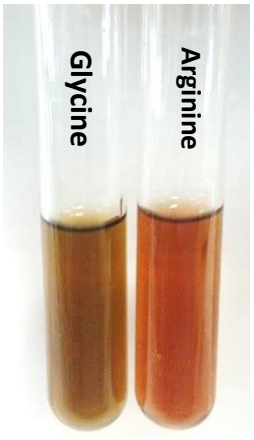
Experiment 4 : Sakaguchi Test

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	



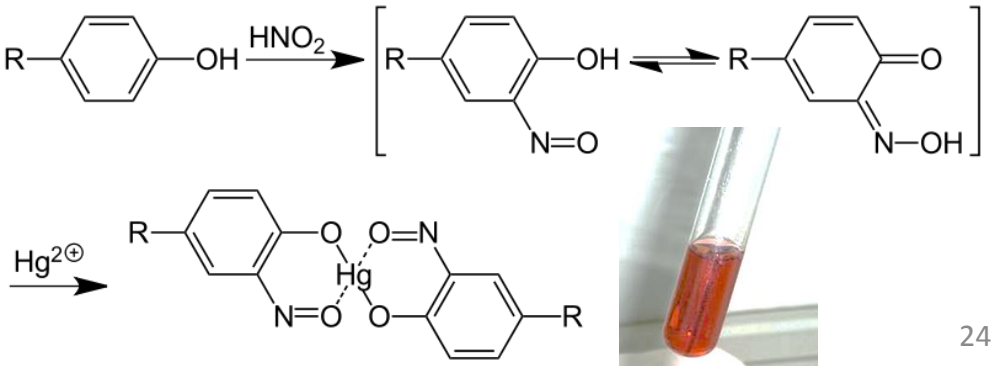
Experiment 5 : Millon's test

Objective:

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

Principle:

- The phenol group of tyrosine is first **nitrated** by nitric acid in the test solution.
- Then the nitrated tyrosine complexes mercury ions in the solution to form a brick-red solution or precipitate of nitrated tyrosine, in all cases, appearance of red color is positive test.



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

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 can be detected by the precipitation of PbS (lead sulfide) from an alkaline solution when adding lead acetate $(CH_3COO)_2$ Pb .