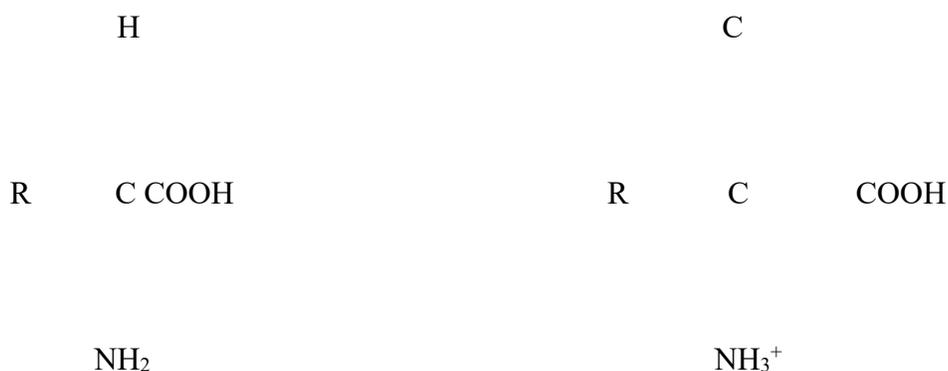


## Aim - Qualitative Determination of Proteins and Amino Acids

Proteins are among the most abundant organic molecules in living systems. Proteins are of great nutritional value and are directly involved in the chemical processes essential for [life](#). Proteins are chains of amino acids, linked by covalent bonds, called peptide (-CO-NH-). Proteins are compounds of carbon, hydrogen, oxygen, nitrogen and occasionally sulphur. All proteins consist of long chains of  $\alpha$ -amino (alpha amino) acids. The  $\alpha$ -amino acids are so called because the  $\alpha$ -carbon [atom](#) in the molecule carries an amino group (-NH<sub>2</sub>) and a carboxyl group (-COOH). The distinct R-groups distinguishes one amino acid from another, also are attached to the alpha-carbon (except in the case of glycine where the R-group is hydrogen). The fourth substitution on the tetrahedral  $\alpha$ -carbon of amino acids is hydrogen. There are two types of amino acids- essential and non-essential amino acids consisting of 10 numbers each respectively. The essential or dietary indispensable amino acids are those which cannot be synthesized by the animal organism out of materials ordinarily available at rates commensurate, with the demands for normal growth. Several tests for detection of amino acids in a test sample is given below:

The general structure of amino acids is:



### (a) Ninhydrin Test:

**Principle:** This test is a general test for all amino acids. This test is due to a reaction between amino group of free amino acid and ninhydrin. Ninhydrin (triketohydrinedene hydrate) is a powerful oxidizing agent and in its present amino acids undergo oxidative deamination liberating ammonia, carbon dioxide, corresponding aldehyde and reduced form of ninhydrin (hydrindantin). The reduced ninhydrin condenses with ammonia and non-reduced ninhydrin

molecule which leads to the formation of the violet-blue condensation product called **Ruhemann's purple** (diketohydrin). However, the imino acid like proline and hydroxyproline gives a different product of bright yellow colour. Asparagine which has a free amide group reacts to give a brown coloured product. This test is also given by protein and peptides.

### **Materials:**

- Boiling water bath
- Weight 0.2g. of ninhydrin and dissolve in 10 ml of acetone or ethanol (2% ninhydrin solution)
- Test solution of individual amino acids (50µg/ml)
- Dry test tubes
- Pipettes

### **Method:**

- Take 1 ml of the test solution in a dry test tube and another 1ml distilled water in another test tube as control. .
- Add about 4-5 drops of ninhydrin solution in both the test tubes.
- Keep the test tubes in boiling water bath for 5 minutes
- Look for the development of blue or violet colour.
- But in the case of proline and hydroxyproline, yellow color will develop instead of blue color. Similarly, asparagine will give brown color.

### **(b) Xanthoproteic Test:**

**Principle:** Xanthoproteic test is used to detect amino acids containing an aromatic nucleus (**tyrosine, tryptophan and phenylalanine**) in a protein solution which gives yellow colour nitro- derivatives on heating with concentrated nitric acid where the aromatic benzene ring undergoes nitration to give yellow colored product. Phenylalanine though this amino acid contains aromatic nucleus gives a negative or weakly positive reaction because it is difficult to nitrate under normal condition. Addition of an alkali to these nitro derivative salts, the colour change from yellow to orange. This test is used to differentiate aromatic amino acids which give positive result from other amino acids.

**Materials:**

- Boiling water bath
- Conc. HNO<sub>3</sub>
- NaOH: 40% solution (w/v)
- Test solution of aromatic amino acids (50µg/ml)

**Method:**

- Take 1 ml of the test solution in a test tube.
- Another 1 ml of distilled water as control.
- Add 1 ml of conc. HNO<sub>3</sub> in all test tubes and mix thoroughly.
- Keep the test tubes in boiling water bath for 2 minutes.
- Then cool in a tap water.
- Add 40% of NaOH solution slowly to all the test tubes.
- Look for color development in the mixture.
- The appearance of red colour gives the positive result.

**(c) Biuret Test:**

**Principle:** This is a general test for compounds having a [peptide](#) bond (CO-NH group). Compounds containing two or more peptide bonds when treated with alkaline copper sulphate give a violet colored product. As all proteins and peptides possessing at least two peptide linkage i.e. tri peptide gives positive biuret test. This is due to formation of co-ordination complex of cupric ions with unshared electron pairs of peptide nitrogen and O<sub>2</sub> of water. Biuret test is conveniently used to detect the presence of proteins in biological fluids.

**Materials:**

- Copper sulphate: 1% (w/v) solution of CuSO<sub>4</sub>, 5H<sub>2</sub>O in distilled water.
- NaOH:40% solution (w/v)
- Test solution: 0.5 % (w/v) solution of casein or bovine serum albumin (BSA) in NaOH (0.1N).
- Test tubes

**Method:**

- Take 1ml of the test solution in a test tube and take 1 ml distilled water as control.
- Add about 0.5 ml of 40% solution of NaOH to it and mix well.
- Add 4-5 drops of copper sulphate solution
- Observe for the development of color.
- Pink or velvet colour denotes the presence of peptides or proteins in a sample