Biochemistry of Proteins BCH 303 [Practical]

Lab (1) Qualitative Tests of Amino Acids

Emtenan Mohammed Alkhudair

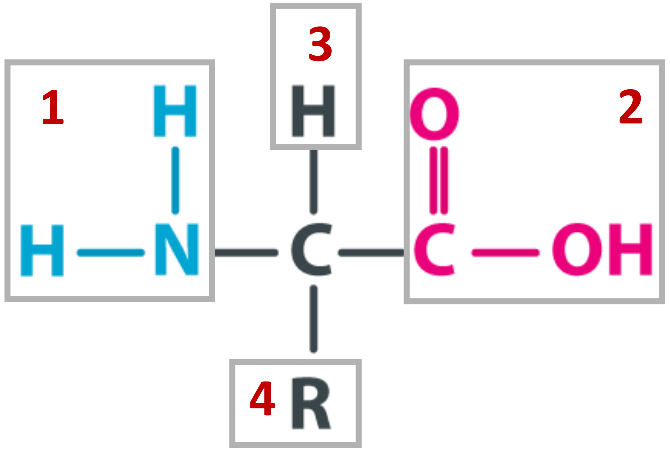
Office: Building 5, 3rd floor, Office No. 269
E.mail: ealkhudair@ksu.edu.sa
Website: http://fac.ksu.edu.sa/ealkhudair

Introduction

- Amino acids play central roles both as **building blocks of proteins** and as intermediates in metabolism.
- There are **20 natural amino acids** found within proteins convey a vast array of chemicals versatility.
- All of them are **L-α amino acids.**

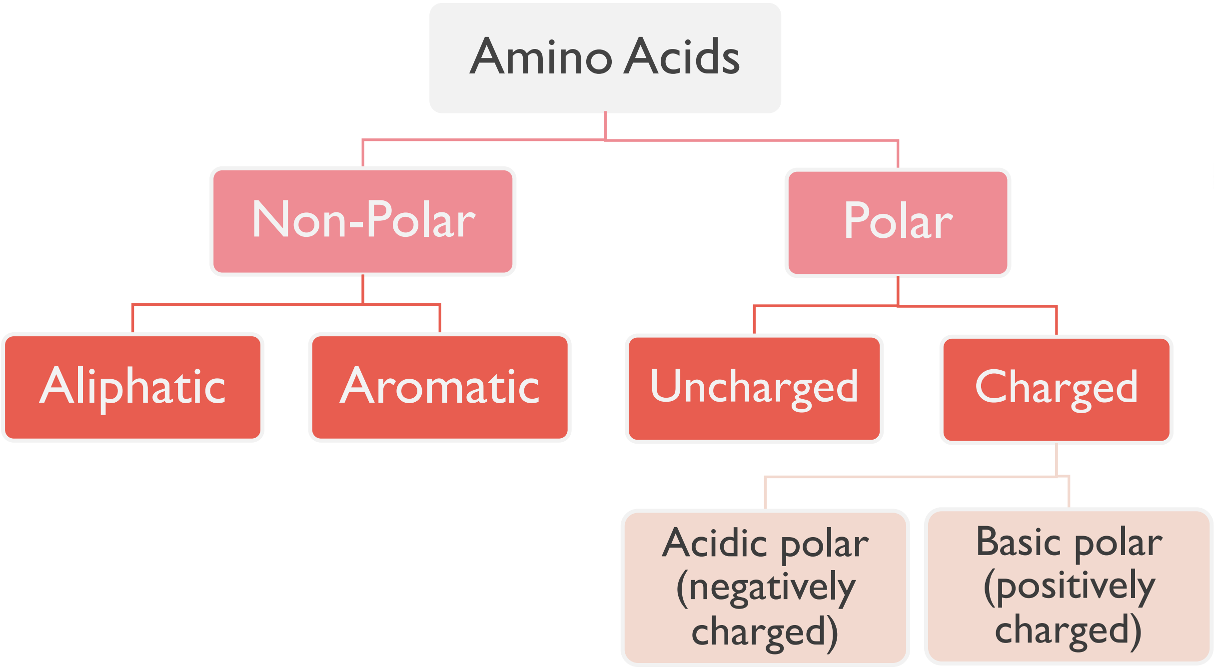
All amino acids found in proteins consist of:

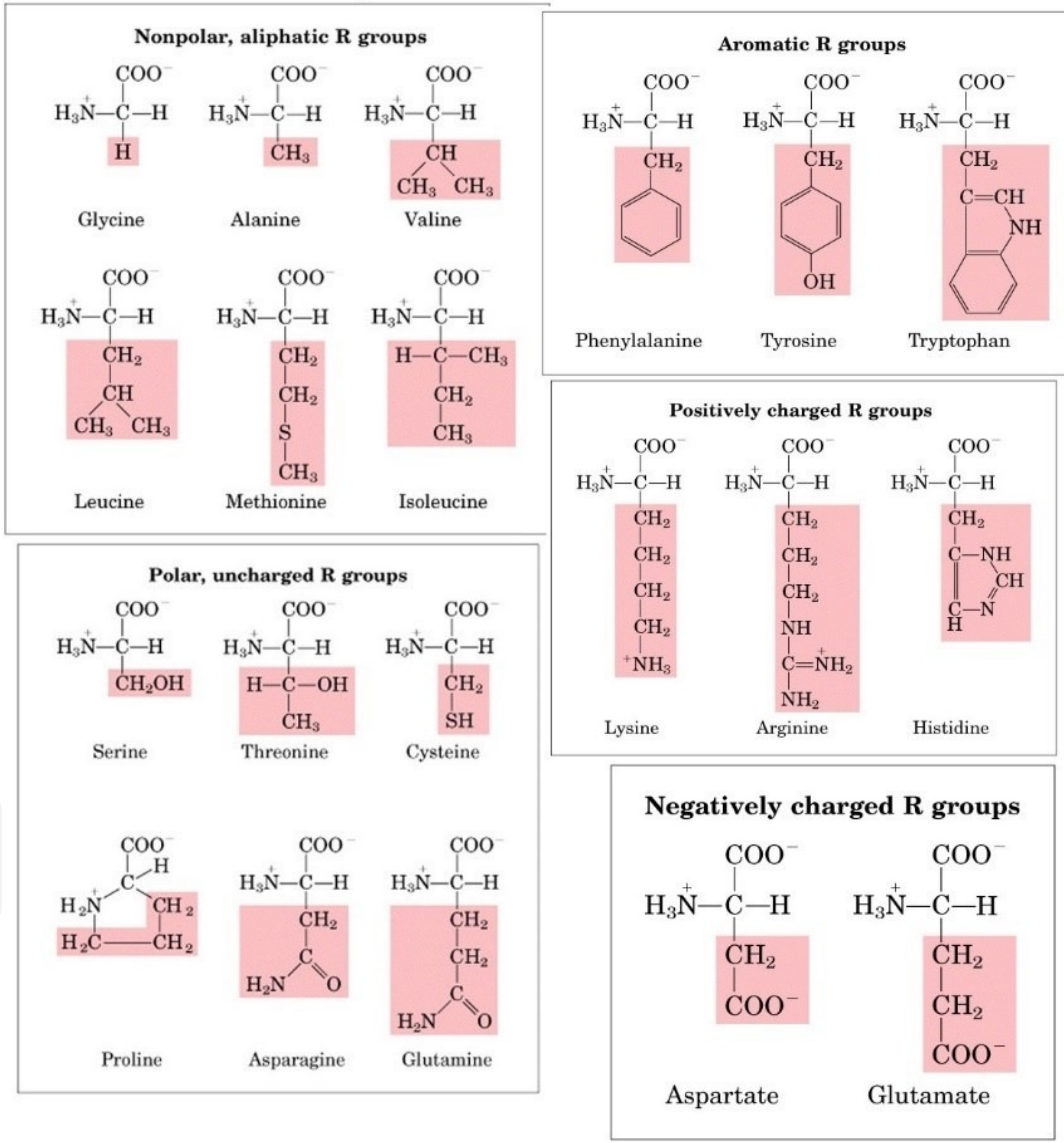
1. A basic amino group (—NH_2)
2. An acidic carboxyl group (—COOH)
3. A hydrogen atom (—H)
4. A distinctive side chain (—R).



- 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:





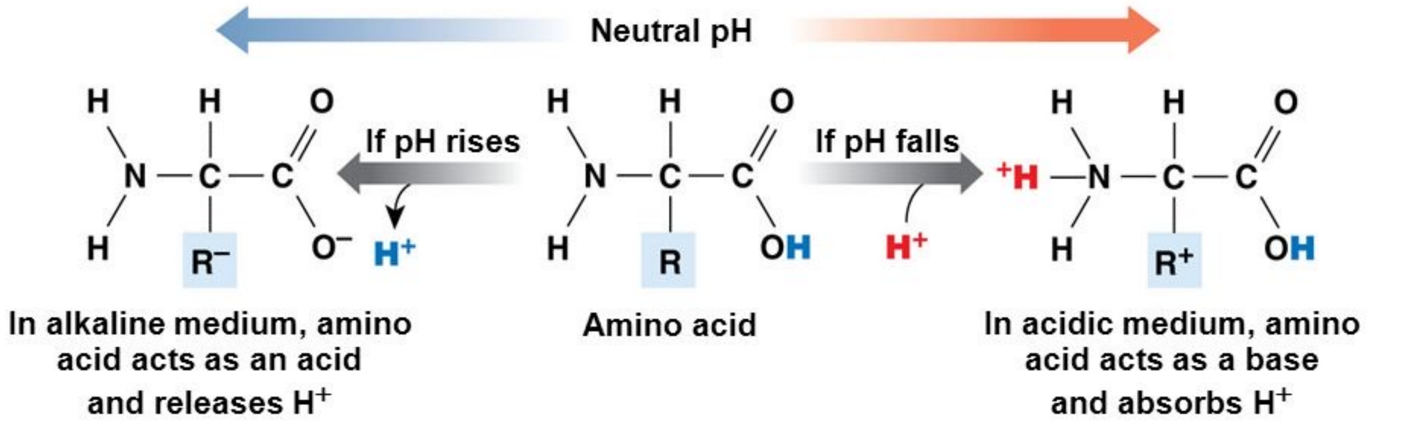
Some properties of Amino Acids:

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

Some properties of Amino Acids:

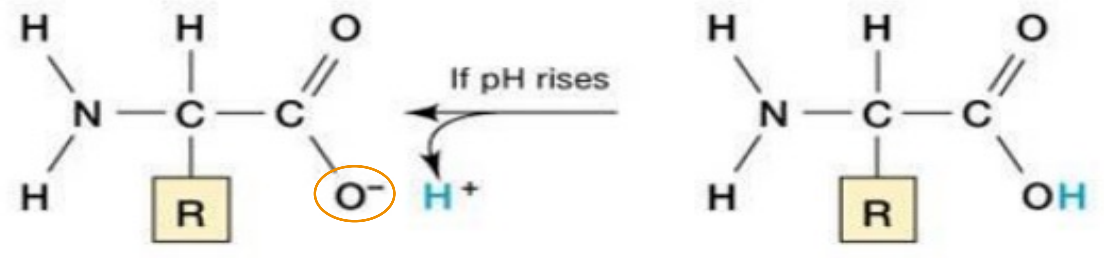
1. Amphoteric Compounds:

- An amphoteric compound is a molecule or ion that can act both as an acid and as a base.
- 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^- :

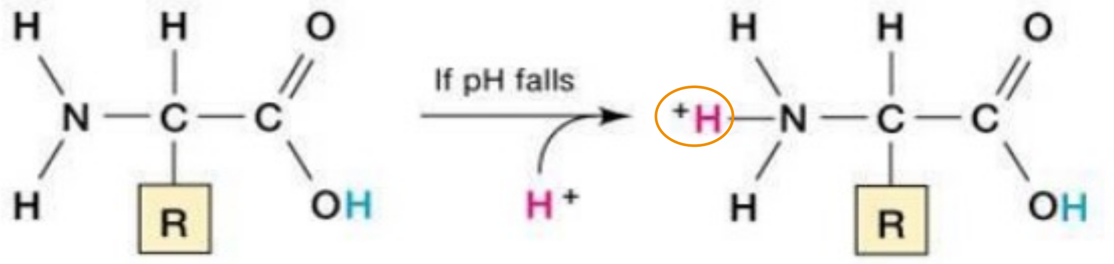
COOH ➔ COO^-



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 ➔ NH_3^+

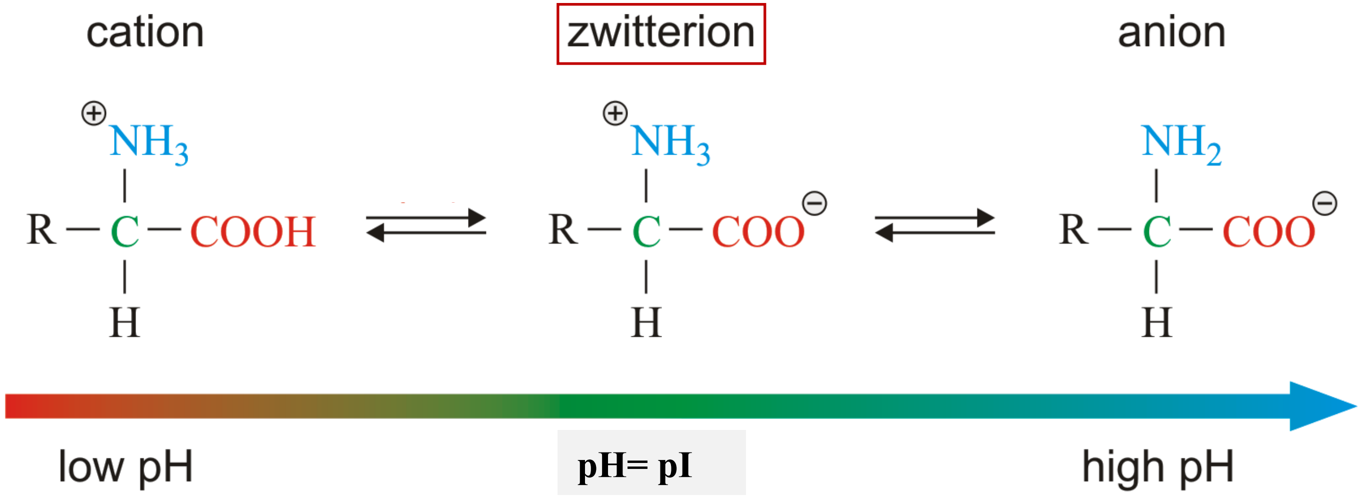


Low pH ➔ Act as **Base**

Some properties of Amino Acids:

2. Isoelectric point (pI):

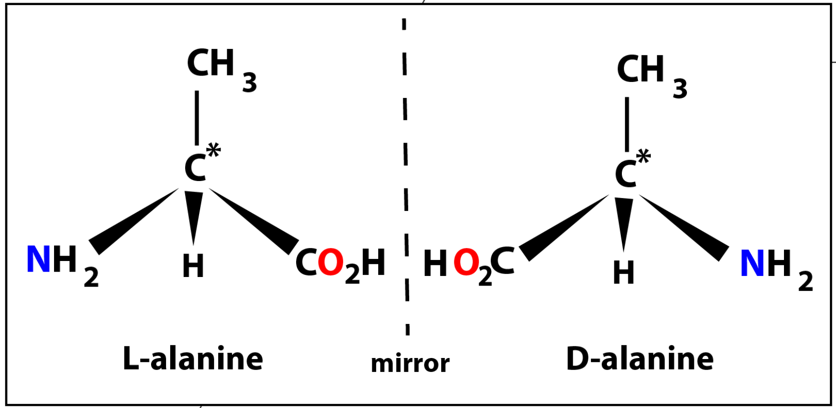
- It is the <u>pH value</u> at which the positive charge <u>equals</u> the negative charge (i.e. the net charge of this molecule equals <u>zero</u>) ➔ Zwitter ion
- Isoionic or isoelectric point of the amino acid.
- Each amino acid has a different pI (*Based on what?*).
- At this point, its solubility is minimal and it does not migrate when placed in an electric field (unlike the cation and the anion) (Why?).

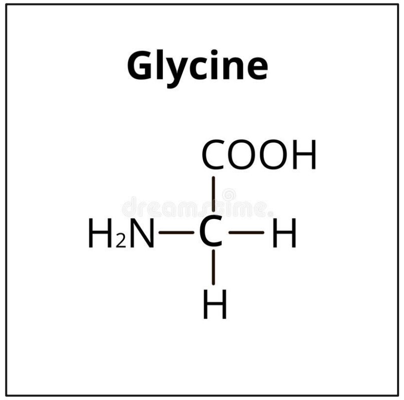


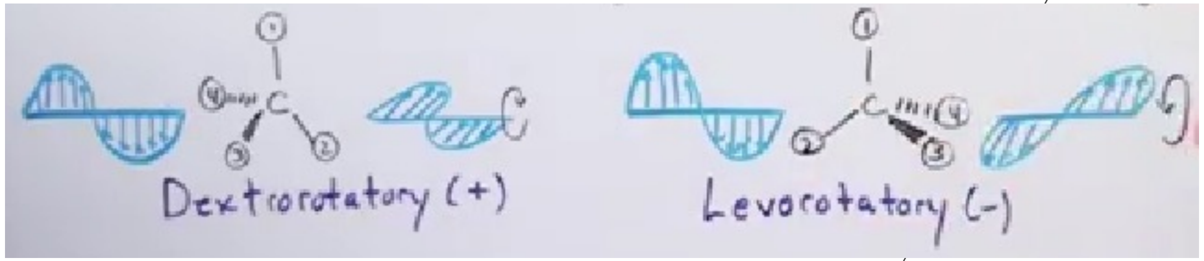
Some properties of Amino Acids:

3. Optical Activity:

- Amino acids are able to rotate polarized light either to:
  - The left (*Levorotatory*) ➔ (-) – Amino acid
  - The right (*Dextrorotatory*) ➔ (+) – Amino acid
- Asymmetric C atom: a carbon atom linked to 4 different groups *(what are they ?)*.
- *What about glycine ?*



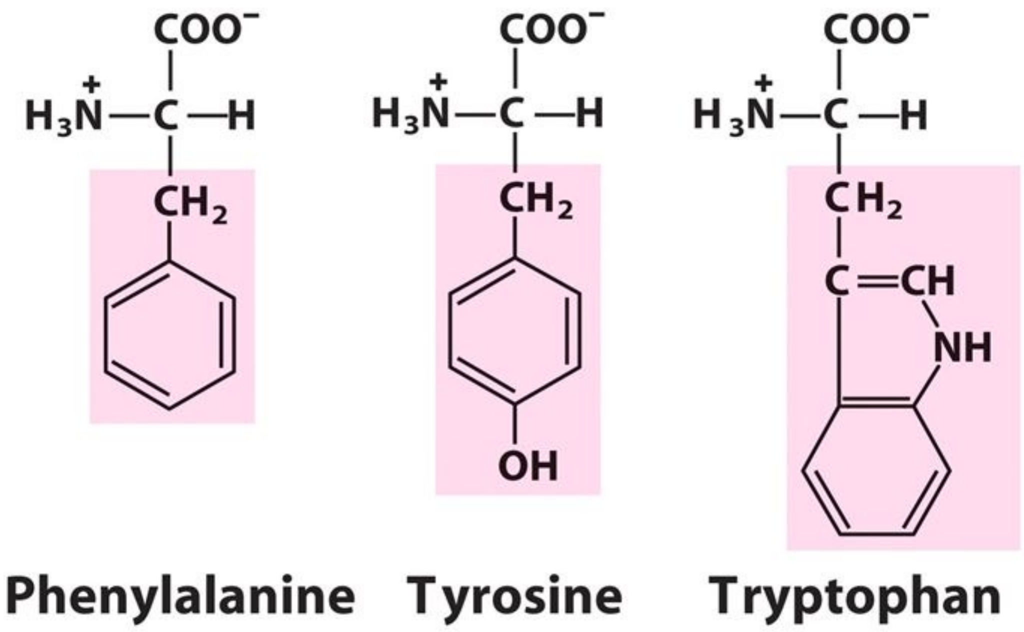




Some properties of Amino Acids:

1. Light Absorption:

- The aromatic amino acids absorb ultraviolet light at 280nm.
- *What about 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 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.

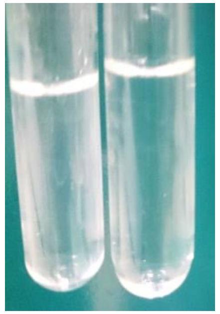
Experiment (1): Solubility Test

Method:

1. Add 2 ml of different solvents in 3 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

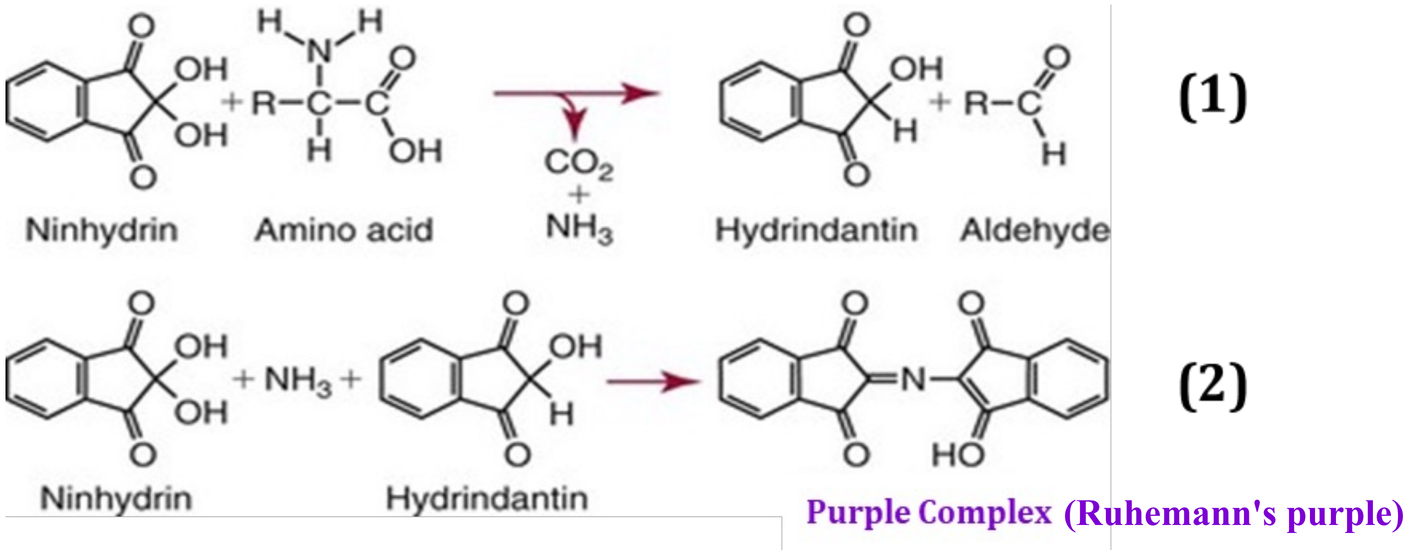
Experiment (2): Ninhydrin test

Objective:

- To detect α-L-amino acids.

Principle:

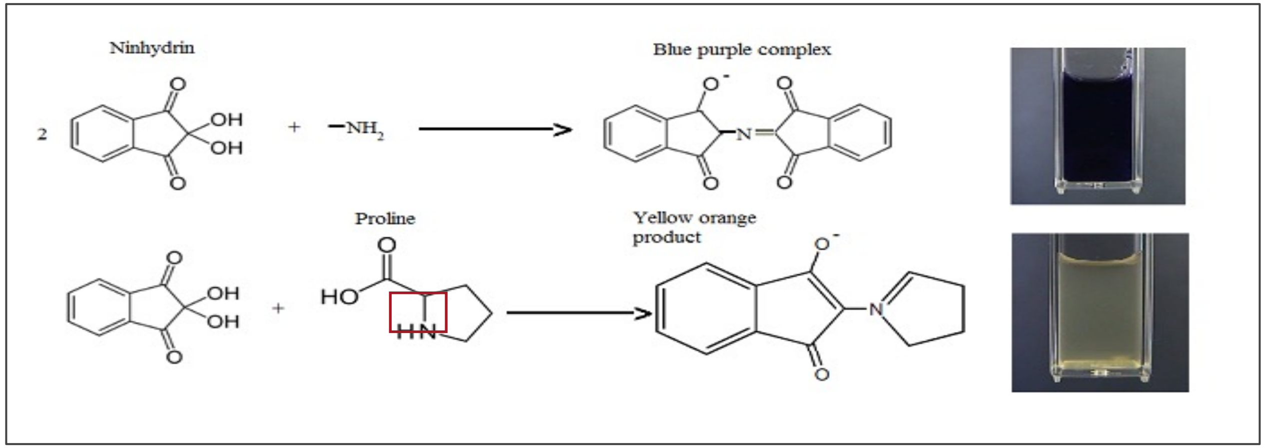
1. In the pH range of 4-8, ninhydrin (triketohydrindene hydrate) degrades amino acids into aldehydes, ammonia and CO_2 → *hydrindantin*
2. More ninhydrin condenses with ammonia and hydrindantin → intensely blue or purple pigment (diketohydrin), *Ruhemann's purple*
3. The color varies slightly from acid to acid.



Experiment (2): Ninhydrin test

Principle:

- All amino acids that have a free amino group (NH_2) will give (purple color).
- While not free amino group-proline and hydroxy-proline (imino acids) will give a (yellow color), because the N is not available for the reaction as it is locked in the ring structure, therefore no ammonia is produced.



Note:

All primary amines and ammonia react similarly and produce blue/purple product but without the liberation of carbon dioxide.

Experiment (2): Ninhydrin test

Method:

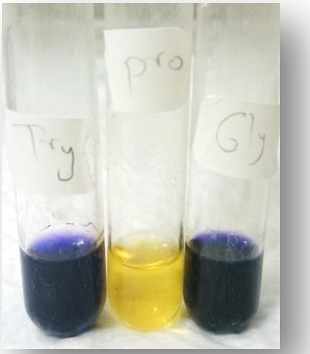
1. Place 1 ml of each of the solutions in a test tube and add 1 ml of ninhydrin solution.
2. Boil the mixture over a water bath for 2 min.
3. Allow to cool and observe the blue-purple color formed.
4. Record your results.

⚠ CAUTION

Ninhydrin is a strong oxidizing agent, it should be handled with care, and applied apart from contact with skin or eyes, gloves and mask is a must, using hood is required, if accidently get in touch with the skin, the resulting stains is a temporarily one, that will be eliminated within 24 hours.

Results:

Tube	Observation
Glycine	
Tryptophan	
Proline	



Experiment (3): Xanthoproteic test

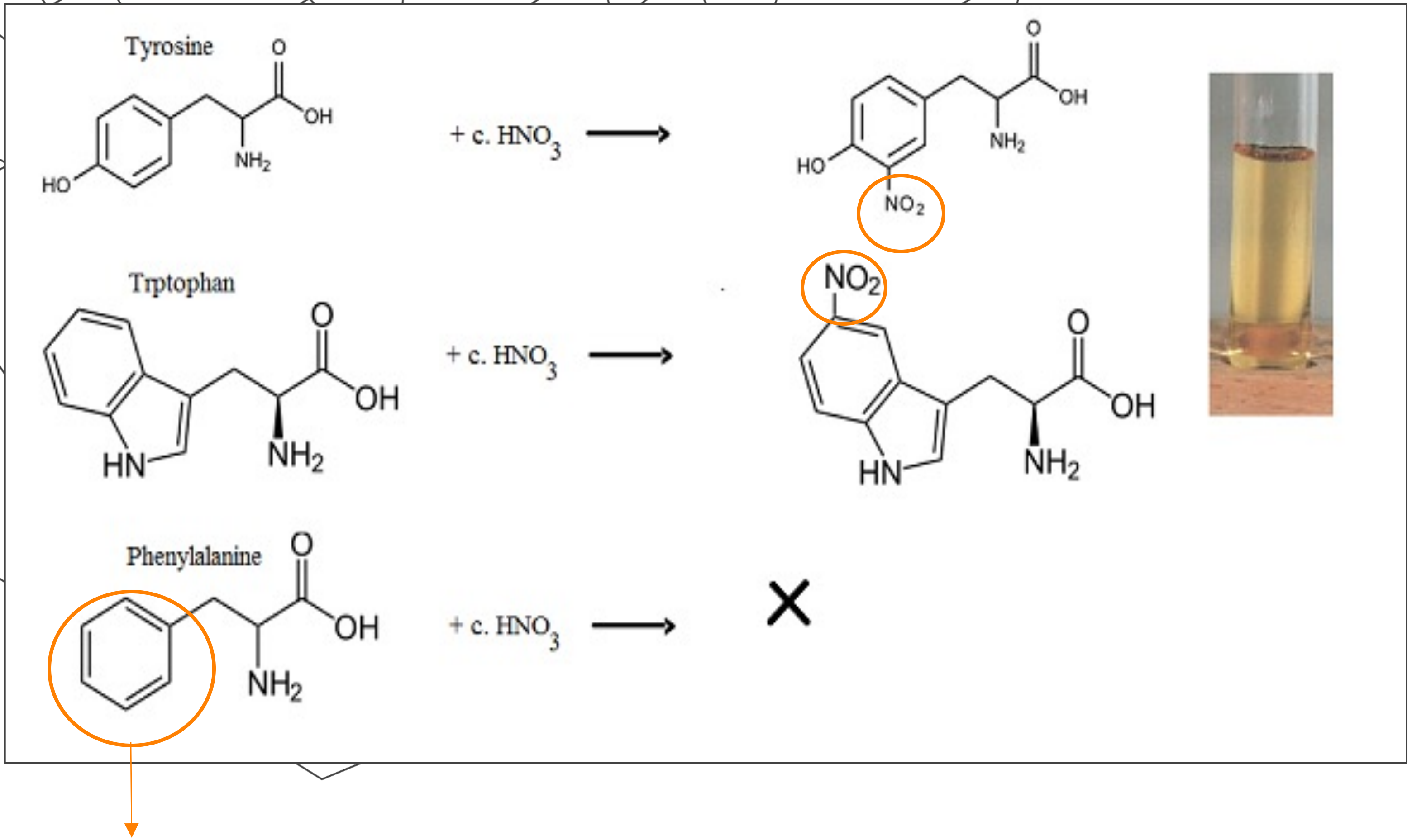
Objective:

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

Principle:

- In the presence of concentrated nitric acid (HNO_3), the aromatic phenyl ring is nitrated to give nitro-derivatives, [nitration reaction] ➔ giving the solution yellow color.
- At alkaline pH, the color changes to orange due to the ionization of the phenolic group.
- Amino acids tyrosine and tryptophan → contain activated benzene rings → 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

⚠ CAUTION

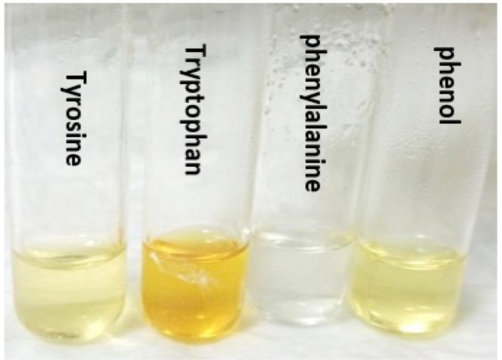
Concentrated HNO_3 is a toxic, corrosive substance that can cause severe burns and discolour your skin. Prevent eye, skin and cloth contact. Avoid inhaling vapors and ingesting the compound. Gloves and safety glasses are a must; the test is to be performed in a fume hood.

Method:

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 results.
3. Now COOL THOROUGHLY under the tap and CAUTIONLY 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		



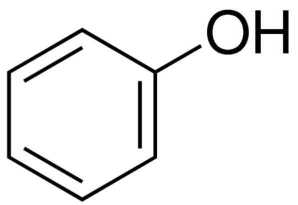
Experiment (4): Millon's test

Objective:

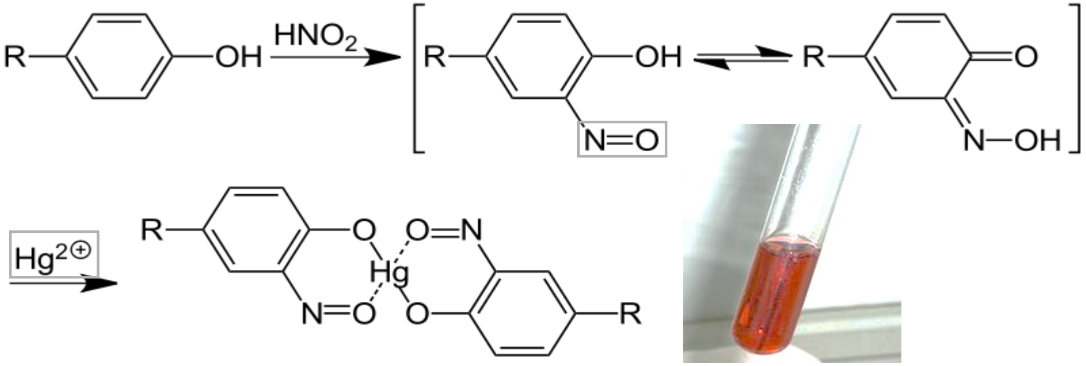
- This test is specific for Tyrosine ➔ because it is the only amino acid containing a phenol group.

Principle:

- The phenol group of tyrosine is nitrated by nitric acid.
- Nitrated tyrosine complexes mercury ions in the solution to form a brick-red solution or precipitate of nitrated tyrosine.
- Appearance of red color → positive test.



Phenol group



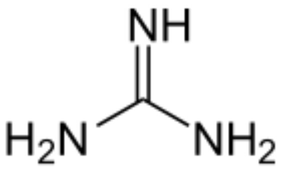
Note:

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

Experiment (5): Sakaguchi Test

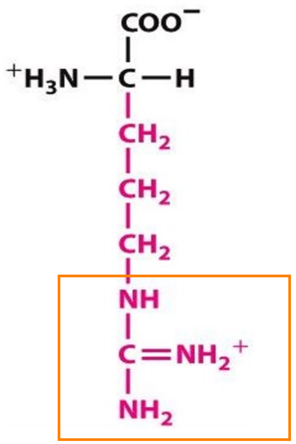
Objective:

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



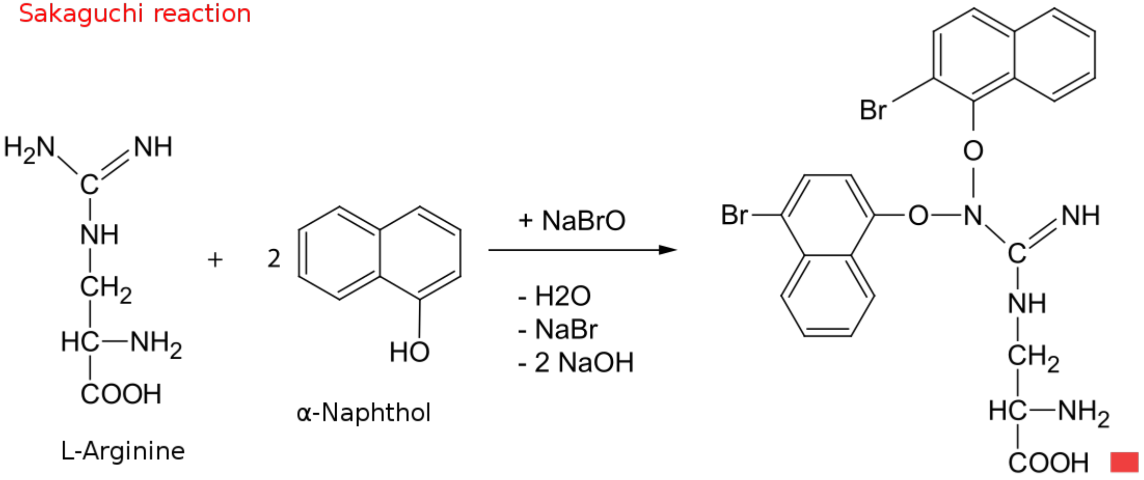
Principle:

- In alkaline solution, mono-substituted guanidine compound like (arginine) react with α-naphthol and sodium hypobromite/chlorite as an oxidize agent, to form red complexes as a positive result.



Guanidium group

Sakaguchi reaction

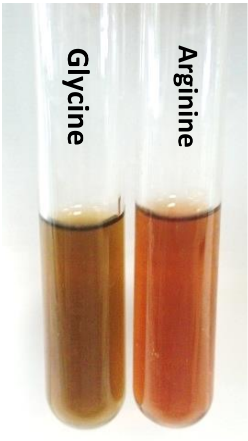


Red compound

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 Sulfide Test

Objective:

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

Principle:

- Sulphur in cysteine, is converted to sodium sulfide by boiling with 40% NaOH.
- The Na_2S can be detected by the **black precipitate** of PbS (lead sulfide) from an alkaline solution when adding lead acetate $(CH_3COO)_2Pb$.

$$\text{Cysteine (S)} + 2\,NaOH \xrightarrow{\text{heat}} Na_2S$$

$$Na_2S + (CH_3COO)_2Pb \longrightarrow \boxed{PbS} + 2CH_3COONa$$

Homework

- Are D-amino acids present naturally? where in nature?
- What is the difference between Xanthoproteic test and Millon's test?