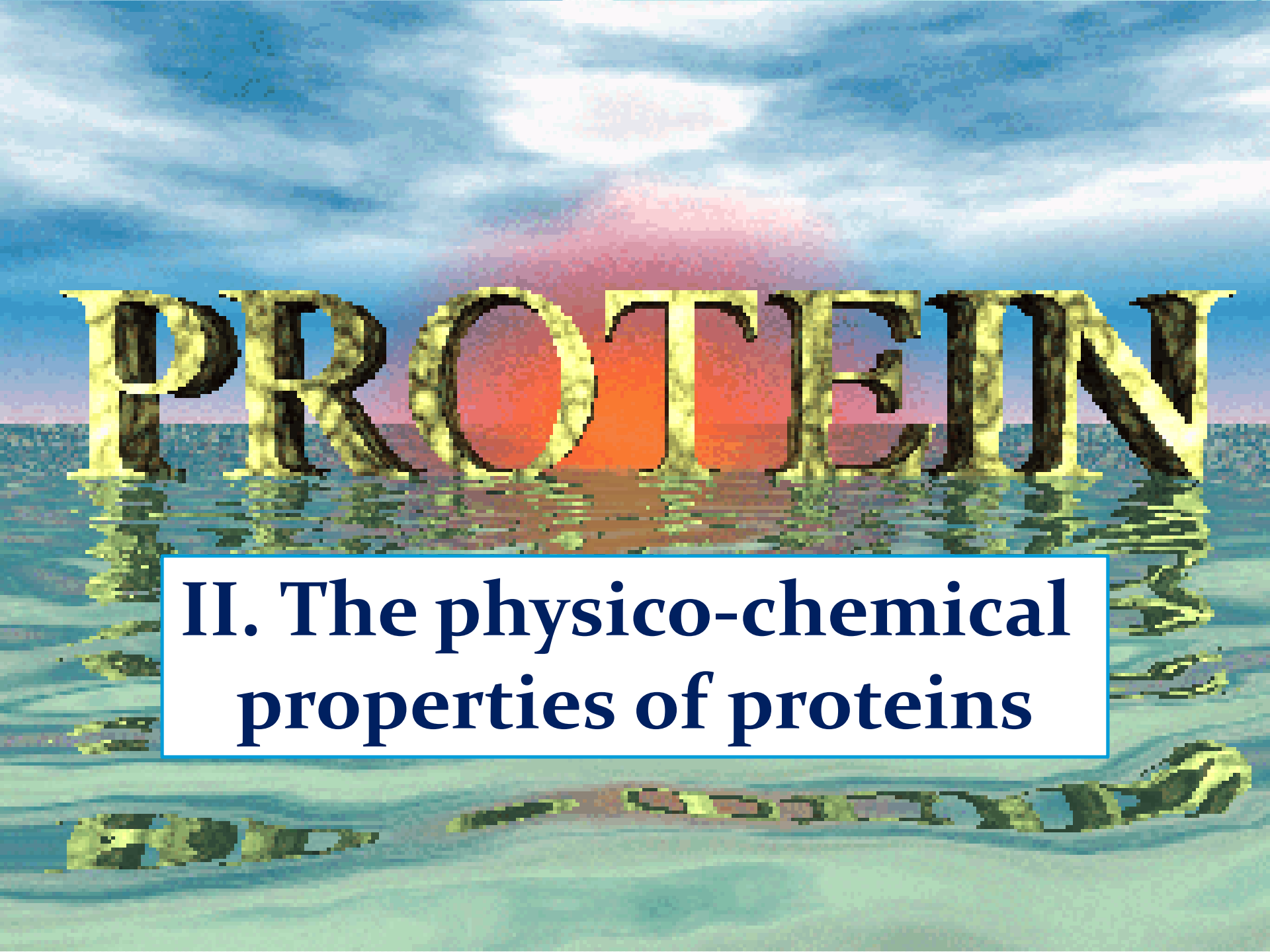


II. The physico-chemical properties of proteins

Proteins differ by there physical and chemical properties:

- **Molecular mass**
- **Total electrical charge**
- **Termolability**
- **Solubility**

Molecular weight of the proteins

Proteins are **high molecular compounds**

- with molecular weight from **5 000 to 1 000 000 Da** (Daltons)

in dependence of the number of amino acid residues and of the number of protomers.

Molecular weight of some proteins, Da

Protein	Protein source	Molecular weight
Lactalbumin	milk	17.000
Insulin	pancreas	12,000
Hemoglobin	erythrocytes	68.000
Myosin	muscle	850.000
Pepsine	stomach	36.000
Peroxidase	kidney	44.000

Total electrical charge of proteins

- Proteins are amphoteric polyelectrolytes
- The total charge of proteins depends on 2 main factors:

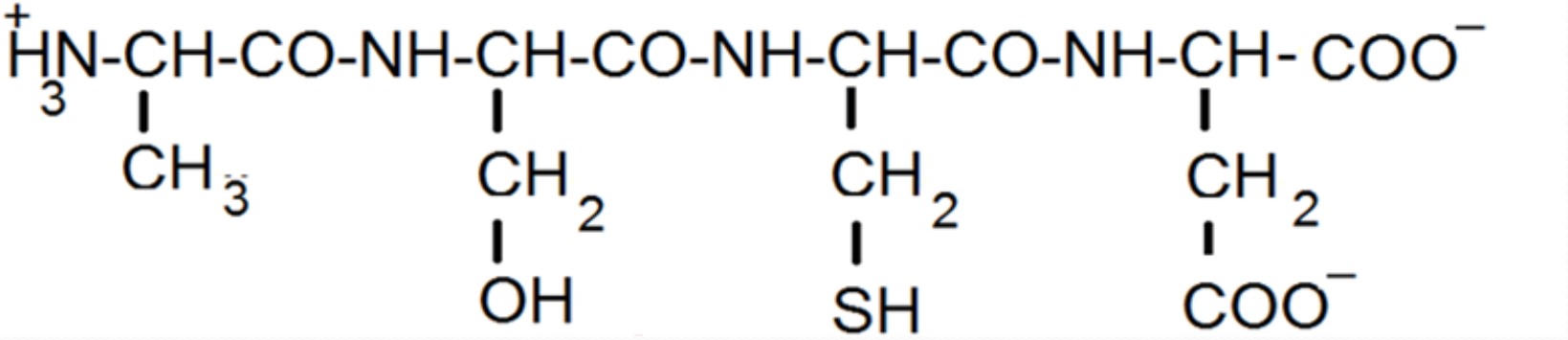
1. amino acid composition

2. pH of the medium

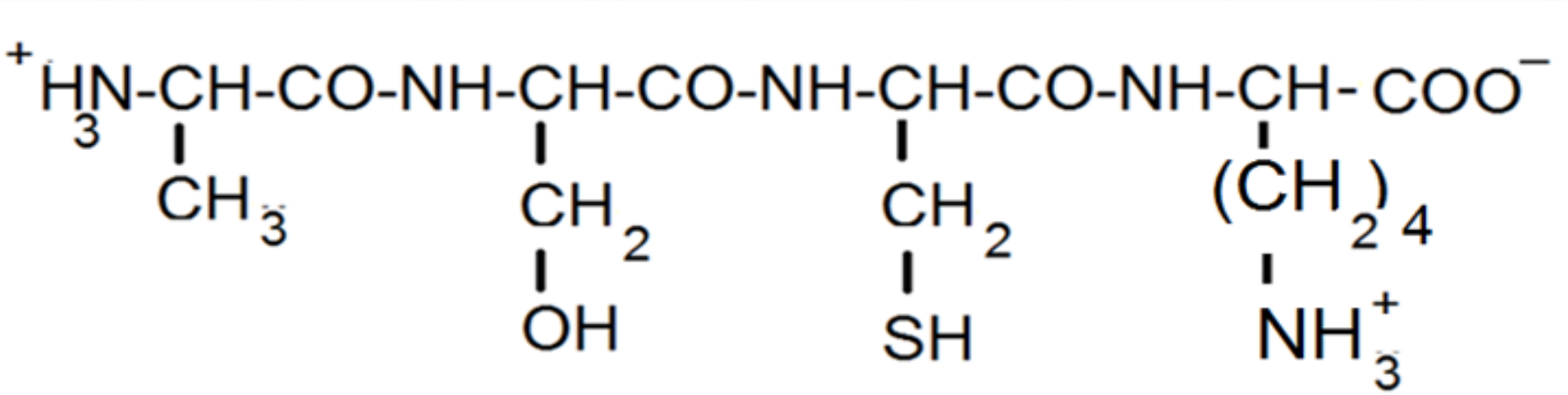
Total electrical charge of proteins:

1. depends on the amino acid composition - on the presence and correlation of charged radicals of amino acids;

- If the protein has more negatively charged amino acids (Glu and Asp)– in aqueous medium its total charge will be negative (for example - albumin).



- If the protein has more positively charged amino acids (Lys, Arg and His)– its charge will be positive (like in histones).



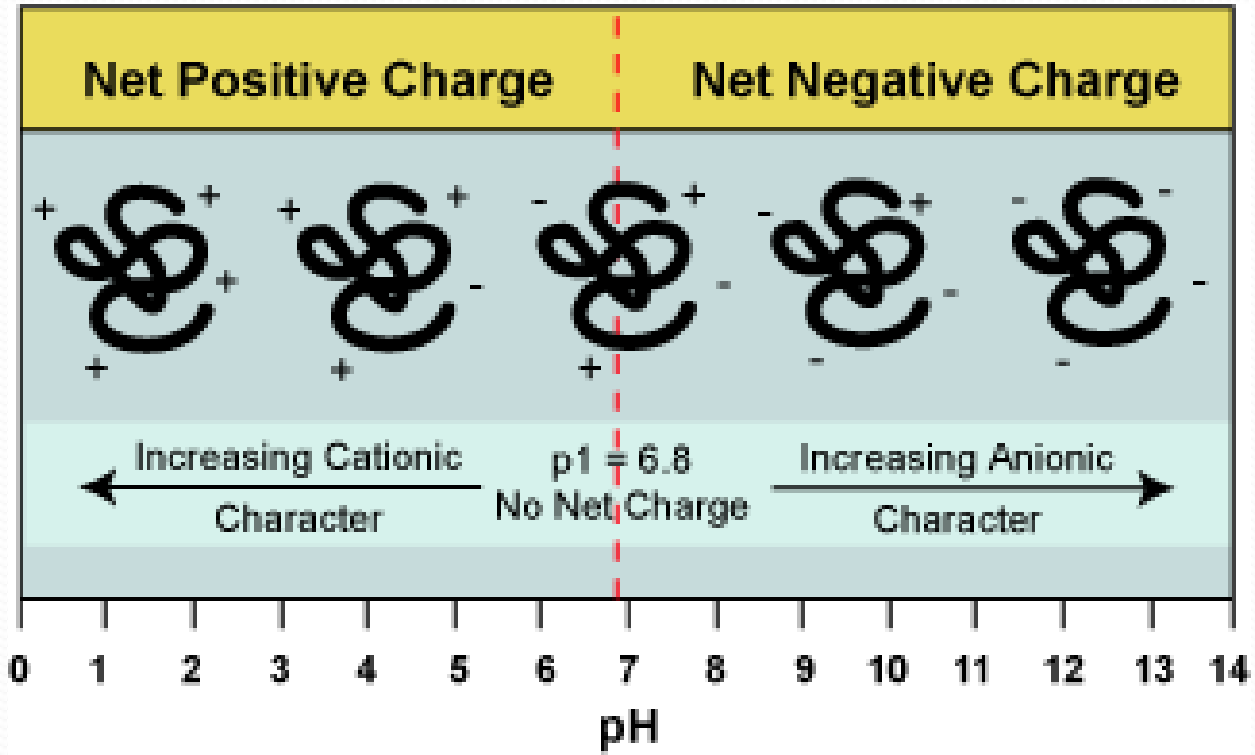
Total electrical charge of proteins:

2. depends on the pH of the medium

In acid medium the concentration of H^+ is high and neutralizes the COO^- - groups of amino acids - the negative charge decreases;

protein becomes positively charged – becomes a cation, and migrates to the cathode in the electric field.

In **basic medium** the concentration of OH^- is high and neutralizes the positive charge of amino groups $-NH_3^+$ - the positive charge decreases, the protein becomes **negatively charged** - becomes **an anion**, and migrates to **the anode** in the electric field.



Isoelectric state and isoelectric point

- The state of the protein when its total electrical charge is equal to zero is called **isoelectrical state**.
- The value of pH when the protein is in the isoelectrical state is called **isoelectrical point.**
- The proteins in the isoelectrical state have a **low solubility** in water medium and can easy precipitate.

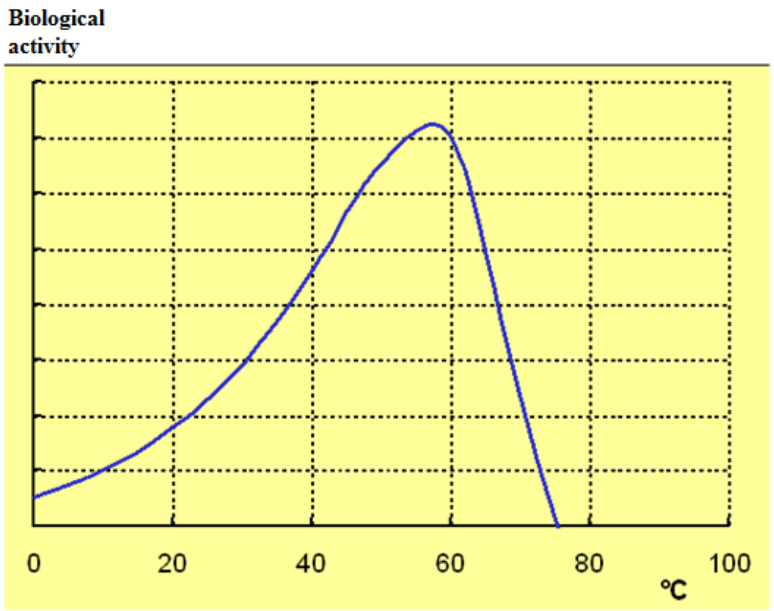
Isoelectric point

- Proteins that contain more acidic amino acids, with negatively charged residues (Glu, Asp) - have the isoelectric point in the acidic medium;

- Proteins that contain more basic amino acids, with positively charged residues (Lys, Arg, His) - have the isoelectric point in the basic medium.

Termolability

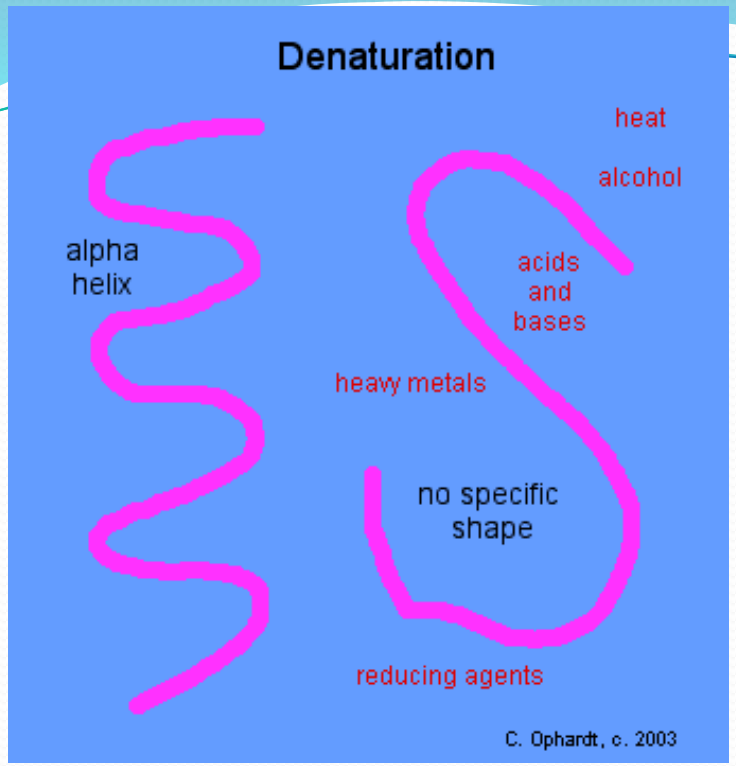
- - is the property of protein to maintain the biological activity in narrow limits of temperature (from 10 to 40°C)
- If the temperature is higher then 50-60°C the protein **denatures** – loses its native conformation. The destruction of all the structural levels of protein (except the primary) takes place.



Termolability

- There are several exceptions – **the termostable proteins** (tripsin, lyzozime, tag-polymerase) – stable at high temperature.

- If the temperature is low - the protein structure doesn't change, but the protein becomes biologically inactive.



Denaturation

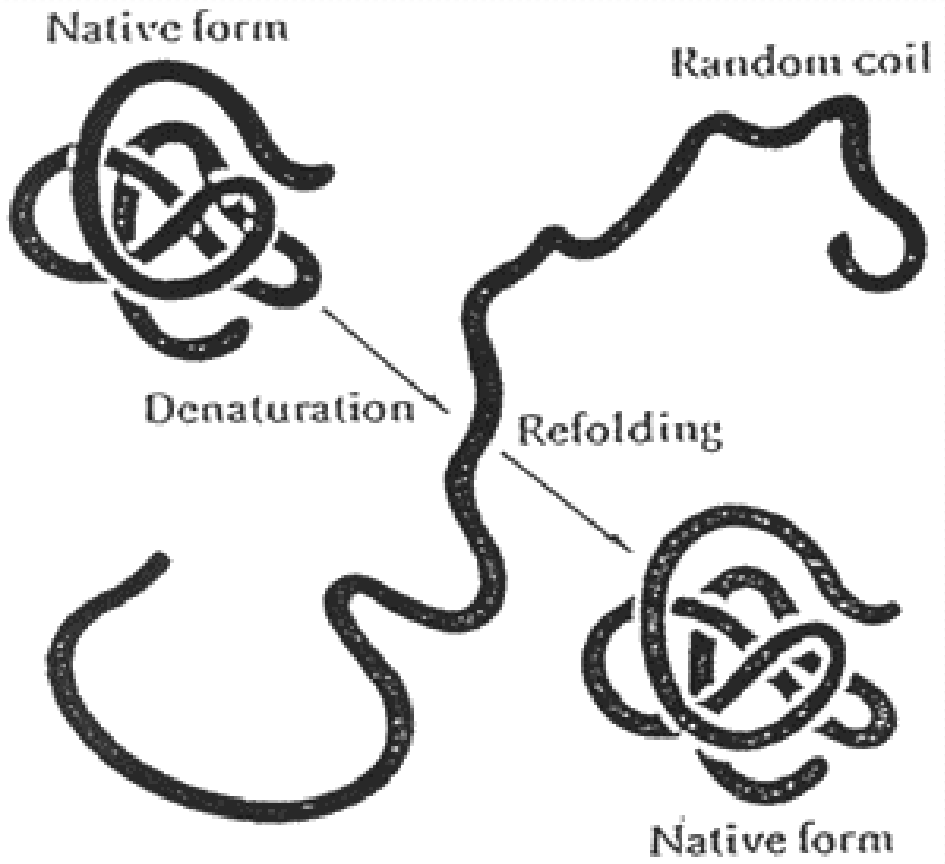
The native conformation of proteins can be lost as the result of **denaturation**: the destruction of the secondary, tertiary and quaternary structures at extreme pH values, at high temperatures, and in the presence of organic solvents, detergents, and other denaturing substances.

Since denaturation reactions are not strong enough to break the peptide bonds, the primary structure (sequence of amino acids) remains the same after a denaturation process.

Effects of Denaturation

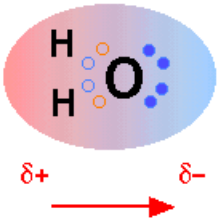
- Loss of biological activity
- Decreased solubility

Refolding or renaturation

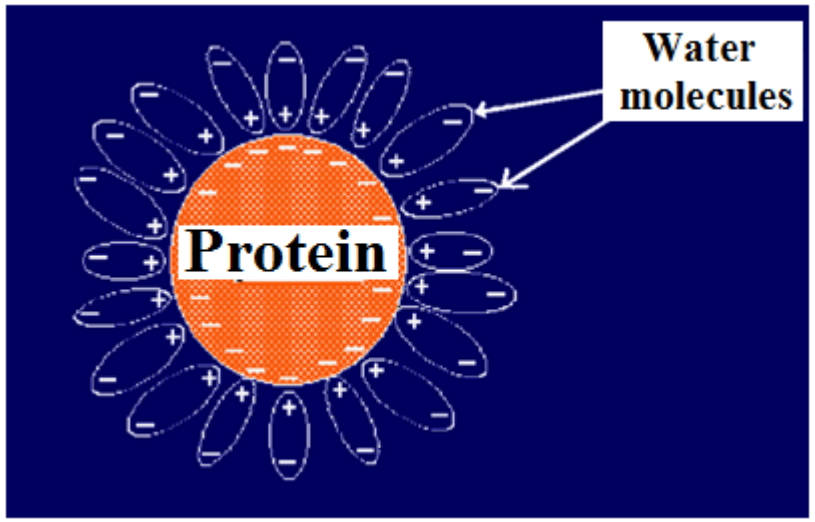


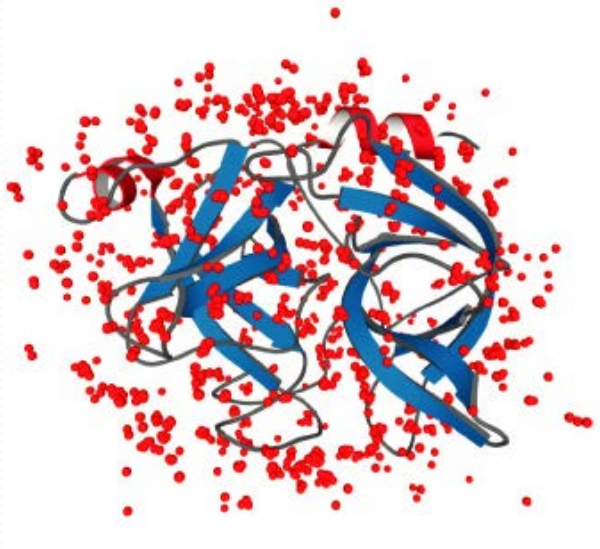
The denatured protein can spontaneously return to its native conformation – **renaturation** can takes place, but only if the denaturating agent **was not strong enough and its action was of short duration**.

Solubility of proteins



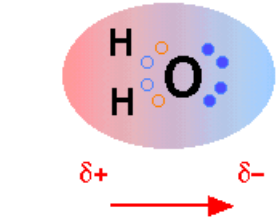
- The most of proteins are hydrophilic compounds and are soluble in water.
- Water interacts with the polar groups of proteins and forms an aqueous membrane - **hydration shell** - at the surface of the protein.





Hydration shell

- - is formed from interaction of polar groups of protein with water dipoles:
- -COO^- group interacts with 4 molecules of H_2O,
- -NH_3^+ group interacts with 3 molecules of H_2O;
- -OH and -NH groups interact with 2 molecules of H_2O.
- Water that enters in the composition of the hydration shell is called "**bound water**"



Solubility of proteins depends on:

1. presence of the **polar groups,** including those with electric charge;
2. presence of the **hydration shell**;
3. **shape of the molecule** – the globular proteins are more soluble then fibrous;

Solubility of proteins depends on:

4. **Solvent** – for example: albumins are soluble both in water and salt solution of different concentration, but globulins are not soluble in water and soluble only in weak salt solution;
5. **pH of the medium** – pH influence on the charge of the protein; in the isoelectrical point the solubility decreases;
6. **Temperature.**

The protein solution are **colloidal solution**

Classification of solutions based on:
- **molecular mass (M);**
- **diameter (Φ) of the dispersed phase particles**

Molecular:	$M < 10^3$ Da, $\Phi < 10$ Å
Colloidal:	10^3 Da $< M < 10^8$ Da, 10 Å $< \Phi < 10^3$ Å
Suspensions:	$M > 10^8$ Da, $\Phi > 10^3$ Å

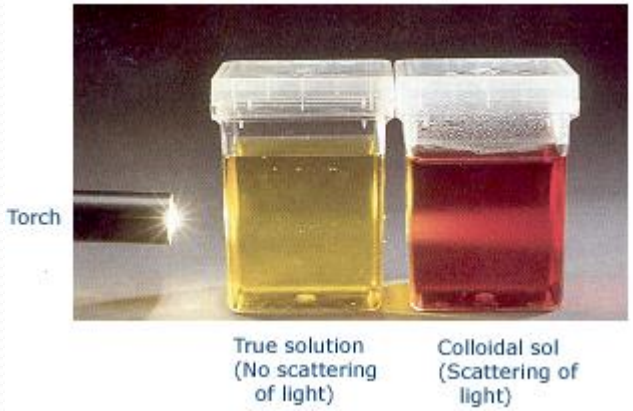
- In contrast to the other colloidal solutions, protein solutions do not require the presence of the stabilizer. Protein solutions are stable and do not precipitate over time.

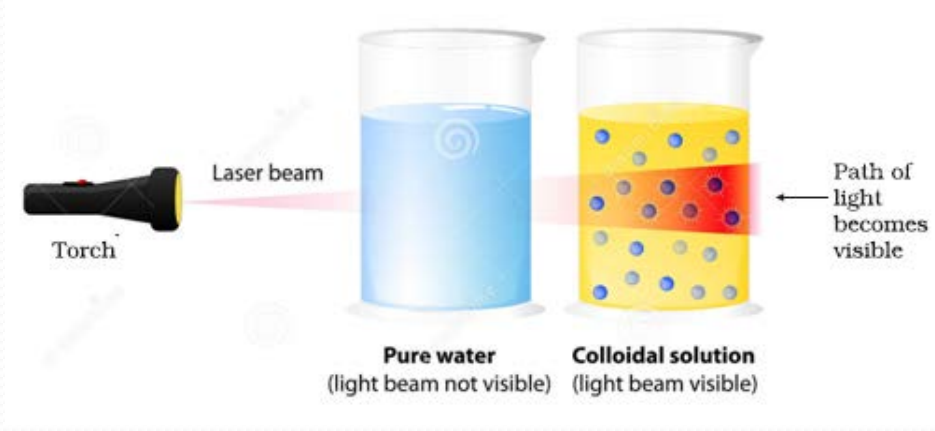
Properties of protein solutions as colloidal solutions:

- Optical properties – Tindal effect
- A low speed of diffusion
- Osmotic (oncotic) properties
- A high viscosity of the solutions
- A capacity to form gels – structural grating with water inside

Optical properties

- When the protein solution is illuminated, the light beam becomes visible, forming a light cone – the **Tindal effect**. This effect is explained by the scattering of light beam by particles in the solution.





The ability of proteins to disperse light is used for:

- the quantitative determination of protein by nephelometric method
- the microscopic study of cellular structures.

Low speed of diffusion

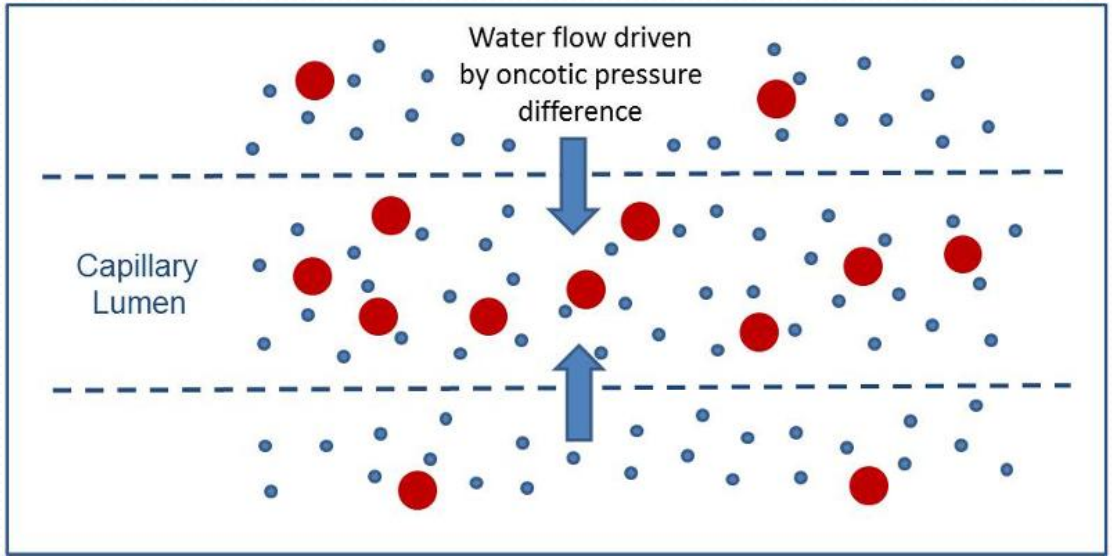
- **Diffusion** – a spontaneous movement of the solute molecules due to the concentration gradient (from regions with higher concentrations toward those with lower concentrations).

- **The speed of proteins diffusion depends more on the shape of the molecule, than on its mass.**

- The intracellular distribution of proteins occurs by diffusion. Since the diffusion rate is low, the speed of the processes is limited by diffusible proteins.

Osmotic (oncotic) properties

- the protein macromolecules is not able to diffuse through the semipermiabile membrane; it results in the phenomenon of **osmosis** (movement of H_2O molecules through the membrane towards the protein in solution). The movement of water is restricted by the hydrostatic pressure called the **osmotic pressure**;
- Osmotic pressure depends on the molar protein concentration and temperature.
- The osmotic pressure caused by proteins is called **oncotic pressure**.

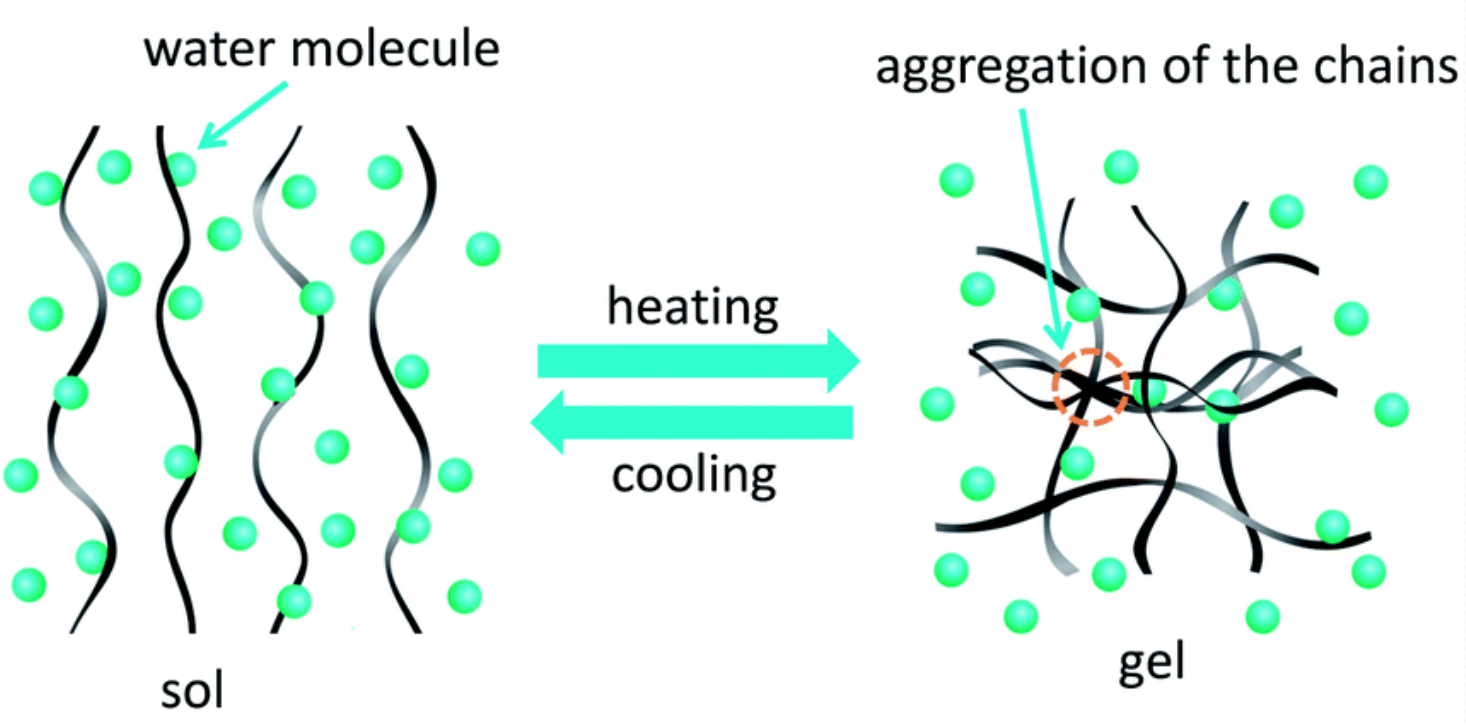


High viscosity of the solutions

- **Viscosity** - the force of cohesion between protein molecules - is dependent on the mass and shape of molecules
- A high protein concentrations lead to a high solution viscosity
- The fibrillar proteins are more viscous than the globular.
- Viscosity is dependent on:
  - **temperature** (at high temperature the viscosity decreases);
  - **presence of electrolytes** (eg. Ca^{2+}salts increase the viscosity by the formation of Ca^{2+}bridges)

Capacity to form gels

- Protein molecules interact one with another to form structural networks with immobilized water inside.
- Gelatinization occurs easier in fibrous protein solutions



- Gel formation is observed in blood coagulation (fibrin network formation).
- In aging gels **syneresis** takes place - the expulsion of water molecules due to contraction of the molecules of the network.
- **Gel formation depends on:**

  - concentration of the solution;
  - temperature (lower temperature favors gel formation);
  - the concentration of hydrogen ions (in the isoelectric point the rate of gel formation is maximal);
  - the presence of electrolytes

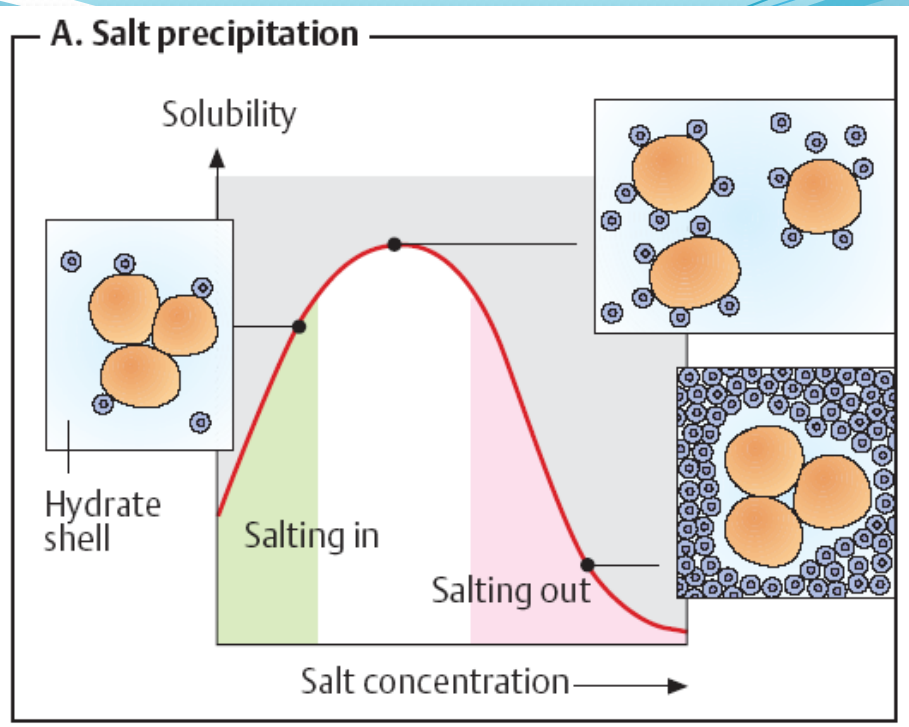
Xerogel-

- it is a dry (waterless) gel.
- is obtined by the **liophilic drying up** of the colloidal solutions
- The **liophilic drying up** is the water removing under vacuum from the frozen colloidal solution.
- Can be kept for a long time – it has a practical importance in the industry of proteic medicines production (e.g.: different proteins - albumin, gamma-globulins and others).

Methods of purification, fractionation and analyzing proteins:

- Salt precipitation
- Dialysis
- Electrophoresis
- Gel-filtration

Salt precipitation



- it is a method of precipitating proteins from the solution under the action of neutral salts in high concentrations (ammonium sulfate, etc.)
- it is a reversible process
- the protein doesn't lose the activity.

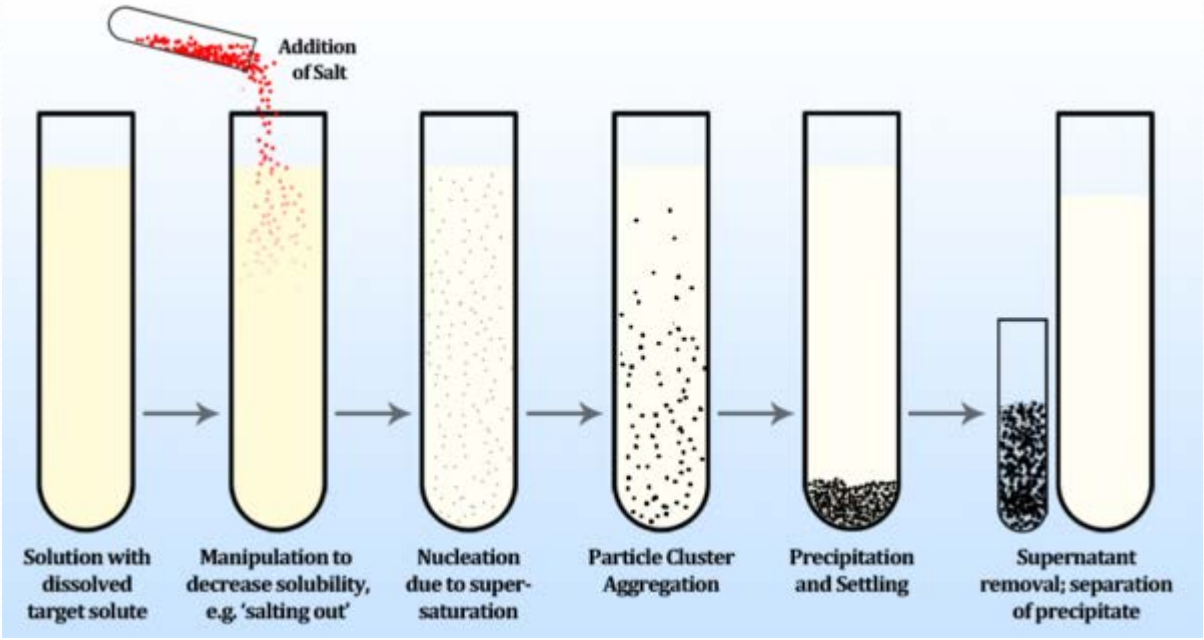
The mechanism of salt precipitation:

- breakdown of the hydration shell
- removing the electric charge.

Salt precipitation

- The solubility of proteins is strongly dependent on the salt concentration (*ionic strength*) of the medium.
- Proteins are usually poorly soluble in pure water. Their solubility increases as the ionic strength increases, because more and more of the well-hydrated anorganic ions are bound to the protein's surface, preventing aggregation of the molecules (**salting in**).

- At very high ionic strengths, the salt withdraws the hydration shall from the proteins and thus leads to aggregation and precipitation of the molecules (**salting out**). For this reason, adding salts such as ammonium sulfate $(NH_4)_2SO_4$ makes it possible to separate proteins from a mixture according to their degree of solubility (**fractionation**).



- The rate of precipitation of proteins by salt precipitation depends on a number of factors such as hydrophilic properties of the protein, its molecular weight, electric charge; thus the salt precipitation of various proteins occurs in various salt concentrations.

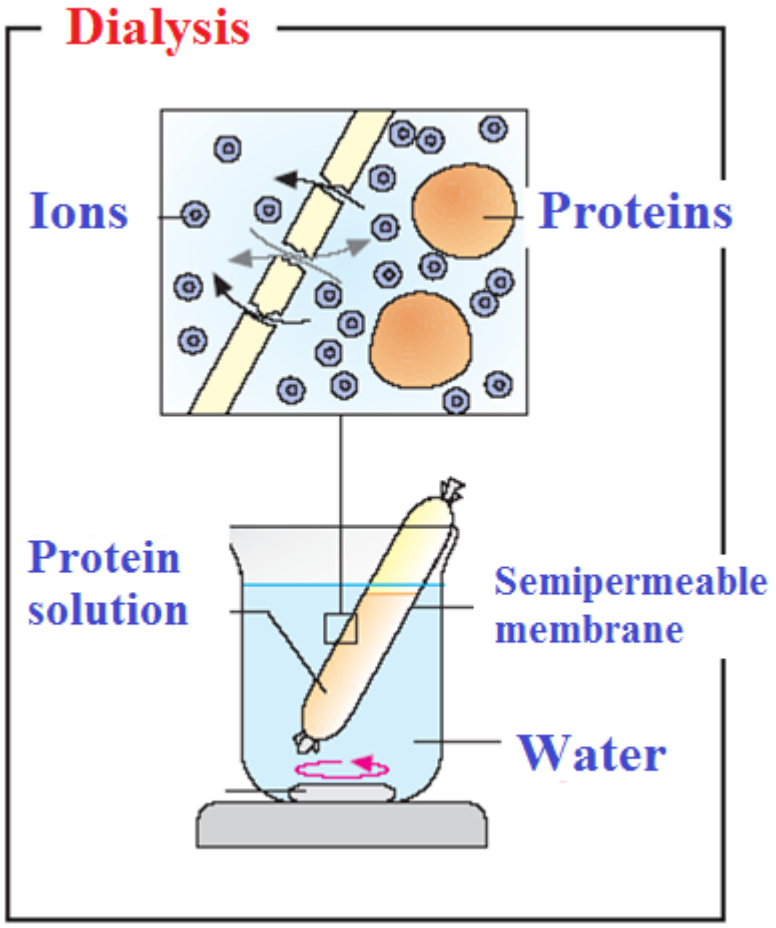
- For example, albumin is precipitated in a saturated solution of ammonium sulphate, whereas globulins - the half-saturated

Dialysis-

- – is the process of separating molecules in solution by the difference in their rates of diffusion through a semipermeable membrane

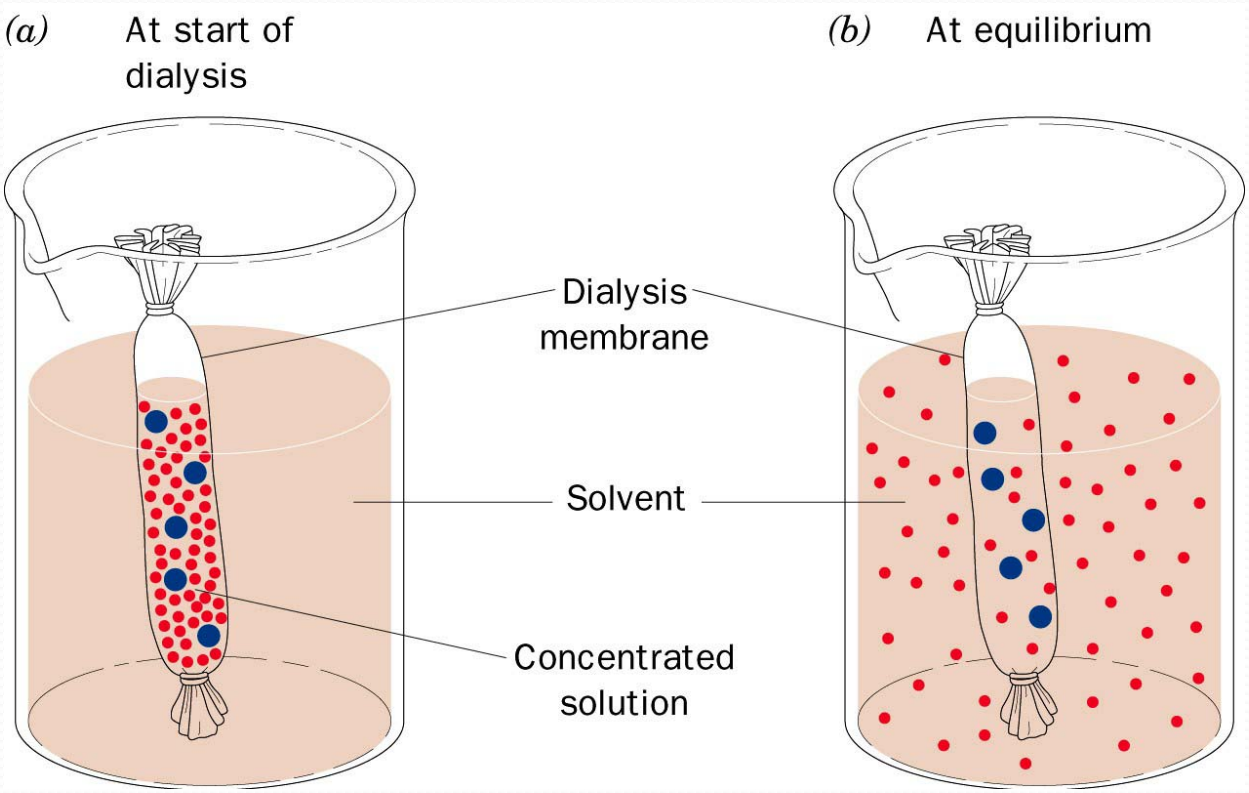
- - method is used for macromolecular compounds (proteins) separation and purification of micromolecular compounds with the help of semipermeable membrane (cellophane, parchment, etc.).

- - through the pores of this membrane can pass only micromolecular compounds that have low molecular weight and small size.

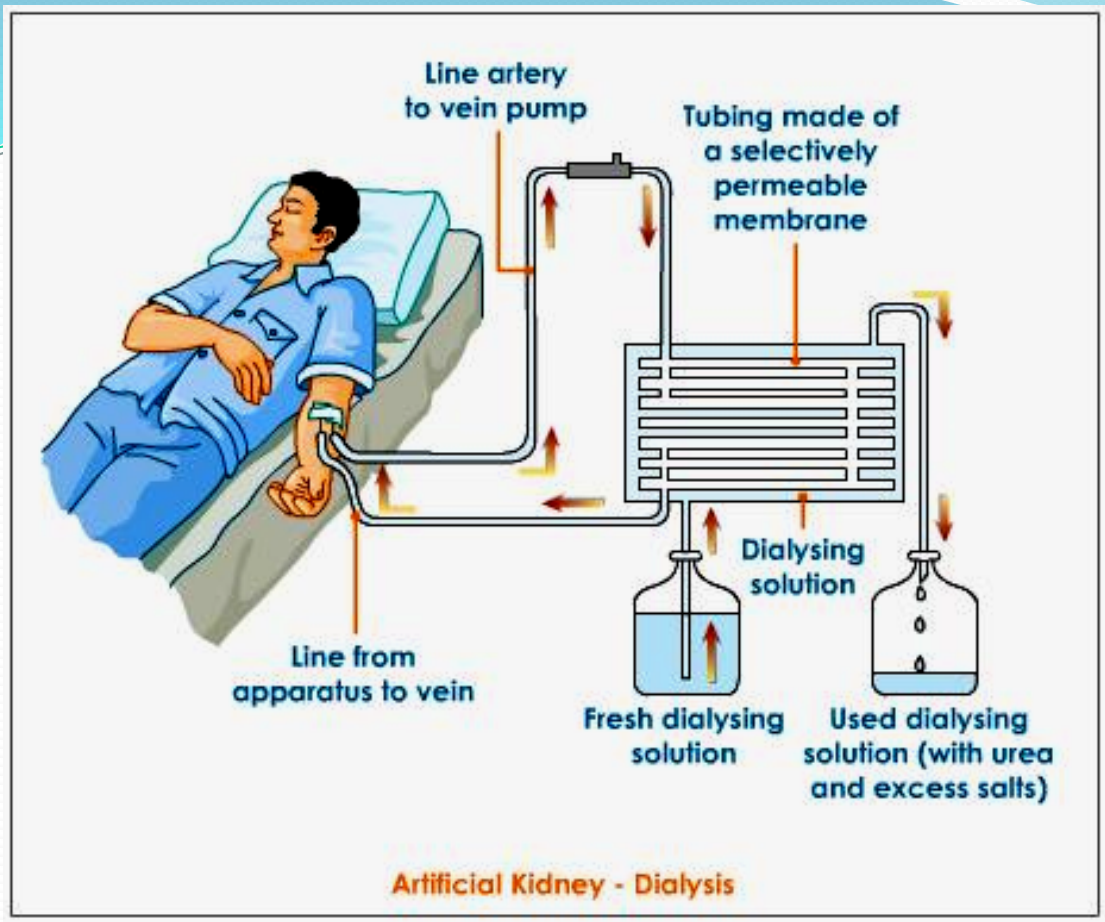


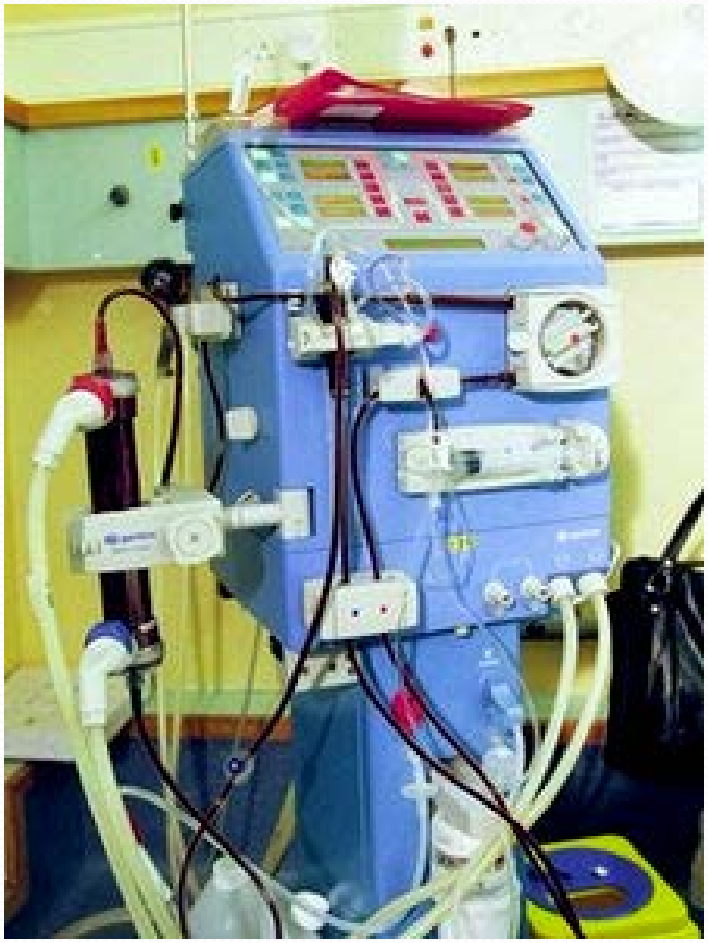
Dialysis

Due to their size, protein molecules are unable to pass through the pores of a **semipermeable membrane**, while lower-molecular substances are able. Thus, dialysis can be used to remove lower-molecular components from protein solutions.



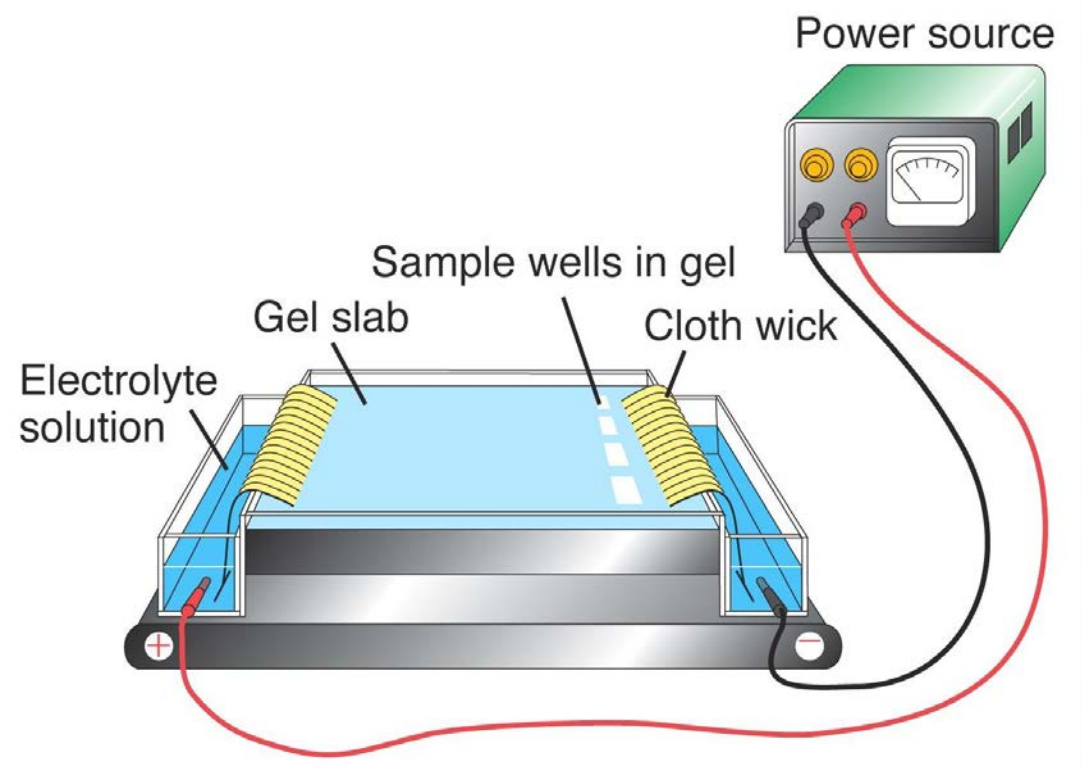
Dialysis in medicine





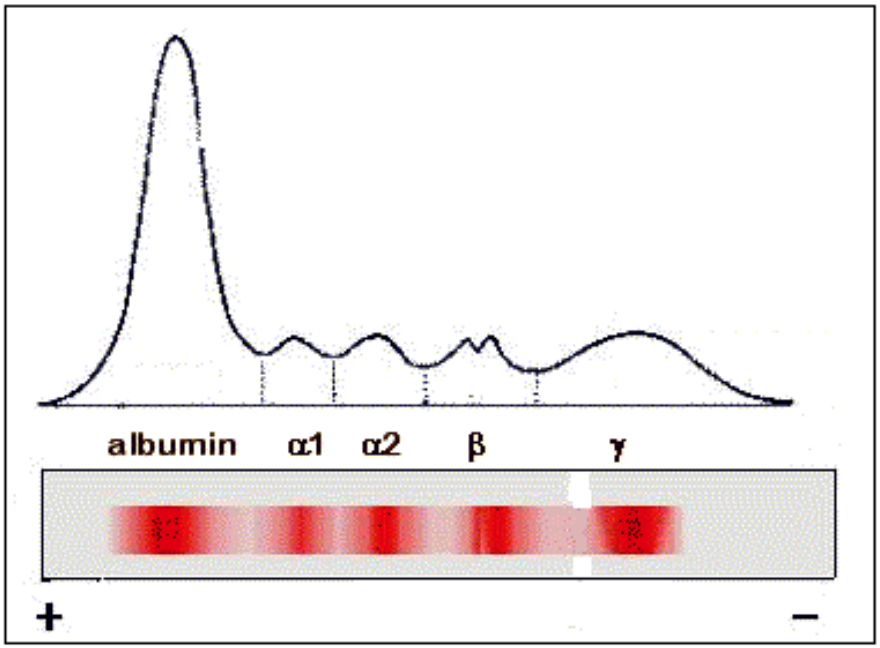
Electrophoresis

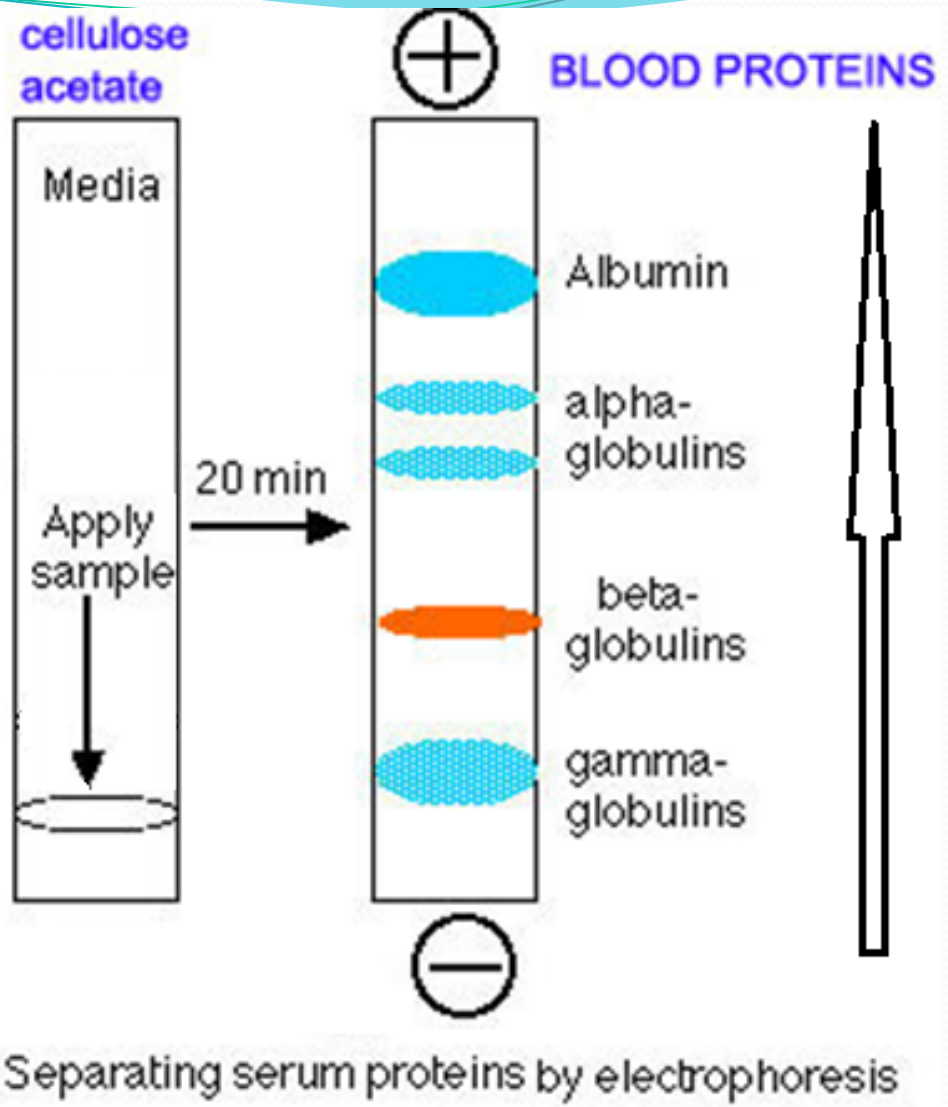
- is based on the ability of particles possessing electric charge, including proteins, to migrate in continuous electric field.



Electrophoresis

- is a technique used to separate different elements (fractions) of a blood sample into individual components. Serum protein electrophoresis is a test that measures the major blood proteins by separating them into five distinct fractions: albumin, alpha1, alpha2, beta, and gamma proteins.





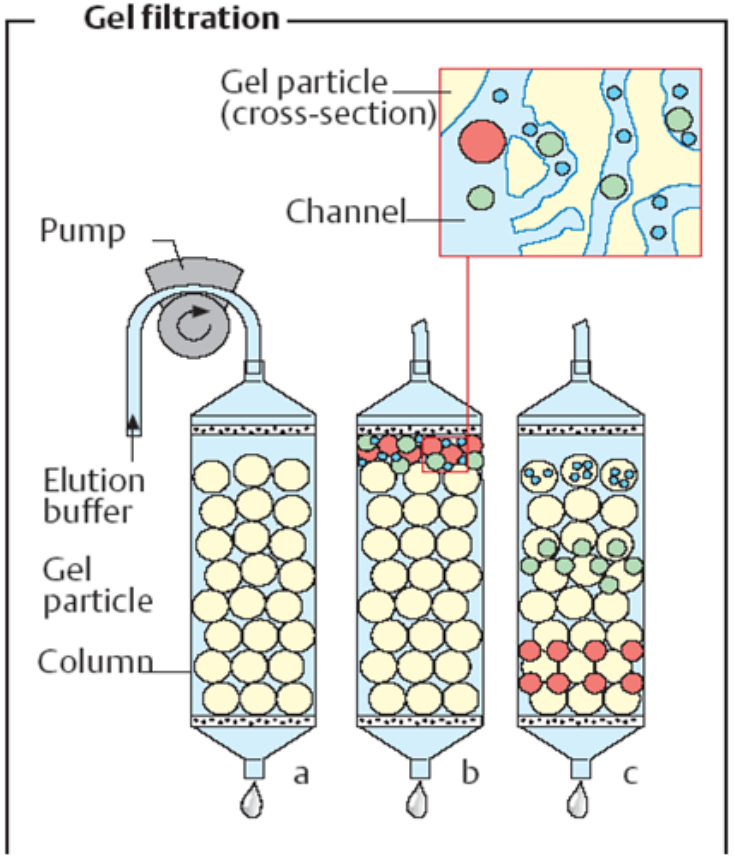
Separating serum proteins by electrophoresis

Gel-filtration (gel-chromatography)

- is one of the main chromatographic methods for the proteins purification.
It requires:
- **Stationary phase: molecular sieve**
- **Mobile phase: buffer**

• The molecular sieves consist of granules of inert hydrated polysaccharide gel. The granules have pore with different diameter.

• Small size micromolecules penetrate these pores, the macromolecules – don't.



• The speed of micromolecules migration through the column is less than of macromolecules – it allows to purify proteins of micromolecular compounds.

• The speed of proteins migration through the column depends on their mass and size - **those which have larger mass and size move faster .**