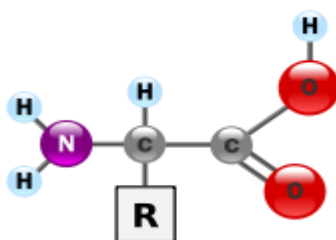


## **EXPERIMENT 2- QUALITATIVE ANALYSIS OF AMINO ACIDS AND PROTEINS**

**Amino acids** are molecules containing **an amine group, a carboxylic acid group** and a side chain that varies between different amino acids. Amino acids of the general formula  **$RCH(NH_2)COOH$**  are amphoteric, behaving as amines in some reactions and as carboxylic acids in others. At a certain pH known as the **isoelectric point** an amino acid has no overall charge, since the number of protonated ammonium groups (positive charges) and deprotonated carboxylate groups (negative charges) are equal. Since the amino acids at their isoelectric points have both negative and positive charges, they are known as **zwitterions**.



**Amino acids** are critical to life. They have particularly important functions like being the **building blocks of proteins** and being the **intermediates in metabolism**.

Amino acids are generally classified by the properties of their side chain into four groups. The side chain can make an amino acid a weak acid or a weak base, and a hydrophile if the side chain is polar or a hydrophobe if it is nonpolar.

**Proteins** (also known as **polypeptides**) are organic compounds made of amino acids arranged in a linear chain. The amino acids in a polymer are joined together by the **peptide bonds** between **the carboxyl and the amino groups** of adjacent amino acid residues.

Like other biological macromolecules such as polysaccharides and nucleic acids, proteins are essential parts of organisms and participate in virtually every process within cells.

**Proteins are important in:**

- catalyzing biochemical reactions (enzymes)
- structural and mechanical functions (actin and myosin)
- cell signaling
- immune responses
- cell adhesion
- cell cycle

## **TESTS ON AMINO ACIDS:**

### **1) Ninhydrin Test:**

Ninhydrin (triketohydrindene hydrate) is a chemical used to detect ammonia or primary and secondary amines. Amino acids also react with ninhydrin at pH=4. The reduction product obtained from ninhydrin then reacts with  $\text{NH}_3$  and excess ninhydrin to yield a blue colored substance. This reaction provides an extremely sensitive test for amino acids. Apply this test to **any of the amino acids you choose.**

**WARNING:** Avoid spilling ninhydrin solutions on your skin, as the resulting stains are difficult to remove. (Ninhydrin is the most commonly used method to detect fingerprints, as the terminal amines or lysine residues in peptides and proteins sloughed off in fingerprints react with ninhydrin).

### **Procedure:**

- To 1 mL amino acid solution add 5 drops of 0.2% ninhydrine solution in acetone.
- Boil over a water bath for 2 min.
- Allow to cool and observe the blue color formed.

### **Questions:**

- ✓ Write the reaction(s) involved in Ninhydrin Test.

## **2) Specific Reactions for Individual Amino Acids:**

**WARNING:** Please DO NOT use vast amounts of solution for these tests, since most of the amino acids are very expensive!!

### **a) Ehrlich Test:**

Aromatic amines and many organic compounds (indole and urea) give a colored complex with this test. Apply this test to **tryptophan, urea and glycine.**

#### **Procedure:**

- Put 0.5 mL of the amino acid solution to a test tube.
- Add 2 mL Ehrlich reagent and observe the color changes.
- Repeat the test with urea solution.

#### **Questions:**

- ✓ What chemicals are found in Ehrlich's reagent.
- ✓ Explain the reaction involved in Ehrlich Test.
- ✓ Explain your observation for the urea solution when it is tested with Ehrlich's reagent.

**b) Nitroprusside Test:**

The nitroprusside test is specific for cysteine, the only amino acid containing sulfhydryl group (-SH). This group reacts with nitroprusside in the presence of excess ammonia. Apply this test **cysteine, cystine and methionin.**

**Procedure:**

- Put 2 mL amino acid solution into the test tube.
- Add 0.5 mL nitroprusside solution and shake thoroughly.
- Add 0.5 mL ammonium hydroxide.
- Observe the color change.

**Questions:**

- ✓ Write the reaction(s) involved in Nitroprusside Test.
- ✓ Is there any difference in the test results of cystine and cysteine? If there is, explain the reasons by giving the related structures.