QUALITATIVE TEST OF PROTEIN
Experiment-2

To detect the presence of peptide bonds or proteins in the sample

Using Biuret Method

Protein precipitation and denaturation

Salt
Strong Acid
Heavy metals
Heating
BIURET TEST

Objective:
- To detect the presence of a protein or peptides.

- Positive result (purple color) will be given if the substance has **two or more peptide bonds** (three or more amino acids).

**Note:** Despite its name, the reagent does not in fact contain biuret ((H2N-CO-\(\_\_\)2NH). The test is so named because it also gives a positive reaction to the peptide-like bonds in the biuret molecule.
**PRINCIPLE:**

- In this reaction, proteins form a pink-purple colored complex with CuSO$_4$ in a strongly alkaline solution.

When *proteins and peptides* (i.e., peptide bonds) treated with an **alkaline solution of dilute copper sulfate** a **violet color** is formed. A positive test is indicated by the formation of a **violet color**.

![Chemical diagram of the reaction](image)

The reaction is represented by the following equation:

$$\text{R}-\text{NH}_2 + \text{Cu}^{+2} + \text{OH}^- \rightarrow \text{Biuret complex}$$
**METHOD:**

1. Add 3ml of protein Albumin
2. Add 1 ml of 10M NaoH
3. Add 0.5 ml of CuSO4 and mix well.

<table>
<thead>
<tr>
<th>protein</th>
<th>Observation</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2-PROTEIN PRECIPITATION

- The solubility of proteins is affected by pH, temperature, salts, heavy metal salts etc.
- The change of one of these factors will lead to protein precipitation and/or denaturation.
- Proteins will get denatured while using some factors that lead to precipitation.

- Is widely used in downstream processing of biological products in order to concentrate proteins and purify them from various contaminants.
DENATURATION OF PROTEINS

- **Denaturation** is a process in which the proteins lose its quaternary structure, tertiary structure and secondary structure, by application of some external factor or compound such as a strong acid or base, an organic solvent (e.g., alcohol or chloroform), or heat.

- Protein will become more viscous, decreased solubility and aggregation, and protein become inactive.
EXPERIMENT (2): EFFECT OF SALT CONCENTRATION ON THE PROTEIN SOLUBILITY:

Objective:

- To investigate the effect of different salt concentration on protein solubility.

- When low concentrations of salt is added to a protein solution the solubility increases (This is called salting in)

- At some point, solubility begins to decrease as salt increases-"salting out”

- Each protein can be precipitated at specific salt concentration.

- It is Reverse process, the protein can again become soluble when we add water

- It could used in the process of protein isolation
**PRINCIPLE: SALTING IN**

- Low concentrations of salt → the solubility increases. This could be explained by the following:
- Salt molecules stabilize protein molecules by:
- Decreasing the electrostatic energy between the protein molecules which increase the solubility of proteins.
**PRINCIPLE: SALTING OUT**

High concentration of salts → the solubility decreases, and protein precipitates.

This could be explained by the following:

because the excess ions (not bound to the protein) compete with proteins for the solvent. The decrease in solvation allows the proteins to aggregate and precipitate.
**METHOD:**

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Take 2 ml of your <strong>albumin</strong> sample</td>
<td>On the same tube</td>
</tr>
<tr>
<td><strong>Add of 0.1M NaCl solution</strong></td>
<td>Add a few amount of 100% solid (NH4)2SO4</td>
</tr>
<tr>
<td>Concentrate your vision on the tube while adding</td>
<td>Shake it well and write your observation</td>
</tr>
<tr>
<td>record your observation</td>
<td>Compare between the tube of A and B</td>
</tr>
</tbody>
</table>
## RESULTS:

<table>
<thead>
<tr>
<th>Tube</th>
<th>Observation</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin+NaCl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Albumin+ 100% saturated)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(NH4)2SO4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discuss each result and compare between them what and why you obtain it ...
EXPERIMENT(3): ACID PRECIPITATION OF PROTEINS

**Objective:**
To investigate the *effects of strong acids* on the protein solubility.

**Applications:**
- Separation and purification
- Detection of small amount of protein in urea sample
- Stop the enzyme reaction
PRINCIPLE:

- This test depend on affecting solubility of the protein as a function of changes in pH in highly acidic media, the protein will be positively changed, which is attracted to the acid anions that cause them to precipitate.
**METHOD**

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>In a test tube, put 3ml of conc. nitric acid carefully</td>
<td>Put 3 ml of the albumin solution</td>
</tr>
<tr>
<td>Using a dropper add to (albumin) on the inner wall of the tube to form a layer up the acid</td>
<td>add 5-7 drops of T.C.A solution carefully</td>
</tr>
<tr>
<td>Record your observation</td>
<td>Record your observation</td>
</tr>
</tbody>
</table>
RESULTS:

<table>
<thead>
<tr>
<th>Tube</th>
<th>Observation</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc. HNO(_3) + Albumin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin + TCA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discusses each result what and why you obtain it ...
EXPERIMENT(4): PRECIPITATION OF PROTEINS BY SALTS OF HEAVY METALS:

Objective:

to identify the effect of heavy metal salt on protein
Heavy metal salts usually contain Hg$^{+2}$, Pb$^{+2}$, Ag$^{+1}$, Tl$^{+1}$, Cd$^{+2}$ and other metals with high atomic weights.

Heavy metal salt will neutralize the protein.

By the negative charge of protein will bind with positive charge of metal ion. Then the protein will precipitate as insoluble metal protein salt.

**Application:**

To eliminate the poisoning by palladium Pb$^{++}$, ...... mercury salts Hg$^{++}$
## Method

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In a test tube, put 1 ml of Albumin sample</td>
<td>In a test tube, put 1 ml of Albumin sample</td>
</tr>
<tr>
<td></td>
<td>Using a dropper add to (albumin) few drops of AgNO3</td>
<td>Using a dropper add to (albumin) few drops of HgCl2</td>
</tr>
<tr>
<td></td>
<td>Record your observation</td>
<td>Record your observation</td>
</tr>
</tbody>
</table>
# RESULTS:

<table>
<thead>
<tr>
<th>Tube</th>
<th>Observation</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin + AgNO3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discusses each result what and why you obtain it …
EXPERIMENT(5): PROTEINS DENATURATION BY HEATING

Non-covalent bond can be broken by heating, leading to protein denaturation and the precipitation.
METHOD:

1- Take 3 ml of protein Albumin
2- Place it in a boiling water bath for 5-10 minutes
3- Remove aside to cool to room temperature.
4- Note the change

Result:

<table>
<thead>
<tr>
<th>protein</th>
<th>Observation</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin+</td>
<td>heating</td>
<td></td>
</tr>
</tbody>
</table>