MINISTRY OF HEALTH OF UKRAINE
ODESA NATIONAL MEDICAL UNIVERSITY
Department of Medical Biology and Chemistry

CONFIRMED by
Vice-rector for scientific and pedagogical work
Eduard BURIACHKIVSKYI
"1" 09 2024

METHODOLOGICAL DEVELOPMENT FOR INDEPENDENT WORK OF STUDENTS IN THE DISCIPLINE "MEDICAL CHEMISTRY"

Department, course **International faculty, 1st course**

Specialty: **221 "Dentistry"**

Discipline **Medical chemistry**

The program was approved:
at a meeting of **the Department of Medical biology and chemistry** Odesa National Medical University
Minute № 1 dated 26 august 2024
Head of Department _____ Hennadii STEPANOV
(signature)

Developers:
associate professor of the department, Ph.D., Assoc. Burdina Y.F.
associate professor of the department, Ph.D. Grekova A.V.
associate professor of the department, Ph.D., Assoc. Shirykalova A.O.
assistant of the department Gridina I.R.

Practical lesson 1

Topic: Subject and tasks of medical chemistry. Classification of nutrients.

Relevance of the topic: The biogenicity of chemical elements are the properties of the elements that the human body needs for the construction and normal functioning of all organs and cells. Biogenic elements, depending on their classification, are divided into: 1) macro-, micro-, ultramicroelements; 2) s-elements, p-elements, d-elements and f-elements. The content of biogenic chemical elements is determined by the influence and distribution in the body of the chemical elements themselves, and also depends on the environmental conditions of the body. Established 30 biogenic elements. Therefore, this topic is the basis for the subsequent study of complex metabolic processes in the organism and their correction with drugs.

Lesson objective: To form in medical students a systematic knowledge of chemical elements in the human body, evaluate the importance of these elements on the cellular level, correctly interpret the results of exposure to chemicals on the body, which is necessary for studying other disciplines and the acquisition of professional medical skills.

Basic definitions: V.I. Vernadsky's doctrine, biogenic elements, nutrients, macro-, micro-, ultramicroelements, s-elements, p-elements, d-elements.

Plan and organizational structure of the lesson:

1. Chemistry in medicine.
2. Classification of biogenic elements.
3. The concept of biogenic elements. Organogens.
4. Quantitative and qualitative composition of biogenic elements in the human body.
5. The essence of V.I. Vernadsky and his school about the biosphere and biochemistry, the concept of biogeochemical provinces and endemic diseases.
6. Classification of biogenic elements according to the electronic structure of atoms of s-, p-, d-blocks, according to the quantitative content in the human body, physiological activity, knowledge, biological role.

Content of the topic

M.V Lomonosov, considering the commonwealth of sciences as a necessary condition for the development of natural science, said that a physicist is blind without mathematics, a dry hand without chemistry.

Many years later, an outstanding scientist of the XX century. V.I.Vernadsky noted that the development of scientific knowledge is rapidly reducing the distance between individual sciences, and scientists are increasingly specializing not in sciences, but in problems. This makes it possible, on the one hand, to delve into the study of phenomena, and on the other hand, to cover them as widely as possible from all points of view.

This specialization creates the most important prerequisite for the interaction and interpenetration of sciences. The mutual influence of the natural sciences is a natural phenomenon characteristic of the entire history of natural history. The places of collision between separate sciences were bridges over which there was an interpenetration of one knowledge into another. The study of the process of interaction between chemistry and related sciences helps to understand it in the system of natural sciences in the development of natural science.

E. Fisher (1849) wrote that no other science of the naturalist is connected with medicine with such a strong and deep connection as chemistry.

The formation of the foundations of scientific, experimental and theoretical chemistry in the XVII century (new ideas about chemical elements, the first quantitative laws, the use of methods - weight and gas analyzes; the creation of devices designed to study the thermal effects of chemical reactions, etc.) led to its further rapid development and active interaction of chemistry with biology.

Concerning the problem of interaction between chemistry and biology, organic chemistry is of greatest interest.

J. Berzelius (1807) proposed to call substances that belong to living nature organic, and substances that belong to inanimate nature – inorganic.

The first attempts to apply methods from physics to solve environmental problems were made in the XVIII century, after E. Euler found out the movement of blood in the

vessels. Outstanding physiologist I. M. Sechenov studied the problem of gas absorption by blood and saline solutions. Investigating the state of CO_2 in the blood, I.N Sechenov (1886) discovered the law of dissolution of gases in aqueous solutions of electrolytes, which are the basis of the modern theory of the respiratory function of blood.

At the beginning of the XX century. arose immunochemistry – a science that studies the chemical processes of immune phenomena. In the meantime, the attention of chemical scientists was attracted by the problem of the interaction of antigen - antibody and toxins - antitoxins. Outstanding chemists P. Yerlich and S. Arrhenius were engaged in its solution. Most of the substances of an animal organism, osmotic and capillary phenomena play an essential role in life processes, therefore biologists have paid special attention to physical chemistry, its laws and theories.

Early XX century was marked by the emergence of a new fundamental concept of the geochemical role of substances in geological processes, which formed the basis of biogeochemistry – a science that studies the chemical processes of the earth's crust, depending on the development of the organic world. V.I. Vernadsky proved that living matter, living organisms, in the process of life, carried out and are carrying out large-scale transformations of the earth's crust. For example, "biogenic migration of atoms" covers part of the atmosphere, hydrosphere, and upper lithosphere. Just ten years ago, at the crossroads of inorganic and biological chemistry, a new related field was born - bioinorganic chemistry. Within such a short time, inorganic chemistry has become an independent science, which continues to develop rapidly. The objects of her research are biocomplexes. The complexes include biometals – "metals of life" (Cu, K, Ca, Mn, Co, Mo, etc.), and various atoms, molecules, and ions are used as ligands.

<u>Bioinorganic chemistry is developing in such directions:</u>

1. Research of biologically active substances as carriers of oxygen and ions (ionophores).
2. Research of antitoxins and migration of toxic metals in nature.
3. Study the structure and mechanism of action of metalloenzymes.
4. Research and reconstruction of the most important biochemical processes (biochemical modeling of biological structures).

Classification of nutrients

The scientific substantiation of the doctrine of chemical elements was obtained in the works of Academician **V.I. Vernadsky**, who showed a close relationship between the chemical composition of the earth's crust, the world's oceans and a living organism. He believed that living organisms and the earth's crust represent a single system, and living organisms participate in the geochemical processes of the distribution of chemical elements in the earth's crust.

The close connection of the living with the inanimate is manifested primarily in the generality of the elemental composition. Substances of animate and inanimate nature consist of the same chemical elements, the same forces of chemical interaction act between them. The elemental composition of a living organism completely coincides with the elements found in sea water, and the latter almost correspond to the composition of the earth's crust.

Studying the migration of elements, V.I. Vernadsky established that the migration, dispersion and concentration of elements depends on the atomic mass of a chemical element, the size of atomic and ionic radii, and also on the ability of elements to form chemical compounds.

All chemical elements that are involved in the biological processes of living organisms are called **biogenic elements.**

The chemistry of biogenic elements was further developed in the works of A.P. Vinogradov, V.V. Kuznechny, A.I. Venchikov, K. B. Yatsimirsky, M. Dixon and E. Webb, A. I. Voinar, E. Underwood, G.A. Babenko and others.

The quantitative content of chemical elements in living matter (living matter is the totality of all living organisms) is inversely proportional to their serial numbers in the periodic table of elements, that is, the quantitative chemical composition of living matter is a periodic function of the serial number of an element. However, this pattern is violated for the elements of the main subgroups I, II and VII groups. Violation of the noted pattern is observed due to the fact that they are included in the molecules of bioorganic compounds.

The quantitative content of covalently bonded atoms of elements decreases with an increase in the charge of atoms in a group (for example, N, P, As, Sb), and elements that are in the body in the form of ions (s-elements of groups I and II, p-elements of group VII) – increases (to the optimal ionic radius), and then decreases. For example, during the transition from beryllium to calcium, the content of the element in a living organism increases and then decreases; in the transition from fluorine to chlorine, it also increases, and then decreases (Table 1).

Table 1

Dependence of the quantitative content of chemical elements in living organisms on their nuclear charge

Element	Content, mass fraction, %	Element	Content, mass fraction, %
$_{4}$Be	10^{-7} -10^{-4}	$_{9}$F	10^{-5}
$_{12}$Mg	$2{,}7 \cdot 10^{-2}$	$_{17}$Cl	$1 \cdot 10^{-1}$
$_{20}$Ca	2,0	$_{35}$Br	10^{-4} - 10^{-3}
$_{3S}$Sr	10^{-3} - 10^{-2}	$_{53}$I	10^{-5} -10^{-3}
$_{56}$Ba	10^{-5} -10^{-4}		

Of all biogenic elements, eleven (O, H, N, S, Ca, Mg, K, Na, Cl, P, C) make up 99.5% of the body's mass. The content of all other elements is less than 0.5%. Natural selection of elements was due to such factors:

- the ability to form strong (energy-intensive) ties;
- the ability to form chains;
- lability of connections;
- "lability" of atoms, for example: S, P, Fe (by J. Bernal);
- the formation of compounds readily soluble in water, which contributed to their concentration in the body;
- the tendency to form stable coordination compounds with biological molecules.

Human organs concentrate different chemical elements in themselves in different ways, i.e. micro- and macroelements are unevenly distributed between

different organs and tissues. Most trace elements accumulate in the liver, bone and muscle tissue. These tissues are the main depot (storehouse) for many trace elements. Elements can show specific affinity for certain organs and are contained in them in high concentrations. It is well known that zinc is concentrated in the pancreas, iodine – in the thyroid, fluor – in the enamel of teeth, aluminum, arsen, vanadium accumulate in hair and nails, cadmium, mercury, molybdenum – in the kidneys, stanum – in the intestinal tissues, strontium – in the prostate gland, bone tissue, barium – in the pigment retina of the eye, bromine, mangan, chromium – in the pituitary gland, etc. Hydrogen and oxygen are macroelements. They are part of water, which in the body of an adult contains about 65% on average. Water is unevenly distributed over human organs, tissues and body fluids. So, in gastric juice, saliva, blood plasma, lymph, water is from 90 to 99.5%. In urine, gray matter of the brain, liver, skin, spinal cord, muscles, lungs, heart - 70-80%. Least of all – 40% of water is contained in the skeleton.

Macronutrients – C, H, O, N, S, P – are part of proteins, nucleic acids and other biologically active compounds of the body. C, H and O are also part of carbohydrates, the content of which in the tissues of animals and humans is small (about 2%). These elements are part of lipids (fats). In addition, phospholipids contain phosphorus in the form of phosphate groups. Lipids are most concentrated in the brain (12%), and then in the liver (5%), milk (2-3%) and blood serum (0.6%). However, the main part of phosphorus – 600 g – is contained in bone tissue. This is 85% of the mass of all phosphorus in the human body. Phosphorus is also concentrated in the hard tissues of the teeth, in which it is included together with calcium, chlorine, fluorine in the form of hydroxyl, chlorine, fluorapatites of the general formula $Ca_5(PO_4)_3X$, where X = OH-, Cl-, F-, respectively. Calcium is predominantly concentrated in bone tissue, as well as in dental tissue. Sodium and chlorine are mainly found in extracellular fluids, while potassium and magnesium are found in intracellular fluids. In the form of fluorides, sodium and potassium are found in bone and dental tissue. Magnesium in the form of phosphate $Mg_3(PO_4)_2$ is found in the hard tissues of the tooth. Some macronutrients (magnesium, calcium) and most trace elements are contained in the body in the form of complexes with bioligands – amino acids, proteins, nucleic acids, hormones,

vitamins, etc. For example, the Fe^{2+} ion as a complexing agent is part of hemoglobin, Co^{2+} is a vitamin B12, Mg^{2+} – to chlorophyll. Numerous biocomplexes of other elements (Cu, Zn, Mo, etc.) are known that play an important biological role in the body.

Developing the ideas of V.I. Vernadsky about the role of the elemental composition of soil in the evolution of organisms, **A.P. Vinogradov** developed a study on biogeochemical provinces – areas with a high or low content of any element in them - and endemic diseases caused by the associated content **of elements in the human body**.

V.V.Kovalsky created the science of geochemical ecology – biochemical and physiological adaptations of the organism to the chemical elements of a given environment. According to Kovalsky, most organisms adapt to the unusual content of certain elements and develop normally. And only from 5 to 20% of organisms in these conditions suffers from endemic diseases.

There are a number of different classifications of chemical elements that are found in the human body.

By their quantitative content, they are divided into:

- ❖ macronutrients (10% or more) are C, H, O, P, N, Na, K, Ca, Mg, Cl;
- ❖ trace elements (10^{-3} -10^{-12}%) – Mn, Zn, Cu, Co, Fe, I, Mo, Ba, As, Br etc .;
- ❖ ultramicroelements (less than 10^{-12}%) –-Ra, etc.

However, such a classification does not indicate the role and significance of this or that element in the body. **V.V.Kovalsky**, according to their knowledge and significance, divided chemical elements into three groups:

- ✓ elements that are constantly present in a living organism, participate in the metabolism, are irreplaceable;
- ✓ elements that are constantly in the body, but their biological role is poorly understood;
- ✓ elements that are constantly in living organisms, but their biological role has not been clarified.

A.I Venchikov believed that chemical elements, regardless of their quantitative content, should be called biotic elements if their physiological activity has been proven. According to A.I. Venchikov, biotics are chemical elements of exogenous origin, which are part of the biochemical structures and systems of the body, which take part in biochemical and physiological processes and are able to increase the body's resistance to the action of harmful agents. It follows from this definition that both macro- and microelements that are part of vitamins, enzymes and other substances that are necessarily involved in metabolic processes can be classified as biotics.

In accordance with this classification, elements that play the role of a plastic material in the body are distinguished into a separate group. This group, in addition to C, N, O, H, S, P includes the macronutrients Na, Ca, K, Mg, Cl,.

The next group includes elements that activate the enzymatic processes of the body or are included in the structure of enzymes (Zn), vitamins (Co), hormones (I).

The third group includes so-called reticuloendothelial elements (As, Hg, Sb, etc.), which suppress the vital activity of microbes.

A.P. Vinogradov proposed a fundamentally new classification, based on which the biological role of elements depends on the electronic structure of their atoms, that is, on the position in the periodic system of DI Mendeleev. Based on the electronic structure of atoms, elements of s-, p-, d-blocks are referred to as biogenic elements. The electronic structure of an atom determines its behavior in chemical reactions, affects the types of chemical bonds formed by it in compounds.

Practical lesson 2

Topic: Typical chemical properties, biological role and application in medicine of biogenic s-, p- and d-elements. Qualitative reactions for the determination of s-, p-, and d-elements. Degree and constant of hydrolysis. The role of hydrolysis in biochemical processes.

Relevance of the topic: Of the 117 elements in the periodic system, 62 of them have a biological function. And 12 elements are vital. These elements are part of hormones, enzymes, vitamins. They participate in their biosynthesis, increase and regulate their activity. Physiological action of elements depends on their concentration. Studying this topic, students acquire knowledge that will help them to study biochemical processes that occur in the human body, human physiology in conditions of ecological crisis on the planet, as well as the use of derivatives of chemical elements as medicines.

Lesson objective: to form a systematic knowledge of students about the physical, chemical and biological properties of s-, p- and d-elements, their important compounds, their use in medicine; the ability to work with literature, chemical glassware and reagents; to learn to identify biogenic metal cations and salt anions.

Basic definitions: s-, p-, d-elements, solubility, solubility product, salt hydrolysis, analytical reactions.

Plan and organizational structure of the lesson:

1. Position of s-elements in the Periodic Table of Elements. Characteristics of biogenic s-elements. The biological role of s-elements.
2. Solubility. Solubility product
3. General characteristic of p-elements. Electronic configuration of atoms p-elements.
4. General characteristic of d-elements. Electronic configuration of atoms d-elements.
5. Hydrolysis of water solution of d-elements compounds.

6. Analytical reactions for the determination of ions of s-elements: (K^+, Mg^{2+}, Ba^{2+}, Ca^{2+}). Qualitative reactions to CO_3^{2-}, SO_4^{2-}, NO_2^-, $S_2O_3^{2-}$ ions.

Content of the topic

Chemical elements in whose atoms the *s*-sublevel is filled with electrons by the latter are called *s*-elements. Since there can be a maximum of 2 electrons on the s-sublevel, each period of the PES begins with two *s*-elements, and in general they form two groups: I-A and II-A. *S*-elements also include hydrogen and helium.

Table 2

General characteristics of s-elements

Group characteristics	**IA**	**IIA**
s-Elements – Metals	Li, **Na, K**, Rb, Cs, Fr	Be, **Mg, Ca**, Sr, Ba
External level electronic structure	ns^1	ns^2
Oxidation scheme for chemical interactions	Me - 1ē → Me^{+1}	Me - 2ē → Me^{+2}
Oxidation state	+1	+2

Elements of groups I–A and II–A are metals, exhibit reducing properties, high chemical activity, in complex substances the oxidation state is +1 (group I – A), +2 (group II – A). Group II – A elements are weaker reducing agents than Group I – A elements. The resulting *s*-element cations have stable shells such as inert gases.

Ions of *s*-elements can form unstable complex compounds with organic and inorganic ligands in solutions. With an increase in the charge and a decrease in the cation radius, the stability of the complexes increases. Thus, the complexing ability of s-elements is low, since:

a) metal cations have a complete electron shell of the previous inert gases, therefore, there are no vacant electron orbitals and the role of these cations as acceptors of electron pairs of ligands is difficult;

b) according to Pearson's theory, low-polarizable Na^+ and K^+ cations are "hard" acids, therefore, in physiological solutions, they practically do not form stable complexes with biosubstrates containing soft readily polarizable groups –COO–, –

NH_2 and –SH. They form relatively stable complex compounds practically only with macrocyclic ligands. The stability of the resulting complex compounds depends on the ratio of the ionic radius and the diameter of the inner cavity. The correct selection of macrocyclic ligands allows selective binding of either Na^+ or K^+. For example, the valinomycin present in the body binds K^+ ions 20 times more strongly than Na^+, since the valinomycin molecule does not allow the ligands to come close enough around the Na^+ ion having a smaller radius. The many alkyl groups on the surface of the valinomycin molecule provide it with a hydrophobic (lipophilic) character, due to which it can easily pass through cell membranes, transferring K^+ ions against the concentration gradient.

Less "hard" s-elements of II – A groups, in particular Mg^{2+} and Ca^{2+}, are more prone to complexation. So, for example, Ca^{2+} forms chelate complexes with EDTA, used as antidotes, and complexes of Mg^{2+} with porphyrin are the basis of chlorophyll. In physiological media, cations Mg^{2+} and Ca^{2+} are found both in ionized form and in the form of complexes with proteins and nucleic acids.

Most of the salts formed by cations of ns^1-elements are readily soluble in water, form precipitates only with large, easily polarizable anions, for example, urates $Na_2C_5H_2N_4O_3 \cdot H2O$. These salts are deposited in gout on the bones of the foot. Cations of ns^2-elements have a high polarizing ability, which determines the presence of a larger, in comparison with ns1-elements, amount of poorly soluble compounds (with SO_4^{2-} anions; $C_2O_4^{2-}$; CO_3^{2-}; $Cr_2O_4^{2-}$, etc.) Solubility of precipitates of ns^2 salts-elements decreases with increasing cation radius.

Ions of *s*-elements are resistant to the action of oxidants and reducing agents; redox reactions (ORR) are not typical for them. In nature (the earth's crust) s-elements-metals are found only in the form of chemical compounds. Na, K, Mg, Ca are widespread. The rest of the elements are rare and scattered. Elements of groups I – A are found in nature mainly in the form of soluble salts (in the water of rivers, seas, oceans) and solid deposits. Group II – A elements (except magnesium) – mainly in the form of insoluble compounds: carbonates, sulfates, fluorides. S-elements widespread in nature are at the same time biogenic.

Table 3

Topography of biogenic *s*-elements in the body

Element	Content in the body	Topography
Na	ω(Na) = 0,08%, with a body weight of 70 kg sodium weight 60 g	Na^+ – the main extracellular ion, distributed throughout the body, is found in blood serum, cerebrospinal fluid, in the eye fluid, in digestive juices, bile, kidneys, skin, bone tissue, lungs, brain in the form of soluble salts NaCl, NaH_2PO_4, NaH_2PO_4,$NaHCO_3$.
K	ω(K) = 0,23%, the mass of potassium in the body 160 g	K^+ – the main intracellular ion, 98% of potassium is contained in intracellular fluids; distributed throughout the body, found in all organs and tissues.
Mg	ω(Mg) = 0,027%, mass of magnesium in the body 20 g	Mg^{2+} – intracellular ion. Dentin and enamel of teeth, bone tissue, pancreas, skeletal muscles, kidneys, liver, heart.
Ca	ω(Ca) = 1,4%, body calcium mass ≈ 1,5 kg	Ca^{2+} – extracellular ion. Bone and dental tissue; heart, blood, kidneys, liver.

The biological role of s-elements and the use of their compounds in medicine

Hydrogen. There is practically no free hydrogen in the biosphere. The main forms of its presence in the biosphere are natural waters, gases and organic matter. In organisms, hydrogen is a part of hydrocarbons, carbohydrates, proteins, fats, nucleic acids. Hydrogen does not participate in the formation of the skeleton of organic compounds. The majority of hydrogen atoms are contained in water, which accounts for more than 90% of the mass of a living cell. All cell chemistry is based on the fact that water serves as a solvent in cellular systems. The human body weighing 70 kg contains approximately 45 liters of water. There are two main types of fluid in the body with different composition of electrolytes, namely: intracellular, in which the predominant cation is potassium, and extracellular, with sodium predominance. Another hydrogen

compound is hydrogen peroxide H_2O_2, which is formed in all cells of the body during various redox processes as a byproduct of metabolism and immediately decomposes under the influence of the enzyme catalase into water and molecular oxygen.

Lithium. Microelement is a permanent component of living organisms. The lithium ion, which has the smallest radius among alkali metals, is so strongly hydrated in aqueous solutions (13 water molecules are retained in the hydrate ion) that its size in the hydrated state is much greater than the radii of hydrated Na^+ ions (retains 8 water molecules) and K^+ (retains 4 water molecules). This prevents the penetration of Li^+ through the ion channels of cell membranes. Li^+ ions, influencing the activity of some enzymes, regulate the ionic Na^+-K^+ balance of the cells of the cerebral cortex. That is why lithium-containing drugs are widely used in a psychiatric clinic.

Sodium and potassium. Ions Na^+ and K^+ are distributed throughout the body, with the former being part of the predominantly intercellular fluids, and the latter being mainly inside the cells. The intracellular concentration of sodium ions is less than 10% of its content in the extracellular fluid, while the concentration of potassium ions inside cells is almost 30 times higher than outside the cell. If we evaluate the absolute values, then about 95% of sodium ions involved in metabolism are outside the cells and about the same proportion of potassium ions – inside the cells. Na^+ ions are associated with osmotic pressure of liquids, water retention by tissues (15 g of NaCl retains up to 2 liters of fluids in the human body), maintenance of acid-base balance in the body ($NaHCO_3$ – alkaline reserve of blood – a component of the hydrocarbonate buffer system), transfer of amino acids and sugars through cell membrane. Na^+ and K^+ ions activate (Na^++ K^+) – adenosine triphosphatase of cell membranes, which pumps Na^+ ions out of the cell and ensures the conjugate accumulation of K^+ ions in the cell. Different concentrations of these two ions on different sides of the membrane cause the appearance of a transmembrane potential difference (up to 100 mV), which ensures the existence of an easily accessible energy source for many processes associated with the functioning of membranes.

Beryllium. The biological role has not been clarified. Beryllium compounds are poisonous. Volatile beryllium compounds and dust containing beryllium and its compounds are especially toxic. The presence of even small amounts of beryllium in the environment leads to the disease of beryllium (beryllium rickets). Be^{2+} ions displace Ca^{2+} ions from bone tissue, causing it to soften.

Magnesium. In total, the human body contains about 40 g of magnesium, of which more than half is in the bone tissue. Most of the magnesium found outside the bones is concentrated inside the cells. Mg^{2+} ions are the second most abundant intracellular cations after K^+ ions. Therefore, Mg^{2+} ions play an important role in maintaining osmotic pressure inside cells. In humans and animals, Mg^{2+} ions are one of the main activators of enzymatic processes. Magnesium ions, injected subcutaneously or into the blood, cause depression of the nervous system and lead to a narcotic state, a decrease in blood pressure, etc.

Calcium. It is one of the five (O, C, H, N, Ca) most common elements in the human body (1.5%). The bulk of the calcium in the body is found in bones and teeth. The composition of the dense bone matrix includes a thermodynamically and kinetically stable form of calcium phosphate – $Ca_5(PO_4)_3OH$ at pH 7.40. The extraosseous calcium fraction, although only 1% of the total body calcium, is very important for its effects on blood clotting, neuromuscular excitability and heart muscle.

Barium. The biogenic role of barium has been little studied so far. All barium salts soluble in water and acids are highly toxic. Water and acids insoluble $BaSO_4$, absorbs X-rays well, and therefore it is used to study the human gastrointestinal tract.

Strontium. In the body of animals and humans, it accumulates in large quantities in bone tissue and affects the process of bone formation. Its excess causes fragility of bones, "strontium rickets". The reason is the substitution of strontium for calcium in the bone substance. It is almost impossible to extract strontium from bones. An increase in the radioactive background of the biosphere can cause the appearance of a fission product of heavy elements $_{90}Sr$ in the atmosphere. By settling in the bone, the latter irradiates the bone marrow and disrupts bone marrow hematopoiesis.

Table 4

Medicines containing s-elements

Element	Finding and role in the body	Medicinal substances
H	Organogenic element	**H_2O_2** (3%) – antiseptic, topical hemostatic; **HCl** (8,2-8,3%) – with low acidity of gastric juice.
Li	Trace element	**Li_2CO_3** – used in the treatment of mental illness and schizophrenia.

Na	Extracellular cation. Buffer systems, osmosis, K,Na-pump.	**NaCl** (0,9%) – physiological solution (isotonic solution) – the simplest blood substitute; for the preparation of solutions of medicinal substances; **NaCl** (3-10%) – hypertonic solution. Outwardly in compresses for the treatment of purulent wounds. Osmosis results in the separation of pus from the wound and plasmolysis of bacteria; as well as in case of poisoning with salts of argentum: $Ag^+ + NaCl \rightarrow AgCl\downarrow + Na^+$ insoluble, non-toxic **$NaHCO_3$** – baking soda, antacid; 4% solution intravenously for acidosis: $NaHCO_3 + RCOOH \rightarrow RCOONa + H_2O + CO_2$ with urine through the lungs; weak antiseptic, because as a result of hydrolysis, the baking soda solution has an alkaline reaction of the medium: $NaHCO_3 + H_2O \rightarrow H_2CO_3 + NaOH$ **$Na_2SO_4 \cdot 10H_2O$** (Glauber's salt) – laxative, antidote for poisoning with barium salts and plumbum.
K	Intracellular cation. Buffer systems, osmosis, K,Na-pump.	Tartaric salt **$KHC_4H_4O_6$** – mild laxative; potassium acetate **CH_3COOK** – diuretic, works well for cardiac and renal edema; potassium iodide **KI** – treatment of eye diseases – cataracts, glaucoma. Often potassium iodide is used for poisoning with mercury salts; **KCl** – intravenously for cardiac arrhythmias (relaxes the heart muscle), edema; **$KMnO_4$** – strong oxidizer, antiseptic, topically for washing wounds.
Ca	Bone and dental tissue in the form of compounds:	**$CaCl_2 \cdot 6H_2O$** – antiallergic, anti-inflammatory drug; increases blood clotting; **Ca-gluconate** – antiallergic, anti-inflammatory

	$Ca_5(OH)(PO_4)_3$ or $CaCO_3 \cdot 3Ca_3(PO_4)_2 \cdot H_2O$	effect; **$2CaSO_4 \cdot H_2O$** – burnt plaster for plaster castings. When mixed with water, an insoluble $CaSO_4 \cdot 2H_2O$ is formed.
Mg	Intracellular ion; anti-spasmodic action	**$MgSO_4$** – 25% solution, strong laxative; **MgO** – burnt magnesia, antacid effect; **$MgCO_3 \cdot Mg(OH)_2 \cdot 3H_2O$** – white magnesia, antacid effect; **$3MgO \cdot 4SiO_2 \cdot H_2O$** – talcum powder adsorbent.
Ba	Retina	**$BaSO_4$** – fluoroscopy contrast agent.

Solubility. Solubility product

Solubility – the ability of a substance to form homogeneous systems with other substances – solutions in which the substance is in the form of individual atoms, ions, molecules or particles. Solubility is expressed by the concentration of a solute in its saturated solution as a percentage in weight or volume units, referred to 100 g or 100 cm^3 (ml) of the solvent. The solubility of gases in a liquid depends on temperature and pressure. The solubility of liquid and solid substances is practically only a matter of temperature. Solubility can be quantified using the coefficient of solubility (k_s). The solubility coefficient is the mass of a solute that at a given temperature can dissolve in a certain volume of solvent to form a saturated solution. According to their ability to dissolve, all substances are divided into:

- Soluble (k_s> 1 g / 100 g solvent);
- Slightly soluble (1> k_s> 0.1 g / 100 g solvent);
- Slightly soluble or practically insoluble (k_s <0.1 g / 100 g solvent).

Solubility depends on: solute, solvent, temperature, pressure, presence of other substances in the solvent.

A poorly soluble electrolyte can be in dynamic heterogeneous equilibrium with its saturated solution:

crystalline (amorphous) precipitate (k) ↔ saturated solution (l).

For example, $AgCl_{(к)} \leftrightarrow Ag^{+}_{(l)} + Cl^{-}_{(l)}$

In such a solution, the concentration of ions is very low and, as a result, their interaction with each other is practically absent.

Equilibrium constant for a heterogeneous system:

$$К = \frac{[Ag^{+}] \cdot [Cl^{-}]}{[AgCl_k]}$$

solid phase concentration $AgCl_к$ can be considered constant, then:

$$К \cdot [AgCl_k] = [Ag^{+}] \cdot [Cl^{-}] = ПР_{AgCl} = const$$

or

$$C_{Ag^{+}} \cdot C_{Cl^{-}} = ПР_{AgCl} = const$$

In general form for the equation:

$$A_n \cdot B_n \leftrightarrow nA_p^{+m} + mA_p^{-n}$$

$$PS = [A^{+m}]^n \cdot [B^{-n}]^m$$

In a saturated solution of a poorly soluble strong electrolyte, the product of the concentration of its ions in powers of stoichiometric coefficients at a given temperature is a constant value called the product of solubility (PS).

The solubility product characterizes the solubility of a poorly soluble electrolyte at a given temperature. Of the two salts of the same type, for example, $CaSO_4$ with a PS = $2.5 \cdot 10^{-5}$ and $BaSO_4$ with a PS = $1.1 \cdot 10^{-10}$, the salt with a higher PR is more soluble.

The concentration of each ion in a saturated electrolyte solution can be changed, but the concentration of another ion also changes so that the product of the concentrations remains the same. Therefore, if a certain amount of one of the ions that make up the electrolyte is introduced into a saturated electrolyte solution, then the concentration of the other ion should decrease and part of the dissolved electrolyte will precipitate, that is, the electrolyte solubility decreases from the introduction of the same ions into the solution.

In general, the solubility L of the poorly soluble electrolyte AnBm is determined by the ratio:

$$L_{A_nB_m} = \sqrt[n+m]{\frac{PS}{n^n \cdot m^m}}$$

PC – the product of ion concentrations in powers corresponding to the stoichiometric coefficients for the system in a nonequilibrium state. Knowing the PC and comparing it with the PS, it is possible to establish whether it will dissolve or precipitate at a given temperature:

1. If PS = PC – the system is in equilibrium (saturated solution).
2. If PC < PS – the process of dissolution of the sediment occurs spontaneously.
3. If PC > PS – only the process of sediment formation is possible.

p-Elements *are chemical elements in which the p-sublevel of the external energy level is filled.* These are elements of III-A, IV-A, V-A, VI-A and VII-A of groups of the periodic system of D.I. Mendeleev. The electronic configuration, i.e., the distribution of electrons over energy levels for atoms and the possibility of the formation of cations or anions, can be shown for some elements in Table 5.

Table 5

Electronic structure of atoms of p-elements

Chemical symbol	Serial number	Electronic structure of the external energy level	Possible oxidation states in compounds
III A group			
B	5	$2s^2 2p^1$	+3
Al	13	$3s^2 3p^1$	+3
Ga	31	$4s^2 4p^1$	+3
In	49	$5s^2 5p^1$	+3
Tl	81	$6s^2 6p^1$	+1, +3

IV A group			
C	6	$2s^2 2p^2$	+2, +4, -4
Si	14	$3s^2 3p^2$	+2, +4, -4
Ge	32	$4s^2 4p^2$	+2, +4
Sn	50	$5s^2 5p^2$	+2, +4
Pb	82	$6s^2 6p^2$	+2, +4
V A group			
N	7	$2s^2 2p^3$	+1, +2, +3, +4, +5, -1, -2, -3
P	15	$3s^2 3p^3$	+3, +5, -3
As	33	$4s^2 4p^3$	+3, +5, -3
Sb	51	$5s^2 5p^3$	+3, +5, -3
Bi	83	$6s^2 6p^3$	+3, +5, -3
VI A group			
O	8	$2s^2 2p^4$	+1, +2, -1, -2
S	16	$3s^2 3p^4$	+4, +6, -2
Se	34	$4s^2 4p^4$	+4, +6, -2
Te	52	$5s^2 5p^4$	+4, +6, -2
Po	84	$6s^2 6p^4$	+2, -2
VII A group			
F	9	$2s^2 2p^5$	-1
Cl	17	$3s^2 3p^5$	+1, +3, +5, +7, -1
Br	35	$4s^2 4p^5$	+1, +3, +5, +7, -1

I	53	$5s^25p^5$	+1, +3, +5, +7, -1
At	85	$6s^26p^5$	+1, +3, +5, +7, -1
VIII A group			
Ne	10	$2s^22p^6$	-
Ar	18	$3s^23p^6$	-
Kr	36	$4s^24p^6$	-
Xe	54	$5s^25p^6$	-

In periods from left to right, the atomic and ionic radii of p-elements decrease with increasing nuclear charge, the ionization energy and electron affinity as a whole increase, electronegativity increases, the oxidative activity of elemental substances and non-metallic properties increase.

In groups, the radii of atoms and ions of the same type, in general, increase. The ionization energy decreases during the transition from 2p-elements to 6p-elements, since as the number of electron shells increases, the screening of the nuclear charge by electrons preceding the outer electrons increases.

With an increase in the ordinal number of the p-element in the group, the non-metallic properties weaken, and the metallic ones increase.

The properties of *p*-elements and their compounds are influenced by both the appearance of new sublevels on the outer electron shell and the filling of the sublevels of the inner electron shells. *p*-Elements of the second period – B, C, N, O, F – differ sharply from the elements of the following periods. So, starting with the p-elements of the third period, a low-lying free d-sublevel appears, to which electrons can pass from the p-sublevel upon excitation of atoms. Fully filled 3d-sublevels in d-elements of the fourth period – Ga, Ge, As, Se, Br – determines the difference in their properties from elements of the third period. The maximum filling of the 4f-sublevel in the sixth period similarly affects the difference in the properties of *p*-elements of the sixth and fifth periods.

In the period, the p-elements have a decrease in the ability to form positively charged ions with a charge corresponding to the group number. On the contrary, the ability to form negative ions with a charge equal to the difference (8 – No. of the group) increases with movement along the period.

p-Elements form diatomic E2 molecules, differing in stability. The most stable molecules are E2 of the elements of the second period – N_2, O_2 and F_2. The exception is the clorie molecule (239 kJ/mol), which has greater strength compared to the fluorine molecule (153 kJ/mol) due to the formation of additional bonds along the vacant d-orbitals. When passing from IIIIA- to IVA- and VA-groups, the stability of the molecules increases, and then when passing to VIIIA-group decreases. In groups, when moving down, the strength of the E - E bond decreases.

p-Elements of the second period – nitrogen, oxygen and fluor – have a pronounced ability to participate in the formation of hydrogen bonds. Elements of the third and subsequent periods lose this ability.

The similarity of the p-elements of the second period with the p-elements of subsequent periods is mainly only in the structure of the outer electron shells and those valence states that arise due to unpaired electrons in unexcited atoms. Boron, carbon, and especially nitrogen are very different from the rest of the elements of their groups (that have d- and f-sublevels).

In the transition from the p-elements of the second period to the p-elements of the third and subsequent periods, all types of bonds characteristic of the elements of the second period remain, and new types of chemical bonds appear. In this direction, the tendency of elements to form complex compounds increases, and the coordination numbers increase.

When going down the group, the stability of the maximum positive oxidation state for p-elements decreases and the stability of the lowest oxidation states increases. So, for example, for carbon the stable oxidation state is +4, and for plumbum +2, for aluminum +3, and for thallium +1.

The physical properties of simple substances of p-elements are very different. Some substances (gases) – oxygen, nitrogen – boil and melt at very low temperatures,

others – boron, carbon – at very high temperatures. By groups and periods, physical properties change nonmonotonically, and it is not always easy to associate the nature of changes with the structure of the electron shells of atoms, the type of chemical bond, and the coordination number of the atom.

All p-elements and especially p-elements of the second and third periods (C, N. P, O, S, Si, Cl) form numerous compounds with each other and with s-, d- and f-elements. ***Most of the compounds known on Earth are compounds of p-elements.***

Consequently, the study of p-elements is especially important for physicians, since five of them – C, N, P, O and S – are ***organogens*** and form the basis of living systems, and a number of others – Cl, I – are irreplaceable trace elements

Table 6

Topography of macro- and micro-p-elements in the body

Element	Content in the body	Element topography
C	ω(C) = 21%	It is found in all organs and tissues. Is the basis of proteins, fats, carbohydrates and nucleic acids.
O	ω(O) = 62%	Found in all organs and tissues.
N	ω(N) = 3%	Found in all organs and tissues.
P	ω(P) = 1,16%	Most of the phosphorus (85-90%) is found in bones and teeth, the rest is in soft tissues and fluids. About 70% of the total phosphorus in the blood plasma is included in organic phospholipids, about 30% is represented by inorganic compounds (10% compounds with protein, 5% complexes with calcium or magnesium, the rest are orthophosphate anions).
S	ω(S) = 0,25%	Sulfur is an indispensable component of cells, organ tissues, nervous, bone and cartilage tissue, as well as human hair, skin and nails.
Cl	ω(Cl) = 0,1%	Chlorine is present in all organs and tissues, in particular, bone tissue, blood, extracellular fluid of the body, but its

		main part (30-60%) is concentrated in the epithelium.
Si	$\omega(Si) = 1 \cdot 10^{-3}\%$	Most of all Silicium is in the liver, adrenal glands, hair, lens.
Sn	$\omega(Sn) = 1 \cdot 10^{-3}\%$	The highest concentration of stanum is found in the lungs, heart, kidneys, small intestine and bones. With age, the concentration of stanum in the body, especially in the lungs, increases, and in the body of a newborn it is close to zero.
F	$\omega(F) = 1 \cdot 10^{-5}\%$	Found in bones and tooth enamel.
Br	$\omega(Br) = 1 \cdot 10^{-5}\%$	The pituitary gland and other endocrine glands in the body are in the form of hydrated Br^- ions.
I	$\omega(I) = 4 \cdot 10^{-5}\%$	Thyroid gland, blood. In the thyroid gland, iodine is in a bound form – in the form of the hormone thyroxine and triiodothyronine – (15 mg) and about 1% in the form of iodide ion. The rest of the iodine is found in other organs. The iodine content in the blood is maintained constant at 10^{-4}-10^{-5}%. This is an iodine mirror of blood.
Se	$\omega(Se) = 2 \cdot 10^{-5}\%$	Part of over 30 proteins and 200 hormones and enzymes.
B	$\omega(B) = 1 \cdot 10^{-5}\%$	Boron is concentrated mainly in the lungs (0.34mg), thyroid (0.30mg), spleen (0.26mg), liver, brain (0.22mg), kidneys, heart muscle (0.21mg).
Al	$\omega(Al) = 1 \cdot 10^{-5}\%$	Aluminum is concentrated mainly in blood serum, lungs, liver, bones, kidneys, nails, hair, and is included in the structure of the nerve membranes of the human brain.
As	$\omega(As) = 1 \cdot 10^{-5}\%$	It is concentrated in the liver, kidneys, spleen, lungs, bones, hair, brain tissue, and muscles.

Biological role of *p*-elements of III-group

Boron

- affects the activity of some enzymes (inhibits catalase), vitamins (inactivates vitamins B2 and B12);
- enhances the effect of insulin;
- necessary for the absorption of Ca;
- has a gonadotropic effect;
- preserves bones in old age;
- necessary for the regulation of respiration processes;
- teratogen (causes a violation of embryonic development).

Aluminum

- reduces the activity of a number of enzymes (lactate dehydrogenase, alkaline phosphatase, catalase, etc.);
- blocks the active centers of enzymes involved in hematopoiesis;
- affects metabolism, especially mineral;
- participates in the regulation of the functions of the nervous system;
- affects the reproduction and growth of cells, directly acting on nuclear chromatin;
- competitor of P and Ca, Fe;
- affects reproductive capacity, embryonic and postembryonic development;
- is able to accumulate in the body;
- mutagen.

The biological role of p-elements of group IV.

Carbon

- is a part of proteins, fats, carbohydrates, nucleic acids, hormones, enzymes, vitamins, etc .;
- blood carbon dioxide stimulates the respiratory center, expands the cerebral vessels, increases the excitability of the heart muscle;
- bicarbonates of potassium and sodium are part of the buffer systems of blood and tissues, maintaining the constancy of the body's pH;

- acetic acid takes part in the synthesis of cholesterol

Plumbum

- can accumulate in the skeleton, replacing Ca;
- is a hemolytic poison: it reduces the hemoglobin content in the blood by 50%, causing lead anemia;
- is able to "turn on" into various cellular enzymes, disrupting their functioning (inhibiting);
- teratogen; carcinogen.

The biological role of p-elements of the V-group.

Nitrogen

- is a part of proteins, amino acids, nucleic acids, ATP and other important substances;
- is part of the exchange products;
- ammonia neutralizes excess acids in the body.

Phosphorus

- is a part of proteins, vitamins, nucleic acids, enzymes, phospholipids, etc .;
- participates in the formation of high-energy compounds (ATP, creatine phosphate);
- participates in the formation of bone tissue ($Ca_5(OH)(PO_4)_3$, $CaCO_3$) and teeth ($Ca_5F(PO_4)_3$);
- actively participates in the metabolism of proteins, fats, carbohydrates;
- plays a leading role in metabolic processes in muscles;
- hydrogen phosphates K and Na are part of the buffer systems of blood and tissue fluids that maintain the pH of the body;
- necessary for the normal functioning of the central nervous system;
- stimulates the processes of memorization;
- enhances potency, its deficiency negatively affects the quantity and quality of sperm

The biological role of p-elements of the VI-group.

Oxygen

- essential for all forms of life:
- is a part of water, proteins (17.9%), fats (22.4%), carbohydrates (49.38%), nucleic acids, etc .;
- participates in tissue and cellular respiration: oxidizes fats, proteins and carbohydrates supplied with food;
- water is the main component of the body (60-80% of the total mass);
- neutralization of toxins in the body.

Sulfur

- is a structural component of almost all proteins, some amino acids (cysteine, cystine, methionine), vitamins, biologically active substances (histamine, biotin, etc.);
- is a structural component of insulin and other hormones, thereby participating in the metabolism;
- activates some proteolytic enzymes, enters the active centers of molecules of a number of enzymes in the form of SH-groups;
- essential for normal liver function;
- participates in blood clotting;
- participates in the synthesis of collagen, is a part of cartilage tissue, hair, nails;
- has medicinal effects (sulfuric ointments are used for skin diseases).

Selenium

- is an antioxidant, protecting the body from the action of active radicals, including atomic oxygen and peroxides;
- has a positive effect on the cardiovascular system, promotes blood supply to the heart;
- stimulates the formation of erythrocytes;
- builds "strength ligaments" in the right places of the muscles;
- increases the immune properties of the body;
- fights against harmful substances in the liver;

- can replace S in various organic compounds;
- protects vitamin E and lipids of biological membranes from destruction;
- stimulates the synthesis of sulfur-containing amino acids and proteins;
- is necessary for the manifestation of sexual desire and normal potency;
- carcinogen, teratogen;
- has a rejuvenating effect, making the skin smooth and shiny hair.

The biological role of p-elements of the VII-group.

Fluor

•participates in the formation of bones and teeth ($Ca_5F(PO_4)$) and tissues of ectodermal origin (hair, nails, epidermis);

•influences enzymatic processes: reduces the metabolism of carbohydrates, fats, normalizes phosphorus-calcium metabolism;

•inhibits tissue respiration;

•affects the immunobiological state of the body;

•influences the level of biologically active substances (kinins, catecholamines) in the body;

•inhibits the function of the thyroid gland, since it is an antagonist of I;

•inhibits the biosynthesis of saccharides necessary for bacteria that contribute to the development of caries;

•enhances adaptation to cold;

•prevents the accumulation of Sr.

Chlorine

- activates some enzymes;
- affects the electrical conductivity of cell membranes;
- takes part in the regulation of osmotic blood pressure and normalization of water metabolism, maintains acid-base balance;
- serves as a source for the formation of hydrochloric acid in the stomach;
- participates in the transmission of nerve impulses;
- promotes the deposition of glycogen;

- affects the growth of hair and nails;
- is able to disrupt the structure of the double helix of DNA and cause its denaturation;
- has a bactericidal effect.

Iodine

- is a part of thyroid hormones - thyroxine, diiodotyrosine, triiodothyronine, etc., which affect the activity of the central nervous system, growth and general development of the body;
- regulates the level of basic metabolism;
- increases the body's resistance to various diseases and cold;
- is necessary for the manifestation of sexual desire and normal potency, and conception;
- is able to disrupt the structure of DNA and cause its denaturation.

Medical applications and drugs based on *p*-elements

Boron

- Boric acid **H_3BO_3**; antiseptic, is part of various ointments; in the form of solutions (1-3%) is used for rinsing the mouth and in ophthalmic practice;
- sodium decahydrate of tetraborate $Na_2B_4O_7 \cdot 10H_2O$ (borax); antiseptic; indications for use are the same.

Aluminum

- Potassium alum **$KAl(SO_4)_2 \cdot 12H_2O$** – has an astringent, cauterizing and hemostatic effect;
- aluminum hydroxide **$Al(OH)_3$**; has an absorbent and enveloping effect, lowers the acidity of gastric juice, is a part of the combined preparation "Almagel".

Carbon

- Activated carbon (carbolene); adsorbent for poisoning with alkaloids, salts of heavy metals, etc .; finds wide application in hemo- and lymphosorption;
- sodium bicarbonate $NaHCO_3$; lowers the acidity of gastric juice; aqueous solutions are used for rinsing and lotions.

Plumbum

Plumbum compounds are used only externally as antiseptic and astringent agents. Plumbum oxide PbO is a part of a lead plaster used for inflammatory skin diseases, furunculosis. Plumbum additives are used in the manufacture of clothing for the medical staff of X-ray rooms (aprons, mittens, helmets), since lead absorbs X-rays γ-rays.

Nitrogen

- Nitrogen oxide (I), or "laughing gas", N_2O; mixed with oxygen is used as a narcotic;
- aqueous ammonia solution (ammonium hydroxide, ammonia) NH_4OH; used to stimulate breathing and remove patients from fainting

Phosphorus

Adenosine triphosphoric acid is prescribed for chronic coronary insufficiency, muscular dystrophy and atrophy, peripheral vascular spasms.

Oxygen

Oxygen is used for inhalation in diseases accompanied by oxygen deficiency, in case of poisoning with carbonic oxide (II), hydrocyanic acid, etc. A mixture of 95% oxygen and 5% carbon dioxide (carbogen) is often used. In anesthetic practice, oxygen is widely used in a mixture with inhaled drugs. For medicinal purposes, oxygen can be injected under the skin, as well as in the form of an oxygen cocktail into the stomach.

Sulfur

- Purified sulfur; used as a laxative, as well as externally in the form of ointments and powders for the treatment of psoriasis, seborrhea, scabies, etc.;
- sodium, magnesium, barium, calcium sulfates

Chlorine

- Diluted hydrochloric acid (8%); taken orally in drops and mixtures (often together with pepsin) with insufficient acidity of gastric juice;
- sodium, potassium, calcium chloride;
- bleach $Ca(ClO)_2$; used in sanitary engineering as a disinfectant

Bromine

Ammonium bromides (NH_4Br), potassium (KBr) and sodium are used (NaBr).

Iodine

- Radioactive iodine (isotopes $_{131}I$, $_{132}I$, $_{125}I$); due to the short half-life of these isotopes, they are used for the treatment and diagnosis of thyroid diseases;
- alcohol solution of iodine (5 or 10%); used externally as an antiseptic, internally prescribed for the prevention of atherosclerosis (1-10 drops of a 5% solution);
- Lugol's solution (iodine solution in KI aqueous solution); used to lubricate the mucous membrane of the pharynx and larynx; potassium iodide KI, sodium iodide NaI; prescribed for endemic goiter; tablets called "antistrumin" containing KI are used to prevent endemic goiter.

Hydrolysis of salts

Salt hydrolysis refers to exchange reactions in which water is one of the reagents. The essence of hydrolysis is the interaction of a weak acid anion or a weak base cation with water molecules. Hence, it follows that not dissolved salts are subjected to hydrolysis, but only certain ions that make up their composition. These ions must correspond to either weak acids or weak Brønsted bases.

Anions of strong acids and cations of strong bases do not participate in the hydrolysis process.

Depending on the nature of the cation and anion, the average salts can be divided into four groups with respect to hydrolysis.

1. Salts formed by the anion of a strong acid and a cation of a strong base, for example NaCl, K_2SO_4, completely dissociate in solution and do not undergo hydrolysis, and the only protolytic equilibrium in such a solution is the ionization of water: $H_2O_{(ж)} \leftrightarrow H^+ + OH^-$

The medium in solutions of such salts is neutral, pH = 7.

2. Salts formed by a strong acid anion and a weak base cation (NH_4Cl, $CuSO_4$) are *hydrolyzed at the cation*. For example, in a solution of NH4Cl, the following protolytic equilibrium takes place:

$$NH_4^+ + H_2O \leftrightarrow NH_4OH + H^+$$

The balance is significantly shifted to the left. The medium in solutions of such salts is acidic The balance is significantly shifted to the left. The medium in solutions of such salts is acidic, pH < 7.

3. Salts formed by the anion of a weak acid and a cation of a strong base (NaF, K_2SO_3) are *hydrolyzed at the anion*. For example, in a NaF solution, the following protolytic equilibrium exists:

$$F^- + H_2O \leftrightarrow HF + OH^-$$

Again, the balance is significantly shifted to the left. The medium in solutions of such salts is alkaline., pH > 7.

4. Salts formed by the anion of a weak acid and a cation of a weak base, for example CH_3COONH_4, $(NH_4)_2SO_3$, are *hydrolyzed by both the cation and the anion*. For example, for CH_3COONH_4 in solution, the following protolytic equilibria can be written:

$$CH_3COO^- + H_2O \leftrightarrow CH_3COOH + OH^-$$

$$NH_4^+ + H_2O \leftrightarrow NH_4OH + H^+$$

In the case of a salt of this type, hydrolysis occurs as much as possible, since the cation and anion are reactive and react with water to form H^+ and OH^- ions. The resulting solution, as a rule, remains neutral, but it can be either weakly acidic or slightly alkaline if both reactions occur at different rates.

LABORATORY WORK

«Determination of cations and anions of s- and p-elements»

I. Qualitative reactions to cations

1. s- Elements.

Volatile salts of *s*-elements color the flame of a gas burner in the following colors: lithium – carmine red; sodium – yellow; potassium – purple; calcium – brick red; strontium – carmine red; barium – yellow-green.

Performance:

Volatile salts of s-elements are introduced on a thin platinum or nichrome wire, first into the base of the flame, and then into its upper part. Appropriate coloration is observed.

Create a table with a record of your observations!

2. Cation K^+

Sodium hexanitrocobaltate (III) $Na_3[Co(NO_2)_6]$ in a neutral or acetic acid solution gives a yellow crystalline precipitate of potassium-sodium hexanitrocobaltate with potassium ions (III):

$$2KCl + Na_3[Co(NO_2)_6] = K_2Na[Co(NO_2)_6]\downarrow + 2NaCl$$

in ionic form:

$$2K^+ + Na^+ + [Co(NO_2)_6]^{3-} = K_2Na[Co(NO_2)_6]\downarrow$$

Performance:

Place 3-4 drops of a potassium salt solution in a test tube, add 2-3 drops of a reagent solution. If no sediment occurs, let the mixture stand. In an alkaline environment, the reaction cannot be carried out, since the reagent itself decomposes. The reaction is sensitive, it is used to precipitate the K^+ ion from the blood serum for the permanganatometric determination of potassium in the blood.

3. Cation Ca^{2+}

Ammonium oxalate $(NH_4)_2C_2O_4$ with calcium salts gives a white fine crystalline precipitate of calcium oxalate CaC_2O_4:

$$CaCl_2 + (NH_4)_2C_2O_4 = CaC_2O_4\downarrow + 2NH_4Cl$$

in ionic form:

$$Ca^{2+} + C_2O_4^{2-} = CaC_2O_4\downarrow$$

Performance:

Add 5-6 drops of reagent to 5-6 drops of calcium salt solution. Observe the precipitation. The precipitate CaC_2O_4 is insoluble in acetic acid, but easily soluble in mineral acids (test and write equations). The considered reaction is used to precipitate Ca^{2+} ions in the determination of calcium in urine and blood by the permanganatometric method.

4. Cation Mg^{2+}

Sodium hydrogen phosphate Na_2HPO_4 in the presence of $NH\cdot 3H_2O$ and NH_4Cl precipitates a white crystalline precipitate of magnesium ammonium phosphate from solutions of magnesium salts:

$$MgCl_2 + Na_2HPO_4 + NH_3\cdot H_2O = MgNH_4PO_4\downarrow + 2NaCl + H_2O$$

in ionic form:

$$Mg^{2+} + HPO_4^{2-} + NH_3 \cdot H_2O = MgNH_4PO_4\downarrow + H_2O$$

Performance:

Add 2 drops of NH_4Cl solution to 1-2 drops of magnesium chloride solution; 2M ammonia solution and 1 drop of Na_2HPO_4 solution. The reaction of formation of $MgNH_4PO_4$ is used in biochemical analysis to determine magnesium in the blood.

5. Cation Ba^{2+}

a) Sulfate acid forms with Ba^{2+} cations, precipitates insoluble in acids and alkalis.

$$BaCl_2 + H_2SO_4 \rightarrow BaSO_4\downarrow + 2HCl$$

in ionic form:

$$Ba^{2+} + SO_4^{2-} \rightarrow BaSO_4\downarrow$$

Performance:

To 2 drops of a barium chloride solution, add 2 drops of a dilute sulfate acid solution. Observe the formation of a white crystalline precipitate.

б) Potassium chromate K_2CrO_4 precipitates barium salts from solutions, forming a yellow precipitate:

$$BaCl_2 + K_2CrO_4 \rightarrow BaCrO_4\downarrow + 2\ KCl$$

in ionic form:

$$Ba^{2+} + CrO_4^{2-} \rightarrow BaCrO_4\downarrow$$

Performance:

To 2 drops of barium chloride solution add 2 drops of potassium chromate solution. Observe the formation of a yellow precipitate.

II. Qualitative reactions to anions

1. Anion HPO_4^{2-}

The action of barium salts $BaCl_2$ gives a white precipitate of barium hydrogen phosphate with a solution of Na_2HPO_4:

$$BaCl_2 + Na_2HPO_4 = BaHPO_4\downarrow + 2NaCl$$

in ionic form:

$$Ba^{2+} + HPO_4^{2-} = BaHPO_4\downarrow$$

The precipitate is soluble in mineral acids (except for H_2SO_4) and in acetic acid.

Performance:

Add 5-6 drops of $BaCl_2$ solution to 4-5 drops of Na_2HPO_4 solution. Test the precipitate for solubility in hydrochloric and acetic acids.

2. Anion $C_2O_4^{2-}$

The action of potassium permanganate $KMnO_4$ in the presence of sulfate acid at low heating oxidizes $C_2O_4^{2-}$ ions to carbonic anhydride CO_2, and itself is reduced to a colorless ion Mn^{2+}:

$$5Na_2C_2O_4 + 2KMnO_4 + 8H_2SO_4 =$$
$$2MnSO_4 + K_2SO_4 + 5Na_2SO_4 + 10CO_2\uparrow + 8H_2O$$

in ionic form:

$$5C_2O_4^{2-} + 2MnO_4^{-} + 16H^+ = 2Mn^{2+} + 10CO_2\uparrow + 8H_2O$$

Performance:

Add 5-6 drops of sulfate acid solution to 4-5 drops of $Na_2C_2O_4$ solution. Heat the mixture slightly. Add the reagent solution dropwise to the resulting solution. Observe the discoloration of the solution. The formation of CO_2 and the discoloration of $KMnO_4$ is evidence of the presence of oxalates in the analyzed solution.

3. Anions Cl^-, Br^-, I^-.

Halide ions can be determined using argentum nitrate $AgNO_3$.

Performance:

Add Argentum nitrate to 2 ml of solutions of sodium chloride NaCl, sodium bromide NaBr, potassium iodide KI. In test tubes, curdled precipitates of insoluble argentum halides appear.

Argentum chloride precipitate - white

$$NaCl + AgNO_3 = AgCl\downarrow + NaNO_3$$

in ionic form:

$$Cl^- + Ag^+ = AgCl\downarrow$$

Argentum bromide sediment – pale yellow

$$NaBr + AgNO_3 = AgBr\downarrow + NaNO_3$$

in ionic form:

$$Br^- + Ag^+ = AgBr\downarrow$$

Argentum iodide precipitate – yellow

$$KI + AgNO_3 = AgI\downarrow + KNO_3$$

in ionic form:

$$I^- + Ag^+ = AgI\downarrow$$

4. Anion I^-

The action of solutions of plumbum salts $Pb(CH_3COO)_2$ with I^- ions gives a yellow precipitate of plumbum iodide PbI_2, which dissolves in water when heated (colorless solution), and when cooled, precipitates in the form of golden flakes:

$$2KI + Pb(CH_3COO)_2 = PbI_2\downarrow + 2CH_3COOK$$

in ionic form:

$$2I^- + Pb^{2+} = PbI_2\downarrow$$

Performance:

To 4-5 drops of plumbum salt solution add (carefully) dropwise a solution of potassium iodide until a precipitate forms. The precipitate is dissolved in an excess of the reagent, forming complex compounds:

$$PbI_2 + 2KI = K_2[PbI_4]$$

in ionic form:

$$PbI_2 + 2I^- = [PbI_4]^{2-}$$

Add 5-6 drops of water to the resulting PbI_2 precipitate. Heat the mixture until the precipitate is completely dissolved. Cool the solution with water under a tap. PbI_2 precipitates in the form of golden-yellow scales, the luster of which is clearly visible when the liquid is stirred. Add a few drops of potassium iodide solution to PbI_2. Observe the dissolution of the precipitate.

5. Anion NO_3^-

The action of a solution of diphenylamine $(C_6H_5)_2NH$ is oxidized by NO_3^- ions to a product with a dark blue color.

Performance:

Add 5-6 drops of concentrated H_2SO_4 and 2 drops of sodium nitrate to 2-3 drops of diphenylamine solution. Observe the appearance of an intense blue coloration. The reaction is very sensitive.

6. Anion $B_4O_7^{2-}$

The action of barium salts $BaCl_2$ precipitates a white precipitate of barium metaborate in concentrated solutions of borax $Ba(BO_2)_2$:

$$Na_2B_4O_7 + BaCl_2 + 3H_2O = Ba(BO_2)_2\downarrow + 2H_3BO_3 + 2NaCl$$

in ionic form:

$$B_4O_7^{2-} + Ba^{2+} + 3H_2O = Ba(BO_2)_2\downarrow + 2H_3BO_3$$

The Ba $(BO_2)_2$ precipitate is soluble in dilute acetic, hydrochloric and nitrate acids.

Performance:

Add 5-6 drops of $BaCl_2$ to 4-5 drops of borax solution. Test the solubility of the resulting precipitate in HCl and HNO_3.

After completing the laboratory work, it is necessary:

- draw up a protocol;
- write down the reaction equations in molecular, full and abbreviated ion-molecular forms.

General characteristics of biogenic d-elements

32 elements of periodic system belong to the d-block, they are in the IV-VII long periods. d-Elements are also called transitive. This name is explained by their position between the s-elements that begins each period, and p-elements that finishes each period. According to capacity of d-orbital, in each long period 10 transition elements are present. All transition elements are metals.

Features of chemical properties of d-elements allow considering them as unitary group. These features are:

- all d-elements differ from s-elements by their smaller reduction ability and bigger chemical inertness;
- the majority of d-elements have two or more of oxidation in compounds numbers, practically equal on thermodynamic firmness in usual conditions, so a big variety of oxidation-reduction reactions is typical;
- compounds of many d-elements in higher oxidation numbers show acid properties, and in lower oxidation state slightly basic;
- the most typical feature of d-metals is ability to form various coordination compounds, many of which are steady in aqueous solutions.

Complexes of 3d-metals are of great biochemical importance thanks to a variety of coordination spheres, lability and abilities to change oxidation-reduction properties.

Electronic Configuration

The general electronic configuration of transition elements is **$(n-1)d^{1-10}ns^{1-2}$**. The (n-1) stands for inner shell and the d-orbitals may have one to ten electrons and the s-orbital of the outermost shell (n) may have one or two electrons. After filling of 4s orbital successively with two electrons at atomic number 19 and 20, the next incoming electron goes to 3d orbital instead of 4p, as the 3d orbital is of lower energy than the 4p orbital. In the case of next nine elements following calcium, the incoming electron is filled in the d- subshell.

Since half filled and completely filled subshells are more stable than the one in which one electron is short, an electron gets transferred from 4s to 3d in case of the elements. d-Block elements occupy the middle portion of the periodic table – between s- and p- block elements. They include elements from groups 3 to 12. In these elements the outermost shell contains one or two electrons in their outer most ns orbital but the last electron enters into the inner d-subshell – (n-l) d orbital.

The elements of the d-block are metallic in nature. Their general characteristic properties are intermediate between those of the s block elements, on one hand and of the p-block elements on the other. Transition elements are elements in which the d subshell is partially filled either in atomic state or in ionic state.

There are four series of transition elements in the periodic table. The first transition series begins with scandium (At. No. 21) and ends at copper (At. No. 29) whereas the second, third and fourth series begin with yttrium (At. No. 39), lanthanum (At. No. 57) and actinium (At. No. 89) and end at silver (At. No. 47), gold (At. No. 79) and at the element having atomic number 112 (a synthetic element), respectively. These series are also referred to as *3d*, *4d*, *5d* and *6d* series, respectively.

Zinc, cadmium and mercury do not have partially filled d subshell either in the elemental state or in any of their common ions. Therefore, these elements are not transition elements. However, zinc, cadmium and mercury are considered along with d- block elements.

Electronic configuration of d-elements of IV period

Element	Symbol	Z	Electronic Configuration
Scandium	Sc	21	$1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^6\ 3d^1\ 4s^2$
Titanium	Ti	22	$1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^6\ 3d^2\ 4s^2$
Vanadium	V	23	$1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^6\ 3d^3\ 4s^2$
Chromium	Cr	24	$1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^6\ 3d^5\ 4s^1$
Manganese	Mn	25	$1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^6\ 3d^5\ 4s^2$
Iron	Fe	26	$1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^6\ 3d^6\ 4s^2$
Cobalt	Co	27	$1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^6\ 3d^7\ 4s^2$
Nickel	Ni	28	$1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^6\ 3d^8\ 4s^2$
Copper	Cu	29	$1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^6\ 3d^{10}\ 4s^1$
Zinc	Zn	30	$1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^6\ 3d^{10}\ 4s^2$

Table 7

Common Oxidation States of the First-Row Transition Metals*

	Sc	**Ti**	**V**	**Cr**	**Mn**	**Fe**	**Co**	**Ni**	**Cu**	**Zn**
electronic structure	$s^2\ d^1$	$s^2\ d^2$	$s^2\ d^3$	$s^1\ d^5$	$s^2\ d^5$	$s^2\ d^6$	$s^2\ d^7$	$s^2\ d^8$	$s^1\ d^{10}$	$s^2\ d^{10}$
oxidation states									I	
				II	II	II	II	II	II	II
	III	III		III	III	III	III	III		
		IV	IV		IV					
			V							
				VI	VI	VI				
					VII					

	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn
***The convention of using roman numerals to indicate the oxidation states of a metal is used here.**										

The acid–base character of transition-metal oxides depends strongly on the oxidation state of the metal and its ionic radius. Oxides of metals in lower oxidation states (less than or equal to +3) have significant ionic character and tend to be basic. Conversely, oxides of metals in higher oxidation states are more covalent and tend to be acidic, often dissolving in strong base to form hydroxy anions

Table 8.

Degree of oxidation and connections d-elements

Element	The degree of oxidation	Properties	Oxide	Acid	Base	Salts
Mn	+2	Basical	MnO	–	$Mn(OH)_2$	$MnCl_2$
	+4	Amphoteric	MnO_2	–	$Mn(OH)_4$	K_2MnO_3
	+7	Acidic	Mn_2O_7	$HMnO_4$	–	$KMnO_4$
Fe	+2	Basical	FeO	–	$Fe(OH)_2$	$FeCl_2$, $FeSO_4$
	+3	Amphoteric	Fe_2O_3	–	$Fe(OH)_3$	$FeCl_3$, $Fe_2(SO_4)_3$
	+6	Acidic	–	–		$NaFeO_2$, K_2FeO_4
Zn	+2	Amphoteric	ZnO	–	$Zn(OH)_2$	$ZnCl_2$, Na_2ZnO_2

Table 9.

Redox properties of d-elements

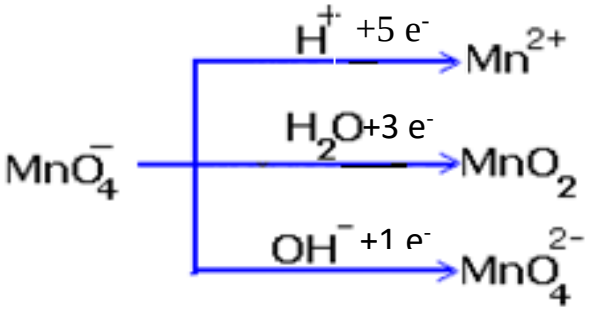
Element	The degree of oxidation	Oxidant or reductant
Fe	+2	reductant
	+3	oxidant and reductant
	+6	oxidant
Cr	+2	reductant
	+3	reductant and oxidant
	+6	strong oxidant
Mn	+2	reductant
	+4	oxidant and reductant
	+6	oxidant and reductant
	+7	strong oxidant

Table 10.

Acid-base properties of oxides of d-elements

Element, degree of oxidation	Oxygen and hydrogen compounds	Acid, bases	Salt
Cr, +3 +6	Cr_2O_3 CrO_3	$Cr(OH)_3$ H_2CrO_4, $H_2Cr_2O_7$	$CrCl_3$, $Cr_2(SO_4)_3$, $NaCrO_2$ K_2CrO_4, $K_2Cr_2O_7$
Mn, +2 +7	MnO Mn_2O_7	$Mn(OH)_2$ $HMnO_4$	$MnCl_2$, $MnSO_4$ $KMnO_4$
Fe, +2 +3	FeO Fe_2O_3	$Fe(OH)_2$ $Fe(OH)_3$	$FeCl_2$, $FeSO_4$ $FeCl_3$, $Fe_2(SO_4)_3$, $NaFeO_2$
Cu, +2	CuO	$Cu(OH)_2$	$CuCl_2$, $CuSO_4$
Zn, +2	ZnO	$Zn(OH)_2$	$ZnCl_2$, $ZnSO_4$, Na_2ZnO_2

Influence of solution acidity



Hydrolysis of d-elements salts

As d-element bases are weak electrolytes their salts are hydrolyzed with acidic medium formation.

There are 3 types of hydrolysis description. For example for the first stage equation of $CuCl_2$ hydrolysis:

a) Molecular form:

$$CuCl_2 + H_2O \leftrightarrow CuOHCl + HCl$$

basic salt

b) Ionic form:

$$Cu^{+2} + 2Cl^- + HOH \leftrightarrow CuOH^+ + Cl^- + H^+ + Cl^-$$

$$\text{Or } Cu^{+2} + HOH \leftrightarrow CuOH^+ + H^+ \quad (pH<7)$$

c) Protolytic form:

$$[Cu(H_2O)_4]^{+2} + HOH \leftrightarrow [Cu(OH)(H_2O)_3]^+ + H_3O^+$$

Hydrolysis constant of the salt which is form by weak base and strong acid may be described by formula:

$$K_{hydrolysis} = \frac{K_W}{K_{base}}$$

where K_W - ionic product of water, K_b - basic constant of weak base.

d-Elements belong to the microelements.

Metals-microelements have definite general properties:

- they are spread enough, i.e. are accessible to extracting from soil;
- have high complexing ability according to different donor atoms, possess various steady oxidation numbers and easily transform from one oxidation state into another.

These properties of microelements ensure their active participation in the major processes that occur in cells, such as:

- enzymatic catalysis reactions of synthesis and reactions of cellular energetics;
- transfer of electrons, ions, molecules and molecular enzymes;
- regulation of the activity of mechanisms and cells systems.

Free ions of d-metals do not exist in an organism; more often d-elements take part in biochemical reactions in a form of bioinorganic metal complexes.

Vital elements Zn, Cu, Fe, Mn, Co, Mo are called *life metals.*

Copper is a necessary microelement of vegetative and animal organisms. Now it is known about 25 copper-containing proteins and enzymes.

Part of enzymes catalyzes the interaction of oxygen with a substratum. They are members of group so-called oxygenase.

There is a big group of copper-containing proteins, so-called oxidases, which catalyze oxidation-reduction reactions with the transfer of protons or electrons immediately from substance which is oxidized into molecular oxygen. It is called oxidizes. For them is typical a high affinity to oxygen, and also high value of oxidation-reduction potentials. Among oxidizers is the major respiratory enzyme cytochrome oxidase (CCO) which catalyzes the final stage of tissues breathing.

There are known copper-containing proteins, for example superoxide dismutase (SOD) which accelerates decomposition reaction of superoxide ion O_2 . This ion, by entering into interaction with organic components of a cell, destroys it:

$$[SOD \cdot Cu^{2+}] + O_2^- \rightarrow [SOD \cdot Cu^+] + O_2;$$
$$[SOD \cdot Cu^+] + O_2^- + 2H^+ \rightarrow [SOD \cdot Cu^{2+}] + H_2O_2.$$

Thus, SOD converts superoxide ion O_2 into hydrogen peroxide a rather weaker oxidizer that quickly decomposes in an organism under the influence of catalase enzyme.

Copper together with iron participates in hematopoiesis. At deficiency of copper in the organism the iron exchange between blood plasma and erythrocytes is broken that can lead to destruction of erythrocytes. In experiments on animals it was shown, that the deficiency of copper leads to heavy deviations in a metabolism as: copper anemia, exotic ataxia, etc. Requirement of the person for copper (2- 3 mg a day) is completely provided by food.

There is known the illness of Konovalov-Wilson that is connected with the surplus content of copper in an organism. It is considered that abnormalities in the synthesis of ceruloplasmin cause a copper surplus that is why the removal of copper surplus that arrives with food is not provided.

In big concentrations soluble salts of copper are toxic. Copper (II) sulphate (blue vitriol) mass under 2 g causes a strong poisoning with a possible lethal outcome. It is explained that copper forms with proteins insoluble bioinorganic chelates-albuminates, so it coagulates proteins.

Zinc is a member of more than 40 metalloenzymes catalyzing hydrolysis of peptides, proteins, some ethers and aldehydes. Constant oxidation number defines its role in the reactions of hydrolysis occuring without transfer of electrons.

One of the most studied zinc-containing enzymes is carbonic anhydrase. This blood enzyme is contained in erythrocytes and can be found in three forms with different activity. This enzyme consists approximately 260 amino acid residues and represents a bioinorganic compound in which coordination number of zinc is 4: three coordination places are occupied by amino acid residues, the fourth zinc orbital connects water (or OH- group).

The presence of zinc in enzyme is a necessary condition for catalytic activity of carbonic anhydrase, which ensure hydration of CO_2:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3\,(CO_2 \cdot H_2O) \rightleftharpoons H^+ + HCO_3^-).$$

It must be noted that there is no single opinion about the mechanism of action of this enzyme.

Another zinc-containing enzyme is carboxypeptidase (COP) which exists in several forms that differ in number of amino acid residues and molar mass. Carboxypeptidase participates in reactions of peptide bonds hydrolysis. The mechanism of action of COP up to the end is not found out.

There are enzymes that participate in hydrolysis of dipeptides, they are called dipeptidases, and their structure without fail includes zinc. Zinc is a member of an insulin hormone which has influence on the sugar content in blood. Meat, liver, milk, eggs are richest in zinc.

Manganese is necessary for normal proceeding of processes in animals and vegetable organisms. In human organism manganese forms complexes with proteins, nucleic acids (RNA and DNA) and amino acids. These complexes are members of metalloenzymes (arginase, choline esterase, phosphoglucomutase, pyruvate carboxylase, etc.).

It is proved that manganese by participating in biochemical processes, as a rule, does not change oxidation number. It, possibly, is related with that in an organism strong oxidizers are absent, and also that ligands, by chelate effect and ligands field, stabilize a state of manganese (II).

Arginase as an enzyme, participates in a cycle urine formation, phosphoglucomutase participates in a carbohydrate exchange on the stages of glycogen decomposition.

Manganese participates in synthesis of vitamins C and B, chlorophyll synthesis. It is known that system ATP-ADP is a carrier and the accumulator of chemical energy in the organism. There are such enzymatic reactions in which MnATP2 complex is the donor of phosphatic groups. Thus, manganese participates in such vitally important process, as accumulation and transfer of energy in an organism. The

daily requirement for manganese of 5-7 mg is satisfied with food. Tea, beet, carrots, liver, and potato are richest in manganese.

Permanganates are poison for an organism.

Most part of iron is concentrated in blood hemoglobin (< 70 %). Iron is a member of some enzymes (cytochrome, catalase, peroxidase, etc.). The connected form of iron is in some proteins which perform a role of iron carriers.

One of the most important intracomplex natural compounds of iron is hemoglobin. It is a complex protein that contains nonprotein (prosthetic) group heme, whose mass fraction is 4 %. The prosthetic group is a biocomplex of iron (II) with polycyclic organic substance porphyrin. This is the group that is named heme (from Greek *haema* - blood). Heme has a flat structure. In heme the Fe^{2+}ion forms four bonds with atoms of nitrogen in the plane of porphyrin ring. The fifth bond the iron ion forms with an atom of nitrogen of imidazole group histidine which is an amino acid residue of globin. The iron (II) ion in heme has also the sixth orbital which in hemoglobin is used in the process of oxygen bonding. Same orbital participates in formation of bond with carbon (II) oxide.

Physiological function of hemoglobin consists in ability to connect reversibly oxygen and to transfer it from lungs to tissues. The hemoglobin which has attached oxygen, is called oxyhemoglobin, and hemoglobin that has given oxygen is dezoxyhemoglobin. $[Hb \cdot Fe^{2}+] + O_2 \rightleftarrows [HbFe^{2+} \cdot O_2]$.

Hemoglobin has a structure characterized by the least affinity to electron, where the iron atom of heme overhangs the plane of porphyrin ring. At the same time in oxyhemoglobin iron atom is in the plane of porphyrin ring.

Hemoglobin interacts also with carbon (II) oxide, and forms a macrocyclic complex carboxylhemoglobin: $[Hb \cdot Fe^{2+}] + CO \rightleftarrows [HbFe^{2+} \cdot CO]$.

At inhalation of carbon (II) oxide most part of hemoglobin transforms into carboxylhemoglobin, as a result the transferring of oxygen from lungs to tissues is disturbed and organism poisoning takes a place.

There is a group of iron-containing enzymes that catalyze the process of transferring electrons in mitochondria; these are so-called cytochromes (CC). In total it is

known 50 cytochromes. Cytochrome S is the the most studied. The transferring of electrons in the reduction-oxidation chain with participation of this enzyme is carried out at the expense of change of the iron state:

$$CC \cdot Fe^{3+} + \bar{e} \rightleftarrows CC \cdot Fe^{2+}.$$

Peroxidase enzyme accelerates reactions of oxidation of organic substances by hydrogen peroxide.

In organs and tissues there is a so-called deposition iron used at iron deficiency. It is deposited by means of protein ferritin with the molecular mass 460000, which is a biocluster. The lack of iron and cobalt in an organism leads to disturbances of hemoglobin synthesis. It causes blood diseases as an anemia.

In an organism iron can be transported in a form of amino acid complexes formed at the expense of coordination bond of iron atom with nitrogen atoms of peptide groups. Formation of such bioinorganic complexes makes possible passage of ions through cellular membranes.

Cobalt in the role of a microelement carries out various functions as it forms catalytic active centers of many enzymes that are necessary for DNA synthesis and metabolism of amino acids. Some of its complexes with proteins are carriers of molecular oxygen.

In an organism cobalt is present in a form of vitamin B_{12} ($C_{63}H_{90}N_{14}O_{14}PCo$). It is a bioinorganic coordination compound of porphyrin series in which the complex-ing agent is Co^{3+}. In a molecule of vitamin B_{12} the coordination number of cobalt is 6 according to its d^2sp^3-hybridization orbitals. There are enzymatic systems in which free structure of vitamin B_{12} does not operate, but it does so-called B_{12} coenzyme (cofactor). Cofactor is the active part of enzyme that can be easily separated. The inactive protein part that remains is called *apoenzyme.* As a coenzyme B_{12} takes part in two processes:

- transfer methylic CH_3-group (reactions of methylation);
- transfer of hydrogen ions.

Cobalt influences on carbohydrate, mineral, protein, lipid exchange, and also participates in process of hemopoiesis. The isotope of radioactive cobalt ^{60}Co has

found application in treatment of malignant tumors, and a complex of cobalt with nicotinic acid (coamide) in anemia treatment.

It is known that the enzymes which contain molybdenum participate in reactions connected with transfer of oxogroups. It is caused by ability of molybdenum to form stable oxocomplexes [MoO (oxalate) $(H_2O)_2]^{2-}$ or $[MoO_3(En)_2]$, where En is ethylenediamine. Molybdenum does not form in biological systems steady cations in low oxidation state. In an organism it exists exclusively in a form of complexes, in which oxidation number of Mo is +5 and +6. In complexes molybdenum is bonded, as a rule, with oxygen atom.

Molybdenum is a member of some enzymes, that catalyze oxidation-reduction reactions in vegetative and animal organisms. Xanthine dehydrogenase, xanthine oxidase, aldehyde oxidase belong to them. These enzymes catalyze reactions connected with transfer of oxygen. Xanthine oxidase catalyzes the oxidation of xanthine by oxygen into uric acid.

The detachment of electrons and protons from a substratum occurs with molybdenum participation.

Molybdenum is of importance in the process of soft fixing of nitrogen of the air. The enzymes that contain molybdenum catalyse processes of transformation of molecular nitrogen into ammonia and the other products containing nitrogen. That is why molybdenum is an important microelement for vegetative organisms.

Vanadium is a member of one of the major enzymes nitrogen-fixing microorganisms of the sol, the vanadium nitrogenase that reduces molecular nitrogen into ammonia.

As a microelement chrome is studied insufficiently, but its essential biogenic-role in vegetative and animal organisms does not cause doubts. It is a member of some enzymes which realize oxidation-reduction reactions in cells. Chrome is also a member of pepsin that breaks up proteins in a digestive tract of animals, participates in regulation of assimilation of glucose by tissues of animals. Chrome that is contained in yeast in the form of a complex with nicotinic acid and aliphatic amino acids, is considered «as the factor of tolerance to glucose», necessary for a normal

carbohydrate exchange in a human body. Its action consists in strengthening hypoglycemic insulin actions.

The metal details containing chrome do not show appreciable toxic action, but the metal dust irritates lung tissue that can lead to disease. It is known that bonds of chrome (VI) are more toxic, than bonds of chrome (III). All compounds of chrome cause the irritation of a skin and leads to occurrence of dermatitis. There is data that derivatives of chrome (VI) show cancerogenic properties.

Nickel in comparison with iron and cobalt plays more modest role in an organism. However there is data that nickel, like cobalt, participates in hemopoiesis, has influence on carbohydrate exchange. For Ni^{2+} is typical the formation of compounds with amino acids, carboxylic acids and other biologically active complexes that have N- or O-containing donor groups of atoms. Obviously, by formation of numerous compounds nickel stimulates synthesis of amino acids in a cell, accelerates regeneration of proteins of blood plasma, normalizes the hemoglobin contents in blood.

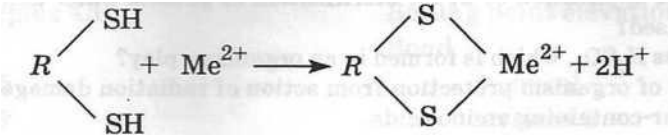
Silver is an impurity microelement of vegetative and animal organisms. As well as the majority of heavy metals, this element does not play an important role, but, as all heavy metals, after getting into an organism, shows toxic action: by joining proteins that contains sulphur, inactivates enzymes, destroys and coagulates proteins, forms insoluble albuminates. The same property to form albuminates, predetermines bactericidal properties of silver and its compounds. Already at the content of 10^{-8} mmol/1 of silver water possesses bactericidal action. All preparations of silver used in medicine are preparations of external action whose application is based on cementing, cauterizing and bactericidal properties. With that end in view from the inorganic connections silver nitrate (lunar caustic).

Bioinorganic complexes of silver with proteins (the proteinates) are colloid solutions. Colloid preparations of silver do not cause sedimentation of tissues proteins; they are used for treatment of conjunctivitis, infectious diseases of mucous membranes, venereal diseases and illnesses of a skin. From colloid silver preparations are most known protargol (a protein complex of silver) and collargol (colloid silver).

In small amounts it is applied for preparation of alloys (copper, silver, tin) that are used in stomatology. At ingress in an organism of big doses of soluble salts of silver a sharp poisoning accompanied by dying of a mucous membrane of a gastrointestinal tract can happen. The first aid at a silver poisoning is washing of the stomach by sodium chloride: the formed insoluble silver chloride is removed from an organism.

High toxicity of cadmium compounds is explained, first of all, by replacement of zinc ions by cadmium ions from many enzymes, and also by competition with calcium ions in bone tissues (calcium and cadmium ions have close sizes of radiuses).

Mercury compounds are more toxic than cadmium compounds. Already in small doses mercury affects a brain and nervous system. Corrosive sublimate ($HgCl_2$) is one of the strongest poisons. Serious poisonings are also caused by eva- portion of metalic mercury. Compounds of Cd and Hg cause damages of protein exchange and leads to a taking out of plasma proteins by kidneys (proteinuria). This toxic action is explained also by interaction of ions of these metals with sulphur of sulfhydric groups of proteins, enzymes and some amino acids. The blocking of sulfhydric groups leads to oppression of activity of enzymes and to coagulation of proteins according to the scheme:



Those compounds which are dissolved in lipids and easily get through a membrane into a cell are most toxic.

Practical lesson 3

Topic: Structure and classification of complex compounds. Complex formation in biological systems. Metalloligand homeostasis. Violation of homeostasis.

Relevance of the topic: Complex compounds are of great interest, they combine organic and inorganic chemistry. Almost all of the d-elements ions are in a bound state under body conditions, because they form complex compounds with various bioligands. For example, the formation of metallopolynucleotide complexes stabilizes the double helix of DNA, enzymes. In medicine, complex compounds are used as stimulators of biochemical processes.

Lesson objective: to form a systematic knowledge of the theory of complex compounds by A. Werner; regularities of the course in living organisms of formation and destruction of complex compounds and maintenance of metal ligand homeostasis.

Basic definitions: theory of coordination compounds, central atom (ion), ligand, coordination number, ligand denticity, dissociation constant.

Plan and organizational structure of the lesson:

1. A.Werner's theory of coordination compounds.
2. Structure of coordination compounds – central atom (ion), ligands.
3. Coordination number. Spatial character of ligand coordination by the central atom.
4. Ligand denticity. Examples of mono- and polydentate ligands. Macrocyclic ligands and chelation effect. Antidotes.
5. Types of coordination compound isomerism.
6. Mechanism of covalent bond formation between central atom and ligands.
7. Stability of complex ion. Instability constant.

Content of the topic

d-Elements. Coordination compounds. General characteristic of d-elements

In 1893 Swiss chemist Alfred Werner proposed a theory explaining features of the coordination compound structure. In honor of the author the theory was named *Werner's theory of coordination compounds.* Werner's ideas underlie the modern theory; according to it, coordination compounds are complex compounds or contain complex ions. A complex is any species involving a *central atom (ion)* and attached ligands. A central atom (ion) is also called a *complexing agent.* Around the central atom (central ion) other ions, atoms or molecules, named *ligands*, are located (coordinated) in a certain order. The word *ligand* is derived from a Latin word *ligare* that means to bind.

A complexing agent is connected with ligands by covalent polar bonds formed by the donor-acceptor mechanism. Together they form *the inner sphere of the coordination compound.* In formulas of coordination compounds the inner sphere is placed in square brackets. It may be neutral or have a positive or negative charge. In the last case, around the inner sphere an outer sphere is formed. Ions of the outer sphere are not connected directly with the central atom or the central ion. Between the inner and outer spheres of a complex there is, as a rule, an ionic bond.

The number of points around the central atom (ion), by which ligands are bonded to it, is called the *coordination number* of the complexing agent.

Hence, the structure of coordination compounds, for example $K_4[Fe(CN)_6]$, maybe represented in the following way: Fe^{2+} is the central ion (complexing agent); CN^- ions are the ligands; $[Fe(CN)_6]^{4-}$ is the inner sphere, its charge is 4-; K^+ ions are the outer sphere; the coordination number of Fe^{2+} is 6.

Practically all the elements of the periodic system of Mendeleev can be complexing agents. Many complexes are formed by transition metals such as the platinum metals (Pt, Pd), elements of the iron series (Fe, Co, Ni), copper subgroup (Cu, Ag, Au), zinc subgroup (Zn, Cd, Hg), which are elements with incomplete or 18-electron outer energy level. The least complexation ability is observed in alkali and alkaline earth metals.

Central atoms in complexes can also be nonmetals, for example boron ($K[BF_4]$), silicon ($K_2[SiF_6]$), phosphorus ($K[PF_6]$), etc.

The coordination number of the central atom (ion) is the major characteristic of complexes. The coordination number depends on the nature and radius of the central ion and ligands, the external sphere of a compound, the nature of the solvent and external conditions, under which the complex is formed.

Complexes with coordination numbers 2, 4 and 6 are most common. Very seldom there are complexes with large coordination numbers such as 8 and more.

Coordination numbers specify not only the number of ligands placed around the central atom, but also their location in space, which means that the coordination number has spatial interpretation.

The major conclusion of A. Werner's coordination theory is the notion of the spatial character of ligand coordination by the central atom.

Complexes with the coordination number 4 may have the form of three geometrical figures: a square, a tetrahedron and a tetragonal pyramid.

Octahedron geometrical configuration belongs to complexes with the coordination number 6.

The number of complexes with other coordination numbers is much less. For complexes with the coordination number 2 there are two possible geometrical configurations: linear and angular.

Denticity is defined by the number of sites occupied by ligands in the internal sphere of a coordination compound. In other words ligands may occupy one, two, three and more places, that is they may connect to the central atom by means of one or several atoms. There are monodentate (literally «one tooth») ligands – F^-, Cl^-, Br^-, I^-, CN^-, NH_3^0, H_2O^0, and polydentate («many teeth») ligands, for example: CO^{2-}, SO_4^{2-} ions, ethylendiamine molecule (ethane-1,2-diamine) $H_2N—CH_2—CH_2— NH_2$, that can form more than one bond with a complexing agent, they are attached to the central ion at two points (bidentate ligands).

Bioorganic molecules are polydentate ligands. These are, first of all, proteins, particularly enzymes and nucleic acids.

A primary role in the vital activity of plants and animals belongs to bioinorganic compounds with *macrocyclic* ligands. Tetradentatemacrocycles such as por-

phyrins are the most common in nature. They form stable coordination compounds with cations of different elements. Thus the central atom can be Mg^{2+} (chlorophyll), Fe^{2+} (hemoglobin). The Fe^{2+} ion in the hemoglobin molecule, as well as the Mg^{2+} ion in chlorophyll, is in the center of the porphyrin macrocycle being bound to four nitrogen atoms of tetrapyrrole macrocycle.

Iron- and cobalt-porphyrin complexes are also part of catalase, cytochromes, vitamin B_{12}. All of them have an octahedral structure.

The heightened stability of complexes with polydentate ligands in comparison with complexes with monodentate ligands is known as *chelation effect* (the word *chelate* comes from the Greek word *chela* that means crab's claw). This is the reason for polydentate ligands (complexones) wide application for the maintenance of the metal-ligand homeostasis and removing toxic metal ions from the organism.

In medical practice salts of ethylenediaminetetraacetic acid (EDTA) are widely used as *antidotes.*

Such a method of complexone application, based on the formation of stable complexes by metal ions with an anion of EDTA and other complexones, is not only used for treating some cases of metal poisoning, but also for delivering the body from radioactive isotopes.

Depending on the electric charge of the inner sphere all known coordination compounds can be divided into:

♦Coordination compounds containing complex cations (the inner sphere has a positive charge). For example: $[Zn(NH_3)_4]Cl_2$ – tetraamminezinc (II) chloride.

♦Coordination compounds containing complex anions (the inner sphere has a negative charge). For example: $K_3[AI(OH)_6]$ – potassium hexahydroxoaluminate (III).

♦Neutral complexes. For example: $[Pt(NH_3)_2Cl_2]$ –diamminedichloro-platinum.

Depending on the nature of ligands, complexes are classified into:

♦Acidocomplexes containing residues of acids as ligands. For example: $[Fe(CN)_6]^{4-}$ - hexacyanoferrate (II) ion.

♦Aquacomplexes containing water molecules as ligands. For example: $[Cr(H_20)_6]^{3+}$ - hexaaquachromium (III) ion.

♦Hydrocomplexes containing hydroxide ions as ligands. For example: $[Zn(OH)_4]^{2-}$ - tetrahydroxozincate ion.

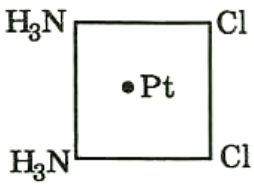
♦Ammonia complexes containing ammonia molecules as ligands. For example: $[Cu(NH_3)_4]^{2+}$ - tetraamminecopper (II) ion.

There are various types of *isomerism* in coordination compounds. As it is known, isomerism is existence of different compounds having the same molecular formula, but different structural formulas.

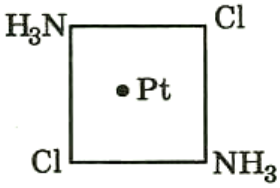
For complex connections such kinds of isomerism are known: structural isomerism (difference in the basic structure or bond type) and stereoisomerism (difference in the way of space location of ligands around the central atom or ion).

Structural isomerism includes ionization, coordination and linkage isomerism. Stereoisomerism may be represented by geometric and optical isomerism.

Geometric isomerism in coordination compounds is observed in the case when ligands in complex compounds are not identical, so may be situated in different ways in relation to each other. For example, in the compound $[Pt(NH_3)_2Cl_2]$ - diamminedichloroplatinum (II), that has a square planar structure and is characterized by the coordination number 4, two identical ligands can either be along the same edge of the square (cis-isomer) or on the opposite corners (trans-isomer):



cis-isomer



trans-isomer

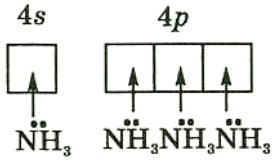
As it has been already mentioned, *donor-acceptor* is the mechanism of covalent bond formation between complexing agent and ligands. Ions of metals such as Zn^{2+}, Ag^{+}, Au^{3+}, Cu^{2+}, Hg^{2+}, Co^{3+}, Fe^{2+}, Fe^{3+} with vacant orbitals, and also nonmetals, for example boron in a compound H[BF] or silicon in a compound $H_2[SiF_6]$ are most often acting as acceptors of electrons. Neutral molecules (H_20, NH_3, ethylenediamine) or negatively charged ions (F^-, Cl^-, Br^-, I^-, CN^-, COO^-, $C_20_4^{2-}$, etc.) can be donors of electrons.

Formation of a donor-acceptor bond may be considered using the ion $[Zn(NH_3)_4]^{2+}$:

$$ZnSO_4 + 2NH_3 \cdot H_2O \rightarrow Zn(OH)_2\downarrow + (NH_4)_2SO_4;$$
$$Zn(OH)_2\downarrow + 4NH_3 \cdot H_2O \rightarrow [Zn(NH_3)_4](OH)_2 + 4H_2O.$$

The electronic formula of a zinc atom (No. 30): $1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^6\ 3d^{10}\ 4s^{i!}$.The formula of a zinc ion may be represented as: $1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^6\ 3d^{10}\ 4s^0\ 4p^0$

A zinc ion has four vacant orbitals (one 4s and three 4*p),* which can receive four lone pairs of electrons of nitrogen atoms.



According to the given scheme, three Zn—N bonds based on the 4p-orbitals of a zinc ion, are mutually perpendicular (the bond angle is 90°). The fourth Zn—N bond would be directed at whatever position in the complex ion, however it has been experimentally determined that H—N—H bond angles are approximately 109°. In other words, all the four bonds formed by a zinc ion with nitrogen atoms are directed in a tetrahedral fashion. It may be explained by modification of the atomic orbitals of the bonded atoms, proposed by Linus Pauling (1931).

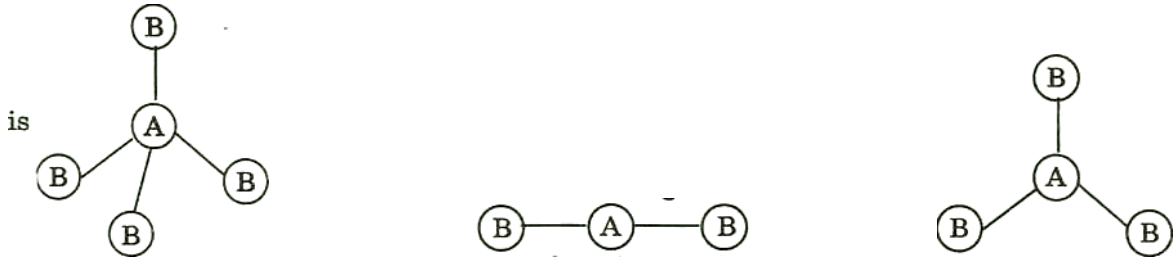
In accordance with the theory of L. Pauling, new orbitals having identical form and energy maybe produced by a linear combination of atomic orbitals with different forms and energy. These produced orbitals are called *hybrid orbitals* and the mathematical process of replacing pure atomic orbitals with a hybrid is called *hybridization.*

In case of Zn—N bonds in $[Zn(NH_3)_4]^{2+}$ ion, a combination of one 4s and three 4*p* orbitals of a zinc ion leads to formation of four sp^3 hybrid orbitals, sp^3-*Hybridization* has a tetrahedral structure:

It should be noted that the molecular geometry of NH_a also explained by sp^3 hybridization (the bond angle is 109°28^{l}).

Other combinations of *s* and *p* orbitals lead to the formation of *sp* and sp^2 hybrid orbitals.

From one *s* and one *p* orbital two hybrid orbitals are formed. *sp-Hybridization* corresponds to linear configuration (the bond angle is 180°):



For example, sp-hybridization takes place in the complex ion $[Ag(NH_3)_2]^+$.

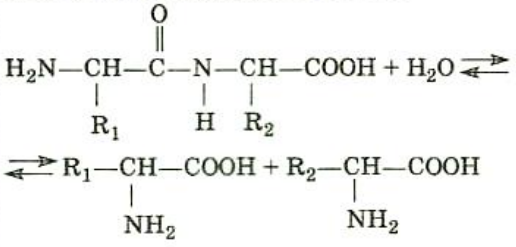
The combination of one s and two *p* orbitals leads to the formation of three sp^2hybrid orbitals located at an angle of 120°.

sp^2-Hybridization corresponds to trigonal-planar geometry:

Except for the cases considered above, other types of hybridization with participation of d and f orbitals are possible, for example: *d^2sp-*, *sp^3d^2-*, *sp^3d^2f-*, etc. $[Pt(NH_3)_2Cl_2]$ may be an example of a complex with *d^2sp* hybridization of the central ion orbitals. sp^3d^2-Hybridization takes place in the complex ion $[Al(OH)_6]^{3-}$.

In biohemical processes d-elemens take part as biocomplexes of metals (*Table)*

Characteristics of some metalloenzymes

Metal-loenzyme	Cent-ral atom (ion)	Ligands	Object of concentration (content)	Enzyme action
Carbonic anhy-drase	Zn^{2+}	Amino acid residues	Erythrocytes	Catalyzes reversible hydration of carbon dioxide: $CO_2 + H_2O \rightleftarrows H_2CO_3 \rightleftarrows H^+ + HCO_3^-$
Carboxy-peptidase	Zn^{2+}	Amino acid residues	Pancreas, intestines	Catalyzes splitting of proteins by hydrolysis of peptide groups of the ends of the polypeptide chain: 

Catalase	Fe^{3+}	Amino acid residues, tyrosine, histidine	Blood	Catalyzes hydrogen peroxide decomposition: $H_2O_2 = H_2O + O$
Peroxi-dase	Fe^{3+}	Proteins	Tissues, blood	Catalyzes oxidation of substrates (RH_2) by hydrogen peroxide: $RH_2 + H_2O_2 = R + 2H_2O$
Cyto-chrome oxydase	Cu^{2+}, Fe^{3+}	Amino acid residues	Heart, liver, kidneys	Catalyzes reduction of oxygen in the mitochondrial respiratory chain
Pyruvate carboxy-lase	Mn^{2+}	Tissue proteins	Liver, thyroid gland	Catalyzes the process of pyruvic acid carboxylation
Ribonuc-leotide reductase	Co^{2+}	Tissue proteins	Liver	Takes part in the biosynthesis of ribonucleic acids

Acid residues, peptides, proteins, hormones, nucleic acids and some other biologically active substances can be ligands in biocomplexes. Biocomplexes of d-metals with protein molecules are called *bioclasters.* In a bioclaster molecule a metal ion is located in the cleft. It forms bonds with donor atoms of such groups of proteins and amino acids as hydroxylic OH^-, carboxylic COO^-, amino group NH_3 .

Depending on the biological function such kinds of metal biocomplexes are distinguished: transport, accumulators, biocatalysts, activators of inert processes.

Instability (dissociation) constant

The equilibrium of complex ion dissociation is characterized by the equilibrium constant, which is named the instability (dissociation) constant, K_{inst}:

$$Ag(NH_3)_2^+ \rightleftarrows Ag(NH_3)^+ + NH_3;$$
$$Ag(NH_3)^+ \rightleftarrows Ag^+ + NH_3.$$

The summary equation of dissociation:

$$[Ag(NH_3)_2]^+ \rightleftarrows Ag^+ + 2NH_3;$$
$$K_{inst._1} = \frac{[Ag(NH_3)^+] \cdot [NH_3]}{[Ag(NH_3)_2^+]};$$

The general instability constant:

$$K_{inst.2} = \frac{[Ag^+] \cdot [NH_3]}{[Ag(NH_3)^+]} \cdot \qquad K_{inst.} = \frac{[Ag^+] \cdot [NH_3]^2}{[Ag(NH_3)_2^+]} \cdot$$

Beside the instability constant, the constant of stability (formation), K_{st}, is used to characterize complex compound stability. It is a value opposite to the instability constant:

$$K_{st.} = \frac{1}{K_{inst.}} \cdot$$

Nomenclature of coordination compounds

IUPAC has suggested the following rules for naming complex compounds.

1. In ionic complex the cation is named first than anion. Non – ionic complexes are given in a one word name.

2. In naming the coordination sphere the ligands are named first and then the central metal ion.

3. The name of ligands:

Negative ligand	Name	Negative ligand	Name	Neutral ligand	Name
Cl^-	Chloro	NO_2^-	Nitro	H_2O	Aqua
Br^-	Bromo	NO_3^-	Nitrato	NH_3	Ammine
F^-	Fluoro	SCN^-	Thicocyanato	NO	Nitrosyl
CN^-	Cyano	OH^-	Hydroxo	CO	Carbonyl

4.The ligands are named, IUPAC rules have recommended that all ligands whether negative, neutral or positive be arranged alphabeticaily whithout any preference order.

5. When more than one ligands of a particular kind are present in the complex, the prefixes:

number of ligands (coordination number)	prefixes
2	di-
3	tri-
4	tetra-
5	penta-
6	hexa-

6. When the complex is anionic, the name of the central metal atom end is -ate. For cationic and neutral complexes the name of the metal is written without any characteristic ending.

For example:

- the cationic complex $[Co(NH_3)_6]^{+3}Cl_3$ is named hexaamminecobalt(III) chloride.

- the anionic complex $K[PtCl_5(NH_3)]^{-1}$ is named with potassium amminepentachloroplatinate(IV);

$Ca_2[Fe(CN)_6]$ is named as calcium hexacyanoferrate (II).

7. The oxidation state of the central ion is designated by a Roman numeral

(such as II, III,IV) in the parentheses at the end of the name of the complex without

a space. Some more examples are given below:

$K_4[Fe(CN)_6]$ - Potassium hexacyanoferrate (II)

$[Fe(CO)_5]$ - Pentacarbonyliron(0)

Practical lesson 4

Topic: Elements of quantitative analysis. Method of neutralization. Determining the concentration and titer of an alkali solution using an acid solution. Method of neutralization. Determining the concentration and titer of an alkali solution using an acid solution.

Relevance of the topic: Solutions are one of the most important states of matter that are of great importance in human life and practical activities. The processes of food assimilation by humans and animals are connected with the transfer of nutrients into solutions. Biological fluids (blood, lymph, etc.) are solutions. Knowledge of how to express the concentration of solutions and the ability to prepare solutions of a certain concentration are needed for students to study biochemistry, hygiene, pharmacology, and for doctors to interpret laboratory analysis data and calculate the dosage of drugs.

Lesson objective: To form a systematic knowledge of students about solutions, their classification, methods of expressing concentration, properties; to learn to use Van Hoff's law to calculate the corresponding properties of dilute solutions, including biological fluids.

Basic definitions: solution, solvent, concentration, mass fraction, neutralization method, alkalimetry, acidimetry, solution titer.

Plan and organizational structure of the lesson:

1. Solutions and their composition.
2. Physical and chemical theory of solutions
3. Ways to express the composition of the solution.
3. Solubility. Factors affecting solubility.
4. Concentration. Types of concentration. Ways to express concentrations.
5. The role of solutions in nature, living organisms, science and technology. The biological role of solutions.
6. Hydrates, crystalline hydrates, crystallization water.
7. Medical solutions. Doses Calculation of the dosage of the drug.

Content of the topic

1.1. Composition of solutions.

Solutions are of great practical importance. Many chemical and biochemical reactions proceed in solutions.

Solutions are homogeneous systems of variable composition, consisting of two or more substances (components).

A true solution is a homogeneous system formed by at least two components. The composition of the solution can be changed continuously within certain limits. The true solution differs from colloidal solutions and mechanical mixtures (suspensions, emulsions, aerosols). Colloidal solutions are multicomponent heterogeneous systems. The homogeneity of the solutions makes them similar to chemical compounds and distinguishes them from mechanical mixtures.

Solutions can be in three states of aggregation: gaseous, solid and liquid.

In solid solutions, particles of one substance are randomly distributed among particles of another solid. For example, hydrogen is well soluble in some metals (platinum, palladium), and this is a solid solution. Mixtures of gases (e.g. air) are not called solutions. The fact is that an important property of solutions is a noticeable interaction between solvent particles and dissolved substances, and in gases such interaction is practically absent. In liquid solutions, the solvent and the solute are distinguished.

A solvent is a substance that is in the same state of aggregation as the solution. If the aggregate state of the substances that make up the solution is the same (for example, alcohol and water), then the solvent is a substance that is present in excess compared to other components. The remaining components, which are in solution in a smaller amount, are called solutes. According to modern theory, solutions are liquid dissociated systems formed by particles of solvent and solute and those uncertain compounds that form between them.

Dissolution is a physicochemical process. When dissolving, heat is always released or absorbed, and a change in volume occurs. This indicates a chemical interaction between the solute and the solvent. As a result of this interaction, solvates

or hydrates (solvent- water) are formed. On the other hand, solutions do not obey the law of constancy of composition; they, like mixtures, can be divided by physical methods into their constituent parts.

Most hydrates and solvates are weak compounds that decompose readily. However, hydrates can also be strong; they can be easily isolated during crystallization. Examples of crystalline hydrates: $CuSO_4 \cdot 5H_2O$, $CaSO_4 \cdot 2H_2O$, $BaCl_2 \cdot 2H_2O$.

Such medicinal substances also include glucose, terpinghydrate, magnesium sulfate, copper sulfate, alum, codeine, etc., which are crystalline hydrates with different contents of crystallization water. When water is removed from crystalline hydrates, they change the appearance and individual properties (copper sulfate, gypsum, crystalline soda, etc.). In the hydrated state, individual ions of salt dissolved in water also remain, which is crucial for many properties of salt solutions.

In chemical practice, the most important are liquid solutions.

Qualitatively, by the ratio of the dissolved substance and the solvent, the solutions are divided into:

- concentrated – solutions in which there is a lot of dissolved substance;
- diluted – solutions in which there is little solute. But these concepts are conditional, relative and indefinite.

The following classification of solutions is more defined.:

- saturated solution is a solution in which at a given temperature the substance no longer dissolves, i.e. in this case the solubility product (SP) is equal to the product of ion concentrations in degrees equal to stoichiometric coefficients;
- unsaturated solution is a solution in which at a given temperature there is less soluble substance than in a saturated solution, i.e. in this case the solubility product (SP) is less than the product of ion concentrations in degrees equal to stoichiometric coefficients ;
- supersaturated solution is a solution in which at a given temperature in the dissolved state there is more substance than in its saturated solution under the

same conditions, ie an in this case the solubility product (SP) is bigger than the product of ion concentrations in degrees equal to stoichiometric coefficients ;

- . Oversaturated solutions are very unstable. They are able to exist only in the absence of a solid phase of a dissolved substance. Crystallization can be caused by the addition of crystalline, shaking, friction with a stick on the walls of the vessel.

1.2. Solubility

*Solubility i*s the ability of a substance to dissolve in a particular solvent.

Solubility is a spontaneous physicochemical process. It occurs due to the diffusion of molecules or ions from a region with a higher concentration to a region with a lower concentration. As a result, the substance is evenly distributed throughout the solution. Solubility is a bidirectional process: a solid goes into solution, and a dissolved substance goes into a solid. Therefore, both dissolution and crystallization occur simultaneously. These processes proceed with the same speeds over time - dynamic equilibrium sets in. In this case, the concentration of the soluble substance remains constant without changing the conditions. This condition is called the saturation state, and the solution is called saturated. The ability of various substances to dissolve in a particular solvent is called solubility. A measure of solubility is the princiconcentration of a saturated solution at a given temperature and pressure. The dynamic equilibrium Le Chatelier`s principle is applicable to the dissolution process. Solubility depends on temperature, external pressure, nature of the solute and solvent.

If more than 10 g of a substance is dissolved in 100 g of water, then such a substance is called highly soluble. If less than 1 g of the substance is dissolved, the substance is sparingly soluble. A substance is considered practically insoluble if less than 0.01 g of the substance passes in solution. Absolutely insoluble substances do not exist. Even when we pour water into a glass vessel, a very small part of the glass molecules inevitably passes into solution.

Solubility expressed by the mass of a substance that can dissolve in 100 g of water at a given temperature is also called the solubility coefficient.

The mutual solubility of liquids or solids in liquids depends on a number of properties of these substances: chemical nature, value and structure of particles, electric charge (in the case of ions), dipole moment and others.

Unlimited solubility is when two liquids dissolve one into the other in any ratio. *Limited* solubility is one liquid that dissolves in another in a certain concentration. With limited mutual solubility of the two liquids A and B, each of them dissolves. However, after settling, two layers are formed, which are arranged one above the other in the order of decreasing density.

Information on the solubility of drugs is given in pharmacopeia articles and reference tables..

1.3 Concentration of solution

The main characteristic of the solution is its concentration. In chemistry, the concentration of a solution is the quantity of a **solute** that is contained in a particular quantity of **solvent** or solution. Knowing the concentration of solutes is important in controlling the stoichiometry of reactants for solution reactions. Chemists use many different methods to define concentrations, some of which are described in this section.

Quantitatively, the concentration of the solution is expressed in many ways. Six methods for expressing the concentration of solutions are most commonly used.:

mass fraction..... ω, %

molar concentration.....C_M, M

molar concentration of equivalent(normality)…….…....C_N, C_{eq}, N

molality …….....Cm

titre .....T

mole fraction.....N

In practice, you often have to work with solutions with a strictly defined content of solute in them. For example, the preparation of various medicinal solutions, the preparation of solutions with a given concentration, chemical reactions in solutions.

1.4. Methods for expressing the concentration of solutions.

Mass fraction (w or W). The mass fraction of component X of the solution is defined as the ratio of its mass to the mass of the entire solution m. Mass fraction – dimensionless quantity, it is expressed in fractions of one:

$$\omega(X) = \frac{m(X)}{m} \quad (0 < \omega < 1). \qquad (1)$$

The mass fraction of solute is also called the percentage concentration of the solution.

Percentage concentration is mass fraction multiplied by 100:

$$\omega(X),\% = \frac{m(X)}{m} \cdot 100 \quad (0\% < \omega < 100\%), \qquad (2),$$

where w (X) is the mass fraction of the component of solution X; m (X) is the mass of the component of solution X; m is the total mass of the solution.

The volume fraction (φ) of the solution component is defined as the ratio of the volume of a given component X to the total volume of solution V. Volume fraction is a dimensionless quantity, it is expressed in fractions of unity:

$$\varphi(X) = \frac{V(X)}{V} \quad (0 < \varphi < 1). \quad (3)$$

Volume percent is volume fraction multiplied by 100.

The molar fraction (N) of the solution component is equal to the ratio of the amount of the substance of this component X to the total amount of the substance of all components in the solution.

For a binary solution consisting of solute X and a solvent (e.g. H_2O), the mole fraction of solute is:

$$N(X) = \frac{n(X)}{n(X) + n(H_2O)}. \qquad (4)$$

Molar concentration(molarity) (C_M) is defined as the ratio of the amount of solute X to the volume of solution V:

$$c_M(X) = \frac{n(X)}{V}. \qquad (5)$$

The basic unit of molar concentration is mol/l. Example of recording molar concentration: $C_м(H_2SO_4)$ = 0,8 mol/l or 0,8M.

The normality or molar concentration of equivalent (C_{eq}) is defined as the ratio of the number of equivalents of dissolved substance X to the volume of solution V:

$$C_{equiv} = \frac{n_{equiv}(X)}{V} \qquad (6)$$

The basic unit of equivalent concentration is mol-eq/l. For example, $C_н(H_2SO_4)$ = 0,8 mol-eq/l or 0,8N.

The titre (T) shows how many grams of dissolved substance X is contained in 1 ml or in 1 cm^3 of solution:

$$T = \frac{m(X)}{V}, \qquad (7)$$

где m(X) –solute (X) mass ,g; V – solution volume, ml.

The molality (m) of solution m indicates the amount of solute X in 1 kg of solvent:

$$m = \frac{n(X)}{m_o}, \qquad (8)$$

where n (X) is the number of moles of dissolved substance X, and mo is the mass of the solvent in kg.

Calculation of concentrations. Examples of calculationon the topic "Solutions"

Task 1. Calculate molar concentration, equivalent concentration, molality, titer, mole fraction for 40 % Sulfuric acid solution, if the density of this solution is 1.303 g / cm^3.

Solution to the problem.

The mass of 1 liter of solution is M = 1000·1,303 = 1303,0 г.

The mass of sulfuric acid in this solution: m = 1303·0,4 = 521,2 г.

Molar concentration $c_м$ = 521,2/98 = 5,32M.

Equivalent concentration c_N = 5,32/(1/2) = 10,64N.

Titre of this solution T = 521,2/1000 = 0,5212 g/sm^3.

Molality m = 5,32/(1,303 – 0,5212) = 6,8 mol/kg of water.

Note that in concentrated solutions, molality (m) is always greater than molarity (C_M). In dilute solutions m ≈ C_M.

Mass of water in solution: m = 1303,0 – 521,2 = 781,8 g.

Amount of water substance: n = 781,8/18 = 43,43 mol.

The molar fraction of sulfuric acid: N = 5,32/(5,32+43,43) = 0,109. The mole fraction of water is 1– 0,109 = 0,891.

Methods of solution preparation.

Example 1. For the treatment of hypertension, 25% solution of magnesium sulfate $MgSO_4$ is used.

Decision: This means that 100 g of this solution contains 25 g of $MgSO_4$.

Method of solution preparation: To prepare a 25% solution, it is necessary to weigh 25 g of anhydrous magnesium sulfate on a scale and measure 75 ml of water with a beaker (or weigh 75 g of water on a scale. The density and weight of water are equal under normal conditions.). Then magnesium sulfate must be poured into water and mixed until completely dissolved. Get 100 g of solution (25 g + 75 g = 100 g), in which the mass fraction of magnesium sulfate is exactly 25%.

$$w = \frac{25\ г}{100\ г} \times 100\% = 25\%$$

* If anhydrous salt is not found for weighing 25 g of $MgSO_4$, and only the more common crystalline hydrate $MgSO_4 \cdot 7H_2O$ is available, then another calculation needs to be done. Preliminarily, it is necessary to calculate how much $MgSO_4 \cdot 7H_2O$ contains 25 g of $MgSO_4$ and weigh precisely this calculated amount of $MgSO_4 \cdot 7H_2O$. Accordingly, less water will be used to prepare such a solution, because part of it already exists in crystalline hydrate.

Example 2. To fill in a new car battery you need 36% solution of sulfuric acid. This means that 100 g of such a solution contains 36 g of sulfuric acid and 64 g of water (100 g - 36 g = 64 g). The mass fraction of sulfuric acid in such a solution is 36%.

$$w = \frac{36\ г}{100\ г} \times 100\% = 36\%$$

1.5. Medical solutions.

Medical solutions are homogeneous systems containing at least two substances, one of which is a drug substance. The solvent used is water, oils, water-alcohol solutions.

Other solvents and cosolvents are also used: glycerin, propylene glycol, isopropyl alcohol.

The solutions are diverse in composition. There are solutions of individual substances or compositions of medicinal substances.

In addition to medicinal substances, auxiliary substances may be present in medical solutions: flavoring agents, odors, preservatives, dyes, stabilizers, buffer systems. Medical solutions for oral use (syrups, aromatic water, etc.) are usually prepared in purified water, solutions for external use (lotion rinses, drops, etc.) are prepared in purified water and other solvents (ethyl alcohol, glycerin, fatty and mineral oils, DMSO, silicones, etc.).

Depending on the solvent, medical solutions are divided into:

- aqueous solutions;
- alcohol solutions;
- glycerin solutions;
- oil solutions;
- sugar solutions (syrups);
- aromatic waters.

Water as a solvent.

Most often, water of the Purified Water category is used as a solvent for the preparation of medical solutions.

Advantages of water as a solvent:

- high bioavailability of aqueous solutions of medicinal substances;
- cheapness;
- ease of receipt.

Disadvantages:

- chemical instability of drugs during storage (hydrolysis, oxidation);

- susceptibility to microbial contamination;

- the need to use packaging made of chemically resistant glass to prevent leaching.

Non-aqueous solvents

The quality of non-aqueous solutions, as well as technological methods for their manufacture, are largely determined by the physicochemical properties of the solvents. Non-aqueous solvents differ in their chemical structure, dielectric constant, and, consequently, their ability to dissolve medicinal substances.

Classification of non-aqueous solvents.

The solvents used to obtain non-aqueous solutions are divided into volatile and non-volatile.

To obtain medical solutions, volatile solvents are often used, which include: ethyl alcohol, medical ether.

As non-volatile solvents, glycerol, fatty oils, liquid paraffin, etc. are used.

Such a classification is important from a technological, pharmacological, consumer point of view and for the proper observance of production safety regulations.

Some medicinal substances do not dissolve in specific solvents to prepare a solution of the required concentration. Combined solvents (solvent mixtures) are used to dissolve such substances. As an example, mixtures of ethanol with glycerol, glycerol with dimexide, etc.

The use of combined solvents also allows combining several medicinal substances with different solubilities in aqueous dosage form.

Co-solvents are substances used in complex solvents to increase the solubility of certain difficultly soluble drugs. These include benzyl benzoate, which is used to increase solubility in oils, as well as ethanol, propylene glycol, glycerol, which are used to increase the solubility of a drug substance in water.

Dissolution in viscous liquids (glycerin, fatty oil, liquid paraffin) is often carried out at elevated temperatures to reduce viscosity and accelerate diffusion (solutions of boric acid, borax in glycerin, camphor in oil, etc.).

Alcohol solutions are prepared without heating with strict observance of the safety rules, labor protection and fire protection.

Solutions are cleaned by settling and filtering.

In recipes the concentration of solutions is indicated by the following methods:

1. Indicate the concentration of the drug in percent (which shows the weight amount of solute in grams in 100 ml of solution).

Rp.: Solutionis Kalii iodidi 2 % 200 ml Da. Signa.

2. Amounts of drug and solvent indicated.

Rp.: Kalii iodidi 4,0

Aquae purificatae 200 ml Misce. Da. Signa.

3. Indicate the amount of drug substance and the total volume of the solution, which is achieved by adding the prescribed solvent

Rp.: Kalii iodidi 4,0

Aquae purificatae ad 200 ml Misce. Da. Signa.

4. Indicate the ratio of the amount of prescribed medicinal substance to the total amount of the resulting solution using lat. *ex - from*.

Rp.: Solutionis Kalii iodidi ex 4,0 — 200 ml Da. Signa.

Despite the different methods of prescribing potassium iodide solutions, its volume is 200 ml, the amount of the drug substance is 4.0 g.

5. Indicate the degree of dilution of the drug substance, for example, 1:1000, 1:5000, 1:10000 and the volume of this solution.

Rp.: Solutionis Furacilini (1:5000) 200 ml Da. Signa.

Of all the above methods, the method of designating the solution concentration in percent is most often used.

1.6. Calculation of the amount of prescribed fluid

1. Pharmacopoeial liquid is prescribed under the chemical name.

If the pharmacopoeial liquid concentration is indicated, then the calculations proceed from the actual substance content in a standard solution according to the formula:

$$X = V * A / B,$$

X is the volume of standard liquid, ml;

V is the volume of the solution to be prepared, ml;

A is concentration prescribed in the recipe,%;

B is the actual concentration of the standard fluid, %.

A) Calculation of doses in various dosage forms

In medicine, three basic metric units are used: Gram (g) is a measure of mass, Meter (m) is a measure of length, Liter (l) is a measure of volume

Unit ratio:

1 gram = 1 000 milligram (mg) 1 gram = 1 000 000 micrograms (mcg) 1 kg= 1 000 gram (г) 1 gram = 0,001 kilogram (kg)	1 liter = 1 000 milliliters (ml) = 1 dm^3 1 milliliters = 1 sm^3
	Teaspoon Volume = 5 ml Dessert spoon volume = 10 ml Tablespoon volume = 15 ml In 1 ml of 20 drops of solution

Dose is the amount of a substance intended for one dose. The effectiveness and safety of treatment depends on the dosage. The dose of the substance must be carefully selected, otherwise the drug will either not provide the desired effect, or cause poisoning.

In the prescription, a single dose (SD), daily dose (DD), course dose are indicated in terms of weight or volume of the drug substance (DS) is grams, fractions of a gram, milliliters, drops. In the signature of the prescription, the dose of the drug for admission is indicated in pieces (tablets, capsules, etc.), spoons, drops, so that it can be understood by an unprepared patient.

If the appointment is made in the DD, then the frequency of reception is indicated SD is found by dividing the daily dose by the number of doses.

Giving the patient pills and capsules, you need to remember a few rules:

• only specially labeled tablets or notched tablets can be accurately separated; Coated tablets, drages, capsules cannot be divided into parts!

• the dosage of the drug you have and the dosage prescribed by your doctor must be in the same units.

So, if the doctor prescribed 1 g of the drug, and the nurse has 500 mg of the drug, then she should know that 1 g = 1000 mg and give the patient 2 tablets

To calculate the dose of the drug, you can use the following formula:

- $$SD = \frac{The\ required\ dose\ (doctor's\ prescription)}{amount\ of\ drug\ per\ tablet}$$

Example 3. Assigned to 0.5 g of drug for 4 doses per day. Then SD = 0,5/4 = 0,125 (125 mg).

If a dose is prescribed per unit of weight per day or at a dose, SD and DD are calculated by multiplying the dose by the weight of the patient.

Example 4. Appointed 50 mg / kg for 2 doses to a patient weighing 50 kg. Then DD = 50 mg • 50 kg = 2500 mg/day (2,5 g/day); SD = СД/2 = 2,5/2 = 1,25 (1250 mg).

Course dose is the product of the daily dose for the duration of the course of treatment in days.

Example 5. A patient is prescribed a dose of 600 mg per dose. The drug is packaged in grams. How many grams should be given to the patient?

Solution: Compose the proportion: *1 g – 1000mg*
X g – 600mg

2) Now solve this proportion

$$X = \frac{1\ g\ \cdot 600 mg}{1000\ mg} = 0.6\ g$$

Answer: it is necessary to give the patient 0.6 g of the drug.

B) Calculation of the dose of solutions

Features:	**Remember!**
	-- the volume of prepared solutions can be from 2 ml (volume of a syringe) to 500 ml (volume of a bottle);
	-- the source line of the proportion will most often be matching: In 100 ml of a solution, as many grams as percent are

	Example: in 100 ml 1,5% solution – 1,5 g substance 3 % - 3 g 20 % - 20 g

Example 6. The doctor's prescription is 250 ml of sterile 5% *Glucosum* solution for intravenous drip. How many grams of glucose in solution?

Solution : 100 ml (5%) – 5 g

250 ml – x g $x = \frac{250 \cdot 5}{100} = 12.5\ g$

Now we will deal with solutions whose concentration is given by the ratio, for example, 1: 5.

Convert to %.

Example 7. The solution is set 1:1. That is, for example, one part of alcohol and one part of water.

Part may be a drop or a teaspoon, or a glass, etc. Now we decide. Together it turned out 2 parts. **According to proportion:**

2 parts – 100%

1 part – x % x=50%, the solution has a concentration 50%

Example 8. The solution is set 1:3.

According to proportion:

4 parts – 100%

1 part – x % x=25%, the solution has a concentration 25%

B) Dilution of antibiotics

PLEASE NOTE: Some drugs are not dosed in units of the metric system (for example, grams), but in AU action units ED or IU.

Units (units of action) measure, for example, antibiotics, insulin, pancreatin, nystatin, botox. Grams or milligrams, or AU, can be indicated on an antibiotic vial.

IU (international unit) is in pharmacology, this is a unit of measurement of the amount of a substance based on biological activity. It is used for vitamins, hormones, certain drugs, vaccines, blood constituents and similar biologically active substances.

The drug content in grams or units is indicated on the package, for example, sodium benzylpenicillin - 1000 000 units. According to the instructions, add a certain amount of solvent (for example, 10 ml) to a dry drug. In the calculations, we also make proportions

Example 9. Appointed: sodium salt of benzylpenicillin 750,000 units.
Proportion:

1000000 AU – 10 ml

750000 AU – *x* ml

Then x = 750000 • 10/1000000 = 7,5 ml.

If a solution of benzylpenicillin sodium salt is prescribed, for example, to a child weighing 6 kg, then a volume of 7.5 ml will be traumatic. To reduce the input volume, it is necessary to prepare a more concentrated solution, i.e. dilute benzylpenicillin sodium salt not in 10, but in 5 ml of solvent. Then the injection volume will be calculated from the proportion:

1000000 ЕД – 5 ml

750000 ЕД – *x* ml

x = 750000 • 5/1000000 AU = 3,25 ml.

Practical lesson 5

Topic: Hydrogen index of biological fluids. Definition of K and α weak acid.

Relevance of the topic: The concentration of hydrogen ions [H^+] in cells and biological fluids is one of the most important parameters for homeostasis. The concentration of H+ ions significantly affects all vital functions. For example, kinetics of enzymatic reactions, physicochemical and structural state of membranes, conformation of macromolecules, hemoglobin affinity to oxygen, receptor sensitivity to biologically active substances, intensity of generation of reactive oxygen species. Deviations [H^+] from the optimal range lead to disorders of metabolism, cellular activity (up to their death), tissues, organs and the body as a whole.

Lesson objective: to consider the theory of acids and bases and the properties of strong and weak acids; to study methods for determining pH; learn how to calculate the degree and dissociation constant and the pH of a solution. Apply the basic principles of acid-base equilibrium to living organisms.

Basic definitions: electrolytes, degree of dissociation, pH, ionic product of water, alkalosis, acidosis, Ostwald's law.

Plan and organizational structure of the lesson:

1. Electrolytes in the organism of a human body. Acid and base theories.
2. The dissociation degree and constant of weak electrolytes dissociation.
3. Ostwald's law for dilute weak electrolytes.
4. Solution properties of strong electrolytes.
5. Activity and coefficient of activity.
6. Ionic strength of solution.
7. Hydro-electrolytic balance – necessary condition of homeostasis.
8. Normal and pathologic pH intervals.
9. Acidosis. Alkalosis.
10. The role of electrolytes in processes of vital activity.
11. Acid-base equilibration in solutions of electrolytes.
12. Determination of dissociation constant and dissociation degree of weak electrolyte

Content of the topic

Electrolytes are substances (acids, bases, salts) which, when dissociate into ions (cations and anions) in melt state and water solutions conduct electricity. Cations are ions with positive charge. Anions are ions with negative charge.

Acids and bases play important roles in living organisms. Strong acids and bases can be quite harmful; they will destroy tissue by dissolving protein material and drawing out water. Concentrated bases will react with the fats that make up the protective membranes of cells, destroying such membranes and causing even more widespread destruction to tissues than wilt acids. Biological molecules, such as proteins and nucleic acids, bear numerous functional groups, such as carboxyl (-COOH) and amino ($-NH_2$) groups, that can undergo acid- base reaction. Many properties of not only these molecules therefore vary with the acidities of the solutions in which they are immersed.

The stomach living excretes about 2 liters of HCl solution over a 24-hour period, most coming after meals.

Even small changes in acid- base concentration can be of great importance to living cells and critical in many fields of scientific investigations. Acids and bases are critical to all living organisms. Estimation of acid- base equilibrium, is the source of clinical information used to diagnose and treat infections, metabolic abnormalities, and various diseases.

A quantitative measure of the extent of dissociation is **the degree** of **dissociation** *(a)*

$$\alpha = \frac{Ndis}{Ntot}$$

Where N_{dis} - number of molecules split into ions, N_{tot} - total number of molecules dissolved.

α depends upon temperature, concentration and the nature of the solute and the solvent.

Examples of strong and weak electrolytes are given below:

Strong Electrolytes (α >30%)	strong acids	HC1, HBr, HI, HNO_3, $HC1O_3$, $HC1O_4$, and H_2SO_4
	strong bases	NaOH, KOH, LiOH, $Ba(OH)_2$, and $Ca(OH)_2$
	salts	NaCl, K_2S, NH_4Cl and all soluble salts
Middle Electrolytes (3%< α <30%)	middle acids	H_3PO_4, HF, H_2SO_3
Weak Electrolytes (a<3%)	weak acids	CH_3COOH, H_2CO_3, H_2S, HCN, H_2SiO_3
	weak bases	NH_4OH, $Mg(OH)_2$ all insoluble bases of p- and d-elements

Ionic product of water

Water is universal solvent

Pure water is a weak electrolyte:

$$\mathbf{2H_2O \leftrightarrow H_3O^+ + OH^-}$$

$$\mathbf{H_2O \leftrightarrow H^+ + OH^-}$$

According to Mass action law, the dissociation of water can be represented as

$$\mathbf{K_d} = \frac{[H^+][OH^-]}{[H_2O]}$$

If temperature is a constant concentration of water $[H_2O]$ is constant too, then

$$\mathbf{K_d \cdot [H_2O] = [H^+]\cdot[OH^-] = K_w}$$

At equilibrium, the product of $\mathbf{[H^+]\cdot[OH^-]}$ is a constant. K_w is called the ionic product of water. At 298° K the H^+ concentration is equal the OH^- concentration are respectively $1\cdot10^{-7}$ mol/L.

The acid- base balance of the body is basically the metabolism of hydrogen ions (H^+). One convenient way to express the H^+ concentration is by using pH.

$$\mathbf{pH = -log[H^+]}$$

$$\mathbf{pOH = -log[OH^-]}$$

$$\mathbf{[H^+]\cdot[OH^-] = 10^{-14}}$$

Taking logarithm of both sides:

$$\mathbf{-log([H^+]\cdot[OH^-]) = - (log\ [H^+] + log\ [OH^-]) = -lg10^{-14} = 14}$$

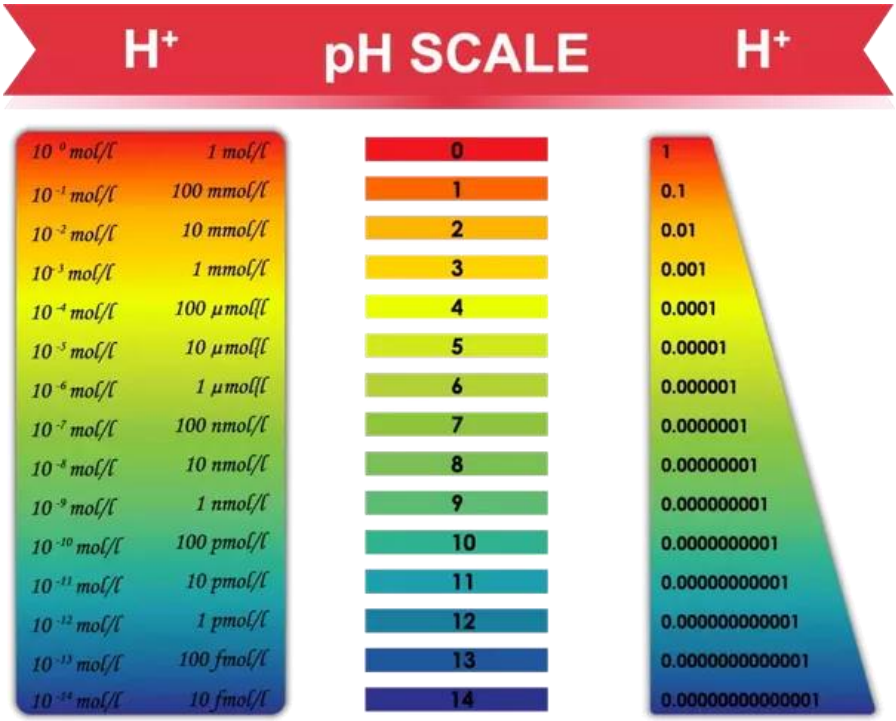
According to the definition of pH and pOH, the above equation is simplified to

pH + pOH=14

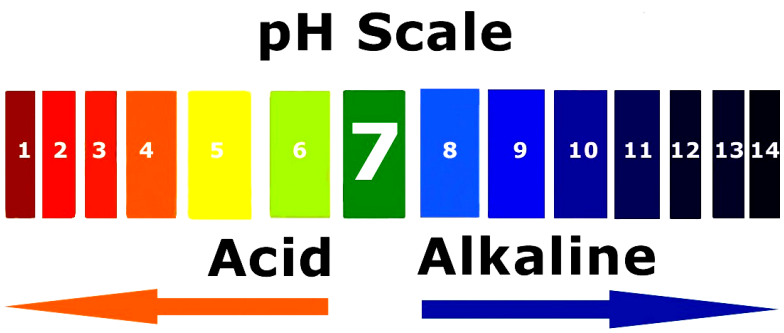
In acidic solutions $[H^+] > [OH^-]$ or pH < 7.0

In neutral solutions $[H^+] = [OH^-]$ or pH = 7.0

In basic solutions $[H^+] < [OH^-]$ or pH > 7.0.



A pH scale is shown below:



Normal pH range of body fluids

Gastric juice	1,0-3,0	Pancreatic juice	8,0
Saliva	6,5 -7,5	Tears	7,2
Urine	5,5-7,0	Vaginal secretions	7,7
Blood	7,35 -7,45	Intestinal secretions	7,7
Bile	7,8-8,8	Milk	7,1

Contemporary Theories of Electrolytes

A substance, that dissolves in water to give an electrically conducting solution is called an electrolyte. A substance that dissolves in water to give nonconducting or very poorly conducting solutions is called a nonelectrolyte.

When electrolytes dissolve in water they produce ions, but they do so to varying extents. A strong electrolyte is an electrolyte that exists in solution almost entirely ions. A weak electrolyte is an electrolyte that dissolves in water to give equilibrium between a molecular substance and a small concentration of ions.

According to **Svante Arrhenius** concept:

Acid is electrolyte that when dissolved in water increase the concentration of hydrogen ion H^+.

Base is electrolyte that, when dissolved in water, increase the concentration of hydroxide ion OH^-.

$$NaOH \rightarrow Na^+ + OH^-$$

$$HCl \rightarrow H^+ + Cl^-$$

The most shortcomings of Arrhenius concept:

1. Arrhenius concept (theory) does not explain the cause of dissociation of electrolytes on ions.

2. Arrhenius concept (theory) does not explain an acid or base property of organic substances, which not produced ions in water solution.

3. Arrhenius concept (theory) does not take account of interaction between solvent and dissolved substance.

4. Arrhenius concept (theory) can be used only in water solutions.

Protolytic theory (1923)

According to **Johannes N. Brønsted and Thomas M. Lowry** concept:

Acid is the species (molecule or ion) that donates a proton to another species in a proton-transfer reaction.

Base is the species (molecule or ion) that accepts a proton in a proton-transfer reaction.

$$H_2SO_4 \leftrightarrow H^+ + HSO_4^-$$

acid conjugated base

$HSO_4^- \leftrightarrow H^+ + SO_4^{2-}$

acid conjugated base

$H_2O \leftrightarrow H^+ + OH^-$

acid conjugated base

$H_2O + H^+ \leftrightarrow H_3O^+$

acid conjugated acid

H_2O is ampholyte

$NH_3 + H_2O \rightarrow NH_4^+ + OH^-$

base I acid II acid I base II

A conjugate acid-base pair consists of two species in an acid-base equilibrium, one acid and one base, which differ by the gain or loss of a proton. The acid in such a pair is called the *conjugate acid* of the base, whereas the base is the *conjugate base* of the acid.

The Brønsted-Lowry concept of acids and bases has greater scope than the Arrhenius concept:

1. A base is a species that accept protons; the OH^- ions is only one example of a base.

2. Acids and bases can be ions as well as molecular substances.

3. Acid-base reactions are not restricted to aqueous solutions.

4. Some species can act as either acids or bases, depending on what the other reactant is.

Such species, which can act either as an acid or a base (it can lose or gain a proton), called an amphiprotic species:

$HCO_3^- + HF \rightarrow H_2CO_3 + F^-$

Base I acid II acid I base II

$HCO_3^- + OH^- \rightarrow CO_3^{2-} + H_2O$

acid I base II base I acid II

Protolytic theory can be used for water and non-water solutions.

Electronic theory (1923)

According to **G. N. Lewis** concept:

Lewis acid is a species that can form a covalent bond by accepting an electron pair from another species.

Lewis base is a species that can form a covalent bond by donating an electron pair to another species.

$$H^+ \quad + \quad :NH_3 \quad \longrightarrow \quad NH_4^+$$

electron-pair acceptor	electron-pair donor
Lewis acid	Lewis base

The Lewis and the Brønsted-Lowry concepts are simply different ways of looking at certain chemical reactions. The Lewis concept could be generalised to include many other reactions, as well as proton-transfer reactions.

Acids and bases are classified as strong or weak.

Strong acids are acids that ionise completely in water (that is, they react completely to give ions) ($\alpha > 30\%$).

Weak acids are acids that are only partly ionised as the result of equilibrium reaction with water ($\alpha < 3\%$).

Strong bases are bases that are present in aqueous solution entirely as ions, one of which is OH^- ($\alpha > 30\%$).

Weak bases are bases that are only partly ionised as the result of equilibrium reactions with water ($\alpha < 3\%$).

The strongest acids have the weakest conjugate bases, and the strongest bases have the weakest conjugate acids. The terms *strong* and *weak* are used only in a comparative sense. The strengths of acids and bases are relative. In acid base interaction the water (or another solvent) exhibits a *levelling effect* on the strength of the strong acids.

Acid and base with water produce hydrogen ion or hydroxide ion (relatively) and its conjugated ions. The process is called electrolyte ionisation or electrolyte dissociation.

For the strong electrolyte (acid or base), which completely ionise in solution, the concentration of ions are determined by the stoichiometry of the reaction from the initial concentration of electrolyte:

$$[H^+] \approx [HA]$$

$$[OH^-] \approx [BOH]$$

Calculation of pH of weak acid and base solutions

The weak electrolyte (acid and base) ionises or dissociates to a small extent in water (about 1 % or less, depending on concentration of electrolyte). For the weak electrolyte (acid or base) the concentration of ions in solution are determined from the acid ionisation (or dissociation) constant (K_a) or the base ionisation (or dissociation) constant (K_b), which is the equilibrium constant from the ionisation of a weak electrolyte.

$$K_a = \frac{[H^+]\cdot[A^-]}{[HA]} \qquad K_b = \frac{[B^+][OH^-]}{[BOH]}$$

The degree of ionisation (α) of a weak electrolyte is the fraction of molecules that react with water to give ions. This also may be expressed as a percentage, giving the percent of ionisation:

$$\alpha_a = \frac{[A^-]}{[HA]} \qquad [H^+] = [A^-] = \alpha\cdot[HA] = \alpha\cdot C_a$$

$$\alpha_b = \frac{[OH^-]}{[BOH]} \qquad [OH^-] = [B^+] = \alpha\cdot[BOH] = \alpha\cdot C_b$$

In water solution weak acid dissociates according to equation:

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

According to active mass law:

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

At dynamic equilibrium:

$$c_{dis} = [H^+] = [A^-] = c_0\alpha,$$

where c_0 is initial concentration of acid. So,

$$K_a = \frac{c_0\alpha * c_0\alpha}{c_0 - c_0\alpha} = \frac{c_0\alpha^2}{1-\alpha}$$

For dilute weak electrolyte $\alpha \to 0$, and $(1 - \alpha) \approx 1$; percent of ionisation can be shown approximately on Ostwald's dilution rule:

$$K_a = \frac{C\alpha^2}{1-\alpha} \qquad \alpha = \sqrt{\frac{Kc}{C}}$$

The aqueous solutions of strong electrolytes and concentrated solutions of weak electrolytes not submit to classic law of mass action in full. Peter Debye and Erich Hückel were able to show that the properties of electrolyte solutions could be explained by assuming. The electrolyte is completely ionised in solution but that the activities, or effective concentrations, of the ions are less than their actual concentrations as a result of the electrical interaction of the ions in solution. The **Debye-Hückel** theory allows us to calculate these activities. When this is done, excellent agreement is obtained for dilute solutions:

$$a = C\cdot\gamma \qquad \lg\gamma = -Az^2\sqrt{I},$$

where:

a – active concentration of ions;

C – relative concentration of ions;

γ – activity index;

A – value, calculate theoretically, depends from temperature, ion-dipole force etc.;

for water solutions at t = 25 °C A = 0,509;

I – ionic strength;

z - charge of ion.

In solutions ion is a charged particle, surrounded *ionic atmosphere* with solvent ions. Ionic atmosphere parameters are definite by ionic strength:

$$I = \frac{1}{2}\sum_i c_i z_i^2,$$

where c_i – molality of electrolyte ions.

Thus, we have seen that equilibrium-constant of electrolyte solutions change value accordance to activities of ions and depend from ionic strength of solution.

Determination of the total (general) acidity, pH, K and ά of the weak acid.

5,0 mL of the analyzed weak acid are pipetted out and transferred completely in a clean conical flask. 1-2 drops of phenolphthalein indicator solution are then added to it and the solution well-mixed by slowly stirring. A colorless solution results. The flask with its contents is slowly and gradually added to the solution in the flask, stirring the solution. The end point is indicated by the change of the colorless solution to light pink by the addition of the last drop of the NaOH solution.

Three such titrations are generally carried out by filling the burette up to the zero mark after each titration.

$$V(NaOH) = \frac{V1(NaOH) + V2(NaOH) + V3(NaOH)}{3} \text{ [ml]}$$

The total (general) acidity (C_o) of the weak acid is

$$Co = \frac{C(NaOH) \bullet V(NaOH)}{V\ (acid)}$$

Where C(NaOH) = 0,1 M and V(acid) = 0,5 mL

Calculate the ά and K using Oswald`s law for dilute weak electrolyte:

$$ά = \frac{[H^+]}{c_0} \qquad K = ά^2C$$

Where active concentration of weak acid is calculated from the pH of the weak acid.

Practical lesson 6

Topic: Colligative properties of solutions. Osmometry, cryometry, ebuliometry. The role of osmosis in biological fluids.

Relevance of the topic: Osmosis and osmotic pressure play an important role in the regulation of biological processes. In the body, osmotic pressure is an important factor determining the distribution of water and nutrients between organs and tissues. Calculation of osmotic pressure is used in the manufacture of drugs, such as eye drops. In addition, the study of this topic is necessary to understand the pathological processes: violation of water and ionic balance, pathophysiology of the blood system, including the mechanisms of inflammatory processes and their elimination.

Lesson objective: To master theoretical material on the colligative properties of dilute solutions; to learn how to perform appropriate calculations; to learn how to operate formulas and use them to solve situational problems on the topic of the class; to learn to solve problems, calculate the osmotic pressure of solutions, the molecular weight, etc.

Basic definitions: dispersion system, diffusion, osmosis, osmotic pressure, turgor, Fick's law, Van't Hoff's law.

Plan and organizational structure of the lesson:

1. Classification of dispersion systems. Molecular and ionic solutions.
2. Colligative properties of dilute solutions of nonelectrolytes and electrolytes.
3. Diffusion. Fick's law.
4. Osmosis and osmotic pressure. Vant-Hoff`s law and Vant-Hoff`s law consequents.
5. Biological importance of diffusion and osmosis. Isotonic, hypotonic and hypertonic solutions. Plasmolysis and hemolysis.
6. Cryoscopy and ebullioscopy application in biological researches.
7. Medical application of colligative properties of dilute solutions in medical-biological researches.

Content of the topic

Some properties of diluted solutions depend on the number of particles of dissolved substances and do not depend on the chemical composition of these particles. They are called *colligative* (from Latin *colligatus).* So, colligative properties of solutions depend on the number of kinetic units of the system (molecules or ions) and don't depend on the identity of the solute. They include: diffusion, osmosis, lowering of the vapor pressure of the solvent above the solution, boiling-point elevation, freezing- point depression, and some other properties.

Diffusion – the spontaneous mixing of particles, from regions of higher to regions of lower concentration.

Fick's equation

$$V = \frac{\Delta m}{\Delta \tau} = -DS\,\frac{\Delta c}{\Delta x}$$

V is the rate of diffusion

D is a coefficient of diffusion

τ is the time of investigation

$\frac{\Delta c}{\Delta x}$ is concentration gradient

Einstein's equation:

$$D = \frac{kT}{6\pi\eta r}$$

k is the Boltsman's constant

T is the absolute temperature

n is the viscosity of the medium

r is the radius of particles

Osmosis and osmotic pressure. Van't Hoff's law

An important colligative property of solutions in biology and medicine is *osmosis* (from Greek *osmos* - push, pressure). For osmosis occurrence it is necessary to bring solutions of different concentration in contact through a semipermeable membrane. In biological systems there may be membranes permeable only for sol-

vent molecules. In this case the solute molecules can't diffuse through a membrane, but solvent molecules can move in both directions, besides, the number of solvent molecules that got into a more concentrated solution is greater than the number of solvent molecules that pass into the opposite direction, where there is a more diluted solution.

Osmosis – the movement of water molecules through a differentially permeable membrane from a region of lower solute concentration to a region of higher solute concentration.

Or **one-sided diffusion of solvent molecules through a semipermeable membrane to a more concentrated solution is called osmosis.**

The property of semipermeability is typical of animal and vegetative membranes, and also of artificial porous polymeric films, for example, films of collodion, cellophane.

A vessel with a solution separated with a semipermeable membrane from the solvent is called an osmotic cell *(Fig. 1.).* As a result of osmosis the solution volume in the osmotic cell increases and its concentration gradually decreases. The volume increase of solution leads to expansion of liquid column *h* in the apparatus for measurement of osmotic pressure. Thus, hydrostatic pressure rises. This pressure rise leads to increase of the number of solvent molecules moving through the membrane from solution to solvent. Hydrostatic pressure and solution dilution will gradually reach sizes at which the number of solvent molecules moving in both directions will be equal and osmotic balance will be established. The excess of hydrostatic pressure occurring as a result of osmosis and being enough to stop one-sided diffusion is equal to osmotic pressure. So,

Osmotic pressure is the external pressure on a solution, at which osmotic equilibrium (through a semipermeable membrane) between the solution and a pure solvent is established.

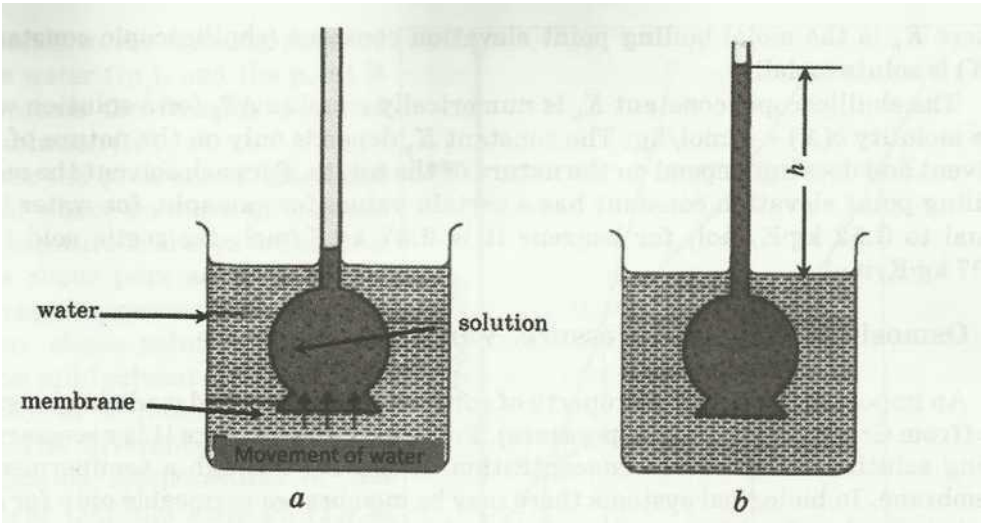


Fig. 1. Laboratory osmotic cell: *a* — at the beginning of the experiment; *b* — equilibrium state at the end of the experiment, when hydrostatic pressure of the solution column *h* counterpoises pressure

Studying of osmotic pressure laws has found their full analogy to gas laws.

For diluted solutions of non-electrolytes Van't Hoff *proposed the next law:*

Osmotic pressure of a solution is equal to the pressure that a solute would have if it were in a gaseous state at a given temperature and occupied the same volume that the solution occupies.

The mathematical expression of this law is:

$$\pi = c(X) \cdot R \cdot T$$

where *n* is osmotic pressure; *c(X)* is the molarity of the solute *X; R* is the universal gas constant *(R* = 8.31 J/ mole· K); *T* is temperature by Kelvin.

Consequents from Van't Hoff's law:

if $T_1=T_2$ → $C_1/C_2 = \mathbf{P_1/P_2}$

if $C_1=C_2$ → $T_1/T_2 = P_1/\mathbf{P_2}$

if $C_1=C_2$ and $\mathbf{T_1=T_2}$ → $P_1=\mathbf{P_2}$ **(isotonic solutions)**

Van't Hoff's law for diluted solutions of non-electrolytes has another formulation:

Osmotic pressure of diluted solutions of non-electrolytes is directly proportional to solution molarity, its absolute temperature, and does not depend on the nature of the solute.

Exact measurements of osmotic pressure of solutions have shown that Van't Hoff's equation may be applied only to highly diluted solutions of non-electrolytes.

Colligative properties of diluted solutions of electrolytes. Isotonic factor

For solutions of electrolytes (salts, acids, bases) the experimentally received values ΔT_f, ΔT_b and *n* are much bigger than the expected ones (calculated by using formulas). Van't Hoff's suggested to use the correction multiplier named *the isotonic factor i* for accounting of such derivations, which gives a possibility to apply Raoult's and Van't Hoff's laws for diluted solutions of electrolytes:

ΔT_f (measured) = i ΔT_f (expected)

ΔT_b (measured) = i ΔT_b (expected)

$\boldsymbol{\pi}$ = (measured) = i π (expected)

Numerical values of the isotonic factor depend on the nature of electrolyte and its concentration in the solution. So, for example, isotonic factors for solutions of some electrolytes with the molality $b(X)$ = 0.1 mol/kg have such numerical values: i(KCl) = 1.85; i($Ba(OH)_2$) = 2.54; i(H_2SO_4) = 2.2; i(CH_3COOH) = 1.01. When solution is diluted, the *i* value increases gradually approximating to whole numbers:

♦for binary electrolytes, such as KCl, $MgSO_4$, i = 2;

♦ for ternary electrolytes, such as $MgCl_2$, H_2SO_4, i = 3.

Such features of the colligative properties of electrolyte solutions were used by first researchers of this phenomenon, in particular by S. Arrhenius, to develop the electrolytic dissociation theory. S. Arrhenius arrived at the conclusion that the number of particles in an electrolyte solution is higher than the number of molecules, because electrolytes in the process of dissolution dissociate into smaller charged particles named ions.

Osmotic pressure – the amount of pressure that would have to be applied to a solution to prevent osmosis if the solution were separated from pure water by a differentially permeable membrane

P=CRT (for nonelectrolytes)

P= iCRT Vant Hoff s law (for electrolytes)

P is the osmotic pressure

C is the molarity

T is the absolute temperature

R is the solution constant

Isotonic, hypertonic, and hypotonic solutions

Solutions with an identical value of osmotic pressure are called *isotonic* (from Greek *isos* - equal, identical). Solutions with a higher osmotic pressure than that of a standard solution of comparison are called *hypertonic*, and solutions with a lower osmotic pressure are *hypotonic*. In medicine the osmotic pressure of the human blood plasma at 37°C is taken as standard, which is equal to 770 kPa = = 7.7 -10^5 Pa (7.6 atm).

In biological liquids (plasma, lymph, saliva, urine, etc.) osmotic pressure is created by dissolved inorganic and organic substances in the form of ions, molecules and colloidal particles. Their total concentration, which can create certain osmotic pressure, is called *osmotic concentration* (*osmolarity* or *osmolality).* In physiology the unit *osmole* - a unit of a number of substances osmotically active in a solution –

is used. One osmole contains 6.02-10^{23} particles (ions and molecules). Thus, osmolarity is analogous to molar concentration (molarity), and osmolality corresponds to molality. In practice, one thousandth of an osmole is used, it is called milliosmole. For biological liquids osmolarity is expressed in mosm/l, and osmolality - in mosm/kg. Osmolality of body liquids is equal to 292 ± 12 mosm/kg. Solutions with the same osmolality are isoosmotic as well as isotonic. 0.9 % or 0.15 molar NaCl solution in water is isotonic to the human blood plasma. It is often named a physiological solution.

It is known that in various living organisms different types of cells may have different osmotic pressure. For example, in tissues of plants osmotic pressure is equal to 500-2000 kPa, and in plants growing in deserts it may exceed 10000 kPa. Therefore the same solution for one type of cells may be hypotonic, and for another type it may be isotonic, and for yet another one it may be hypertonic.

Role of osmosis and osmotic pressure in biological systems

Osmosis is of great importance for vegetable and animal organisms. It promotes the normal course of various physical and chemical processes: hydration and dissociation of substances, reactions of hydrolysis, reduction-oxidation, etc. The osmotic pressure arising in organs and tissues causes cell turgor. Turgor (from Latin *turgeo* - to swell, to fill) is a state of tension of the cellular cover caused by the osmotic pressure of the cell contents. Turgor promotes maintenance of tissue elasticity and resiliency, preservation of a certain form of organs.

The human body maintains some physical and chemical indices of the internal environment, including osmotic pressure. The osmotic pressure of the human blood in norm is equal to 770 kPa (7.7 atm) at 37°C. The constancy of these indices is named *isotonicity*. A decrease in osmotic pressure caused by introduction of an excessive amount of water or as a result of intensive loss of salts causes vomiting, spasms, loss of consciousness and other disorders up to death. An attempt to increase osmotic pressure by introducing a considerable amount of salts leads to water redistribution. Water accumulates in the tissues containing excessive amounts of salts, this makes them edematic (first of all the hypodermic tissue). Thus there is dehydration of the mucous membranes, a feeling of thirst, the normal activity of the nervous system and other vitally important organs is disturbed.

Thus, preservation of constant osmotic pressure (isotonicity) is an important property of the organism. The ability of some tissues (hepatic, hypodermic) to accumulate or deposit excessive amounts of water and salts, and also the capacity of the organism to remove these substances quickly in the urine and sweat, are mechanisms that take part in isotonicity maintenance. An especially important role in isotonicity support belongs to the kidneys. The processes of isotonicity in the body are regulated first of all by the nervous system and endocrine glands. Fluctuations of blood osmotic pressure in the body are insignificant (about one tenth of atmosphere), even under conditions of severe pathology. However in limited sections of tissues osmotic pressure changes may be considerable. So, in local inflammatory processes protein molecules break down into a great number of small fragments that increase the concentration of particles in the inflammation area. Water from the surrounding

tissues and vessels flows to this area leading to edemas. For example, in the place of suppurative inflammation the patient feels excessive pressure. When such inflammation is cut or punctured, purulent liquid flows out from it as a stream under considerable pressure.

It is possible to introduce into the human body and animals only considerable amounts of isotonic solutions. In case of need, some liters of such solutions are introduced into a patient within one day, for example, after difficult operations for compensation of liquid loss with the blood. During surgical operations the intestinal loops and other internal organs extracted from the abdominal cavity are protected from drying by covering them with gauze napkins moistened with a physiological solution.

In clinical practice hypertonic solutions are also applied. Hypertonic bandages in the form of gauze strips moistened with hypertonic NaCl solution and introduced into purulent wounds are used in surgical practice. In accordance with the osmosis laws liquids are directed from a wound to the gauze. It promotes evacuation of pus, microorganisms, and disintegration products from the wound.

Hypertonic solutions of $MgSO_4$ and Na_2SO_4 salts are poorly absorbed in the alimentary canal, and so are used as purgative remedies. Taking of $MgSO_4$ and Na_2SO_4 solutions causes transition of a significant amount of liquid from the mucous membrane into the intestines. The feces contained by the intestines are diluted and easily removed from the organism.

One should obligatorily use isosmotic (isotonic) nutrient mediums and solutions during experimental researches in organs and tissues removed from the organism.

Plasmolysis and hemolysis

Each living cell has a membrane or surface protoplasm layers having the property of semipermeability. If animal or vegetative cells are placed into distilled water, the water will pass into the cells. Thus the cells will swell and then their membranes will rupture leading to outflow of the cellular contents into the environment. If in such an experiment erythrocytes are used, water is dyed red by hemoglobin. A similar

destruction of cells by means of rupture of their membrane (or of surface protoplasm layers) is named *lysis*, and in the case of erythrocytes, it is *hemolysis.*

In concentrated (hypertonic) solutions of salts, on the contrary, shrinking of cells *(plasmolysis)* occurs. It is caused by loss of water, which passes from cells into a more concentrated external solution.

The effect of microorganism cell plasmolysis is used, for example, in food canning. For preservation of food (meat, fish) a large amount of table salt is used, and for preservation of berries and fruit sugar is added. As a result of plasmolysis harmful microorganisms are destroyed.

Isotonic solution – a solution with a solute concentration equal to the standard solution

Hypotonic solution – a solution with a lower solute concentration than the standard solution

Hemolysis – the rupturing of red blood cells that results when the cells are placed in a hypotonic solution (or from other causes).

Hypertonic solution – a solution with a higher solute concentration than the standard solution.

Crenation – the shrinking of red blood cells when they are placed in a hypertonic solution.

Osmometria – determination of molecular weight (M) by measurement of osmotic pressure. The osmotic pressures of aqueous solutions can be measured by Berkeley and Hartley's method. They used the method of applying to the solution a pressure which was just sufficient to stop osmosis.

Let’s consider the laws, which determine the changes of saturated vapor pressure above pure solvents and solutions. Vapor pressure above pure solvents depends only on temperature. Temperature rise leads to an increase in saturated vapor pressure. When saturated vapor pressure reaches the value of atmospheric pressure, the liquid begins to boil.

If at a given temperature a nonvolatile substance, such as glucose, is dissolved in a liquid, then pressure of saturated vapor decreases. In 1886 French scientist F.

Raoult established that lowering of saturated vapor pressure above a solution depends on the number of dissolved substance particles in the solution. If saturated vapor pressure above a pure solvent is designated as P_0, and saturated vapor pressure of a solvent above a solution is designated as P, then the difference between the pressure of vapor above the pure solvent (P_0) and the pressure of solvent vapor above the solution (P) is designated as AP and called *lowering of solvent vapor pressure above the solution:*

$\Delta P = P_0 - P$

The rations called *fractional vapor pressure lowering of solvent above a solution.*

$$P = \frac{\Delta P}{P_0}$$

Fractional lowering of the vapor pressure of a solvent above a solution is equal to the mole fraction of the dissolved substance.

Raoult's law may be formulated as:

$\frac{\Delta P}{P_0} = \chi(X)$ or $\Delta P = P_0 \cdot \chi(X)$

where P is lowering of the saturated vapor pressure of a solvent above a solution; P_0 is saturated vapor pressure above a pure solvent; *%(X)* is the mole fraction of a dissolved substance.

Thus, fractional lowering of the vapor pressure of a solvent above a solution depends on the number of particles of the dissolved substance and does not depend on the nature of the dissolved substance.

Raoult's law may be used only for the so-called ideal solutions or highly diluted real solutions, whose properties approximate to ideal solutions.

The freezing point of a nonvolatile substance solution is always lower than the freezing point of a solvent, and the boiling point of a nonvolatile substance solution is always higher than the boiling point of a solvent. It is possible to explain these laws using a phase diagram *(Fig. 2).*

The curve OA shows the dependence of the pressure of saturated water vapor on the temperature of pure water, the curve BC shows the dependence of the pressure

of saturated water vapor on the temperature of solution. The point O in Fig. 2 corresponds to the freezing point of pure water (fp_o), and the point B represents the freezing point of the solution (fp).

As the pressure of saturated vapor above solution at a given temperature is always lower than it is above pure solvent, so the curves of pressure of saturated vapor above solution (BC) and above solid solvent (OB) are intersected at a lower temperature *(fp).* The difference between crystallization temperatures of the solvent *(T_Q)* and solution *(T_f)* is called *depression of the freezing point of a solution.*

So, freezing point depression of a solution is proportional to the molality of the dissolved substance and does not depend on its nature (Raoult's law):

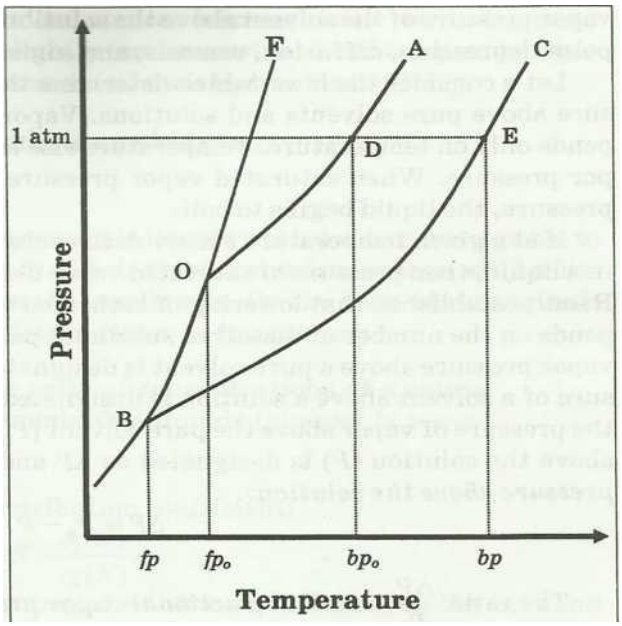


Fig 2. Lowering of vapor pressure by a nonvolatile solute

$\Delta T_f = K_f \cdot b(X)$, $\Delta T_f = T_0 - T_f$

where K_f is the molal freezing point depression constant (cryoscopic constant); $\chi(X)$ is solute molality.

The cryoscopic constant K_f is numerically equal to ΔT_f for a solution with the molality $b(X)$ = 1 mol/kg. The constant K_f depends only on the solvent nature and does not depend on the solute nature. For each solvent the molal freezing point depression constant has a certain value, for example, for water it is equal to 1.86 kg·K/mol, for benzene it is 5.12 kg·K/mol, for acetic acid it is 3.90 kg·K/mol.

Liquid boils at such a temperature when the pressure of saturated vapors reaches external atmospheric pressure *(P* = 101.3 kPa). From the diagram *(Fig. 2)* it is obvious that the boiling point of a solution is higher than the boiling point of a solvent. The difference between the boiling point of a solution *(T_b)* and the boiling point of a solvent *(T_o)* is called *boiling point elevation of a solution:*

$\Delta T_b = T_b - T_0$

Elevation of solution boiling point ΔT_b is as dependent on molality as depression of freezing point ΔT_f is.

According to Raoult's law, *boiling point elevation of a solution is proportional to the molality of the dissolved substance and also does not depend on its nature:*

$\Delta T_b = K_b \cdot b(X)$, where K_b is the molal boiling point elevation constant (ebullioscopy constant); *b(X)* is solute molality.

The ebullioscopy constant K_b is numerically equal to ΔT_b for a solution with the molality *b(X)* = 1 mol/kg. The constant K_b depends only on the nature of the solvent and does not depend on the nature of the solute. For each solvent the molal boiling point elevation constant has a certain value, for example, for water it is equal to 0.52, for benzene it is 2.57 kg·K/mol, for acetic acid it is 3.07 kg·K/mol.

Cryometry and ebulliometry, their application in medical and biological research

Cryometry (cryos - cold, frost) is a method of researching liquid solutions of nonvolatile substances, based on the measurement of freezing-point depression of solutions (ΔT_f).

Ebulliometry (ebullio boiling) is a method of measuring boiling-point elevation of solutions (ΔT_b).

These methods, in particular cryometry, are widely used. Freezing-point depression (ΔT_f) orboiling-point elevation (ΔT_b) are used to calculate the molar mass of a solute, the degree of dissociation of weak electrolytes.

In medical and biological research cryometry is used more often because the majority of biological objects are destroyed at the boiling point of water. Osmotic

pressure of biological liquids, molar masses of dissolved proteins may be calculated by means of cryometry.

For very diluted solutions c(X) a *b(X)*, then:

M(X) may be calculated according to $\pi = \frac{\Delta T_f RT}{K_f}$ the formula:

$$M\ (X) = \frac{K_f \cdot m(X)}{\Delta T_f \cdot m(solvent)}$$

Ebullioscopy law – elevation in boiling point depends upon the relative number of moles of solute and solvent but does not depend upon nature of solute , so it is a **colligative property**

$\Delta T_b = K_b \cdot C$

ΔT_b is the elevation in boiling point

C is molality of the solution

K_b is ebullioscopic constant

Ebullioscopic constant (K_b) – is the elevation of the boiling point of a solvent when 1 mole of a non- volatile solute is added to 1000g of the solvent. It has a unit of

C $mol^{-1}kg^{-1}$. K_b can also be expressed as $^{\circ}C$ mol^{-1} $100g^{-1}$. This is the elevation of boiling point when 1 mol of the solute is added to 100g of the solvent. The table below lists the ebullioscopic constant for some solvents:

Water, H_2O	0,52
Ethanol, C_2H_5OH	1,15
Ethanoic acid, CH_3COOH	3,07
Propanone, CH_3-CO-CH_3	1,72
Benzene, C_6H_6	2,57
Chloroform, $CHC1_3$	3,63
Camphor	5,95

The ebullioscopic constant for example, for water is 0,52 $^{\circ}C$ $mol^{-1}kg^{-1}$

(or 5,2 $^{\circ}C$ $mol^{-1}kg^{-1}$). That is the boiling point of water will increase by 0,52$^{\circ}C$ when 1 mol of any non- volatile solute is added to 1000g of water.

Ebulliometria (Ebullioscopic method) or determination of molecular weights by boiling point elevation.

If K_b, b, and **a** are known, molecular weight of non- volatile solute can be determined. The elevation of boiling point produced when a solute is dissolved in a solvent is usually measured by Landsberger's method.

Procedure. A few milliliters of the solvent (7-10ml) are placed in the graduated boiling tube and solvent vapours from the distillation flask are passed through it. The latent heat of vaporization liberated by the condensed vapour raises the temperature of the solvent in the boiling tube continuously. Finally the solvent begins to boil. Steady temperature of the thermometer is recorded. This is the boiling point of the solvent. The supply of the vapour is stopped. A known quantity of the solute (0,2-0,3g) is now dissolved in the solvent. The solvent vapours are again passed through the solution. The boiling point of the solution is again determined. The rose head is raised and the volume of the solution recorded. The weight of the solvent (**a**) is calculated by multiplying the volume by its density. The difference in the two boiling points represents the elevation of boiling point, ΔT . The molecular weight of the solute can be determined by substituting the values of K_b, b, ΔT and **a** (experimental data) in the formula:

$$M_b = \frac{1000 \cdot K_b \cdot b}{\Delta T \cdot a}$$

b - is the weight of the solute, gr

a - is the weight of the solvent, gr

Cryoscopic law – depression in freezing point depends upon the relative number of moles of solute and solvent but does not depend upon nature of solute, so it is a **colligative property**

$\Delta T_f = K_f C$

$\Delta \mathbf{T_f}$ is the depression in freezing point

C is molality of the solution

$\mathbf{K_f}$ is cryoscopic constant

Cryoscopic constant (K_f) – is the depression of freezing point when lmol of a solute is dissolved in 1kg (or 100g) of solvent. It has a unit of $^{\circ}C\ mol^{-1}kg^{-1}$ or $°C\ mol^{-1}100g^{-1}$. The table below lists the cryoscopic constant of some solvents :

Water, H_2O	1,85
Ethanoic acid, CH_3COOH	3,90
Benzene, C_6H_6	5,12
Camphor	39,7
Chloroform, $CHC1_3$	4,70
Ethanol, C_2H_5OH	1,99

Cryometria (Cryoscopic method) or determination of molecular weigh by depression of freezing point.

If K_f, **b**, ΔT and **a** are known, the molecular weight of non-volatile solute can be determined. The experimental determination of freezing point depression is made by the Beckmann's method.

Procedure. A known weight (about 15-20g) of the pure solvent is placed in the freezing- point tube. A Beckmann thermometer is suspended inside the tube so that its bulb is immersed in the solvent. The tube is fitted inside the air jacket and this assembly immersed in a suitable cooling bath. When the temperature of the pure solvent has fallen to about *0,5 °C* below its freezing point, the solvent is stirred vigorously to induce crystallization. When the solvent begins to freeze the temperature rises sharply to the true freezing point due to the release of the latent heat of fusion. The steady temperature attained is noted. The freezing- point tube is then removed and warmed slightly to melt the crystals and the process is repeated until two concordant results are obtained. A weighed pellet of solute is then introduced through the side tube, and allowed to dissolve in the solvent. The freezing point of the solution is determined in the same way. The procedure is then repeated with several successive additions of the solute. The difference in the two freezing points represents the depression of freezing pointy)

The molecular weight of the solute can be determined by substituting the values of K_f , **b**, ΔT and **a** (experimental data) in the formula

$$M_b = \frac{1000 \cdot \mathrm{K_f} \cdot b}{\Delta T \cdot a}$$

Van't Hoff s factor or isotonic factor (i)

$$i = \frac{\text{observed value of colligative property}}{\text{calculated value of colligative property assuming no dissociation}}$$

i = 1 + (m-1)α

$$\alpha = \frac{i - 1}{M - 1}$$

The measurements of colligative properties i.e., depression of freezing point, elevation of boiling point, osmotic pressure and vapour pressure lowering of the solutions may also give abnormal values of molecular weights in certain cases. This is because these properties depend upon the number of solute particles in a given volume of solution and not upon their nature. If a solute dissociates in solution, the total number of particles is increased, each ion formed by the dissociation exerts same colligative effect as the undissociated molecule. Hence the molecular weight of the solute as determined by the measurement of these properties will be lower than that calculated from its chemical formula. On the other hand, if the solute associates in the solution, forming a single complex molecule, the total number of particles is decreased. The molecular weight, thus determined by these measurements will be higher than the true molecular weight.

To summarise, the colligative methods for determining the molecular weights of solutes are applicable only when the solutes neither are fully soluble in a given solvent, neither associate nor dissociate and the solution of the solute must be fairy dilute.

Practical lesson 7-8

Topic: Buffer solutions, classification and mechanism of action.Buffer capacity. The role of buffer systems in maintaining the acid-base balance of the body. Determination of buffer capacity.

Relevance of the topic: The concentration of hydrogen ions [H^+] in cells and biological fluids is one of the most important parameters for homeostasis. The concentration of H^+ ions significantly affects all vital functions. For example, kinetics of enzymatic reactions, physicochemical and structural state of membranes, conformation of macromolecules, hemoglobin affinity to oxygen, receptor sensitivity to biologically active substances, intensity of generation of reactive oxygen species. Deviations [H^+] from the optimal range lead to disorders of metabolism, cellular activity (up to their death), tissues, organs and the body as a whole.

Lesson objective: In the body, biological fluids, tissues have appropriate pH values, which are maintained at a constant level with the help of buffer systems of organic and inorganic nature. The purpose of the body's buffer systems is to maintain homeostasis. An understanding of buffer systems, knowledge of their composition, mechanisms of pH maintenance are important for understanding the mechanisms of buffer systems action and regulation of basic physiological processes in the body. The mechanism of buffer systems action is widely used in the study of biochemistry course, normal and pathological physiology.

Basic definitions: acid buffer, buffer systems, buffer capacity, pH, dissociation, electrolytes, dissociation degree.

Plan and organizational structure of the lesson:

1. Electrolytes in the organism of a human body. Acid and base theories.
2. The dissociation degree and constant of weak electrolytes dissociation.
3. Ostwald's law for dilute weak electrolytes.
4. Solution properties of strong electrolytes.

5. Activity and coefficient of activity.
6. Ionic strength of solution.
7. Hydro-electrolytic balance – necessary condition of homeostasis.
8. Normal and pathologic pH intervals.
9. Acidosis. Alkalosis.
10. The role of electrolytes in processes of vital activity.
11. Acid-base equilibration in solutions of electrolytes.
12. Determination of dissociation constant and dissociation degree of weak electrolyte

Content of the topic

Buffer solution or simply a **buffer** – a solution whose pH value should not change on keeping or when it is diluted or when a small amount of a strong acid (H^+ ions) or a strong base (OH^- ions) is added to it.

Buffer action – the capacity of a buffer solution to resist the change of its pH value.

Acid buffer – a pair of weak acid and its salt with a strong base or a weak acid and its conjugate base.

Acetate buffer	$\frac{CH_3COOH}{CH_3COONa}$
Bicarbonate buffer	$\frac{H_2CO_3}{NaHCO_3}$

Basic buffer – a pair of weak base and its salt with a strong acid or a weak base and its conjugate acid

Ammonium buffer $\frac{NH_4OH}{NH_4Cl}$

Henderson's equation for acid buffer

$$\mathbf{pH = pKa + \log \frac{[salt]}{[acid]}}$$

Henderson's equation for basic buffer

$$\mathbf{pH = 14 - pK_b - \log \frac{[salt]}{[base]}}$$

Buffering capacity – Van Slyke's buffer value (B) which is the number of moles of a strong monoacidic base or a strong monobasic acid required to be added to 1 L of the buffer to change its pH by 1

$$\mathbf{\beta = C/pH}$$

Buffer systems of the body fluids - the main buffers which help in maintaining the pH in extracellurar and intracellular fluids are

- bicarbonate buffer,
- phosphate buffer,
- protein buffer,
- hemoglobin buffer,
- oxyhemoglobin buffer.

Acidosis (respiratory and metabolic) – a disturbances in acid- base equilibrium that occurs when the blood pH falls below 7,2.

Alkalosis (respiratory and methabolic) – a disturbances in acid- base equilibrium that occurs when the blood pH rises above 7,5.

Bicarbonate buffer (H_2CO_3/HCO_3^-). It is the most important buffer system of non-volatile acids entering the extracellular fluids because of two reasons:

1) It is present in high concentration than the other buffer systems

2) The production of H_2CO_3 is effectively buffered and is disposed by the lungs as CO_2.

This buffer system acts in the blood to prevent both acidosis and alkalosis.

For the dissociation $H_2CO_3 \leftrightarrow H^+ + HCO_3^-$ the Henderson- Hasselbalch equation indicates that the buffer ratio of 20 gives a pH of 7,4 to the solutions of bicarbonate buffer and this is the normal blood pH

$$\mathbf{pH = pK_a + \log \frac{[HCO_3^-]}{[H_2CO_3]}}$$

or $$\mathbf{pH = 6,1 + \log 20 = 7,4}$$

Bicarbonate buffer is of great importance in the acid- base balance of the extracellular fluid and in the maintenance of the blood pH within normal limits. This buffer has far less importance inside the cell because cells contain much lower amounts of HCO_3^-.

The bicarbonate system is of prime physiological importance and acts cooperatively with other buffers.

Phosphate buffer ($H_2PO_4^-/HPO_4^{2-}$). It plays a minor part in blood and is active mainly within the cells (maximum buffering action at a pH of 7,2)

$$H_2PO_4^- \leftrightarrow HPO_4^{2-} + H^+$$

Adding strong acid to this system will drive the reaction to the left, increasing the concentration of $H_2PO_4^-$, which is weakly acidic. Large amounts of $H_2PO_4^-$ will result in acidosis, but the body will eliminate the excess in the urine. Adding strong base to the system will drive the reaction to the right, as the hydrogen ions react with the base to form water. Large amounts of HPO_4^{2-} would be found in alkalosis, but under normal kidney function the HPO_4^{2-} is also excreted in the urine.

Phosphate buffer is of importance in raising the plasma pH through excretion of $H_2PO_4^-$ by kidney. It is an important urinary buffer and works cooperatively with the bicarbonate system.

Hemoglobin (HHb/Hb^-) and **Oxyhemoglobin ($HHbO_2/HbO_2^-$) buffers** are of prime importance in the erythrocytes. Hemoglobin is a better buffer than most proteins at pH 7,4 because of relatively high concentration of imidazole group (**pKa**~7) of the constituent histidine molecules.

Particularly due to reversible changes in the buffering capacity of hemoglobin on oxygenation and deoxygenation, it plays the major role in buffering CO_2 inside erythrocytes. Deoxyhemoglobin is a weaker acid (**pKa =** 8,18) and consequently possesses a much higher capacity than oxyhemoglobin

(**pKa** = 6,62) for accepting H+ and buffering CO_2 On entering the erythrocytes in tissue capillaries, CO_2 combines with H_2O to form H_2CO_3 under the action of carbonic anhydrase H_2CO_3 remains 95% dissociated into H^+ and HCO_3^- at the blood pH of 7,4 and consequently needs immediate buffering. Side by side, oxyhemoglobin

(HbO_2^- or $HHbO_2$) has lost O_2 to form deoxyhemoglobin (Hb^- or HHb). But while $HHbO_2$ remains about 85% ionized as HbO_2^- at pH 7,4 85% of Hb^- remains as undissociated HHb by accepting H^+ from the ionization of H_2CO_3. Thus, Hb^- buffers H_2CO_3 in erythrocytes

$$HbO_2^- \leftrightarrow Hb^- + O_2$$

$$Hb^- + H_2CO_3 \leftrightarrow HHb + HCO_3^-$$

Some of the HCO_3^- ions, thus formed, diffuse out into the plasma to maintain the balance between intracellular and plasma bicarbonates. This results in a simultaneous influx of some Cl^- into erythrocytes along the electrical gradient produced by the HCO_3^- outflow (chloride shift). Subsequent oxygenation of HHb in lungs produces $HHbO_2$ which immediately ionizes into H^+ and HbO_2^- to a large extent due to its much lower pKa. The released H^+ ions are buffered by HCO_3- inside erythrocytes to form H_2CO_3 which is dissociated by carbonic anhydrase into H_2O and CO_2. The latter diffuses out from erythrocytes and escapes in the alveolar air. Some HCO_3^- ions return from the plasma to erythrocytes in exchange of Cl^- ions and are in turn changed to CO_2.

$$HHb + O_2 \leftrightarrow HHbO_2 \leftrightarrow HbO_2^- + H+$$

$$HCO_3^- + H+ \leftrightarrow H_2CO_3 \rightarrow H_2O + CO_2$$

In a mixture of several buffer pairs, alterations in the ratio of any one pair are accompanied by parallel changes in the ratios of all other pairs. So, the pH of the entire mixture may be controlled by regulating the ratio of any one buffer pair, say that of the bicarbonate buffer in the blood.

Protein buffers (HPt/Pt^-). At the pH of the blood, the plasma proteins are anions but act as weak acids.

$$HPt \leftrightarrow H^+ + Pt^-$$

In plasma protein buffers play a much smaller part than bicarbonate buffer but in the cells proteins form the most important buffering system. Many of the proteins in the plasma are acidic proteins with acidic isoelectric pI. So, at the blood pH of 7,4,

these exist as anions to serve as conjugate bases (Pt^-) and may accept H+ ions to form the corresponding conjugate acids (HPt). Protein buffers may even buffer some H_2CO_3 in the blood.

$$H_2CO_3 + Pt^- \leftrightarrow HCO_3^- + HPt$$

Laboratory work

Determination of the buffering capacity (β_a and β_b) of acetate buffer

CH_3COOH/ CH_3COONa

a) Determination of the buffering capacity on acid (β_a)

Using a clean pipette, transfer 5 mL of acetate buffer solution (pH_0 = 4,8) to a conical flast. Add 1- 2 drops of universal indicator. Standard solution of HCl (0,1 M) added from burette until the color of the contents of flask becomes pink (pH_1=2). Three such titration are generally carried out. Concentration of buffer solution (C') is calculated from the formula (I). The buffering capacity **βa** is calculated from the formula (I').

b) Determination of the buffering capacity on base (βb) Using a clean pipette, transfer 5 ml of acetate buffer solution (pHo=4,8) to a clean conical flask. Add 1-2 drops of universal indicator. Standard solution of 0,1 M NaOH added from burette until the end point is reached (the color of the contents of flask becomes green (pH_1=8) of blue (pH_1=10). Concentration of buffer solution (C') is calculated from the formula(II). The buffering capacity **βb** is calculated from the formula (II').

Determination of the buffering capacity (βa and βb) of phosphate buffer

(NaH_2PO_4/Na_2HPO_4)

a) Determination of the buffering capacity on acid (βa)

Using a clean pipette, transfer 5 ml of phosphate buffer solution (pHo=6,68) to a conical flask. Add 1 -2 drops of methyl orange indicator solution.

The clean burette (V=25 ml) filled with the given standard solution 0,1 M HCl. The flask with its content placed below the burette and acid solution from it is slowly

and gradually added to the solution in the flask, stirring the solution. The end point is indicated by the change of the yellow color solution to light gold-pink by the addition of the last drop of HC1 solution.

Three such titrations are generally carried out by filling the burette upto the zero mark after each titration. The mean of the readings are taken.

b) Determination of the buffering capacity on base (βb)

Using a clean pipette, transfer 5 ml of phosphate buffer ion (pHo=6,68) to a conical flask. Add 1 -2 drops of phenolphthalein indicator solution.

The clean burette (V=25 ml) filled with the given standard solution NaOH 0.1 M. The flask with its component placed below the base solution from it is slowly added to the solution in the flask, stirring the solution. The end point is indicated the change of the colorless solution to light violet-pink by addition of the last drop of the NaOH solution. Three such titrations are carried out. The mean of the three readings are taken. The buffering capacity βb is calculated from the formula (II').

Calculations

1	$C' = \frac{V(HCl) \cdot C\ (HCl)}{V\ (buf)}$	C' – concentration of buffersolution on acid
2	$C'' = \frac{V(NaOH) \cdot C\ (NaOH)}{V\ (buf)}$	C'' – concentration of buffer solution on base
1'	$\boldsymbol{\beta}a = \frac{C'}{pHo - pH_1}$	**β**a – buffering capacity on acid
2'	$\boldsymbol{\beta}b = \frac{C''}{pH_1 - pH_0}$	**β**b – buffering capacity on base

Practical lesson 9

Topic: **Basic concept of chemical thermodynamics. Theoretical basis of bioenergetic.**

Relevance of the topic: all chemical reactions are accompanied by the transformation of chemical energy into other types of energy (heat, electricity, mechanical, etc.). Chemical reactions are characterized by chemical and physical phenomena that are closely related to each other. Such regularities are studied by thermodynamics. The basic laws of thermodynamics are common to all macrosystems regardless of the nature of the particles that form them and the nature of the interaction between them.

Lesson objective: to integrate systemic knowledge of the subject and tasks of thermodynamics, thermodynamic systems, theoretical principles of thermochemistry, state functions; to interpret basic concepts of chemical thermodynamics (internal energy of the system, enthalpy, heat and work); to apply laws and principles of thermochemical calculations to form a holistic approach to the study of chemical and biological processes.

Basic definitions: thermochemistry, thermal effect of chemical reaction, Hess's law, consequences of Hess's law, first law of thermodynamics, enthalpy, entropy, Gibbs energy.

Plan and organizational structure of the lesson:

1. Types of thermodynamic systems.
2. Thermodynamics functions and parameters of system.
3. The first law of thermodynamics. Internal energy. Enthalpy.
4. Heat of isobaric and isochoric process. Standard heats of the substance formation and combustion.
5. Thermochemistry. Hess law. Thermochemical transformation.
6. Thermochemical calculation and their use for energetic characteristics of biochemical process.
7. Second law of thermodynamic. Entropy. Gibbs energy.
8. Chemical equilibrium. Thermodynamic conditions of equilibrium.

Content of the topic

All chemical reactions are accompanied by transformation of chemical energy to other forms of energy – thermal, electrical, mechanical, etc.

Thermodynamics is the branch of physical science that studies all forms of energy and their mutual transformations; therefore it is sometimes called energetics. Bioenergetics is a field of thermodynamics that deals with biosystems.

Classical thermodynamics is based on propositions which are confirmed by experiment and does not use knowledge about the molecular structure of substances.The energy of reactions is studied by the branch of thermodynamics which is called **thermochemistry** or **chemical thermodynamics**.

In thermochemistry two types of chemical reactions are distinguished: **exothermic** (are accompanied by heat release) and **endothermic** (are accompanied by heat absorption). There are reactions (not so numerous), which are not accompanied by heat exchange.

Chemical reactions can occur at a constant pressure (for example in an open flask) – these are **isobaric processes**, at a constant volume (in a closed flask or an autoclave) – the se are **isochoric processes**, or at a constant temperature - these are **isothermal processes** (the names are derived from the Greek words isos - identical, baros - pressure, chorus - space, thermos - heat).

Thermodynamics deals with the study of properties of various thermodynamic systems and processes occurring in them.

A thermodynamic system is anybody or totality of bodies being in interaction with each other, which may be separated (conditionally or practically) from the surroundings for studying by thermodynamic methods.

Different types of thermodynamic systems are known:

1. **Homogeneous system** – it is uniform in all its parts. For example an aqueous solution of ethanol, or a mixture of gases.

2. **Heterogeneous system** – it is not uniform and consists of two or more phases, e.g. water-benzene. The term phase means a part of a system with a characteristic chemical composition and macroscopic properties. Phases are separated

from each other by physical surfaces, and at transition of these surfaces the properties sharply vary.

3. **Physical system** – it is a system, in which processes are accompanied by energy change, but the chemical nature of a substance is invariable. For example, changing of the modular condition of a substance at its melting (or crystallization) temperature; condensation of liquid vapor at its boiling temperature (water boils at 373 K).

4. **Chemical system** – it is a system, in which both phenomena take place: change of the energy content, change of the chemical nature of the system components. For example, interaction of zinc with sulfuric acid and other chemical reactions.

5. **Open system** – it is a system that may exchange both energy and substance with the surroundings. For example, a bio system - a living organism.

6. **Closed system** – it is a system that may exchange only energy with other systems, but not substance. For example, an electric range.

7. **Isolated system** – it is a system, which doesn't exchange energy or substance with the surroundings. It is very difficult to create an absolutely isolated system. Reactors with good thermoisolation may be reckoned among these systems.

At each moment of time the state of a system is characterized by physical properties that do not depend upon the previous history of the system state, for example: temperature T, pressure P, volume V, energy E, mass m, internal energy U, enthalpy H, entropy S, Gibbs energy G, Helmholtz energy F, etc.

Thermodynamics is of great importance for medicine since it helps:

- To generate scientific representation of the energy balance of a living organism.
- To establish connection between the caloric content of food and energy expenses of the organism.
- To develop objective criteria for determining the possibility of realization of separate processes in the human body without carrying out tests.

There are some formulations of the first law of thermodynamics. Such concepts as «heat and work», on the one hand, and «internal energy and enthalpy», on the other hand, underlie it.

Heat and work are different forms of energy transmission. In thermodynamics heat and work are algebraic values that may be positive and negative. Work is measured in joules. Heat is also expressed in joules in the SI, the unit calorie is also applied. The connection between a joule and a calorie is: 1.00 cal = 4.184 J. When heat is absorbed by a system from the surroundings, it has a positive value, if heat is released by a system into the surroundings, it is taken as negative.

Using the concepts ***heat and work*** the first law of thermodynamics may be formulated as:

Energy can neither be created nor destroyed, but only can be converted from one form into another (including heat and work), without changing quantitatively.

In fact it is the law of conservation of energy, which was formulated by M. Lomonosov as long ago as 1748.

Other formulation of the first law is:

It is impossible to develop a perpetuum mobile of the first kind (i.e. a machine producing work without expenditure of energy).

It is possible to formulate the first law of thermodynamics on the basis of other reasons, introducing the concepts *internal energy* and *enthalpy*.

Internal energy may be considered a sum of different types of energy from atoms, ions and molecules (energy of molecular motion, of intermolecular interaction, etc.).

According to the law of energy conservation, the heat that is absorbed by a system is spent to change its internal energy and to produce work:

$$Q = \Delta U + A \qquad (1)$$

For chemical processes the work against external forces is work against external pressure and it is equal:

$$A = p(V_2 - V_1) = p\,\Delta V \qquad (2)$$

For an isochoricprocess (V— const):

$A = 0$ and $Q_v = U_2 - U_1 = \Delta U$ (3)

It means that the system doesn't produce external work that is associated with a volume change, and all heat that is released or absorbed is spent on changing the internal energy of a system.

For an *isobaric process (p — const),* excluding internal energy changes, certain work is carried out as a result of volume change in a system, which is equal to the product between pressure (p) and change of the system's volume (F):

$\Delta F = \Sigma F_{prod.} - \Sigma F_{reac}$, (4)

$Q_p = \Delta U + p \Delta V$ (5)

$Q_p = (U_2 - U_1) + p(V_2 - V_1)$ (6)

or $Q_p = (U_2 + pV_2) - (U_1 + pV_1)$ (7)

Assuming that $U + pV = H$ (8)

the heat of the processes taking place at constant temperature and pressure (the most widespread chemical processes) may be represented as:

$Q_p = H_2 - H_1 = \Delta H$, (9)

where H is the enthalpy of a system.

The positive value of enthalpy change $(\Delta H > 0)$ corresponds to enthalpy increase or to heat absorption by a system (an *endothermic process).* The negative value of enthalpy change $(\Delta H < 0)$ corresponds to enthalpy decrease or to heat release by a system (an *exothermic process).*

So in an isochoricprocess the heat of a reaction is equal to external energy change ΔU*:*

$Q_V = \Delta U$ (10)

and in an isobaric process heat is equal to a change of system's enthalpy:

$Q_p = \Delta H$ (11)

It must be noted that $Q_p > Q_v$ on $p\Delta V$ value which is the work of expansion.

As well as the internal energy of a system U, enthalpy H is also a state function of a system. U and H may be considered as a measure of heat transportation at certain conditions: U at V = const, H at p = const.

There is a relationship between internal energy and enthalpy of a system:

$\Delta H = \Delta U + p\Delta V$

From the equation (5) it follows:

$\Delta U = Q_p - p\Delta V$ (12)

The equation (12) may be interpreted as a mathematical expression of the first law of thermodynamics.

An increase of the internal energy of a system is equal to the heat, which is received by the system from the outside, except for the work produced by the system against external forces.

All thermochemical calculations are based on Lavoisier and Laplace's law and Hess's law.

The law of Lavoisier and Laplace (1780):

The heat of decomposition of a chemical compound into simple substances is numerically equal and opposite in sign to the heat of formation of this compound from simple substances.

Hess's law (1840):

The heat of a reaction is independent of the way, in which this reaction occurs, and only depends upon the initial and final states of a system.

The consequences of Hess's law are of great importance for thermochemical calculations.

Consequences of Hess's law:

1. Enthalpy of a forward reaction is equal and opposite in sign to enthalpy of a reverse reaction. $\Delta H_{forward} = \Delta H_{revers}$

2. Reaction enthalpy is equal to the sum of enthalpies of reaction products formation minus the sum of enthalpies of reactant formation.

$$\sum_{i=1}^{n} \Delta H_{f\,prod} - \sum_{i=n}^{n} \Delta H_{f\,reag} = \Delta H_f$$

3. Enthalpy of a combustion reaction is equal to the sum of enthalpies of reactant combustion (ΔH_c) minus the sum of enthalpies of product combustion.

$$\Delta Hc = \Delta H_{react} - \Delta H_{prod}$$

For a reaction nA + mB = qC + pD

$\Delta H_f = [q\Delta H°_f C + p\Delta H°_f D] - [n\Delta H°_f A + m\Delta H°_f B];$

$\Delta H_c = [n\Delta H_c A + m\Delta H_c B] - [q\Delta H_c C + p\Delta H_c D].$

The data on the thermal effects of reactions are used for: calculation of the thermal balances of technological processes, determination of the energy of inter-atomic and intermolecular bonds, ascertainment of the structure and reactionary ability, establishment of the direction of chemical processes, description of the energy balance of an organism.

Second law of thermodynamics

All the processes connected with transition of one type of energy to another refer to the first law of thermodynamics, the law of energy conservation. However, it is important to know not only the energy of processes (for example, the heat of formation or substance decomposition), but also which factors influence the direction and depth of the proceeding of chemical reactions. Another no less important question is whether a given reaction will occur spontaneously, without external intervention, or not. The answers to these questions are given by the second law of thermodynamics.

There are some formulations of the second law:

It is impossible to construct a perpetuum mobile of the second kind, i.e. it is impossible to transform heat into work completely. (W. Thomson)

The work of each electric power station leads to thermal contamination of the surroundings because some part of energy is being lost.

It is impossible to transfer heat from a cooler body to a hotter body without performing work. (E.R. Clausius)

The refrigerator's temperature will stay less than the outdoor temperature only if electric energy is spent.

A process, which under particular conditions occurs by itself without an extraneous source of energy, is called spontaneous.

For example: falling of a stone from hands to the floor, expansion of an ideal gas, melting of ice, dissolution of salts, evaporation of liquids, etc.

In the adduced examples the motive power of the processes consists in transition from a thermodynamic system with a more regulative state into a less regulative state. Reverse transition of a system is hardly probable.

For a quantitative estimation of the probability of a system state or for an estimation of the disorder degree a thermodynamic function as entropy S has been proposed. Entropy is a measure of a system disorder. Entropy is a state function: its change (ΔS) depends only on the initial and final states of a system.

Entropy is connected with the thermodynamic probability of the realization of some particular system state by L. Boltzmann's equation:

$$\mathbf{S = KlnW,}$$

where K is Boltzmann's constant, W is the thermodynamic probability or the number of possible microstates which may be realized for a particular system macrostate.

Entropy is measured in J/mol • K.

From Boltzmann's law it follows that the entropy of a pure ideal crystal is equal to zero at the temperature of absolute zero (W = 1 for it, then S = K In 1 = 0). It is the most regulated system. In other systems the value W is greater and S > 0. The bigger the system disorder, the higher its entropy. Entropy is connected with the thermal characteristic of a system by the following correlation:

$$\Delta \boldsymbol{S} = \frac{\Delta \boldsymbol{Q}}{\boldsymbol{T}}$$

The product TΔS is called connected energy.

The concept of entropy underlies the second law of thermodynamics:

In isolated systems, processes occur spontaneously on condition of entropy increase.

In the real world isolated systems are found very rarely. In real systems processes may be accompanied by both an increase and a decrease of entropy.

An important conclusion follows from the second law of thermodynamics:

The total change in entropy that is necessary for the formation of a human body and maintenance of its life and the life of any other living system is always positive.

The human body is a complex, highly organized, and very regulated system. Its entropy is much less than the entropy of the same quantity of CO_2, H_2O and some other substances, which compose the organism. But proceeding of many thousands of chemical reactions that are necessary for the recreation and vital activity of the organism are accompained by a substantial increase of entropy in the environment.

All human activity aimed at ruling the world that surrounds us demands high energy expenditure, which eventually increases disorder.

At various transformations it is important to know not the absolute value of entropy, but its change ΔS. As well as enthalpy change (ΔH), entropy change (ΔS!) may be calculated using the following equation:

$$\mathbf{\Delta S^0 = \Sigma S^0_{prod} - \Sigma S^0_{react}}$$

To compare the entropy of different substances, these, as well as enthalpy of formation, are given under standard conditions (S°):

T = 298 K, p = 101.3 kPa, n(x) = 1 mol.

The value of entropy allows:

1) to forecast which processes may occur spontaneously and which cannot;
2) to predict the direction of possible transformations and to control them.

The dependence of entropy on temperature is formulated in the third law of thermodynamics, or Nernst's heat theorem, or Planck's postulate:

The entropy of a pure ideal crystal at absolute zero is equal to zero.

Application II law of thermodynamics to biological systems

A. Although living organisms is open, nonequilibrium systems are applicable to them, and I and II of the laws of thermodynamics, as biochemical processes are irreversible, occur spontaneously. In other words living organisms is stationary systems. Part of the energy that is released during the oxidation of food, irreversibly converted into heat, which dissipates into the surrounding space.

B. In the body, all the processes are spontaneous and therefore the entropy S increases. But the body temperature does not rise and does not come "heat death" because body consumes a substance with low entropy (IUD), and highlights the decay products

with high entropy (small molecules). As a result, the entropy of an open system - is'a constant value.

Gibbs equation

The behavior of chemical processes is influenced by two factors: enthalpic and entropic. The process is promoted by a combination of conditions: $\Delta H < 0$ and

$\Delta S > 0$, i.e. a decrease of enthalpy and an increase of entropy. Therefore the behavior of a reaction depends on what factors prevail in the current case.

The summary effect of the action of enthalpic and entropic factors may be transmitted by the Gibbs energy (which is also called the Gibbs free energy, isobaric-isothermal potential):

$$\mathbf{G = H - TS}$$

The Gibbs equation:

$$\mathbf{\Delta G = \Delta H - T\Delta S}$$

The minus sign before $T\Delta S$ testifies that entropy counteracts enthalpy. ΔG is measured in kJ/mol.

The Gibbs energy is a state function as it is equal to the difference between two state functions. ΔH is the maximum energy that is evolved or absorbed during a chemical reaction; $T\Delta S$ is the bound energy which cannot be used for work. ΔG depends on the nature and physical state of reactants and products and is independent of the behavior of a process (i.e. intermediate stages of a reaction).

The Gibbs equation is one of the most important equations in chemical thermodynamics. The character of Gibbs energy change allows concluding that reaction realization is principally possible:

$\Delta G < 0$ - the process is possible, occurs spontaneously;

$\Delta G = 0$ - the process is impossible, a reverse process occurs spontaneously;

$\Delta G > 0$ - the system is in an equilibrium state.

To compare ΔG of different processes their values are introduced under standard conditions and represented by ΔG

The change of Gibb senergy $\Delta G°$ may be calculated similar to ΔH:

$$\mathbf{\Delta G = \Delta H - T\Delta S}$$

$$\Delta G^\circ = \Sigma \Delta G^\circ_{prod} - \Sigma \Delta G^\circ_{react}$$

A criterion of process spontaneity is: $\Delta G < U$.

If $\Delta G > 0$, a process is non-spontaneous.

In biochemical systems association of two or more reactions is required to make the proceeding of a non-spontaneous chemical process possible.

For example, in the process of glucose oxidation a large quantity of free energy is liberated; the energy is spent to realize certain reactions (e.g. the transformation of ADP into the more power-intensive substance ATP). Then ATP molecules are used as an energy source for the transformation of simple molecules into more complex components of a living cell. An ATP molecule turns into an ADP molecule by releasing free energy. Such mutual transformations ATP ↔ ADP are used by the organism as a way of energy accumulation and its liberation for realizing necessary reactions (all mentioned processes occur in the presence of catalysts-enzymes).

In the study of biochemical reactions in bioenergetics it is accepted to use the values of ΔG°at pH=7.

By the ΔG value the processes in the human body are divided into:

1. Exergonic: $\Delta G < 0$ (respiration processes, food assimilation, that is oxidation).
2. Endergonic: $\Delta G > 0$ (are provided with energy by exergonic processes).

Thus it is possible to draw the following conclusions:

1. The motive force of a chemical reaction under isobaric-isothermal conditions is the Gibbs energy:
2. Spontaneous proceeding of a reaction under isobaric-isothermal conditions is possible only if ΔG value is negative: $\Delta G < 0$
3. The higher decrease of G, the higher the probability of a direct reaction.
4. If $\Delta H < 0$ and $\Delta S > 0$, a reaction is possible without restrictions. If $\Delta H > 0$ and $\Delta S < 0$, a reaction is impossible.

The Helmholtz energy F is used for isochoric-isothermal processes (T, V = const):

$$F = U - TS$$

$$\mathbf{\Delta S = \Delta U - T\Delta S}$$

where F is the Helmholtz energy, which is also named the isochoric-isothermal potential; U is the internal energy; S is entropy.

Isochoric-isothermal processes are observed extremely seldom.

Practical lesson 10

Topic: Physico-chemical bases of kinetics of biochemical reactions. Kinetics of complex reactions. Catalysis. Features of enzymes.

Relevance of the topic: Chemical kinetics is a branch of physical chemistry that studies the concept of rates and mechanisms of chemical reactions, as well as the factors affecting them. The laws of chemical kinetics are used to explain the mechanisms of biochemical reactions (normal and malignant tissue growth), kinetic evaluation of treatment efficacy, studying the distribution of drugs introduced into the body and their half-life. Currently, chemical kinetics has become one of the effective "tools" for studying catalytic reactions, including enzymatic ones, occurring in the human body.

Lesson objective: Learn the most important concepts and laws of chemical kinetics; analyze the effect of various factors (concentration, pressure, temperature) on the rate of a chemical reaction; interpret the effect of catalysts on the rate of chemical processes and explain the mechanism of their action; know the features of enzymatic catalysis.

Basic definitions: chemical reaction rate, reaction molecularity, simple and complex chemical reactions, catalyst, promoter, the Michaelis-Menten equation.

Plan and organizational structure of the lesson:

1. The subject of chemical kinetics.
2. Factors that affect the rate of chemical reaction.
3. Molecularity and order of reaction. Simple and complex reactions.
4. Arrhenius theory. Activation energy. Transition state.
5. Van't Hoff's rule. Effect of temperature on reaction rate.
6. Catalysis and catalysts. Features of the catalysts.
7. Homogeneous, heterogeneous and microheterogeneous catalysis.
8. Acid-base catalysis. Autocatalysis. Mechanism of action of catalysts.
9. The promoter and catalyst poisons. The idea of the kinetics of enzymatic reactions. Enzymes as biological catalysts.
10. Activation and inhibition of enzymes.

Content of the topic

Chemical reactions proceed at different rates. Some of them are fully finished in fractions of seconds, other last hours, days, decades and even yet greater periods of time. Note should be taken that the same reaction may proceed quickly under certain conditions, and more slowly under other conditions.

Most bioprocesses are slow chemical processes. These are biosynthesis, including photosynthesis, fermentation, etc. Thus, half of proteins are renewed during 70 days, and the inorganic basis of the bone tissue is fully renewed in the course of 4-7 years.

Homogeneous and heterogeneous reactions are distinguished.

A *homogeneous reaction* takes place in a homogeneous system and is carried out in the bulk of a system.

A *heterogeneous reaction* is possible between substances forming a heterogeneous system. It takes place only in the interphase. For example:

$Fe + 2HCl \rightarrow FeCl_2 + H_2$

Dissolution of metal in acid occurs only on the surface of the metal, because only there both reactants come into collision with each other.

Simple and complex reactions are distinguished. *Simple* (elementary) *reactions* are single-stage reactions. For example:

$H_2 + I_2 \rightarrow 2HI$

$CH_3–N=N–CH_3 \rightarrow C_2H_6 + N_2$

Complex reactions are multistage reactions. There are few simple reactions, most reactions are complex.

The rate of a chemical reaction is the rate of concentration change of any substance – a reactant or a product of the reaction – per unit of time.

Concentration is measured in mol/1, and time - in seconds, minutes, hours, years, etc.

During a reaction the concentration of reactants diminishes, and the concentration of reaction products increases. For a hypothetical reaction:

$$A+B \rightarrow AB$$

the average rate of reaction is:

$$\vartheta = -\frac{\Delta C_A}{\Delta t} \qquad \text{or} \qquad \vartheta = +\frac{\Delta C_{AB}}{\Delta t}$$

in general view:;

where $\Delta c = c_1 - c_0$ $\Delta t = t_1 - t_0$ $\vartheta = \pm \frac{\Delta C}{\Delta t}$

To describe a chemical process it is enough to know the change of the concentration of the substance entering into reaction or the substance formed as a result of reaction. The rate of reaction may be found by measuring changes in some property of the reactant or product, for example, changes in the absorption spectrum at some time after reaction beginning.

During reaction concentrations of the substances *A, B, AB* are changing continuously, that is why it would be reasonable to consider the instantaneous (veritable) rate of the process: $\vartheta = \pm \frac{dc}{dt}$

In case of a heterogeneous reaction, the reaction rate is measured by the unit of the surface area of the phase.

There are many factors that affect the rate of reaction. Some important factors are: the nature of reactants, their concentration, temperature, the presence of catalysts in a system. The rate of heterogeneous reactions depends also on the surface area of the phase, in which the reaction occurs, and on its nature.

Dependence of reaction rate on reactant concentration.

Mass action law

A necessary condition for chemical interaction between particles (molecules, ions) of reactants is their collision with each other: in order to react particles must collide. The number of collisions grows as concentration of each of the reactants increases.

The dependence of the reaction rate on the concentration of reactants is described by the law of mass action discovered by N. Beketov, C. Guldberg and P. Waage in 1865-1867:

At constant temperature the rate of chemical reaction is in proportion to the product of reactant concentrations.

In general view for a homogeneous reaction

$$aA + bB \rightarrow c\ AB$$

$$\upsilon = k\ [A]^n \cdot [B]^m$$

where *k* is the coefficient of proportionality, which is named *the rate constant of reaction*; [A] and [B] are molar concentrations of reactants, mol/1; *n* is the order of reaction relative to *A; m* is the order of reaction relative to *B; (n+m)* is the general order of reaction; u = *k* if [A] = [*B*] =1 mol/1.

The rate constant of a reaction at constant temperature may be considered as the rate of a chemical reaction, if the concentration of each reactant is equal to 1 mol/1.

The rate constant of a reaction depends on the nature of reactants, temperature, presence of catalysts, but does not depend on the concentration of reactants.

The law of mass action is just for simple (single-stage) reactions. For a simple reaction $aA + bB \rightarrow c\ AB$ the order of reaction respectively to a given substance is equal to the stoichiometric coefficient: $\upsilon = k\ [A]^a \cdot [B]^b$

If reactions are complex and may be considered as a totality of consecutive single-stage processes, the law may be applied only to these processes individually.

In the case of heterogeneous reactions only concentrations of substances being in the gas phase or in solution must be included in the equation of the law of mass action. For example:

$$S_{(s)} + O_{2(g)} \rightarrow SO_{2(g)}$$

$$\upsilon = k \cdot [O_2]^m$$

If the order of reaction is indicated respectively to each reactant, then the mathematical expression of the law of mass action is named the kinetic equation (rate equation) for a given reaction.

Molecularity and order of reaction

All chemical reactions may be classified into different types according to molecularity and order of reaction.

The number of reactive particles (atoms, ions, molecules or free radicals) taking part in an elementary act of a reaction is called *molecularity* of the reaction. Depending on the number of particles that participate in the collision leading to product formation, reactions may be divided into *monomolecular*, *bimolecular* and *trimolecular*. The probability of simultaneous collision of three particles is very small; tetramolecular reactions are unknown.

An example of a monomolecular reaction may be:

$$CH_3–N=N–CH_3 \rightarrow C_2H_6 + N_2$$

bimolecular: $H_2 + I_2 \rightarrow 2HI$

As to the order of reaction, the *overall order of reaction*, as it has been already mentioned, is equal to the sum of the exponents, on which the particle concentrations are raised in the kinetic equation of a reaction. The order of reaction does not follow from its stoichiometric equation: $\upsilon = k\,[A]^n \cdot [B]^m$

It may be found only from experimental data. As appears from the experiments, there are zero-order, first-order, second-order and third-order reactions.

If one of the reactants is present in excessive quantity, in other words, its concentration practically does not change, the order of reaction respective to a given substance results with a zero. The rate equation for a zero-order reaction is $\upsilon = k \cdot [A]^0 = k$. So rate is independent of concentration.

For example, in a reaction of sucrose hydrolysis:

$$C_{12}H_{22}O_{11} + H_2O \leftrightarrows C_6H_{12}O_6 + C_6H_{12}O_6$$

Sucrose Glucose Fructose

water is present in excessive quantity and in the process of reaction its concentration is practically constant ([H_2O] = const.). The reaction is considered as zero-order reaction relative to water. As to sucrose, the order of reaction, as appears from experimental data, is correspondingly equal to 1. The overall order of reaction appears to be

$n + m = 0 + 1 = 1$.

The kinetic reaction for the given reaction looks like:

$\upsilon = k [C_{12}H_{22}O_{11}]$

For a first-order reaction the rate of reaction is directly proportional to the reactant concentration:

$\upsilon = k [A]$

For a second-order reaction the rate of reaction is:

$\upsilon = k [A] \cdot [B]$ or

$\upsilon = k [A]^2$ or

$\upsilon = k [B]^2$

The reaction between hydrogen and iodine ($H_2 + I_2 = 2HI$) is a first-order reaction in respect to each reactant: $\upsilon = k [H_2] \cdot [B_2]$

The overall order of the given reaction is

$m + n = 1+1=2$

Many fermentative processes, where the number of substrates is excessive and the amount of enzyme is limited, can be referred to zero-order reactions.

In most cases fermentative processes, reactions of antigens with antibodies, reactions of isomeric transformation, hydrolysis, etc., are first-order reactions. Investigations show that reactions, whose order exceeds two, are not found among biochemical processes.

As appears from above, the order of reaction (empirical result) determines the dependence of the reaction rate on the concentration of reactants. Molecularity gives a molecular-kinetic description of the process.

Only in simple (elementary) reactions molecularity and order numerically correspond to each other.

In kinetic investigations of the order of reaction the concept of half-life ($\tau_{1/2}$) is often used.

The time taken by a reaction to proceed to half-completion is called the half-life of the reaction.

In other words, the half-life of a substance is the time taken for its concentration to reduce by half.

$\tau_{1/2}$ is often used to describe the processes of radioactive decay. These processes are described by an equation of a first-order reaction.

Simple and complex reactions

According to the mechanism of their process, all chemical reactions can be classified as simple and complex.

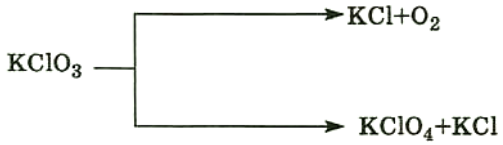
Simple (elementary) reactions are characterized as processes taking place in one stage. For example:

$$H_2 + I_2 = 2HI$$

The majority of reactions are complex.

Complex (multistage) reactions can be parallel (simultaneous), consecutive, conjugated, chain, photochemical and so on.

When a substance (substances) reacts simultaneously to give different products, we speak about *parallel reactions.* For example, during thermal decomposition of potassium chlorate two transformations occur simultaneously:



In the organism simultaneously with biological oxidation of glucose its lactic acid or alcoholic fermentation may occur. There are many cases like that in bio systems. An organism must find the optimum portion of each direction.

If a reaction consists of a number of consecutive stages, it is called a *consecutive (series) reaction.* Products formed during the first stage are initial substances for the second stage and so on:

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C \xrightarrow{k_3} D.$$

Biological oxidation of glucose, hydrolysis of ATP, etc. can be referred to such type of reaction. The rate of a process is determined by the rate of the slowest stage which is named the rate determining stage.

Conjugated reactions may be considered as special cases of parallel reactions:

$$1.\ A + B \rightarrow E,$$
$$2.\ A + C \rightarrow F,$$

where the first reaction occurs only jointly with the second reaction, i.e. it stimulates the second reaction. The first reaction does not take place until substance C (inductor) enters the system. Russian scientist A.N. Shilov was the first to investigate the chemical induction phenomenon in 1905.

In biological systems all endergonic reactions proceed according to the mechanism of conjugated reactions. Catalytic oxidation of carbohydrates and lipids in the organism leads to the synthesis of adenosine-triphosphate, which stimulates other transformations, in particular, biosynthesis of proteins and nucleic acids.

Chain reactions are those reactions that proceed with participation of free radicals (residua of molecules having unpaired electrons and displaying very high reaction activity relative to this).

Reaction of hydrogen chloride synthesis can be an example of chain reaction:

$$H_2 + Cl_2 \rightarrow 2HCl$$

Under the action of a quantum of energy the molecule of Cl_2 forms two radicals. A reaction begins at irradiation of the reactant mixture with ultraviolet light:

$$Cl_2 \xrightarrow{h\nu} Cl^{\cdot} + Cl^{\cdot}$$ (initiation of the chain).

Further there is chain development:

$$Cl^{\cdot} + H_2 \rightarrow HCl + H^{\cdot}$$
$$H^{\cdot} + Cl_2 \rightarrow HCl + Cl^{\cdot}$$ (chain propagation reactions)

These stages are repeated many times. A chain reaction is finished by a combination of free radicals:

$$Cl^{\cdot} + Cl^{\cdot} \rightarrow Cl_2;$$
$$H^{\cdot} + H^{\cdot} \rightarrow H_2;$$
$$Cl^{\cdot} + H^{\cdot} \rightarrow HCl$$ (chain termination reactions)

It is an example of a nonramified chain reaction.

In a ramified chain reaction:

$$2H_2 + O_2 \rightarrow 2H_2O$$

interaction of free radicals with a molecule of the initial substance causes generation not of one, but of two or more new radicals:

$$H_2 + O_2 \rightarrow {\cdot}OH + {\cdot}OH$$
$${\cdot}H + O_2 \rightarrow OH^{\cdot} + {\cdot}O^{\cdot}$$

$${\cdot}OH + H_2 \rightarrow H_2O + H^{\cdot}$$
$${\cdot}O^{\cdot} + H_2 \rightarrow HO^{\cdot} + H^{\cdot}.$$

The chain is ended by recombination of free radicals, and also by their interaction with foreign substances.

The action of toxic substances on the human body is usually described by a chain mechanism. It stipulates irreversible changes in the organism. The substances, which can break a ramified oxidation chain and thus prevent oxidation processes, are called *antioxidants.* Vitamin E may be an example of an antioxidant preventing oxidation of unsaturated lipids and protecting biological membranes from destruction in the organism. Its biological activity is based on the ability to

form stable free radicals as a result of removing a hydrogen atom from a hydroxyl group. These radicals interact with other free radicals, which contribute to organic peroxide formation.

Chain reactions play an important role in a number of pathological bioprocesses such as carcinogenesis, radiation disease, etc. Nuclear reactions, explosions, reactions of polymerization and other belong to chain processes. The theory of chain reactions was developed by Nobel Prize laureates N. Semenov and C. Hinshelwood.

Photochemical reactions are a kind of chain reactions. They proceed according to a chain mechanism and receive required energy in the form of electromagnetic vibrations of different frequencies. For example, the process of photosynthesis occurs in plant leaves under sunlight:

$$6CO_2 + 6H_2O \xrightarrow{h\nu} C_6H_{12}O_6 + 6O_2.$$

Some biochemical reactions may be referred to photochemical processes.

For example, physiological icterus of prematurely born children is a disease conditioned by accumulation of bilirubin in the blood: bilirubin is not excreted from the organism because of the underdeveloped liver. As it was established that the reaction of bilirubin oxidation is photochemical and oxidation products are harmless, low-energy irradiation of children became the method of medical treatment for physiological icterus.

Effect of temperature on reaction rate

As temperature rises, the rate of chemical processes usually increases. In 1879 Dutch scientist J.H. van't Hoff formulated an empirical rule:

As temperature increases by 10 K, the rate of chemical reactions increases by 2—4 times.

Van't Hoff's rule may be represented mathematically as:

$$\gamma_{10} = \frac{k_{T+10}}{k_T}$$

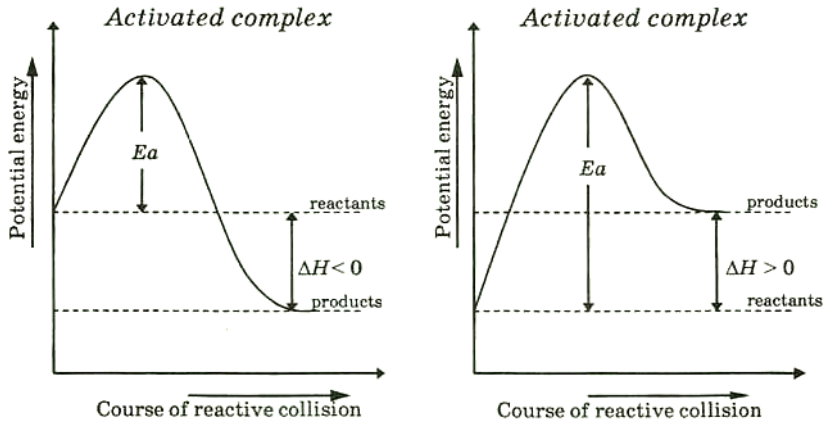
Where k_T is the reaction rate constant at a temperature T;

k_{T+10} is the reaction rate constant at a temperature $T + 10$;

γ_{10} is Van't Hoff's temperature coefficient. Its value ranges from 2 to 4. For biochemical processes γ_{10} ranges from 7 to 10. Body temperature in most living organisms varies from 35 to 40 °C. In the case of warmblooded organisms this temperature is kept constant as a result of thermoregulation of proper biosystems. To study biosystems the temperature coefficients γ_2, γ_3, γ_5 are used. For comparison they are reduced to γ_{10}.

For any chemical process it is possible to distinguish initial, intermediate and final states. On the top of the energy barrier reactants are in the intermediate state, which is called the *activated complex* or *transition state.* The difference between the energy of the activated complex and the initial energy of reactants is equal to E_a and the difference between the energy of reaction products and the energy of initial substances (reactants) is equal to the thermal effect of a reaction, *ΔH.*

Activation energy, unlike *ΔH,*is always positive. For an exothermic reaction *(Fig.1. a),*products are located at a lower energy level than reactants *(E_a< ΔH).*



a b

Fig. 1. Energy profiles for: a) an exothermic reaction; b) an endothermic reaction

In the case of an endothermic reaction *(Fig. 1. b)* the potential energy of products is higher than the energy of reactants *(.*$E_a > \Delta H$*).*

E_a is the main factor determining the reaction rate: if E_a> 120 kJ/mol (there is a high energy barrier and a minor fraction of active particles in the system), a reaction proceeds slowly; and vice versa, if E_a< 40 kJ, the reaction will proceed at a high rate.

Svante Arrhenius proposed an equation establishing a connection between the reaction rate constant, energy activation, and temperature:

$$k = A \cdot e^{-\frac{E_a}{RT}} \quad \text{or,}$$

$$k = \frac{A}{e^{E_a/RT}}$$

where e is the base of natural logarithms; k is the reaction rate constant; A is the frequency factor that takes into account the number of collisions between particles per unit of time in a unit of volume; R is the gas constant; T is temperature; E_a is activation energy.

It is evident from the Arrhenius equation that when temperature rises, a chemical reaction proceeds faster. On the other hand, a reaction proceeds faster when activation energy is lower.

As to reactions with participation of complex biomolecules, one must take into account that in the activated complexes that are formed by collision of particles, molecules must be favorably oriented, because in this way the only reacting part of a molecule (small in relation to all its size) is exposed to transformation.

If the rate constants k_1 and k_2 at temperatures T_1 and T_2 respectively are known, it is possible to calculate activation energy E_a.

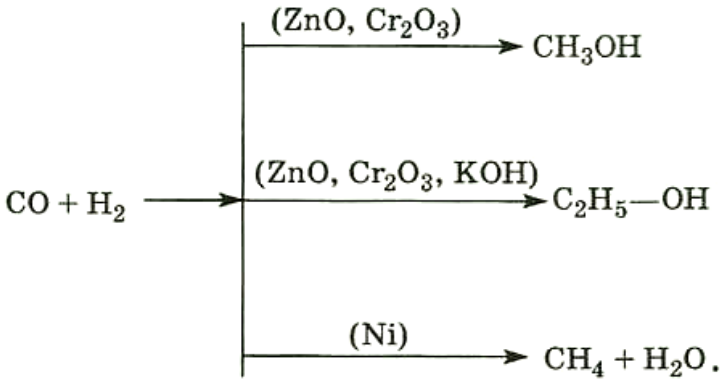
In biochemical processes activation energy is lower than in the inorganic ones by 2-3 times. At the same time E_a with participation of foreign substances, xenobiotics, considerably exceeds the E_a of ordinary biochemical processes. This fact is natural bioprotection of a system from foreign substances. It means that natural reactions of the organism take place under favorable conditions with low E_a, and for foreign substances E_a is high. It is a genetic barrier characterizing one of the basic features of biochemical process proceeding.

The phenomenon of increasing the rate of biochemical reactions by catalysts is named *catalysis.*

A catalyst is a substance that increases the rate of chemical processes without changing its own chemical composition.

If a catalyst takes part in a chemical reaction, such a reaction is called a *catalytic reaction.*

A catalyst can change not only the rate of a chemical process but also its direction, for example:



Depending on the nature of a catalyst different products are formed from the same substances.

When a catalyst is one of reaction products, the process is described as *autocatalysis.*

The majority of biochemical processes are catalytic processes, in which specific substances called *ferments* or *enzymes* take part as catalysts. That is why such processes are named *fermentative (fermentative catalysis).*

Enzymes differ from other types of catalysts because they are highly selective and efficient. Thus it is known that extremely small quantities of enzymes can increase the reaction rate by thousand or million times as compared with uncatalyzed reactions. It should be noted that the activity of biocatalysts depends on temperature (the optimum temperature is about 37 °C) and pH.

Researches of fermentative processes both under normal and pathological conditions are necessary for a deeper understanding of the features of metabolism process specificity in a living organism with the purpose of managing them.

Enzymology (the study of enzymes) has found practical application for enzymes in different spheres of national economy such as food industry, agriculture and especially medicine.

A new sphere of medicine has appeared – that is *medical enzymology* including the following sections: *enzymodiagnostics*(analysis of enzymes in biological liquids

and tissues with diagnostic and prognostic purposes); *enzymopathology* (application of enzymes for the analysis of disease pathogens); *enzymotherapy* (medical application of enzymes, their activators and inhibitors).

A new type of enzymotherapy is *system enzymotherapy*, in which combined enzymes of animal and vegetable origin are used orally.

Heterogeneous catalysis and homogeneous catalysis are distinguished. In the case of *homogeneous catalysis*a catalyst and reactants form a single phase. For example, decomposition of hydrogen peroxide, catalyzed by ions of copper (II) in an aqueous solution:

$$2H_2O_2 \xrightarrow{Cu^{2+}} 2H_2O + O_2.$$

If a catalyst and reactants are present in the system in different phases, this type of catalysis is called *heterogeneous catalysis.* Oxidation of SO_2 to SO_3 in the presence of solid V_2O_5 or Pt as catalysts may be an example of this type of catalysis:

$$2SO_{2(g)} + O_{2(g)} \xrightarrow{V_2O_{5(s)} \text{ or } Pt_{(s)}} 2SO_{3\,(g)}.$$

Enzymes are referred to microheterogeneous catalysts as long as they agree with the sizes of dispersed colloid particles. The role of enzymes is often implemented by proteins, which have metal ions in their active centers (metallo enzymes). For example, insulin contains a zinc ion, vitamin B_{12} contains a cobalt (III) ion.

The action of catalysts may be explained by a supposition that they lower the energy of reaction activation. In the presence of a catalyst a reaction passes through other intermediate stages, than in its absence. In the presence of a catalyst other activated complexes are formed, because the energy required for their formation is lower than the energy needed for their formation in the absence of the catalyst *(Fig. 1.).*

Participation of the catalyst K changes the reaction mechanism:

$$A + K \longrightarrow \underset{\textit{transition state 1}}{A \ldots K} \longrightarrow AK;$$

$$AK + B \longrightarrow \underset{\textit{transition state 2}}{AK \ldots B} \longrightarrow AB + K.$$

There are substances that slow down the action of catalysts, they are called *catalytic inhibitors* or *catalytic poisons.* Among fermentative process inhibitors

it is possible to differentiate natural or synthetic compounds oppressing the activity of enzymes.

There are substances that activate catalysts and especially biocatalysts. They are named *activators.* There are substances that renew the action of catalysts; they are called *reactivators of catalysts.*

Activation and inhibition have become apparent in fermentative processes. For example, spatially screened phenols inhibit the development of malignant tumors. The presence of NaCl admixtures in the reactionary medium accelerates the process of starch hydrolysis to glucose by salivary enzymes such as amylase. The presence in such an environment, for example, of $CuSO_4$ inhibits the catalytic process of starch hydrolysis by enzymes of saliva, because their molecules are denaturized and hydrolysis does not take place.

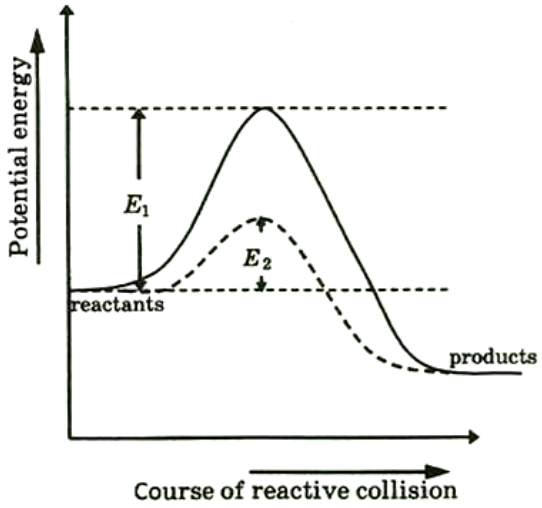


Fig. 1. Energy profiles for uncatalyzed and catalyzed reactions. E_1 is the activation energy of an uncatalyzed reaction; E_2 is the activation energy of a catalyzed reaction.

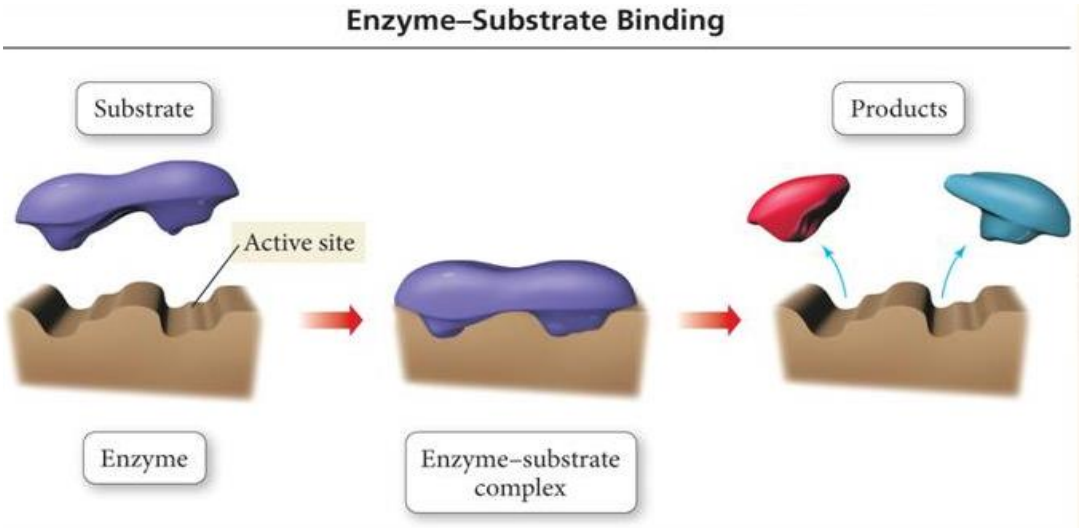
Many drugs acting as inhibitors are known now.

Conformity to the laws of chemical kinetics is used in pharmacokinetics and toxicokinetics to study rates of action and obtain remedies and take out poisons from the organism, respectively.

An ordinary way of remedy action in the organism may be considered a sequence of two processes: absorption from the stomach into the blood (is characterized

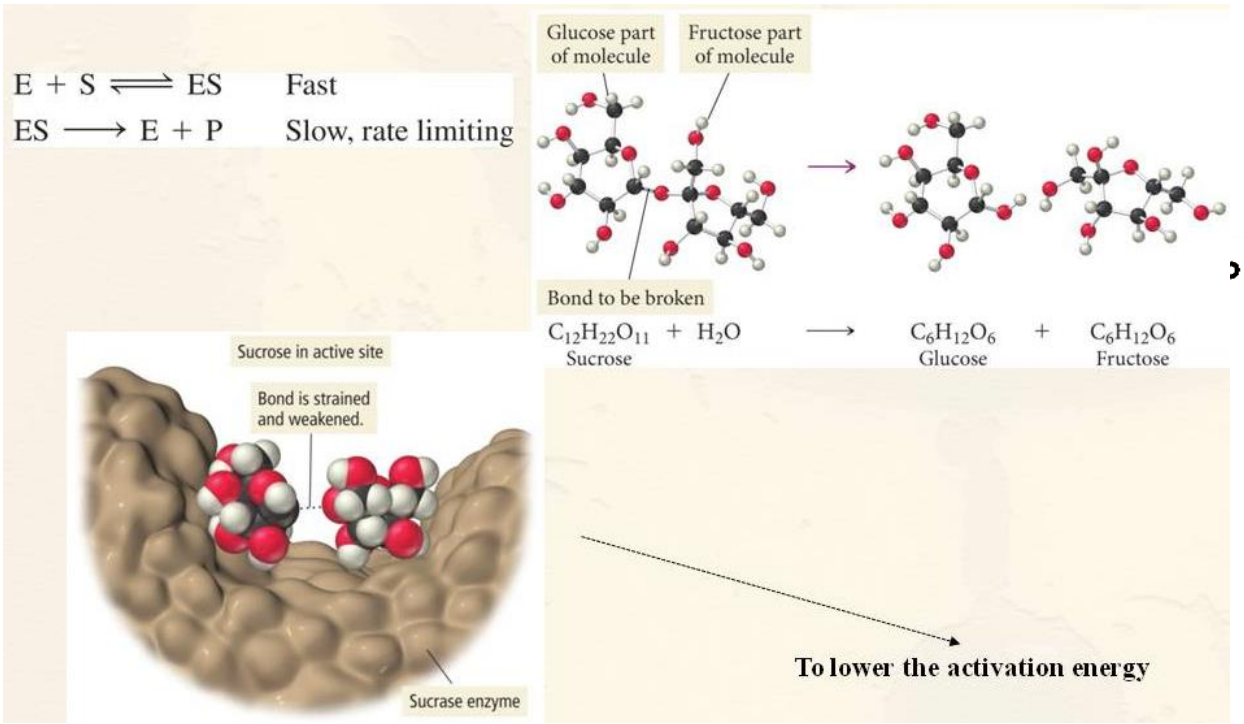
by the absorption rate constant k_s), and removal (elimination) from the blood into the urine (is characterized by the removal rate constant):

The kinetics of mass change of a drug in the stomach m_a, blood $m_h$and urine $m_u$is described by a system of three different equations that are solved graphically. On the basis of this calculation the therapeutic dose of a drug m_0 and the time of intake of the next dose may be determined.

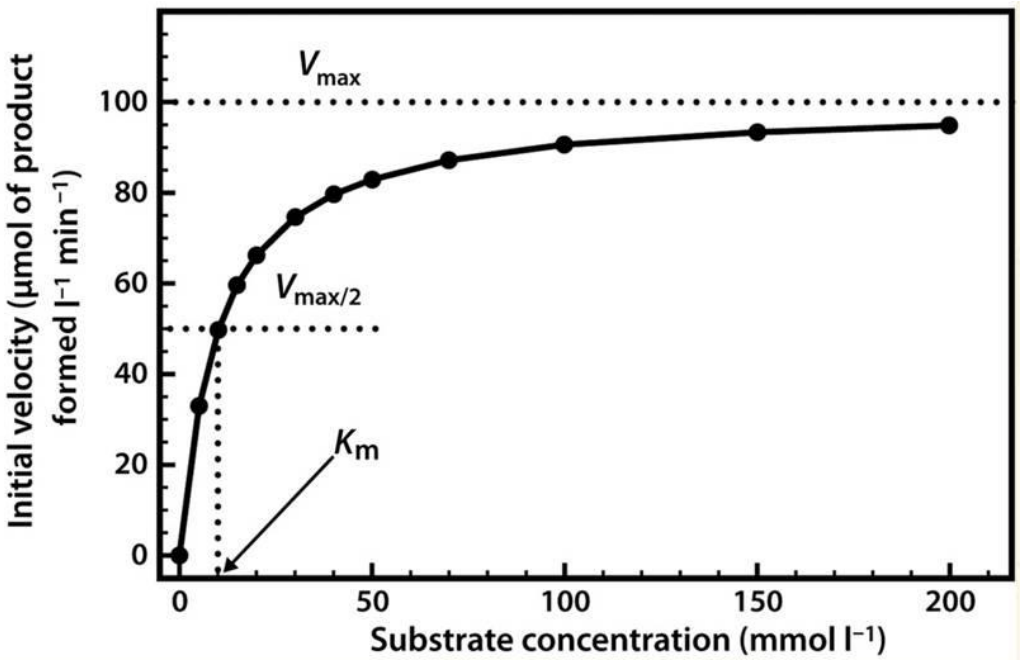


Binding is H bonds or weak covalent bonds.

Enzymatic hydrolysis of sucrose



k_1, k_{-1}, k_2, k_{-2} - rate constant - indicate the speed or efficiency of a reaction



The Michaelis-Menten equation

The basic equation derived by Michaelis and Menten to explain enzyme-catalyzed reactions is

$$v_o = \frac{V_{max}[S]}{K_m + [S]}$$

K_m - Michaelis constant;

V_o - initial velocity caused by substrate concentration, [S];

V_{max} - maximum velocity

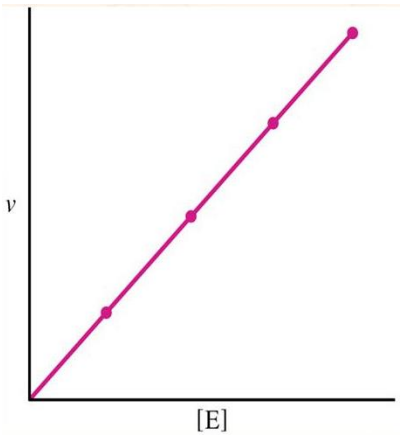
_ At a fixed enzyme concentration [E], the initial velocity Vo is almost linearly proportional to substrate concentration [S] when [S] is small but is nearly independent of [S] when [S] is large

- Rate rises linearly as [S] increases and then levels off at high [S] (saturated)

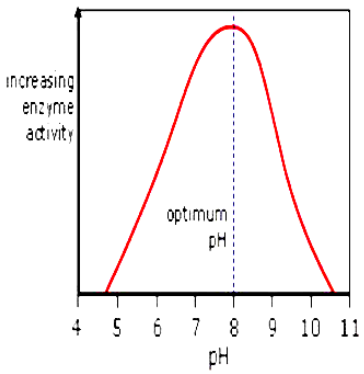
Effect of enzyme concentration [E] on velocity (v).

In fixed, saturating [S], the higer the concentration of enzyme, the greater the initial reaction rate.

This relatiomship will hold as long as there is enough substrate present.



Effect of pH on enzyme activity



- Hydrogen ion concentration also have an influence on enzyme activity.
- For most enzymes, the effective pH range is 4.0-9.0.
- Beyond these limits, denaturation of enzymes take place.
- Optimum pH for pepsin is 2.0 and for trypsin 8.0

Practical lesson 11

Topic: Chemical equilibrium. Equilibrium constant. The product of solubility. Heterogeneous equilibrium with the participation of salts in the general homeostasis of the organism.

Relevance of the topic: The concept of equilibrium states is one of the sections of thermodynamics. A particular case of thermodynamic equilibrium state is chemical equilibrium. Chemical equilibrium contributes significantly to the homeostasis of the living organism. Exposure to various factors (concentration of substances, pressure, temperature) can shift the position of equilibrium. Knowledge of the laws of equilibrium in homogeneous and heterogeneous systems allows us to analyze the conditions of formation and dissolution of sediments, such as the formation of kidney stones or in the gallbladder.The value of the equilibrium constant characterizes the completeness of the chemical reaction. Le Chatelier's principle: the system always tends to counteract influences from the outside in the opposite direction, the equilibrium shifts in the same direction.

Lesson objective: to remember the concept of chemical equilibrium and the main factors influencing the state of chemical equilibrium; learn to apply in practice the Le Chatelier principle and determine and explain the direction of chemical equilibrium shift.

Basic definitions: irreversible reaction, reversible reaction, equilibrium state, equilibrium constant, Le Chatelier’s principle, solubility.

Plan and organizational structure of the lesson:

1. Equilibrium state. Equilibrium concentrations.
2. Thermodynamic criteria of the condition of chemical equilibrium.
3. Constant of equilibrium.
4. Le Chatelier’s principle. Factors which affects the shift of chemical equilibrium.
5. Heterogeneous equilibrium by salt action in the general homeostasis of the body.
6. Laboratory work.

Content of the topic

If Gibbs free energy diminishes as a result of a process ($\Delta G<0$), chemical processes take place spontaneously. If enthalpy and entropy factors operate in concord guiding the reaction toward product formation, the reactants are fully converted into products of reaction. Such reactions are called *irreversible reactions.* For example:

$$2KClO_3 = 2KCl + 3O_2$$

$$Mg + 2HCl = MgCl_2 + H_2$$

If reaction products may interact forming initial substances, the reaction proceeds in both forward and reverse directions. Such reactions are named *reversible.* For example:

$$H_2 + I_2 \leftrightarrow 2HI$$

$$N_2 + 3H_2 \leftrightarrow 2NH_3$$

A forward reaction occurs at the rate (corresponds to the rate constant and *a reverse reaction* proceeds at the rate v_2 (corresponds to the rate constant k_2). When the rates of forward and reverse reactions become equal, the *state of chemical equilibrium* is reached in the system. The kinetic condition of chemical equilibrium is equality of the forward and reverse reaction rates. It appears from this that chemical equilibrium has a dynamic nature.

A quantitative characteristic of equilibrium can be the equilibrium constant. For example, for a reversible reaction:

$$H_2 + I_2 \underset{v_2}{\overset{v_1}{\rightleftharpoons}} 2HI$$

the rates of forward and reverse reactions according to the law of mass action may be represented as:

$$v_1 = k_1[H_2] \cdot [I_2]; \qquad v_2 = k_2[HI]^2.$$

At equilibrium the rates of forward (u,) and reverse (uj reactions are equal:

$$k_1[H_2] \cdot [I_2] = k_2[HI]^2, \text{ or } \frac{k_1}{k_2} = \frac{[HI]^2}{[H_2] \cdot [I_2]}.$$

The ratio of the rate constants of forward and reverse reactions at any given temperature is a constant. It is called *the equilibrium constant* of a given reaction *(K_e):*

$$\frac{k_1}{k_2} = K_e; \quad K_e = \frac{[HI]^2}{[H_2]\cdot[I_2]}.$$

Concentrations of substances in this equation are called equilibrium concentrations (concentrations established in the state of equilibrium).

In general, for a reversible homogeneous reaction:

$$aA + bB \leftrightarrows cC + dD$$

the equilibrium constant may be written as:

$$K_e = \frac{[C]^c \cdot [D]^d}{[A]^a \cdot [B]^b}.$$

The given ratio is a mathematical expression of the law of mass action for reversible reactions:

In the state of chemical equilibrium at a given temperature, the ratio of the product of product concentration and the product of reactant concentration at exponential degrees that are equal to coefficients of a balanced equation is a constant value and is called the equilibrium constant.

The equilibrium constants expressed through equilibrium concentrations in moles per liter are designated as K_c.

For a reaction with participation of gases the equilibrium constant is expressed through partial pressure of gases - K_p.

If the product of reactant concentrations approaches zero, $K_c \to \infty$, such a process is reversible. If product concentrations approach zero, $K_c \to 0$, it means that the process does not occur. If $K_e >> 1$, it means that in the state of chemical equilibrium products predominate.

If $K_e << 1$, reactants predominate. In practice for equilibrium processes: $0 < K_e < \infty$

Equilibrium concentrations of substances participating in a reaction are connected with each other, therefore if the concentration of the substance A is changed, the concentrations of the substances *B, C* and *D* are changed too. However, their ratio remains the same.

The equilibrium constant does not depend on the concentration of substances participating in a reaction, but depends on their nature and the temperature of the process. The presence of a catalyst does not alter the equilibrium constant, so far as it increases the rates of both forward and reverse reactions. The effect of a catalyst consists in decreasing the time needed for the system to reach the state of equilibrium.

It should be noted that for heterogeneous reactions the concentrations of only the substances that are in a gaseous or liquid phase must be represented in the expression of the equilibrium constant. Forexamüle:

$$CO_{2(g)} + C_{(s)} \rightleftarrows 2CO_{(g)}$$

$$K_c = \frac{[CO]^2}{[CO_2]} \quad \text{or} \quad K_p = \frac{P^2_{CO}}{P_{CO_2}}$$

One of the types of equilibrium constants encountered in physical and analytical chemistry is the dissociation constant K_d that characterizes the equilibrium processes of weak electrolyte dissociation. For example:

$$H_2SO_3 \rightleftarrows H^+ + HSO_3^-; \qquad K_{d_1} = \frac{[HSO_3^-]\cdot[H^+]}{[H_2SO_3]};$$

$$HSO^-_3 \rightleftarrows H^+ + SO_3^{2-}; \qquad K_{d_2} = \frac{[H^+]\cdot[SO_3^{2-}]}{[HSO_3^-]}.$$

The summary equation of dissociation:

$$H_2SO_3 \rightleftarrows 2H^+ + SO_3^{2-}.$$

The general dissociation constant: $$K_d = \frac{[H^+]^2 \cdot [SO_3^{2-}]}{[H_2SO_3]}.$$

In practice pK_d is often used instead of K_d:

$$pK = -\lg \bar{K}_d.$$

The equilibrium of complex ion dissociation is characterized by the equilibrium constant, which is named the instability (dissociation) constant, K_{inst}:

$$Ag(NH_3)_2^+ \rightleftarrows Ag(NH_3)^+ + NH_3;$$
$$Ag(NH_3)^+ \rightleftarrows Ag^+ + NH_3.$$

The summary equation of dissociation:

$$[Ag(NH_3)_2]^+ \rightleftarrows Ag^+ + 2NH_3;$$
$$K_{inst._1} = \frac{[Ag(NH_3)^+] \cdot [NH_3]}{[Ag(NH_3)_2^+]};$$

The general instability constant:

$$K_{inst._2} = \frac{[Ag^+] \cdot [NH_3]}{[Ag(NH_3)^+]}. \quad K_{inst.} = \frac{[Ag^+] \cdot [NH_3]^2}{[Ag(NH_3)_2^+]}.$$

Beside the instability constant, the constant of stability (formation), K_{gt}, is used to characterize complex compound stability. It is a value opposite to the instability constant:

$$K_{st.} = \frac{1}{K_{inst.}}.$$

Some of other equilibrium constants will be represented in the following chapters of this book.

Displacement of chemical equilibrium

If a system is in the state of chemical equilibrium, it will be in this state until external conditions change. If these conditions are altered, the position of equilibrium shifts. Displacement of equilibrium occurs according to *Le Chatelier's principle:*

If a system in the state of equilibrium is disturbed by a change in some external factors, the position of equilibrium will shift in the direction that tends to counteract the applied alteration.

Such external factors may be: changes in temperature, pressure (if one of the reactants or products is a gas), or concentration of reactants or products.

In case of an increase in concentration of any substance taking part in the process, the position of chemical equilibrium shifts in the direction of the consumption (decrease) of this component and vice versa. For example, if in the system $H_2 + I_2 \rightleftarrows 2HI$ that is in the state of equilibrium, the concentration of hydrogen is increased, the position of equilibrium will shift to the right, in the direction of HI formation. When the rates of forward and reverse reactions become equal again, a new equilibrium is achieved.

If pressure increases in a mixture that is in the state equilibrium and contains gases as reaction participants, the position of equilibrium shifts in the direction of reduction in the number of gas molecules, i.e. in the direction of pressure lowering, and vice versa. For example, in the reaction:

$$2CO + O_2 \rightleftarrows 2CO_2,$$

from three molecules of the initial gaseous substances two molecules of $C0_2$ are formed, therefore at pressure increase the position of equilibrium will shift in the direction of $C0_2$ formation.

As it has been mentioned, the thermodynamic condition of equilibrium is $\Delta G = 0$. It means that at chemical equilibrium the entropic and enthalpy factors are equal: $\Delta H = T\Delta S$

To compensate the increasing temperature in such a system, it is necessary to increase the enthalpy factor. It is possible if heat is absorbed, i.e. $\Delta H > 0$. An endothermic process must take place in this case. And vice versa, the system compen-

sates temperature decrease by evolving heat, in other words by an exothermic reaction. For example, in the system: $2CO + O_2 = 2CO_2$; $\Delta H < 0$ in case of temperature lowering the equilibrium shifts to the right, in the direction of the exothermic reaction, and in case of temperature rise it shifts to the left, in the direction of the endothermic reaction.

Le Chatelier's principle is applied not only to chemical but also to physical and biochemical equilibriums. Disturbance of equilibrium owing to a change in the conditions of such processes as boiling, crystallization, dissolution, occurs in accordance with Le Chatelier's principle.

Interaction between hemoglobin (Hb) and oxygen (O_2) leading to the formation of oxyhemoglobin (HbO_2) takes place according to the equation:

$$Hb + O_2 \rightleftarrows HbO_2;$$

$$K_e = \frac{[Hb \cdot O_2]}{[Hb] \cdot [O_2]} = 1300.$$

An increase of O_2 concentration results in equilibrium displacement to the right, in the direction of oxyhemoglobin formation.

On the other hand, if carbon monoxide reacts with hemoglobin, a more stable complex is formed, the concentration of hemoglobin is diminished in this case and the position of equilibrium shifts to the left, in the direction of oxyhemoglobin destruction.

If some of the products are taken out of the sphere of reaction, i.e. their concentration decreases, the position of equilibrium shifts to the right. It occurs if there forms a gas, a precipitate or slightly soluble substances. For example, in the stomach a reaction reducing the acidity of its contents proceeds:

$$NaHCO_3 + HCl \rightarrow NaCl + H_2O + CO_2\uparrow.$$

The possibility of Le Chatelier's principle application allows predicting many changes in the body caused by external influences and managing them.

Solubility of substances

Solubility is the ability of a substance to dissolve in a given solvent. *Substance solubility under given conditions is measured by its content in a saturated solution.*

For practical purposes, solubility may be expressed as the maximum amount of the substance in grams that can be dissolved under certain conditions in 100 g of the solvent. Solubility expressed in such a way is named *the solubility coefficient.*

Solubility of various substances in water has different values. According to the ability of substances to dissolve in water, such groups of substances are distinguished:

- Soluble substances;
- Slightly soluble substances;
- Practically insoluble substances.

If in 100 g of water more than 1 g of a substance is dissolved, such a substance is named soluble in water; if there is dissolved from 0.01 g to 1 g of a substance, it is a slightly soluble substance. If in 100 g of water less than 0.01 g of a substance is dissolved, it is called insoluble.

Solubility of substances depends on the properties of the solvent and the substance that is dissolved, on temperature, and also on pressure (for gases). Pressure rise increases solubility of gases. Solubility of liquids and solid substances in liquids practically doesn't depend on pressure.

Laboratory work:

1. Chemical equilibrium. Influence of concentration.

Exchange reaction between Ferrum (III) chloride and potassium thiocyanate or ammonium thiocyanate is reversible.

$$FeCl_3 + 3KSCN \leftrightarrow Fe(SCN)_3 + 3KC1$$

$$K = \frac{[Fe(SCN)_3]\cdot[KCl]^3}{[FeCl_3][KSCN]^3}$$

The solution Ferrum (III) rhodanide $Fe(SCN)_3$, which was derivated as a result of the reaction, has a red color, intensity which depends on the concentration. The displacement of equilibrium is easy to define because of the change of intensity in the coloring of the solution.

Fulfillment of activity. In a beaker pour 20 ml of water and add 1-2 drops of saturated solutions of $FeCl_3$ and KSCN. The displacement of equilibrium is easily determined by the change of intensity in the coloring of the solution.

In the first test tube add 2-3 drops of saturated solution $FeCl_3$. What has changed? Give an explanation from the equation of equilibrium constant.

In another test tube, add a little firm crystalline of potassium chloride and mix. Compare the color of solutions in the 3rd and 4th test tubes. *Give* explanations. Record the outcomes of observations in the table.

№ of Test tube	Additional compound	Intensity of coloring	Direction of the displaced equilibrium
1			
2			
3			

2. The influence of temperature.

At interaction of Iodine with Amylum forms clathrate type inclusion compound

Iodine + Amylum ↔ iodoamylum

Fulfillment of work. In 2 test tubes pour 4-5ml of starch solution and add 3-4 drops of iodine concentration solution 0,1 Mol/L. Heat one of the test tubes, and then cool. Abandon the second tube for comparison. What happens? Is iodoamylum formation an exo- or endothermic reaction?

Practical lesson 12

Topic: The mechanism of electrode potential formation and their classification. Redox potentials. Potentiometry. Determination of pH of biological fluids.

Relevance of the topic: the laws that are associated with the transformation of chemical and electrical forms of energy study the thermodynamics and kinetics of electrode processes. The study of these phenomena is of great importance for understanding the mechanism of action of drugs. The study of electrical properties of cells and tissues is important for understanding their structure and physicochemical properties. Conductometry and potentiometry are widely used in medicine to solve diagnostic and research problems. The study of the mechanism of appearance of electrode, membrane and redox potentials explains the regularities of biochemical reactions in the body.

Lesson objective: be able to explain the mechanism of formation of electrode potentials; analyze the principles of potentiometry method and draw conclusions about their use in medical and biological research; measure redox potentials and predict the direction of redox reactions; calculate electrode potentials, make diagrams of electrodes and galvanic elements.

Basic definitions: electrode, galvanic cell, diffusion and membrane potentials, Nernst equation.

Plan and organizational structure of the lesson:

1. Electrode potential and mechanism of their occurrence. Nernst equation.
2. Normal hydrogen electrode.
3. Galvanic cell. Measurement of electrode potentials.
4. Classification of electrodes:

   a)Electrodes of the first kind.

   b)Electrodes of the second kind.

   c)Ion selective electrodes. Glass electrode.

   d)Inert redox electrodes.

5. Potentials of biological systems. Diffusion potential (damage potential), membrane potential, phase potential.
6. The biological role of diffusion and membrane potentials.

Content of the topic

If a plate of any metal, for example zinc, is dipped into water, the ions of zinc being part of the metal crystal lattice, under the influence of polar molecules of water are hydrated, their bond with the lattice becomes weak and some amount of them coming out from the metal surface passes into water. An equivalent number of electrons stays on the metal:

$$Zn \rightarrow Zn^{2+} + 2e^{-}.$$

Between the metal cations, which have passed into water, and the negatively charged plate an electrostatic attraction arises. As a result, a reverse process - transition of metal ions into the plate - takes place. Metal ions will gain electrons from the metal plate and change into metal atoms. So a dynamic equilibrium is established in the system:

$$Zn \rightleftarrows Zn^{2+} + 2e^{-}.$$

It means that zinc ions pass from the plate into the solution and settle from the solution on the plate with identical rate. A double electrical layer is formed on the interface between the metal and the solution and a potential difference arises.

If the liquid is water, then any metal is charged negatively and the layer of liquid adjoining to it is charged positively. A different picture is observed if a metal plate is immersed not into pure water, but into a solution of this metal's salt. If an active metal is in contact with solution of its own salt, then its surface is charged negatively, and the value of this negative charge decreases with increasing of active concentration (activity) of its ions in the solution. If the metal is inactive, then the process of solution ion reduction will prevail and a plate of such metal will develop a positive charge.

So, at immersing of a metal plate into a solution of its own salt, a potential difference develops in the place of contact between the metal and solution. Its value and sign depend on the chemical nature of the metal and activity of its own ions in the solution.

A conductor (metal) immersed in a solution of electrolyte is called an *electrode.*

Schematically an electrode may be written down as $Cu|Cu^{2+}$, $Ag|Ag^{+}$.

Potential difference arising on the electrode-solution interface is called *electrode potential.*

The value of electrode potential can be calculated according to the *equation of W. Nernst* (a German scientist) (1889):

$$E = E^0 + \frac{2.3 \cdot RT}{nF} \cdot \lg a(Me^{n+}),$$

where E is electrode potential under given concentration of Me^{n+} ions; R is the universal gas constant, 8.314 J /mol · K; T is temperature, K; $F$is the Faraday constant, 96500 C/mol; n is the number of electrons involved in an electrode reaction; $a(Me^{n+})$ is the activity of metal ions in a solution; $E°$ is normal (standard) electrode potential. Standard electrode potential, $E°$, is the potential measured under standard conditions: the activity of metal ions in solution is equal to 1 mol/1, temperature is 298 K.

At a temperature of 298 K the Nernst equation may be represented as:

$$E = E^0 + \frac{0.059}{n} \cdot \lg a(Me^{n+}).$$

The science does not have any methods allowing to measure the absolute value of potentials of individual electrodes, it is possible to measure only the potential difference between electrodes. For this purpose it is necessary to use some electrodes as reference electrodes. A commonly accepted reference electrode is the normal (standard) hydrogen electrode. The *normal hydrogen electrode* represents platinized platinum (a platinum plate covered with black platinum) immersed in an acid solution where the activity of H^+ ions is equal to 1 mol/1. Carefully cleared hydrogen gas at a

pressure of 101325 Pa (1 atm) is run over this solution. The platinum surface becomes covered with a layer of gaseous hydrogen. An the interface between gaseous hydrogen and hydrogen ions equilibrium is established:

$$H_2 \rightleftharpoons 2H^+ + 2e^-.$$

Apparently, platinum does not react, but provides a surface for electrode process. Potential of the standard hydrogen electrode is conditionally taken as zero.

Hydrogen electrode may be attributed to gas-ion electrodes, because they consist of gas in contact with its cation (or anion) in the solution.

So, for determining the electrode potential of any electrode, a cell comprising the electrode under investigation as one of the electrodes and a standard hydrogen electrode as the reference electrode must be assembled. Electrodes in this electrochemical cell must be joined with a wire, and solutions must be joined with a salt bridge that conducts electricity, but prevents the ions from mixing among themselves in the solution.

Electrons flow from the electrode where oxidation takes place (anode), to the electrode where reduction occurs (cathode). Thus the electrochemical cell produces electricity as a result of spontaneous chemical process. Such system is called the *galvanic cell.*

Potential difference between electrodes is measured by a potentiometer. Difference between potentials of electrodes is known as *electromotive force (EMF)* of the cell. (It should be more exactly noted that EMF is the maximum voltage obtainable from the cell).

EMF is always calculated in the following way:

$$\text{EMF} = E_{\text{cathode}} - E_{\text{anode}}.$$

If the normal hydrogen electrode is used as the reference electrode, whose electrode potential is equal to zero, EMF of the cell will immediately give the electrode potential of the other electrode.

Electrode potential is called *oxidation potential* if oxidation process occurs on the electrode relative to the standard hydrogen electrode, and on the contrary, it is called *reduction potential* if reduction takes place on the electrode relative to the standard hydrogen electrode. It means that the electrode where reduction takes place relative to the standard hydrogen electrode has a positive reduction potential. The electrode where oxidation occurs relative to the standard hydrogen electrode has a negative reduction potential. Reduction potential is equal to oxidation potential, but an opposite sign. In practice electrode potentials are expressed as reductions potentials.

As it has been mentioned, if a metal is immersed in a solution of its own ions in concentration of 1 mol/1 at 298 K, then the potential arising under these conditions is named the *standard electrode potential* of this metal. When all the metals are arranged in the order of their standard reduction potentials increase, an electrochemical series of metals is obtained:

K	Ca	Al	Zn	Fe	$1/2H_2$	Cu	Ag	Hg	Au
− 2.92	− 2.84	− 1.66	− 0.76	− 0.44	0	0.34	0.80	0.85	1.42
K^+	Ca^{2+}	Al^{3+}	Zn^{2+}	Fe^{2+}	H^+	Cu^{2+}	Ag^+	Hg^+	Au^{2+}

The electrochemical series of metals coincides with the activity series empirically established by Russian scientist N. Beketov by investigating metal displacement from their compounds by other metals.

Relative activity of metals can be estimated by comparison of their oxidation potentials, because metallic properties are characterized by the ability of metals to lose electrons. So, a metal with a greater oxidation potential will be more active than a metal with a lower oxidation potential. That is why any metal of the electrochemical series can displace metals that are on its right side from the salt solution.

Classification of electrodes

All electrodes may be divided into 4 basic types:

1. Electrodes of the first kind, reversible relative to cation or anion.
2. Electrodes of the second kind, reversible relative to both cation and anion.
3. Ion-selective electrodes.
4. Inert oxidation-reduction electrodes.

Electrodes of the first kind.

Metal immersed in a solution of its own salt can be an example of an electrode of the first kind, reversible relative to cation: $Cu|Cu^{2+}$; $Zn|Zn^{2+}$; $Ag|Ag^{+}$. Gas-ion electrodes such as: (Pt) $1/2H_2|H^{+}$; (Pt) $C1^{-}|1/2C1_2$ maybe also an example of the first-kind electrodes.

Their potential is equal to:

$$E = E^0 + \frac{2.3 \cdot RT}{nF} \cdot \lg a(\text{cat.}), \text{ or } E = E^0 - \frac{2.3 \cdot RT}{nF} \cdot \lg a(\text{an.}),$$

where a(cat.) and a(an.) are the activities of cation and anion, mol/1, respectively.

Electrodes of the first kind are often used as indicator electrodes. Since such electrodes rapidly respond to the concentration of the analyte ion in a solution, it is possible to calculate activity of its ions in a solution. For example, the concentration of H^{+} ions may be determined using the hydrogen electrode. The Nernst equation for the hydrogen electrode can be written as:

$$E = 0.059\ a(H^{+}) \text{ or } E = -0.059\text{pH}.$$

Electrodes of the second kind.It is metal covered with a layer of its insoluble salt being in contact with a solution containing an anion of the salt. For example, the so-called silver/silver chloride electrode: Ag|AgCl, KC1.

It is a silver wire covered with *silver chloride* and immersed in a solution of potassium chloride. During its work reactions occur:

$$E_{Ag|AgCl} = E^0{}_{Ag|AgCl} + \frac{2.3 \cdot RT}{nF} \cdot \lg \frac{K_{sp\,AgCl}}{a(Cl^{-})}.$$

$$E_{gl.} = E^0{}_{gl.} - 0.059\text{pH}.$$

$$E_{Ag|AgCl} = E^0{}_{Ag|AgCl} + \frac{2.3 \cdot RT}{nF} \cdot \lg a(Ag^{+}).$$

$$Ag \rightarrow Ag^{+} + e^{-} \text{ and } Ag^{+} + Cl^{-} \rightarrow AgCl,$$
$$\text{or } Ag + Cl^{-} \rightarrow AgCl + e^{-}.$$

The potential arises at the $Ag|Ag^{+}$ interface. The potential of silver/silver chloride electrode has a sign «+» in relation to the normal hydrogen electrode.

If a solution of KC1 is saturated, in other words, a system consists of the silver electrode immersed in a solution saturated with both silver chloride and potassium chloride, the activity of Ag^+ ions is determined by the solubility product constant K_{spAgCl} of AgCl:

Consequently,

$$K_{\text{sp AgCl}} = a(\text{Ag}^+) \cdot \text{a}(\text{Cl}^-).$$

Then:

$$a(\text{Ag}^+) = \frac{K_{\text{sp AgCl}}}{a(\text{Cl}^-)}.$$

The saturated silver/silver chloride electrode is often used as a reference electrode ($E_{Ag^+/AgCl}$ = 0.201 V) instead of the standard hydrogen electrode, which is difficult to operate.

The saturated calomel electrode $Hg|Hg_2Cl_2$, KCl_{satd} is also referred to electrodes of the second kind, and is also used as a reference electrode.

Ion-selective electrodes. An ion-selective electrode consists of an ionite, material capable of exchanging ions with solution, and electrolyte solution. The potential at the interface of phases arises at the expense of ion-exchange processes between ionite and solution. When exchange process equilibrium is achieved, the surface of ionite and solution acquire electric charges of an opposite sign, at the ionite-solution interface potential difference is generated. The most important representative of ion-selective electrodes is the *glass electrode.* Now it is the most widespread indicator electrode for pH measurement.

The potential of the glass electrode depends on the activity of hydrogen ions:

The glass electrode has a number of advantages in comparison with the hydrogen electrode. The potential of the glass electrode is established instantly, it is independent of the presence of oxidizers, reducers and other substances. By means of the glass electrode it is possible to measure pH from 0 to 12 (by the hydrogen electrode – from 2 to 9). A disadvantage of glass electrode is its fragility and high

resistance. Therefore glass electrodes are made of special glass with a heightened electrical conductivity, and measurements are carried out by means of amplifiers.

There are also ion-selective electrodes used for determination of the content of such ions as Na^+, K^+, NH_4^+, Ca^{2+}, NO_3^-, Ag^+, Cl^-, etc. in a solution.

Lately, modified ion-selective electrodes have appeared: enzyme electrodes, bacterial electrodes and immune electrodes, named *biological sensors.* They are used for determination of organic substances concentration.

Inert oxidation-reduction electrodes. The inert oxidation-reduction electrode is one that consists of an inert metal – platinum, iridium – immersed in a solution containing oxidized and reduced forms of a substance. During operation of this electrode oxidation-reduction reactions proceed in a solution without participation of the substance. The electrode serves only as a conductor of electrons, as their reservoir. Oxidation or reduction products are not liberated on the electrode, but remain in the solution. For example, if to place a platinum plate in a solution containing Fe^{2+} and Fe^{3+} ions, the following process is possible in this solution:

$$Fe^{2+} \rightleftarrows Fe^{3+} + e^-.$$

Fe^{3+} ions can oxidize various substances removing electrons from them. If such substances are not present in a solution, oxidation does not occur, but the tendency of removing electrons by Fe^{3+} ions remains. Platinum in this case serves as a reservoir of free electrons. Fe^{3+} ions are converted into Fe^{2+} ions by taking away electrons from platinum, as a result the electrode is charged positively and anions are collected close to its surface in a solution. These anions are surplus anions because a Fe^{3+} ion can bond three Cl^- ions, but Fe^{2+} ions formed in the solution can bond only two Cl^- ions.

The arising potential difference characterizes the oxidizing ability of a solution. The value of the redox system potential is defined according to the *Nernst- Peters equation:*

$$E_{red/ox} = E^0_{red/ox} + \frac{2.3 \cdot RT}{nF} \cdot \lg \frac{a(ox.)}{a(red.)}$$

where a(ox.) and a(red.) are activities ofthe oxidized and reduced forms; $E^0_{red/ox}$ is normal (standard) electrode potential of a redox system. $E°_{red/ox}$ is equal to the

potential of an electrode if a(ox.) = a(red.).The potential of a redox system depends on the ratio $\frac{a(ox.)}{a(red.)}$. An increase of $\frac{a(ox.)}{a(red.)}$ increases the potential, intensifies oxidizing action. A decrease of this ratio reduces $E_{red/ox}$, in other words, strengthens reducing action.

The Gibbs free energy or isobaric- isothermal potential is one of the most important thermodynamics state function. The character of Gibbs energy change allows concluding that reaction realization is principally possible : $\Delta G = -W_{electricity}$

$\Delta G = -nF\Delta E$, where n- number of electrons, F=96500 C/mol, Faraday constant.

$\Delta G < 0$ – spontaneous process condition, consequently electromotive force(EMF):

$\Delta E > 0$ .EMF is always calculated in the following way :

$\Delta E = E^0_{ox.ag} - E^0_{red.ag}$.

If $E^0_{ox.ag} > E^0_{red.ag}$ the process is possible, occurs spontaneously.

For example

$Cl_2 + 2e^- \leftrightarrow 2Cl^-$ $E^0 = 1,36$ V – oxidizing agent (1,36 V > 1,06 V)

$Br_2 + 2e^- \leftrightarrow 2Br^-$ $E^0 = 1,06$ V – reducing agent

$2Br^- - 2e^- \leftrightarrow Br_2^-$ - oxidation

$Cl_2 + 2e^- \leftrightarrow 2Cl^-$ -reduction

The $E°_{red/ox}$ value serves as a measure of the oxidizing or reducing ability of a system: the greater the value $E^0_{red/ox}$means the better its oxidizing action, and vice versa. $\pm E°_{red/ox}$ allows to draw a conclusion about the direction of a reaction: if the $E°_{red/ox}$ value for one system in a redox reaction is larger than for another system, then this system is a better oxidizing agent.

Thermodynamics of galvanic cell

Electric work in isobaric-isothermal process is accomplished due to the loss of Gibbs energy

$$\Delta G^0 = -nF\Delta E^0$$

Where E^0 – standard e.m.f. with average activities of all ions in solution equal to one.

To determine the equilibrium constant use the equation

$$\Delta G^0 = -RT\ln K_{eq}$$

The equilibrium constant of the reaction occurring in a galvanic cell is calculated by the following formula $\ln K_{eq} = \frac{nF\Delta E^0}{RT}$ or $\lg K_{eq} = \frac{nF\Delta E^0}{2{,}3RT}$

Diffusion and membrane potentials

At the interface between solutions of different nature or different concentration there is potential difference as a result of dissimilarity of cation and anion diffusion speed. More mobile ions gain advantage over less mobile ones. Two waves of discharged ions are formed. It is similar to discharged plates of condensator. In the case of two solutions with different concentrations, the more diluted solution gets a potential with the sign of the charge of the «fast» ions, and the more concentrated one gets a potential with the sign of the charge of the «slow» ions. On the solutions interface diffusion potential arises averaging the speed of ions movement, restoring the electro neutrality of the solution.

Potential difference on the interface between solutions, which differ in composition or concentration, is called diffusion potential.

For example, at contact of solutions with different concentration of HC1 diffusion of ions takes place. Transference speeds of H^+ (H_3O^+) and Cl ions are unequal. Since H^+ ions move faster, the number of these ions passed through the contact border is bigger than the number of Cl^- ions. Therefore a front of H^+ ions is formed, and a front of Cl^- ions moves behind it. As a result a double electrical layer of ions is formed.

If solutions with different concentrations are separated with a special membrane, which is permeable only for ions of one sign, membrane potential is generated.

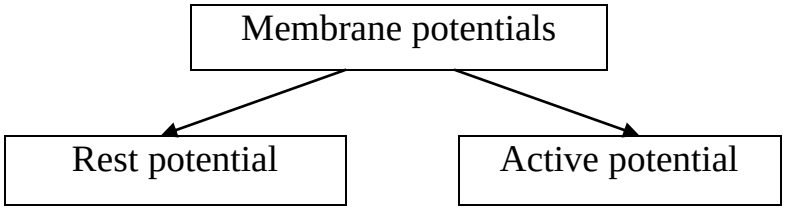
In this case ions of this sign of charge (for example, positively charged ions) pass through the membrane from the more concentrated solution toward the less concentrated one. As a result the more diluted side becomes positively charged, therefore the more concentrated side acquires a negative charge relative to the other side of the membrane because of an excess of negatively charged ions. Resulting potential difference prevents further crossing of the membrane by positively charged ions. In other words, it prevents from the tendency to concentrations equalization. As a result equilibrium between these processes is established.

Membrane potentials are maintained unaltered for a long period of time. In tissues of vegetable and animal organisms there are membrane and diffusion potentials caused by chemical and morphological heterogeneity of intracellular contents. Diverse reasons, which change properties of cell microstructures, lead to generation of various bio potentials and bio currents. The role of bio currents is still not studied completely, but available experimental data testify to their great importance in self-regulation processes of living organisms.

Potentials in biological systems

All the reactions occurring in a living organism are accompanied by electrochemical phenomena which may be divided in two groups:

♦Processes accompanied by ions transferring without changes of their charge and formation of three types of *bioelectric potentials*: *diffusion*, *membrane* and *phase*;



Rest and active potential equations:

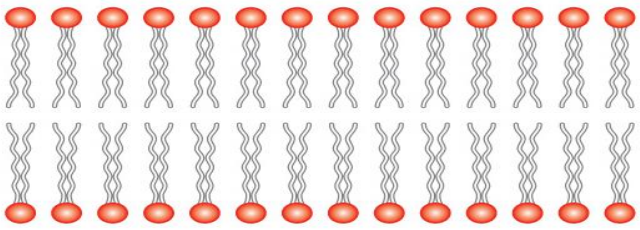
$$E_{rest} = \frac{RT}{F} ln \frac{[K^+]cell}{[K^+]out\ of\ cell} \qquad E_{active} = \frac{RT}{F} (\ln \frac{[K^+]cell}{[K^+]out\ of\ cell} + \ln \frac{[Na^+]out\ of cell}{[Na^+]cell})$$

Where $\frac{[K^+]cell}{[K^+]out\ of\ cell}$ = 20 ÷ 40 times Where $\frac{[Na^+]out\ of cell}{[Na^+]cell}$ = 10 ÷ 20 times

E_{rest} = 50 ÷ 100 mV E_{active} = 90 ÷ 130 mV

Time of membrane depolarization is 10^{-4} s

$K^+K^+K^+K^+K^+K^+K^+K^+$



anions of aspartic acid, acetic acid etc.

♦Processes caused by intermolecular electrons transferring, generation of *oxidation-reduction potentials* and formation of energy required for organism vital activity.

These processes take place in organs, tissues and cells. A cell is a heterogeneous multiphase system. If in one phase cations are more dissoluble and in another one anions are more dissoluble, then on the phases interface a double electrical layer is generated, as a result phase potential arises. In biological objects diffusion potentials may generate, for example, at damaging of cell membranes. On account of the damage, selectivity of their permeability increases and electrolytes begin diffusing into the cell and from it, depending on the concentration difference. Following electrolytes diffusion, the potential of damage arises. Resulting potential can reach 30-40 millivolts. A cell contains a complex of biological membranes for protective and metabolic purposes. The chemical composition of intracellular and intercellular liquids is unequal.

Practical lesson 13

Topic: Sorption of biologically active substances. Fundamentals of occupational therapy. Adsorption of electrolytes. Chromatographic methods of analysis of mixtures of biologically active substances.

Relevance of the topic: Surface phenomena are processes that occur at the boundary of two phases and depend on the features and structure of their surface. Biological objects are heterogeneous systems. Any living organism contains a large number of heterogeneous systems, at the interface of which the most important biochemical processes take place. Biochemical reactions occur on the interface at high rate due to low activation energy. Interactions of enzymes with substrates, antibodies with antigens are regarded as sorption processes.

Lesson objective: To form systematic knowledge about sorption processes; to find examples of practical application of sorption processes in professional activities and in life; to evaluate the surface properties of substances based on the structure of their molecules; to be able to explain the behavior of biologically active substances in terms of surface activity; to interpret the use of adsorbents for analytical and medical purposes.

Basic definitions: surface tension, diffusion, aggregate state, interface, adsorption, sorption, sorbent, adsorbate, Duclos-Traube rule, double electric layer, selective adsorption, Paneth-Fajans rule, chromatography.

Plan and organizational structure of the lesson:

1. Surface phenomena and their significance in biology and medicine.
2. Surface tension of liquids and solutions. Isotherm of surface tension.
3. Surface active and surface-inactive substances. Surface activity. Duclos-Traube rule.
4. Adsorption at the interface of liquid-gas and liquid-liquid. Gibbs equation.
5. Orientation of surfactant molecules in the surface layer.

6. Adsorption at the interface solid-gas. Langmuir equation.
7. Physical and chemical adsorption.
8. Physical and chemical basis of adsorption therapy (hemosorbtion, plasma adsorption, adsorption lymphocytes, enter sorption, application therapy). Immunosorbent.
9. Adsorption of electrolytes: specific (selective) and ion exchange. Paneth-Fajans rule.
10. The role of adsorption and ion exchange in the vital processes of plants and organisms.
11. Chromatography. Classification of chromatographic analysis based on aggregate state phases, technique and distribution mechanism.

Content of the topic

The state and properties of liquid molecules in the surface layer, i.e. the layer, which appears on the interface, essentially differ from the state and properties of the same liquid molecules in the volume. Inner molecules are attracted equally in all directions, that is why the resulting force amounts to zero. On the other hand, surface molecules are subjected to attraction forces by the molecules below them, but the opposing forces from the molecules above them are absent. This results in net force being directed perpendicular to the surface and inside the liquid. In order to increase the surface area of the liquid, molecules must move from its inside part to the surface against attraction molecular forces. Thus work is performed to increase the surface area. As a result there forms a surface layer with a *surplus of energy which is the surface Gibbs free energy* G_g.

The surface Gibbs energy is proportional to the interphase area:

$$G_s = \sigma \cdot S.$$

The coefficient of proportion a is called *surface tension and is defined as the surface Gibbs energy per unit of the surface area:*

$$\sigma = \frac{G_s}{S}.$$

Surface tension (σ) **is equal to the work, which is required to increase the surface area of a liquid per unit.**

Surface tension is measured in kJ/m^2 or in N/m. In accordance with the second law of thermodynamics any system tries to pass spontaneously to such a state, where the Gibbs energy is minimal. The surface Gibbs energy can decrease in two ways: *by decrease of the surface (S) or by decrease of surface tension* (σ). The *first way* is observed in pure liquids. As their surface tension does not change at a constant temperature this lessening of surface tension occurs by surface decrease: any liquid which flows out slowly tries to take a form of a sphere.

The *second way* of lessening of the Gibbs energy happens due to decrease of surface tension and is observed in solutions.

Surface tension changes at dissolution of this or that substance in water. Depending on the influence of different substances on surface tension all substances are divided in two groups. The first group includes substances that diminish the surface tension of water even at small solute concentrations. They are called *surface-active substances* (SAS). These are: carboxylic acids, alcohols, amines. Substances which increase surface tension are named *surface-inactive substances* (SIS). Some strong electrolytes (inorganic acids, bases, salts) and also polar organic compounds, such as glycerin, belong to this class of substances. Substances that don't influence the surface tension of water are also named surface-inactive substances. For example, addition of saccharose or some other carbohydrates to water does not change its surface tension.

The graphic relation of surface tension to solute concentration at a stationary temperature is called a surface tension isotherm.

The isotherms of surface tension for both cases are represented in *Fig. 1.*

Surface activity is the measure of the solutes ability to change the surface tension of a liquid; it quantitatively characterizes a change of surface tension at a change

of concentration. For surfactants the surface activity is more than 0, for surface-inactive substances it is less or equal. The surface activity of surfactants depends on their molecular mass.

According to the *Duclos-Traube rule,***in a homologous series of carboxylic acids, alcohols, amines at extension of the length of the hydrocarbon chain by one CH_2 group the surface activity of substances increases by about 3—3.5 times.**

The relation of surface tension to the concentration of lower homologs (C_7-C_g) of aliphatic carboxylic acids, alcohols and some other saturated compounds is rather accurately represented by the empirical *equation of* ***B.I.*** *Shishkovsky:*

$$\sigma_0 - \sigma = B \cdot \ln(1 + A \cdot c),$$

where σ_0 is the surface tension of a pure solvent; a is the surface tension of a solution; *B* is the constant for this homologous series; A is a value, which characterizes the change of surface activity in the homologous series according to the Duclos-Traube rule; *c* is the concentration of surface-active substances.

Fig. 2. illustrates the character of surface tension relation to concentration for a homologous series of aliphatic carboxylic acids.

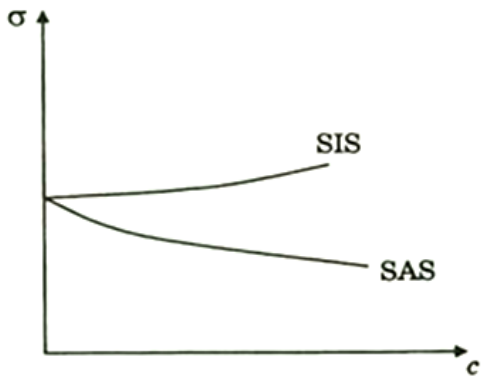


Fig.1. Relation of surface tension to the concentration for surface-active substances

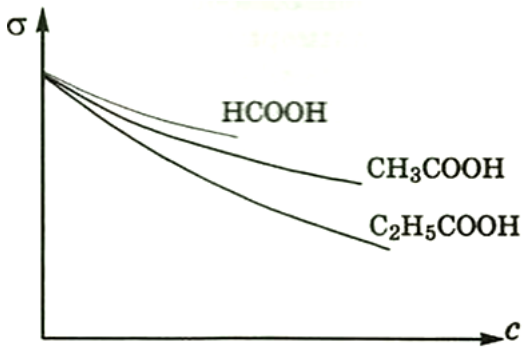


Fig. 2. Surface tension isotherms of aliphatic carboxylic acids

Adsorption at the liquid-gas and liquid-liquid interfaces

Comparison of the structure of surface-active agents with the structure of surface inactive molecules shows that all the surfactants are characterized by having a

hydrophobic part (hydrocarbon radical) and a hydrophilic part, that is a polar functional group (for example, —COOH, —OH, —NH_2), their ends are joined together. That is why molecules of surfactants after getting in water are pushed out to the surface layer, and oriented to water by the polar groups and towards air the nonpolar hydrocarbon radicals, as a result surface tension is diminished.

The majority of substances that enter into the composition of the human body are surface-active. For this reason such substances as fatty acids, steroids, which are contained in biological liquids, accumulate near walls of vessels, cellular membranes. It facilitates their penetration through these membranes.

The hydrocarbon radical is the hydrophobic part of natural surface-active substances. It may have a linear structure, the amount of carbon atoms in its structure, as a rule, is not less than twelve. On the other hand, it may be a polycyclic radical of steroid series, for example bile acids which emulsify fats because of their high surface activity and thus promote fat assimilation in the human body.

The hydrophilic part of natural surfactants contains a polar acid residue.

Depending on the dissociation ability of surface-active substances they may be electrolytes or nonelectrolytes. The former are called ionogenic, the latter - nonionogenic. Among ionogenic surfactants there are cation-active, anion-active and amphoteric. It must be noted that cationic and anionic surface-active substances are used in surgery as antiseptics. For example, the antimicrobial action of quaternary ammonium compounds is almost 300 times more effective than the action of phenol. The antiseptic action of surfactants is explained by their influence on the permeability of cellular membranes, and also inhibiting action on the fermentative systems of microorganisms.

Moreover, surface-active substances are actively used in pharmaceutical practice as the basis for preparation of suppositories and ointments, as stabilizers of emulsions, and as solubilizers. The preparation of solubilized medicines of fat-soluble vitamins, hormones and some other preparations is based on dissolution of organic substances in the hydrocarbon part of surfactants micelles.

As has been mentioned above, the diminishing of surface tension of liquids at addition of surfactants is conditioned by their accumulation in the surface layer.

The spontaneous process of solute accumulation at the interface is named adsorption.

Adsorption may be considered a surplus of a substance in the surface layer (as compared with the content of this substance in the volume) per unit of surface area. The value of adsorption is designated as JP (greek *gamma)* and is equal to $\Gamma = \frac{n}{S}$, where *n* is the amount of substance (mole), and *S* is the surface area (m^2). In 1878 Gibbs deduced an equation in theoretical (thermodynamic) way; the equation allows defining the value of adsorption in solutions on the basis of experimental data in relation to the change of surface tension at the change of solute concentration.

The Gibbs equation may be represented in such a way:

$$\Gamma = -\frac{C_p}{RT} \cdot \frac{\Delta\sigma}{\Delta c},$$

where *T* is the value of adsorption, mol/m^2; C_p is the equilibrium substance concentration, mol/1;

$-\frac{\Delta\sigma}{\Delta c}$ is the surface activity of a substance, J m/mol; *R* is the universal gas constant equal to 8.31 J/mol-K; *T* is absolute temperature, K.

As appears from the equation, the value of adsorption is more than zero for surface-active substances. For surface-inactive substances the value of adsorption is $\Gamma < 0$, which means adsorption is absent.

Orientation of molecules of surface-active substances in the surface layer

A molecule of surfactant may be represented as d), where the hydrophilic part and the hydrocarbon chain (hydrophobic part) of the molecule are marked. Orientation of such molecules in the surface of an aqueous solution will depend on their concentration. At small concentrations of a surfactant the polar groups are submerged in a polar liquid and the hydrocarbon radicals almost «lie» on the surface. With the increase of surfactant concentration the chain rises and, as a result, acquires a vertical orientation at a certain concentration of the surface-active substance.

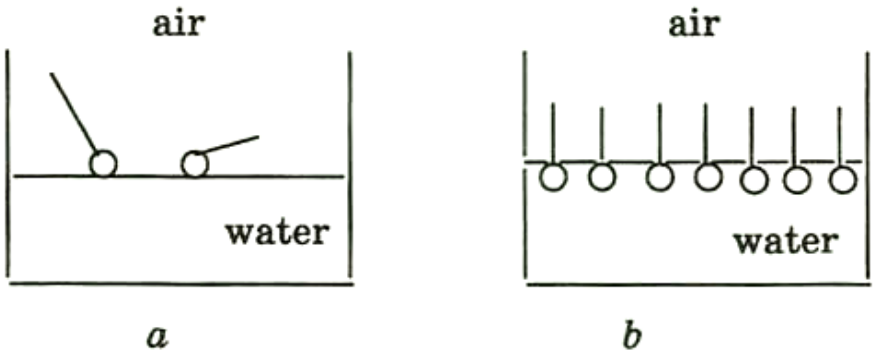


Fig. 3. Orientation of surfactant molecules in unsaturated (a) and saturated (b) surface layer

As appears from *Fig. 3*., the surfactant molecules may form a *monolayer on the surface*. Its thickness is equal to the length of one molecule. It is called *«Langmuir's palisade»*. Such filling of the surface corresponds *to maximum (limiting) adsorption* r_{max}. r_{max} represents the concentration of adsorbed substances on the surface, when one complete monolayer of coverage is achieved. Proceeding from F_{max} it is possible to calculate the length of a molecule *(I)* and its cross section area (S), in other words, the area which is occupied on the surface by one molecule of a surface-active substance.

If the amount of molecules adsorbed on a unit of the surface area at formation of a monolayer from SAS molecules is equal to $r_{max} \bullet N_A$, where N_A is the Avogadro constant, then *the area occupied by one molecule* may be calculated according to the formula:

$$S_0 = \frac{1}{\Gamma_{max} \cdot N_A}.$$

Experimental determination of the cross section area of fatty acid molecules is in full accordance with the theory, so molecules which have one polar group occupy an identical area on water surface, irrespective of the length of the hydrocarbon chain. Molecules that have two polar groups occupy an area two times larger.

The molecule length (monolayer thickness) is calculated by using the substance mass. The substance mass of the surface layer may be calculated according to this

expression: $m(x) = \Gamma_{max} \cdot M(x) \cdot S,$ where r_{max} is the maximum absorption, *M(x)* is the molar mass of a substance, S is the surface area.

On the other hand, the mass of a substance may be defined according to the formula: $m(x) = l \cdot S \cdot \rho,$ where *I* is the length of a molecule, S is the surface area, p is the density of a substance.

The *length of a molecule* may be found by equating the expressions for mass:

$$l = \frac{\Gamma_{max} \cdot M(x)}{\rho}.$$

Langmuir isotherm equation

The value of maximum adsorption r_{max} is found from the adsorption equation of Langmuir.

Proceeding from the results of the study of gas and vapor adsorption on the surface of solids, Langmuir supposed that adsorption occurs at definite localized ♦sites» on a surface. Secondly, it is assumed that each site can bind only one molecule of the adsorbing substance. When every side is occupied, ♦ Langmuir's palisade» is formed. The so-called *Langmuir isotherm equation* is:

$$\Gamma = \Gamma_{max} \cdot \frac{C}{C + K},$$

where *r* is adsorption, r_{max} represents the maximum absorption, C is the equilibrium concentration of a substance, *K* is the constant determined by experimental data. *K* is quantitatively equal to the concentration, when adsorption equalizes to half of maximum adsorption.

In accordance with the equation the dependence of adsorption on equilibrium concentration at a constant temperature has a shape that is shown in *Fig. 6.4.*

Analysis of Langmuir's equation shows that in the field of small concentrations (C << *K*, and so it is possible to neglect C in the denominator) it assumes the form:

$$\Gamma = \Gamma_{max} \cdot \frac{C}{K}.$$

That is why the dependence of adsorption on concentration has a linear character in this field. In the field of large concentrations (C >>*K*, and so it is possible to do neglect *K* in the denominator), Langmuir's equation of adsorption will look as:

$$\Gamma = \Gamma_{max}.$$

So, at large concentrations of a substance adsorption reaches the maximum value that corresponds to formation of a monolayer from molecules which are absorbed on the surface. Thus with the increase of surface concentration the value of adsorption increases not continuously, but asymptotically approaches to the maximum value. For determination of maximum adsorption it is necessary to divide *C* into both parts of Langmuir's equation:

$$\frac{C}{\Gamma} = \frac{K}{\Gamma_{max}} + \frac{1}{\Gamma_{max}} \cdot C.$$

It is the equation of straight line in the coordinates $\frac{C}{\Gamma} - C$ *(Fig. 5.).*

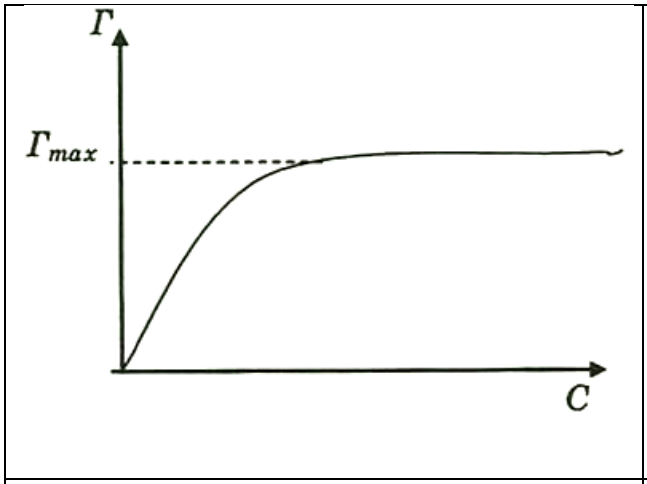


Fig.4. Langmuir isotherm equation

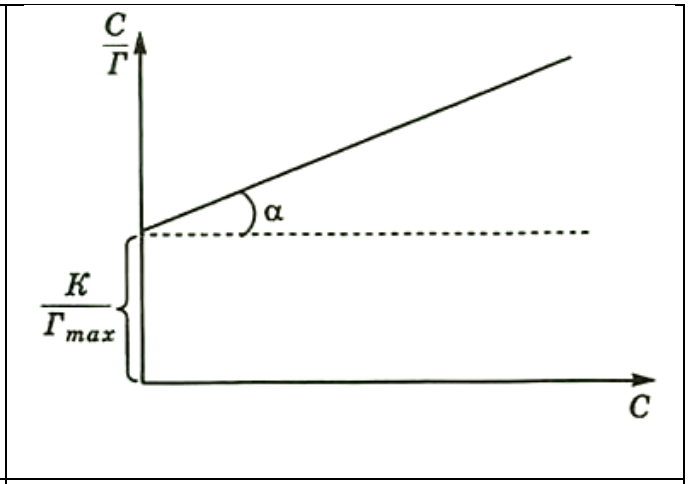


Fig.5. Graphical determination of the Langmuir adsorbtion equation constant

Using the angle of line inclination it is possible to calculate the maximum adsorption: $\mathrm{ctg}\alpha = \Gamma_{max}.$

The segment which is intercepted on the y-axis is equal to $\frac{K}{\Gamma_{max}}$. Thus it is possible to define the constant *K*. The constant *K* is quantitatively equal to equilibrium concentration when only half of adsorption centers of the surface are occupied by adsorbed molecules, i.e. adsorption is equal to half of maximum adsorption.

Adsorption of gases on a solid surface plays an important role in the processes of gas exchange of the organism with the environment. The principle of action of gas-masks, systems of life-support in submarine boats and spaceships is based on the adsorption of gases and vapors on a solid surface. As to gas-masks, the activated carbon, on whose surface adsorption occurs, performs a role of a catalyst in decomposition reactions of such poisonous substances as phosgene or chloropicrin.

Structure of biological membranes

Langmuir's representation of the structure of surface layers underlies the formation of *biological membrane models. A* modern point of view on the membranes structure is based on the orientation of lipid molecules which form a structural basis of a membrane. Lipids of phosphatidylcholine have a hydrophilic group, which is joined to hydrophobic hydrocarbon radicals. If such lipids get into water, spontaneously a two-layer film 4-5 nm thick is formed, in which hydrophilic groups are turned into an aqueous medium and hydrophobic carbon radicals are disposed in two rows creating a waterless lipid phase. Cellular membranes are represented by a double layer of lipids of the same type *(Fig. 6.).* They contain gly- colipids, cholesterol and phospholipids. The hydrophilic part of glycolipids is formed by oligosaccharides.

It should be noted that only double-stranded diphilic lipid molecules are able to form flat bimolecular layers. Single-stranded diphilic lipid molecules have a tendency to form globular structures in an aqueous medium.

A double lipid layer also contains protein molecules. Some of them pierce through the membrane from its external to internal surface, and other are adsorbed on both surfaces of the membrane. They are oriented in such a way that their

hydrophobic groups are submerged in the lipid layer, and their polar hydrophilic groups are submerged in the aqueous phase.

The majority of proteins that are located on the external surface of a membrane are glycoproteins which contain hydrophilic saccharide groups. These very groups are turned towards the extracellular medium.

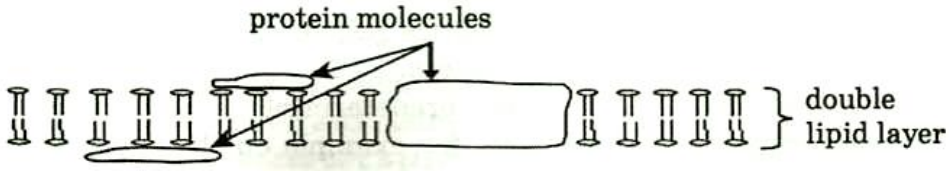


Fig. 6. Schematic representation of a membrane.

Solute adsorption at solid interface

Adsorption of solute molecules on the surface of a solid is rather a complex process, as at least three, but not two components are its participants.

It is important to distinguish the process of accumulation of one substance on the surface of another from *adsorption.* Attraction and retention of gas or vaporous molecules may not be limited only by their accumulation in the surface layer – they can diffuse into the bulk of a liquid or solid. This process is named *absorption.* Dissolution of a gas in a liquid with formation of a homogeneous solution may be an example of absorption. Absorption may be also observed when gases are merged by solids. For example, 1 cm^3 of spongy platinum absorbs 800 cm^3 of hydrogen. This ability of spongy platinum is used in technologies of hydrogen electrode manufacture.

It is difficult to define a clear boundary line between adsorption and absorption in practice, and that is why the names of both processes are united in one term that is *sorption.*

The substance, on whose surface adsorption occurs, is named an adsorbent.

The substance, which is adsorbed on the surface of an adsorbent, is called an adsorbate.

Together with the term «adsorbent» the term «sorbent» is rather widely used.

Solid sorbents are natural materials or materials of artificial origin that have a developed surface. Coal, silica gel, different natural silicates, alumogel, aluminosilicates, etc. belong to them. Common coal, which is produced from mineral coal or wood, has a comparatively small specific surface. A considerable increase of surface and, accordingly, of adsorbability is conditioned by its activation. Activation consists in heat treatment of coal in the presence, for example, of water vapor or carbon dioxide. As a result pores of different size in the structure of coal become free. Before activation these pores were to a large extent filled with resins. A consequence of the activation process is formation of activated carbon that has a specific surface as large as 1000 m^2/g.

Technical progress made it possible to produce activated carbon not only from natural but also from synthetic raw material.

The range of sorbents, which are now used in practice, and in medicine too, is wide enough. Besides the already mentioned activated carbon, these are also sorbents on the basis of amorphous silicates (silica gel, aerosil, aluminosilicates, hydrophobic kinds of silica), aluminum oxide, etc.

Physical heterogeneity of solid surface predetermines its energetic heterogeneity. On certain sections of the surface, for example on the edges of crystals, there is an uncompensated power field. On such centers, which have a surplus of the Gibbs energy, adsorption occurs more often. These sections of surface are named *adsorption centers.*

Depending on the nature of forces that cause adsorption of molecules on the surface, *physical and chemical adsorptions* are distinguished.

Physical adsorption is conditioned by van der Waals forces of physical nature.

A leading role is performed by electrostatic effects: at adsorption of polar substances on polar adsorbents the decisive role belongs to the orientation and induction effects, and at adsorption of nonpolar substances on nonpolar adsorbents, which is observed more often, the decisive role belongs to the dispersive effect. These forces

are weak enough. The heat of physical adsorption stays within the range from -4 to -40 kJ/mol.

Chemical adsorption (chemisorption) is conditioned by forces of chemical nature, moreover, surface compounds are formed between an adsorbent and an adsorbate at the interface.

Unlike physical adsorption, forces that ensure chemisorption are substantial enough: the heat of chemisorption is almost 10 times more than the heat of physical adsorption.

At formation of surface compounds, connections between surface atoms of an adsorbent and other atoms, which are included in its structure, are not destroyed; this is a characteristic feature of these compounds. It is impossible to clearly differentiate an adsorbent and a surface chemical compound, i.e. to select a surface compound as a separate phase. That is the principal *difference between chemisorption and chemical reaction.*

It should be emphasized that in real conditions only these two mechanisms may take place simultaneously or in a certain sequence. For example, at low temperatures adsorption of oxygen on activated carbon occurs by the *physical adsorption* mechanism. The mechanism of *chemisorption* is realized with temperature rising, and at further temperature increase a chemical reaction of producing carbon (IV) oxide takes place. Findings allow considering *chemisorption an intermediate stage of a heterogeneous chemical reaction.*

Laws of solute adsorption at solid interface

Analysis of the results of research on substances adsorption from solutions on the surface of a solid allows ascertaining that *adsorption is a selective process.* It means that *polar substances are better adsorbed on polar adsorbents, and nonpolar substances – on nonpolar adsorbents.*

This rule has a general character: it is also applied to adsorption of gases and vapors.

So, on the surface of a polar adsorbent, for example on silica gel $Si0_2$, water, alcohols, amines and other polar compounds are well adsorbed. On the other hand,

nonpolar molecules of solutes are better adsorbed on the surface of carbon adsorbents. Besides, adsorption of such substances on coal or soot increases with rising of their molecular mass.

As it has been already mentioned, solutes adsorption becomes more complicated in the presence of a solvent as a third component. It is logical to suppose that depending on the nature of the solute, it may compete with adsorbate molecules for a place on the surface adsorption center («site»). P. Rebinder has formulated the *rule of polarity leveling.* In accordance with this rule polar substances are better adsorbed on polar adsorbents from nonpolar or low-polar solvents, and nonpolar substances are better adsorbed on nonpolar adsorbents from polar solvents.

Shilov's rule is in complete accordance with these ideas:

The better a substance is dissolved in a solvent, the worse it is adsorbed on a solid surface. And on the contrary, the worse a substance is dissolved, the better it is adsorbed.

It has been established that displacement of some substances from the surface layer and replacement of them with other substances are observed during sorption from multicomponent solutions. That is why during adsorption of a substances mixture each component is absorbed in less quantities than from individual solutions with the same concentration. Freundlich formulated the *rule of displacement.* According to this rule, the ability to displace is conditioned by the surface activity of a substance:

The better a substance is adsorbed from a solution, the better it is absorbed from a mixture, thus it can displace other substances.

In the majority of fermentative processes the surface activity of decomposition products is less than the surface activity of initial substances. Considering that, it is possible to explain why exactly on the surface of an enzyme the decomposition products are substituted by new portions of substrate macromolecules.

Adsorption is a reversible process. Process reversibility is characteristic of physical adsorption. Chemical adsorption is an irreversible process. Reversibility means that adsorbed molecules do not remain fixed on the adsorbent surface, they

leave it in hundredth or thousandth fractions of a second, giving new molecules a possibility to occupy the vacant places. The process of moving away from the surface of adsorbed molecules is named *desorption.* Consequently, always two processes take place simultaneously on the adsorbent surface – these are adsorption and desorption.

Adsorption equilibrium is such a state of the adsorbent-adsorbate system, when the rate of adsorption is equal to the rate of desorption.

At adsorption equilibrium ELS many molecules are absorbed by the surface, ELS many molecules leave it (are desorbed) per unit of time. Thus the *kinetic condition* of adsorption equilibrium may be formulated. From the position of thermodynamics approach adsorption equilibrium may be characterized by the condition $\Delta G = 0$ (the *thermodynamics condition* of adsorption equilibrium). The concentration of adsorbed substance in a solution, which stays in dynamic equilibrium with the adsorbent, is called *equilibrium concentration.*

Adsorption depends on temperature. As physical adsorption is an exothermic process, heating promotes a reverse process, i.e. desorption. As to chemisorption, the influence of temperature is not so simple.

Most accurately the *dependence of substance adsorption on its concentration in a solution* is described by the empirical *Freundlich isotherm equation:*

$$\frac{x}{m} = k \cdot c^{\frac{1}{n}},$$

where x is the amount of the adsorbed substance; m is the mass of the adsorbent; c is the equilibrium concentration of the adsorbate; k and are empirical constants. $\frac{1}{n}$

Depending on the properties of an adsorbent, an adsorbate and temperature the constant k choses over a wide range. It is numerically equal to adsorption if equilibrium concentration is 1 mol/1. Values of the $\frac{1}{n}$ constrict:

$$0 < \frac{1}{n} < 1.$$

Graphical relation of adsorption to equilibrium concentration at a constant temperature looks like a parabola *(Fig. 8.).* The degree of the curvature is characterized by $\frac{1}{n}$.

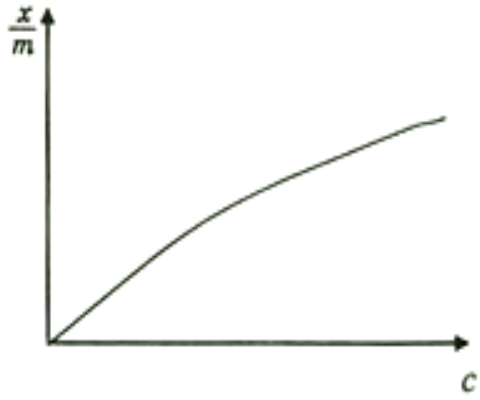


Fig.8. Freundlich isotherm of adsorption

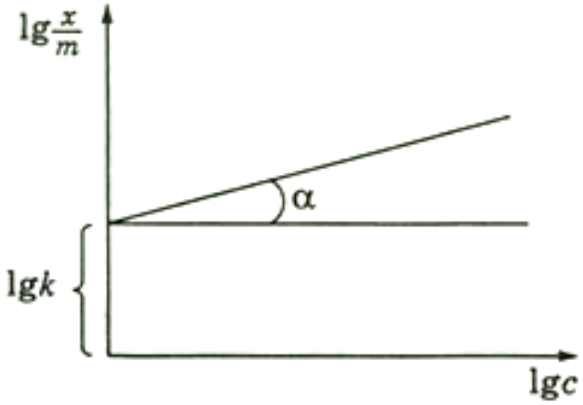


Fig.9. Freundlich adsorption isotherm in logarithmical coordinates

In order to estimate if the Freundlich equation fits for description of the adsorption process it is represented in logarithmical coordinates:

$$\lg\frac{x}{m}=\lg k+\frac{1}{n}\lg c.$$

When joined, the points corresponding to experimental data must form a straight line *(Fig. 9.)*

It should be noted that the Freundlich equation rather accurately describes adsorption from solutions in the range of average concentrations of a solute.

For certain systems adsorbent-adsorbate multilayer adsorption is possible.

The theory of multilayer adsorption was developed by S. Brunauer, P.H. Emmett and E. Teller. It gives five types of adsorption isotherms that have an S-shape. They are known as the BET isotherms according to the first letters of the authors' surnames.

Adsorption therapy fundamentals

The development of civilization, first of all the growth of industrial production, chemicalization of agriculture and conditions of life, intensive use of mineral fuels, have resulted in the appearance of numerous toxic for human health substances. Tens of thousands of foreign compounds get in the internal environment of man in our days. That is why in the last decades a new direction in medicine has been developed. It is called *efferent medicine* (from Latin *efferens* - bear off). Unlike the traditional methods of treatment based on the introduction of medicines into the organism, efferent medicine is based on the application of sorbents for removal of harmful and toxic substances from the human body's internal environment. Methods of efferent medicine cause purification of the organism not only from toxic substances which got there from the outside, i.e. toxins of exogenous origin, but also from toxins of endogenous origin (toxic substances which are formed and accumulated in the human body, for example, in case of burns, radiation sickness, nephritic or hepatic insufficiency, etc.).

Depending on specific aims sorption detoxication is carried out by means of hemosorption, plasmosorption, lymphosorption, enterosorption and application

Fig. 6.9. Schematic representation of the procedures of hemosorption (*a*), enterosorption (*b*), application sorption (*c*)

sorption *(Fig. 9.).*

Hemosorption, or hemoperfusion, is a method of immediate purification of the blood, which provides removal of toxins from the blood by passing it, within an extracorporeal circuit, over microcapsules containing adsorbent.

Hemosorption is an effective mode of treatment of serious cases with endogenous and exogenous intoxications of different origin. At present it is successfully used for treatment of patients with exogenous poisonings, hepatic and renal insuf-

ficiency, autoimmune and allergic diseases, surgical patients with severe endotoxicosis, patients with toxic forms of schizophrenia. It is necessary to note that hemosorption is used in medical emergency, dermatology (treatment of psoriasis and neurodermatosis), gynecology, pediatrics (in critical states), in treatment of cardiovascular and oncological diseases.

In opinion of clinicians, the application of hemosorption is the most reasonable at the first stages of poisoning, when the quantity of poison circulating in the blood is maximal.

Hemosorbents that are recommended for application in clinical practice must meet clear requirements. They mustn't substantially influence the rheological characteristics of the blood, must be characterized by high mechanical durability, chemical stability, homogeneous granule composition, high adsorbability in relation to one or another toxin. Besides, they must be biologically compatible with the blood and mustn't contain particles of sorbent in the form of dust.

Plasmosorption is an effective method of organism detoxication, whose escence consists in passing of the plasma that has been preliminary separated from blood corpuscles through a column with a sorbent, after that the cleared plasma joins with the blood corpuscles and returns into the bloodstream.

Plasmosorption is used for treatment of patients with severe forms of poisoning by organophosphorous insecticides, barbiturates, antidepressants, chlorinated hydrocarbons etc. It is applied instead of hemosorption in the cases, when it is impossible to use hemosorption because of such phenomena as anemia recess, strengthening of extravasation because of a blood corpuscles injury, damages of the blood coagulation system.

Lymphosorption is a type of organism sorptional detoxication that consist in passing of the lymph that has been removed from the organism through the thoracic lymphatic duct on the neck, through a column with a sorbent, and subsequent introduction of the lymph, deprived of toxic substances, into the vascular system of a patient.

Lymphosorption is the most effective method of organism detoxication when such toxic substances of exogenous origin as deadly amanita's alkaloids, tetrachlorated carbon, etc. get into the organism. Being relatively nontraumatic (absence of blood corpuscles injuries, damages of blood coagulation processes, changes in the cardiovascular system) is one of the advantages of this method. But extensive use of lymphosorption is limited by insufficient speed of the formation and outflow of the lymph.

The method of liquorosorption is less widespread because of its complicated procedure. Liquorosorption is a kind of organism detoxication when the spinal fluid is passed through a layer of sorption material, after which it returns purified into the spinal canal.

Application sorption is one of the techniques of sorption detoxication, which promotes healing of infected wounds and burns, renewal of the integrity of the skin and mucous membranes by adsorption of toxins from the wounds or burn area.

The use of application sorption therapy contributes to intensification of the regeneration process of tissues, as by means of applications the wound quickly becomes free of protein decomposition products. At the same time general intoxication of the organism diminishes.

The essence of application therapy technique consists in application of a gauze napkin with a granular sorbent or a bandage of carbon fiber material (charcoal dressing) on a wound or burn area. The application material is easily regenerated after use and so can be used repeatedly.

Original stock for preparation of porous fiber carbon materials may be synthetic or natural fibers which first undergo carbonization (in an inert atmosphere) at a temperature of 500-1000°C, then are exposed to high temperature activation in an atmosphere of gases-oxidizers or aqueous vapor. The specific surface of fiber carbon materials reaches 2500 m^2/g.

Special attention is given to the application of fiber carbon adsorbents with immobilized proteolytic enzymes on their surface that accelerate healing of wounds. It should be noted that fiber carbon materials as well as activated carbon can also be

used as bearers of different antiseptics, antibiotics and other antibacterial preparations. It guarantees constancy of the medicinal substance, concentration in the place of its contact with a wound surface.

Application sorption dates back to the times of Hippocrates. Since old times coal was used as therapeutic adsorption material. Ancient Egyptian papyruses which have been saved to our days are evidence of its application in medical practice. Having such deep historical roots coal adsorbents are experiencing new birth now. Synthetic materials are used as original stock for the preparation of high absorption activated carbon. The development of research in this branch has become the base of the creation of high absorption combined (with addition of natural components, for example pectins) adsorbents based on activated carbon, adsorbents with prolonged action, etc. The sphere of medical application of coal adsorbents is expanding constantly.

In spite of numerous convincing facts that indicate the benefit of these techniques of biological liquid sorption purification, we must mention certain lacks of these methods. First of all, all of them are impossible without efficient access to the bloodstream of the patient, their realization requires a comparatively sophisticated apparatus which is capable to provide the given rate of biological liquid movement. Moreover, a sorbent must be biologically compatible with a medium and, besides that, must be in the form which doesn't contain dust particles. The consequences of operative interference include damages of vessels, disturbance of membranes permeability, destruction of blood corpuscles (erythrocytes, thrombocytes and leukocytes), proteins, enzymes, etc.

Softer in this respect is the enterosorption technique (from Greek *entera* - intestine).

Enterosorption is a type of sorption detoxication of the organism, in which a sorbent gets into the oral cavity and by passing with different speed through parts of the digestive system it adsorbs toxic substances and products of metabolism.

This method is based on a conception that the diminishing of toxic substances quantity in one of the parts of the organism (in this case in the stomach and intestine) causes lowering of their concentration in the entire organism. Due to adsorption of toxins by sorbents in the intestine their content in the blood decreases, and the load on such detoxication organs of the organism as the liver, kidneys, etc. diminishes.

Enterosorption has established a good reputation for the removal of alkaloids, barbiturates, narcotic substances, different toxins of bacterial, vegetable, animal origin, etc. from the organism. Enterosorption is also used in antitumoral chemo-therapy, treatment of hepatic insufficiency, allergic diseases. Expedience of its use is confirmed in the treatment of advanced and elderly age diseases (a kind of "gerontosorption" therapy), and it results in slowing down the aging rate.

Elaboration of enterosorbents based on food fibers (phytosorbents), which can adsorb heavy metals, radionuclides, bile acids, etc. on their own surface is promising.

Summarizing the short comparative analysis of organism purification methods it is necessary to emohasize that one of the main criteria of choice and estimation of a sorbent, irrespective of a specific method of application, is the high *sorption capacity* in relation to a given substance. Such primary information is first got in laboratory conditions where adsorption isotherms are built and their character is analyzed (a more comfortable way of comparing sorption isotherms is their construction in logarithmical coordinates). It is necessary to note that not only the *chemical nature* of a sorbent surface but also the *accessibility of its pores* for substances that must be removed from the organism influence the adsorption capacity of the sorbent. It means that the size of molecules that are being removed from the organism must not be bigger than the diameter of sorbent pores. It is interesting to note that the features of a porous structure also help to create sorbents with a prolonged sorption action.

Unlike the described molecular adsorption, by whose mechanism adsorption of non-electrolyte and weak electrolyte molecules occurs, the *adsorption of strong electrolytes* has its own features. In the adsorption of electrolytes either cations or anions are adsorbed. The solution remains electrically neutral.

Depending on the character of the process two types of adsorption of strong electrolytes are distinguished: *selective* adsorption and *ion exchange* adsorption. A common feature for both types of adsorption is the fact that in both cases the formation of a *double electric layer* in the solid-liquid interface is present. It arises at the expense of prevalent adsorption of ions of the same charge sign on the solid surface or at the expense of electrolytic dissociation of certain (ion exchange) groups located on the surface. In both cases, independently of the mechanism, the solid surface acquires a certain charge. As a result ions of the opposite charge sign are attracted to it from the solution.

Selective adsorption

Selective adsorption is realized in accordance with *the Paneth-Fajans rule:*

Ions identical to those that build the lattice of a crystal or similar (isomorphic) to them are preferentially adsorbed on the crystal surface from a solution forming with the crystal ions slightly soluble compounds.

The isomorphism of ions means that they are similar in their size and structure. So, I" ions are isomorphic in relation to Br^-, Cl^-, CN^- ions, but they aren't, for example, similar to NO^- ions. Consequently, in accordance with the Paneth-Fajans rule, on the surface of an Agl crystal all enumerated anions may be adsorbed except for the last one *(Fig. 1.).*

The effectiveness of the Paneth-Fajans rule may be represented in the reaction of ion sorption on Agl precipitate from a solution:

$$AgNO_3^- + KI = AgI\downarrow + KNO_3.$$

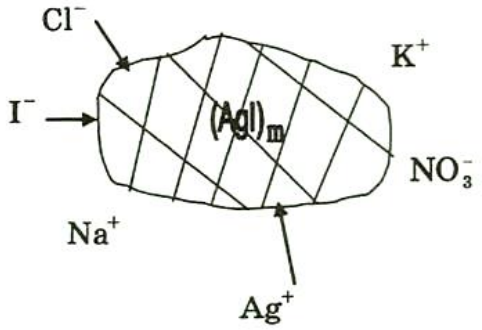


Fig. 1. Selective adsorbtion of ions on the surface of an AgI crustal

If initial substances are taken in equivalent amounts, then the surface of the derived precipitate is electroneutral. If the reaction takes place in conditions of $AgNO_3$ surplus, then the surface charges positively due to adsorption of Ag^+ ions on the crystal surface. On the other side, at surplus of KI, K^+ ions are adsorbed on the surface charging it negatively. Connection between adsorbed ions and a surface is very durable, that is confirmed by researches of ion sorption kinetics: the speed of sorption increases with rise in temperature. The results of similar researches allow formulating a conclusion that the forces causing adsorption of ions are not only electrostatic but also chemical by their nature.

Ions of the opposite sign of charge are collected near the charged surface of a crystal forming as a result a *double electric layer* (detailed description of the mechanism of its origin is adduced below in the chapter 7 «Physical chemistry of disperse systems»).

Ion exchange adsorption

Ion exchange adsorption is a process, in which ions from an adsorbent are replaced with equivalent amounts of ions of the same sign of charge from a solution.

Materials capable of exchanging ions with a solution are known as *ion exchangers.* A characteristic feature of all ion exchangers is the presence of *ionogenic groups* on the surface (they are named *functional*), they are capable of being dissociated. As a result a double electric layer is created. Its external facing is held near the interface by electrostatic forces. Ions of the external layer, unlike ions of the internal one, are relatively mobile and so may be exchanged for the ions of the same sign which are in the solution. Natural and synthetic ion exchangers are distinguished. For example, clay and different minerals are natural ion exchangers. Among synthetic (*ionites)* a leading place is occupied by *ion exchange resins.*

Ion exchange resins are insoluble high molecular synthetic compounds with a three-dimensional coherent structure that is formed by a lattice of carbon atoms on which ionogenic groups are irregularly located. These groups are covalently bounded to the organic polymeric matrix. There are cationites and anionites.

Cationites are ion exchangers capable of exchanging cations with a solution.

The groups —SO_3 and —COOH are frequently functional groups of cationites. A conditional high molecular polyvalent anion (matrix of the ion exchanger) may be designated as *R.* If there are mobile H^+ ions near a surface formed as a result of dissociation of the mentioned functional groups, such cationite may be schematically designated as RH^+ (cationite in the H^+-form).

Anionites are ionites able to exchange anions with a solution.

The groups —NH_2, =NH, =N may be the functional groups of anionites. Due to interaction with water the group —NH_2, for example, transforms into — NH_3^+ OH^-» as a result the ionite has the ability to replace the OH^- ion with ions of a solution. Such ionite may be designated as ROH~ (anionite in the OH~-form).

Depending on the ionization ability of functional groups there are distinguished strong-acid and weak-acid cationites, accordingly, strong-basic and weak-basic anionites. As functional groups of strong-acid cationites maybe residues of sulfate and phosphate acids; as to weak-acid cationites, they may be carboxylic, sulfhydric and other groups. As regards anionites, strong-basic are those of them, which contain groups of ammonium and sulfonic bases. Weak-basic anionites are anionites, whose functional groups are amino-groups of different substitution degree, pyridic bases.

The quantitative characteristic of the ability of ionites to exchange is the *ion exchange capacity.* It is expressed in millimoles of ions that are taken from a solution by one gram of dry ion exchanger at equilibrium conditions.

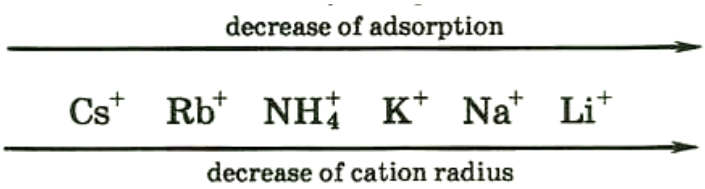
An ion exchange capacity depends on the nature of an ion exchanger, on the properties and concentration of ions, which are absorbed by the ion exchanger, on the pH of medium.

The ability of ions to be adsorbed on a surface depends on the value of their charge. Multivalent ions are adsorbed better than univalent. The comparison of the adsorbability of same charge ions indicates a dependence of sorption on the size of ions and their hydratability (solvation). Ions of greater radius are better polarized and that is why they are stronger attracted to the surface of an adsorbent. On the other hand, with growth of the ions radius their hydratability is being reduced, which

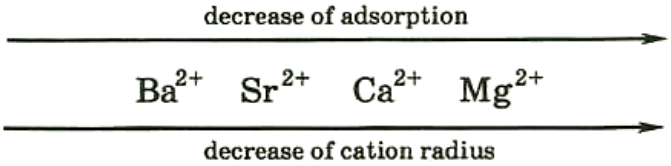
eventually increases adsorption of ions on a surface, as a large hydrate sheath impedes ion adsorption.

According to the ability of being adsorbed on a surface, ions may be arranged in the so-called *lyotropic series (Hofmeister series).*

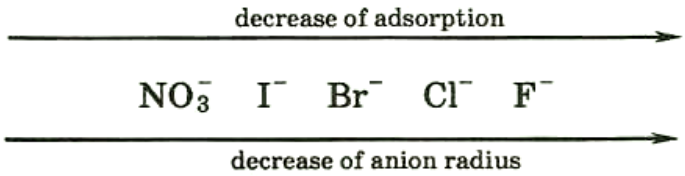
Singly charged cations create such lyotropic series:



The lyotropic series of doubly charged ions looks like:



As regards anions, their lyotropic series is the following:



Ionic exchange plays a great role in vital functions of the organism. Ion exchange properties are inherent to the structural elements of cells, namely to the nuclei, mitochondria, membranes, microsomes, sarcolemmas.

Ion exchangers are used in the process of baby food preparation, i.e. ionite milk. They are also applied in the blood preserving process. Ion exchangers are used in medical practice for prevention and treatment of edemas caused by decompensation of cardiac activity, for prevention of acidosis, for determination of gastric juice acidity without a stomach probe. The expedience of their use with the purpose of detoxication in poisonings with toxic electrolytes is proved by practice. Ion exchangers are also applied as antiacid preparations (preparations that reduce gastric acidity).

Special attention is deserved by application of ion exchangers with the purpose of demineralization of water. So, after an introduction of aqueous solution of NaCl into a column filled with an H^+-form of cation exchanger, the equilibrium will be established:

$$RH^+ + Na^+ + Cl^- \rightarrow RNa^+ + H^+ + Cl^-.$$

Continuing by introduction of the solution that flows out of this column into another column with an OH"-form of anion exchanger, a new equilibrium will be achieved:

$$ROH^- + H^+ + Cl^- \rightarrow RCl^- + \underbrace{H^+ + OH^-}_{H_2O}$$

The action of each ion exchanger is schematically described in *Fig. 2.*

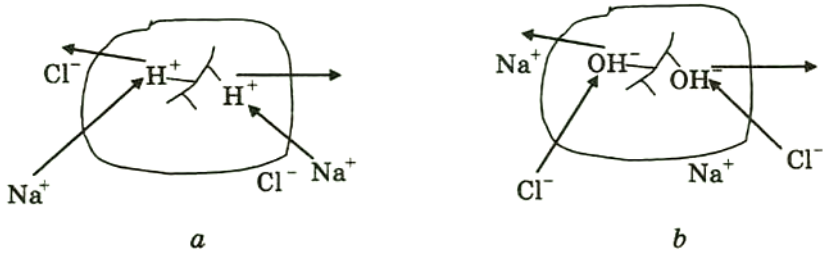


Fig. 2. Ion exchange on the granules of a cationite in the H+ form (a) and an anionite in OH- form (b)

(H+ and OH-are ions that are exchanged for N_A^+ and Cl^- ions respectively)

Consequently, in such way it is possible to prepare pure water (without salts). Water, cleaned by the method of ion exchange, is much cleaner than that produced by distillation.

The used ion exchangers may be regenerated by NaOH and HC1: an anionite is treated by an alkali solution and a cationite - by an acid solution. Ions which were absorbed by the ion exchanger easily pass to the solution. So, the same ion exchanger may be reused.

Chromatographic methods of analysis

Chromatography is a physicochemical method of analysis and separation of components of a mixture, based on their different distribution between two phases,

one of which is immobile (solid or liquid) and the other is mobile (gas or liquid), filtered through the immobile phase.

The creadit of discovering chromatography is given to Russian botanist M. Tswet (1903). By making an alcoholic extract of chlorophyll pass through a column filled with calcium carbonate, he got eight stripes of different colors corresponding to that or another pigment. Taking into consideration the coloration of zones, M. Tswet proposed to name the obtained picture of pigments distribution a chromatogram (from greek *chromos* - color). That is why the method that was suggested by Tswet was called *chromatography*. It should be noted that the author of the method also prognosticated a possibility of separation of colorless components mixtures.

Thanks to technical progress chromatography has became a unique method of analysis *(analytical chromatography)* and separation *(preparative chromatography)* of any mixtures of substances, the most important one being of substances, which are very similar in their structure and properties.

A substantial advantage of the method is that analysis and separation of mixtures of components take place, as a rule, in mild conditions: at comparatively low temperatures and in the absence of aggressive media.

Principles of chromatographic methods classification

According to the *aggregate state of phases*, mobile and immobile (it is also called the stationary phase), *gas* and *liquid* types of chromatography are distinguished.

The mobile phase in gas chromatography is a *gas carrier* (hydrogen, helium, argon, nitrogen), to which a gaseous or vaporous mixture of analyzed substances is added. The immobile phase may be a *non-volatile liquid* (glycerin, polyethylene glycol), which is spread on a solid inert adsorbent (solid carrier), for example, glass, teflon, that fills a column. A mixture of gases is moved into a column under certain pressure. Mixture components are variously distributed between both phases by moving through the column.

The presence of components in the current gas carrier that goes out from the column is registered by a detector. Signals of the detector are written down as a chromatogram by an automatic potentiometer. A separate peak corresponds to each of the mixture components on the chromatogram. Modern detectors allow recording even 10^{12} g of a substance. The described variety of gas chromatography is called *gas-liquid chromatography*, and the instrument that analyzes a mixture is named a gas chromatograph.

If a *solid adsorbent* (silica gel, aluminium oxide), not a liquid, is used as an immobile phase instead of a liquid, then such variant of chromatography is named *gas adsorption* chromatography.

Gas chromatography is applied for separation of mixtures of volatile thermally steady substances with a molecular mass below 300.

As to *liquid* chromatography, a *liquid* is used as a mobile phase in this case, and the immobile phase is a *liquid* that is bounded to an inert solid support or a *solid.* Among liquid chromatography varieties of special attention, according to medical and biological practice, is the *method of high-performance liquid chromatography* (HPLC). In this method a mobile phase is a specially selected mixture of solvents which is introduced at a high pressure (about 200 atm.) into a chromatographic column.

Various chromatographic methods are also classified according to the *technique of mixture components separation.* There may be *column*, *capillary* or *planar* (*thin-layer* and *paper)* chromatography.

In *column* chromatography a mobile phase is passed through an immobile phase that fills the column.

Capillary chromatography is based on the use of capillaries, in which a liquid immobile phase is applied to their inner walls and a mobile phase passes through it.

In *paper* chromatography separation of a components mixture, which is in the liquid phase, takes place on a special prepared paper. In this technique a more polar component of the solvents mixture becomes bonded to paper cellulose and thereby

forms a stationary (immobile) phase. The less polar solvent forms the mobile phase by migrating on the paper.

Thin-layer chromatography is based on the use of a thin layer of solid material (immobile phase) that is spreaded on a chromatographic plate made of an inert material (glass, aluminium foil).

Depending on the dominant *mechanism of the process of mixture components separation*, the chromatographic methods may be classified as: adsorption, partition, ion exchange, gel permeation, affinity, precipitation and thermochromatography.

The essence of *adsorption chromatography* consists in separation of substances of mixtures based on their different adsorbability on that or another adsorbent (immobile phase). During analysis, as a consequence of numerous continuous processes of adsorption-desorption, components that have a great adsorbability would move slower together with a mobile phase, than those that are badly adsorbed. As a result there is observed the motion of areas, each of them mainly containing a pure substance, along the adsorbent layer. The analysis of components that go out in a certain sequence from an adsorbent is realized by detectors or by fraction sampling of the solution. The selected fractions are analyzed by corresponding methods (spectrophotometry, refractometry, etc.).

Ion exchange chromatography is based on a different ability of ions that are in the analyzed mixture to exchange with ions that enter into the composition of an ion exchanger (immobile phase). Zeolites, resins, aluminium hydroxides, ferrum hydroxides, etc. perform a function of the immobile phase. As well as in adsorption chromatography isolation of ions from a solution occurs.

The property of substances to be distributed between the immobile (liquid) and mobile (gas or liquid) phases in accordance with their *partition coefficient* is assumed as a basis of *partition chromatography*, for its discovery American scientists A. Martin and R. Sting were awarded the Nobel Prize.

An immobile phase may be fixed on a chromatographic paper (paper chromatography), on a thin layer of adsorbent (TLC), or may be dispersed in a volume of a solid carrier (column chromatography).

According to the Nernst distribution law, at a constant temperature the ratio of substances concentration distributed between two immiscible liquids is a constant value, and it serves as a theoretical basis of partition chromatography.

In partition chromatography the partition coefficient is designated as R_f. In the case of paper chromatography for determination of R_f at first one measures distance from the start line (place of substance application on the paper) to the center of the spot, which corresponds to this substance on the chromatogram. The distance (covered by a solvent) from the start line to the finish line is also measured. The ratio of the distance covered with a substance to the distance covered with a solvent is equal to the partition coefficient R_f of this substance between the

mobile and immobile phases. Consequently, R_f serves as a qualitative characteristic of a substance. The comparison of partition coefficients that were obtained by means of an experiment with reference data enables to identify substances that enter into a composition of a mixture.

Separation of mixtures into their components by *gel permeation* chromatography, which is also called *size exclusion* or *molecular sieve* chromatography, is based on the different ability of substances to penetrate into pores of a gel that plays a role of the immobile phase. In this case gel works as a molecular sieve by letting pass (retaining) only those molecules that due to their size and shape are able to diffuse into its pores. Molecules of small size are able to penetrate deeper into gel and stay there longer than large molecules.

Affinity (biospecific) chromatography is based on the abilities of some compounds to «recognize» in a solution only their «own» substances and to interact with them. So if a substrate is an immobile phase, than it «recognizes» its enzyme. In the other case, if an enzyme is an immobile phase, than it would seek «its» substrate in the mixture of components. Similarly, an antigen «recognizes» an antibody, and a hormone does it with «its» receptor.

Precipitation chromatography is based on a difference in the solubility of precipitates which are obtained as a result of chemical interaction of mixture compo-

nents with a substance of the immobile phase. For this method a mixture is passed through a carrier that has been impregnated with a precipitator.

In *thermochromatography* the division of a components mixture occurs due to a change of their adsorbability at temperature variation.

Application of chromatography in biology and medicine

The chromatographic methods of analysis are widely used in medical-biological researches and in clinical practice.

It should be noted that chromatography is applied with a diagnostic aim. Thanks to this method it is possible to reveal various microcomponents in biological fluids (even such ones which cannot be detected by other methods), that appear in this or that pathology.

A chromatogram of human body metabolites gives a possibility to establish the type of microorganisms that have produced a disease. By the instrumentality of, for example, gas chromatography it is feasible to realize an accelerated identification of microorganisms by the spectrum of specific components of their membranes and specific products of pyrolysis. It is possible to control the process of antibiotics action by analyzing the fatty acids content in the purulent discharge from the lungs of a patient that is affected with an anaerobic infection, because the action of antibiotics eliminates all carbonic acids of an inflammation zone, except for acetic acid that is a natural metabolite.

Chromatography has a great role in the diagnosis of congenital and acquired metabolism dysfunctions, such as diabetes mellitus, podagra and other diseases.

The advantages of the thin-layer chromatography method, which is quite simple technically and a very sensitive express analysis of substances contained in biological fluids, allow not only to give a quick diagnosis in severe chemical poisoning, but also to control the process of organism detoxification.

The application of chromatography enables to define almost instantaneously the blood content of alcohol, drugs, volatile substances that cause toxicomania. This method is also used for dope control (detection of stimulant substances in the organism of a sportsman).

Chromatographic researches of the lipid content in the blood have promoted the determination of the causes of origin, ways of prophylaxis and treatment of atherosclerosis, which is a disease that leads to the development of ischemic heart disease, disturbance of blood circulation in the brain. The results of determination of the fatty acid composition of lipids favor the cognition of the structure and functions of biological membranes, of intracellular metabolism processes. The chromatographic analysis of carboxylic acids of the Krebs cycle that is responsible for providing cells with energy, helped to deeper understand intracellular metabolism processes in different pathological states.

Practical lesson 14

Topic: **Colloidal solutions. Molecular-kinetic, optical and electrokinetic properties**.

Relevance of the topic: Blood, biological membranes, fibers, genes, viruses are colloidal formations. Colloid-chemical processes underlie nutrition, growth and development of plant and animal organisms. The study of the properties of colloidal systems and methods of their production allows us to understand the complex processes of life activity of organisms. The study of methods of purification of colloidal solutions contributed to the introduction into medical practice such methods of diagnosis and treatment as electrophoresis, compensatory dialysis, vivialysis.

Lesson objective: To form a systematic knowledge of the basic laws of colloidal chemistry, their close relationship to the vital activity of biological systems; consideration of the colloid-chemical aspects of transformations of the molecule-cell-organism; disclosure of the content of the basic laws, understanding their principal possibilities in solving specific problems.

Basic definitions: disperse systems, dialysis, electrodialysis, ultrafiltration, brownian motion, diffusion, osmotic pressure, electropherogram.

Plan and organizational structure of the lesson:

1. The organism as a complex set of dispersed systems.
2. Classification of disperse systems by the degree of dispersion.
3. Lyophilic and lyophobic colloidal systems.
4. The structure of colloidal particles. Electric double layer.
5. Methods of obtaining and purification of colloidal solutions.
6. Dialysis, electrodialysis, ultrafiltration, dialysis compensation, vivi dialysis.
7. Molecular-kinetic properties of colloidal systems.
8. Brownian motion, diffusion, osmotic pressure.
9. Optical properties of colloidal systems.
10. Application of electrophoresis in research and clinical laboratory practice. Electropherogram.

Content of the topic

Classification of disperse systems

Disperse systems are classified according to a number of characteristics.

Classification according to the degree of dispersion is given in *Table.1*

It should be noted that transition from coarsely dispersed to molecular dispersed systems is continuous. However, the latter are not studied by colloid chemistry, because they are homogeneous, i.e. the concepts *disperse phase* and *disperse medium* are not applied to them.

Among other classifications of disperse systems the most widespread is this one: coarsely dispersed systems (the value of particles of the disperse phase makes more than 10^{-5} m); microheterogeneous (10^{-7} – 10^{-5} m); ultramicroheterogeneous (10^{-9} –10^{-7} m).

Table 1

Class	Range of particle size, d	Characteristics of system
Coarse dispersion	d> 0.5 μm (>0,5.10^{-6} m)	Don't diffuse, visible under microscope (Suspension & emulsion)
Colloidal dispersion	1.0 nm <d>0.5 μm (0,5.10^{-6}÷10^{-9} m)	Diffuse slowly, visible in electron microscope, ultramicroscope (colloidal Ag sol)
Molecular dispersion (true solutions)	d<1.0 nm (1.10^{-9} m)	Rapid diffusion, invisible in electron microscope (Ordinary ions)

The particles of the disperse phase of coarsely dispersed systems settle in the gravitational field, do not pass through a paper filter, they are visible under an ordinary microscope. The particles of fine-grained systems pass through ordinary. In this classification some systems have special names. So, fine-grained systems are named sols. Depending on the nature and aggregate state of the disperse medium, hydrosols (the disperse medium is water), organosols (organic liquid) and aerosols

(gas) are distinguished. Coarsely dispersed systems of the S/L type are called suspensions, and systems of the L/L type are emulsions. Liquid crystals are referred to disperse systems of the L/L type, and are used as a basis for researching structures of many biological systems.

Table 2.

Classification of disperse system according to the nature and aggregate state of the disperse medium

DP	DM	Designation	Example
Solid	Gaseous	S/G	Aerosols (dust, smoke, smog)
	Liquid	S/L	Sols (sols of metals in water, suspended particulate matter in natural waters - WA), suspension - DG
	Solid	S/S	Solid colloidal solutions (concrete, alloys, tinted glass, minerals - gems)
Liquid	Gaseous	L/G	Aerosols (fog, clouds)
	Liquid	L/L	Emulsion (milk, crude oil, cream)
	Solid	L/S	Fluid in porous solids (adsorbents, soil)
Gaseous	Gaseous	G/G	System with the density fluctuations (the atmosphere)
	Liquid	G/L	Gas emulsion, foam
	Solid	G/S	Porous and capillary body (adsorbents, catalysts, pumice, activated carbon)

Accordance with this classification, the colloidal state of matter is intermediate. For example, urine depending on the state of the organism (norm or pathology) changes through the colloided state from a true solution to a suspension.

Classification according to interphase interaction.

Between a disperse phase and a disperse medium there is an interaction; however the degree of manifestation significantly differs in different systems. P. Rehbinder offered to divide all disperse systems depending on the degree of interphase interaction into *lyophilic (lyo* - dissolve, *philo* - love) and *lyophobic*

(phobos - fear). If a disperse medium is water, then the systems are named hydrophilic and hydrophobic respectively.

In lyophilic systems, interaction of particles of the disperse phase with the disperse medium is rather strong, that means solvation (hydration) of particles occurs. They appear by spontaneous dispersion, are thermodynamically steady ($\Delta G < 0$). In lyophobic systems interaction is slight. They do not appear as a result of spontaneous dispersion, are thermodynamically unstable ($\Delta G > 0$).

Among lyophilic systems there are solutions of high molecular compounds: albumens, nucleic acids, polysaccharides, polyesters, etc.

Among lyophobic disperse systems there are sols of noble metals, sols of a variety of inorganic compounds, emulsions, suspensions.

Methods of colloid system preparation

Colloid systems may be prepared both by grinding of coarsely dispersed particles and by association (coalescence) of molecules, atoms, ions into colloid particles, because they occupy an intermediate position between coarsely dispersed and molecular dispersed systems according to the sizes of disperse phase particles. As a result, the *dispergation* and *condensation* methods of colloid system preparation are distinguished.

For preparation of steady colloid solutions (sols) the following conditions are required:

a) presence of two mutually insoluble or slightly soluble components for the formation of a disperse phase and a disperse medium;

b) achievement of the colloidal degree of dispersion ($10^7 – 10^9$ m^{-1});

c) presence of a stabilizer conditioning certain stability of the system.

Methods of dispergation

Dispergation methods consist in commination of large particles into smaller ones. Under laboratory and industrial conditions these processes are carried out in crushers, millstones and grinding mills of different constructions. For preparation of emulsions and suspensions ultrasound is widely applied.

To dispergation methods it is possible to refer a method based on the formation of metal vapor in an electric arc with its subsequent condensation in a chilled solvent. This method combines signs of the dispergation and condensation methods. It is used in medicine for drug preparation.

Method of peptization

Peptization is a process of converting a fresh precipitale into a colloid sol under the influence of an external factor.

First this method was applied in biochemistry for disintegration of complex albumens into simpler and very soluble ones (peptones) with the enzyme pepsin, that's why the method got its name.

Peptization can be caused by washing a fresh precipitate with a pure solution, changing pH of the environment, adsorption of surface-active substances, action of electrolytes, etc. Substances, under whose influence peptization occurs, are named *peptizers.*

Methods of condensation

Condensation methods consist in formation of large particles from smaller ones.

1. *Method of physical condensation by solvent substitution.* For example, if alcoholic solution of rosin is poured into water, a sol of rosin is formed in water.
2. *Methods of chemical condensation.* There are applied any chemical reactions for preparatin of colloid systems, as a result of which slightly diluted substances are formed. The stabilizer is usually one of the reacting substances, which is taken in surplus.

For generation of a sol, but not a precipitate, from a saturated solution, simultaneous formation of an enormous amount of disperse phase nuclei is needed. Thus the speed of nucleus formation must be larger than the speed of crystal growth. It is obtained by inflowing of a concentrated solution of one component into a much more diluted solution of another one at strong interfusion.

Examples of reactions used for receiving colloid solutions:

- reactions of hydrolysis: $FeCl_3 + 3H_2O \rightarrow Fe(OH)_3 + 3HC1$;

- redox reactions: $2H_2S + O_2 \rightarrow 2S + 2H_20$;

$2KAuO_2 + 3HCHO + K_2CO_3 \rightarrow 2Au + 3HCOOK + KHCO_3 + H_2O$;

- double-exchange reactions:

$BaCl_2 + K_2SO_4 \rightarrow BaSO_4 + 2KC1$;

$AgNO_3 + KC1 \rightarrow AgCl + KNO_3$.

All considered methods are used for *lyophobic sols* preparation. Lyophilic systems, in particular solutions of HMC, are spontaneously formed at dissolution in a certain solvent.

Methods of colloid system purification

The disperse systems prepared by this or another method, and also the disperse systems of natural origin - latexes, vaccines, serums etc. are cleared of admixtures (molecules, ions). Purified disperse systems become steady and can stay in metastable state for certain time. Different methods are used for purification of disperse systems: dialysis, vividialysis, electrodialysis, ultrafiltration.

T. Graham was the first to apply *dialysis* for purification of colloid solutions. A dialyzer *(Fig. 1)* consists of two vessels separated with a semipermeable membrane which is pervious to low-molecular ions and molecules and impervious to disperse particles. As membranes one may use parchment, cellophane, collodion, etc. The internal vessel is filled with a sol, and a clean solvent circulates through the external vessel. All components that can pass through membrane pores are withdrawn from the sol into a running solvent. As a rule, purification by dialysis lasts few days. Rise in temperature facilitates acceleration of the process as result of diffusion speed increase.

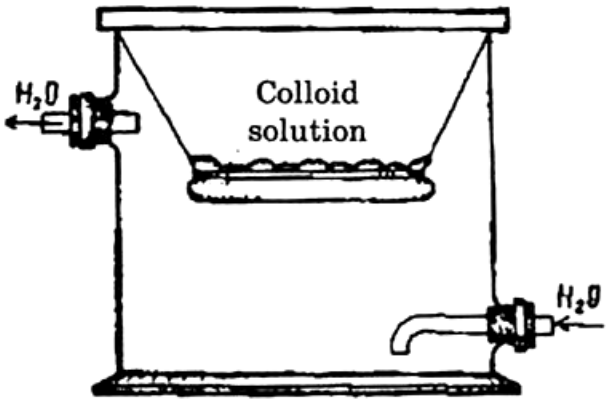


Fig. 1. Graham's dialyzer

If admixtures are only electrolytes, they are eliminated by the method of *electrodialysis* which is dialysis by the action of electric current.

Extraction of low-molecular substances from biological liquids is often carried out by the method of *compensation dialysis.* The essence of this method consists in washing the liquid in a dialyzer not by a clean solvent, but by solutions of low-molecular substances with a concentration that must be preserved in the colloid solution.

Vividialysis is a method of low-molecular substances extraction from physiological liquids with the purpose of their purification or analysis, at which a biological liquid passes through a dialyzer and then returns into a living organism.

The «artificial kidney» device works by the vividialysis principle, it is used in acute renal insufficiency, for example, in poisoning with corrosive sublimate, sulfanamide preparations, in uremia after blood transfusion, severe burns, toxicosis in pregnancy, etc. The device is connected to the blood circulation system of a patient. The blood under the pressure caused by a pulsating pump is pushed into capillaries made of a semipermeable material and washed externally by a solution with the same electrolytic composition as the blood. Purification of the blood (hemodialysis) from metabolites (urea, uric acid, surplus of potassium chloride ions, etc.) is carried out during 3-4 hours. Disposable hemodialyzers made of polymeric materials such as: polyethylene, polyvinylchloride, polystyrene, polysiloxane, etc. have been widely used recently.

Ultrafiltration is filtration of colloid solution through a membrane at increased external pressure or under vacuum. In biochemical practice cellulose acetate, nitrocellulose, fiberglass are applied as membrane filters.

Molecular-kinetic properties of disperse systems

Molecular-kinetic properties are properties conditioned by chaotic thermal motion of particles.

Molecular-kinetic properties of disperse systems are the function of the dispersion degree. The differences between molecular-kinetic properties of true solutions and disperse systems have only quantitative character.

Brownian motion. The chaotic thermal motion of disperse particles was named *Brownian* in honor of English botanist R. Brown.

Colloid particles are hit a countless number of times by solvent molecules being in a constant thermal motion. If a particle is small, then the number of hits from different sides is usually different, and it gets impulses that move it in various directions.

Diffusion is a process of spontaneous balancing of the concentration of ispersed particles as a result of chaotic thermal motion of system particles. The speed of diffusion is always increased with rise in temperature. Colloid particles are both in values and mass considerably larger than molecules and ions, therefore the speed of their thermal movement and, accordingly, the speed of diffusion are much lower.

Osmotic pressure. The osmotic pressure of colloid solutions as well as of true solutions is calculated according to the van't Hoff equation. However, for colloid solutions instead of molar it is accepted to write down particle concentration (the number of particles in a unit of volume: v = c(*x*) N_A):

$$\pi_{osm.} = \frac{\nu}{N_A} RT = \nu kT,$$

where v is particle concentration; N_A is the Avogadro number; *R* is the gas constant; *T* is temperature.

The particle concentration is insignificant as compared with the concentration of molecules in true solutions because of particles largeness. Hence the low values of osmotic pressure, for example, the osmotic pressure of silver sol with co(Ag) = 1 % is equal to 0.045 kPa, while for a saccharose solution with a>($C_{12}H_{22}O_{11}$) = 1 % it is 72.5 kPa.

Besides that, the magnitude of osmotic pressure changes with time because of colloid system instability.

Sedimentation equilibrium

The process of settling of disperse phase particles under the action of forces of different nature (gravitational, centrifugal, electrical) is called sedimentation.

Coarsely dispersed particles with a large radius settle with a greater velocity. Colloid dispersed particles under the action of gravitational force settle very slowly. Determination of particle settling velocity serves as a basis of sedimentation analysis that is used in medicine for quantitative characteristic of the functional state of red corpuscles. Erythrocyte sedimentation rate (ESR) considerably changes in different diseases and allows a doctor judging about the state of the patient's organism.

Optical properties of colloid systems

Optical properties of disperse systems are conditioned by their heterogeneity.

At passing of a light wave through a disperse system, light can be refracted, absorbed, reflected or dispersed by disperse phase particles.

The advantage of some of these phenomena depends, mainly, on the correlation of the light wave length and the values of disperse phase particles.

Light scattering. To some extent, dispersion of light is observed in any system. More intensively light disperses in colloid disperse systems, where the value of disperse phase particles makes 10^{-9}-10^{-7} m. In the case, when the linear values of particles are less than the wavelength of incident light, *diffraction* is observed - light waves round the particles and change the initial direction. Diffraction underlies *opalescence* that is a lusterless luminescence, more frequently blue. Opalescence is the cause of turbidity of lyophilic colloid solutions and solutions of HMC at their side illumination, and also it is the cause of different coloration of the same colorless colloid system at transmitted and reflected light.

The phenomenon of diffraction scattering of light in heterophase systems in the form of a cone was first observed by Tyndall. If a beam of light is transmitted through a colloid solution in a dark room, a luminous cone is visible. It appears as a result of light diffraction. The presence of the *Tyndall effect* is an important sign for detection of the colloid state. On the basis of this phenomenon some scopes, which allow differentiating colloid and true solutions, determining the form and value of

colloid particles, studying their physical and chemical properties, are constructed. Ultramicroscope, nephelometer and photocolorimeter in the first place belong to this kind of devices.

The theory of light scattering was developed by English scientist I. Rayleigh (1871). He formulated a law:

Intensity of scattered light (/) depends on the difference bet- wen refraction indexes of disperse phase and disperse medium (K), is in direct proportion to the intensity of incident light (J_0), to the quantity of disperse phase particles in a unit of volume (n), to the square of volume of particles, which scatter light, (V^2) and is in inverse proportion to the length of a light wave raised to the fourth power ($A,^4$):

$$I = K\frac{I_0 n V^2}{\lambda^4}.$$

The constant *K* contains the indexes of refraction of the disperse phase and disperse medium.

This law is valid for spherical particles not conducting electric current, small as compared to the wave length of incident light, colorless and fine-grained.

I. Rayleigh, on the basis of light scattering theory, explained the color of the sky and sea water at different times of the day. The reason for the blue color of the sky during daytime is scattering of short waves of sunlight by the particles of the Earth's atmosphere. The intensity of light scattered in 1 cm^3 of air or water is very small. It becomes appreciable due to the enormous thickness of the atmosphere and fluctuation of gas molecules. The red or orange color of the sky in the morning or in the evening is explained by the fact that at rising or setting of the sun, the light that got through the atmosphere is generally observed.

Double electrical layer. Structure of colloid particles. Electrokinetic phenomena

The presence of a double electrical layer in particles of colloid solutions determines their existence, and also their behavior under various conditions.

On the surface of a solid at its contact with a liquid, there spontaneously appears surplus electric charge of a certain sign because of the formation of a voltage-determining ions layer, which is compensated by ions of the opposite sign, the

counterions. As a result, at the solid-liquid interface a double electric layer (DEL) appears. Its formation occurs spontaneously.

The modern theory of DEL structure was created by Guye, Chapman, Stern, Frumkin, Graham. In accordance with this theory the structure of DEL is conditioned by three factors: forces of adsorption interaction of ions with the surface of the solid phase, electrostatic interaction of ions with a charged surface and with each other, and also thermal movement of ions.

In accordance with the modern ideas of DEL structure, the *internal facing of DEL* is made of voltage-determining ions, relatively firmly bounded with the disperse phase. *External facing* is formed by a layer of counterions consisting of two parts. One part is located directly near the charged solid surface and is held by adsorption forces. Such layer is named *adsorptive* or Helmholtz layer. The thickness of the adsorptive layer (S) is equal to the diameter of hydrated ions /*see Fig. 7.2.).* This is the *compact part of DEL.* Other counterions that are necessary for compensation of the surface charge, as a result of thermal motion and mutual repulsion, move away at some distance from the surface forming a *diffuse layer*. Counterions of the diffuse layer are not fixed; they are able to move within its limits deep into the liquid, that is, from the high concentration area into the low concentration area. The thickness (A-) of the diffuse layer depends on the ionic force of an electrolyte *(Fig. 2.).*

Any point of the electric field within the limits of DEL is characterized by definite values of the potential. In accordance with the Stern theory, potential drop of an electric field depending on the distance to the interphase occurs steeply, along a straight line in the adsorptive layer, and more flatly, along a curve, - in the diffuse layer *(Fig. 7.2.).*

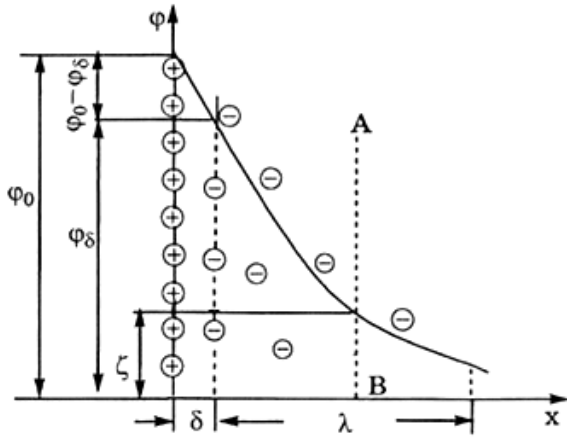


Fig.2. DEL and potential drop in it

φ-potential (interphase potential) is a difference of electric potentials between the disperse phase deep under the surface and the disperse medium outside DEL, in the state of thermodynamic equilibrium.

Surface potential <p_0 is a potential of the solid phase surface in relation to the disperse medium outside DEL.

The modem theory of DEL structure allows explaining the *electrokinetic phenomena* conditioned by relative displacement of the disperse phase and disperse medium. Their intensity is determined by the *electrokinetic potential*, or *zeta* potential, which is designated with the symbol *C*,.

Relative displacement of charged particles and liquid takes place not at the solid-liquid interface, but out of it, at the *sliding interface* (the AB line in *Fig. 7.2.).* The sliding interface is in the liquid medium at some short distance from the interphase surface, but within DEL.

The potential appearing at the sliding interface is named electrokinetic or the φ-potential *(Fig. 2.).* Zeta-potential is a very important characteristic of DEL: it determines the possibility and speed of relative displacement of the disperse phase and disperse medium, the intensity of electrokinetic phenomena, the stability of sols and destruction of disperse systems by electrolytes.

The theory of double electrical layer allowed explaining the structure of micelles that are basic structural units of colloid solutions.

Micelle is an electrically neutral particle of the disperse phase with the double electrical layer surrounding it.

Inside a micelle there is a microscopic crystal formed as a result of chemical or physical processes from molecules, atoms or ions that form the disperse phase; this part of micelle is named *aggregate.* There are *potential-determining ions* located on the aggregate surface. The aggregate together with potential-determining ions constitutes the *micelle nucleus.* The nucleus together with counterions of the compact part of DEL forms a *granule. A granule is actually a colloid particle, it is always charged,* the sign of its charge is determined by the sign of the potential-determining ions charge. A granule is surrounded by counterions of *diffuse layer*; together they form a *micelle* which is always electroneutral.

Let's consider, for example, the structure of a sol's micelle Agl obtained in $AgN0_3$ surplus by a double exchange reaction (by the method of chemical condensation): $\mathbf{AgNO_3 + KI \rightarrow AgI\downarrow + KNO_3.}$

The aggregate of this micelle consists of *m* molecules of Agl (more precisely microscopic crystals of insoluble Agl) that forms a nucleus together with a layer of potential-determining ions (aAg^+); the nucleus with part of counterions *(n - x)* NO_3^- which are located closer to it due to electrostatic and adsorption forces (the adsorption layer of counterions), form a colloid particle, or a granule proper. In the external electric field the adsorption layer of NO_3^- counterions moves together with the nucleus to the cathode. Other counterions (xNO_3^-) are placed in the diffuse layer of the micelle, because of thermal motion they are comparatively weakly bounded to the nucleus. The granule together with the diffuse layer of counterions forms the micelle. The structure of the micelle can be presented with a formula and a diagram *(Fig.3.).*

The ζ-potential of colloid particles is an important characteristic of colloid systems, as in many cases there is a correlation between the change of this value and sol stability. The value of the electrokinetic potential, and sometimes its sign, can change under the influence of external factors (addition of electrolytes, dilution, change of temperature and medium pH, etc.).

The electrokinetic potential is part of the (p_0-potential; it is always less than the surface potential. If the value of the latter depends on the amount of potential-

determining ions, the value of the electrokinetic potential depends on the amount of counterions. The value of the cp_0-potential is experimentally impossible to measure, but the electrokinetic potential is determined directly from experimental data.

The concentration and valence of counterions that are present in the disperse medium have a great influence on the ζ-potential. Since for compensation of the charge ions the same quantity of counterions is always necessary, at increasing of their concentration the thickness of the diffuse layer diminishes, and as a result the potential decreases. An increase of counterions charge stipulates their stronger attraction to the charged solid surface, this also results in DEL compression and ζ - potential decrease.

Variation of the electrolyte composition of a disperse medium is accompanied by an ion exchange and causes a change of the DEL structure. The nature of DEL changing is determined by the properties of electrolyte ions added to the colloid solution, foremost by their ability to be included in the solid phase compound by specific adsorption at the interface, and also by correlation of the charges of electrolyte ions that were again introduced and DEL-forming ions (mainly counterions).

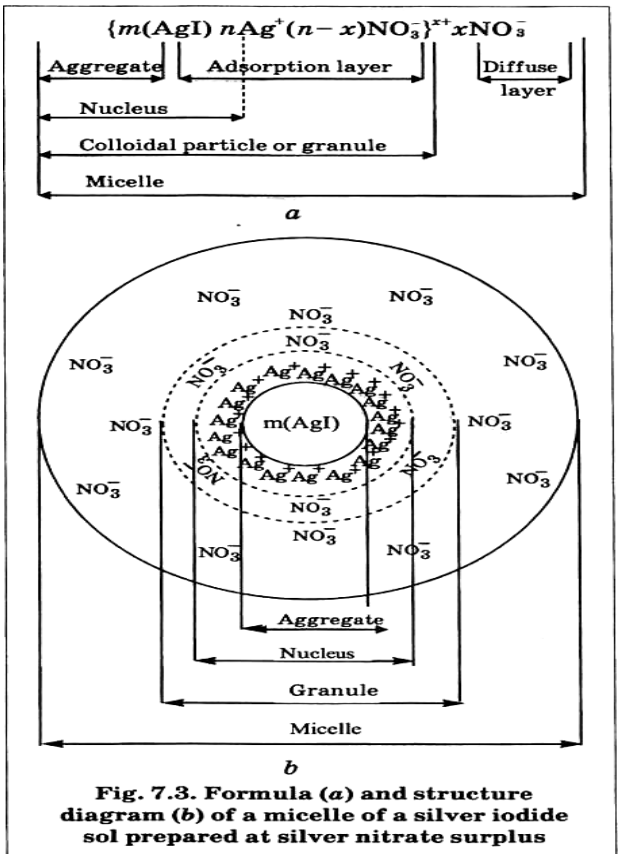


Fig. 7.3. Formula (*a*) and structure diagram (*b*) of a micelle of a silver iodide sol prepared at silver nitrate surplus

Electrokinetic phenomena

Electrokinetic phenomena are phenomena of relative displacement of disperse phase or disperse medium particles at imposition of an external difference of potentials on the disperse system. Arising of potential difference at displacement of disperse phase particles in relation to the disperse medium or vice versa, is also referred to electrokinetic phenomena.

Electrokinetic phenomena were discovered by professor of Moscow University F. Reuss (1808) at researching of electrolysis laws. For prevention of interaction of electrolysis products Reuss divided cathodic and anodic spaces in a U-shaped tube with a diaphragm of quartz sand *(Fig.4., a).*

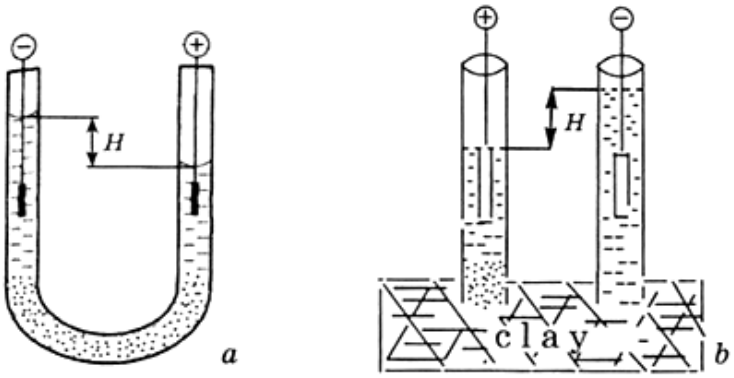


Fig. 7.4. Reuss's experiments:
***a*) diagram of an electroosmosis device;**
***b*) diagram of a device for electrophoresis observation**

At transmission of constant electric current he discovered transfer of liquid from anodic into cathodic space. This phenomenon was called *electroosmosis.*

Electroosmosis is displacement of the disperse medium in relation to the immobile disperse phase (porous material, diaphragm) under the action of external potentials difference.

Electroosmosis has found wide application in dehydration processes and drying of many porous materials or concentrated colloid systems, wood, soils. Electroosmosis is used for purification of medicinal serums. An opposite phenomenon to electroosmosis is *electrophoresis.*

Electrophoresis is a directed motion of disperse phase particles in relation to the immobile disperse medium under the action of external electrostatic field.

The phenomenon was discovered by Reuss in similar experiments, where the role of a porous diaphragm was performed not by coarsely dispersed sand, but by fine-grained clay. Reuss immersed in a moist lump of clay two glass tubes filled with water, containing electrodes connected to a constant-current source. Thus, he noticed rising of liquid near the cathode and emergence of a suspension of particles in the anodic space that were moving to the anode *(Fig. 4., b). A* diagram of electrophoresis is presented in *Fig. 5.*, where a disperse phase particle is represented scaled-up.

The diagram shows that at imposition of an electrostatic field disperse phase particles move to the electrode, whose sign of charge is opposite to the colloid particle sign of charge (the direction of particle motion is shown with a long arrow in the diagram). Motion of particles in electrophoresis is conditioned by attraction of opposite charges.

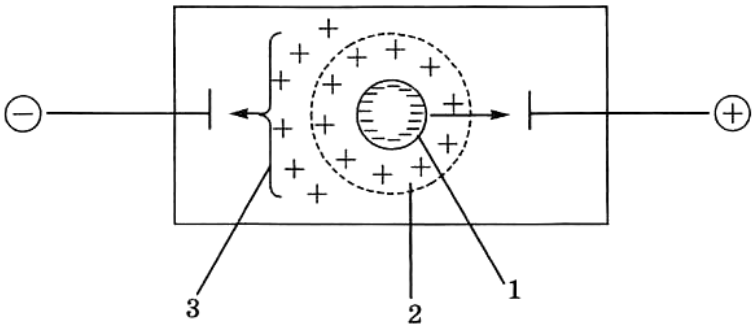


Fig. 7.5. Electrophoresis diagram: 1 – layer of potential-determining ions; 2, 3 – counterions of the adsorption (2) and diffusion (3) layers

In the process of electrophoresis the spherical symmetry of the diffuse layer of counterions is being disturbed, and it begins moving to the side opposite to the particles motion (a short arrow in *Fig. 7.5.).* The oppositely directed flow of counterions of the diffuse layer hampers the particles movement. This effect is named *electrophoretic deceleration.*

The presence of a double electrical layer in colloid particles is the reason for all electrokinetic phenomena. At application of external potential difference electrokinetic potential appears at the sliding interface. The intensity of electrokinetic phenomena is determined by the value of the potential, therefore this very value is used for estimation of electrokinetic effect.

The dependence of electrophoresis velocity on the value of the electrokinetic potential is described by the Helmholtz-Smoluchowski equation: $v = \frac{\varepsilon \cdot \varepsilon_0 \cdot \zeta \cdot E}{\eta}$, where v is linear speed, m/s; ζ is the zeta potential, V; E is external electric field intensity, *V/m* ($E = \Delta\varphi/l$, where $\Delta\varphi$ is the difference of potentials between electrodes, l is the distance between electrodes); η is viscosity of the disperse medium; ε is relative dielectric permeability of the disperse medium; ε_0 is absolute dielectric permeability of vacuum or the electric constant that is equal to 8.85 10~^{12} F • m^{-1}.

The equation is very important, as it is applied for calculating electrophoresis velocity of particles of any form in concentrated solutions of electrolytes (i.e. in the case of thin DEL). Still, the Helmholtz-Smoluchowski equation cannot be used if particles have a thick DEL (i.e. in dilute solutions of electrolytes). To date there is no quantitative theory, which may describe electrophoresis velocity of particles of complex form in dilute solutions of electrolytes. It should be emphasized that such theory has been developed for particles of spherical form. It allows calculating electrophoresis velocity of spherical particles with arbitrary thickness of DEL

Electrophoresis is widely used in medicine for segregation and analyses of proteins. Components of the blood plasma have different electrophoresis mobility, therefore in the process of long-term electrophoresis they are spatially separated in a U-shaped tube by complex optical circuits. As a result an electrophoregram is obtained, which is a curve with peaks that correspond to different blood components. Electrophoregrams of the blood plasma of all healthy people have a practically identical picture. However in the case of pathological processes electrophoregrams have another aspect that is typical of each disease. The height of peaks in an electrophoregram quantitatively characterizes the content of every blood plasma fraction. Therefore this method is used fo diagnosis and control of disease progress. An electrophoregram of the blood plasma in norm (a) and in nephritis (*b*) is represented in *Fig. 6.*

Electrophoresis is also used for separation of mixtures of amino acids, antibiotics, enzymes, antibodies, blood corpuscles, bacterial cells, for determination of purity of protein preparations.

Electrophoresis is used in pharmaceutical industry for purification of different medicinal preparations. By the homogeneity of electrophoresis mobility it is possible to establish the purity degree for a variety of antibiotics, vitamins and other compounds.

Paper electrophoresis is used for separation of mixtures of different medicinal substances and biologically-active compounds.

Transport of medicinal preparations through the skin is carried out by electrophoresis. Electrophoresis of medicinal substances is used for a treatment of burns, atherosclerosis, rheumatism, neuropsychic diseases, etc. Introduction of medicinal preparations through the skin creates a depot, which for a long time influences the sick person's organism. In addition, at application of a difference of potentials on the skin an electroosmotic transfer of liquid through its pores occurs, as the air is removed, that is why permeability of the skin is increased.

It was researching of electrophoresis that helped to establish that all biological surfaces are negatively charged.

Practical lesson 15

Topic: **Kinetic and aggregate stability of dispersed systems. Condensation method of sol formation**.

Relevance of the topic: Aggregative stability of a colloidal system is the resistance of the system to sticking of its particles due to interaction. The problem of aggregative stability of highly dispersed systems is a central research problem in colloid chemistry, since its solution leads to the solution of many problems. For example, coagulation of cholesterol in blood leads to formation of cholesterol plaques on the inner surface of blood vessels. Doctors constantly deal with the phenomenon of erythrocyte coagulation due to a decrease in their zeta potential in clinical laboratories (method of determination of erythrocyte sedimentation rate).

Lesson objective: on the basis of a systematic study of the coagulation process of sols, learn to predict the effect of various factors on the stability of biologically important colloidal systems; consider factors influencing stability and coagulation of disperse systems.

Basic definitions: disperse medium, micelle, ζ-potential, methods of chemical condensation. electro osmosis.

Plan and organizational structure of the lesson:

1. Conditions for obtaining sols using condensation method.
2. Features of the lyophobic sol structure. Micelle examples.
3. Determination of the charge on a colloidal particle.
4. Stability of the colloidal systems. Stability factors. Isoelectric point. Isoelectric state.

Content of the topic

Methods of colloid system preparation

Colloid systems may be prepared both by grinding of coarsely dispersed particles and by association (coalescence) of molecules, atoms, ions into colloid

particles, because they occupy an intermediate position between coarsely dispersed and molecular dispersed systems according to the sizes of disperse phase particles. As a result, the dispergation and condensation methods of colloid system preparation are distinguished.

For preparation of steady colloid solutions (sols) the following conditions are required:

a) presence of two mutually insoluble or slightly soluble components for the formation of a disperse phase and a disperse medium;

b) achievement of the colloidal degree of dispersion (10^7-10^9 m^{-1});

c) presence of a stabilizer conditioning certain stability of the system.

Methods of condensation

Condensation methods consist of large particles formation from smaller ones.

1. Method of physical condensation by solvent substitution. For example, if alcoholic solution of rosin is poured into water, a sol of rosin is formed in water.

2. Methods of chemical condensation. There are applied any chemical reactions for preparation of colloid systems, as a result of which slightly diluted substances are formed. The stabilizer is usually one of the reacting substances, which is taken in surplus.

For generation of a sol, but not a precipitate, from a saturated solution, simultaneous formation of an enormous amount of disperse phase nuclei is needed. Thus the speed of nucleus formation must be larger than the speed of crystal growth. It is obtained by inflowing of a concentrated solution of one component into a much more diluted solution of another one at strong interfusion.

Examples of reactions used for obtaining colloid solutions:

- reactions of hydrolysis: $FeCl_3 + 3H_2O \rightarrow Fe(OH)_3\downarrow + 3HC1$;
- redox reactions: $2H_2S + O_2 \rightarrow 2S\downarrow + 2H_2O$.
- double-exchange reactions:

$BaCl_2 + K_2SO_4 \rightarrow BaSO_4\downarrow + 2KC1$;

$AgNO_3 + KC1 \rightarrow AgCl\downarrow + KNO_3$.

All considered methods are used for lyophobic sols preparation. Lyophilic systems, in particular solutions of HMC, are spontaneously formed at dissolution in a certain solvent.

Double electrical layer. Structure of colloid particles. Electro kinetic phenomena

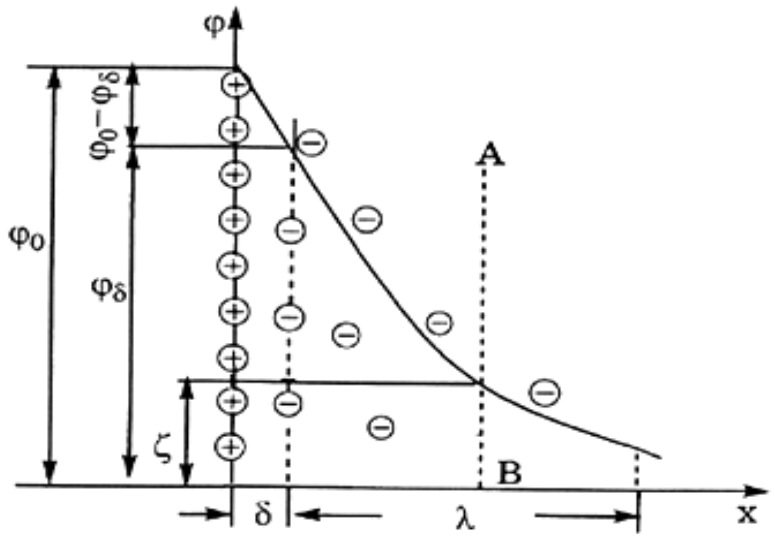
The presence of a double electrical layer in particles of colloid solutions detemines their existence, and also their behavior under various conditions.

On the surface of a solid at its contact with a liquid, there spontaneously appears surplus electric charge of a certain sign because of the formation of a voltage-determining ions layer, which is compensated by ions of the opposite sign, the counter ions. As a result, at the solid-liquid interface a double electric layer (DEL) appears. Its formation occurs spontaneously.

The modern theory of DEL structure was created by Guye, Chapman, Stern, Frumkin, Graham. In accordance with this theory the structure of DEL is conditioned by three factors: forces of adsorption interaction of ions with the surface of the solid phase, electrostatic interaction of ions with a charged surface and with each other, and also thermal movement of ions.

In accordance with the modern ideas of DEL structure, the internal facing of DEL is made of voltage-determining ions, relatively firmly bounded with the disperse phase. External facing is formed by a layer of counter ions consisting of two parts. One part is located directly near the charged solid surface and is held by adsorption forces. Such layer is named adsorptive or Helmholtz layer. The thickness of the adsorptive layer (S) is equal to the diameter of hydrated ions. This is the compact part of DEL. Other counter ions that are necessary for compensation of the surface charge, as a result of thermal motion and mutual repulsion, move away at some distance from the surface forming a diffuse layer. Counter ions of the diffuse layer are not fixed; they are able to move within its limits deep into the liquid, that is, from the high concentration area into the low concentration area. The thickness (A-) of the diffuse layer depends on the ionic force of an electrolyte.

Any point of the electric field within the limits of DEL is characterized by definite values of the potential. In accordance with the Stern theory, potential drop of an electric field depending on the distance to the interphase occurs steeply, along a straight line in the adsorptive layer, and more flatly, along a curve, - in the diffuse layer.



1 **Fig. 7.2. DEL and potential drop in it**

φ-Potential (interphase potential) is a difference of electric potentials between the disperse phase deep under the surface and the disperse medium outside DEL, in the state of thermodynamic equilibrium.

Surface potential φ_0 is a potential of the solid phase surface in relation to the disperse medium outside DEL.

The modem theory of DEL structure allows explaining the electro kinetic phenomena conditioned by relative displacement of the disperse phase and disperse medium. Their intensity is determined by the electro kinetic potential, or zeta potential, which is designated with the symbol ζ.

Relative displacement of charged particles and liquid takes place not at the solid-liquid interface, but out of it, at the sliding interface.

The sliding interface is in the liquid medium at some short distance from the interphase surface, but within DEL.

The potential appearing at the sliding interface is named electro kinetic or the ζ-potential. Zeta-potential is a very important characteristic of DEL: it determines the

possibility and speed of relative displacement of the disperse phase and disperse medium, the intensity of electro kinetic phenomena, the stability of sols and destruction of disperse systems by electrolytes.

The value of the ζ-potential is possible to calculate by experimental data. Its sign is determined by the sign of charge of potential-determining ions layer: if the potential-determining layer is formed of anions, the sign is negative, if it is formed of cations, the sign is positive.

The value of the ζ-potential depends on the nature of the disperse phase and disperse medium, concentration of an electrolyte and a colloid solution, pH medium, and temperature.

By adding an electrolyte to a colloid solution that contains ions of identical with the DEL counter ions sign of charge, the diffuse layer becomes compressed as a result of transferring of counter ions from it into the adsorption layer. It stipulates

ζ-potential decline. If the amount of counter ions in the adsorption layer becomes sufficient for full compensation of the charge of the potential-determining layer, the ζ-potential decreases to zero (isoelectric state).

The ζ-potential depends on the concentration of colloid solution. A decrease of concentration causes an increase of diffuse layer thickness, as a result the

ζ-potential increases. But simultaneously desorption of potential-determining ions from the surface of sol particles can take place, that will cause a decrease of the φ_0- and ζ-potentials. Thus, potential changing will depend on predomination of one of these effects.

Temperature also influences the potential. The intensity of Brownian motion of counter ions grows with rise in temperature, this leads to an increase of the diffuse layer thickness, and consequently, to the augmentation of the potential value. At the same time, desorption of potential-determining ions can occur, which is the cause of φ_0- and ζ-potential diminishing.

The ζ-potential decreases at a decline in dielectric permeability of the disperse medium.

The ζ_0-potential depends on pH of the disperse medium, which is conditioned by great adsorptivity of H^+ and OH^- ions.

The theory of double electrical layer allowed explaining the structure of micelles that are basic structural units of colloid solutions.

Micelle is an electrically neutral particle of the disperse phase with the double electrical layer surrounding it.

Inside a micelle there is a microscopic crystal formed as a result of chemical or physical processes from molecules, atoms or ions that form the disperse phase; this part of micelle is named aggregate. There are potential-determining ions located on the aggregate surface. The aggregate together with potential-determining ions constitutes the micelle nucleus. The nucleus together with counter ions of the compact part of DEL forms a granule. A granule is actually a colloid particle, it is always charged, the sign of its charge is determined by the sign of the potential-determining ions charge. A granule is surrounded by counter ions of diffuse layer; together they form a micelle which is always electroneutral.

Let's consider, for example, the structure of a sol's micelle AgI obtained in $AgNO_3$ surplus by a double exchange reaction (by the method of chemical condensation):

$$AgNO_3 + KI \rightarrow AgI + KNO_3.$$

The aggregate of this micelle consists of m molecules of Agl (more precisely microscopic crystals of insoluble AgI) that forms a nucleus together with a layer of potential-determining ions (nAg^+); the nucleus with part of counter ions (n - x) NO_3^- which are located closer to it due to electrostatic and adsorption forces (the adsorption layer of counter ions), form a colloid particle, or a granule proper. In the external electric field the adsorption layer of NO_3^-~ counter ions moves together with the nucleus to the cathode. Other counter ions (x NO_3^-) are placed in the diffuse layer of the micelle, because of thermal motion they are comparatively weakly bounded to the nucleus. The granule together with the diffuse layer of counter ions forms the micelle. The structure of the micelle can be presented with a formula and a diagram (Fig. 2).

The ζ-potential of colloid particles is an important characteristic of colloid systems, as in many cases there is a correlation between the change of this value and sol stability. The value of the electro kinetic potential, and sometimes its sign, can change under the influence of external factors (addition of electrolytes, dilution, change of temperature and medium pH, etc.).

The electro kinetic potential is part of the φ_0-potential; it is always less than the surface potential. If the value of the latter depends on the amount of potential-determining ions, the value of the electro kinetic potential depends on the amount of counter ions. The value of the electro thermodynamic potential is experimentally impossible to measure, but the electro kinetic potential is determined directly from experimental data.

The concentration and valence of counter ions that are present in the disperse medium have a great influence on the ζ-potential.

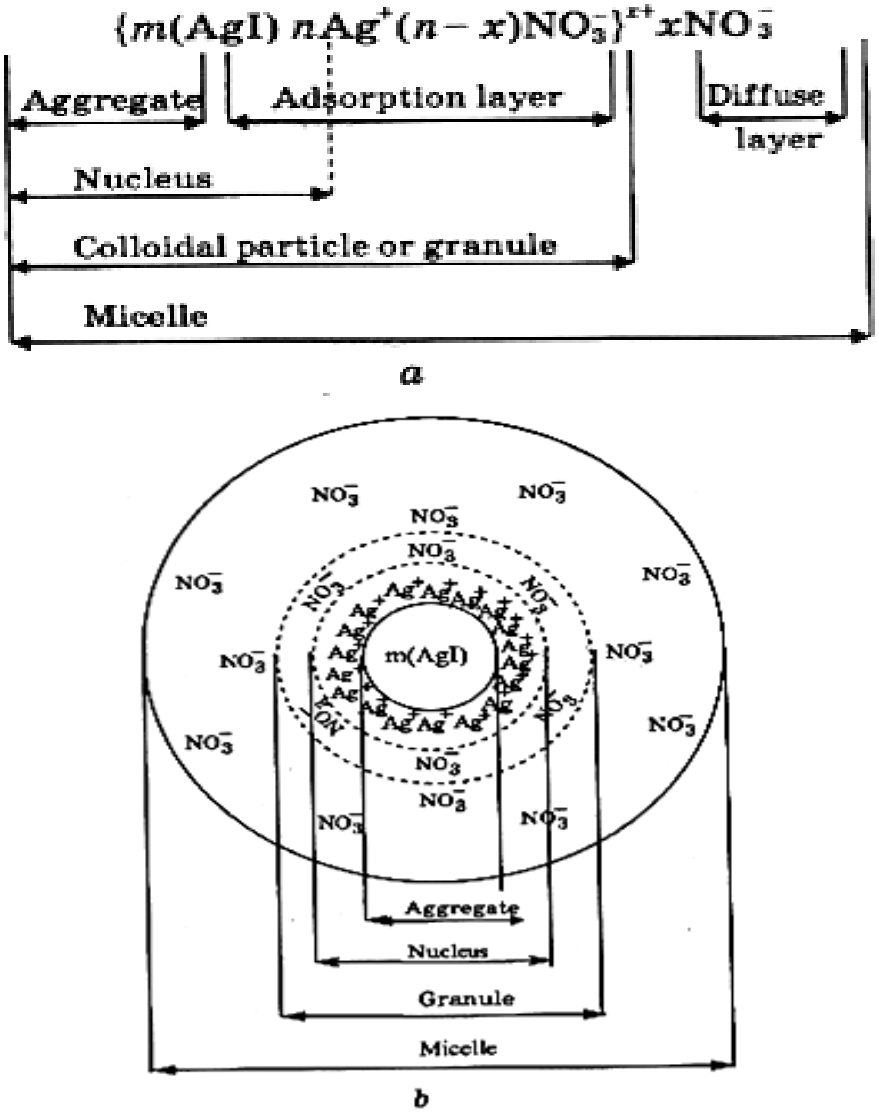


Fig. 2. Formula (a) and structure diagram (b) of a micelle of a silver iodine sol prepared at silver nitrate surplus.

Electro kinetic phenomena

Electro kinetic phenomena are phenomena of relative displacement of disperse phase or disperse medium particles at imposition of an external difference of potentials on the disperse system. Arising of potential difference at displacement of disperse phase particles in relation to the disperse medium or vice versa, is also referred to electro kinetic phenomena.

Electro kinetic phenomena were discovered by professor of Moscow University F. Reuss (1808) at researching of electrolysis laws. For prevention of interaction of electrolysis products Reuss divided cathodic and anodic spaces in a U-shaped tube with a diaphragm of quartz sand (Fig. 3, a).

At transmission of constant electric current he discovered transfer of liquid from anodic into cathodic space. This phenomenon was called electro osmosis.

Electro osmosis is displacement of the disperse medium in relation to the immobile disperse phase (porous material, diaphragm) under the action of external potentials difference.

Electro osmosis has found wide application in dehydration processes and drying of many porous materials or concentrated colloid systems, wood, soils. Electro osmosis is used for purification of medicinal serums. An opposite phenomenon to electro osmosis is

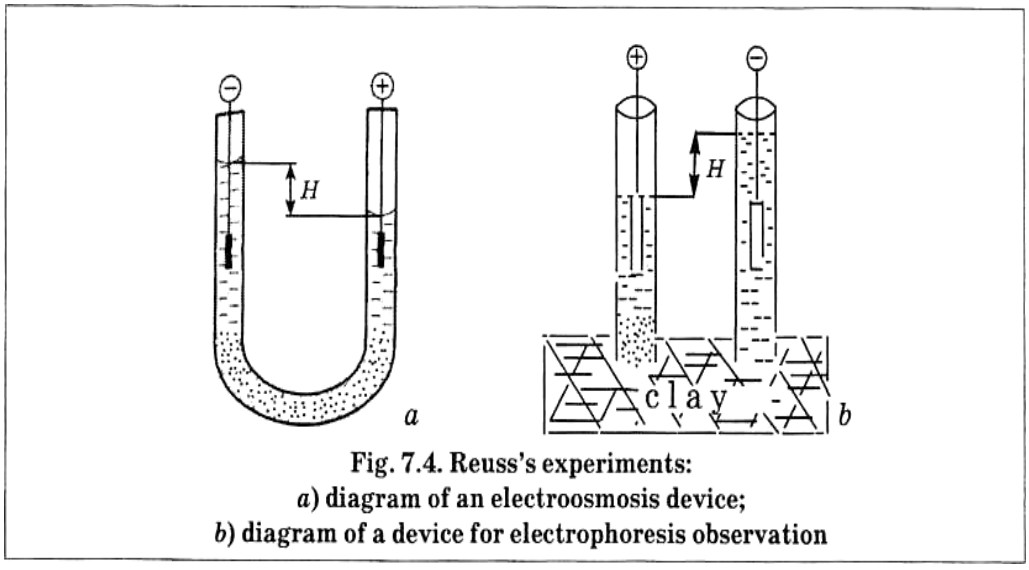


Fig. 7.4. Reuss's experiments:
***a*) diagram of an electroosmosis device;**
***b*) diagram of a device for electrophoresis observation**

electrophoresis.

Electrophoresis is a directed motion of disperse phase particles in relation to the immobile disperse medium under the action of external electrostatic field.

The phenomenon was discovered by Reuss in similar experiments, where the role of a porous diaphragm was performed not by coarsely dispersed sand, but by fine-grained clay. Reuss immersed in a moist lump of clay two glass tubes filled with water, containing electrodes connected to a constant-current source. Thus, he noticed rising of liquid near the cathode and emergence of a suspension of particles in the anodic space that were moving to the anode (Fig. 3, b). A diagram of electrophoresis is presented in Fig. 4, where a disperse phase particle is represented scaled-up.

The diagram shows that at imposition of an electrostatic field disperse phase particles move to the electrode, whose sign of charge is opposite to the colloid particle sign of charge (the direction of particle motion is shown with a long arrow in

4

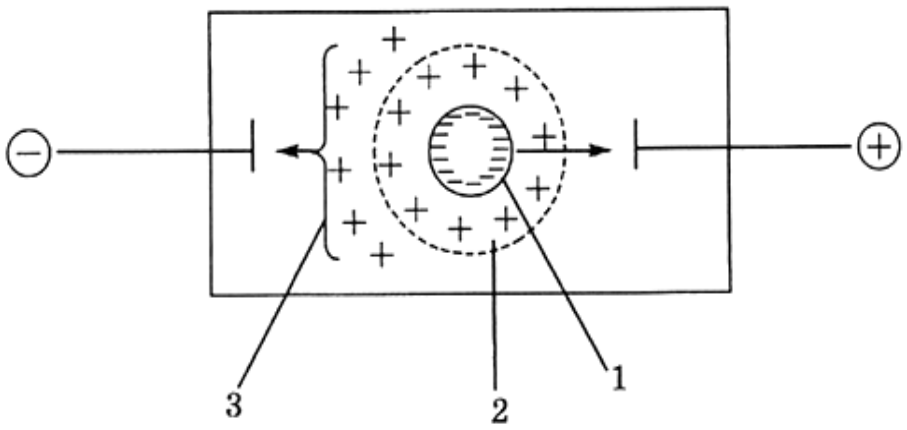


Fig. 7.5. Electrophoresis diagram: 1 – layer of potential-determining ions; 2, 3 – counterions of the adsorption (2) and diffusion (3) layers

the diagram). Motion of particles in electrophoresis is conditioned by attraction of opposite charges.

In the process of electrophoresis the spherical symmetry of the diffuse layer of counter ions is being disturbed, and it begins moving to the side opposite to the particles motion (a short arrow in Fig. 4). The oppositely directed flow of counter ions of the diffuse layer hampers the particles movement. This effect is named electrophoretic deceleration.

The presence of a double electrical layer in colloid particles is the reason for all electro kinetic phenomena. At application of external potential difference electro

kinetic potential appears at the sliding interface. The intensity of electro kinetic phenomena is determined by the value of the potential, therefore this very value is used for estimation of electro kinetic effect.

The dependence of electrophoresis velocity on the value of the electrokinetic potential is described by the Helmholtz-Smoluchowski equation:

$$\upsilon = \frac{\varepsilon \cdot \varepsilon_0 \cdot \zeta \cdot E}{\eta},$$

where v is linear speed, m/s; ζ is the zeta potential, V; E is external electric field intensity, V/m (E = Δφ/l, where Δφ is the difference of potentials between electrodes, l is the distance between electrodes); η is viscosity of the disperse medium, Pa· s; ε is relative dielectric permeability of the disperse medium; s0 is absolute dielectric permeability of vacuum or the electric constant that is equal to 8.85 10^{-12} F · m^{-1}.

The equation is very important, as it is applied for calculating electrophoresis velocity of particles of any form in concentrated solutions of electrolytes (i.e. in the case of thin DEL). Still, the Helmholtz-Smoluchowski equation cannot be used if particles have a thick DEL (i.e. in dilute solutions of electrolytes). To date there is no quantitative theory, which may describe electrophoresis velocity of particles of complex form in dilute solutions of electrolytes. It should be emphasized that such theory has been developed for particles of spherical form. It allows calculating electrophoresis velocity of spherical particles with arbitrary thickness of DEL.

Electrophoresis is widely used in medicine for segregation and analyses of proteins. Components of the blood plasma have different electrophoresis mobility, therefore in the process of long-term electrophoresis they are spatially separated in a U-shaped tube by complex optical circuits. As a result an electrophoregram is obtained, which is a curve with peaks that correspond to different blood components. Electrophoregrams of the blood plasma of all healthy people have a practically identical picture. However in the case of pathological processes electrophoregrams have another aspect that is typical of each disease. The height of peaks in an electrophoregram quantitatively characterizes the content of every blood plasma

fraction. Therefore this method is used for diagnosis and control of disease progress. An electrophoregram of the blood plasma in norm (a) and in nephritis (b) is represented in Fig. 5.

Electrophoresis is also used for separation of mixtures of amino acids, antibiotics, enzymes, antibodies, blood corpuscles, bacterial cells, for determination of purity of protein preparations.

Electrophoresis is used in pharmaceutical industry for purification of different medicinal preparations. By the homogeneity of electrophoresis mobility it is possible to establish the purity degree for a variety of antibiotics, vitamins and other compounds.

Paper electrophoresis is used for separation of mixtures of different medicinal substances and biologically-active compounds.

Transport of medicinal preparations through the skin is carried out by electrophoresis. Electrophoresis of medicinal substances is used for a treatment of burns, atherosclerosis, rheumatism, neuropsychic diseases, etc. Introduction of medicinal preparations through the skin creates a depot, which for a long time influences the sick person's organism. In addition, at application of a difference

of potentials on the skin an electroosmotic transfer of liquid through its pores occurs, as the air is removed, that is why permeability of the skin is increased.

It was researching of electrophoresis that helped to establish that all biological surfaces are negatively charged.

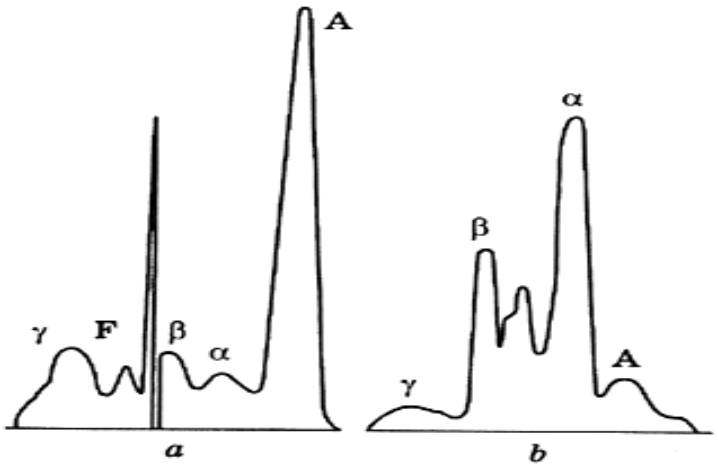


Fig. 7.6. Electrophoregram of blood plasma: ***a*** **– norm,** ***b*** **– nephritis; A is albumin, α, β, γ are globulins, F is fibrinogen**

The investigations of bacterial cells electrophoretic mobility showed that its electro kinetic potential may change from zero to some tens of millivolts. For some bacterial cells it depends on pH of the medium, presence of proteins in the medium, and also the age of cells: the older the cell, the less the value of its

ζ-potential. Researches of pH influence on the ζ-potential of bacterial cells has allowed dividing them in two groups: bacteria of protein and nonprotein nature (whose surface consists of polysaccharides).

Measurement of ζ-potentials of different cells has showed that its value changes from -10 to -30 mV. Investigations of red corpuscles electro kinetic potential appeared to be particularly interesting. It was established that the value of red corpuscles ζ-potential is characteristic of each type of living organisms (Table 1.).

Table 1.

ζ-Potential and electrophoresis velocity of erythrocytes of different living organisms

Living organism	**Electrophoresis velocity, $\upsilon \cdot 10^6$ m/s**	**ζ-Potential, mV**
Rabbit	0.55	-7.0
Pig	0.98	-12.5
Cavy	1.11	-14.2
Man	1.31	-16.8
Rhesus monkey	1.33	-17.0
Cat	1.39	-17.8
Rat	1.45	-18.6
Dog	1.65	-21.1

The potential of human erythrocytes is a constant value and it is equal to -16.8 mV. The isoelectric state of red corpuscles is observed at pH = 1.7. A permanent negative value of electro kinetic potential of red corpuscles, and also transition to an isoelectric state in a very acidic medium is explained by dissociation of phospholipid acid groups.

It was also established that for every kind of living organisms the electro kinetic potential of red corpuscles is more than that of leukocytes. For example, electrophoresis velocity of horse's red corpuscles, which correlates with the potential, is equal to $1.01 \cdot 10^{-6}$ m/s, and for leukocytes it is equal to $0.49 \cdot \bullet\ 10^{-6}$ m/s.

It is known that in inflammatory process of any human organ a migration of leukocytes to the area of inflammation occurs. There is an opinion that a negative electro kinetic potential of leukocytes takes part in that process, because leukocytes start moving as a result of potential difference that appears between the inflamed and healthy tissue areas.

On the biological membrane surface a double electrical layer is formed due to the presence of negatively charged functional groups and attraction to them of positively charged ions from the intercellular environment. The electro kinetic potential (ζ-potential) appears.

Stability and coagulation of disperse systems

Stability of disperse systems is one of their most important characteristics.

*Stability is the ability of a system to keep invariable or practically invariable the value of particles of the disperse phase and their even distribution in the volume of the disperse medium***.**

The problem of colloid systems stability is the most difficult problem of colloid chemistry.

N. Peskov (1920) divided the stability of disperse systems into two kinds: kinetic (sediment) and aggregation.

Kinetic (sediment) stability is determined by the capacity of systems to counteract settling (sedimentation) of particles under the action of gravitational force (gravity). Sedimentation is the reason for destruction of a disperse system, namely its stratification into two separate phases: a disperse phase and a disperse medium.

Fine-grained systems are sedimentary steady, coarsely dispersed systems (emulsions, suspensions) are sedimentary unsteady.

Aggregation stability is the ability of a system to keep invariable the primary size of disperse phase particles, that is to resist their adhesion. Interaction and ag-

glutination of solid particles leads to formation of aggregates that are able to settle or emerge. Aggregation (adhesion) of solid particles is called coagulation. Enlargement (coagulation) of particles leads to spontaneously, as it is accompanied by reduction of the specific surface of the disperse phase and respective diminishing of free surface energy at the interphase boundary. Not only coagulation, but also coalescence (fusion of particles) belongs to the processes of disperse systems destruction that lead to decreasing of free surface energy.

These very processes are observed in lyophobic disperse systems having a considerable surplus of free surface energy. At the same time, many lyophobic disperse systems can be aggregation steady.

Stability factors of disperse systems

Aggregation stability is determined by the time of process proceeding caused by a surplus of surface energy of a disperse system. Therefore this stability has a kinetic character, so it can be defined by the time and speed of coagulation.

Fundamental thermodynamic imbalance of lyophobic disperse systems conditioned by a surplus of free surface energy, initiates the proceeding of processes that change with time their structure and lead to their destruction. The speed of destruction processes is determined by nature, phase state, composition of the disperse phase and disperse medium, and also by dispersity and concentration of the disperse phase. Different factors of stability can operate in a lyophobic system: both of thermodynamic and kinetic nature, slowing or practically fully stopping the process of destruction.

Among the thermodynamic factors of stability the next ones are reckoned:

1. Electrostatic. The role of this agent consists in the fact, that at approaching of two similarly charged particles of the disperse phase the diffuse layers of their DEL overlap, consequently electrostatic repulsion force appears between the particles.

2. Solvate - it is conditioned by formation of solvate layers on the surface of particles from molecules of disperse medium, and in case of water - by formation of hydrate shells. P.A. Rehbinder established that these solvate layers have a high

viscidity, resiliency, displacement resistance, they are able to render a disjoining pressure at approachment of particles that is the reason for repulsive forces between them.

3. Entropic factor appears as a result of approaching of particles with surface- active substances or high-molecular compounds adsorbed on their surface. At approachment of particles with such adsorption layers, entropy of a disperse system sharply diminishes, and the Gibbs free energy is increased ($\Delta G > 0$), which counteracts the fusion of particles.

To the kinetic factors of stability that decrease aggregation (coagulation) speed of disperse phase particles belongs the structural mechanic factor, it arises at adsorption of such SAS which are able to form gelatinous structured layer at the interphase boundary. To such substances belong proteins, glycosides, cellulose derivatives (carboxymethylcellulose), soaps formed by polyvalent metals, polymers, i.e. substances that form two-dimensional structures – they are called stabilizers. At overlapping of these adsorption layers a structure emerges having certain resiliency and durability. In other words, there appears a structural mechanic barrier that counteracts particle fusion.

Theory of coagulation and stability of disperse systems

There are a couple of theories of disperse systems stability that explain reasons for coagulation from different positions.

The modern theory of the coagulating action of electrolytes, created by B. Deryagin, L.D. Landau, and later, independently, by Dutch physicists-chemists E. Verwey and J. Overbeck (DLFO), is based on comparison of molecular interaction of disperse phase particles in a disperse medium with electrostatic interaction of diffuse ions layers, considering the thermal (Brownian) motion of particles of the disperse phase.

According to this theory, at approaching of two particles at a certain distance the thickness of the inter layer (film) of liquid between them diminishes. At decreasing of the film thickness the diffuse layers of DEL begin to overlap. In

addition, at close distances molecular attraction forces start acting. The combined action of these two factors causes the origin of disjoining pressure.

Disjoining pressure is that surplus of pressure which must be applied to surfaces limiting a thin film, so that its thickness remained constant or could be reversibly changed.

More frequently the change of aggregation stability is carried out by addition of electrolytes that are coagulants. They change the structure of DEL and its diffuse layer (compact), diminish the potential and electrostatic repulsion, this causes coagulation.

Coagulation of sols by electrolytes was investigated by F. Selmi, T. Graham, I. Borschev. M. Hardy (1900) established that coagulating action isn't developed by all ions of an electrolyte, but only by those which have a charge opposite to the sign of the colloid particle charge. Even before that H. Schultze (1882) discovered that coagulation force of an electrolyte ion increases with rise in its valence. These generalizations were called the Schultze-Hardy rule.

At a gradual increase of electrolyte concentration, coagulation becomes evident only when the concentration is higher than some critical concentration which is named the coagulation threshold. $\left(C_c\left(\frac{1}{z}x\right)\right)$.

The coagulation threshold is calculated according to the formula:

$$C_c\left(\frac{1}{z}x\right)=\frac{C\left(\frac{1}{z}x\right)\cdot V(\text{electrolyte})}{V(\text{sol})+V(\text{electrolyte})},$$

Where $\left(C_c\left(\frac{1}{z}x\right)\right)$. is molar concentration of the electrolyte equivalent; V(sol) is the sol volume; V (electrolyte) is the electrolyte solution volume.

The coagulation threshold is measured in mmol/1.

The inverse value to the coagulation threshold is named coagulating power ($V_c(x)$) and is expressed in 1/mmol:

$$V_c(x)=\frac{1}{C_c\left(\frac{1}{z}x\right)}.$$

On the basis of numerous experiments Schultze and Hardy established that approximately coagulate powers of one-, two- and three-charge counterions correlate as 1: 25 : 500.

This empirically established law has got a theoretical justification within the DLFO theory. In accordance with this theory, for strongly charged particles of sols, the coagulation threshold is in inverse proportion to the coagulate ion charge raised to the sixth power.

So, in accordance with the theory of DLFO, for one-, two- and three-charge counter ions, their coagulate powers are co related as 1: 64 : 729.

Some discrepancy in this information is explained by an increasing role of specific adsorption of multiply charged ions, which is not taken into account by the DLFO theory.

It was also determined that near the coagulation threshold the absolute value of the potential, independently of the charge sign of a colloid particle, is $\zeta cr \approx 30$ mV (in the range from 25 to 50 mV).

Researches of coagulate actions of various electrolytes with an identical charge of the coagulating ion have shown that they form lyotropic series. Ions of alkaline metals are arranged in a lyotropic series by their coagulating power:

Coagulating power →

Li^+ Na^+ K^+ Rb^+ Cs^+

← Hydration degree

Coagulating power depends on the hydration degree of ions: the larger is the hydration degree, the weaker the coagulating action of these ions.

Anions also form similar series, but the difference in their coagulating power is insignificant:

Coagulating power →

Cl^- Br^- I^- SCN^-

← Hydration degree

Laboratory work

I. Condensation method of sols preparation (exchange reactions).

(The sols obtained in procedures 1, 2 and 3 are used then for the further determination of a colloidal particle charge).

1. Preparation of ferric ferrocyanide (prussian blue) sols.

a) Colloidal solution of a deep-blue coloured ferrocyanide sol is formed when 1 drop of the 2% ferrum(III) chloride ($FeCl_3$) solution is added with energetic stirring to a 3 ml $K_4[Fe(CN)_6]$ (potassium ferrocyanide).

b) Colloidal solution of a green coloured sol is formed when 1-2 drops of the 0,1% $K_4[Fe(CN)_6]$ solution is added with shaking to a 3 ml $FeCl_3$ solution.

Write down the formulas of the blue and green sols.

2. Preparation of copper ferrocyanide sols.

Add 1-2 drops of a 0,1% solution of copper sulfate ($CuSO_4$) to a 3 ml 0,1% solution of potassium ferrocyanide ($K_4[Fe(CN)_6]$). A red-brown coloured sol is formed.

Write down the micelle formula.

Condensation method of sols preparation (hydrolysis reaction).

3. Preparation of ferrum(III) hydroxide ($Fe(OH)_3$) sol.

Add dropwise quickly 5 drops of concentrated aqueous solution of ferric chloride ($FeCl_3$) to a large volume (3 ml) of boiling water with constant stirring. $FeCl_3$ is hydrolyzed to ferric hydroxide ($Fe(OH)_3$) which remains in solution in the colloidal form and produces a red-brown sol of ferric hydroxide.

$FeCl_3+3H_2O \rightarrow Fe(OH)_3+3HCl$

Write down the micelle formula.

II. Determination of the electric charge on hydrophobic colloids by the capillarization method.

Most of the colloidal particles are electrically charged. Some of them are charged with positive electricity and some with negative electricity. These charges are required through adsorption during their formation and these charges are mainly responsible for the stability of the colloid.

A drop of the sol to be investigated (see procedures 1, 2 and 3; use also some organic dyes - thymol blue, phenol red, methyl red, methyl orange, bromothymol blue, bromocresol purple, etc.) is placed with a micropipette on the filter paper. The filter paper wetted by sols with liquid dispersion medium is charged negatively. The charge on hydrophobic sol can be identified by the character of shape it forms on the filter paper.

Recommended literature

Basic literature:

1. Medical Chemistry: textbook / V.Y. Tsuber, A.A. Kotvytska, K.V. Tykhonovych et al. - – Kyiv, AUS Medicine Publishing, 2022. – 392 p.

2. Medical chemistry: a textbook for universities / V. O. Kalibabchuk, I. S. Chekman, V. I. Galynska and others; for ed. Prof. V. O. Kalibabchuk – 4th ed. – K. VSV "Medicine", 2019 – 336 p.

3. Medical chemistry / V.O. Kalibabchuk, V.I. Halynska, L.I. Hryshchenko et al. – Kyiv, AUS Medicine Publishing, 2020. – 224 p.

4. General and Inorganic Chemistry: textbook / V.O. Kalibabchuk, V.V. Ohurtsov, V.I. Halynska et al. – Kyiv, AUS Medicine Publishing, 2019. – 456 p.

Additional literature:

1. Medical chemistry: a textbook / V. P. Muzychenko, D. D. Lutsevich, L. P. Yavorska; for order. B. S. Zimenkovsky. – 3rd ed., Ed. – K.: BCB «Medicine», 2018. – 496 p.

2. Mironovich L. M. Medical Chemistry: A Textbook. – Kyiv: Karavella, 2008. – 159 p.

3. Moroz A. S. Medical chemistry: a textbook / D. D. Lutsevich, L. P. Yavorska. – Vinnytsia: New book, 2006. – 776 p.

4. Gotsulyak L. O., Mardashko O. O., Yerigova S. G., Kuzmenko G. I., Kuzmina A. V., Zhilinskaya K. I. Bioinorganic, physicoloid and bioorganic chemistry. Teaching. manual. Odessa. Odessa State Medical University 1999. – 248 p.

5. Textbook of Medicinal Chemistry / V. Alagarsamy // CBS Publishers & Distributors Pvt Ltd, India; 3rd edition, 2018 – 584 p.

6. Richard Post. Chemistry: Concepts and Problems / Richard Post, Chad Snyder, Clifford C. Houk // A Self-Teaching Guide, Jossey-Bass, 2020. – 432 p.

7. Darrell D. Ebbing. General Chemistry / Darrell D. Ebbing, Steven D. Gammon. – Boston: Cengage Learnin, 2017. – 1190 c. – (Eleventh Edition)