Qualitative tests of amino acids

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Amino Acid

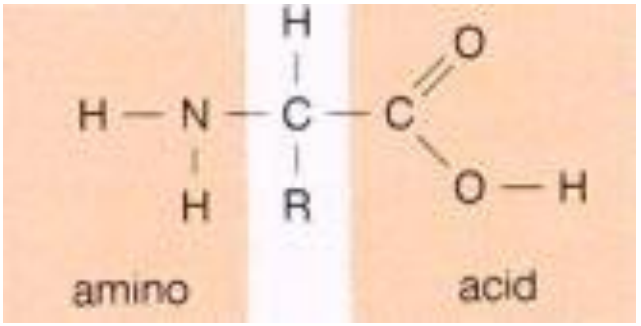
Amino acids play central roles both as

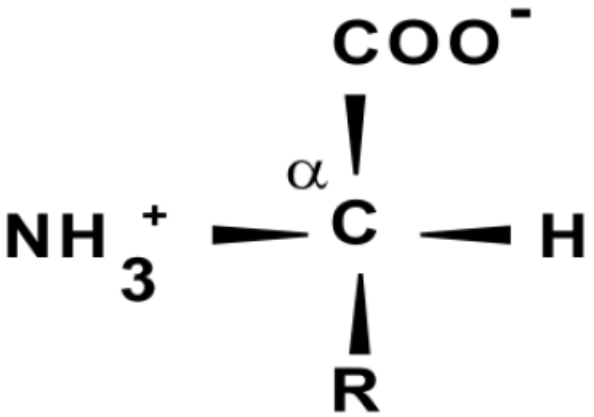
-building blocks of proteins

- as intermediates in metabolism.

There are **20** natural amino acids that are found within proteins convey a vast array of chemicals versatility.

All of them are L-α amino acids.

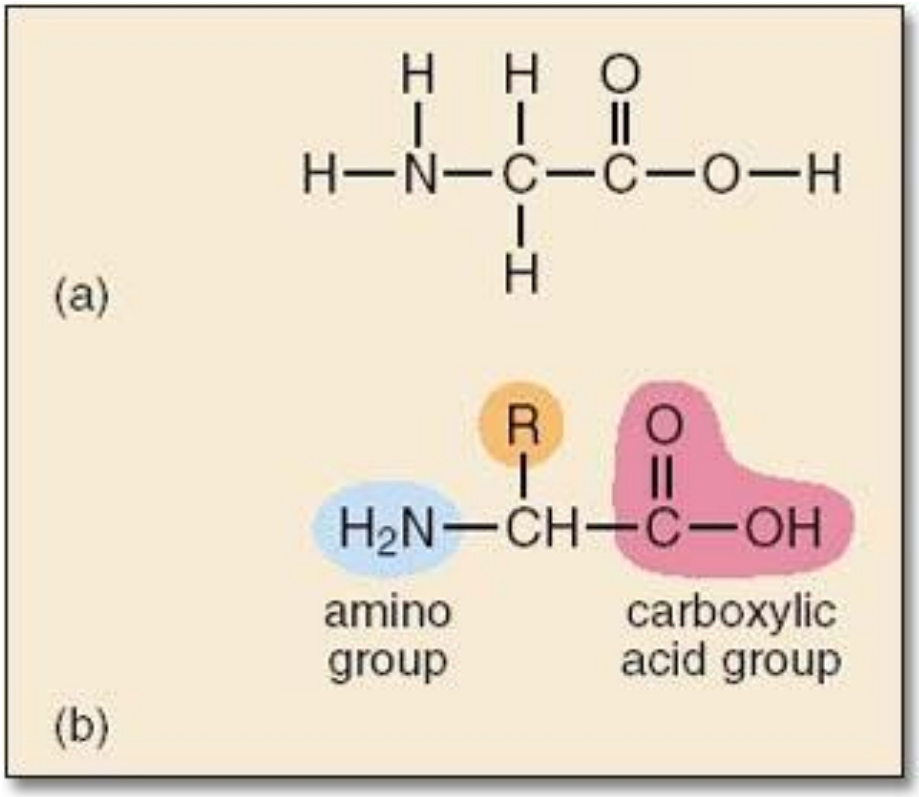




amino acids structure

All found in proteins have this basic structure, 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:

1- Polar amino acids:

a-Polar chraged

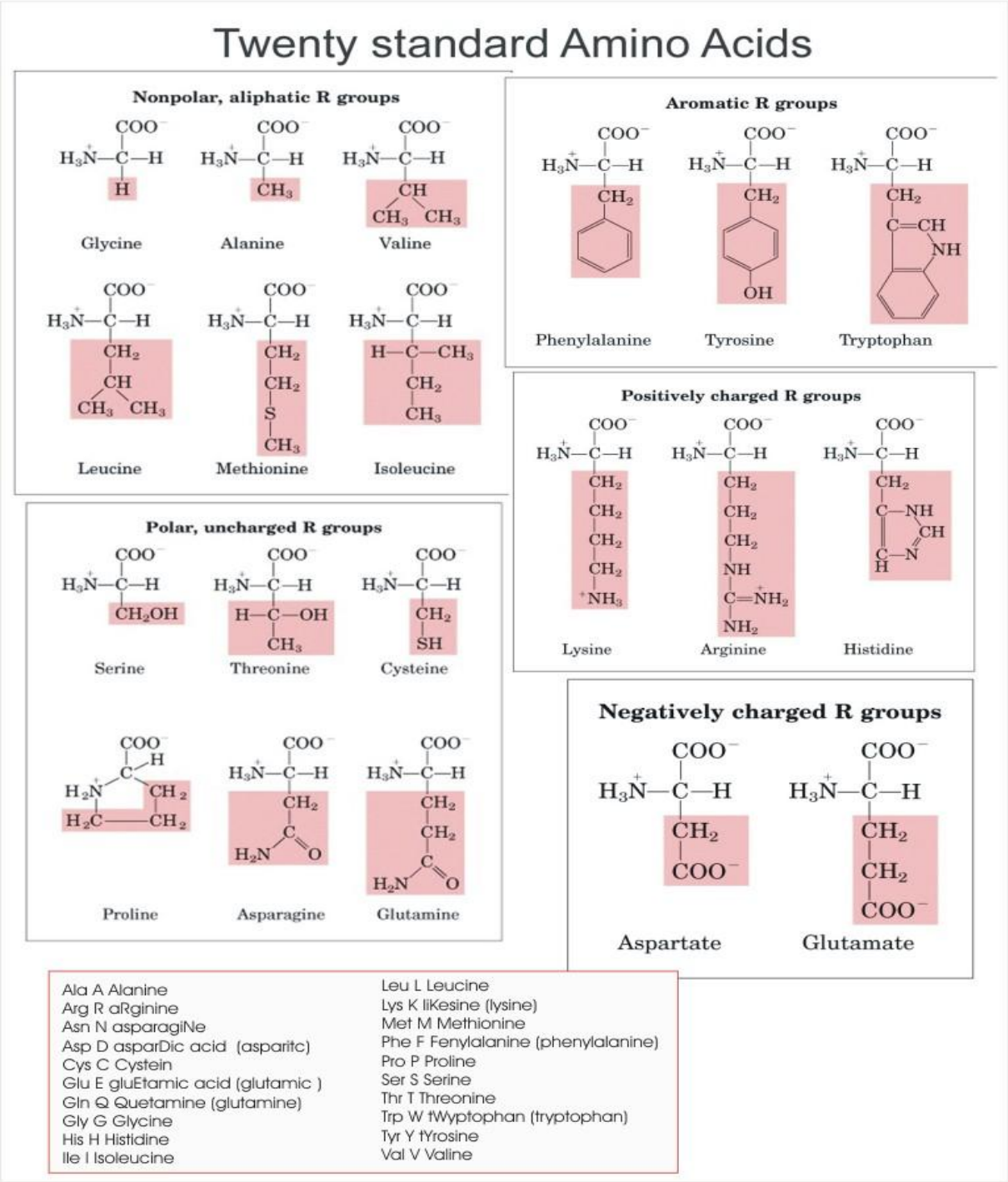
i-Acidic amino acids (-ve)

ii-Basic amino acids(+ve)

b-Polar uncharged amino acids

2-Non polar/ aliphatic/neutral

3- Aromatic amino acids



Ala A Alanine
Arg R aRginine
Asn N asparagiNe
Asp D asparDic acid (asparitc)
Cys C Cystein
Glu E gluEtamic acid (glutamic)
Gln Q Quetamine (glutamine)
Gly G Glycine
His H Histidine
Ile I Isoleucine
Leu L Leucine
Lys K liKesine (lysine)
Met M Methionine
Phe F Fenylalanine (phenylalanine)
Pro P Proline
Ser S Serine
Thr T Threonine
Trp W tWyptophan (tryptophan)
Tyr Y tYrosine
Val V Valine

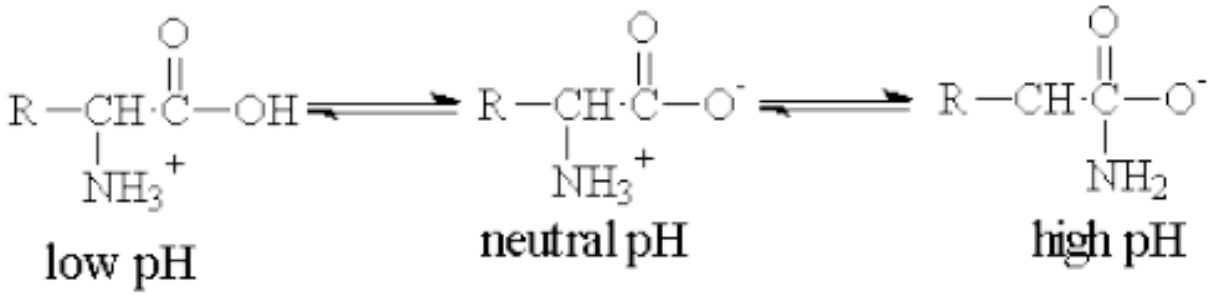
Polar amino acids are more soluble in water than non-polar, due to presence of amino and carboxyl group which enables amino acids to accept and donate protons to aqueous solution, and therefore, to act as acids and bases**.**

Some properties of Amino Acids

1- Amphoteric Compounds:

which mean they can **act as acids and bases, due to** presence of **carboxyl group COOH** that able to **donate** proton(H^+), and convert to **COO^-**

Also presence of **amino group NH2** which is enable to **accept this** proton(H^+) and convert **into $NH3^+$**



2- PI point

Iso electric point (PI) :

It is the pH value at which concentration of anionic and cationic groups are equal **(i.e. the net charge of this molecule equals zero)**

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

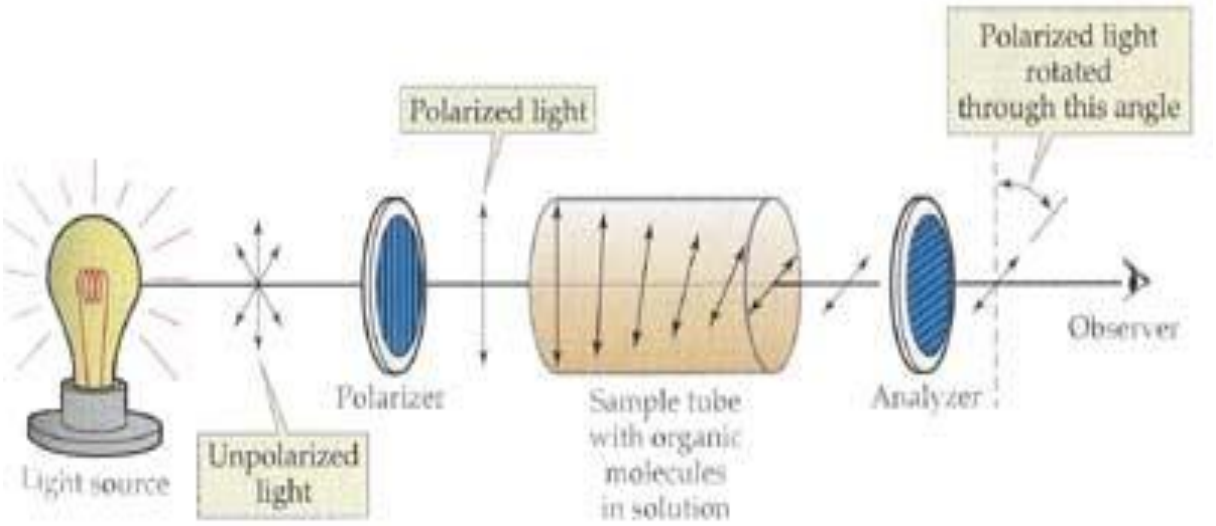
Amino Acid	Abbreviation		pK_1	pK_2	pK_R	pI
	3-Letters	1-Letter	-COOH	$-NH_3^+$	R group	
Alanine	Ala	A	2.34	9.69	-	6.00
Arginine	Arg	R	2.17	9.04	12.48	10.76
Asparagine	Asn	N	2.02	8.80	-	5.41
Aspartic Acid	Asp	D	1.88	9.60	3.65	2.77
Cysteine	Cys	C	1.96	10.128	8.18	5.07
Glutamic Acid	Glu	E	2.19	9.67	4.25	3.22
Glutamine	Gln	Q	2.17	9.13	-	5.65
Glycine	Gly	G	2.34	9.60	-	5.97
Histidine	His	H	1.82	9.17	6.00	7.59
Isoleucine	Ile	I	2.36	9.60	-	6.02
Leucine	Leu	L	2.36	9.60	-	5.98
Lysine	Lys	K	2.18	8.95	10.53	9.74
Methionine	Met	M	2.28	9.21	-	5.74
Phenylalanine	Phe	F	1.83	9.13	-	5.48
Proline	Pro	P	1.99	10.60	-	6.30
Serine	Ser	S	2.21	9.15	-	5.58
Threonine	Thr	T	2.09	9.10	-	5.60
Tryptophan	Trp	W	2.83	9.39	-	5.89
Tyrosine	Tyr	Y	2.20	9.11	10.07	5.66
Valine	Val	V	2.32	9.62	-	5.96

From Lehninger Principle of Biochemistry.

3-Rotate the polarized light

Amino acids are able to

rotate polarized light either to the left **(livo) L- a.a** or to the right (**dextro) D- a.a** , since **they have an asymmetric C atom** (a carbon atom linked to 4 different groups), **except glycine** which lacks asymmetric C atom (has 2 H+ on α-C) .



Qualitative tests of amino acids

1.Solubility test:

2. Ninhydrin test for α-L amino acids

2. Xanthoproteic test for Aromatic amino acids

3. Millon's test for amino acids containig hydroxy phenyl group

4.Sakaguchi Test

5. Detection of amino acids containing sulfhydral group (- SH)/ Lead Sulfite Test

1.Solubility test:

The physical proprieties of amino acid are mainly result of their structure, both the solid state and in various solutions

Objective:

investigate the solubility of selected amino acid in various solutions

Principle:

Polar amino acids are more soluble in water than non-polar, due to presence of amino and carboxyl group which enables amino acids to accept and donate protons to aqueous solution

Materials:

Different amino acid solutions: glycine , lysine, glutamine.

Solvents:

H2O

-HCL

– NaOH

-chloroform

Test tubes

Water bath

Method:

Place 0.5ml of amino acid sample in 4 test tubes clean, dry containing 4ml of different solvents (chloroform and hot water),

Shake the tubes thoroughly, then leave the solution for about one minute,

Record your result

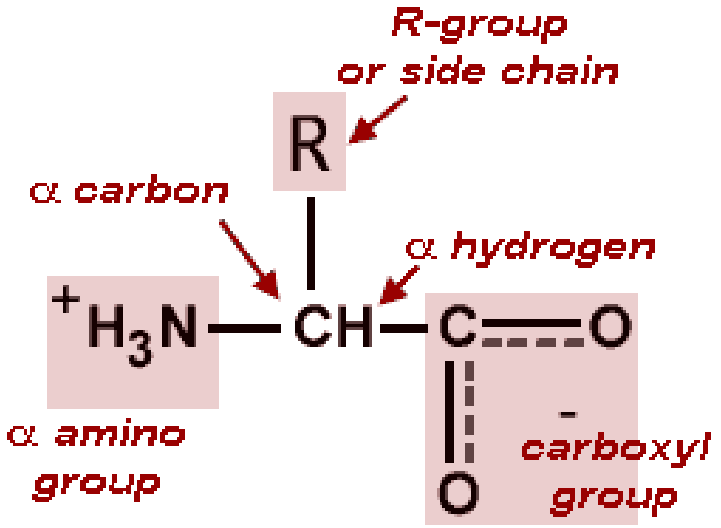
Results:

Tube / Amino acids	Result		
	glycine	lysine	glutamine
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**)

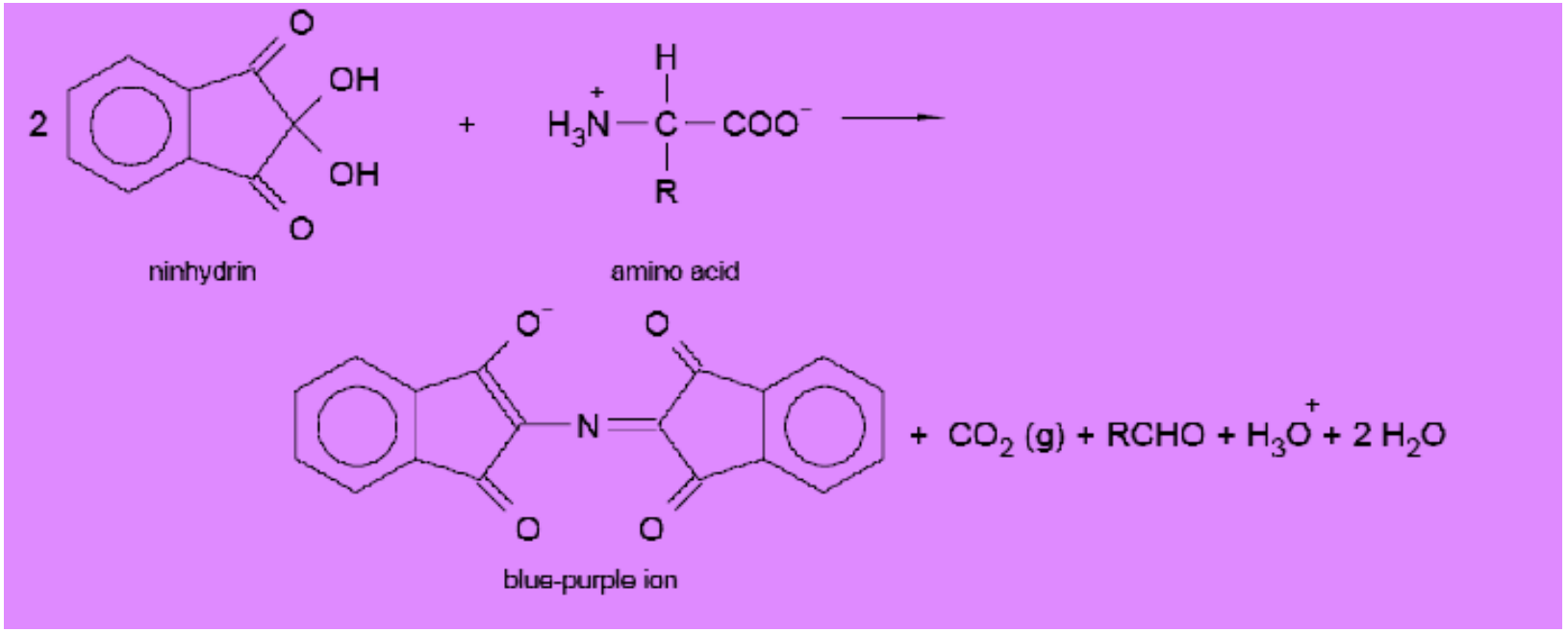
2. Ninhydrin test

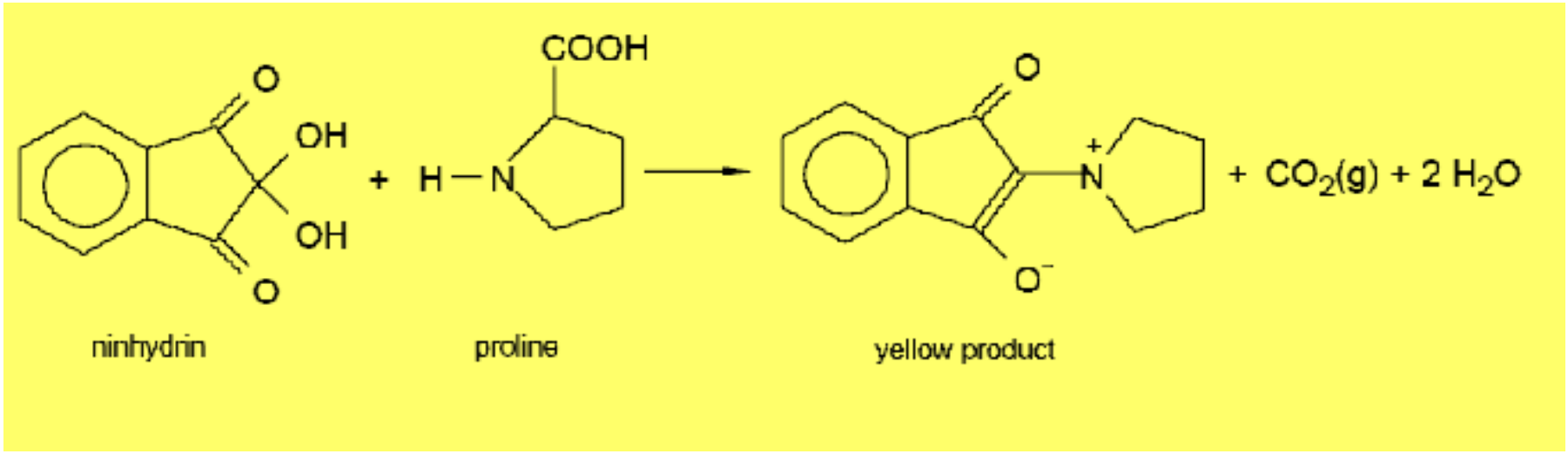
***Principle*:**

-Ninhydrin (*tri ketohydrindene hydrate*) degrades a.a into ***aldehydes***, ***ammonia*** *and* ***CO2*** (on pH range 4-8) though a series of reactions.

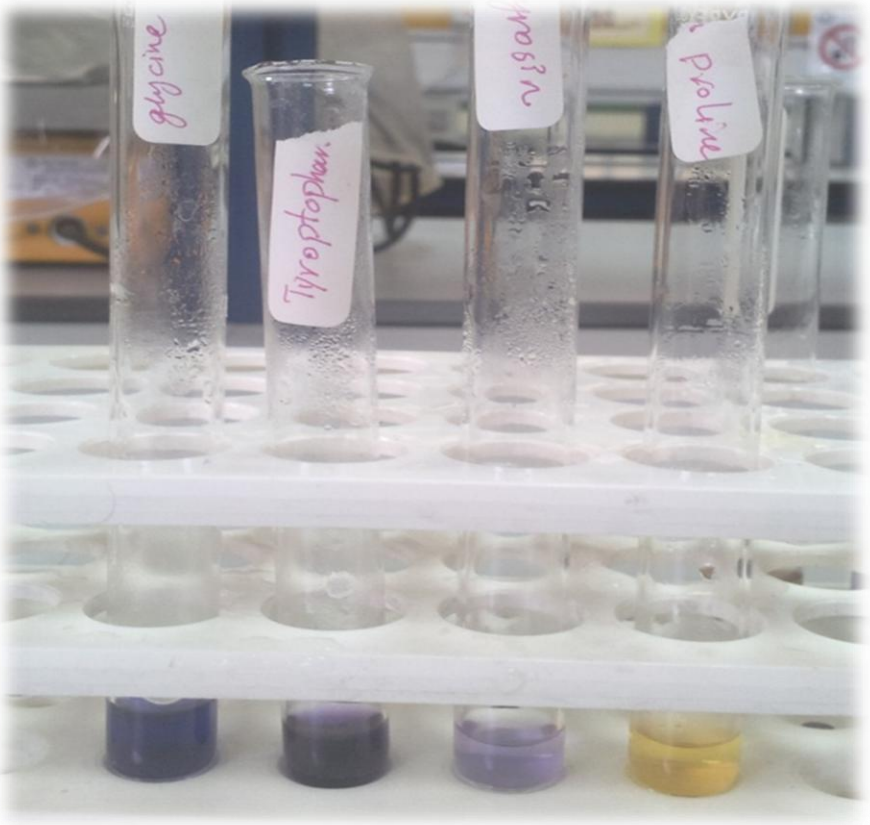
The net result is ninhydrin in a partially reduced from hydrindantin.

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


2
O
OH
OH
O
ninhydrin
+
H
H_3N
C
COO^-
R
amino acid
O^-
O
N
O
O
blue-purple ion
+ CO_2 (g) + RCHO + H_3O^+ + 2 H_2O


O
OH
OH
O
ninhydrin
+
COOH
H
N
proline
O
N
O^-
yellow product
+ CO_2(g) + 2 H_2O

The color varies slightly from acid to acid. **Proline** and **hydroxy-proline** (amino acids) give **yellow** color. 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.



⚠ 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 2 drops of ninhydrin solution.

2- Boil the mixture over a water bath for 2 min.

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		

Discussion

Comment on each result , and give reasons .

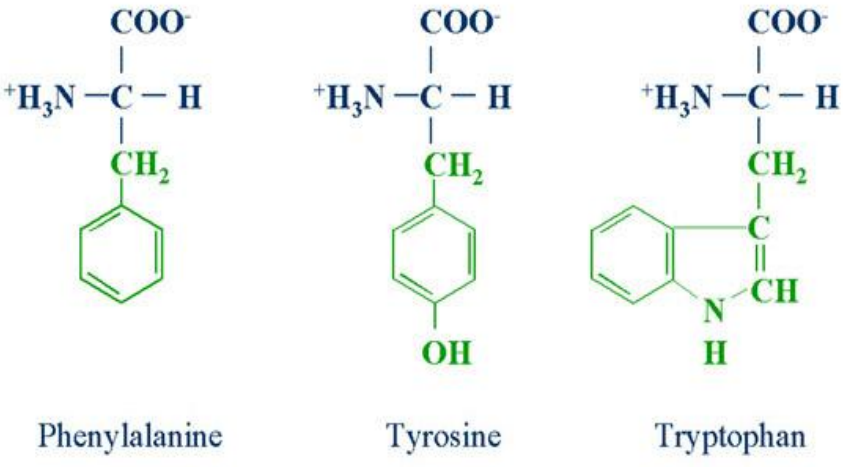
2.Xanthoproteic test

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.

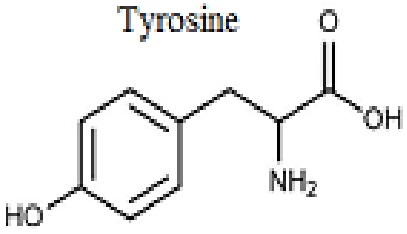
The salts of these derivatives are orange in color.



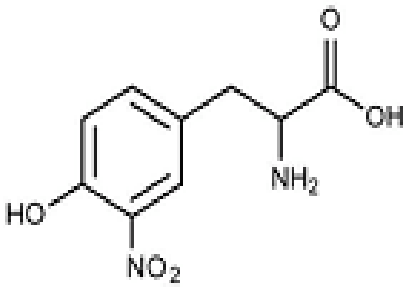
Principle:

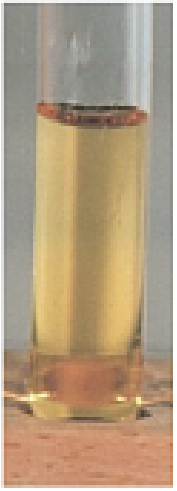
Conc. HNO3 reacts with the aromatic rings that are derivatives of benzene giving the characteristic ***nitration reaction.*** Amino acids tyr. and typ. contain ***activated benzene*** rings which are easily nitrated to **yellow colored** compounds. The aromatic ring of **phenyl alanine dose not react** with nitric acid despite it contains a benzene ring, but it is **not activated**, therefore it will not react.

Tyrosine

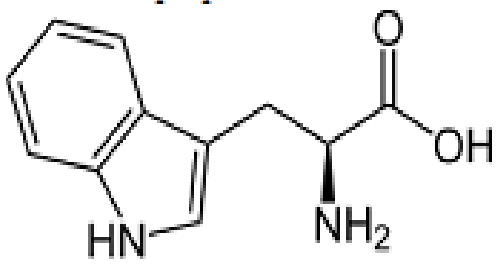


+ c. HNO_3 ⟶

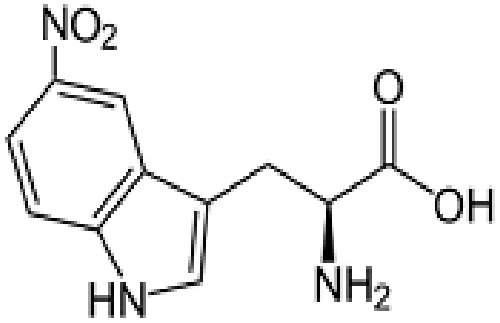




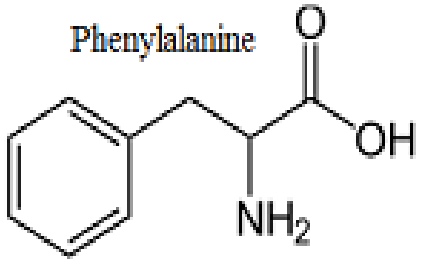
Trptophan



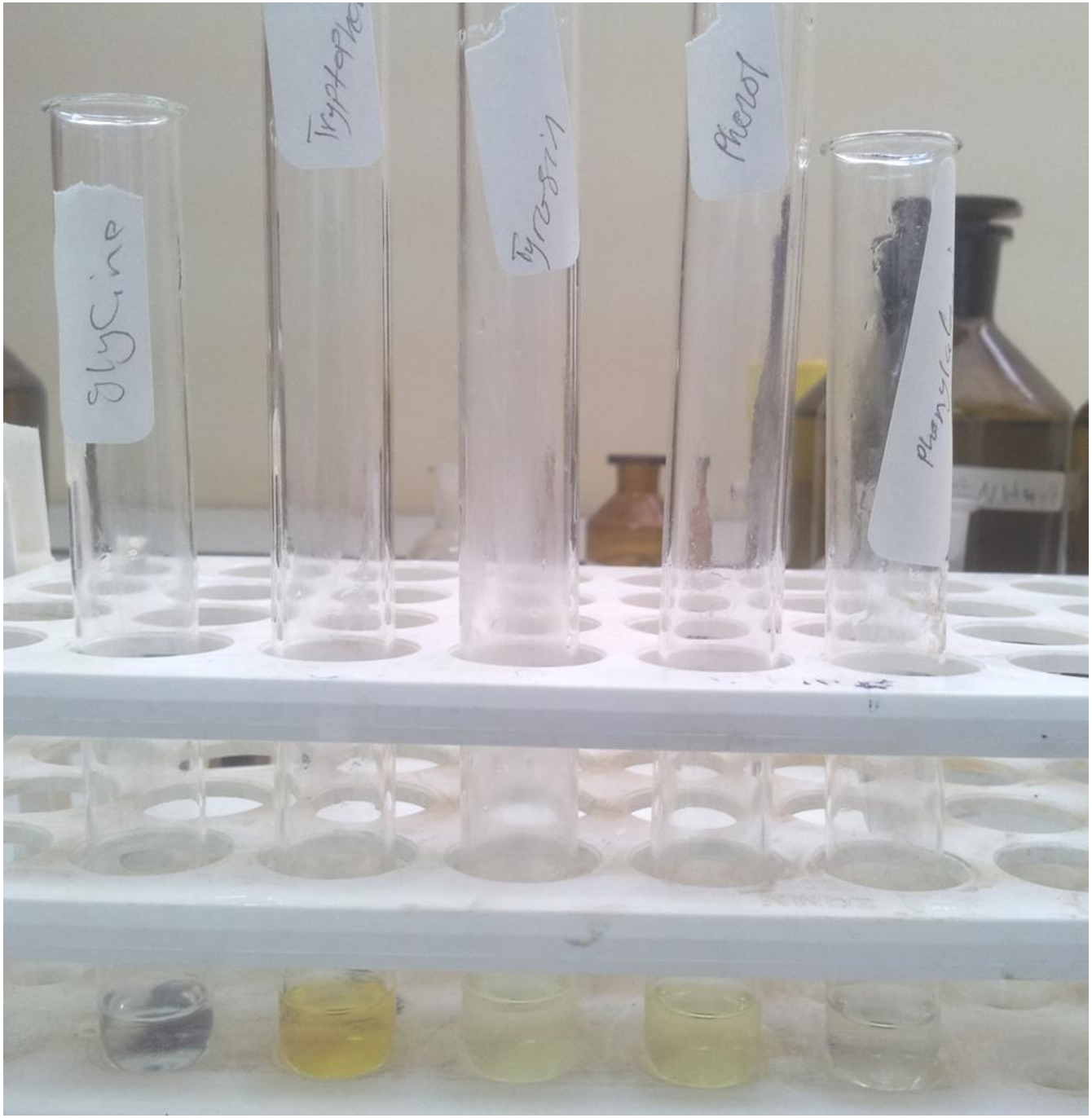
+ c. HNO_3 ⟶



Phenylalanine



+ c. HNO_3 ⟶


Glycine
Tryptophan
Tyrosin
Phenol

⚠ 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:

1-Label five tubes (1 - 5), then add 0.5 ml of each amino acid solution and phenol solutions to those test tubes each alone.

3-Add a few drops of concentrated HNO_3.

5-Compare the color with that given by blank using water instead.

6-Now COOL THOROUGHLY under the tap and CAUTIOSLY add 5 drops of 10M NaOH to make the solution strongly alkaline.

Result:

Tube	Observation	
	+ HNO3	+ NaOH
Tyrosine		
Tryptophan		
plenylalnin		
phenol		

Discussion

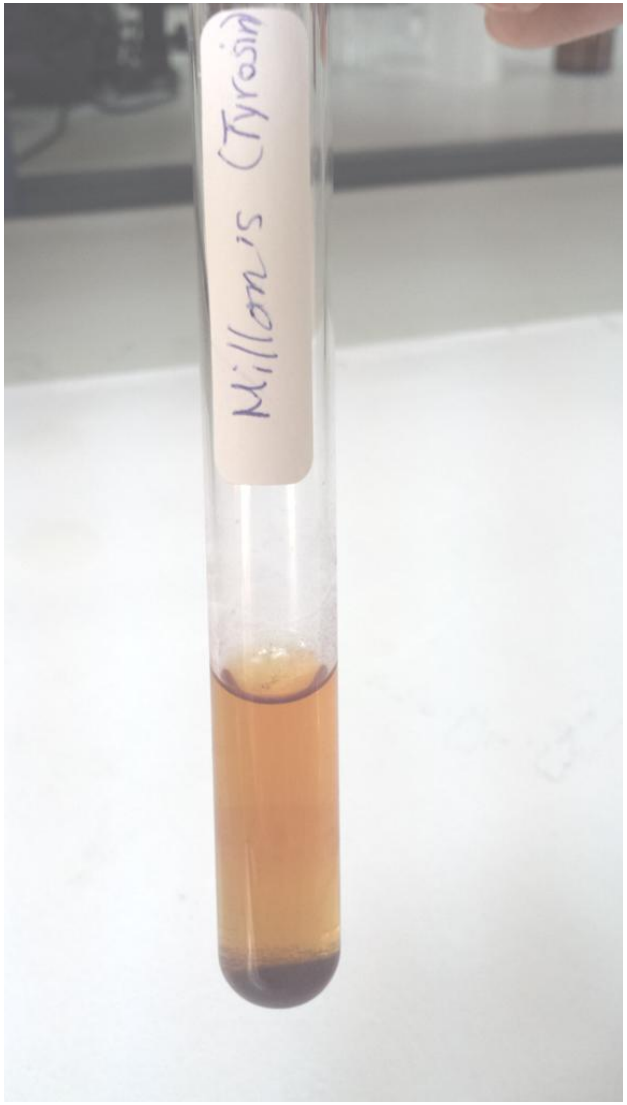
Comment on each result , and give reasons .

3.Millon's test

Objective:

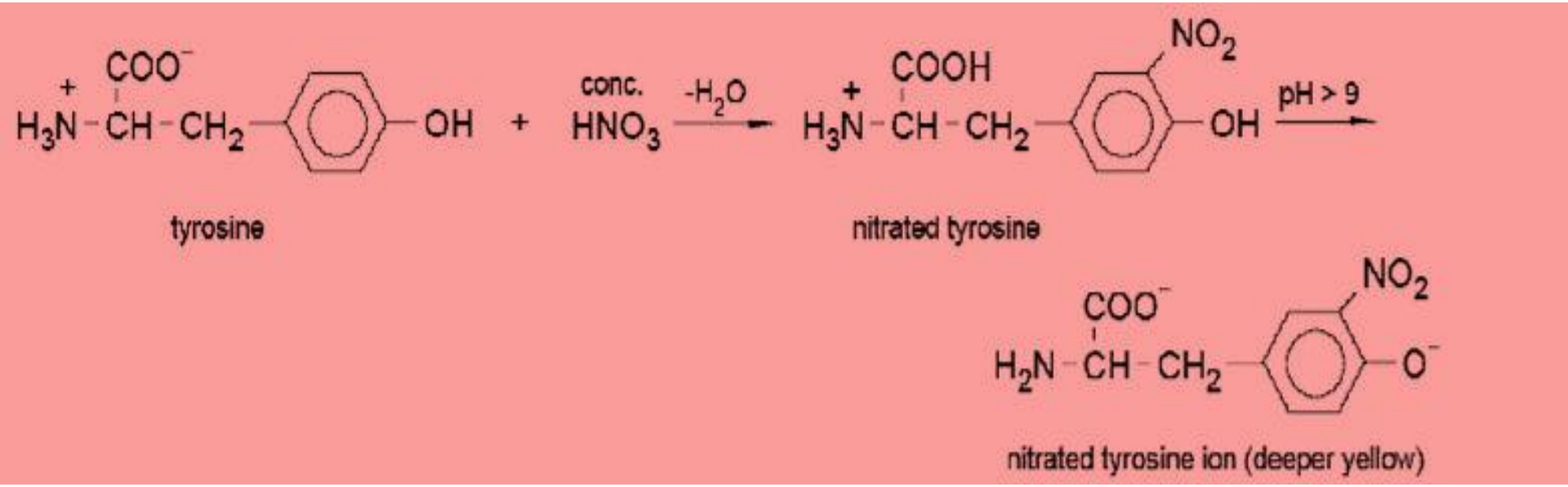
This test is specific for **tyrosine**, the only amino acid **containing a phenol group**, a hydroxyl group attached to benzene ring.

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 (1 - 5).

2-Add 1 ml of test solutions in separate tubes and the phenol solution in one tube.

3-Add to each tube 2 ml Millon’s reagent and shake it well.

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

5-Write your observation in the following table.

⚠ CAUTION

MILLON'S REAGENT IS HIGHLY TOXIC AND HIGHLY CORROSIVE

مادة سامة

Toxic

مادة كاوية وحارقة

Corrosive

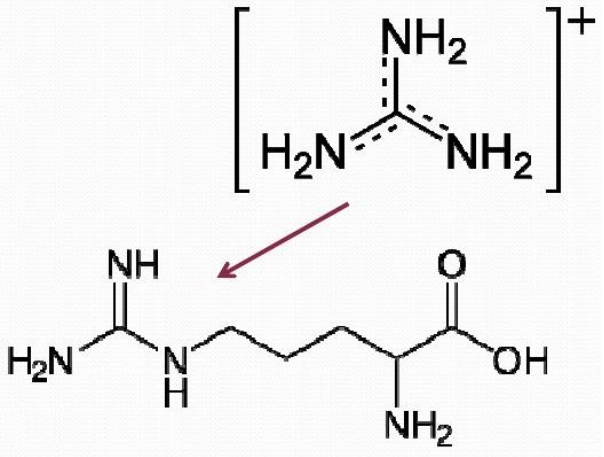
Result:

Tube	Observation	Conclusion
Tyrosine		
phenol		

Discussion

Comment on each result , and give reasons .

5. 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,
- tryptophan
- arginine
- 10%NaOH
- α-naphthol in 10% ethanol
- 5%sodium hypobromate (or sodium hypochlorite)

Method:

- Label 3 test tube and put in each one 1 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		
Tryptophan		

Discussion

Comment on each result , and give reasons .

5- **Detection of amino acids containing sulfhydral group (- SH)- Lead Sulfite Test**

Objective:

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

Principle:

- Some of **sulfur** in cystine, is converted to **sodium sulfide** by boiling with **40% NaOH**.

- The **Na2S** can be detected by the precipitation of **PbS** from an alkaline solution. -

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**.

sulfur in cystine **+ 40% NaOH → heating→ Na2S**

Na2S + Pb (CH3COO)2 →(black precipitate) PbS

Thank You