Ministry of Health of the Republic of Belarus
Educational institution
"Gomel State Medical University"

Department of General and Bioorganic Chemistry

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# METHODOLOGICAL MANUAL

for conducting the laboratory class with the first-year students
of the Faculty of International Students
studying in the specialty 7-07-0911-01 "Medical business" (FIS)
English-speaking students
in the discipline "Medical chemistry"

## Topic 17: Physico-chemistry of biopolymers and their solutions

Time: 2 hours

2023

## THE TRAINING AND EDUCATIONAL GOALS, MOTIVATION TO STUDY THE TOPIC

**The purpose of the class:**

To acquaint medical students with the preparation and physico-chemical properties of solutions of high molecular compounds (HMC), as well as with various methods for determining the isoelectric point of proteins. To consider the theoretical bases of electrophoresis of HMC solutions, as well as its application areas.

**The tasks of the class:**

As a result of the class, the student ***must know:***

1) general characteristic of the HMC;
2) the concept of biopolymers and synthetic HMC, their use in medicine;
3) methods of HMC obtaining, specific examples;
4) theoretical bases of the HMC dissolution process;
5) comparative characteristics of the properties of HMC solutions and colloidal solutions;
6) theoretical bases of HMC swelling and factors affecting swelling;
7) the concept of polyelectrolytes and proteins as amphoteric polyelectrolytes;
8) the concept of the isoelectric point of the protein and methods of its determination;
9) theoretical basis of electrophoresis of HMC solutions and its applications.

The student ***must be able to:***

1) compare the degree of swelling and the degree of coagulation of the protein at different pH values, knowing its isoelectric point;
2) determine in what form (cationic or anionic) a protein with a known isoelectric point value will exist if placed in a solution with a certain pH value;
3) indicate the direction of protein movement during electrophoresis in a solution with a certain pH value;
4) knowing the isoelectric point of the enzymes, determine the pH values at which the mixture of enzymes can be separated by electrophoresis.

**Motivation to study the topic:**

High-molecular compounds (HMC, polymers) are substances whose macromolecules consist of a large number of monomeric units with a high molecular mass from few thousand to several million.

Proteins, nucleic acids, polysaccharides, as well as an increasing number of synthetic polymers, are high-molecular compounds.

Synthetic polymers are widely used in medicine for the production of prostheses, vessels, joints, heart valves, eye lenses, various tissues. Synthetic polymer fibers are used as suture material in surgical practice. Special polymers have been created, from which blood transfusion equipment is made.

HMC of wildlife are called biological or biopolymers. These are proteins, carbohydrates, nucleic acids, complex peptides. Biopolymers are formed during biosynthesis in cells and are the most important components of all living organisms. Gels and jellies include muscle fibers, cartilage, cellular and other membranes and membranes in the body. The study of the structure and properties of biopolymers is necessary for the knowledge of the most important biological processes – the basis of life. Familiarity with the nature of the phenomena of swelling and gelatinization contributes to a better understanding of many physiological processes: inflammation, edema formation, tissue regeneration, etc.

Many HMC have physiological activity and are used as substitutes for blood plasma (polyvinyl alcohol), in the treatment of burns (polyvinylbutyl ether), as prolongators of the action of drugs (cellulose derivatives, polyethylene glycols, polyacids, etc.), to stabilize medicinal colloids, suspensions and emulsions.

The main task is to study the physico-chemical properties of these compounds that are important in the activity of a doctor.

## NECESSARY EQUIPMENT

1. Methodological manual for students on the topic "Physico-chemistry of biopolymers and their solutions".

2. Reference materials of physico-chemical constants for the 1st year education international students.

3. Chemical reagents and equipment necessary for laboratory work.

## CONTROL QUESTIONS ON THE TOPIC OF THE CLASS

1. General characteristic of high molecular compounds (HMC) and their classification.

2. Polymers' solutions.

3. Polyelectrolytes. Isoelectric point and methods of its determination.

## COURSE OF THE CLASS

**The theoretical part**

### 1. GENERAL CHARACTERISTIC OF HIGH MOLECULAR WEIGHT COMPOUNDS (HMC) AND THEIR CLASSIFICATION

**High molecular compounds (HMC)** are substances having a high molecular mass from few thousand to several million. The length of HMC macromolecules in the elongated state is about 1,000 nm.

Classification of high molecular compounds:

*1. According to their origin:*

▪ natural HMC (biopolymers) are substances of plant and animal origin: proteins, nucleic acids, polysaccharides (starch, cellulose, dextrans), natural rubbers;

▪ synthetic HMC – polyethylene, dacron, polystyrene, nylon, etc. – are products of polymerization and polycondensation reactions.

*Polymerization reactions*:

a) polyethylene: $nCH_2 = CH_2 \rightarrow [–CH_2–CH_2–]_n$

Polyethylene is applied in medicine to prepare films, tubes and bottles.

b) teflon: $nCF_2 = CF_2 \rightarrow [–CF_2–CF_2–]_n$

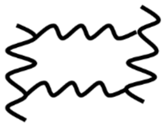
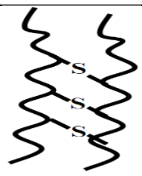
Teflon is applied to prepare cooking utensils, electrical insulation, and also blood vessels and heart's vents for transplantation.

*Polycondensation reactions*: nylon is a polycondensation product of adipic acid and hexamethylenediamine:

$$nHOOC–(CH_2)_4–COOH + nH_2N–(CH_2)_6–NH_2 \rightarrow$$
$$\rightarrow [–CO–(CH_2)_4–CO–NH–(CH_2)_6–NH–]_n + nH_2O$$

*2. According to the configuration of macromolecules* (Table 1):

Table 1 – Classification of high-molecular compounds according to the configuration of macromolecules

| Type of configuration | Representation of configuration | Examples of HMC |
|---|---|---|
| ▪ linear (long threadlike straight molecules) |  | gelatin, cellulose, natural rubber |
| ▪ branched-chain |  | amylopectin of starch, glycogen |
| ▪ three-dimension |  | phenol formaldehyde resins |
| ▪ cross-linked |  | vulcanized rubber |

*3. According to the values of molar masses:*

▪ monodisperse are polymer systems whose molecules have the same molar mass (hemoglobin and other proteins);

▪ polydisperse are polymer systems whose molecules have different molar masses (DNA, rubbers).

The molar mass for such polymers can be determined as:

$$M = \frac{n_1 \times M_1 + n_2 \times M_2 + ...}{n_1 + n_2 + ...}$$

$M_1$ and $M_2$ are molar masses of polydisperse polymer molecules;
$n_1$ and $n_2$ are the numbers of molecules with molar masses $M_1$ and $M_2$, respectively.

*4. According to elemental composition:*

▪ homochain: polymer chains consist only of carbon atoms; they are obtained by polymerization reactions (teflon);

▪ heterochain: contain not only carbon in the chain, but also heteroatoms (N, S, etc.); they are obtained by polycondensation reactions (nylon).

HMC molecules have a number of specific properties, among which the most important are: the flexibility of chains, the presence of strong intermolecular bonds [1-3].

## 2. POLYMERS' SOLUTIONS

Polymers can form true and colloidal solutions. True solutions are formed when a polar polymer dissolves in a polar solvent (protein in water) or when a nonpolar polymer dissolves in a nonpolar solvent (rubber in benzene).

The first step of the polymer's dissolution process is swelling. Swelling is the spontaneous irreversible diffusion of solvent molecules into the polymer phase. Polymer's molecules do not diffuse into the solvent due to their low mobility caused by high molar mass and strong intermolecular forces.

Swelling leads to an increase in the volume and mass of the polymer. The degree of swelling (α) can be calculated as:

$$\alpha = \frac{V - V_o}{V_o} \times 100 \quad \text{or} \quad \alpha = \frac{m - m_o}{m_o} \times 100$$

$V_o$ is an initial volume of a polymer, ml; $m_o$ is an initial mass of a polymer, g;
V is a volume of a polymer after swelling; m is a mass of a polymer after swelling.

There are two types of swelling: limited and unlimited. Limited swelling leads to the formation of gels (for example, gelatin in cold water). Gel is a state of matter intermediate between solids and liquids. Unlimited swelling leads to the formation of true solutions that are stable systems (for example, gelatin in hot water).

Swelling is an important physiological process. The tissues of the skin, brain and eyes are able to swell. The degree of swelling may change as a result of inflammation or injury. Aging reduces the degree of swelling of tissues.

Swelling is affected by different factors:

a) nature of macromolecules and nature of solvents: «Like dissolves like»;

b) configuration of chains: linear and branched polymers exhibit higher degree of swelling than three-dimension and cross-linked compounds;

c) acidity of a medium (for amphiprotic polyelectrolytes only);

d) temperature: the higher the temperature, the greater the degree of swelling, as increase in temperature leads to increase in the rate of diffusion of solvents into the polymer;

e) presence of electrolytes; they may affect the solubility of polymers in two different ways:

1) *salting-in effect* – the increase in solubility under the influence of electrolytes;

2) *salting-out effect* – the decrease in solubility under the influence of electrolytes [1-3].

## 3. POLYELECTROLYTES. ISOELECTRIC POINT AND METHODS OF ITS DETERMINATION

**Polyelectrolytes** are polymers containing ionizable groups. They are divided into three categories: acidic, basic and amphiprionic.

**Acidic polyelectrolytes** contain ionizable groups that are proton donors:

$$-COOH \rightleftharpoons -COO^- + H^+$$
$$-SO_3H \rightleftharpoons -SO_3^- + H^+$$

**Basic polyelectrolytes** contain ionizable groups that are proton acceptors:

$$-NH_2 + H^+ \rightleftharpoons -NH_3^+$$

**Amphiprotic polyelectrolytes (polyampholytes)** contain both acidic and basic ionizable groups. The bright example of such polyelectrolytes are proteins. The simplified form of the protein can be represented as **$NH_2$ – R – COOH**.

An important feature of proteins and some other biopolymers is that their total charge depends on the pH of medium. Under physiological conditions, proteins exist in the form of dipole ions **$NH_3^+$–R–$COO^-$**, the net electric charge of their molecules is zero. In acidic environments, protons attach to basic groups, and the total charge is positive (cationic form). In basic media, the total charge is negative as a result of the loss of protons (protein turn into anionic form). The acid-base equilibrium in protein solutions is shown in Figure 1.

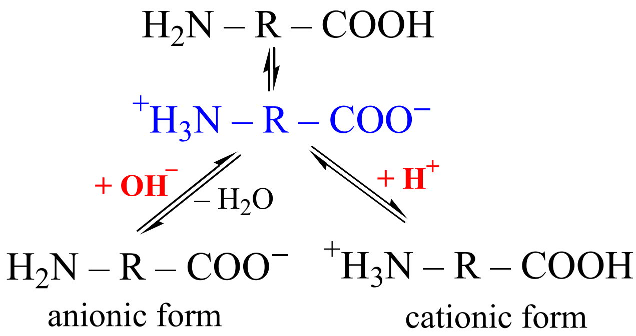



Figure 1 – The acid-base equilibrium in proteins solutions

Proteins exhibit their specific properties **at the isoelectric state**, which is characterized by the zero net electric charge of macromolecules:

$$\mathbf{NH_3^+–R–COO^-}$$

**Isoelectric point (IEP, pI)** is the pH value at which protein is present in the isoelectric state (the total charge of the macromolecule is zero). Isoelectric point is an important characteristic of each protein.

In isoelectric state a protein molecule coils about itself in a spiral manner to form a helix (α-helix configuration). When the pH of the solution is greater than pI or lower than pI, the protein molecule becomes linear due to repulsion of charged functional groups ($NH_3^+$ in acid solutions and $COO^-$ in base solutions) (Fig. 2).

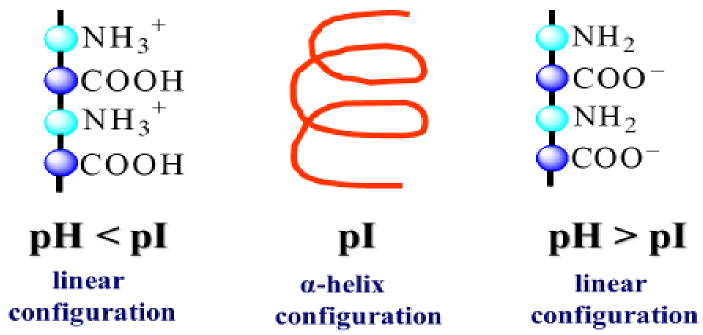



Figure 2 – Configuration of protein molecule in solutions with the difference pH values

A unique configuration of a protein molecule at isoelectric state is responsible for its specific physical and chemical properties. The specific physical properties of proteins at isoelectric state are:

▪ minimum degree of swelling;
▪ maximum degree of coagulation;
▪ zero electrophoretic mobility.

**Methods of experimental determination of pI of proteins:**

**1.** By measuring the *degree of swelling* of proteins in solutions with different acidity (Fig. 3):

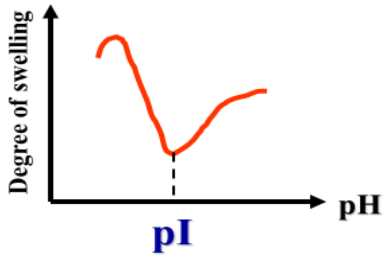



Figure 3 – Degree of swelling of protein in solutions with the different pH values

**2.** By measuring the *degree of protein's coagulation* in solutions with different acidity (Fig. 4):

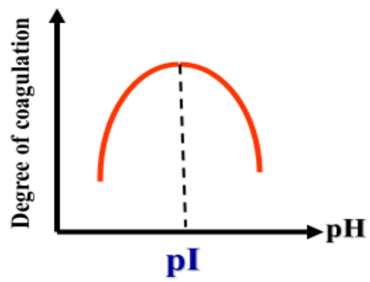



Figure 4 – Degree of coagulation of protein in solutions with the different pH values

**3.** By measuring *the electrophoretic mobility* of proteins (u) in solutions with different acidity.

Electrophoresis is the migration of charged molecules in a solution in response to an electric field. In the isoelectric state, the electrophoresis mobility is zero (u = 0), since the total electric charge of the macromolecule is zero. Positive signs of electrophoresis mobility indicate the movement of charged macromolecules to the cathode, and negative signs of electrophoresis mobility indicate the movement of charged macromolecules to the anode. The curve of electrophoretic mobility of proteins is shown in Figure 5:

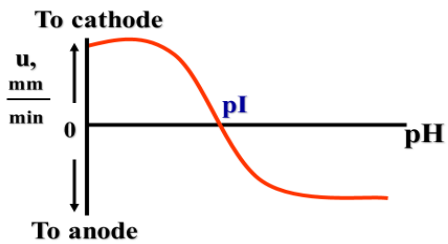



Figure 5 – The curve of protein's electrophoretic mobility

Electrophoresis is used not only to determine pI of proteins, but also to separate their mixtures into fractions [1-3].

**The practical part**

Safety instructions before laboratory work.

**LABORATORY WORK No. 1**

***Test 1.*** *Determining of rubber swelling percent in various solvents*

Weigh four rings of rubber and put them in sample bottles filled with various solvents: a) water; b) benzene; c) turpentine oil; d) hexane. After 30 minutes, take the rings out of the solutions and weigh them again. Write down the initial and final masses of rubber in Table 1. Calculate the degree of swelling of rubber according to the formula:

$$\alpha = \frac{m - m_0}{m_0}$$

$m_o$ – initial mass of a rubber, g;

m – mass of a rubber after swelling.

Table 2– Swelling of rubber

| № | Solvent | Initial mass of rubber **($m_0$)**, g | Mass of rubber after swelling (**$m_2$**), g | Degree of swelling, **α** |
|---|---|---|---|---|
| 1.<br>2.<br>… | | | | |

Make a conclusion about how the polarity of the solvent affects the degree of swelling of the rubber.

***Test 2.*** *Determination of the isoelectric point of the protein*

Take 5 tubes and pour 1 ml of acetate buffer ($CH_3COOH$ + $CH_3COONa$) into each with the following pH values: 3,8; 4,1; 4,7; 5,3; 6,2.

Add 0.5 ml of 1 % albumin solution and 1 ml of acetone (use analytical pipettes) into each test tube. Stir the prepared solutions and determine turbidity in each test tube giving marks from 0 up to 5. Write the obtained data into the Table 2.

Table 3 – Determination of the isoelectric point of the protein

| pH | 3.8 | 4.1 | 4.7 | 5.3 | 6.2 |
|---|---|---|---|---|---|
| Degree of turbidity | | | | | |

The highest turbidity corresponds to the maximum coagulation of the protein and its isoelectric point

**Control over the assimilation of the topic**

It is conducted in the form of an oral conversation with students.

## QUESTIONS FOR SELF-CONTROL OF KNOWLEDGE

1. What substances are called high molecular compounds (HMC)? Give examples of natural and synthetic polymers. Classify macromolecules according to configuration of their chains. What is the difference between homochain and heterochain polymers?

2. What methods are used for HMC preparation?

3. What are the features of dissolving the HMC? What process is called swelling? What factors affect swelling and dissolving of high molecular compounds?

4. What is the difference between polyelectrolytes and polyampholytes? What is the state of the protein called isoelectric? What is the isoelectric point of protein? What methods may be used of its determination?

***Exercises for the self – control***

**1.** The initial mass of a rubber was 10 g. After swelling in benzene, its mass became equal 22 g, and after swelling in toluene – 41.4 g. In what solution is the degree of swelling of rubber is higher?

*Answer: in toluene*

**2.** In which solvent – water, alcohol, benzene or physiological solution – does the gelatin show the highest degree of swelling? Explain your answer.

**3.** pI of gelatin is 4.7. Gelatin was dissolved in a solution with protons ($H^+$) concentration 1000 times higher than that in pure water. What electric charge (positive or negative) will gain gelatin's molecules in this solution?

*Answer: positive*

**4.** In what pH range is it better to separate proteins by electrophoresis, if their isoelectric points are 4.8 and 7.9, respectively. Explain your answer.

*Answer: at pH from 4.8 to 7.9 inclusive*

## QUESTIONS FOR INDEPENDENT WORK OF STUDENTS (IWS)

1. Biological macromolecules: polysaccharides, proteins, nucleic acids. Their monomeric units, spatial structure.

2. Thermodynamic stability of HMC solutions. Methods of proteins precipitation.

## LIST OF SOURCES USED

1. Medical chemistry : textbook for students of higher education establishments – med. univ., inst. and acad. / V.O. Kalibabchuk, V.I. Halynska, L.I. Hryshchenko et al. ; ed. by V.O. Kalibabchuk. – 6th ed., corr. – Kyiv : AUS Medicine Publishing, 2018. – P. 168-183.

2. Основы химии для иностранных студентов = Essential chemistry for foreign students : учебно-методическое пособие / С. В. Ткачёв [и др.]. – 5-е изд. – Минск : БГМУ, 2018. – 168 с. – Режим доступа: http://rep.bsmu.by:8080/handle/BSMU/21054.

3. Общая химия : учеб. пособие для студентов лечебного факультета, обучающихся на английском языке. В двух частях. Часть 2. = General Chemistry : Educational guidance for students medical department in English medium. In two parts. Part 2. – В. А. Филиппова, А. В. Лысенкова, Л. В. Чернышева. – Гомель : УО «Гомельский государственный медицинский университет», 2013. – 180 с. – URI: http://elib.gsmu.by/handle/GomSMU/10939.