Physicochemical properties of proteins: solubility and denaturation

1. Define the isoelectric point of a protein. The isoelectric point of casein is 4.7. What is the net charge (positive or negative) of this protein in a solution of pH 7.4? Which chemical groups are responsible for the ionisation?

2. Why do proteins act as buffers?

3. Proteins solubility depends on (give some examples):
   •
   •
   •
   •

4. Describe the influence of a protein’s dipole moment on its solubility (albumins and globulins).

5. The tertiary structure of a protein involves attractions and repulsions between the side chain groups of the amino acids in the polypeptide chain. What type of interactions would you expect between the R groups of the following amino acids?
   • Phenylalanine and phenylalanine - ..............................................................
   • Serine and threonine - ...........................................................................
   • Two cysteine residues - .................................................................
   • Two leucine residues - ......................................................................
   • Arginine and aspartic acid - .........................................................

6. Explain the term:
   • “salting in”
   • “salting out”
Laboratory activities
1. Fractionation of serum proteins with ammonium sulphate

Scheme of serum protein fractionation with ammonium sulphate
(separation of albumin and globulins)

Calculate the amount of ammonium sulphate necessary to obtain:

- 40% salt saturation using the following equation:

\[ m = \frac{53.3 \times 0.01V \times (s_2 - s_1)}{1 - 0.3 s_2} \]

- 60% salt saturation

\( m \) – amount of ammonium sulphate [g],
\( s_1 \) – starting saturation (in decimal fraction),
\( s_2 \) – final saturation,
53.3 – coefficient of ammonium sulphate solubility,
0.3 – coefficient of volume correction,
\( V \) – sample volume (initial volume is 5 ml).
2. Application of dialysis for desalting a protein preparation

- Explain the term "dialysis"

- Dialyse all three protein preparations (γ globulin, α and β globulin, and albumin fractions) against water to remove the excess salt.

- After half an hour, check if there are salt ions (NH\textsubscript{4}\textsuperscript{+} and SO\textsubscript{4}\textsuperscript{2–}) in the dialysing water. Put a drop of water on a watch glass and add a drop of the appropriate reagent.

- Using Nessler reagent you can detect NH\textsubscript{4}\textsuperscript{+} ions in the dialysis water, write down the reaction

- Using BaCl\textsubscript{2} you can detect SO\textsubscript{4}\textsuperscript{2–} ions in the dialysis water, write down the reaction

- Using Chinese tannin reagent (1% solution in 1 M HCl/phenol) you can check if protein is present in the dialysis water. Compare the result with the same reaction carried out with the protein solution.

3. Denaturation of proteins

- Explain the term:

  “protein denaturation”

  “protein coagulation”
• What sort of chemical and physical agents cause denaturation of protein?

<table>
<thead>
<tr>
<th>Physical agents</th>
<th>Chemical agents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

• Describe alterations in protein structure (bonds disrupted) caused by denaturation.

• Explain the difference between denaturation and salting out.

---

**Laboratory tests - denaturation of proteins**

<table>
<thead>
<tr>
<th>Chemical agent</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td></td>
</tr>
</tbody>
</table>