

МІНІСТЕРСТВО ОХОРОНИ ЗДОРОВ'Я УКРАЇНИ
ХАРКІВСЬКИЙ НАЦІОНАЛЬНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ

**ACID-BASE EQUILIBRIUM IN THE ORGANISM.
pH OF BIOLOGICAL LIQUIDS**

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

**КИСЛОТНО-ОСНОВНА РІВНОВАГА В ОРГАНІЗМІ.
ВОДНЕВИЙ ПОКАЗНИК БІОЛОГІЧНИХ РІДИН**

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

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Acid-base equilibrium in the organism. pH of biological liquids: methodical instructions for 1st year students' self-work in Medical Chemistry / compiled by A. O. Syrovaya, O. L. Levashova, N. N. Chalenko et al. – Kharkiv: KhNMU, 2017. – 24 p.

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Subject “ACID-BASE EQUILIBRIUM IN THE ORGANISM. pH OF BIOLOGICAL LIQUIDS”

1. Number of hours 4.

2. Material and methodological support.

Tables:

1. Scheme of structure of the subject.
2. Tables of logarithms.
3. Mechanism of dissolution process.
4. The ion product of water.
5. Formulas for the pH calculation of different electrolytes.
6. The pH values of body fluids.

Multimedia support (presentation, educational video).

Educational literature:

1. Medical chemistry: textbook / V.A. Kalibabchuk, V.I. Halynska, L.I. Hryshchenko et al.; — Kyiv: AUS Medicine Publishing, 2010. — 224 p.
2. Fundamentals of medical chemistry: manual for students' self-work / A.O. Syrovaya, E.R. Grabovetskaya, L.G. Shapoval. – Kharkiv: KhNMU, 2015.—196 p.
3. Medical chemistry. Adapted concise course: manual for students' self-work / A.O. Syrovaya, E.R. Grabovetskaya, L.G. Shapoval. - Kharkiv: KhNMU, 2013. – 160 p.
4. Medical chemistry: workbook for self-work of first-year students of medical and dentistry faculties / compiled by A. O. Syrovaya, V. N. Petunina, V. A. Makarov et al. – Kharkiv : KhNMU, 2017. – 72 p.
5. Acid-base equilibrium in the organism. pH of biological liquids. – Methodical instructions for 1st year students' self-work in Medical Chemistry / compiled by A.O. Syrovaya, O.L. Levashova, N.N. Chalenko et al. – Kharkiv: KhNMU, 2017. – 24 p.
6. Individual tasks for students' self-control of knowledge in Medical Chemistry / A.O. Syrovaya, L.G. Shapoval, et al. – Kharkiv: KhNMU, 2014. –50 p.
7. Text of lecture.

3. **Substantiation for the subject.** One of the necessary conditions of the living organism is the existence of the relative dynamic constancy of the pH medium – homeostasis. For a better understanding, it is necessary to review the acid-base equilibrium balance in the overall scheme of biochemical processes, characteristic fluids within the blood-stream.

The pH value of the liquid media is one of the main characteristics of the body and under normal conditions varies within a narrow range. Any deviations towards increase or decrease in pH levels cause dysfunction of enzymes and hormones that exhibit biological activity only in strictly defined range of pH values.

Minor changes in the pH of blood and interstitial fluid can lead to death. In many cases, the control of pH value enables to identify various types of pathology and make correct diagnosis.

Acid-base state of the internal environment defines its susceptibility to infectious diseases in some cases. The actuality of the study of this subject is

unquestionable. Understanding of the influence of the pH value on various biochemical and physiological processes, the ability to calculate the pH of acids, bases, and salts solutions allows to evaluate the acidity of biological fluids.

4. The purpose of the subject:

- general: evaluate the acidity of biological fluids based on the knowledge of the pH value;

- specific: make conclusions about the acidity of body fluids based on the pH value;

a) **to know:** properties of electrolyte solutions, water-electrolyte balance in the body, and their impact on the life activity processes.

b) **be able to:** calculate the pH of acids, bases, salts solutions; calculate the concentration of hydrogen ions according to the pH value; evaluate the acidity of liquids based on the pH value.

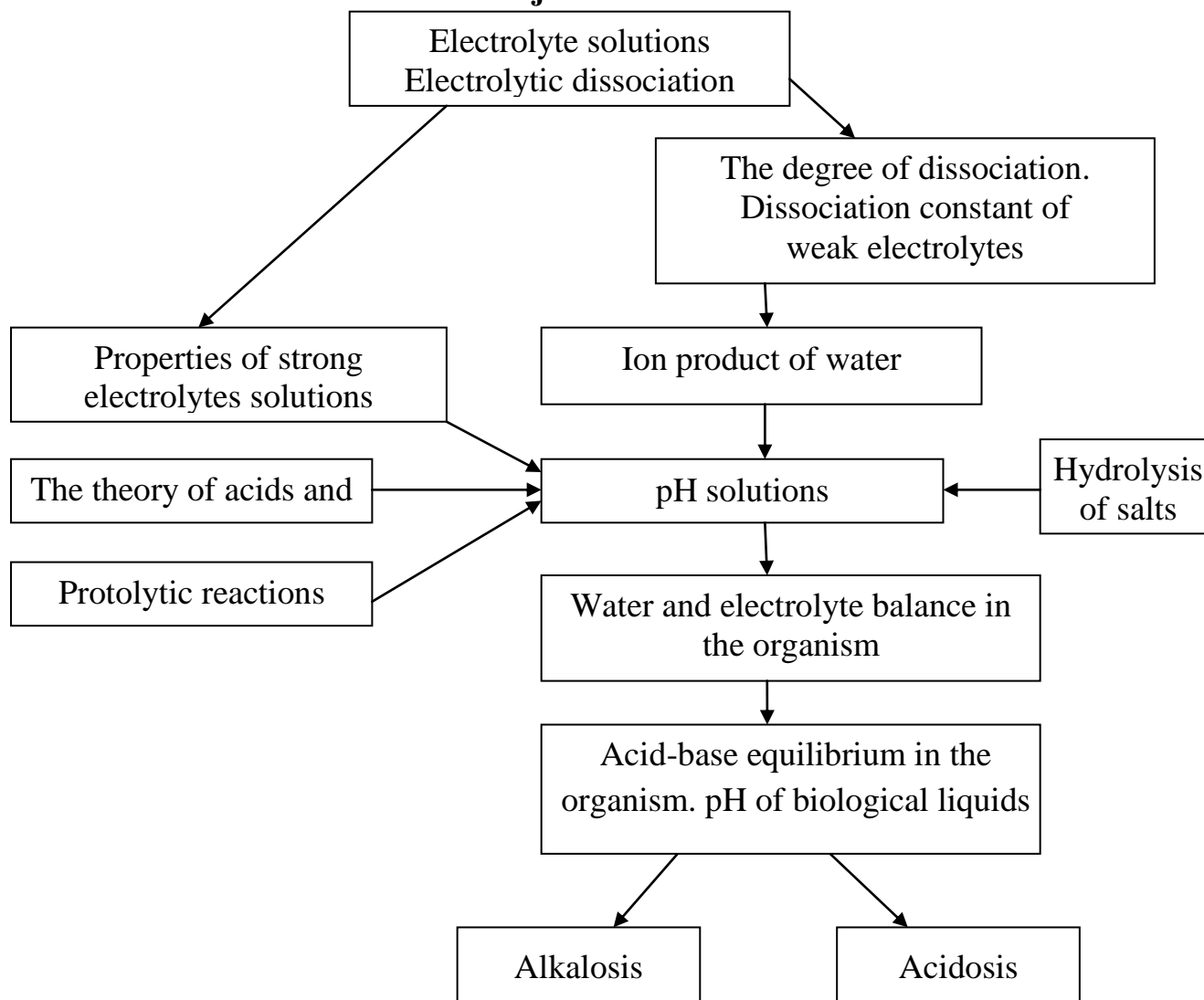
5. Practical skills:

- To make the pH calculation in the solutions of acids, bases and salts;

- To determine the concentration of hydrogen ions according to the pH value;

- To use the knowledge of this subject for the estimation of medium acidity of biological fluids (blood, gastric juice, urine, etc.).

6. Scheme of structure of the subject.



7. Plan of students' work.

№	Stages	Time (min.)	Training and visual aids	Location
1.	Motivation description and plan of topics. Questions and answers	25	Manual	Class room
2.	Incoming control	20		
3.	Independent work of students with methodical literature, the solution of educational problems, filling of self-study guide	60	Methodical instructions for students, text of lecture, manual for students' self-work, work-book, reference data, tables	
4.	Implementation of the laboratory work and writing the report	35	Methodical instructions for students' self-work, work-book, reference data	
5.	Final control	25		
6.	Analysis and conclusions	10		
	Home work	5		

7. Tasks for self-work:

- list of questions to be studied:

1. Solutions of electrolytes. Information on human body electrolytes.
2. The degree of dissociation and dissociation constant for weak electrolytes.
3. Properties of strong electrolytes solutions.
4. Water-electrolyte balance of the organism.
5. Dissociation of water. Ion product of water.
6. pH scale. pH of biological liquids.
7. Theory of acids and bases.
8. Hydrolysis of salts. The degree of hydrolysis. Calculation the pH of salts that undergo hydrolysis.
9. The role of hydrolysis in biochemical processes.

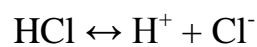
1. Solutions of electrolytes. Information on human body electrolytes.

Compounds whose aqueous solutions conduct electricity are known as electrolytes. Electrolytes are compounds whose molecules are formed by an ionic bond or a polar covalent bond. The process of splitting of the electrolyte molecules into ions under the influence of water molecules is called electrolytic dissociation. This process is a complex physical-chemical interaction of solute (electrolyte) with the solvent (water). It is commonly known that water has a high inductivity (≈ 80),

therefore it influence the weakening of the molecular bonds in the molecules of electrolytes followed by their separation into ions. Ions interact with water molecules to form hydrates. The group of water molecules oriented around an ion is called the hydration shell. The degree of hydration depends on the ionic radius and its charge: the smaller the ionic radius and the higher its charge, the more hydrated the ion. Consider ions having the same charge, the ion is hydrated greater when the smaller is the ionic radius. Thus, Na^+ ion is hydrated more because the specific density of its charge is higher compare to K^+ ion. Dimensions of hydrated ions determine many of their properties: salting-out effect, adsorption capacity, permeability across cell membranes, etc.

One of the properties of electrolytes, which distinguish them from non-electrolytes, is the ability to conduct the electrical current.

The process of electrolytic dissociation is reversible:



The number of positive ions equals to the number of negative ions. Electrolytes, polyelectrolytes, polyampholyte play a significant role in living organisms.

Water-soluble electrolytes (mineral salts) found in various tissues and biological fluids. They help in the osmotic pressure maintenance in cells and body fluids. Thus, they greatly affect the dynamic exchange between cells and intercellular environment.

The pH of biological liquids depends on the concentration of hydrogen and hydroxide ions present in body fluids.

Polyelectrolytes play significant role in living organisms. They are polymers that dissociate in aqueous solution with formation of polycations, polyanions and corresponding to their charge quantity of low molecular weight ions of opposite charge. Polyelectrolyte molecules containing carboxyl groups (pectin, oxidized starch et al.) have acidic properties, those that containe amino group $-\text{NH}_2$ (pyrimidine residue, etc.) have basic properties.

There are also polyampholyte that contain both a carboxyl group and an amino group. In the aqueous solution they dissociate with formation of anions or cations.

Almost all protein compounds (casein, albumin, globulin, and others) have properties of polyampholyte.

Molecules of some substances dissociates completely into ions in aqueous solution. These substances are called strong electrolytes (NaCl , HCl , NaOH , HNO_3 , etc.).

Many other substances (CH_3COOH , NH_4OH , H_2S , etc.) under the same conditions dissociate into ions to a small extent in aqueous solution. This process is reversible and equilibrium is reached after dissociation of a small part of molecules. These substances are called weak electrolytes.

2. The degree of dissociation and dissociation constant for weak electrolytes

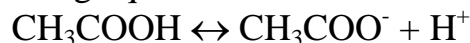
According to the theory of electrolytic dissociation the quantitative characteristic of the electrolyte is the degree of dissociation (α). It is a number of molecules, which have dissociated divided by the total number of molecules in the solution:

$$\alpha = \frac{n_{dis}}{N},$$

where n_{dis} – number of molecules which have dissociated; N – total number of molecules.

All electrolytes by the degree of dissociation are divided into strong electrolytes, moderately weak, and weak electrolytes.

Strong electrolytes have a degree of dissociation ranges from 100 % to 30 %, moderately weak – from 30 % to 3 % in weak electrolytes $\alpha \leq 3$ %. The degree of dissociation increases with rising temperature. It also depends on the nature of the solute, solvent and solute concentration. In addition, the degree of dissociation depends on the presence of other ions in the solution. For example, dilute acetic acid dissociation occurs by the following equation:



When the strong electrolyte NaOH is added to a solution, the concentration of protons reduces due to their interaction with hydroxide ion.

In the process of dissociation of weak electrolyte its molecules exist in the reversible equilibrium between initial molecules and dissociated part. According to Le Chatelier principle the decrease of H^+ ion concentration shifts equilibrium toward increasing concentration, i.e. to the right and the degree of dissociation increases.

Addition of a strong electrolyte to a solution of CH_3COOH solution, which has the same ion such as hydrochloric acid, increases the concentration of protons – the equilibrium shifts to the left, as a result the degree of dissociation decreases. The degree of electrolytic dissociation depends on the concentration: the higher the concentration of the electrolyte, the lower the degree of dissociation and vice versa. In the dilute solution of acetic acid ions are at a distance from each other, forces of electrostatic attraction do not prevent their chaotic motions. So in this case we apply the law of mass action. We write the equation for dissociation constants:

$$K_d = \frac{[\text{H}^+] \cdot [\text{CH}_3\text{COO}^-]}{[\text{CH}_3\text{COOH}]}$$

Electrolytic dissociation constant (K_d) – quantitative characteristic of weak electrolytes, it is the measure of the strength of electrolytes. The higher the dissociation K_d value, the greater ions concentration compare to the concentration of the undissociated electrolyte.

In the dilute solutions K_d is independent of the solute concentration, and depends only on the nature of the electrolyte and temperature.

The relationship between dissociation constant (K_d), the degree of dissociation (α) and the molar concentration of the electrolyte in the solution (s) for binary electrolyte mathematically established W. Ostwald. This is expressed by the equation:

$$K_d = \frac{C \cdot \alpha^2}{1 - \alpha}.$$

For weak electrolytes value of α is very small and therefore $(1 - \alpha) \rightarrow 1$, then the mathematical equation of the Ostwald's law is simplified:

$$K_d = C\alpha^2 \text{ or } \alpha = \sqrt{\frac{K_d}{C}}.$$

W. Ostwald's dilution law: the degree of dissociation of weak binary electrolytes is inversely proportional to the square root of their concentration.

When the concentration decreases, the degree of dissociation increases. At infinitely high dilution the electrolyte dissociates completely ($\alpha = 1$).

3. Properties of strong electrolytes solutions

Strong electrolytes dissociate completely even in concentrated solutions.

The properties of strong electrolytes do not obey the law of mass action. The degree of dissociation and the dissociation constant, which were calculated by equations for weak electrolytes, did not coincide with the experimental data. Strong electrolytes at all dilutions are completely ionized and due to the high ionic concentration the interionic forces (effects) cannot be neglected. Each ion is surrounded by an "atmosphere" of counterions, which creates some ordering in the structure of the solution. The higher the concentration of solution, the denser the "ion atmosphere", and the stronger ionic interactions, which reduce their mobility. This gives the impression that the solution has less quantity of ions than it really has. Therefore, the American scientist G. Lewis suggested using an effective concentration of ions, called the activity (a) instead of the true concentration. *Activity – a real solution concentration*. Also, pH is defined as the negative logarithm of the hydrogen ion activity, not its concentration. The relationship between activity and concentration of a species is expressed by the activity coefficient:

$$\alpha = C \cdot f,$$

where f – an activity coefficient – a value that indicates how much activity (a) is different from the analytical concentration (C), that is the real difference from the ideal solution.

The activity coefficients values can be determined experimentally or calculated theoretically. Many different equations were proposed for its calculation. One of them is the Debye-Huckel equation:

$$\lg \gamma = -0,51 \cdot z^2 \cdot \sqrt{I},$$

where z – charge of ion; I – ionic strength of the solution.

Ionic strength of the solution takes into account the force fields of all ions present in the solution and equals to half of the sum of products molality of each ion by the square of its charge:

$$I = \frac{1}{2} \sum b_i z_i^2.$$

Experimental data shows that the properties of electrolytes in the solution are determined not only by their own ions, but also depend on the presence of different ions. For any electrolyte, whether it is in a solution or mixed with other electrolytes,

applies the ionic strength law: to equal ionic strengths corresponds the equal activity coefficients. Standard solutions that used in medicine are prepared at this time in respect to this law. For example, standard solutions of the following ions Na^+ , K^+ , Ca^{2+} , and others are used for potentiometric determination.

4. Water-electrolyte balance of the organism

The human organism consists of organic substances (proteins, carbohydrates, nucleic acids, etc.) as well as inorganic substances. From all inorganic substances the human body contains the most water (65 %). The mineral content is 5-6 % of body weight. Some minerals exist in dissolved state in biological fluids (electrolytes) and responsible for maintaining homeostasis of the internal environment. Other minerals form compounds with biomacromolecules and low molecular weight compounds.

About two-thirds of the weight of an adult human consists of water. About two-thirds of this water is located within cells, while the remaining third consists of extracellular water, mostly interstitial fluid that bathes the cells, and the blood plasma. The latter, amounting to about 5% of body weight (about 5 L in the adult), serves as a supporting fluid for the blood cells and acts as a means of transporting chemicals between cells and the external environment. The constant dynamic equilibrium between the water intake and output is a necessary condition for living organisms.

Thus, the loss of 2/3 of the extracellular fluids is fatal. Therefore, the imbalance of the body's electrolyte can lead to serious consequences.

The permanent loss of electrolytes through the gastrointestinal tract, kidneys or by sweating continuously replenishes with food and drinking water. Salts maintain osmotic pressure in cells and living environments of the body. They influence the dynamic exchange between the cells and intercellular environment. The main salt that supports osmotic pressure stability is sodium chloride. As the concentration of NaCl decreases the osmotic pressure is significantly reduced, which leads to changes in functions and metabolic processes in the body. The following changes in the cells structure are also possible. When water gets into red blood cells, it leads to hemolysis and blood clotting, which adversely affects the entire dynamics of the blood circulation.

Violation of cations exchange in the body leads to adynamia – muscle weakness, to the standstill or sharp weakening of the motion activity.

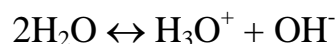
All metabolic processes in living organisms occur under certain pH values. Deviations towards acidity or alkalinity cause dysfunction of enzymes, and as a result chemical reactions behavior. At the same time maintaining the stability pH of biofluids (proton concentration) has the utmost importance for life. Since H^+ ions catalyze many biochemical processes, even small changes in proton concentration in the blood and interstitial fluid substantially affect their osmotic pressure value.

The ability of the body to maintain its optimal concentration of hydrogen ions is one of the most important aspects of homeostasis (homoios – homogenous, permanent; stasis – state).

Hence, water-electrolyte balance is a necessary aspect of homeostasis, which is a prerequisite for the living organism existence.

5. Dissociation of water. Ion product of water

Water is the weak amphoteric electrolyte. Its dissociation occurs by the equation:



or:



The equilibrium constant of this process in this case is the dissociation constant (K_{dys}). According to the law of mass action is:

$$K_{\text{dis}} = \frac{[\text{H}^+] \cdot [\text{OH}^-]}{[\text{H}_2\text{O}]} = 1,8 \cdot 10^{-16} \quad (T = 298^0 \text{ C}).$$

We can neglect the low degree of water ionization due to the extremely small value of the K_{dis} . The equilibrium concentration of undissociated water molecules can be considered constant and equal to the number of moles of water in a 1 liter: $1000/18 = 55,56 \text{ mol/L}$. Applying law of mass action to dissociation equilibrium:

$$K_{\text{dis}} = \frac{[\text{H}^+] \cdot [\text{OH}^-]}{[\text{H}_2\text{O}]} = 1,8 \cdot 10^{-16} \cdot 55,56 = 10^{-14}.$$

Since dissociation of water is too small, undissociated $[\text{H}_2\text{O}]$ can be taken as constant: $K_{\text{dis}} \cdot [\text{H}_2\text{O}] = K_w$. Then for 298 K (25^0 C) we obtain:

$$K_w = K_{\text{dis}} \cdot [\text{H}_2\text{O}] = [\text{H}^+] \cdot [\text{OH}^-] = 10^{-14}.$$

This expression is known as the ion product of water. K_w remains unchanged at constant temperature for water and any aqueous solutions. Such stability means that in any water solution (neutral, acidic or alkaline) there are hydrogen ions and hydroxide ions, and product of their concentrations is always equal to K_w .

For example, hydrochloric acid with a molar concentration $0,01 \text{ mol/L}$ is added to clean water. The hydroxide ions do not disappear from the resulting solution, their concentration is:

$$[\text{OH}^-] = \frac{K_w}{[\text{H}^+]} = \frac{10^{-14}}{10^{-2}} = 10^{-12} \text{ mol/L},$$

i. e. it dropped from 10^{-7} to 10^{-12} mol/L . If we add alkali with molar concentration $0,01 \text{ mol/L}$ to pure water, the $[\text{H}^+] = \frac{10^{-14}}{10^{-2}} = 10^{-12} \text{ mol/L}$. Thus, the concentration of $[\text{H}^+]$ is not zero, it reduced from 10^{-7} to 10^{-12} mol/L . It follows that the concentrations of protons and hydroxide ions are connected.

6. pH scale. pH of biological liquids

In general the molar concentration of hydrogen ions $[\text{H}^+]$ is used to characterize the acidity of the water environment. Since $[\text{H}^+]$ and $[\text{OH}^-]$ ion concentrations are small, for our convenience the logarithmic value pH of hydrogen ions concentrations was introduced by the Danish biochemist Sørensen:

$$\text{pH} = -\lg[\text{H}^+]$$

respectively

$$\text{pOH} = -\lg[\text{OH}^-].$$

pH is a negative logarithm of the molar concentration of H^+ .

The logarithmic form of the ion product of water equation at the temperature of 298 K is:

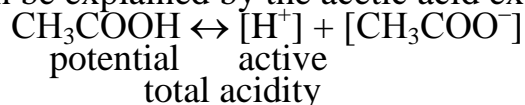
$$\text{pH} + \text{pOH} = 14.$$

In acidic medium $\text{pH} < 7$, $\text{pOH} > 7$; in alkaline medium $\text{pH} > 7$, $\text{pOH} < 7$. There is a relationship between pH and pOH, so it is easier to use only one pH. In neutral medium $\text{pOH} = \text{pH} = 7$.

It is important to distinguish active acidity and total acidity for the evaluation of biological liquids acidity. *Total acidity depends on the content of H^+ ions and undissociated acid molecules.* It can be determined by titration with alkali. *Active acidity depends only on the content of H^+ .* Thus, *pH is a measure of active acidity.*

Potential acidity is a 'reserve' of undissociated acid molecules and can be determine from the total acidity value.

All mention above can be explained by the acetic acid example:



In dilute aqueous solutions (10^{-2} – 10^{-5} mol/ L) strong monobasic acids such as HCl, HNO_3 dissociate almost completely. Water also dissociates with formation of protons: $\text{H}_2\text{O} \leftrightarrow \text{H}^+ + \text{OH}^-$.

However, the concentration of protons obtained under water dissociation can be neglected, because their concentration is much less than protons concentration obtained from the acid dissociation. Thus, the active concentration of protons in solutions of strong monobasic acids is equal to the total (analytical) concentration. By analogy with strong acids solutions it can be considered that:

$$[\text{OH}^-] = C_b,$$

where C_b – molar concentration of a strong base.

Thus, the following formulas are used for calculation pH of strong acids and bases solutions:

$$\begin{aligned} \text{pH} &= -\lg C_a \text{ (for strong acids)} \\ \text{pOH} &= -\lg C_b \text{ (for strong bases)} \\ \text{pH} &= 14 - \text{pOH} = 14 + \lg C_{\text{OH}} \end{aligned}$$

In case concentrated solutions of strong acids the activity coefficients are used for pH calculations.

$$\begin{aligned} \text{pH} &= -\lg \alpha_{\text{H}^+} = -\lg C_a f \\ \text{pOH} &= -\lg \alpha_{\text{OH}^-} = -\lg C_b f \end{aligned}$$

In solutions of weak acids and bases active concentration at the time of dynamic equilibrium is calculated as follows:

$$C_{\text{H}^+} = C_{\text{overall}} \cdot \alpha,$$

where α – degree of dissociation of the electrolyte.

The active concentration of weak acids and bases (if $\alpha \rightarrow 0$, $\alpha < 0,1$) with sufficient accuracy can be calculated knowing the dissociation constant of the electrolyte. Based on the mathematical expression of Ostwald dilution law:

$$\alpha = \sqrt{\frac{K_a}{C_{\text{total}}}},$$

where K_a –dissociation constant of an acid.

Substituting the obtained value for the degree of dissociation α in the formula for calculation the concentration of hydrogen ions in dilute aqueous solutions of weak acids:

$$C_{H^+} = C_{total} \sqrt{\frac{K_a}{C_{total}}} = \sqrt{\frac{K_a}{C_{total}}}$$

Then, taking the log of both sides of the equation:

$$-\lg[C_{H^+}] = -\frac{1}{2} \lg K_a - \lg C_{overall}.$$

and obtain the expression for calculation the pH of dilute aqueous solutions of weak acids:

$$pH = -\frac{1}{2} \lg K_a - \frac{1}{2} \lg C_{overall}.$$

Similarly, we can derive an equation for calculating the $[OH^-]$ in solutions of weak bases:

$$C_{OH^-} = \sqrt{K_b} \cdot C_{total}; \quad pOH = -\frac{1}{2} \lg K_b - \frac{1}{2} \lg C_{overall}.$$

where K_b –dissociation constant base.

$$pH = 14 + \frac{1}{2} \lg K_b + \frac{1}{2} \lg C_{overall}.$$

A monoprotic acid has one proton that can be donated under normal conditions. A diprotic acid has 2, a triprotic 3, etc. A general rule for Bronsted acids is that the first proton to be donated from a multiprotic acid is much more acidic than the second, the second more acidic than the third, etc. In organic acids, the second and third protons may be similar. The conjugate base of the first deprotonation of a diprotic acid is amphoteric, and is both a proton donor and a proton acceptor.

As you know, polyprotic acid dissociates in steps. Analysis of pKa values of phosphoric acid shows that the number of protons, which were formed by dissociation of the second and third steps, makes a significant impact on the equilibrium in the system. Because the first dissociation step is strong, polybasic acid can be considered as monobasic and take into account only the first step of the dissociation. Thus, pH is calculated by pKa₁ of the first dissociation step. Identically calculate the pH of polyprotic bases.

Acids and bases strength that are present in biological objects, determines their behavior, and in some cases properties of biological media. Thus, the strong hydrochloric acid determines the high acidity of gastric juice. Organic derivatives of phosphoric acid, including adenosinetriphosphoric acid (ATP) and nucleic acids are medium-strength acids. This explains the existence of ATP within the cell in dissociated form and determines the ion-ion interaction of DNA with basic proteins that surround this molecule. A large number of mono- and dibasic (acetic, lactic, succinic, etc.) weak acids are common metabolites. Among the number of weak bases in biological media present in trace amounts biogenic amines. Ammonia is produced in the urinary tubules in relatively large quantities. One of the natural amino acids arginine, which contains a fragment of a strong base guanine, shows strong basic properties.

Acidity arises from the concentration of hydrogen ions in solution, and is expresses as pH. The pH value characterizes many vital processes. Cells behavior and their biological activity depend on the pH value. Abnormalities of blood, saliva, and

gastric juice acidity may be a cause of serious diseases. The normal pH of blood plasma is 7,35-7,45. Body waste products are constantly being produced, and affecting the pH of blood.

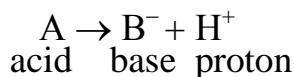
Increase (alkalosis) or decrease (acidosis) pH indicates pathological processes. For example, acidosis occurs in diabetes, coronary heart disease, and myocardial infarction.

The normal range for gastric juice pH is 1,2-3,0. If the pH of gastric juice is less than 1.2 – hyperacidity if the pH of gastric juice is more than 3.0 – hypoacidity. Acidity at pH \approx 6 or more evaluates as “zero acidity”.

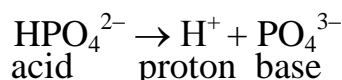
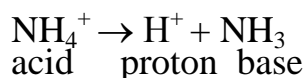
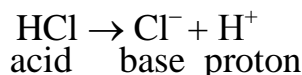
The pH affects the activity of enzymes, protein stability, and the rate of many biochemical processes involving ions. For example, gastric juice pepsin active at pH 1,5-2,0, blood catalase at pH \approx 7, tissue cathepsins catalyze the protein synthesis at pH \approx 7, and its splitting in the acidic medium. Thus, stability of body fluids pH is a guaranty of normal vital activities of the organism. Water and its ionic dissociation products (hydronium ions and hydroxyl ions) are essential factors that determine the structure and biological properties of proteins, nucleic acids and other cellular components.

7. Theory of acids and bases

In 1923, I. N. Bronsted and T. M. Lowry almost simultaneously proposed the protolytic theory of acids and bases. According to this theory, an acid is a substance donating proton in a reaction. A base is a substance accepting the proton in a reaction:

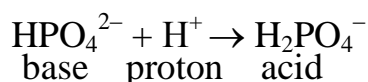
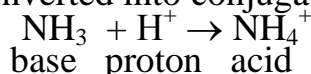


For example:



A substance that acts as an acid, giving a proton is converted into conjugate base. This conjugate base does not have to contain a hydroxyl group, and representatives of acids and bases can be not only neutral molecules, but also ions.

Base attaching a proton is converted into conjugate acid:



During the acid base interaction (neutralization reaction) we obtain an acid-base pair.

The strength of an acid depends on the ability to donate a proton. The strength of a base – to attach it.

Strong acids easily donate a proton and form weak conjugate bases that are poor at accepting proton. Therefore, the dissociation of strong acids is practically irreversible.

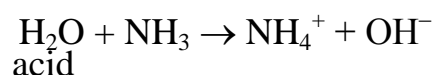
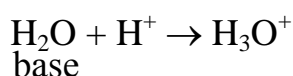
In contrast, weak acids hardly lose a proton and form strong conjugate bases. The dissociation of these acids is a reversible process and the equilibrium shifts towards undissociated form (reactants).

Product of conjugate acid must be weaker and less reactive than the starting acid.

Product of conjugate base must be weaker and less reactive than the starting base.

The same patterns are observed in the case of strong and weak bases.

Some substances can act as acids or bases. Such substances are known as amphiprotic substances, or ampholytes. Water is an ampholyte, so it can act as either an acid or a base:



Typical ampholytes are hydroxides of some metals, hydroanions of polybasic acids and amino acids.

The protolytic equilibrium is established in solutions as a result of the competition for a proton between bases of acid-base conjugate pairs (AH, A and BH⁺, B). The equilibrium shifts towards the formation of a weaker acid. The direction of an acid–base reaction always favors formation of the weaker acid and weaker base.

As you know, the strength of acids and bases of weak electrolytes is determined by the acidity constants K_a or K_b basicity constant. If these constants describe the protolytic interaction of water with an acid or a base, the product of these constants for conjugate acid-base pair is always equal to the ion product of water K_w.

$$K_a(\text{HA}) \cdot K_b(\text{A}^-) = 10^{-14} \text{ or } \text{p}K_a(\text{HA}) + \text{p}K_b(\text{A}^-) = 14$$

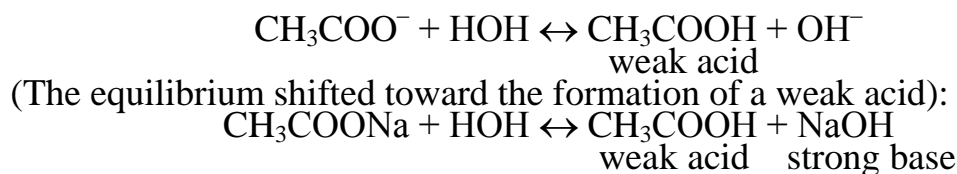
$$K_a(\text{BH}^+) \cdot K_b(\text{B}) = 10^{-14} \text{ or } \text{p}K_a(\text{BH}^+) + \text{p}K_b(\text{B}) = 14$$

Therefore, basicity constant K_b of a weak base can be replaced by an acidity constant K_a of conjugate acid of this base. For example, the strength of ammonia in water as the base (pK_b = 4,76) can be characterized by K_a of its conjugate acid, i.e. ammonium ion – pK_a(NH₄⁺) = 14 – 4,76 = 9,75. The smaller the pK_a value of the conjugate acid, the weaker the conjugate base is. Stronger acids (larger K_a) have smaller pK_a. Weaker acids (smaller K_a) have larger pK_a.

8. Hydrolysis of salts. The degree of hydrolysis.

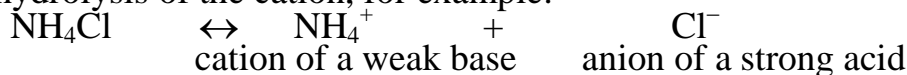
Calculation the pH of salts that undergo hydrolysis

Imbalance of water ionization equilibrium can occur not only by dissolution of acids or bases, but also some salts. The change of the acidity of the solution is caused by the nature of the salt ions. This can be explained in terms of the acidity or basicity of individual ions in the solution.

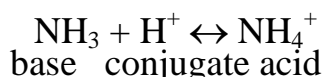


Thus, the anion hydrolysis of the salt produces an alkaline water solution.

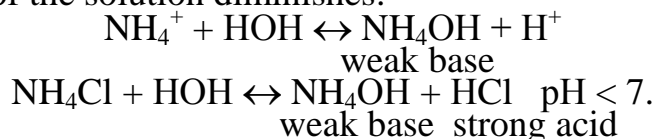
2. Salt made of the cation of a weak base and the anion of a strong acid is acidic because of hydrolysis of the cation, for example:



Cation NH_4^+ is a conjugate acid formed from ammonia (NH_3) by attaching a proton:



In the solution NH_4^+ cation hydrolyzes to give a weak base and H^+ ions. The water dissociation equilibrium shifts to the acidic medium. Proton H^+ concentration increases so the pH of the solution diminishes.

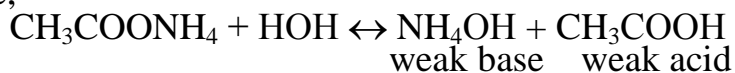


The balance shifted in the forward direction so hydrolysis reaction counteracts to neutralization reaction, which proceed to completion.

As seen from the above, depending on the nature of the salt ion, water acts as an acid or a base.

It is also possible when cation and anion both undergo hydrolysis, in case when the salt formed by the cation of a weak base and the anion of a weak acid. Whether the solution is acidic or basic depends on the relative acid–base strengths of two ions. To determine this, you need to compare the K_a of the cation with the K_b of the anion. If the K_a is larger, the solution is acidic. If the K_b is larger, the solution is basic. If an acid and a weak base close to the strengths ($K_a \approx K_b$), the solution pH may be close to neutral ($\text{pH} \approx 7$).

For example,



As $K_{\text{NH}_4\text{OH}} \approx K_{\text{CH}_3\text{COOH}}$, then the pH of the resulting solution will be approximately equal to seven.

The intensity of any reversible process is characterized by the corresponding equilibrium constants. In the case of salts hydrolysis it is called hydrolysis constant (K_{hydr}).

In case of the hydrolysis of salts formed by anions of weak acids and strong bases residues (anion hydrolysis):

$$K_{eq} = \frac{[\text{HA}] \cdot [\text{OH}^-]}{[\text{A}^-] \cdot [\text{H}_2\text{O}]}$$

Anions in this case are conjugate bases of water. In the competition with water molecules for the proton, they form a weak acid.

The concentration of water for sufficiently dilute solutions can be considered almost constant value, so

$$K \cdot [H_2O] = K_{hydr.} = \frac{[HA] \cdot [OH^-]}{[A^-]}.$$

The multiplication product of the hydrolysis equilibrium constant and the molar concentration of water is called hydrolysis constant.

Express the concentration of hydroxyl ions through the ion product of water:

$$[OH^-] = \frac{K_w}{[H^+]}$$

Substituting in the formula for calculationg the hydrolysis constants:

$$K_{hydr.} = \frac{[HA] \cdot K_w}{[A^-] \cdot [H^+]}$$

The ratio $\frac{[HA] \cdot K_w}{[A^-] \cdot [H^+]} = \frac{1}{K_a}$.

We can see that the hydrolysis constant of salts formed by a weak acid and a strong base (anion hydrolysis) is inversely proportional to the dissociation constant of an acid formed in the hydrolysis:

$$K_{hydr.} = \frac{K_w}{K_a}$$

the product of K_{hydr} and the corresponding value of K_a is the ion product of water:

$$K_{hydr.} \cdot K_a = K_w.$$

A similar conclusion can be made after studying the hydrolysis of salts formed by weak bases and strong acids (cation hydrolysis):

$$K_{hydr.} = \frac{K_w}{K_b} \text{ or } K_{hydr.} \cdot K_b = K_w.$$

Thus, the hydrolysis constant is the ratio of the ion product of water (K_w) to a ionization constant (K_a or K_b) of weak electrolyte formed as a result of hydrolysis. Salts formed by weak electrolytes undergo hydrolysis better.

The degree of hydrolysis increases upon the application of heat, because this increases the K_{hydr} value in dilution, due to the raise of the water concentration.

Hydrolysis constant of salts formed by a weak acid and a weak base is:

$$K_{hydr.} = \frac{K_w}{K_a \cdot K_b}$$

The degree of hydrolysis β – is the ratio of the concentration of the hydrolyzed part of the molecules to the total concentration of the particular salt in solution.

The degree of hydrolysis is associated with a hydrolysis constant and salt concentration by the ratio similar to the law of dilution:

$$K_{hydr.} = \frac{\beta^2 C_c}{1 - \beta}$$

if $\beta \leq 1$, then $K_{hydr.} = \beta^2 \cdot C_c$:

$$\beta = \sqrt{\frac{K_{hydr.}}{C_c}}.$$

Knowing this ratio it is easy to deduce the equation for calculating the pH of salt solutions undergoing hydrolysis. In the case of cation hydrolysis of salt:

$$[H^+] = C_c \cdot \beta = C_c \cdot \sqrt{\frac{K_w}{K_b \cdot C_c}} = \sqrt{\frac{K_w \cdot C_c}{K_b}}$$

or in logarithmic form:

$$pH = \frac{1}{2} pK_w - \frac{1}{2} \lg C_c - \frac{1}{2} pK_b$$

The anion hydrolysis of salt solution produce basic medium:

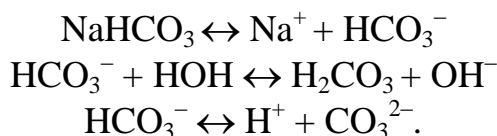
or in logarithmic form:

$$pOH = \frac{1}{2} pK_w - \frac{1}{2} \lg C_c - \frac{1}{2} pK_a.$$

Accordingly, the pH of this solution can be calculated by the following equation:

$$pH = 14 - \frac{1}{2} pK_w + \frac{1}{2} \lg C_c + \frac{1}{2} pK_a = 7 + \frac{1}{2} \lg C_c + \frac{1}{2} pK_a.$$

Hydrolysis of the acidic salts of weak polyprotic acids is a complicated process due to the dissociation of an additional anion. Consider hydrolysis of the bicarbonate ion:



As seen from the equation, bicarbonate ion simultaneously involves in two processes. Hydrolysis increases the concentration of hydroxyl ions and dissociation – hydrogen ions. The pH of the acidic salts solution depends on the intensities of these two processes define by the equilibrium constant and does not depend on the salt concentration. For solutions of salts of diprotic acids, in case when K_{a_1} much less than C_c , acidity can be determined using the following formulas:

$$\begin{aligned} [H^+] &= \sqrt{K_{a_1} \cdot K_{a_2}} \\ pH &= pK_{a_1} + pK_{a_2}. \end{aligned}$$

As you can see from this formula, the pH of acidic salt that hydrolyze significantly does not depend on its concentration, which is consistent with experimental data.

In more complex cases, such as acidic salts of phosphoric acid, an acid dissociation constant is included in a similar formula, which determines the salt hydrolysis constant, and the second that characterize the dissociation of the anion. For example, in the case of hydrolysis dihydrogen phosphate ion the acidity of the medium will be determined by the following formula:

$$pH = \frac{pK_{a_2} + pK_{a_3}}{2},$$

where K_{a_2} – the constant that characterizes the dissociation of the ion and K_{a_3} – constant that determines its hydrolysis.

Solution of acidic salts may have a different pH of the solution. Thus, the solution of sodium hydrogen phosphate yields an alkaline medium, and dihydrogen phosphate solution – acidic.

A solution of sodium bicarbonate – baking soda – slightly alkaline and is used in medicine (e.g. to prevent acidosis) as opposed to technical soda – sodium carbonate, the solution of which is strongly alkaline.

The blood plasma includes soluble salts of carbonic acid. Based on the mention above, it can be stated that due to the strong hydrolysis carbonate ions will not be held and bicarbonate ion is the main component of plasma. Similarly, from all number of phosphate ions, which are components of intracellular fluid, we can neglect the presence of phosphate ions, giving preference to hydrogen phosphate and dihydrogen phosphate ions. If salts undergo cation and anion hydrolysis, the reaction medium is determined by the nature of the strongest electrolyte. Hydrolysis constant and pH in this case is determined by the following formulas:

$$K_{hydr.} = \frac{K_w}{K_a \cdot K_b}$$
$$pH = \frac{pK_a(HA) + pK_a(BH^+)}{2}$$

9. The role of hydrolysis in biochemical processes.

Hydrolytic processes play an important role in metabolism. Biologically active substances such as biopolymers (proteins, fats, nucleic acids, polysaccharides) undergo hydrolysis in the organism. Produced substances are used in the biosynthesis of specific biopolymers. Some of these substances are oxidized in the Krebs cycle serving as a source of energy necessary for biochemical processes. Macroergic compounds such as ATP, GTP also undergo hydrolysis in the body. Breaking phosphoanhydride bond releases energy that spent on all processes occurring in the body from protein synthesis to muscle contraction and electrical activity of nerve cells. The hydrolysis of biologically active substances plays an important role in digestion, in redox processes, in the action of buffer systems.

The action of many chemical therapeutic agents based on their acid-base properties and their tendency to hydrolysis. The possibility of simultaneous prescription of several drugs to the patient is related to these properties.

Since kidneys and lungs are involved into the regulation of acid-base balance, we distinguish metabolic and respiratory acidosis and alkalosis.

Metabolic acidosis – metabolic disorder, when blood pH is low due to the following reasons:

a) administration or excessive formation of stable acids (production of ketoacids in uncontrolled diabetes, fasting or kidney failure, an increased production of lactic acid during the shock, an increased production of sulfuric acid in the process of decomposition of biomolecules, etc.);

b) incomplete elimination of acids in renal failure;

c) excessive loss of HCO_3^- caused by diarrhea, colitis, intestinal ulcer.

Metabolic alkalosis – metabolic disorder, which leads to an increase of blood pH due to the following reasons:

- a) loss of H^+ (prolonged vomiting, intestinal obstruction);
- b) excessive bicarbonate HCO_3^- administration (dehydration, excessive intake of HCO_3^- during metabolic acidosis, excretion of the following organic acids salts: lactic, acetic, citric, etc. which attach H^+).

Respiratory acidosis – uncompensated or partially compensated decrease of blood's pH due to the hypoventilation:

- a) lung and respiratory tract disorders (pneumonia, pulmonary edema, foreign body in the upper respiratory tracts);
- b) weakness of the respiratory muscles;
- c) suppression of the respiratory reflexes by drugs, narcotics, sedatives or anesthetics.

Respiratory alkalosis – uncompensated or partially compensated elevation of blood's pH due to the hyperventilation, fever or hysteria.

For the correction of acid-base equilibrium at acidosis 4 % sodium hydrocarbonate solution, at alkalosis – 5 % solution of ascorbic acid is administered by intravenous injection.

LABORATORY WORK

Experiment 1. pH determination in electrolyte solutions using indicators methyl orange and phenolphthalein.

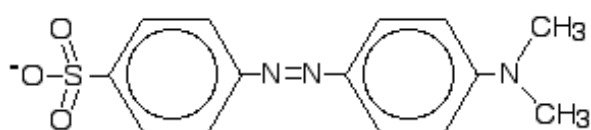
Summary of the method:

One of the most common methods to determine pH of solution is to use an acid base indicator. Acid-base indicators are organic substances that change their color (two-color indicators) or its intensity (monochrome indicators) when the pH is changes. Most of the indicators are themselves weak acids. At a low pH, a weak acid indicator is almost entirely in the HIn form, the color of which predominates. As the pH increases, the intensity of the color of HIn decreases and the equilibrium is pushed to the right. Therefore, the intensity of the color of ionic form In^- increases. An indicator is the most effective when the color change is distinct and over a small pH range. For most indicators the color change range is within ± 1 of the pK_{In} value.

A **universal indicator** is a mixture of indicators which give a gradual change in color over a wide pH range. The pH of a solution can be approximately identified when a few drops of universal indicator are mixed with the solution.

Methyl Orange

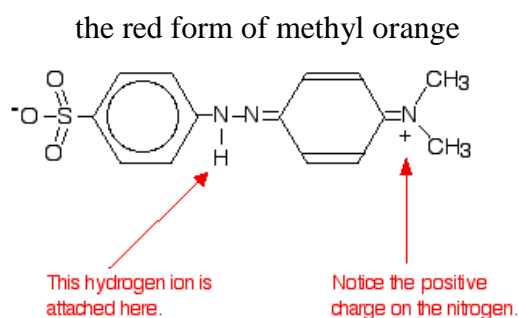
In acidic medium, methyl orange exists in quinonoid form and is red in color whereas in basic medium it exists in benzenoid form and is yellow in color. The methyl orange structure in an alkaline solution is shown below:



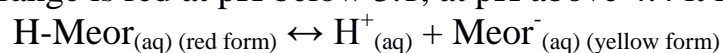
the yellow form of methyl orange

It would be obvious to think that when you add an acid, the hydrogen ion would be picked up by the negatively charged oxygen.

In fact, the hydrogen ion attaches to one of the nitrogens in the nitrogen-nitrogen double bond to give the following structure:

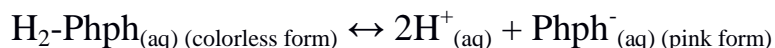


There is equilibrium between two forms of methyl orange, but the colours are different. Methylorange is red at pH below 3.1, at pH above 4.4 it is yellow.



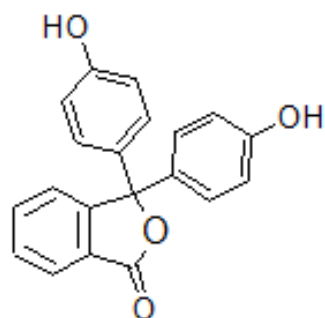
Phenolphthalein

Phenolphthalein is a weak acid used as an acid-base indicator.

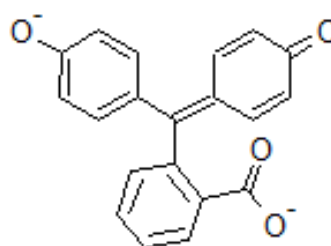


It is colourless in the form of the weak acid and its ionic form is bright pink. Addition of extra hydrogen ions shifts the position of equilibrium to the left, and turns the indicator colourless. Addition of hydroxide ions shifts the equilibrium to the right turning the indicator pink. The transition occurring around pH = 9.

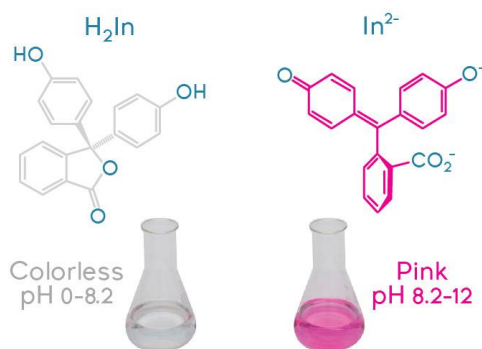
Phenolphthalein structure contains a central 5 membered ring which is somewhat strained. In basic medium the In^- structure opens up and becomes flatter. This allows the electrons more freedom, and the molecule's absorption spectrum now transmits red light. Thus, there is a change in color caused by the change in molecular shape.



Colorless Form



Colored (Pink) Form



Algorithm of the laboratory work

Place 20 drops of each of the following solutions into the test tubes: acetic acid, sodium hydroxide, ammonium hydroxide and water. Add 2 drops of every indicator: methyl orange and phenolphthalein to each of these tubes. Observe colour changing in the solution.

Fill the rapport; record your observations and conclusions into “Work book”.

Experiment 2. Apply 1 drop of every solution, mentioned in experiment 1, on the strip of universal indicator paper. Record changing of coloration and pH value according to the scale of universal indicator.

Fill the rapport; record your observations and conclusions into “Work book”.

8. Tasks for knowledge control

1. Which of the following acids refers to the strong electrolytes:

- A. HNO_2 B. CH_3COOH C. $\text{CH}_2\text{OHCH}_2\text{COOH}$ D. H_2SO_4

2. Determine the pH of a solution of lactic acid if $C = 0,05 \text{ mol/L}$, and constant lactic acid is $1,8 \cdot 10^{-4}$.

- A. 4,58 B. 1,2 C. 2,52 D. 6,84

3. pH of gastric juice is 2. Determine the concentration of hydrogen ions.

- A. 10^{-3} B. 10^{-2} C. 10^{-5} D. 10^{-7}

Answers 1 – D, 2 – C, 3 – B

9. Recommendations for the work results design

Algorithms for solving educational problems of class work and self-work should be recorded in the workbook.

Навчальне видання

**Кислотно-основна рівновага в організмі.
Водневий показник біологічних рідин.**

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

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Комп'ютерний набір та верстка Левашова О.Л.

Ризографія.

Умов. др. арк., тираж 200 прим.

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