Estimation of amino acid by Ninhydrin method

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Introduction: The amino acids are colorless ionic compounds that form the basic building blocks of protein. Apart from being bound as proteins, amino acids also exist in the free form in many tissues and are known as free amino acids. They are mostly water soluble in nature. Very often in plants during disease conditions, the free amino acid composition exhibits a change hence, the measurement of the total free amino acids gives the physiological and health status of the plants.

Principle: Ninhydrin, a powerful oxidizing agent, decarboxylates the alpha-amino acids and yields an intensely colored bluish purple product which is colorimetrically measured at 570nm.

Reaction:

Reagents Required:

1. Ninhydrin: dissolve 0.8g stannous chloride (SnCl$_2$.2H$_2$O) in 500mL of 0.2M citrate buffer (pH 5.0). add this solution to 20g of ninhydrin in 500mL of methyl cellosolve (2 methoxyethanol).

2. 0.2M Citrate Buffer pH 5.0: Solution A: 0.2 M Citric acid. Solution B: 0.2 M Sodium citrate Mix 20.5 ml of solution A with 29.5 ml of solution B and check pH.

3. Diluent Solvent: Mix equal volumes of water and n-propanol, and use.

4. Standard: Dissolve 50mg leucine in 50mL of distilled water in a volumetric flask. Take 10mL of this stock standard and dilute to 100mL in another flask for working standard solution.
Apparatus and Glass wares required: Test tubes, Pipettes, Waterbath, Colorimeter, etc.,

Procedure:
1. Pipette out 0.2, 0.4, 0.6, 0.8 and 1 ml of standard amino acid solution to the respective labelled test tubes.
2. Add distilled water in all the test tubes to make up the volume to 1ml.
3. Add 1ml of distilled water to the test tube labelled Blank.
4. Now add 1ml of ninhydrin reagent to all the test tubes including the test tubes labelled 'blank' and 'unknown'.
5. Mix the contents of the tubes by vortexing/shaking the tubes.
6. Then cover all the test tubes with paper/marble.
7. Place all the test tubes in boiling water bath for 15 minutes.
8. Cool the test tubes in cold water and add 5ml of diluents solvent to each test tube and mix well.
9. Now record the absorbance at 570 nm of each solution using a colorimeter.
10. Then plot the standard curve by taking concentration along X-axis and absorbance at 570 nm along Y-axis.

Result: The given unknown sample contains ----μg amino acid/ml.

Observations and Calculations

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<thead>
<tr>
<th>Volume of standard amino acid (ml)</th>
<th>Volume of distilled water (ml)</th>
<th>Concentration of amino acid (μg)</th>
<th>Volume of Ninhydrin reagent (ml)</th>
<th>Incubate in boiling water bath for 15 min</th>
<th>Volume of solvent (ml)</th>
<th>Incubate at Room temp. for 10 min</th>
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Standard Curve for amino acid by ninhydrin method

![Standard Curve](image-url)