PROPERTIES OF BIOPOLYMERS SOLUTIONS. 
ISOELECTRIC POINT OF PROTEIN.
DEFENCE OF THE COURSE PAPER
«CHEMISTRY OF BIOGENIC ELEMENTS»

Methodical instructions for 1st year students’ self-work
in Medical Chemistry

ВЛАСТИВОСТІ РОЗЧИНІВ БІОПОЛІМЕРІВ.
ІЗОЕЛЕКТРИЧНА ТОЧКА БІЛКА.
ЗАХИСТ КУРСОВОЇ РОБОТИ З «ХІМІЇ БІЮГЕННИХ
ЕЛЕМЕНТІВ»

Методичні вказівки для самостійної роботи студентів 1-го курсу
з медичної хімії

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Subject «PROPERTIES OF BIOPOLYMERS SOLUTIONS. ISOELECTRIC POINT OF PROTEIN. DEFENCE OF THE COURSE PAPER «CHEMISTRY OF BIOGENIC ELEMENTS»»

1. **Number of hours** 4

2. **Material and methodological support**

Tables:

1. Scheme of structure of the subject.
2. The structure of protein molecules.
3. Hydration of polar groups.
4. Isoelectric point of some proteins.
5. Gelation of polymers.
7. Donnan membrane equilibrium.


6. Text of Lecture «Properties of biopolymers solutions. Isoelectric point of protein».

3. **Substantiation for the subject.** High molecular weight natural compounds – biopolymers – are the structural basis of all living organisms. They play an important role in the processes of life. Biopolymers are proteins, polysaccharides, nucleic acids. Biopolymers form during biosynthesis in cells and are involved in the course of vital processes. Thus, proteins constitute the material basis of cell activity. Enzymes, hormones, structural, transport and protective proteins are among them. Animal starch – glycogen performs energetic function as a source of glucose in the organism. Nucleic acids play a major role in the transfer of genetic information and managing the process of protein biosynthesis. Thus, knowledge of the structure and properties of biopolymers is necessary for understanding the essence of the most important biological processes, it will help to understand the phenomena occurring in the organism (inflammation, swelling, and others.).

4. **The purpose of the subject:**
   
   - general: to learn how to interpret the physical and chemical properties of biopolymers due to their biological function.
   
   - specific: to explain physico-chemical properties of proteins, to make conclusions about charge of dissolved biopolymers based on their isoelectric point.

   a) **to know:** classification of HMC, composition of the most important biopolymers, proteins swelling mechanism and physiological significance of this phenomenon, the process of formation and physico-chemical properties of the gels.

   b) **to be able to:** determine the isoelectric point of proteins, degree of swelling, stability of the HMC in respect with the effect of electrolytes, protective action of
HMC solutions; apply Donnan membrane equilibrium for estimation of the distribution of electrolytes in living systems.

c) **practical skills.**

- to identify the isoelectric point of proteins, the degree of swelling, resistance of HMC to the action of electrolytes, the protective effect of the HMC solutions;
- to apply Donnan membrane equilibrium to estimate the distribution of electrolytes in living systems.

5. **Scheme of structure of the subject.**

![Diagram of the subject structure]

- High molecular weight compounds
- Most important biopolymers
- Structure of proteins
- Properties of biopolymers solutions
  - Gelation
  - Swelling
  - Isoelectric state of proteins
  - Donnan membrane equilibrium
- Properties of gels
6. **Plan of students' work.**

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7. **Tasks for self-work:**

- list of questions to be studied:
  
  1. Definition of HMWC and their classification according the basis of origin, spatial structure, chemical composition and mode of synthesis.

3. Properties of HMWC solution: molecular-kinetic, optical and electro-kinetic properties in comparison with true and colloidal solutions.


5. Methods of determination of protein isoelectric point.


7. Influence of HMWC nature, concentration, pH, temperature, electrolytes on the gel formation (jellification).

1. Definition of HMWC and their classification according the basis of origin, spatial structure, chemical composition and mode of synthesis

The word "polymer" (from the Greek polus – many and teros – part, segments) means – many segments. This term covers all substances whose molecules are constructed from a multitude of elements or links.

High molecular weight compounds (HMC), are the substances, which consist of macromolecules having molecular weight of at least 10,000 a.e.m. They are formed from low molecular weight compounds by polymerization or polycondensation and thus they are polymers. The size of the macromolecules is hundreds and thousands times greater than that of ordinary molecules. The most important polymers in the organism are proteins, polysaccharides and nucleic acids.

Polymers or high molecular weight compounds (HMWS) are the substances, with a high molecular weight, which molecules consist of a large number of structural groups (elemental units) that are repeatedly repeated and interconnected by covalent bonds.

For example, in a polyvinyl chloride molecule:

-CH₂-CHCl-CH₂-CHCl-CH₂-CHCl-CH₂-CHCl-CH₂-CHCl-

a repeating link is a fragment:

-CH₂-CHCl-.

Molecular weight of the HMWC is not less than 10000 a.o.m. The size of macromolecules is hundreds or thousands of times larger than conventional molecules, such molecules called macromolecules.
The molar masses of the HMWC are $10^4 < M < 10^6$ g/mol.

*The degree of polymerization* is an important characteristic of the HMWC, which is equal to the number of elementary chains in a macromolecule. For example, the structural formula of polyvinyl chloride can be written in a compact form:

$$(-\text{CH}_2\text{-CHCl}-)_n,$$

where $n$ is the degree of polymerization. Polymers with a low degree of polymerization are called *oligomers*.

*Depending on origin polymers are classified as:*

- natural (biopolymers) – proteins, carbohydrates, nucleic acids, etc.
- artificial – rubber, gutta-percha, cellulose acetate. Artificial polymers are obtained from natural polymers by their chemical modification. For example, when processing cellulose with nitric acid, its ether – nitrocellulose is obtained
- synthetic – polyethylene, polypropylene, synthetic fibers – nylon, polyacrylic, etc. Synthetic polymers are synthesized from low molecular weight substances - monomers. Monomers are substances, each molecule of which is capable of forming one or several components.

*Depending on the method of synthesis:*

- polymerization – polyethylene (-\text{CH}_2\text{-CH}_2-) $n$.
- condensation – for example, proteins, since peptide bond is formed due to interaction of carboxyl and amino groups with release of a water molecule.

*Depending on the method of production:*

- *polymerization*. Polymerization is a reaction of formation of a polymer from monomer molecules without the allocation of low molecular weight by-products. The elemental composition of the monomer molecule does not differ from the elemental composition of the polymer molecule.

*Example.* Ethylene polymerization reaction:

$$n \text{H}_2\text{C}==\text{CH}_2 \rightarrow (-\text{H}_2\text{C}==\text{CH}_2-)_n;$$

- *condensation*. Polycondensation is the reaction of formation of polymer from monomers with the simultaneous formation of secondary low molecular weight reaction products (water, ammonia, alcohol, etc.). The elemental composition of the
monomer molecule differs from the elemental composition of the polymer molecule. For example, a protein is a condensation biopolymer, since the interaction of a carboxyl and amino group forms a peptide bond with the release of a water molecule.

Example. Polycondensation reaction:
\[ nH_2N-R-NH_2 + HOOC-R_1-COOH \rightarrow H[-NH-R-NH-CO-R_1-CO-]_n + (2n-1)H_2O \]
\((-CO-NH-R)\) – elemental link - a repeating group.

If \( R = (CH_2)_6 \) – hexamethylenediamine, \( R_1 = (CH_2)_6 \) – adipic acid, then in the above example nylon is formed – 6,6.

Example. Reaction of formation of phenol-formaldehyde resins (novolak, rezol):

\[ \text{Depending on the chemical composition:} \]

- **homopolymers.** Homochain polymers have a main chain consisting of identical atoms. If it consists of carbon atoms, then such polymers are called carbon chains (polyethylene, polystyrene, etc.):

\[ -CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2- \]

- **heteropolymers.** Heterochain is called polymers whose main chain consists of different atoms. To heterochain polymers are ethers, for example, polyethylene glycol:

\[ -CH_2-CH_2-O-CH_2-CH_2-O-CH_2-CH_2-O-CH_2-CH_2-O- \]

- **copolymers.** Block copolymers: polymer chains can be constructed by combining short chains of different polymers, called blocks (a molecule of one monomer - A of another - B), for example:


*Graft copolymers:* the main chain consists of one monomer A, and side chains with the other B:

Depending on the spatial structure:
- linear (rubber, cellulose);
- branched (starch fraction – amylopectin, some synthetic fibers);
- net or spatial (glycogen, globular and fibrous proteins).

Fig. 1. Schemes of the structure of macromolecules of polymers:
  a – linear; b – branched; c – spatial; d – sewn

**Natural macromolecular compounds.** Natural rubber is a natural polymer, the general formula is \(-(C_5H_8)_n\) with a molecular weight of 15 000 to 500 000, it contains the juice of some tropical trees (Gevei Brazilian, etc.). The juice of trees (latex), for the preparation of rubber, is coagulated in various ways (for example, by the action of acids).

Natural rubber has a linear structure and high elasticity. It was found that the structural unit of natural rubber is an isoprene group:

\[
\text{CH}_2=\text{CH} - \text{C} = \text{CH}_2 \\
\bigg\| \\
\text{CH}_3
\]

By joining together, such groups form a rubber macromolecule:

\[n\text{CH}_2=\text{CH} - \text{C(CH}_3) = \text{CH}_2\rightarrow[\text{CH}_2 - \text{CH} = \text{C(CH}_3) - \text{CH}_2-]_n.\]

**High-molecular carbohydrates (polysaccharides).** High-molecular carbohydrates are the main constituent of organic matter in the biosphere of the
planet. They act as structural components of cells and tissues, energy reserve (starch, cellulose, glycogen, chitin, inulin, gums and pectic substances). Polysaccharides are formed during polycondensation of monosaccharides, mainly glucose and some of its derivatives. In the liver and muscles there is animal starch – glycogen, the monomer of which is α-glucose. An important role in the body is played by heteropolysaccharides of connective tissue (hyaluronic acid, heparin, chondroitin sulfate) formed from the residues of various glucose derivatives.

**Starch** is a naturally occurring polysaccharide that plays the role of a reserve substance in many plants. The composition of starch includes two polysaccharides: amylose (20-30 %) and amylopectin (70-80 %). Polysaccharides are constructed from the residues of α-D-glucose (α, -D-glucopyranose), which are linked together by α-(1,4)-glycoside-glycosidic bonds. Amylose and amylopectin have the same chemical composition and differ in spatial structure. Amylose molecules are linearly constructed, and amylopectin molecules have lateral branches.

**Cellulose** is the main constituent of plant cell membranes. Cellulose is a polysaccharide, which contains the remains of β-D-glucose (β, -D-glucopyranose), which are connected together by β-(1,4)-glycoside-glycosidic bonds. Cellulose is a chemically inert substance.

The difference in the structure of the molecules of cellulose and starch greatly affects their physical and chemical properties. Macromolecular chains of cellulose have a linear structure. Chains are elongated and enclosed by beams in which they are contained with each other due to numerous intermolecular hydrogen bonds between the hydroxyl groups. The linear structure of cellulose leads to the formation of fibrous materials such as cotton and flax.

**Proteins and nucleic acids.** Proteins are natural high-molecular organic substances, the macromolecules of which are built up of a huge number of α-amino acid residues linked together by peptide bonds (–CO-NH–)n. In the human body, there are about 5,000,000 different proteins that differ in the sequence of the amino acid compounds, as well as in the spatial structure of the chains.

**Nucleic acids** are natural HMWC, it functions as storage and transmission of
hereditary information. Molecular weight: 25,000 < M < 1,000,000 (g/mol). Nucleic acids are polymers consisting of nucleotides, which in turn consist of nitrogen bases (uracil, thymine, cytosine, adenine, and guanine), one of the two monosaccharides – ribose or deoxyribose, and phosphoric acid. Composition of ribonucleic acid (RNA) includes ribose and deoxyribonucleic acid (DNA) includes deoxyribose.

**Structural organization of biopolymers.** There are several types of structural organization of polymers. *The primary structure* is determined by the sequence of residues of monomeric molecules in the polymer chain.

The spatial arrangement of the polymer chain determines secondary structure of HMWC. Hydrogen bonds have fundamental significance in the formation of the secondary structure of proteins. Helix is formed if they arise between the peptide bonds in a single chain, and almost flat pleated sheet is formed if hydrogen bonds appear between different chains.

Depending on the shape of macromolecules and solubility in water proteins are divided into two groups – globular and fibrous. This is trivial classification used nowadays.

*Helical structure* is more typical for globular proteins, their chains are bent in space in such a way that macromolecule acquires a shape close to spherical. Globular proteins are soluble in water. Some globular proteins are albumin (egg white), globin (the protein part of hemoglobin), myoglobin, etc.

*Fibrous proteins* have pleated sheet structure. They have a fibrous structure and are not soluble in water. These include widespread proteins – α-keratin (hair, horn tissue), myosin (muscle tissue).

Polypeptide chain with the elements of a particular secondary structure is able to acquire a certain spatial structure, i.e. to form tertiary structure. In this case, radicals of α-amino acids of the polypeptide chain which are approached in space due to its curves, interact. Along with hydrogen bonds involved in the stabilization of secondary and tertiary structure, ionic and covalent bonds, as well as hydrophobic interactions are important.

Several individual polypeptide chains can form more complex structures. Each
individual chain maintaining its characteristic primary, secondary and tertiary structure acts as a subunit of this complex. The quaternary structure of the protein arises on the interaction of multiple subunits. This interaction is provided by hydrogen bonds and hydrophobic interactions.

2. **Swelling and dissolution of polymers. Influence of HMWC nature, t°, pH and presence of electrolytes on the swelling. Degree of swelling**

Polymers are able to dissolve in low-molecular substances. The large size of macromolecules introduces a number of features in the dissolution process and the properties of solutions. The mutual mixing of polymer and solvent molecules always proceeds with a decrease in free energy. Solutions of HMWC, like solutions of low molecular weight compounds, are homogeneous, thermodynamically equilibrium, and aggregatively stable systems. These are the true solutions.

Since the mobility of solvent molecules far exceeds the mobility of macromolecules, in the first stage of dissolution, the solvent penetrates into the polymer, which is accompanied by a significant increase in its volume. However, with a multiple increase in volume, the polymer retains its shape. This phenomenon is known as the swelling. The first step in dissolving any polymer is swelling.

Swelling is an increase in the volume of a polymer when it absorbs a solvent. This process occurs in two stages. *In the first stage*, when the polymer is placed in water, hydration of its polar groups occurs. This stage is characterized by the release of heat and the ordering of the arrangement of water dipoles in macromolecules, as a result of which the bonds between individual polymer chains are weakened. *In the second stage*, a lot of liquid is absorbed without the release of heat. The reason for the swelling is that during dissolution, not only the diffusion of the molecules of the substance into the solvent occurs, but also the diffusion of the solvent molecules into the high-molecular substance. In this case, water molecules fill the space between macromolecules in the loose polymer structure and weaken intermolecular bonds. The gaps that form are filled with new solvent molecules. The distance between macromolecules increases, which leads to an increase in the volume and mass of the
If the polymer is readily soluble in water, then its swelling ends in dissolution. Such a swelling is called unrestricted. If the solubility of the polymer is small, a limited swelling takes place, in which a certain amount of solvent is absorbed, after which the volume ceases to increase. Limited swelling ends in the formation of a gel.

Swelling is an increase of the polymer volume when it absorbs solvent. This process takes place in two stages. On the first stage hydration of polar groups of polymer occurs when it is placed in water. This stage is characterized by the release of heat and orderly arrangement of water dipoles around macromolecules, resulting in weakened links between individual polymer chains. In the second step a lot of liquid is absorbed without heat release. The reason of swelling is the mutual diffusion of solute molecules in the solvent and the solvent molecules inside the macromolecular substance. In this case, water molecules fill the space between the macromolecules in the loose structure of the polymer and weaken the intermolecular bonds. Gaps formed are filled in with new solvent molecules. The distance between the macromolecules increases, thereby increasing the volume and weight of the polymer.

If the polymer is highly soluble in water, its swelling results in complete dissolution. Such swelling is called unlimited. If the solubility of the polymer is poor, limited swelling occurs, at which a certain amount of solvent is absorbed. Limited swelling results in the formation of gel.

Factors affecting the swelling process:

1. Temperature and pressure (according to the Le Chatelier principle). Effect of temperature on swelling is exhibited on the first step. Since hydration is followed by release of heat, the elevation of temperature reduces swelling (Le Chatelier's principle).

2. The nature of the solvent and polymer, that is, their polarity. For good swelling and dissolution, the polarity of the polymer should be close to the polarity of the solvent.

3. The structure of the polymer. Flexible hydrocarbon chains without polar groups unrestrictedly dissolve in nonpolar liquids. Increased rigidity (the appearance
of polar groups) reduces the solubility of HMWS. Crystalline polymers swell and do not dissolve even in solvents that are close in polarity.

4. **Molecular weight of the polymer.** The increase in the molecular weight of the polymer results in a decrease in swelling capacity and dissolution in the same solvent.

5. **pH of the medium.** A smaller swelling and dissolution of polyelectrolytes corresponds to an isoelectric point, swelling and dissolution increases above and below this point.

6. **The presence of electrolytes.** The addition of electrolyte ions to the solvent, which are well solvated, reduces the solubility of polar IUDs and complicates the swelling process. On the influence of the swelling process, the anions are arranged in a certain sequence, called *lyotropic series*:

\[
\text{CNS}^- > J^- > Br^- > NO_3^- > Cl^- > CH_3COO^- > C_2\text{O}_4^{2-} > SO_4^{2-}
\]

The degree of swelling decreases

The quantitative measure of swelling is the degree of swelling \( \alpha \), which can have a volumetric or mass expression:

\[
\alpha = \frac{V - V_0}{V_0} ; \alpha = \frac{m - m_0}{m_0},
\]

where \( V_0, V, m_0, m \) are the volumes and masses of the initial and swollen polymer, respectively.

More precise is the definition of "\( \alpha \)" by its mass expression, since in this case the results of measurements do not depend on the phenomenon of contraction. The contract consists in that the volume of the solution (mixture) of the two liquids is less than the sum of the volumes of the liquids taken.

![Fig. 2. Interaction of solvent with polymer:](image)

1 – interstructural swelling; 2 – internalstructural swelling; 3 – dissolution
The least swelling of proteins occurs in isoelectric state, since in this case the degree of solvation of the ionized groups is minimal.

Different processes in the organism are accompanied by swelling: tissue regeneration, inflammation, edema, acid burns, insect bites. The main cause of swelling in these cases is pH change in the tissues.

2. Properties of HMWC solution: molecular-kinetic, optical and electro-kinetic properties in comparison with true and colloidal solutions

Solutions of high-molecular compounds are true solutions, thermodynamically stable and reversible, requiring no stabilizer. Particles of such solutions do not consist of number of small molecules or ions as in colloids. They are represented by separate molecules. However, the size of such molecules is close, and in some cases is even bigger than the size of colloidal particles, which makes their molecular-kinetic and optical properties similar with colloids.

Properties of solutions of HMWC, characteristic of colloidal solutions:
- the particle size (molecules of HMWC) corresponds to the size of the colloidal particles ($10^{-7}$-$10^{-9}$ m);
- solutions of HMWC do not pass through semipermeable membranes;
- light scattering phenomenon (diffuse Tyndall cone);
- ability to coagulate;
- slow diffusion.

Properties of solutions of HMWC, characteristic of true solutions:
- homogeneity;
- thermodynamic stability;
- spontaneous formation (dissolve in certain liquids, without requiring stabilizers)
- reversibility of coagulation.

Just like colloids, some biopolymers (proteins, nucleic acids) are characterized by the phenomenon of electrophoresis caused by the presence in them of an electric charge in the aqueous medium. However, this charge does not arise because of the
presence of a diffusion layer in the particles, but as a result of the dissociation of the polar groups of molecules in the aqueous solution.

With the help of electrophoresis, it is possible to isolate and investigate individual fractions of plasma proteins. This method is used to diagnose many diseases in which the protein composition of the blood changes.

In the collision of the HMWC molecules due to thermal motion between the individual sections of different macromolecules, bonds are formed, leading to the appearance of associates. These connections are due to various interactions: these can be ionic, covalent, hydrogen bonds, as well as hydrophobic interactions. Associations do not exist constantly: they arise in one place, then break up and re-form in the other. The formation of associates is the main cause of the anomalous viscosity of HMWC solutions: it is much larger than in true and colloidal solutions. The high viscosity of the IUS solutions is due to their high hydrophilicity, the macromolecules are firmly bound to the molecules of the solvent. The viscosity is also affected by the shape of the molecules. If the macromolecules are perpendicular to the flow, the resistance effect is greatest, if the resistance is less along the flow. With increasing pressure, the particles are oriented along the flow and the viscosity decreases. In more concentrated solutions of polymers, spatial structural networks are formed, which increase the viscosity of the solutions.

The viscosity of polymer solutions depends on their molecular weight, so viscosity measurement is used to determine the molecular weights of the HMWC. Unlike colloidal solutions, HMWC solutions are thermodynamically stable, they are formed spontaneously and have much greater stability than colloidal solutions.

HMWC solutions also can scatter light, although in less than typical colloidal systems. Light scattering concentrated solutions of polymers is due to their heterogeneity, which arises as a result of continuous small deviations in concentration, which in turn cause deviations (fluctuations) in the refractive index from its mean value. The phenomenon of light scattering is the basis of the optical method for determining the molecular weight of polymers. The application of the phenomenon of light scattering is not limited at the present time to the determination
of the molecular weight of polymers, but also includes the determination of such important characteristics of the polymer as the size and structure of its macromolecules, polydispersity, thermodynamic parameters of intermolecular interaction in solutions, etc.

3. Stability of polymers solutions. Salting out, coacervation, denaturation

The stability of solutions of polymers can be violated by deterioration of HMWC solubility. This can be achieved by reducing lyophilicity of the polymer due to the removal of solvate shells by the addition of desolvating agents - electrolytes or by adding liquids that poorly dissolve the polymer.

When large amounts of electrolytes are added, the separation of high molecular substances from the solution is observed. Since electrolytes usually use salts, the process is called *salting out.*

Salting is the precipitation of a dissolved substance that is caused by the addition of large quantities to the IUS solution electrolytes (more often salts). If coagulation of sols is negligible a small amount of electrolytes (mmol/l), then for salting out the HMWC very large quantities of salts are being consumed (often concentration reaches saturation). Salting out is explained by a decrease in the solubility of substances in a concentrated electrolyte solution, in analogy with similar phenomena in solutions of low molecular weight substances. When large amounts of electrolytes are added, the hydrate shell of the HMWC molecules breaks down and they are released from the solution. Salting out is at the heart of one of the methods for fractionation of high molecular substances, in particular proteins, since their ability to separate from the solution increases sharply with increasing molecular weight. Applying salts in different concentrations, it is possible to absorb different protein fractions: at a low concentration of salts, the heaviest particles with the lowest charge are deposited, with increasing concentration, more stable fractions fall out. The salting out process is often reversed: after removing the salt from the precipitated protein, it can again be converted into a solution. This principle is based on the
preparation of therapeutic sera and a solution of $\gamma$-globulin.

If the stability of HMWC solutions is disturbed, *coacervate* – a new liquid phase enriched with polymer, coacervate can be in the initial solution in the form of drops or form continuous layer. Coacervation is the fusion of the water shells of several particles without combining the particles themselves.

Fig.3. Coacervation scheme

The emergence of coacervates from protein molecules is an important element in the theory of the origin of life on Earth. Coacervation can be accompanied by the formation of nucleoproteins, lipoproteins and complexes, is of considerable interest for biochemists.

The practical importance of coacervation has increased in connection with the development of microcapsule technology. In the pharmaceutical industry microcapsule is used to protect the drug substance from contact with the environment. Microcapsules are solid, liquid or gaseous encapsulated polymers substance. The shell is formed from adsorbed droplets of the polymer coacervate, which merge into a continuous film and a special treatment are translated into a solid state.

*Denaturation* – under the influence of various physical and chemical factors, the natural spatial structure of the protein molecule is disrupted: the quaternary, tertiary and secondary structures are destroyed (the primary structure does not change). This leads to a decrease or total loss of solubility, specific biological activity, changes in optical properties, viscosity, etc. During ion denaturation, ionic, hydrogen and disulfide bonds are broken, the polypeptide chain is unwound and is either in the unfolded state or in the form of a chaotic coil. For most proteins, this is
an irreversible process, but for some, for example, muscle proteins are reversed. With the aging of the organism, a gradual denaturation of proteins and a decrease in their hydrophilicity occur. Denatured proteins of food are better cleaved by enzymes of the gastrointestinal tract.

4. Methods of determination of protein isoelectric point

The protein molecule has an electrical charge due to the dissociation of ionogenic groups (-COOH) and (-NH₂), which are on the final amino acids, as well as dicarboxylic and diamine amino acids that are in the middle of the polypeptide unit. A bipolar ion is formed in the process of dissociation has a positive and negative charge.

The charge of a protein molecule depends on the pH of the solution in which the protein is located. As the pH decreases, the positive charge increases, and with increasing - the negative charge: the state in which the number of positively and negatively charged groups in the protein molecule is the same and, consequently, the total charge of the molecule is zero, is called the isoelectric state, and the corresponding pH value is the isoelectric point of proteins (pI). In the isoelectric state, the protein molecules do not move in the electric field. The isoelectric point is an important characteristic of proteins. Each protein has its own individual IET.

The reaction of the medium and the nature of the dissociation of the protein molecule determines its shape in the solution. When dissociating ionogenic groups, only the acid charges or only the basic type in the helix of the peptide chain, the same charges appear, distributed over its entire length. Due to electrostatic forces, repulsion takes place, and the spiral turns will move apart, and the macromolecule will expand. In the isoelectric state, the charges of the opposite sign alternate along the peptide chain, facilitating the compression of the molecule and even twisting it into a globule. This means that in the isoelectric state the protein molecules in the solution occupy the smallest volume. With increasing or decreasing pH, the molecules straighten out.
Isoelectric points of some proteins

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<th>pI</th>
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<tr>
<td>Pepsin of gastric juice</td>
<td>2,00</td>
</tr>
<tr>
<td>Casein milk</td>
<td>4,60</td>
</tr>
<tr>
<td>Egg albumin</td>
<td>4,71</td>
</tr>
<tr>
<td>γ- blood globulin</td>
<td>6,40</td>
</tr>
<tr>
<td>Fibrinogen of blood</td>
<td>5,40</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>6,68</td>
</tr>
<tr>
<td>Chymotrypsin pancreatic juice</td>
<td>8,60</td>
</tr>
<tr>
<td>Ribonuclease</td>
<td>9,50</td>
</tr>
<tr>
<td>Cytochrome C</td>
<td>10,70</td>
</tr>
</tbody>
</table>

Methods of determination of protein isoelectric point:

- by electrophoretic mobility: the protein under study is subjected to electrophoresis in buffer solutions with different pH values; in a buffer with a pH value that coincides with the pH of the pI, the latter is electrically neutral and does not move in the electric field;

- according to the degree of coagulation: buffer solutions are poured into tubes with different pH values, equal amounts of protein are added and alcohol is added, the most pronounced turbidity is observed in a tube with a buffer, the pH of which coincides with the pH of the pI;

- on the rate of gelation: buffer solutions are poured into the tubes with different pH values and a concentrated solution of the test protein is added, the gelation of which will occur most rapidly in the solution whose pH is closest to the pH of the pI;

- by the amount of swelling: identical amounts of dry protein are poured into a row of tubes and equal volumes of buffer solutions with different pH values are added. The smallest swelling of the protein will appear in the test tube, where the pH of the medium will be closest to the pH of the pI.

5. Properties of gels and jellies. Phenomena of syneresis and thixotropy

Solutions of the IUS and colloidal solutions under certain conditions are able to turn into a non-flowing structured system - gels and jellies. This is due to the increase in the number of molecular bonds between the particles to form a network structure,
the cells of which are filled with a solvent. It is believed that gels are formed from colloidal solutions, and jelly from solutions of the HMWC. However, the difference between gels and jellies is conditional. The reason for the formation of gels is the appearance of weak bonds between the ends of colloidal particles (where the double electric layer and the hydrated shell are less developed) with the formation of a spatial grid that, like a sponge, captures water molecules. The formation of jelly is caused by the appearance of cross-links between chains of polymers with the formation of a network structure, retains water molecules.

*Gels and jelly are obtained in two ways:*

- *gelatinization (curing).* This is the transition of the liquid solution to gel or gelatin. For example, the hardening of a hot gelatin solution leads to the formation of jelly.

- *swelling of xerogels (solvation of dry gels).* Absorption of water by particles of the HMWC leads to their swelling, with the possibility of merging the swollen particles into a continuous system. So, with the addition of water to starch, dry glue, agar-agar, pectin, the formation of a paste occurs.

*Jells* are polymer-solvent systems characterized by large turnover deformations with almost no viscous flow. They sometimes use the term "gels", which in colloid chemistry means low-coagulated sols. Historically, the term "gel" first appeared in the study of the polymer system (an aqueous solution of gelatin.) Jells differ from viscous solutions of polymers of the same concentration by structural features, which lead to the fact that instead of the flow develops I'm back strain. These structural features are diverse, which allows to classify certain types of jellies.

To the jellies of the first type, mesh polymers swollen in solvents are included, for example, polystyrene with transverse divinylbenzenium "bridges". Their turnover deformation is caused by the entropic effect of straightening and restoring the folded conformation of sections of macromolecular chains located between chemical cross-linking nodes. Since the energy of the chemical bond is very large, such jellies are reversibly deformed over a wide range of temperatures from the crystallization point
of the solvent to the onset of thermal decomposition of the solvent or polymer at high temperatures.

A variety of jellies of the first type are systems in which stable contacts between macromolecules are provided by local crystallization of a group of chains. The segments of macromolecules between the crystal "nodes" are capable of the same conformational transformations under the action of external loads, like chemically cross-linked polymers, but the upper limit of the region of reversible deformation is limited by the melting temperature of the crystalline units. Above this temperature, the jelly turns into a regular polymer solution. An example of jellies of this type are solutions of polyvinyl chloride with a low degree of crystallinity due to low syndiotactic biomolecules. Local crystallization in this case is responsible for the turnover deformation of the viscoplasticizing products from polyvinyl chloride. Analogous jellies are often formed from solutions of copolymers in which, as a result of the heterogeneous distribution of copolymers in the chain, it becomes possible for the local crystallization of a sequence of identical monomers. Local crystallization is also observed for polymers formed by partial polymer-analogous transformations, for example, in the partial saponification of cellulose derivatives.

Jellies of the second type differ from the jelly of the first type by a distinctly expressed two-phase state. They arise as a result of the decomposition of single-phase solutions of polymers into two phases, the first of which contains a large amount of polymer, forms a predominantly continuous framework, and the second phase with a very low concentration of polymer is included in this frame as a dispersion. The properties of this system are determined by the framework polymer phase, which in many cases approximates properties to the solid and is therefore capable of partial elastic bending. In this case, the overall relatively high deformation of the system consists of the sum of small deformations of the individual elements of the spatial grid that forms this structure. In addition, the change in the shape and length of the interphase boundary contributes to the turnover deformation (the interphase energy has a small, but still finite value). Jellies of the second type are often formed from solutions of protein substances, when polymers are deposited during their processing
into articles (for example, in chemical fibers, in particular during maturation of viscose), from aqueous solutions of methyl and hydroxyethylcellulose. The phase decay is associated with a change in the activity of the solvent due to the introduction of a "nonsolvent" or a sudden change in temperature.

A characteristic feature of jelly is *syneresis* – a decrease in their volume, due to the removal of part of the water. Over time, the number of bonds between polymer chains in gels increases. The structural network of the gel is compressed and a part of the solvent containing a small amount of dissolved polymer is released from it. The process of gel aging with the formation of a denser jelly and diluted polymer solution is called syneresis (from the Greek "sinereiso" – constrict). The influence of various factors on the process of syneresis is the same as on gelling. Interestingly, after syneresis, gels and jellies in a reduced form can retain the shape of dishes. The processes of syneresis occur when the bread, jelly, etc. dry up. The syneresis process is observed during the retraction of the blood clot when a significant decrease in the volume of the blood clot occurs.

Syneresis occurs in the aging process of the body. It is established that with age, a gradual change in the spatial structure (tertiary structure) and a decrease in the hydration of protein molecules occurs. As a result, the tissues become more rigid and less elastic.

*Syneresis is promoted by all the factors that contribute to coagulation:*
- increase the concentration of electrolyte in the system;
- temperature increase;
- introduction into the system of desolvating agents.

Most gels can be diluted with stirring, and then go back to gel in a resting state, gelled. This phenomenon is called *thixotropy* (from the Greek words "tixis" – saking and "tropo" – change). Thixotropic transformations can be repeated many times and proceed at a constant temperature. The formation of gels and jellies is reversible if the particles are bound by weak intermolecular forces (hydrogen bonds, Van der Waals forces, hydrophobic interactions). Thixotropy is one of the proofs that the structure formation in jellies and gels occurs mainly due to van der Waals forces.
6. Influence of HMWC nature, concentration, pH, temperature, electrolytes on the gel formation (jellification)

The process of gelification depends on the shape of the macromolecules, the concentration of the solution, the temperature, and the pH of the solution. The gels are the easiest to form polymers with a sharply expressed asymmetry of the particles.

*Effect of polymer concentration.* The increase in concentration promotes gelation of polymer solutions, since it leads to an increase in the frequency of collisions between macromolecules or their sections and an increase in the number of bonds formed per unit volume. Strongly diluted solutions are not capable of forming. In order for it to occur, the concentration of the solution must be above a certain value. The minimum concentration of polymer necessary for the formation of jelly is called the critical concentration of the formation of the gel. Its value depends on the properties of the polymer, temperature. For example, gelatin solutions can form jelly at room temperature and concentration of 1% (by weight), and agar-agar 0.2%. The hardness of the resulting jelly will be the greater, the greater the concentration of the initial solution.

*Influence of the shape and size of the macromolecule.* Macromolecules have the ability to accept a large number of conformations: from an absolutely stretched state to a tight tangle. This is due to the flexibility of polymer chains. The number of bonds that this macromolecule forms with other molecules depends on their shape. Therefore, in a straightened state, access to those parts that can interact can be facilitated. Consequently, for conditions of formation, conditions are necessary in which the macromolecule does not coagulate. Macromolecules having an elongated shape form jellies even in very dilute solutions.

*Influence of temperature.* The temperature has a very strong effect on the structure formation. Since the mobility of the segments of the macromolecule depends on temperature, an increase in temperature interferes with the formation of structural elements, i.e. formation. Lowering the temperature accelerates the process of formation. There is, so-called, the melting temperature of the jelly, at which the jelly passes into solution. For jelly the temperature of melting and solidification
coincide. A certain conditional temperature is adopted for the gelation point, which corresponds to a viscosity that does not allow the system to flow through the capillary, or the temperature at which the meniscus in the tube does not deform when it is tilted.

*The influence of the pH of the medium* occurs when the HMWC is a polyelectrolyte (amphoteric protein). The best gelification occurs at a pH corresponding to the isoelectric point (pH = 4.8). At this pH, the same number of oppositely charged ionized groups are located along the entire length of the molecular chain, which facilitates the connection between individual macromolecules. With a change in pH on both sides of the isoelectric point, the macromolecules acquire the same charge and repulsive forces arise between them, no bonds are formed. When adding excess amounts of acid or alkali, the degree of ionization of ionogenic groups decreases, and the ability to gelify again increases.

*Influence of indifferent electrolytes.* It is determined by their chemical nature. Gelling and swelling processes are directly opposite, therefore electrolytes that promote swelling complicate the formation of the gel or make it impossible. In general, the process of gelling is affected by anions.

*Osmotic pressure of solutions of biopolymers.* The osmotic pressure of solutions of proteins and other HMWC has a significant effect on a number of processes in the body. The osmotic pressure of solutions of colloids and HMWC is less than in ionic solutions (which contain more particles for the same mass of solute).

Oncotic pressure is a part of the osmotic pressure of biological fluids, formed by proteins. Although the oncotic pressure is only 0.5 % of the total osmotic pressure of the blood plasma (0.038 and 7.7 atm, respectively). But it is of great importance for the transport of water and substances between blood and tissues. The protein content in the blood plasma is much higher than in the intercellular fluid, and the walls of the vessels practically do not pass large protein molecules. Since the intercellular fluid contains significantly fewer proteins, its oncotic pressure is also significantly lower than in the blood plasma, which facilitates the movement of fluid
from the intercellular space into the bloodstream. However, the work of the heart, in turn, forms hydrostatic pressure, which in the arterial part of the capillary significantly increases the oncotic pressure of proteins and, thanks to this difference, the vector of fluid movement is directed from the blood into the intercellular fluid. In the venous part of the capillary, the hydrostatic pressure is already less than the oncotic pressure and is about, so the fluid motion vector is directed from the intercellular space to the vascular bed. With a decrease in the number of proteins in the blood (liver disease, fasting), there is a decrease in oncotic pressure and fluid is retained in the tissues, which leads to the formation of oncotic ("hungry" or "kidney") edema in the subcutaneous tissue.

The osmotic pressure in HMWC solutions is largely dependent on temperature and pH. The increase in temperature in solutions of HMWC increases the osmotic pressure to a greater extent than expected from theoretical calculations. This is due to an increase in the degree of dissociation of ionogenic protein groups and the disaggregation of proteins into microglobules.

Donnan membrane equilibrium. Previously it was mentioned that particles of HMWC cannot penetrate through semipermeable membrane as easy as ions of low molecular weight electrolytes can. However, the presence of dissolved proteins substantially influences the distribution of the electrolytes on both sides of membranes.

If cell containing dissolved proteins and low molecular electrolytes is placed in electrolytic solution containing no proteins, then some of the ions pass through the membrane, and equilibrium will be established. Based on the thermodynamic analysis of the process, F.G.Donnan found that in equilibrium the following ratio is maintained:

\[ x = \frac{C_{\text{ext}}^2}{C_{\text{int}} + 2C_{\text{ext}}} \]

where \( x \) – is amount of electrolyte which is passed into solution of protein;
\( C_{\text{ext}} \) – electrolyte concentration in the external liquid;
\( C_{\text{int}} \) – electrolyte concentration within the cell (internal).
This ratio is called Donnan membrane equilibrium. If \(C_{\text{int}}\) is much higher than \(C_{\text{ext}}\), that is, if the concentration of the electrolyte in the external fluid is low, the numerator is small in the given equation. Division gives even smaller number, i.e. in this case, a low molecular weight electrolyte is in the external liquid preferentially.

If \(C_{\text{ext}} \gg C_{\text{int}}\), the \(C_{\text{int}}\) value of the denominator can be neglected, and then:

\[
\frac{C_{\text{ext}}^2}{2C_{\text{ext}}} = \frac{C_{\text{ext}}}{2}
\]

That is, half of the electrolyte from the external fluid will move inside the cell.

When \(C_{\text{int}} = C_{\text{ext}}\)

\[
x = \frac{C_{\text{ext}}^2}{C_{\text{ext}}} + 2C_{\text{ext}} = \frac{C_{\text{ext}}}{3C_{\text{ext}}} = \frac{C_{\text{ext}}}{3}
\]

i.e., in this case, the one third of electrolyte concentration will move into the cell from external liquid.

So, if the content of the electrolyte within the cell and in the external fluid differ insignificantly, some of the electrolyte enters the cell.

For example, if \(C_{\text{int}} = 0.5 C_{\text{ext}}\)

\[
x = \frac{C_{\text{ext}}^2}{0.5C_{\text{ext}}} + 2C_{\text{ext}} = \frac{C_{\text{ext}}}{2.5C_{\text{ext}}} = \frac{C_{\text{ext}}}{2.5} = 0.4C_{\text{ext}}.
\]

that is, 0.4 of electrolyte amount enters the cell from the external fluid. This leads to increase in osmotic pressure and helps to maintain turgor of cells even in hypotonic solutions.

Since osmotic equilibrium is established at uneven distribution of ions on both sides of the membrane, potential difference on both sides of the membrane – the membrane potential arises in the system.

Polymers of medical purpose and application of HMWC in pharmacy. Polymeric materials have come into wide use in medicine, as details of medical devices and tools, parts of blood transfusion systems, nursing supplies, laboratory equipment, rod-piston to single-use syringes, lugs for intestinal lavage, etc. It is also interesting that a new trend in modern macromolecular chemistry is materials for reconstructive surgery. As bioinert polymers, they meet the operational requirements to the best extent, as shown by the experience of many researchers, polyolefins
(polyethylene, polypropylene) fluorinated polyolefins (fluoroplasts), silicone polymers of siloxane type are used.

**Questions for self-examination**

1. What is the main factor of thermodynamic stability of HMWC solutions?
2. What causes the action of high concentrations of neutral salts in HMWC solutions?
3. What is the name of the ability of HMWC solutions to restore the structure of the gel after mechanical stirring?
4. In the process of biosynthesis in the cells of plants and living organisms, macromolecules of proteins are formed. Identify the products whose reactions they have.
5. Macromolecules of albumin albumin are spherical or ellipsoidal in shape, they are folded into a dense, compact structure. Specify the structural type of these macromolecules.
6. Salting and hardening of proteins is most expedient when the biopolymers are in the isoelectric state. Specify the pH value that corresponds to this state.
7. One of the important physicochemical properties of biopolymer solutions is thermodynamic stability. Specify the reason for this property.
8. Electrophoresis of various protein solutions is one of the methods for determining the isoelectric point of a protein. Specify the factor that determines the mobility of the protein macromolecule in this method.
9. With decreasing solubility in solutions of high-molecular compounds, the process of fusion of aqueous shells of soluble molecules of biopolymers is observed. Specify the name of this process.
10. In 1911, Donnan proposed an equation explaining the effect of the concentration of various substances for the vital activity of cells, the magnitude of biopotentials, and so on. Specify the process that describes this equation.
11. Fractional precipitation of blood serum proteins is carried out by seeding with preservation of the native structure. Specify the medications that can be obtained
in this way.

**Algorithm of laboratory work "Protection of colloidal solutions from coagulation by means of HMW compounds"**

1. Preparation of the ferric hydroxide colloidal solutions with addition of various amounts of gelatin solution.
2. Study of coagulation of solutions obtained by electrolyte addition.
3. Calculation of the coagulation threshold in the absence and in the presence of the polymer.

*Method of the experiment*

Fill in 6 test tubes with 5 ml of iron (III) hydroxide or Berlin blue positively charged sol. Add 0,2 ml of 0,1% gelatin solution or 0,5% starch solution to the sol in the first test tube, 0,4 ml – in the second test tube, 0,6 ml – in the third test tube, 0,8 ml – in the fourth test tube, 1,0 ml – in the fifth one. Don’t add polymer solution to the sixth test tube which serves as a control. In 2-3 min add 0,05 M sodium sulfate solution drop wise from burette to each test sol until coagulation occurs.

Calculate coagulation threshold (C), mmol/L, using the formula:

\[
C(\text{Na}_2\text{SO}_4) = \frac{C(\text{Na}_2\text{SO}_4) \cdot V(\text{Na}_2\text{SO}_4) \cdot 1000}{V(\text{sol}) + V(\text{Na}_2\text{SO}_4)}
\]

Compare data obtained and make conclusions about the influence of polymers on sol stability. Write the formula of sol micelle.

- **list of works to be studied:** after studying the subject student should be able to determine the isoelectric point of the protein, the degree of swelling, the stability of the polymer in respect with electrolytes, the protective action of the HMW compounds solutions, to apply Donnan membrane equilibrium for estimation of the distribution of electrolytes in living systems.

8. **Tasks for knowledge control**

1. The main factor of thermodynamic stability of polymers solutions is:
A. High molar mass  
B. Lyophilic property  
C. Lyophobic property  
D. The spatial structure  

2. Addition of high concentrations of neutral salts on HMW compounds solutions causes:  
A. Coagulation  
B. Coacervation  
C. Salting out  
D. Flocculation  

3. The ability of HMW solution to restore gel structure after mechanical demolition is called:  
A. Syneresis  
B. Thixotropy  
C. Coacervation  
D. Salting out  

Answers: 1 - B; 2 - C; 3 - B.  

9. Recommendations for the work results design  
Algorithms for solving educational problems of class work and self-work should be recorded in the workbook. Make a protocol of laboratory work, conclusions about the action of polymers as protective substances on the stability of colloidal solutions.
Defence of the course paper «Chemistry of biogenic elements»

Students should compile a files about biogenic role of the following chemical elements: lithium, sodium, potassium, calcium, magnesium, iron, zink, manganese, phosphorus, sulfur, selenium, chlorine, bromine, iodine, fluorine, copper, chromium, cobalt, aurum, silver, barium, strontium.

Every file must contain the following information about biogenic element:
1. Type of element (micro- or macroelement).
2. Body-element need.
3. Topography of the element in the human body.
5. Element-based medicinal products.
6. Food products that can be used to supplement the content of the element in the body.
Навчальне видання

Властивості розчинів біополімерів. Ізоелектрична точка білка.
Захист курсової роботи з «хімії біогенних елементів»

Методичні вказівки для самостійної роботи студентів 1-го курсу з медичної хімії

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