Precipitation of Proteins

Tests to be done:

- Heavy metals
  - Silver nitrates
  - Lead acetate
- Organic acids
  - Picric acid
  - Trichloro Acetic Acid (TCA)
- Neutral salts (e.g. Ammonium sulphate)
Precipitation of Proteins

1. By Heavy metals:
At pH 7 and above, proteins are usually negatively charged, the positively charged metal ions neutralize the charges of protein causing precipitation of the protein. Precipitation by heavy metals is therefore most effective at neutral to slightly alkaline pH value.

NOTE: The solution must not be too alkaline; otherwise there is a risk of precipitation of metal hydroxides.
I. By Heavy metals:

Heavy metals such as Ag$^+$, Pb$^{2+}$, Hg$^{2+}$, etc. form a complex with the alkaline proteins and precipitate.
Precipitation of Proteins

Procedure for precipitation by Heavy metals:

To 2 ml of protein (e.g., albumin or solution of egg white, gelatin, casein, add a few drops of:

1. Silver nitrate (Ag⁺)
2. Lead acetate (Pb²⁺)

Observe the extent of precipitate in each experiment.
## Proteins precipitation by Heavy metals

### Few drops of B

2 mL of A

**Mix**

C

<table>
<thead>
<tr>
<th><strong>Unknown</strong></th>
<th><strong>Reagent</strong></th>
<th><strong>Observation</strong></th>
<th><strong>Result</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Proteins (e.g. Albumin)</td>
<td>B</td>
<td>White Precipitation</td>
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II. Precipitation by organic acids: e.g. Picric acid & TCA

Organic acids carry a large negative charges which neutralize positively charged protein to form an insoluble salt. The acidic reagents are most effective at acid pH value where proteins are positively charged. The precipitation of proteins by this method is irreversible.

Procedure:

a. To 2ml of albumin solution or egg white solution, add from 5-8 drops of picric acid or Trichloro Acetic Acid (TCA solution).

b. Precipitate is formed in each tube.

c. Slowly add dilute NaOH and observe the results as the pH increases.
Proteins precipitation by Picric Acid or TCA

Few drops of B

2 mL of A

Mix

C

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<th>A</th>
<th>Proteins (e.g. Albumin)</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent</td>
<td>B</td>
<td>1- Picric Acid</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>or</td>
<td>2. TCA</td>
</tr>
<tr>
<td>Observation</td>
<td>C</td>
<td>1. Yellow Precipitation</td>
<td>Proteins</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. White precipitation</td>
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III. Precipitation by neutral salts: e.g. Ammonium sulphate

The charges on a protein solution can also be neutralized by the addition of neutral salt and this also has been used for purification of proteins. Theoretical, any salt can be used but generally ammonium sulphate \((\text{NH}_4)_2\text{SO}_4\) is preferred because it has high solubility and its dissolution in water is endothermic.

Procedure:

a. To 2 ml of albumin solution (egg white + NaCl solution).

b. Add equivalent volume of saturated ammonium sulphate solution or salt.

c. The solution becomes milky or turbid due to the precipitation of globulin, ovamucin and lysozomes.
Proteins precipitation by Neutral salts

2 mL of B

Mix

2 mL of A

C

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<th>Unknown</th>
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<th>Proteins (e.g. Albumin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent</td>
<td>B</td>
<td>Saturated ammonium sulphate or salt</td>
</tr>
<tr>
<td>Observation</td>
<td>C</td>
<td>White Precipitation or white turbidity</td>
</tr>
</tbody>
</table>
# Precipitation of proteins

Proteins can be precipitated by:

**Heavy metals**: Silver nitrate or Lead acetate  
**Organic acids**: Picric acid or Trichloracetic acid  
**Neutral salts**: Ammonium sulfate

<table>
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<tr>
<th>Test</th>
<th>Observation</th>
<th>Result</th>
</tr>
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</table>
| Heavy metals  
Sample solution (2 ml) + Silver nitrate (drops) | White ppt   | Protein precipitates  |
| Heavy metals  
Sample solution (2 ml) + Lead acetate (drops)  | White ppt   | Protein precipitates  |
| Organic acids  
Sample solution (2 ml) + Picric acid (drops) | Yellow ppt  | Protein precipitates  |
| Organic acids  
Sample solution (2 ml) + Trichloro acetic acid (drops) | White ppt   | Protein precipitates  |
| Neutral salts  
Sample solution (2 ml) + Fully saturated ammonium sulfate solution (2 ml) or Ammonium sulfate salt (2-3 pinches) | White ppt   | Protein precipitates  |