Protein solubility

- Proteins are generally insoluble in water or organic solvents, but they form a colloidal solution with water of a certain viscosity.
- Solubility depends on four factors.

1- pH of the Solution.

2- Ionic Strength.

3- Temperature.

4- Protein Concentration.

1- pH of the Solution: The ionization of amino acid side chains can change with pH, affecting the overall charge of the protein. At its isoelectric point (pI), a protein is least soluble.

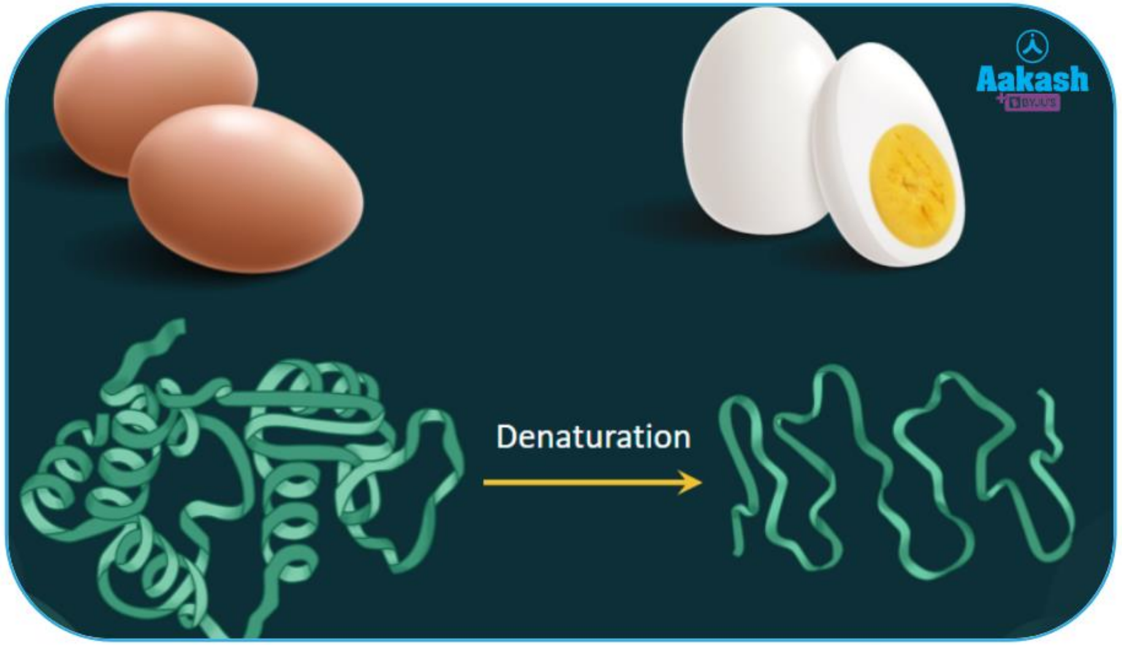
2- Ionic Strength: The presence of salts can stabilize or destabilize proteins.

- High ionic strength can shield charged groups, enhancing solubility.
- while low ionic strength can lead to precipitation.

3- Temperature: Temperature can affect protein solubility; increased temperatures may increase solubility up to a certain point, beyond which denaturation can occur.

4- Protein Concentration: At high concentrations, proteins may aggregate, leading to decreased solubility.

Denaturation is the destruction of the tertiary structure of a protein molecule and the formation of random polypeptide chains.



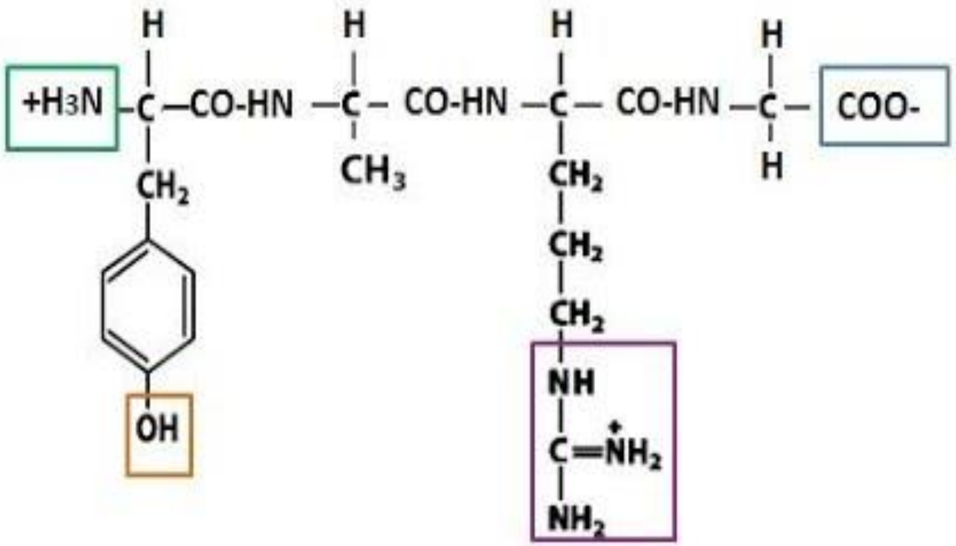
Transformation of Native Protein to Denatured protein

- Proteins differ in their solubility. Albumin is easily soluble in water, while keratin found in nails does not dissolve in water.
- Solubility is at its lowest at the electrical neutrality point, where solubility changes with changes in acidic and basic medium.

isoelectric point (IEP)

- It is the pH value at which the positive and negative electrical charges of the protein are balanced, so the total charges are equal to zero.
- Proteins do not combine at the isoelectric point with an acid or base. Above or below the isoelectric point, the protein is unneutral and combines with ions of the opposite charge to it and forms insoluble salts.

Net charge of the peptide Tyrosine-Alanine-Arginine-Glycine at pH 5



Net charge= (-) + (+) + (0) + (+) = +1

Dr. Karaki

Proteins can be precipitated:

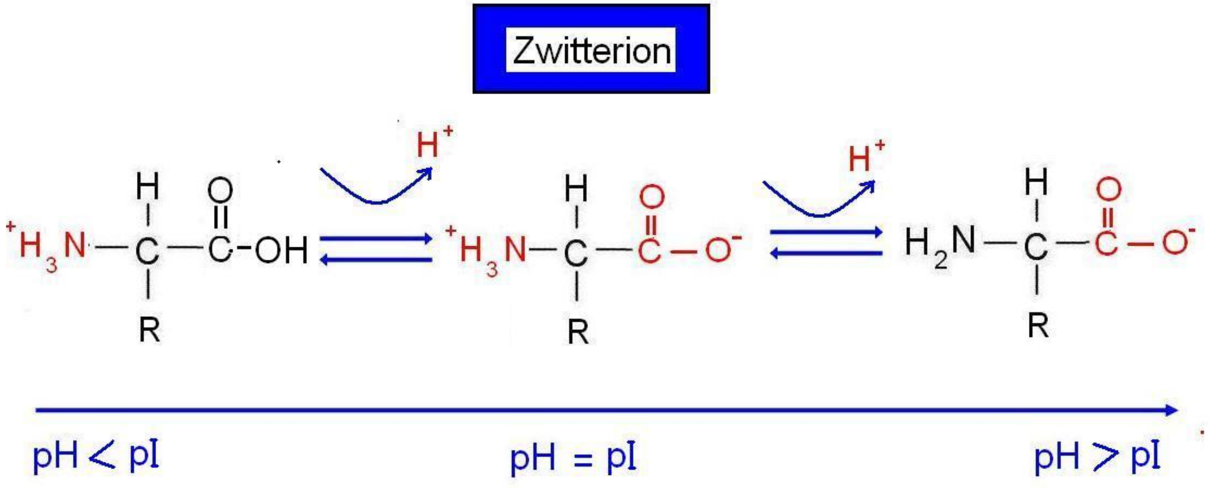
1- by acids such as hydrochloric acid, picric acid, or ferric trichloride.

2- by bases such as sodium hydroxide, calcium hydroxide, and barium hydroxide.

3- by dissolved salts of a metal(Heavy Metals) such as basic lead acetate.

4- by heat

- The theory of precipitation of proteins by bases and acids is based on the amphoteric property of proteins. Proteins form a colloidal solution or emulsion.



Precipitation by Heavy Metals

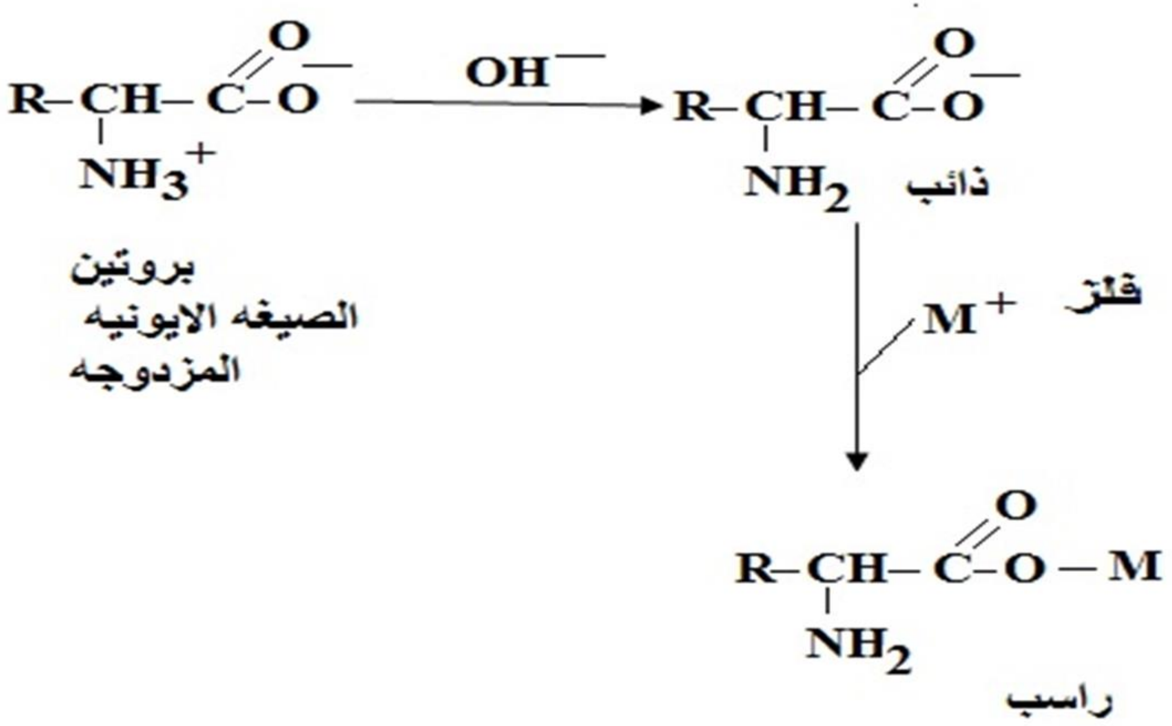
- The isoelectric point of a protein is that pH at which the net charge on the protein is zero. If the pH of the medium is made alkaline the proteins acquire net negative charge and if the pH of the medium is made acidic the proteins acquire net positive charge.
- In this test upon adding NaOH proteins gain negative charge and they form ionic bond with positively charged metal ions leading to precipitation of proteins.

Precipitation by 10% Pb(CH3COO)2 solution , 10% $CuSO_4$ solution and by 10% $FeCL_3$ solution.

1- Take three test tubes and put in each one 1 ml of albumin + 2 drops of sodium hydroxide solution.

2- Add 1 ml of $FeCL_3$ to the first tube, 1 ml of $Pb(CH3COO)_2$ to the second tube and 1 ml of $CuSO_4$ to the third tube.

3- Note the precipitates formed.



OH⁻
ذائب
M +
فلز
بروتين
الصيغه الايونيه
المزدوجه
راسب

Precipitation by Salts

- Inorganic salts when added to the protein solutions, water of hydration around the protein molecules is removed causing aggregation of protein molecules leading to their precipitation.

The effect of salts added to protein depends on their concentration.

- Depending on the concentration of salts, two cases occur.
- salting in.
- Adding low concentrations of salts will increase the solubility of the protein. Because salt ions increase the ionization of the protein and thus increase its solubility.

- <u>salting out.</u>
- Separation and precipitation of protein from solution as a precipitate by adding high concentrations of neutral salts such as NaCl. This salt will compete with the protein for water molecules, causing the protein to precipitate because it is poorly soluble in water.

Full saturation test

- To 5 ml of albumin solution add ammonium sulfate crystals and shake well till some crystals remain at the bottom of the tube. Keep it for 5 minutes and filter. Collect the filtrate.
- Do Biuret test with the filtrate .

Precipitation of proteins by acids

- The theory of protein precipitation by acids is based on the amphoteric property of proteins.
- When adding an acid that carries a quantity of negative charges, it will balance the positive charges of the protein and salts will be formed that will precipitate at the bottom.
- The positive charges of the protein increase as the pH value increases towards acidity.

Precipitation of proteins by heat

- Most types of proteins precipitate when heated to more than 50 ^{0}C, as the structures and molecules of the protein change under the influence of heat.

Procedure:

- Take a test tube and fill protein (albumin) solution up to two thirds.
- Heat the upper one third portion of protein solution column.
- Note whether any precipitate has appeared.
- Irrespective of the presence or absence of the development of the precipitate, add 2% acetic acid drop by drop.
- Note whether the precipitate formed earlier (if any) became intensified or appeared upon adding acetic acid

- White coagulum formed on initial heating intensifies on adding acetic acid
- Albumin is denatured by heating and is precipitated by acetic acid.
- Principle: Heating caused denaturation. Disruption of secondary, tertiary, quaternary structures maintained by noncovalent forces (hydrogen bonds, ionic interactions, van der waals forces, hydrophobic interactions) causes denaturation.