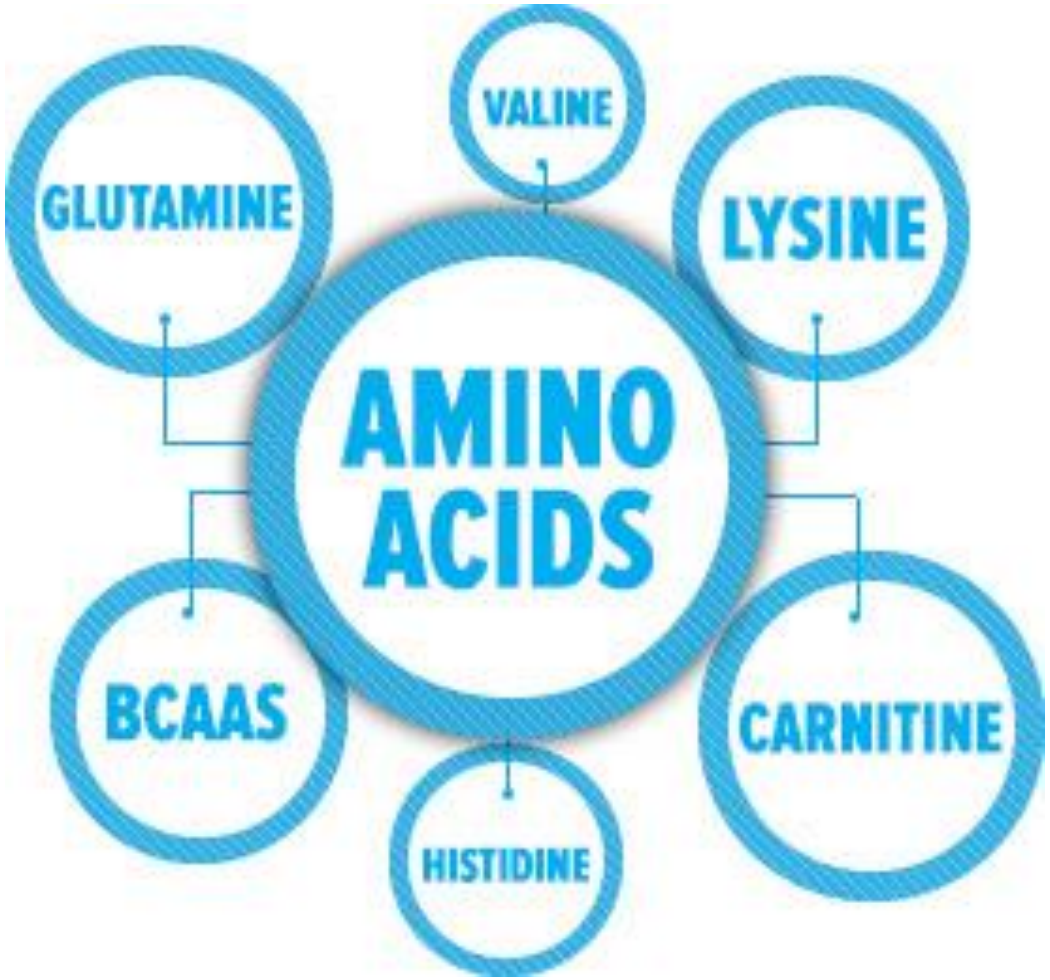
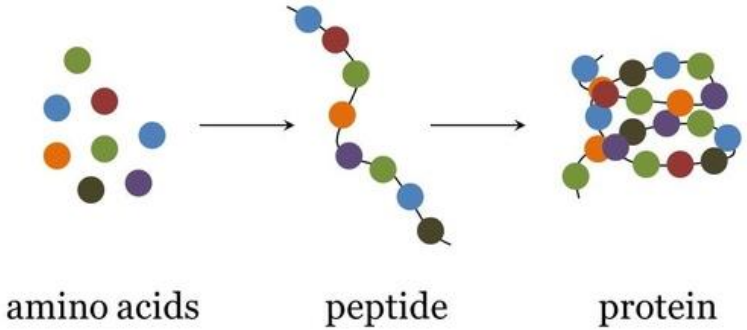



# AMINO ACIDS

Qualitative Tests

# AMINO ACIDS

- Amino acid play A central role as building block of proteins.
- Amino acids also converted to specialized products.




- More than 300 different amino acids have been described in nature,
- only 20 are commonly found in mammalian proteins.

- These 20 amino acids are **L-α-amino acids**

# STRUCTURE OF AMINO ACID THAT FOUND IN MAMMALIAN

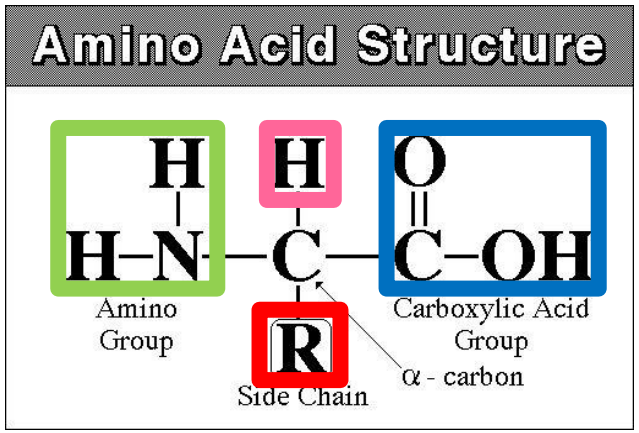
**Mammalian A.A is L-α-amino acids**

**a basic amino group ( —NH 2 )**

**an acidic carboxyl group ( —COOH)**

**a hydrogen atom ( —H)**

**a distinctive side chain ( —R).**




Left-handed Isomer

↑

**L-α-amino acids**

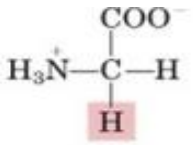
↓

Amino acids having both the amine and carboxylic acid groups attached to the first (alpha-) carbon atom

# CLASSIFICATION OF AMINO ACIDS

*The nature of the side chain (R-group) determine the role of amino acid plays in the protein.*

**So it is useful to classify the amino acid depending on the R-group ionization (polarity) in water:**



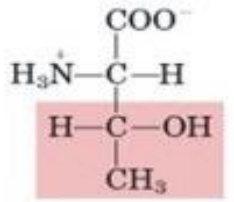
Glycine

**Non-polar side chain:**

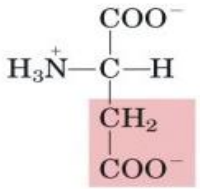
R group does not bind or give off or participate in hydrogen or ionic bonds

**Polar uncharged side chain:**

can participate in hydrogen bond formation



Threonine

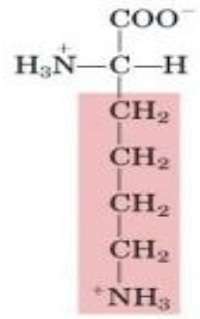


**Acidic side chain: (negative charge):**

Contains –COO- and they are proton donor

**Basic side chain: (positive charge):**

Contains NH3+ and they are proton acceptor



# SOLUBILITY

- **Amino acids** are generally soluble in water

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

**Why?**

- **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.

# AMINO ACID PROSPERITIES

1. Rotate the polarized light

2. Amphoteric compounds

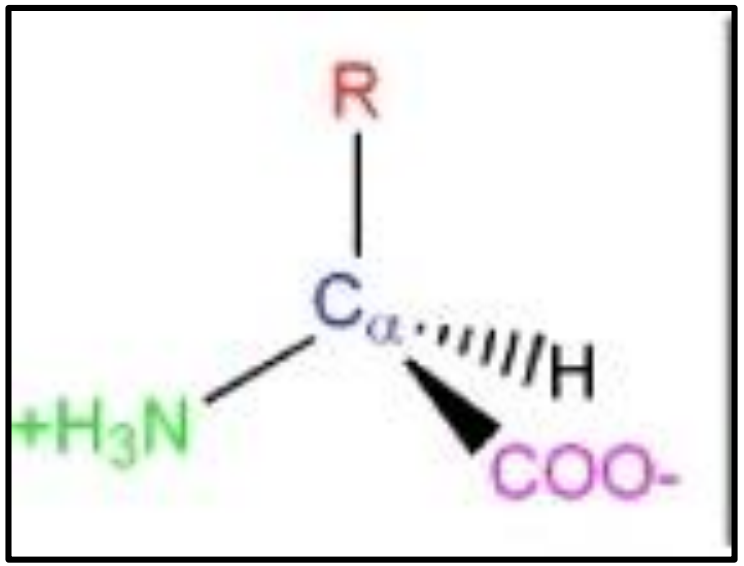
3. Trp, Tyr, and Phe contain conjugated aromatic rings. Consequently, they absorb light in the ultraviolet range (UV).

4. Isoelectric point

# 1-ROTATE THE POLARIZED LIGHT

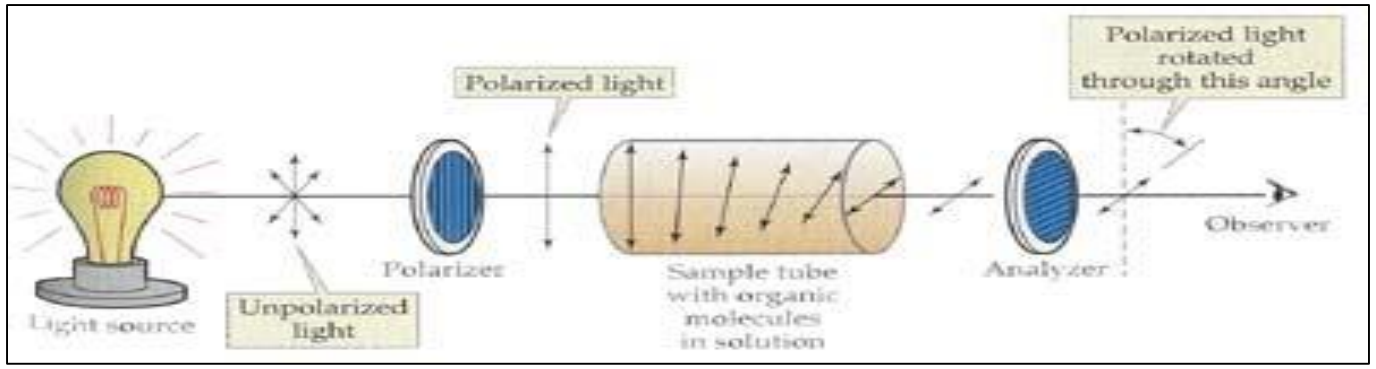
A.As have the ability to rotate the plane of polarized light because the have a chiral carbon(a carbon which link to 4 different groups)

**(Except for glycine)**



# 1-ROTATE THE POLARIZED LIGHT

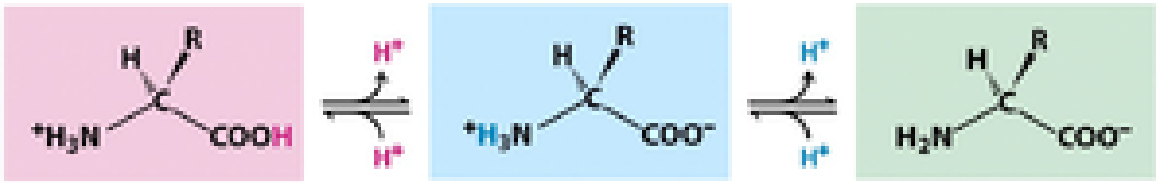
- Clockwise rotation is known as "dextrorotatory" behavior
- Counterclockwise rotation is known as "levorotatory" behavior.
- Some **L-amino acids** are **Levorotatory**, some are actually **Dextrorotatory**
- the optical activities are given as (+) for dextrorotatory, and (-) for levorotatory, for example:
- L(+)-alanine (this is the L-isomere and it is dextrorotatory)



# 2-AMPHOTERIC COMPOUNDS

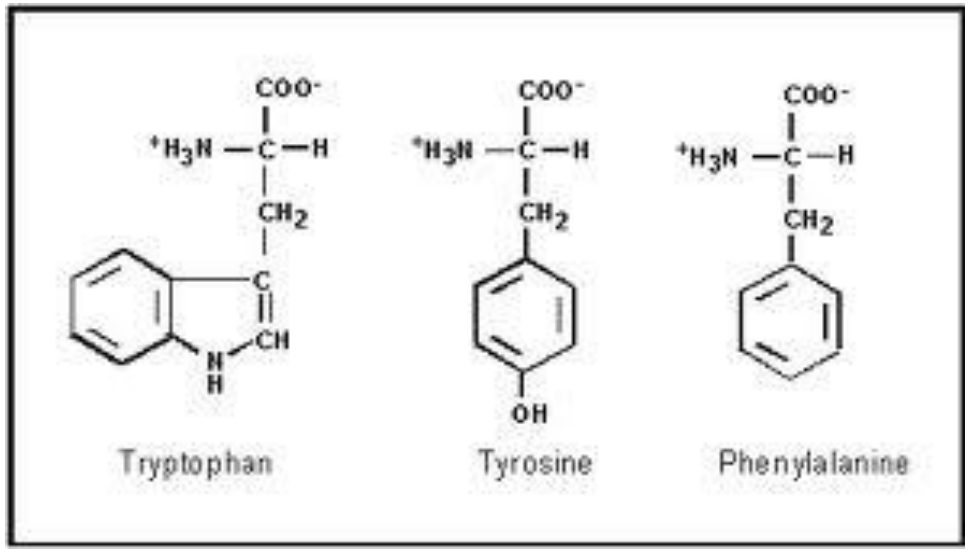
***Amphoteric*** compounds is a molecule that can react as an acid (donate a proton) as well as a base(accept a proton).

**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 .



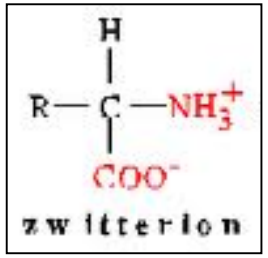
# 3-LIGHT ABSORPTION

The aromatic amino acids tryptophan , tyrosine , phenyl alanine absorb ultraviolet light at 280nm ,which explains the absorption of proteins at 280nm.



# 4-ISOELECTRIC POINT (PI) :

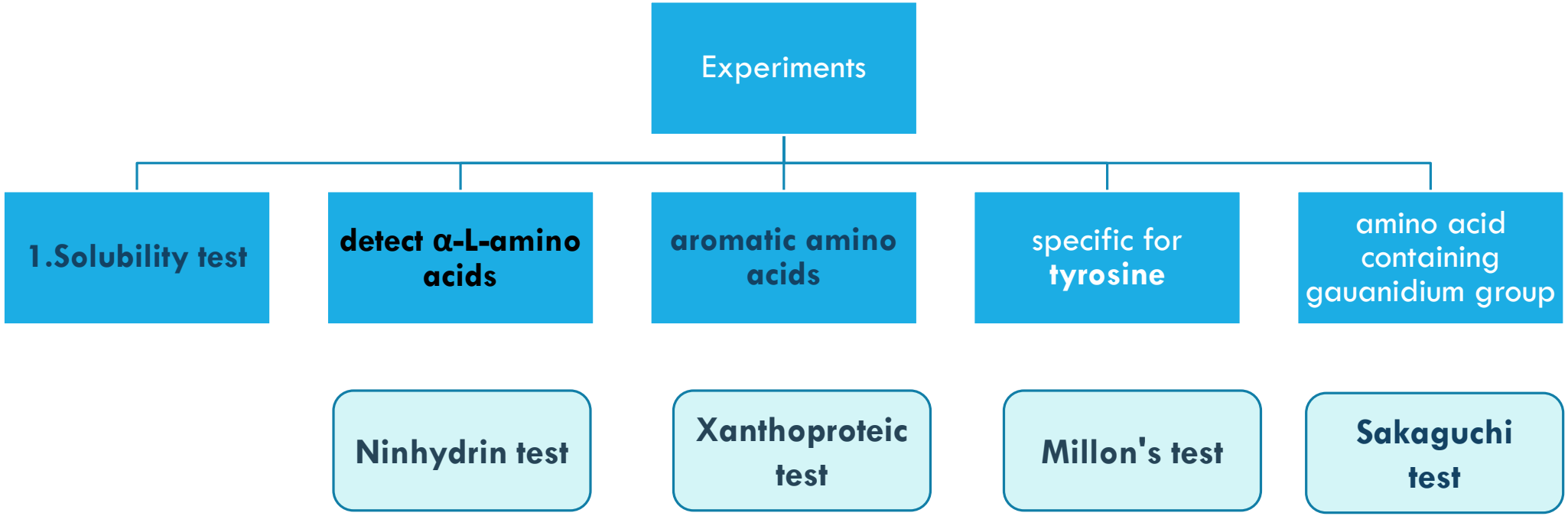
It is the pH value at which the positive charge equals the negative charge (**i.e. the net charge of this molecule equals zero**) **(zwitter ion)**




**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**

# EXPERIMENTS THAT WILL BE DONE



# QUALITATIVE TESTS OF AMINO ACIDS

## 1.SOLUBILITY TEST:

**Objective:**

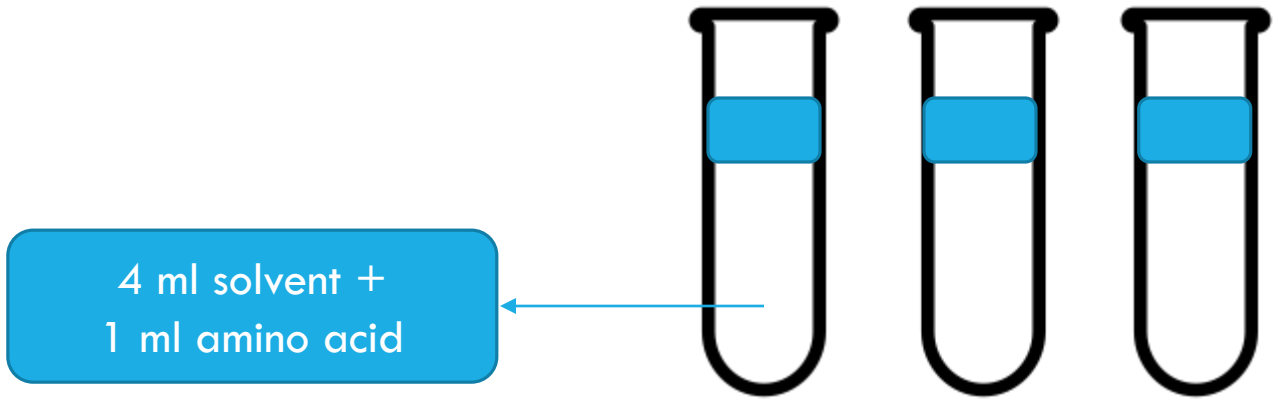
To test the solubility of selected amino acid in (HCl, NaOH, chloroform)

**Materials**:

- Different amino acid solutions:  glycine , lysine, Arginine Solvents:
- HCL
- NaOH
- chloroform
- Test tubes

## Method:

1. Add 4ml of different solvents in 3 clean test tubes then place 1 ml ofglycine, then repeat the experiment with Arginine and lysine
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:

| Tube / Amino acids | Result | | |
|---|---|---|---|
| | glycine | lysine | Arginine |
| HCL | | | |
| NaOH | | | |
| Chloroform | | | |

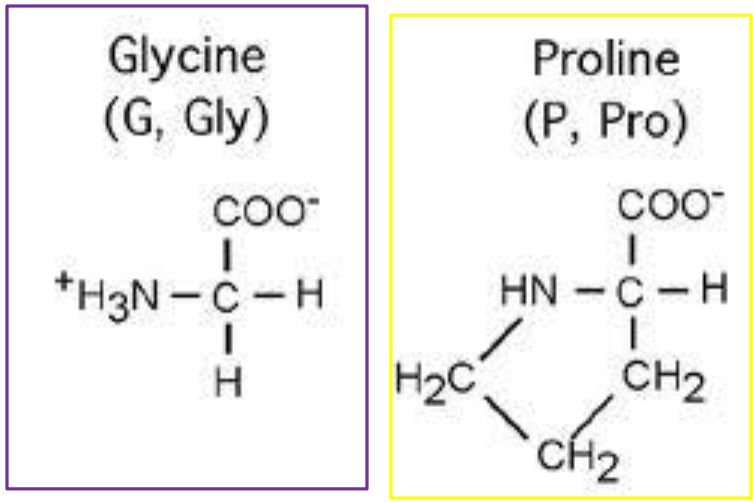
# 2. NINHYDRIN TEST

**Objective:**

- To detect α-L-amino acids

- Can be used also to detect

free amino and carboxylic acid groups on proteins and peptides.

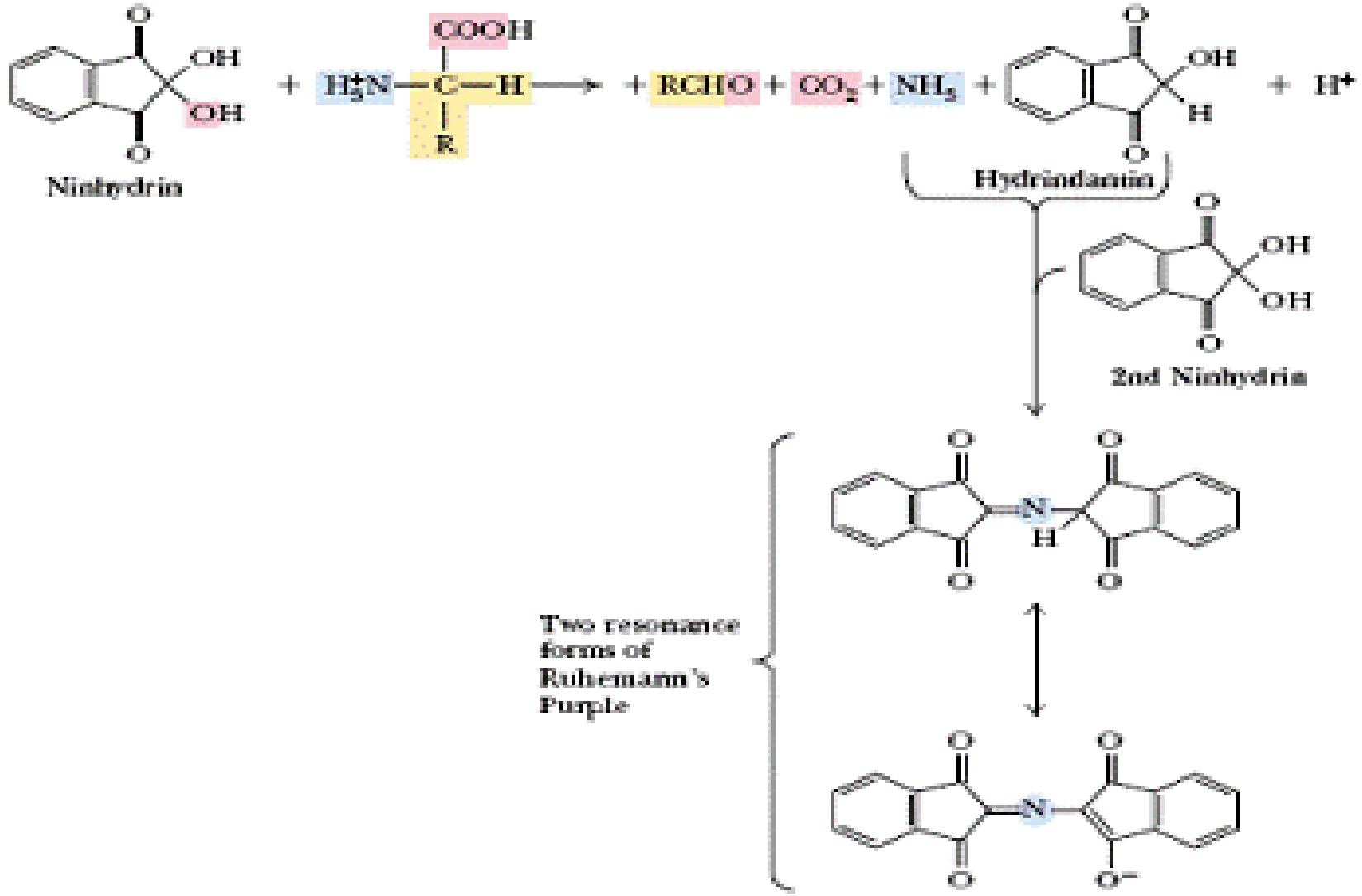



All amino acids that have a **free amino group will give positive result (purple color)** , while **not free** amino group-proline- will give a (**yellow color**)

# 2. NINHYDRIN TEST

## *Principle*:

- Ninhydrin (*tri ketohydrindene hydrate*) degrades a.a into ***aldehydes***, ***ammonia*** *and* ***CO2*** (on pH range 4-8) though a series of reactions.
- The net result is ninhydrin in a partially reduced form hydrindantin.
- **Ninhydrin + amino acid----→ *aldehydes* + *ammonia* + CO2 + hydrindantin**
- Other Ninhydrin molecule then **condenses** with ammonia and hydrindantin to produce an intensely blue or purple pigment, sometimes called **ruhemann's purple**
- Ninhydrin+ **hydrindantin + *ammonia* ------→ ruhemann's purple**


Ninhydrin
Hydrindantin
2nd Ninhydrin
Two resonance forms of Ruhemann's Purple

# ⚠ 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

# 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. (be carful!!!)

3- Allow to cool and observe the blue color formed

4- Complete the below table

## Result:

| Tube | | Observation | Conclusion |
|---|---|---|---|
| A | Glycine | | |
| B | Tryptophan | | |
| C | Proline | | |

# 3.XANTHOPROTEIC TEST

**Objective:**

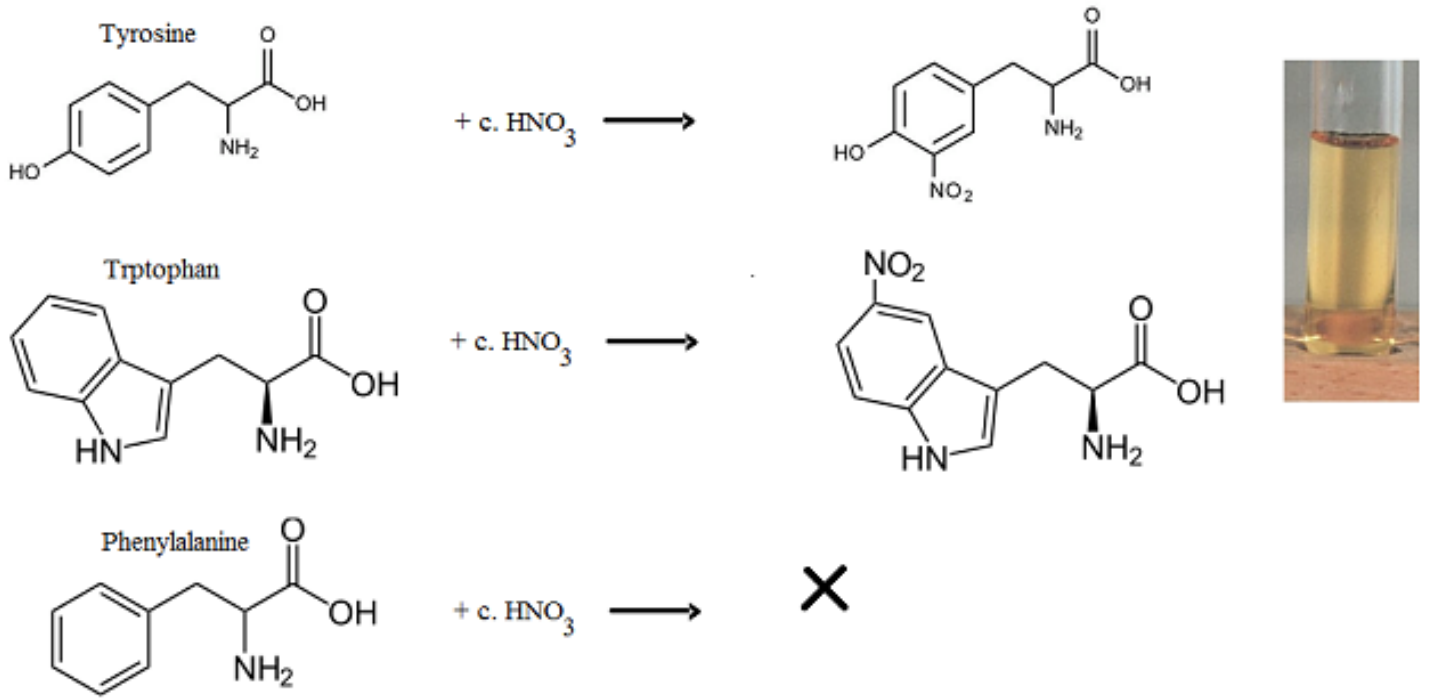
This test is used to differentiate between **aromatic amino acids** which give positive results and other amino acids.

- Amino acids containing an aromatic nucleus form **yellow nitro derivatives** on heating with concentrated HNO3.

# PRINCIPLE:

Conc. HNO3 reacts with the aromatic rings that are derivatives of benzene giving the characteristic ***nitration reaction.*** Amino acids tyr. and typ. contain ***activated benzene*** rings which are easily nitrated to **yellow colored** compounds.

Although **phenylalanine contains benzen ring, it dose not react** with nitric acid because the benzene ring is not activated, therefore it will not react.



# ⚠ CAUTION

Concentrated **HNO3** is a **toxic, corrosive** substance that can cause severe burns and discolor 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.

مادة سامة

Toxic

مادة كاوية وحارقة

Corrosive

# METHOD OF XANTHOPROTEIC TEST:

1-Label five tubes (1 - 5), then add 1 ml of each amino acid solution and phenol solutions 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).

# RESULT:

| Tube | Observation | |
|---|---|---|
| | + HNO3 | + NaOH |
| Tyrosine | | |
| Tryptophan | | |
| plenylalnin | | |
| phenol | | |

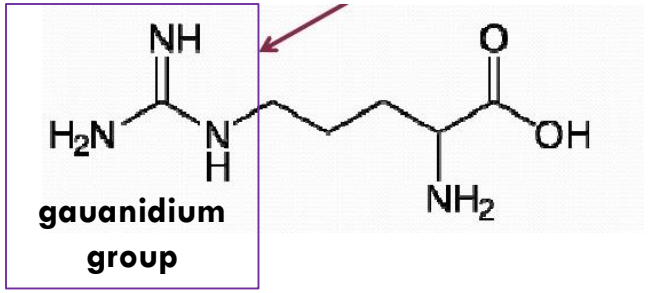
# 4. SAKAGUCHI TEST

**Objective:**

Sakaguchi test is a specific test for detection of amino acid containing gauanidium group [R-NH-C= $(NH2)^{2+}$-NH2]. In other words it's a test for guanidines, i.e 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.



## Material:

glycine,

arginine

10%NaOH

α-naphthol in 10% ethanol

5%sodium hypobromate (or sodium hypochlorite)

## Method:

Label 3 test tube and put in each one 2 ml of the amino acid solution .

Add to each tube 2ml of NaOH solution. Mix well

Add to each tube 2ml of α-naphthol solution. Mix well

Add to each tube 3 drops of sodium hypobromite solution, and record your result

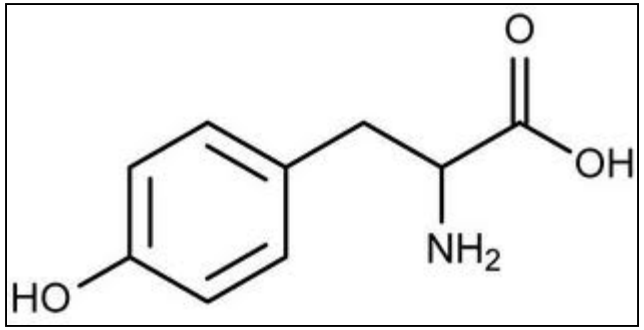
## Result:

| Tube | Observation | Conclusion |
|---|---|---|
| Glycine | | |
| Arginine | | |

# 4.MILLON'S TEST

**Objective:**

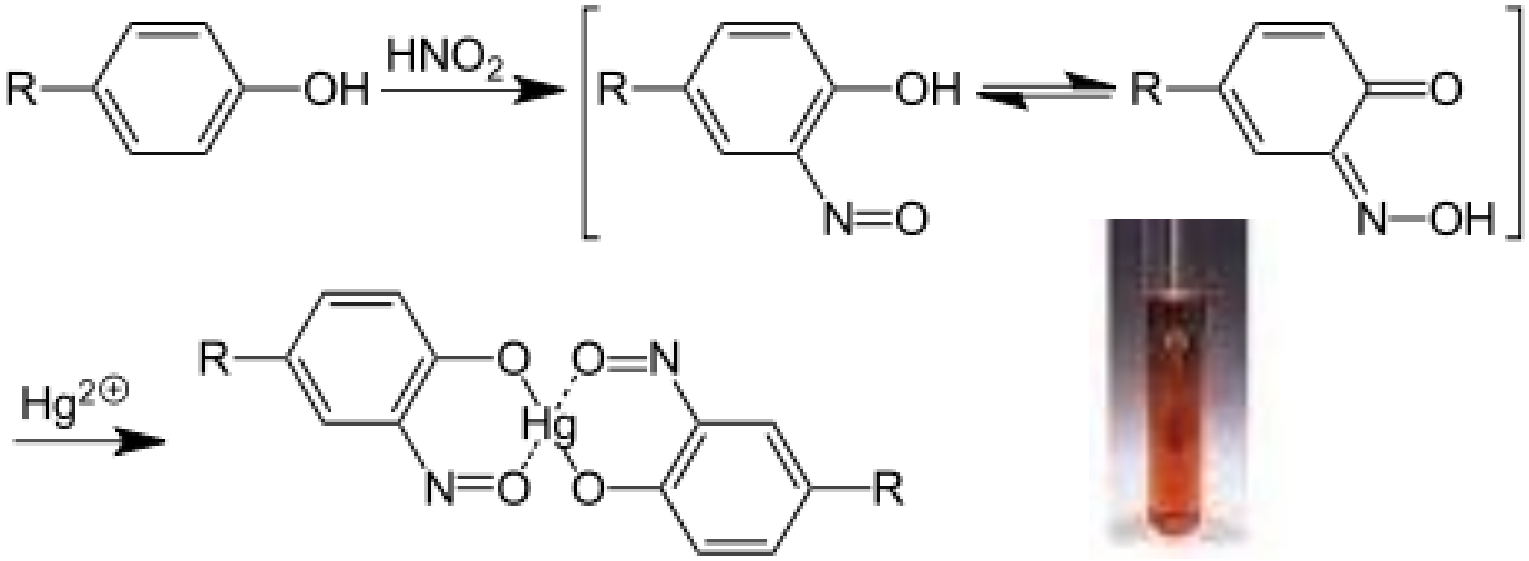
This test is specific for **tyrosine,** the only amino acid **containing a phenol group,**( a hydroxyl group attached to benzene ring)




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

# 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.**



# METHOD:

1-Label 4 test tubes

2-Add 2 ml of each solutions(tyrosine, tryptophan, phenol) to different test tubes.

3-add 1 ml of $HNO_3$

4-Add to each tube 1 ml Millon's reagent and shake it well.

5-add 5 drops of sodium nitrate

6-Place the test tubes in the boiling bath with care, for 10 min.

7-Write your observation in the following table.

# ⚠ CAUTION

**MILLON'S REAGENT IS HIGHLY TOXIC AND HIGHLY CORROSIVE**

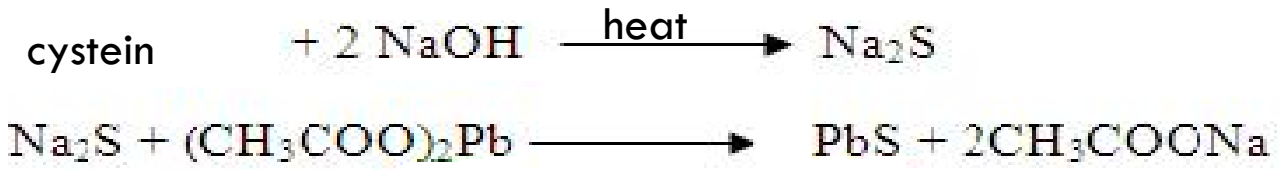

# 6- DETECTION OF AMINO ACIDS CONTAINING SULFHYDRAL GROUP (- SH)- LEAD SULFITE TEST

**Objective:**

This test specific for**–SH** containing amino acid (Cysteine).

**Principle:**

The amino acids containing sulfhydryl group when heated with base, the sulfhydryl group and disulfhydryl are directly converted to inorganic sulfur. Which is confirmed by the black precipitate of PbS (lead sulfide) when adding lead acetate **Pb (CH3COO)2**.



# SUMMARY

| | |
|---|---|
| **Solubility test** | **To test the solubility of selected amino acid in (HCl, NaOH, chloroform)** |
| **Ninhydrin test** | to detect α-L-amino acids |
| **Xanthoproteic test** | To detect **aromatic amino acids (Tyrosine, tryptophan)** |
| **Millon test** | This test is specific for **tyrosine,** the only amino acid **containing a phenol group,** a hydroxyl group attached to benzene ring |
| **Sakaguchi test** | Sakaguchi test is a specific test for detection of amino acid containing gauanidium group |
| **Lead Sulfite Test** | This test specific for**–SH** containing amino acid (Cysteine). |