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Physico-chemical properties of proteins (theoretical part)

Isoelectric point

For each protein particular pH exists when both functional groups are dissociated. This pH is called isoelectric point (pH_i). Protein at this point (pH) is so called „zwitter ion", it is characterised by the smallest solubility and the loss of migration in electric field.

Apart from pH_i isoionic point is defined. It concerns purified proteins. Values of isoionic and isoelectric points are close to each other but not equal.

Henderson – Hasselbalch equation

In order to calculate pH of solution of weak electrolyte the equation of Henderson – Hasselbalch can be used. Weak acid (HA) can dissociate in water solution and liberate proton (H^+) and acidic anion (A^-) in accordance to equation:

$$HA \leftrightarrow H^+ + A^-$$

Constant balance of such reaction:

$$K = \frac{[H^+] * [A^-]}{[HA]}$$

After recalculation, we get:

$$[H^+] = K * \frac{[HA]}{[A^+]}$$

Multiplying by -1 and logarithmic above equation, we get:

$$-\log[H^+] = -\log[K] + \frac{\log([A^-])}{[HA]}$$

Negative logarthm $-\log[H^+]$ from the concentration of hydrogen ions is defined as pH, while negative logarithm from dissociation constant $-\log[K]$ is pK.

$$pH = pK + \frac{\log([A^-])}{[HA]}$$

Equation above is equation of Henderson-Hasselbalch.

Example:

Calculate pH in tube 1 from task 1 (see protocol).

Data: Searches:

$C^1_{CH_3COOH} = 0.01 \frac{mol}{dm^3}$ $pH = ?$

$V^1_{CH_3COOH} = 0.6\ cm^3 = 0.0006\ dm^3$

$C^1_{CH_3COONa} = 0.1 \frac{mol}{dm^3}$

$V^1_{CH_3COONa} = 1\ cm^3 = 0.001\ dm^3$

$V_{probówki} = (0{,}6 + 8.4 + 1) cm^3 = 10 cm^3 = 0.01 dm^3$

pH value in tube 1 is determined by acetate buffer. This buffer consists of the system of weak acid CH_3COOH and its salt CH_3COONa. The dissociation of acetic acid can be presented as:

$$CH_3COOH \leftrightarrow CH_3COO^- + H^+$$

In our case the concentration of $CH3COO^-$ ions equal to the concentration of CH3COONa in the solution. The equation of Henderson-Hasselbalch for acetate buffer can be presented as:

$$pH = pK + \frac{\log([CH_3COONa])}{[CH_3COOH]}$$

pK of acetic acid is 4,65.

The concentration of CH_3COOH in solution can be calculated in the following way:

We calculate the amount of moles CH_3COOH added to tube:

$$n_{CH_3COOH} = C^1_{CH_3COOH} * V^1_{CH_3COOH}$$

We calculate the concentration of solution of CH_3COOH in tube after adding all constituents of solution:

$$C^2_{CH_3COOH} = \frac{n_{CH_3COOH}}{V_{probówki}}$$

Hence the concentration of CH_3COOH in tube can be presented as:

$$C^2_{CH_3COOH} = \frac{C^1_{CH_3COOH} * V^1_{CH_3COOH}}{V_{probówki}}$$

After adding known values, we get:

$$C^2_{CH_3COOH} = \frac{\frac{0.01mol}{dm^3} * 0.0006dm^3}{0.1dm^3} = 6 * \frac{10^{-4}mol}{dm^3}$$

Analogously, we calculate the calculations for CH_3COONa, and we get:

$$C^2_{CH_3COONa} = \frac{C^1_{CH_3COONa} * V^1_{CH_3COONa}}{V_{probówki}}$$

Po podstawieniu:

$$C^2_{CH_3COONa} = \frac{\frac{0.1mol}{dm^3} * 0.006dm^3}{0.01dm^3} = 1 * \frac{10^{-2}mol}{dm^3}$$

After adding obtained values to Henderson-Hasselbalch equation, we get:

$$pH = 4.65 + \log\left[\frac{1 * \frac{10^{-2}mol}{dm^3}}{6 * \frac{10^{-4}mol}{dm^3}}\right]$$

$$pH = 4.65 + \log[1 * 10^{-2}] - \log[6 * 10^{-4}]$$

$$pH = 5{,}87$$

The answer: pH in tube 1 is 5,87.

Solubility

Majority of proteins generally dissolve well in water, some of them dissolve only in diluted solutions of salts, acids and bases. The solubility of proteins is determined by their affinity to water (ability to hydratation) as well as their chemical structure, greater or lesser ease in forming bonds, presence of low salt concentrations in the environment and pH of solution.

Hydratation refers to the binding of water dipoles to the polar groups of proteins, thanks to which protein molecule is surrounded by water shell.

Function group	**-OH**	**-COOH**	**-O-**	**$-NH_4$**	**=NH**	**=N-**
The amount of water molecules	3	4	2	3	2	1

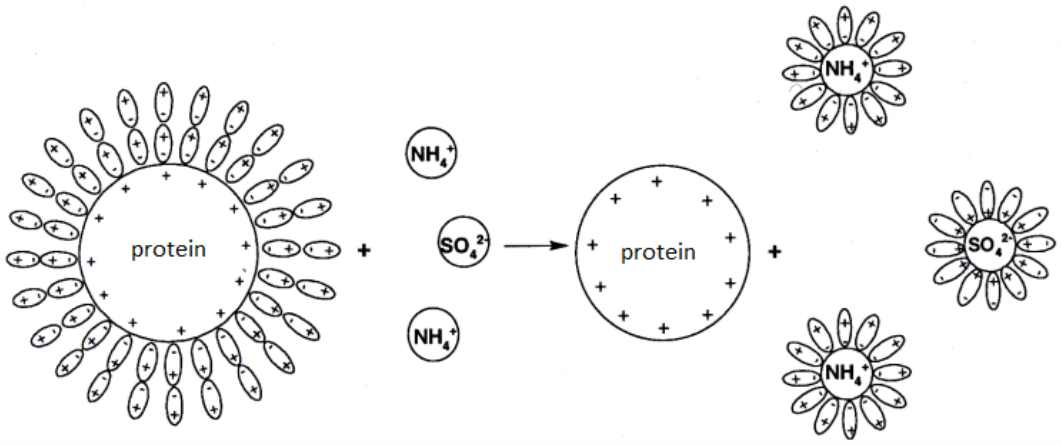
Salting out

Salting out refers to the removal of water shell of protein after adding to protein solution high concentrations of inorganic salts: ammonium sulphate, magnesium sulphate, sodium sulphate.

Molecules deprived of water shell merge into larger conglomerates and as the result fall out into the bottom of the tube. Proteins are the most easily salted out at isoelectric point.

The concentration of salt necessary for salting out depends on the properties of particular protein and pH of environment. Hence, this method can be used to separate proteins that differ in their solubility (separation of albumins from globulins). Salting out is reversible process. After the decrease of salt concentration, precipitated protein can be dissolved back.

Similar effect to salting out can be obtained after adding ethanol or acetone to protein solution. These chemicals, however, should act shortly and in low temperature.



Mechanism of the protein shedding process

Denaturation

It is irreversible process and refers to the damage to spatial structure of proteins (II, III, IV-ordered) leading to the loss of biological activity and physico-chemical properties. It can occur under the influence of physical factors such as temperature or ionizing radiation or chemicalfactors such as concentrates acids, bases, urea, guanidine, acidic amides, acetone, ether. Since denaturation involves spatial structures, this process does not affect peptides as they do not have spatial structures. This is one of the differences between proteins and peptides

Denaturation of proteins is not equal to precipitation from the solution so called coagulation. Denaturated protein may not precipitate if it has an electric charge, i.e. if it is in an

environment with pH other than isoelectric point.

Irreversible denaturation may in certain circumstances be reversed – renaturation – when denaturing chemicals act relatively shortly and did not result in far-reaching structural changes.

Protein precipitation with heavy metal salts

At pH value higher as pHi protein molecules react with cations of heavy metals (Cu^{2+}, Hg^{2+}, Pb^{2+}). Salts with a very low dissociation constant and sparingly soluble are formed. That is why ions of heavy metals are used for the precipitation of proteins from the solution. Cu^{2+} ions form stable complex bindings with proteins with characteristic colour (biuret reaction). Proteins with Hg^{2+} ions form sparingly soluble salts. This observation became the reason for the use of protein as an antidote to mercury poisoning and other heavy metals.