Subject 4. Acid-base equilibrium and pH of biological fluids

1. Importance

The biological fluids of the organism – blood, lymph, gastric juice, urine, saliva etc. normally have different pH values. The pH of biological fluids effects activities of enzymes and hormones that regulate biochemical transformations in cells, tissues, organs. Changes of composition of biological fluids, including changes of pH, characterize abnormalities of organ functioning. Changes of blood pH disrupt structure and functioning of enzymes and hormones and therefore cause disruptions of metabolic regulation, accumulation of incompletely oxidized toxic products, poisoning and, ultimately, death. Monitoring pH helps to reveal pathologies and provide correct disease prevention and treatment.

The potentiometric method of pH measurement is widely applied in biology, medicine and pharmacy. Compared to the indicator method it is more accurate (the accuracy of measurements is 0.02 -0.05 pH units) and renders it possible to measure pH of multicomponent systems and colored solutions.

Competences

Capacity for abstract thinking, analysis and synthesis, capacity to learn.

Ability to apply knowledge in practical situations.

Know standard methods of physical and chemical (laboratory and instrumental) studies of biological systems of the human and the environment. Be able to analyze and evaluate results of physicochemical (laboratory and instrumental) studies of biological systems in the organism and the environment. Be responsible for decisions taken on the basis of evaluation of results of physicochemical (laboratory and instrumental) studies of biological systems of the organism and the environment.

Ability to assess and ensure quality of work.

Ability to communicate in native language both orally and in writing.

Use native language in professional and business communications and preparation of documents.

Ability to choose strategy of communication; ability to work in a team; skills of interpersonal interaction.

Ability to exercise healthy life style.

2. Concrete aims

Measure pH with indicators and a pH-meter.

Analyze the principle of the method of potentiometry and draw conclusions about its applications in medical and biological research.

Make conclusions about acidity of biological fluids from their pH values.

3. Basic knowledge, skills necessary for studying the subject (interdisciplinary integration)

Previous subjects	Obtained skills

1. Biological and bioorganic chemistry	Use theories of acids and bases; know formulas, names and properties of acids and bases. Understand acidity and basicity. Apply the formula of dissociation constant, show examples of dissociation constant. Understand hydrolysis of organic substances. Apply an equation of dissociation of acetic acid.
2. Anatomy	Know about formation of HCl in the stomach.
3. Histology, cytology and embryology	Know about formation of HCl in the stomach; removal of electrolytes by the kidney.
4. Foreign language for professional purposes	Possess basic knowledge of foreign languages. Be able to communicate in a foreign language. Use a foreign language in professional activity.
5. Latin language and medical therminology	Master medical therminology in Latin language.
6. Safety, basics of bioethics and biosafety	Be able to exercise self-control, healthy lifestyle, be able to adapt to and act in new situations. Strive to protect the environment.

4. Tasks for independent work during preparation for the class and in class.

4.1. The list of key terms, parameters, characteristics which the student is to learn while preparing for classes:

Term	Definition
1. Electrolytes: a) strong; b) weak.	**Electrolytes** are substances that conduct electric current both molten and in solutions. - dissociation degree of **strong electrolytes** is close to 1 or 100% (> 30 %): (HCl, H_2SO_4, NaOH etc.); - dissociation degree of **weak electrolytes** is < 3 % (H_2S, HCN etc.).
2. Ionic product of water	**Ionic product of water** is the product of concentrations of H^+ i OH^- : K (H_2O) = [H^+] · [OH^-] = 10^{-14} mol^2/L^2.
3. pH	**pH** is the negative logarithm of molarity of hydrogen ions.
4. Normal pH: a) blood plasma; б) saliva.	pH of blood plasma is 7.36 ± 0.04. pH of saliva is 5.6 − 7.9
5. Neutralization reaction.	**Neutralization reaction** is a reaction of an acid and a base in which salt and water are obtained.
6. Hydrolysis of salts.	**Hydrolysis of a salt** is a reaction of a salt and

	water in which weak electrolytes are obtained.

4.2. Theoretical questions for the lesson:

1. Electrolytes in the organism. Dissociation degree and dissociation constant of weak electrolytes. Properties of solutions of strong electrolytes.
2. Water dissociation. Ionic product of water.
3. pH. Normal and pathological pH values in biological fluids.
4. Types of protolytic reactions. Neutralization, hydrolysis and ionisation reactions.
5. Hydrolysis of salts.
6. Degree of hydrolysis, its dependence on concentration and temperature.
7. Hydrolysis constant.

4.3. Practical work (task) done by students in class

Measurement of pH of biological fluids

Potentiometric measurement of pH is done with an ionometer EV-74.

1. Preparation of the instrument for work

The instrument is prepared as following:

– press the buttons "t" and "-1-19", plug the instrument in and turn it on; warm it up for 30 minutes;

– compose a galvanic chain with a measurement electrode and a comparison electrode.

The measurement electrode is the glass electrode ESL – 43-07 with the hydrogen function. The comparison electrode is the chlorine silver electrode with a constant potential (0.201 ± 0.003V). Fix the electrodes in the holder. Plug them in the respective sockets on the back of the instrument.

The ionometer is standartized with control buffer solutions according to the instruction manual.

2. Measurement of pH of biological fluids

A measurement of pH of a biological fluid is done as following:

– immerse electrodes in a cup with a biological fluid to create a galvanic circle;
– press the buttons "anions/cations", "pX" and the general range "-1-19"**.** Do not press the button "X/X", because the ions to be measured are monovalent;
– determine an approximate pH value on the general range scale;
– press the button of the range which corresponds to the approximate pH value and determine the exact pH value on the scale of the range.

3. Processing of results and writing a report of the laboratory work

1) Fill in Table 5 with the obtained values of pH of biological fluids.
2) Calculate the activity of hydrogen cations a_{H^+} with the values of pH:

$$pH = - \lg a_{H^+}$$

Table 5

№ of measu-rement	Biological fluid	pH	a_{H^+}, mol/L	K (H_2O)	a_{OH^-}, mol/L	pOH	Medium
1							
2							
3							

3) As far as in all water solutions (and biological fluids as well) the ionic product of water K(H_2O) is constant, we can find the activity of hydroxyl ions with the formula (at the standard temperature):

$$K(H_2O) = a_{H^+} \cdot a_{OH^-} = 10^{-14},$$

$$a_{OH^-} = \frac{10^{-14}}{a_{H^+}}.$$

4) Calculate pOH with the formula:

$$pOH = -\lg a_{OH^-}$$

5) Fill in the chart with the results.

2. Determination of pH using indicators:

With the pH values make conclusions about the acidity of the medium in each of the biological fluids.

Contents of the subject (abstract):

1. Electrolytes in the organism. Dissociation degree and dissociation constant of weak electrolytes. Properties of solutions of strong electrolytes.

Electrolytes are substances that conduct electric current both molten and in solutions. These are inorganic and organic acids, bases and salts, e.g., KCl, NaCl, LiF, $CaCl_2$, KOH, NaOH.

Concentration of cations, mostly macroelements Na^+, K^+, Ca^{2+}, Mg^{2+} in blood plasma is 154 mmol/L. Concentration of inorganic anions Cl^-, HCO_3^-, $H_2PO_4^-$, HPO_4^{2-} and SO_4^{2-} is 133 mmol/L, concentration of anions of organic acids and macroions of proteins is 21 mmol/L.

Electrolytic dissociation is splitting of a compound into ions upon dissolving in water.

Degree of dissociation of electrolyte is the ratio of the number of dissociated molecules to the total number of molecules of the electrolyte.

Strong electrolytes (HCl, H_2SO_4, NaOH etc.) – $\alpha > 0.3(30\%)$; weak electrolytes (H_2S, HCN etc.) – $\alpha < 0.03(3\%)$.

The formula of Ostwald's law of dilution for weak electrolytes:

$$K_Д = \alpha^2 C,$$

therefore $\alpha = \sqrt{\frac{K_Д}{C}}$.

The definition of Ostwald's law of dilution:

Degree of dissociation of a weak binary electrolyte increases upon its dilution.

Strong electrolytes

For concentrated solutions of strong electrolytes activity of ions is used instead of their concentrations.

Activity of ions is their effective concentration that is found at certain physical and chemical conditions.

2. Dissociation of water. Ionic product of water.

A simplified equation of dissociation of water: $H_2O \rightleftarrows H^+ + OH^-$.

The product of concentrations of ions H^+ and OH^- is a constant value at a certain temperature and is called **ionic product of water K (H_2O):**

$K(H_2O) = [H^+] \cdot [OH^-] = 10^{-14}$ mol^2/L^2 (t = 25° C).

3. Power of hydrogen or pH. Normal and pathological pH values in biological fluids.

pH is the negative logarithm of molarity of hydrogen ions**.**

$pH = -\lg [H^+]$.

Normal pH of blood plasma is 7.36 ± 0.04.

Зміна pH крові порушує структуру й функції ферментів і гормонів, що порушує регуляцію обміну речовин, викликає накопичення недоокислених токсичних продуктів, отруєння і може призвести до смерті.

Shifts of blood pH alters structure and function of enzymes and hormones, thus disrupting regulation of metabolism and causing accumulation of toxic semioxidized products, poisoning, and can lead to death

Normal pH of saliva is 5.6 – 7.9

Shift of saliva pH towards more acidity causes development of caries, and shift of saliva pH to alkalinity causes development of calculus.

4. Types of protolytic reactions. Neutralization, hydrolysis and ionisation reactions.

1. Neutralization reaction: $HCl + NaOH \rightleftarrows NaCl + H_2O$.
2. Hydrolysis of salts is an interaction of salt and water.
3. Ionization reaction: $CH_3COOH + H_2O \rightleftarrows CH_3COO^- + H_3O^+$.

5. Hydrolysis of salts

1. $CH_3COOK + H_2O \rightleftarrows CH_3COOH + KOH$.
2. $NH_4Cl + H_2O \rightleftarrows NH_4OH + HCl$.
3. $CH_3COONH_4 + HOH \rightleftarrows CH_3COOH + NH_4OH$;

6. Degree of hydrolysis, its dependence on concentration and temperature

$$\alpha_{\text{hydrolysis}} = \frac{n_{\text{hydrolysis}}}{n_{\text{total}}} \quad \text{або} \quad \alpha_{\text{hydrolysis}} = \frac{C_{\text{hydrolysis}}}{C_{\text{total}}},$$

The degree of hydrolysis increases with an increase of salt concentration in the solution.

As the temperature increases the degree of hydrolysis also increases.

7. Hydrolysis constant

$$K_{\text{hydrolysis}} = \frac{K_{H_2O}}{K_a}, \qquad K_{\text{hydrolysis}} = \frac{K_{H_2O}}{K_b}, \qquad K_{\text{hydrolysis}} = \frac{K_{H_2O}}{K_a \cdot K_b}.$$

Materials for self control:

A. Tasks for self control:

1. The relationship between pH and pOH in a solution is:

a) pH = pOH; b) pH+ pOH = 14; c) pH · pOH = 7; d) pH/pOH = 1

2. Choose the correct short ionic equation of the reaction of hydrolysis of sodium carbonate:

a) $Na^+ + H_2O = NaOH + H^+$; c) $Na^+ + OH^- = NaOH$;

b) $CO_3^{2-} + 2H^+ = H_2CO_3$; d) $CO_3^{2-} + H_2O = HCO_3^- + OH^-$.

B. Practical tasks for self control:

1. Effect of alteration of pH in biological liquids:

a) depends on the liquid;

b) with increased pH processes become faster;

c) with increased pH processes become slower, with decreased pH processes become faster;

d) altered pH can change the rate of a process or stop it completely.

2. Why does pH decrease in a zone of inflammation?

a) organic acids as products of non-complete oxidation are formed;

b) metabolic products are evacuated slower;

c) oxidation is supressed and reduction is promoted;

d) pH does not change because it does not depend on the direction of metabolic processes.

3. Choose the group of salts that can be hydrolyzed upon dissolving in water.

a) sodium chloride, ammonium sulfate;

b) potassium cyanide, sodium carbonate;

c) potassium nitrate, calcium acetate;

d) ammonium acetate, sodium sulfate.

4. What type of pH is obtained in dissolving of zinc sulfate in water.

a) acidic; b) basic; c) neutral; d) depends on the concentration of the salt.

5. Explain effect of temperature and salt concentration on hydrolysis degree.

a) hydrolysis degree does not depend of temperature and concentration;

b) hydrolysis degree increases with increase of temperature and concentration;

c) hydrolysis degree decreases with increase of temperature and concentration;

d) hydrolysis degree decreases with increase of concentration and increases with increase of temperature.

Literature

Main:

1. Medical Chemistry: textbook / V.O. Kalibabchuk, V.I. Halynska, V.I. Hryshchenko et al.; edited by Prof. V.O. Kalibabchuk – Kyiv: "Medicine", 2010 –

224 p. (P. 70 – 80).

Informational resources:

2. www. pdmu, edu.ua
https://med-chemistry.pdmu.edu.ua/

(Web page of Poltava State Medical University).