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General instruction for working laboratory

First :Student Instruction

1- The student have to put lab. Robe to product his cloths from stains and corrosion resulting from concentrated and chemical compounds.
2- Never smoke ; eat and drink in side LABS
3- When enter the LAB check the security of gas pipes and electricity connections and run the ventilation system.
4- Decide already the acquired experiments according to definite known steps.
5- When diluting acid …. Add acid to water with small amount and little by little make sure to watch liquid temperature if there any increment in heat (reaction is Exothermic)and never add water to acid .
6- Use the pipette (sucking rubber in the end) to add chemical liquids if this pipette is not available use the regular one but make sure to immerse the far end of tube inside the liquid during suction to keep the pressure steady inside the pipette and the liquid not flow inside your mouth.
7- When you want to get ride from unused chemical liquid like acid or bases …open the water tap in drainage pan before then pour the liquids and keep water going for a while.
8- Don't throw compounds residues that may react with drains pipes directly in drainage but leave inside special trash containers to get ride form it in proper ways.
9- Make sure to read what been written over the chemicals cans or flasks (names – composition – using tips – precautions).
10- Return the flask to their places after use – make sure to close the covers well.
11- Don't waist chemicals (liquids or powders) during measuring or weighting.
12- Write down your notes about experiment and results at each step.
13- After enf the work return the materials you have used during the experiment to their place clean your place well and make sure to close gas pipe.

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Second : instructions inside Labs.

1. Economizing Different chemical compounds by use only small amounts in experiments ... small amounts enough to do experiments.
2. Experiments in large cases fails due to contaminated equipment's and test tubes so make sure to use clean equipment's before you start your work.
3. Make all sufficient precautions when using poisoning compounds - be aware if exposing blasting materials — combustibles like ethers or benzene to direct flame this can result fire.
4. Don't waste reagents just use one or 2 drops which is sufficient for get the desired reaction.
5. In reactions that yield vapors or gases (harmful or poising) or lungs\eyes irritation please use gas hood.
6. Don't put on Lab. Stand (your space)un needed items — put your lab equipment's only.
7. When heating liquids inside test tube make sure that tube opening pointing away your colleagues body or face boiling drops may harm his face.
8. Never heat the standard scaled glasses that will change their size.
9. Many of chemicals are poising therefore make sure to wash your hands with soap and water before you leave the lab.

Third : reagents - directions of use:

Lab experiments performing depends on using reagents and other different chemicals ; in lab there is always specific amount from reagents , some of It have been put on lab tables another kept inside closet,

It is necessary when using these reagents to know their properties combustibility toxic - the ability to make explosives when mix with other reagents.

Dry reagents and liquids kept In glass vessels closed with glass plug or cork with paper carried reagent name and concentration stick to the vessels,

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When using reagent the following instructions have to be follow:

1• If there is no clear instructions about the needed amount of reagent in experiment the minimum amount should be take that can give better result ; save time and reduce compounds wasting.

2- It Is absolutely forbidden return the excess amount of reagent that been taken already from reagent bottle to the same bottle that will cause reagent contamination & spoiledness .

3• Using reagent have to return to their places after finishing.

4• Don't mix reagent bottles plugs or pipettes.

5• Special care and attention should be invoke during dealing with toxic or comestible compounds like barium salts , mercury , arsenic and Di Thio carbon.„etc.

Forth : Preparing Lab Reports

Filing lab reports considered as important part of practical studies when writing a report the following have to be consider :

I-The student must equipped with note book to highlight his notes about the practical lessons in first place.

2. The student has to submit a report about the experiment highlighting the following:

a. Experiment name.
    a. Experiment purpose,
    b. The scientific Idea for experiment with chemical reaction equations.
    c. Observations & conclusions,

d. Difficulties occurred during the experiment.
e. Reasons of experiment fail (if any fail happen).

Biochemistry science consider as modern science in compression with other sciences, and despite of it modernity but it developed in astounding manner on latest decades.

Studying biochemistry became essential to understand the ambiguity of many other sciences like medical ; agriculture ; microbiological sciences and food industries , many problems of these sciences cannot be solve without through study in biochemistry .

Biochemistry as a science for students in medical colleges mainly related to chemical reactions occurred in human body.

In a ward we can say these reactions take place for carbohydrates • lipids and proteins which considering the main components in nutrition and when these components enter the alimentary canal it passes the following stages:-

1. Digestion food digested to simple units or to the simplest forms that would absorb , this process enhance digestion enzymes in mouth ; stomach ; pancreas and intestine ,

2. Absorption the simple units absorb in small intestine.

3.Bacterial action ...take place in large intestine , the following :

a- Fermentation — when bacteria work on carbohydrate.

b- Putrefaction — when bacteria work on protien

4.Remaining food excreted as feces.

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The main aim of Digestion & Absorption is transport the simple units from food to tissues cells.

Some of food meals doesn't need to digestion and absorb as it is like water ; glucose ; vitamins & minerals.

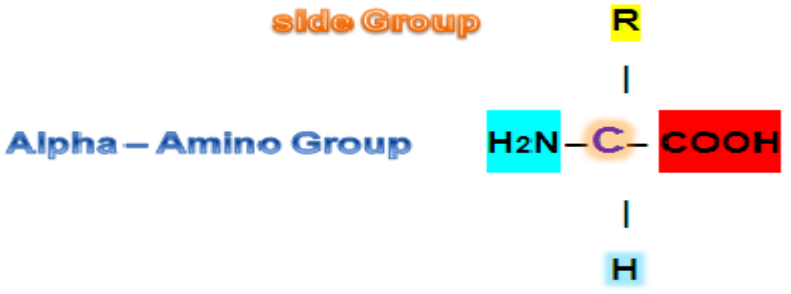
The simple digested & absorbed unites in cells tissues shall be used in one of the following manners :-

1- Breakdown or Catabolic Reaction this process called (Catabolism).
2- Anabolic Reaction or Synthetic Reaction — creating new units , this process called Anabolism or Synthesis.

The Anabolism & Catabolism together called the "Metabolism" therefore this idiom include chain of reactions occurred inside cells tissues whereas the absorption help to metabolize or use simple digested substances because in absorption process the simple units transport from intestine to tissues through blood.

AMINO ACIDS

Amino acid :- are containing amino group and carboxyl group on the same molecule.



General structure of amino acids in proteins

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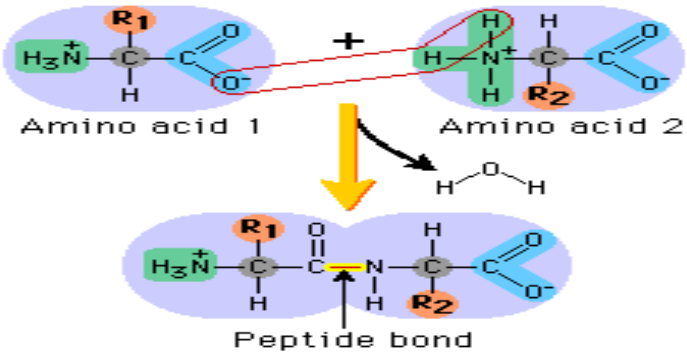
All amino acids found in living systems , plant and animal proteins are L-amino acids. D-amino acid are seen in small amount in microorganism and as constituents of certain antibiotics such as gramicidin-S , polymyxin, actinomycin-D and valinomycin, as well as bacteria cell wall peptidoglycans.

Proteins :- are defined polymers for amino acids connect by covalent bonds contain acidic carboxyl group (-COOH) and alkyl amine group (-NH2).



All amino acids (20 amino acids) have the same structural formula except **Proline** and their derivatives because it consider as secondary amino acid as we will see in amino acids classification latter.

The covalent bond in fact is amido bond resulted from amine group reaction for amino acid with carboxyl group for another amino acid(with losing water molecule) this amido bond called (Peptide bond) this bond is stable against PH changing in solvent or in salt concentration but this bond can break by acidic or base hydrolysis or by defiant enzymes(digestion case).

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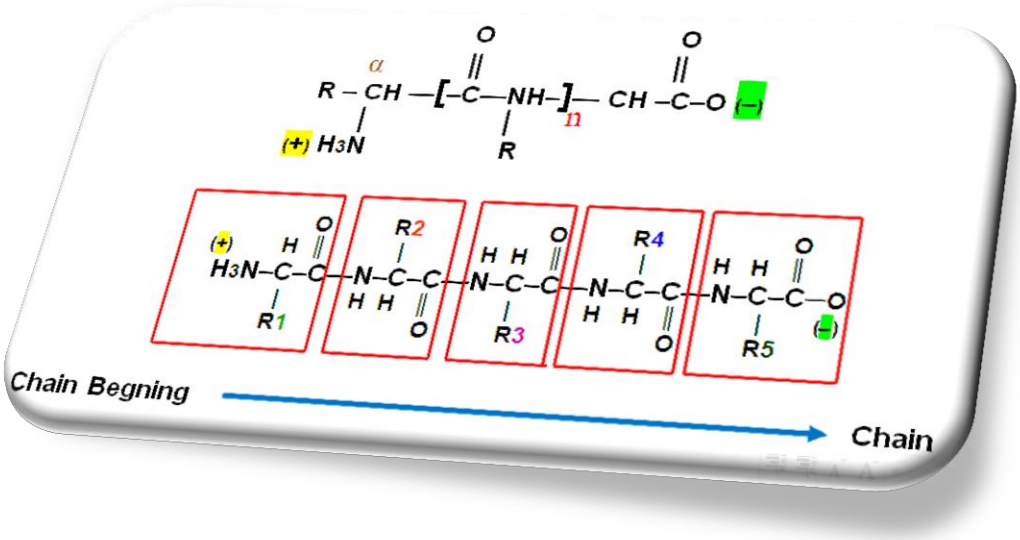
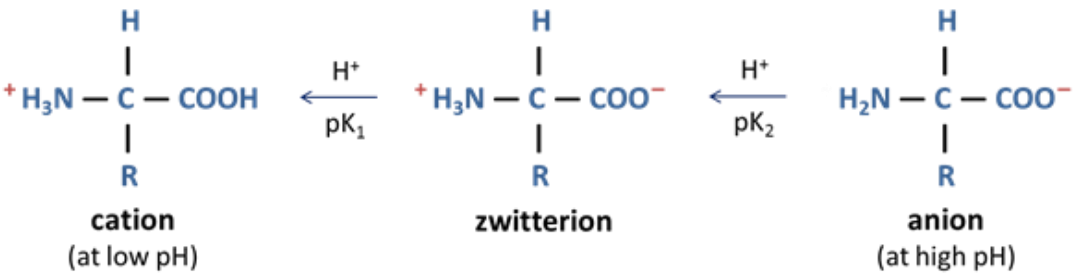


In normal solution an at PH =7 both α-amino and α- carboxyl groups are ionized to give (Zwitter ion) which cannot be migrate in any electrical field .



amino acids resemble inorganic salts; they are readily soluble in aqueous media, but only slightly soluble or insoluble in organic solvents their melting points are very high (over 200c) compared to low molecular weight organic compounds due to the ionic strength of zwitterion dipole.

All amino acids are asymmetric i.e. four different groups are bonded to the α- carbon atom , they are optically active .

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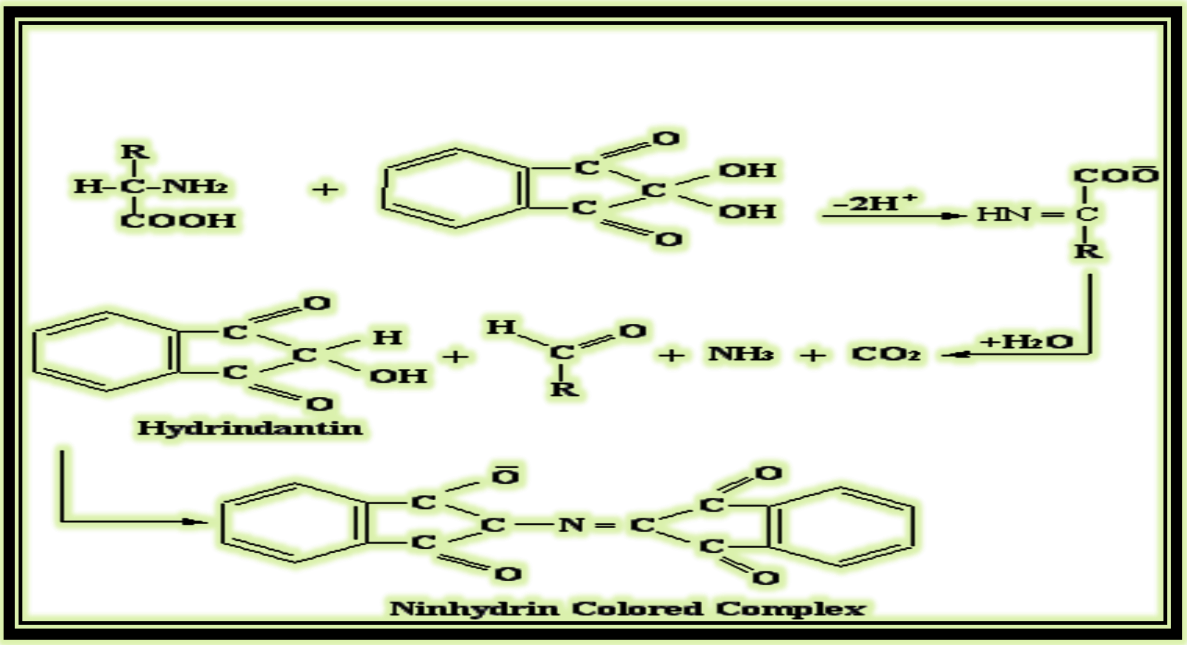
Amino Acids Examinations

1- Ninhydrin test

Test Principle :

This test is general test for all amino acids the reaction illustrate according to following mechanism :

When heating Ninhydrin sol^{n} with amino acid (reduction reagent) , amino acid oxidize when ninhydrin remove the amino group changing to Hydrin Dentine .as reaction continuing with ninhydrine ammonia yielded condensed with hydrin dantin giving violate condensed compound :



Compounds & Reagents :

Ninhydrin reagent prepared by dissolving 2 gm of ninhydrin in 1 litter of acetone

Work method :

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Place 1 ml of amino acid in test tube then add 5 drops of reagent , heat for 5 minutes note sol^{n} color.

Notes :

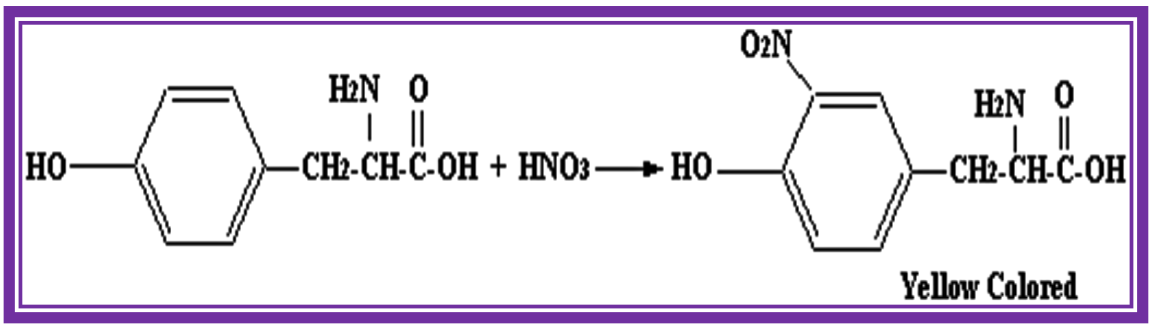
1- Ninhydrin consider as general test for all amino acids.
2- Amino acids (amino type) react with ninhydrin giving violate color.

Amino acid (Imino type) react with ninhydrin giving yellow color.

2-Xanthoproteic Test

Test principle

This test give positive result with aromatic amino acid which contain benzene ring in their composition and as it rarely that proteins not contain one of those aromatic amino acid therefore this test consider as general test for proteins. this experiment stand on Nitrating of benzene aromatic ring in amino aromatic acids,and due to the fact that nitro compounds are yellow therefore this test called Xanthoproteic (Xanthose = Yellow), the cause of yellow color is connecting nitro group NO_2^- with aromatic ring as follows :



Compounds & Reagents

1- Concentrated nitric acid.
2- Sodium hydroxide solution 40%.
3- Amino acids Sol^{ns} Tyrosine , tryptophan ,phenyl alanine 1%.

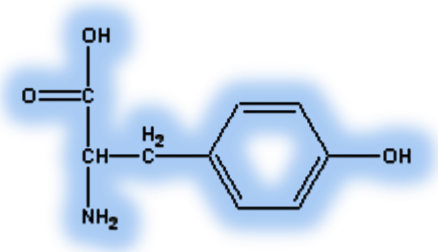
Work method :

Add 1 ml of amino acid to 0.5ml of HNO_3 CONC. Then heat in boiling water bath for 3 minutes note the yellow color, cool the tube then make the solution alkaline by adding sodium hydroxide note color changing from yellow to orang.

Notes :

1- This test indicate amino aromatic acids (tyrosine , tryptophan..etc) this test depend on benzene ring (part of amino acid) nitration.
2- Sometimes we can notice white precipitate appearance once we add the acid to protein solution , this happen because meta protein precipitation in some proteins like albumin, while in some other peptones & protonates this precipitate doesn't appear.
3- This test is not selective to aromatic amino acids because it give positive result with all aromatic compounds & regard the yellow color it stem from aromatic nitro compounds which is yellow or orange.
4- In case some protein traces presence in the sample , after adding concentrated nitric acid to protein soln. & heating the mixture the solution should divide in two parts , the first part left as it while second part treated with ammonia then compare both solutions.
5- Gelatin give negative test because the lack of aromatic amino acids in it.

3-Millon Test



Test principle :

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This test is special for tyrosine because it is the only amino acid which contain PHENOLIC GROUP therefore this test use to detect tyrosine which is found in most proteins, and this test is not special for proteins (tyrosine) but it is general test for phenols.

Compounds & Reagents :

1- Amino acids sample solutions 1%.
2- Millon reagent , prepared by dissolving 150 gm of mercury sulfate in 1 Ltr of diluted sulfuric acid 15%.
3- Sodium nitrate 10 %.

Work Method :

Add 0.5 ml from amino acid to 1 ml of reagent , heat the mix in boiling water bath for 10 minutes then cool the tube and add 4 droops of $NaNO_2$ notice the red color.

Notes :

1- Millon reagent contain mercurous nitrate & mercuric in nitric & nitrous acids.
2- Avoid add excess amounts of millon reagent because the red color will gone by boiling.
3- The red color or red precipitate is the most important thing in this test and no importance for the white precipitate which appear in the beginning of the test.
4- This test indicate the presence of tyrosine which characterized between other amino acids with it phenol group which give the positive test also this test is general test for all phenol compounds not tyrosine only therefore it give positive result with phenol & salicylic acids…etc.
5- This test can't pursued with alkali solutions so it must neutralized first because it give yellow or black precipitant (mercury oxides).
6- This test can apply on solid compounds beside aqueous.
7- Gelatin give negative test with this reagent due to tyrosine absence.

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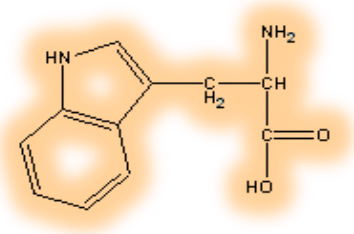
4-Rosenheim Test



Test principle :

This test is special for tryptophan which is amino acid contain Andol group, this A.A condensed with many of aldehydes in strong acidic medium in presence of oxidizing reagent to give violate – red compounds , Rosenheim reagent contain formaldehyde and little $FeCl_3$.

Compounds & reagents :

1- Tryptophan A.A 1%.
2- Conc. Formaldehyde solution.
3- Conc. Sulfuric acid.
4- Ferric chloride 0.01%.

Work method :

Add 3 ml of A.A to test then add 2-3 of formaldehyde, shake the tube well then add similar amounts of H_2SO_4 Conc. (with 2 FeCl3 to increase sensitivity) when add the acid make sure to get the amount slid on the internal surface of test tube,

note the violate ring on separation surface.

Notes :

1- This test indicate tryptophan presence within amino acids entering in protein composition.
2- This test is selective to tryptophan.
3- Gelatin give negative test due to the rarity of tryptophan on it.

4- Pure sulfuric acid must use in this test and experiment could fail if any interferences find with acid.
5- In case of using albumin or blood serum … white ring over the violate ring will appear due to protein precipitation by sulfuric acid forming Metaprotein.
6- This test may fail if any carbohydrate find with protein. Because brown ring will form due to carbohydrate carbonizing by acid.

5-Lead sulfide test

Principle

When alkali sulphur amino acids are heated with strong alkali, lead sulphide is precipitated from the alkaline solution . Organic sulpher containing amino acids converts to inorganic sulpher in the alkalian medium with lead acetate at which the sulpher containing systine and cysteine turns to black p.p.t from lead sulphide.

$$RSH + 2\ NaOH \longrightarrow ROH + Na_2S + H_2O$$

$$Na_2S + (CH_3COO)_2Pb \longrightarrow \underset{\text{Black ppt}}{PbS\downarrow} + 2CH_3COONa$$

Reagents :-

1- NaOH solution 40%
2- Lead acetate 0.1 M
3- Sulpher amino acid (0.1 , cysteine, cysteine and methoninie).

Procedure:-

Add to 3 ml of the amino acid solution above an equal amount of 40% NaOH solution , boil at least for 1 minute, cool then add 2 – 3 drops from lead acetate solution , mix well , then observe a black or drak brown p.p.t from lead sulphide.

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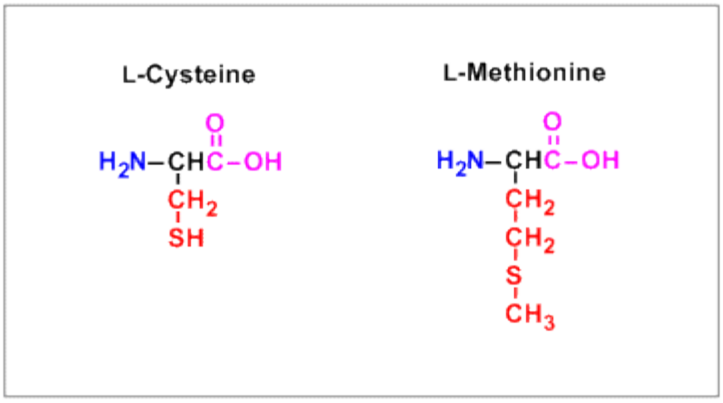
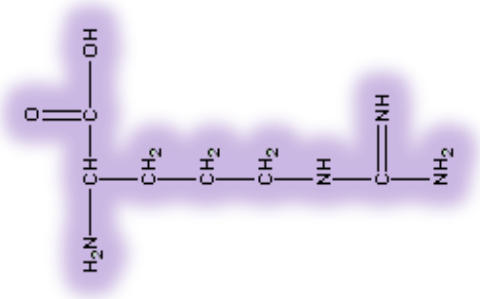
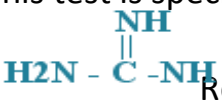


6-Sakaguchi Test



Test principle :

This test is special for Arginine A.A which is ALKALI ACID contain Quinidine group



React with NaOBr & then with α- Naphthol (C10H7OH) giving products with red brick color or crimson red color. NaOBr react as oxidizing agent for arginine

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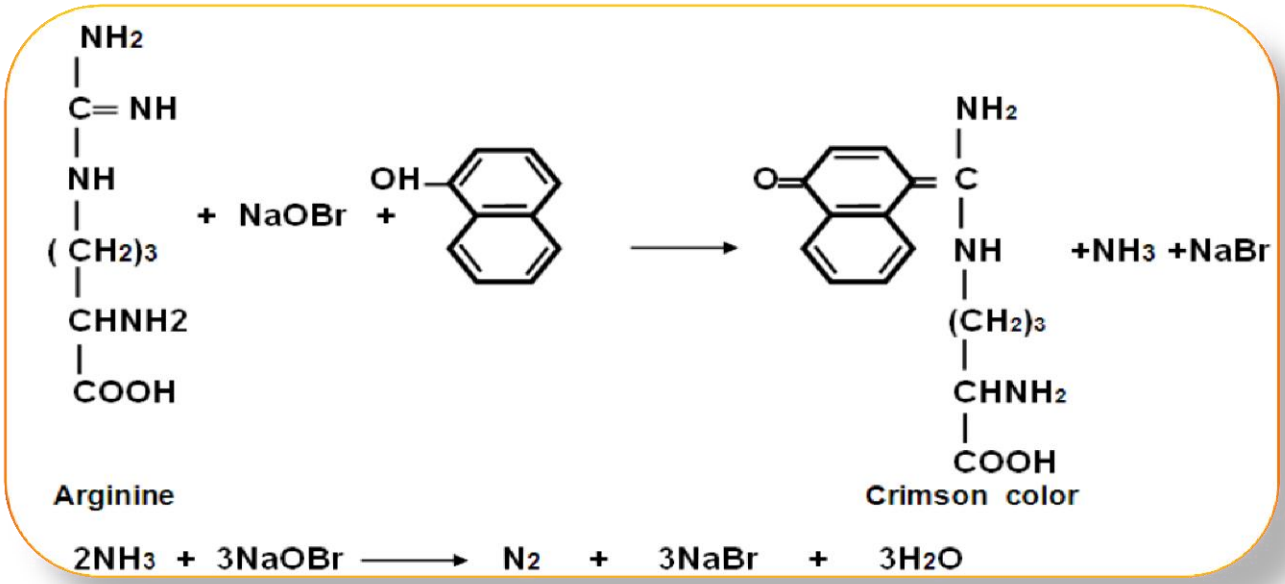
whom loss amino group (=NH) & combine with α- Naphthol :



Compounds & Reagents :

1- Arginine Sol^{n} 1%.
2- Sodium hydroxide 10%.
3- Alpha naphthol 1%.
4- NaOBr 5%.

Working Method :

Take 5ml of A.A then add 1ml of NaOH 10% for it then add 10 drops of α- Naphthol .. mix well , add few drops of NaOBr (prepared newly) to the mix … red crimson color indicate Arginine.

Notes :

1- This test indicate Arginine A.A in protein composition .
2- Ammonia & Ammonium salts conflict with this test.
3- This test depend on Quinidine group presence in A.A molecule Arginine.
4- This test is accurate & sensitive therefore it can consider as general test for proteins that all known proteins contain arginine in sufficient amount giving positive test .

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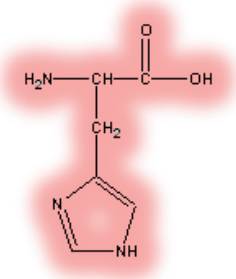
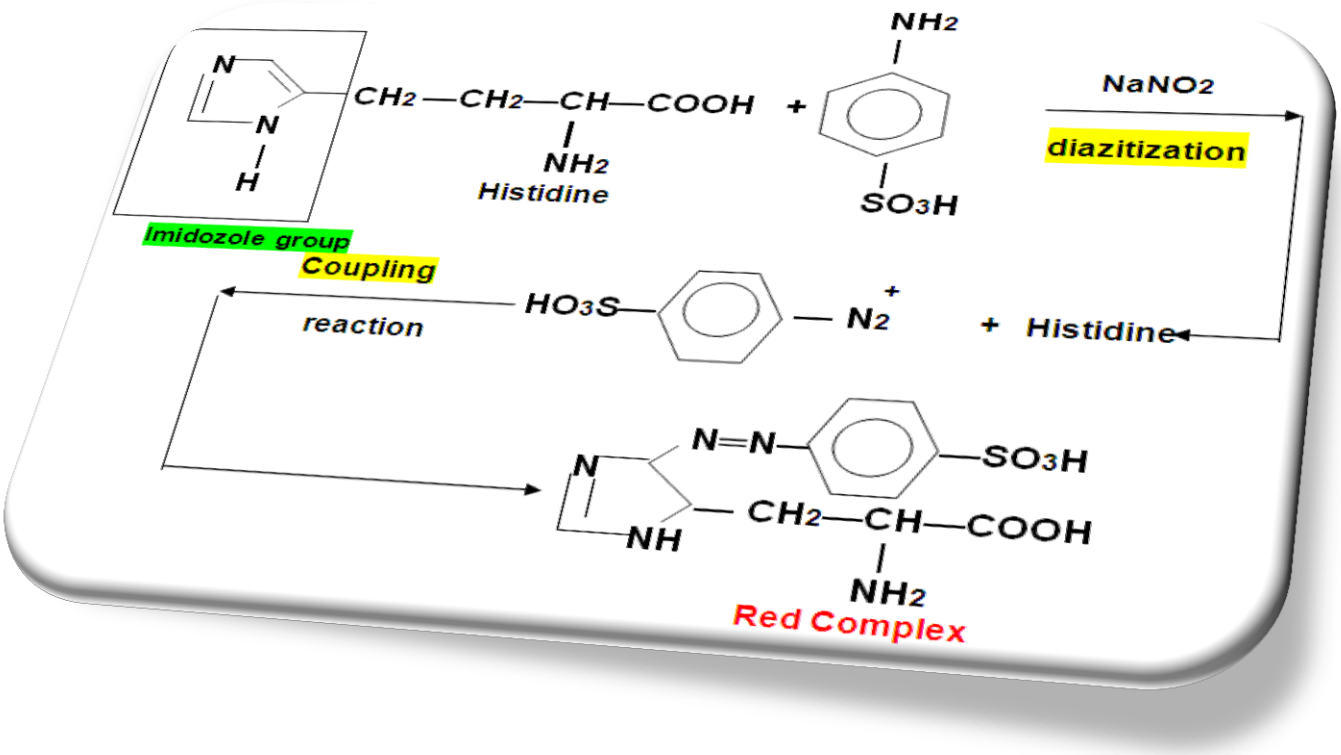
7- Pauly Test



Test principle :

This test is special for Histidine only , reaction explain as Sulfanilic acid coupling with IMIDAZOLE group thus this test give positive result with all compounds contain imidazole in their composition :



Compounds & Regent :

1- Histidine 1% Sol^{n}.

2- Sulphanilic Acid Sol^{n} 5%.

3- Sodium nitrate 5% Sol^{n}.

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4- Sodium Carbonate Sol^{n} 20%.

Work Method :

Place 1ml of histidine solution in test tube then add 1 ml of sulphanilic acid & 1 ml of $NaNO_2$ shake well then leave the mix for 5 minutes then add 3 ml of sodium carbonate , red color appearance indicate to histidine.