**Experiment: 1**

**Color Tests for Proteins and Amino Acids**

**A)General Proteins Color Tests**

**1/Biuret Test:**

\*It is a general test used for detecting the presence of proteins and peptides.

\*Protein sample treated with Copper Sulphate (CUSO4) in an alkaline solution (NaOH) formed a pink-violet colored complex.

\*This color is due to a reaction between Copper ions (CU++) and peptide bonds (CO-NH) in alkaline solution (at least two peptide bonds are required for a positive test).

\*Biuret (H2N-CO-NH-CO-NH2) react with CUSO4 in an alkaline solution and give the same color like protein, that is why the test is called Biuret test.

**Procedure:**

1-Take six separate test tubes. Add 1 ml of : water, 1% egg albumin, 1% alanine, 1% sucrose, 1% glucose , 1% casein respectively.

2-Add 1 ml of 10% NaOH to each tube.

3-Add 5 drops of 0.1% CUSO4 to each tube.

4-Mix, describe any color change that occurred.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| 1%casein | 1%glucose | 1%sucrose | 1%alanine | 1%egg albumin | H2O |
| 1 ml | 1 ml | 1 ml | 1 ml | 1 ml | 1 ml |
| 10% NaOH | | | | | |
| 1 ml | 1 ml | 1 ml | 1 ml | 1 ml | 1 ml |
| 0.1% CUSO4 | | | | | |
| 5 drops | 5 drops | 5 drops | 5 drops | 5 drops | 5 drops |

Mix, describe any color change that occured

**2/Ninhydrin Test:**

\*a.a. (that have α-amino group) react with ninhydrin to form blue colored complex.

\*This color is due to librate NH3 with ninhydrin.

\*Ninhydrin is used to locate the α-amino acid in paper chromatography as a blue to purple spots.

\*Also, permits the quantitative estimation of α-amino acid and peptides in column chromatography.

\*Proline give yellow color due to lack of α-amino group.

**Procedure:**

1-Take seven test tubes. Add 1 ml of: water, 1% egg albumin, 1%alanine, 1% proline, 1% sucrose, 1% casein respectively, and to the seventh tubes add 5 drops diluted ammonia.

2-Add 1 ml of 0.1% aqueous ninhydrin to each tube.

3-Mix, incubate in boiling water bath for 4 minutes, and observe the colors after standing a few minutes.

Describe the color changes that occur in each test tube.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Dilute ammonia | 1%casein | 1%sucrose | 1%proline | 1%alanine | 1%egg albumin | H2O |
| 2 drops | 1 ml | 1 ml | 1 ml | 1 ml | 1 ml | 1 ml |
| 0.1% aqueous ninhydrin | | | | | | |
| 1 ml | 1 ml | 1 ml | 1 ml | 1 ml | 1 ml | 1 ml |

Mix, incubate in boiling water bath for 4 minutes, cool.

Describe the color changes that occur in each test tube.

**3/Xanthoproteic Test:**

\*Nitration of the aromatic rings in Tyrosine and Tryptophan, with concentrated HNO3, produce a yellow color.

Tyrosine or Tryptophan + con.HNO3 heat Yellow color

\*Phenylalanine does not produce the color because the benzene ring is not activated for nitration.

**Procedure:**

1-Take seven test tubes. Add 1 ml of: water, 1% egg albumin, 1% alanine, 1% phenylalanine, 1% sucrose, 1% phenol respectively and 2 ml of 0.02% tryptophan to the seventh tube.

2-Add 1 ml of concentrated HNO3 to each tube.

3-Incubate all the tubes in boiling water bath for 2 mins. Cool. Describe the result in each test tubes, then, add carefully 10% NaOH and see the deepens of yellow color to orange.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| 0.02%tryptophan | 1%phenol | 1%sucrose | 1%phenylalanine | 1%alanine | 1%egg albumin | H2O |
| 2 ml | 1 ml | 1 ml | 1 ml | 1 ml | 1 ml | 1 ml |
| Con. HNO3 | | | | | | |
| 1 ml | 1 ml | 1 ml | 1 ml | 1 ml | 1 ml | 1 ml |

Incubate in boiling water bath for 2 mins. Cool. Describe the change in color in each test tube. Then, add 10% NaOH and see the deepens of yellow color to orange.