University of Baghdad

College of Science for Women

Department of Chemistry

BIOCHEMISTRY LAB

(For Biology students/First class)

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Chapter 2: Amino acids and protein qualitative tests

1- Elements of protein

The main elements of proteins are carbon (50%), hydrogen (7%), oxygen(23%), nitrogen (16%), and sulfur (0-3%).

Protein powder is heated in dry test tube then; nitrogen and sulfur areconverted to NH_3 an H_2S gases respectively.

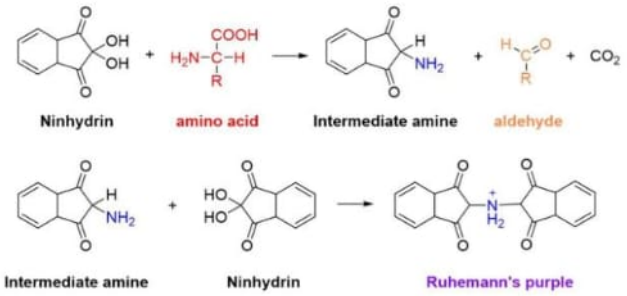
Oxygen and hydrogen are appearing as moisture on the wall of the test tube.

Some of carbon content is liberated as CO_2 gas the other is converted to blackresidue in the bottom of the tube.

Protein + O_2 $\xrightarrow{\Delta}$ **C +CO_2↑+ H_2O↑+** NH_3↑+ H_2S↑

2-Ninhydrin test: (to detection of proteins and amino acid)

Ninhydrin (triketohydrindene hydrate), a powerful oxidizing agent, react with all α-amino acids to give blue or purple-colored compound. The reaction is also given to detect free amino and carboxylic groups containing compounds (proteins, peptides). The imino acids, proline and hydroxyproline, also react with ninhydrin, but in this case a yellow color is formed. Ninhydrin degrades amino acids into aldehyde, ammonia, and CO_2 through a series of reactions; the net result is ninhydrin in a partially reduced form hydrindantin. Ninhydrin then condenses with ammonia and hydrindantin to produce an intensely blue or purple pigment, sometimes called (Ruhemann's purple):



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Method

1. Label 4 clean, test tubes with the names of the following solutions: 2% Gly. , 2% albumin, 2% gelatine and peptone.
2. Add 5 drops of ninhydrin solution to 1ml of the sample
3. Shake it and put in a boiling water bath for 3-5 min.
4. Remove the test tube from the boiling-water bath. Record your observations.

Note: A blue – purple color indicates that we have protein or amino-acid.

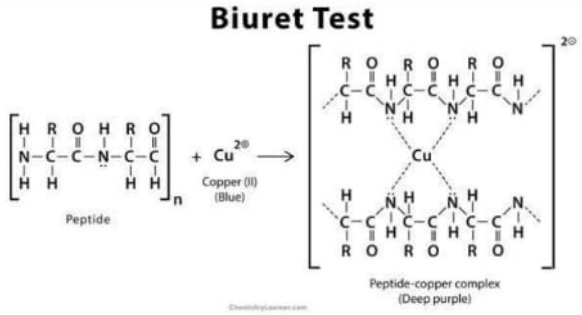
3- Biuret test (General test for proteins)

Copper (II) ion forms a violet-coloured complex in an alkaline solution when mixed with a compound containing tripeptides and larger polypeptides or proteins.

The purple colour is formed when copper (II) ions in the Biuret reagent react with the ione pair of electrons on the N in the peptide bonds to form a complex. Cu^{2+} forms a "tetra dentate" coordination complex through the four nitrogen donor atoms. The test produces the light blue to violet complex.

The intensity of the colour produced is proportional to the number of peptide bonds participating in the reaction. Thus, the biuret reaction is the basis of colorimetric method used to quantitatively to determine total protein concentration.

In spite of its name, the reagent does not in fact contain "biuret" molecule .The test is so named because it also gives positive result with "biuret molecule" at the same of reaction.



Biuret is formed by condensation of two molecules of urea, when heated at 180 °C, note biuret forming a complex with Cu^{2+}.

Note:

1. That Single amino acids (free) and dipeptides do not give the biuret reaction.
2. Other non-protein compounds such as Urea and "Biuret" give a positive test.

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Method:

1. Label 4 clean, test tubes with the names of the following solutions: 1% tyrosine, 2% albumin, 2%gelatine and peptone.
2. Place 15 drops of each in the corresponding test tube.
3. Add 5 drops of 3M sodium hydroxide and 2 drops of 0.1M copper (II) sulfate solution to each test tube.
4. Mix the contents. Record your observations.