МІНІСТЕРСТВО ОХОРОНИ ЗДОРОВ’Я УКРАЇНИ
ХАРКІВСЬКИЙ НАЦІОНАЛЬНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ

BUFFER SYSTEMS, THEIR COMPOSITION, PROPERTIES. BUFFER SYSTEMS OF THE ORGANISM

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

БУФЕРНІ СИСТЕМИ, ЇХ СКЛАД, ВЛАСТИВОСТІ. БУФЕРНІ СИСТЕМИ ОРГАНІЗМУ

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

Затверджено
Вченою радою ХНМУ
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Buffer systems, their composition, properties. Buffer systems of the organism: methodical instructions for 1st year students' self-work in Medical Chemistry / compiled by A.O. Syrovaya, V.N. Petyunina, M.A. Vodolazhenko et al. – Kharkiv: KhNMU, 2017. – 16 p.

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Буферні системи, їх склад, властивості. Буферні системи організму: метод. вказ. для самостійної роботи студентів1-го курсу з медичної хімії / уклад. Г.О. Сирова, В.М. Петюніна, М.О. Водолаженко та ін. – Харьков: ХНМУ, 2017. – 16 с.

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Subject "Buffer systems, their composition, properties. Buffer systems of the organism"

1. **Number of hours** 4
2. **Material and methodological support.**

Multimedia support (presentations, scientific films)

Tables:

1. Graph structure of the subject.
2. Classification of buffer solutions.
3. Study the Henderson–Hasselbalch equation for calculation the pH of buffer solutions.
4. Buffer systems of the organism.

Educational literature:

1. Medical chemistry: textbook / V.O. Kalibabchuk, V.I. Halynska, L.I. Hryshchenko et al. – Kyiv : AUS Medicine Publishing, 2017. – 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. Buffer systems, their composition, properties. Buffer systems of the organism. – Methodical instructions for 1st year students' self-work in Medical Chemistry / complieted by A.O. Syrovaya, V.N. Petyunina, M.A. Vodolazhenko et. al. – Kharkiv: KhNMU, 2017. – 16 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.

Laboratory glassware and reagents for laboratory work "Investigation of the properties of buffer solutions by the potentiometric technique" (stand rods with burette, solutions 0,2M CH_3COOH and CH_3COONa, 0,2M NaH_2PO_4 and Na_2HPO_4, pH-meter, solutions 0,01M HCl and NaOH, pipettes, 50 ml chemical beakers.

3. **Substantiation of the subject.** The pH value of the internal media of the organism is characterized by a significant constancy and stability. It is of great importance, since hydrogen ions have a catalytical effect on many biochemical transformations. Hormones and enzymes are active only at a certain pH value. Even slight changes in hydrogen ion concentration in the blood and intertissue liquid noticeably affect the value the osmotic pressure of these liquids. In normal state, the organism well manages the acid-base variations and maintains the constancy of internal pH environments using physiological (kidneys, liver, lungs, intestines) and physico-chemical (buffer systems of the body) compensation mechanisms.

4. **The purpose of the subject**:

- general: understand the concept of hydrogen balance, to study the mechanism of buffer action and their role in maintaining the acid-base equilibrium in biological systems;

- specific: to explain the mechanism of buffer action of main biological buffer systems and their role in maintaining the acid-base equilibrium in biological systems;

a) **to know:** classification of buffers according to their composition, the mechanism of buffer action of the organism buffer systems and their relationship; buffer capacity and factors it depends on;

b) **to be able to:** to characterize the mechanism of buffer action of the organism buffer systems and their relationship; to explain the effect of the concentration and ratio of the components on the pH and buffer capacity of buffer solutions;

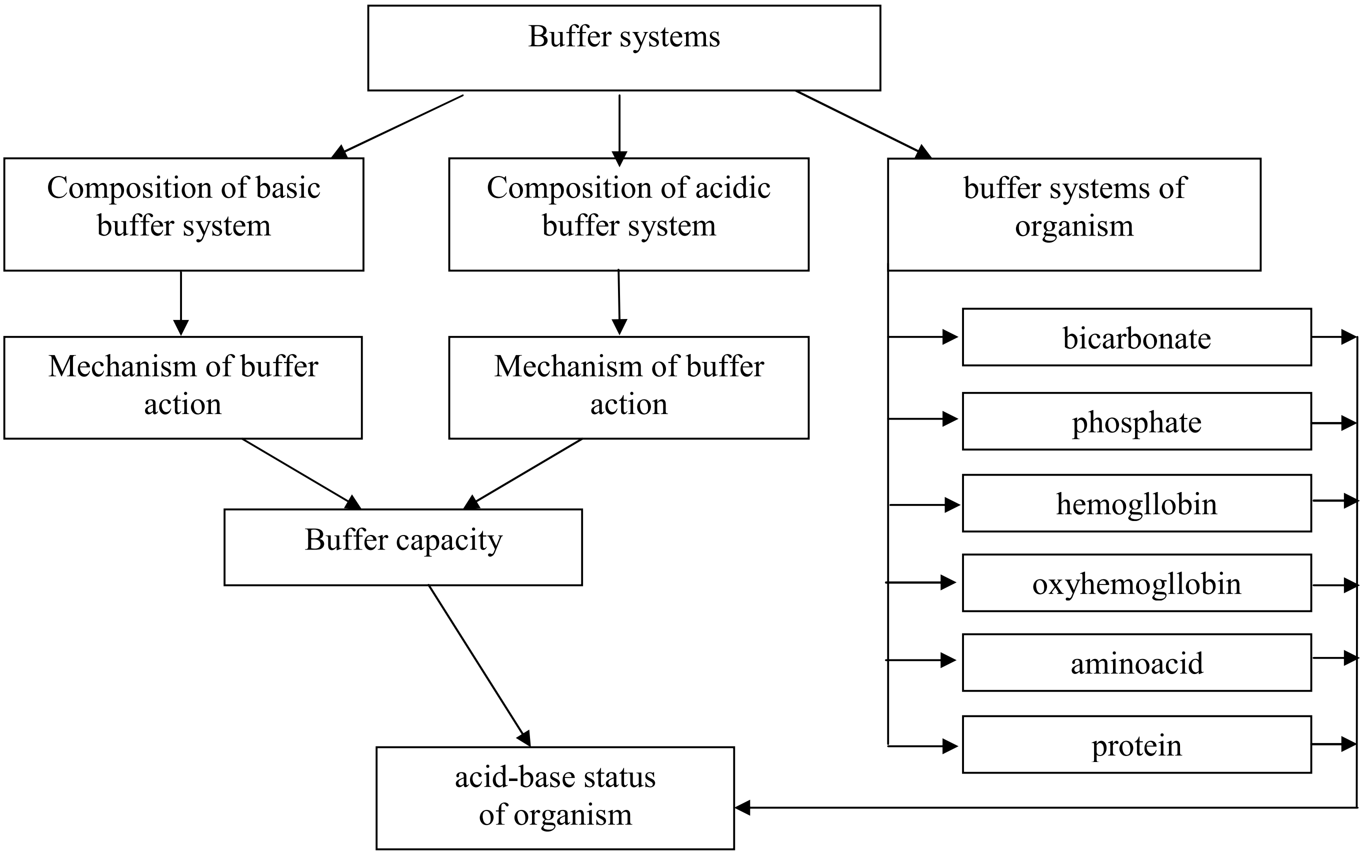
c) **practical skills:**

- to calculate pH of buffer systems, to determine the change in ΔpH of buffer system after addition of strong acid or base;

- to calculate the buffer capacity;

- to calculate the volume of components needed for the preparation of buffer solutions.

5. **Graph structure of the subject.**



6. **Plan of students' work.**

№	Stages	Time	Training and visual aids	Location
1.	Motivational characteristics and plan of the subject. Answers to the students' questions	25	Text book (work book)	Class room
2.	Control of students' knowledge baseline	20		
3.	Students' individual work with methodical literature, solving of educational tasks	55	Methodical instruction for students, lecture notes, text book for students' self-work, reference data, posters. Manuals, reference materials, tables.	
4.	Laboratory work	45	Methodical instruction for students, lecture notes, text book for students' self-work, reference data, posters. Manuals, reference materials, tables.	
5.	Control of knowledge	25		Class room
6.	Analysis and conclusions. Home work	10		

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

- list of questions to be study:

1. Buffer solutions, their classification.
2. The mechanism of the buffer action.
3. The Henderson-Hasselbalch equation for calculation the pH of buffer solutions.
4. The buffer capacity and factors, which it depends on.
5. Buffer system of the organism.
6. The concept of the acid-base balance of the blood.

A buffer solution is one that resists a change in its pH even when a small quantity of a strong acid or a base is added to it. The ability of the buffer solution to resist the changes in pH value upon the addition of small amount of an acid or a base is known as a buffer action.

There are acidic, basic, and ampholytic buffer systems. Acidic buffer solutions are commonly made from a weak acid and its salt with a strong base. Basic buffer solutions – from a weak base and its salt with a strong acid. For example, acetate buffer solution consists of weak acetic acid and sodium acetate; ammonia buffer solution consists of weak ammonium hydroxide and ammonium chloride. Ampholytic buffers - solutions of amino acids or proteins.

Processes that take place in acetate buffer solution and their mutual influence

CH_3COONa is a strong electrolyte, dissociates completely in water:

$$CH_3COONa \leftrightarrow CH_3COO^- + Na^+$$

Acetate ion is the ion of weak acid; in the aqueous solution it undergoes hydrolysis to form acetic acid.

$$CH_3COO^- + H^+ \leftrightarrow CH_3COOH$$

Acetic acid is the weak acid; it dissociates to a very small extent:

$$CH_3COOH \leftrightarrow CH_3COO^- + H^+$$

Dissociation of acetic acid is even more suppressed by the presence of acetate ions from the salt. Thus, the concentration of undissociated acetic acid can be considered equal to its molar concentration in the buffer solution.

Application of the law of mass action to this reaction gives the dissociation constant for acetic acid:

$$K_a = \frac{[H^+][CH_3COO^-]}{[CH_3COOH]}$$

Therefore: $[H^+] = K_a\frac{[CH_3COOH]}{[CH_3COO^-]}$, $[H^+] = Ka \cdot Ca/Cs$.

After taking logs of both sides of the equation we obtain:

$$- \lg [H^+] = - \lg Ka - \lg Ca/Cs \quad \text{or}$$

$$pH = pKa - \lg\ Va/Vs = pKa - \lg \frac{C_a V_a}{C_s V_s}$$

There is the Henderson-Hasselbalch equation for calculation the pH of acidic buffers.

The processes that take place in the ammonia buffer, and their mutual influence.

The ammonia buffer solution consists of a weak base - ammonium hydroxide and a salt - ammonium chloride. Ammonium hydroxide dissociates partially and ammonium chloride dissociates completely.

$$NH_4OH = NH_4^+ + OH^- \quad (1)$$

$$NH_4Cl = NH_4^+ + Cl^- \quad (2)$$

Dissociation constant for the first equation:

$$K_b = \frac{[NH_4^+][OH^-]}{[NH_4OH]}$$

The following expression is correct for the second equation:

$$[NH_4^+] = [Cl^-] = C_s$$

From the expression for the dissociation constant determine the concentration of hydroxide ions:

$$[OH^-] = K_b\frac{[NH_4OH]}{[NH_4^+]}.$$

Since the salt dissociated completely, the concentration of NH_4^+ equals to a salt's molar concentration. The concentration of undissociated molecules of ammonia hydroxide is equal to the initial concentration of the base, because the dissociation is suppressed by the presence of NH^{4+} cations. After taking the logarithm, we obtain the Henderson-Hasselbalch equation for calculation the pH of basic buffers:

$$pOH = pKb - \lg\frac{V_b}{V_s} = pK_b - \lg\frac{C_b\ V_b}{C_s\ V_s}$$

$$pH = 14 - pOH = 14 - pK_b + \lg\frac{C_b\ V_b}{C_s\ V_s}$$

After analyzing the Henderson-Hasselbalch equation, we can conclude that the *pH* value of buffer solution depends on the acid strength (pK), and the ratio of the components concentrations. Therefore, the dilution or concentration of the buffer to certain extent does not change the pH. This property of the buffer solutions is used in the analysis of blood plasma, lymph, and urine.

It should be noted that Henderson-Hasselbalch equation couldn't be applied in the following cases:

- The acid (base) / salts concentration ratio is higher than 100 times;

- Acid (base) is strong, because in this case it is impossible to neglect its dissociation;

- Acid (base) is very weak. The salt hydrolysis cannot be neglected in this case.

Mechanism of buffer action.

When a strong acid is added to acidic buffer solution, it will react with the salt (acceptor of protons) with the formation of equivalent quantity of a weak acid:

$$CH_3COONa + HCl \rightarrow CH_3COOH + NaCl$$

$$CH_3COO^- + H^+ \rightarrow CH_3COOH$$

The strong acid is substituted by the weak acid in the solution resulting in significant decrease in *pH* value.

When a strong base is added to acidic buffer system, it will be neutralized by the weak acid (donor of protons) following by the formation of an equivalent quantity of a salt. The salt hydrolysis is suppressed by the presence of weak acid:

$$CH_3COOH + NaOH \rightarrow CH_3COONa + H_2O$$

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

As a result *pH* doesn't increase very much.

When a strong acid is added to basic buffer solution, the base neutralizes it:

$$NH_4OH + HCl \rightarrow NH_4Cl + H_2O$$

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

When OH^- ions from a strong base are added, they react with the salt. As a result, an equivalent amount of weak base is formed instead of a strong base in a solution.

$$NH_4Cl + NaOH \rightarrow NH_4OH + NaCl$$

$$NH_4^+ + OH^- \rightarrow NH_4OH$$

As a result, the *pH* value in both cases remains almost unchanged.

Calculation the final pH of buffer solution after addition to it a strong acid or base correspond to the mechanism of buffer action.

Addition of a strong acid:

$$NaHCO_3 + HCl \rightarrow H_2CO_3 + NaCl$$

$$pH = pKa - \lg \frac{C_a V_a + C_{HCl} V_{HCl}}{C_s V_s - C_{HCl} V_{HCl}}$$

Addition of a strong base:

$$H_2CO_3 + NaOH \rightarrow NaHCO_3 + H_2O$$

$$pH = pKa - \lg \frac{C_a V_a + C_{NaOH} V_{NaOH}}{C_s V_s - C_{NaOH} V_{NaOH}}$$

From buffer systems of the organism, buffer systems of blood are characterized by the highest buffer capacity. The most important buffer systems of blood are bicarbonate, hemoglobin, oxyhemogllobin, and phosphate.

As a result of metabolism in the body daily produced an amount of acids equivalent 2.5 liters of concentrated HCl. Nevertheless, buffer systems maintain the blood pH at 7,4 ± 0,04.

Carbonic acid – bicarbonate buffer system (pH = 6,0-8,0) consists of weak carbonic acid and hydrogencarbonate ions:

$$H_2CO_3 = H^+ + HCO_3^-$$

$$NaHCO_3 = Na^+ + HCO_3^-$$

The value of blood pH depends on the concentration ratio of free carbonic acid and sodium hydrogencarbonate:

$$pH = pK_1 + \lg \frac{[HCO_3^-]}{[H_2CO_3]}$$

Considering the conditions of blood plasma (at 37°C) pK_1 = 6,1, let's calculate the ratio of concentration at pH = 7,4 according to Henderson-Hasselbalch equation:

$$7{,}4 = 6{,}1 + \lg \frac{[HCO_3^-]}{[H_2CO_3]}$$

$$7{,}4 - 6{,}1 = 1{,}3 = \lg \frac{[HCO_3^-]}{[H_2CO_3]}$$

$$\frac{[HCO_3^-]}{[H_2CO_3]} = \frac{20}{1}$$

Excess of hydrogencarbonate ions is an alkaline reserve of blood. When the number of hydrogen ions in a body's bloodstream increases, the following reaction takes place

$$HCO_3^- + H^+ = H_2O + CO_2$$

The excessive amount of CO_2 is eliminated from blood through the lungs. Thus, the ratio **Ошибка!**and pH value of blood remain unchanged.

Phosphate buffer system

The phosphate buffer system consists of two salts: sodium dihydrogen phosphate (hydrogen-ion donor) and disodium hydrogen phosphate (hydrogen-ion acceptor). It contains in cells, urine, and digestive glands fluid. The mechanism of the buffer action can be represented by the following scheme:

– addition of an acid:

$$Na_2HPO_4 + HCl \rightarrow NaH_2PO_4 + NaCl$$

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

– addition of an alkali:

$$NaH_2PO_4 + NaOH \rightarrow Na_2HPO_4 + H_2O$$

$$H_2PO_4^- + OH^- \rightarrow HPO_4^{2-} + H_2O$$

Determination of phosphate buffer *pH* is calculated as following:

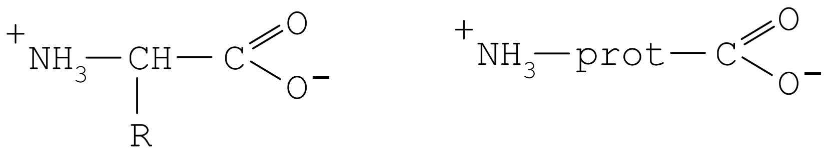
$$pH = 6,8 + \lg \frac{[HPO_4^{2-}]}{[H_2PO_4^-]}$$

The ratio **Ошибка!**in the blood plasma is 4:1 and does not change. The excessive accumulation of any of components results in its urinary excretion.

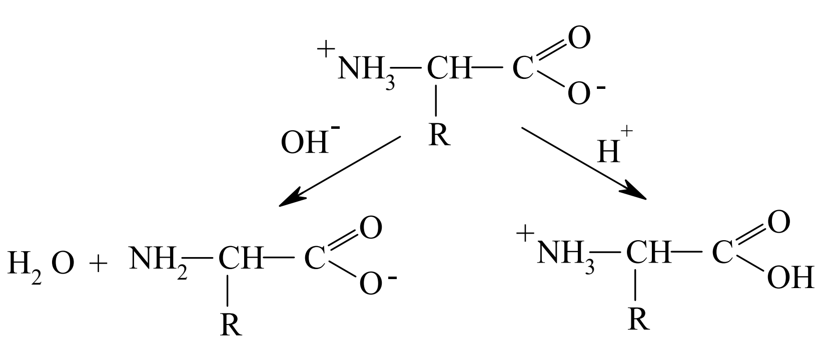
Buffer properties of amino acids and proteins.

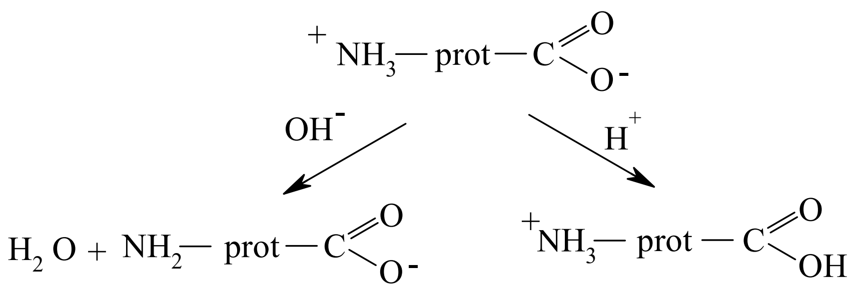
Amino acids and proteins are ampholytes because they contain both functional groups which can be proton donor (–COOH) and proton acceptor ($–NH_2$).

In aqueous solution amino acids can exist in the form of the internal salt (zwitterion):



The mechanism of amino acids and proteins buffer action corresponds to the following scheme:





The pH value, at which amino acid or protein exists in a form of zwitterions, and the molecule is electrically neutral is called isoelectric point (pI). If amino acid or protein molecule has an equal quantity of carboxylic and amine groups, pI ≈ 7. An amino acid (protein) in which amino groups predominate – pI > 7; if carboxyl groups predominate – pI < 7.

In the isoelectric state the proteins solutions are very unstable and easily precipitate. At pH_{medium} > pI proteins exist in the form of polyvalent anions, and at pH_{medium} < pI – in the form of polyvalent cations.

The blood pH is shifted to an alkaline region from the pI of plasma proteins and hemoglobin; therefore, these proteins are found in the blood as polyvalent anions, i.e. have a negative charge.

The buffer systems action is closely linked to the concept of acid-base balance. The acid-base balance - is an integral part of the homeostasis of the organism, which provides optimal conditions for the proper course of metabolism.

Doctors evaluates acid-base balance of the organism using the Henderson-Hasselbalch equation for bicarbonate buffer of blood:

$$pH = 6{,}1 + \lg\,[HCO_3^-]/pCO_2\,,$$

where pK = 6,1 (at T = 311 K), pCO_2 – pressure of carbon dioxide in the blood.

If blood pH is 7.4, the ratio between hydrogencarbonate ions present in the plasma and CO_2 pressure should be 20 : 1.

Bicarbonate buffer system has the highest correlation with all buffer systems of the organism. Abnormalities in any buffer system affect the concentration of the components of bicarbonate buffer system. The change of its parameters can accurately characterize the state of respiratory or metabolic disorders, in other words acid-base balance of the organism. Any violation of the acid-base balance in the body is compensated primarily by the bicarbonate buffer system. This buffer provides 55% of the buffer capacity of the blood.

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

“Investigation of the properties of buffer solutions by the potentiometric technique”

Algorithm of the laboratory work

1. Calculate the volume of _____________ and volume of _____________ in order to prepare 20 cm^3 of ______________ buffer solution where pH = __________.

Concentrations of acid and salts are equal 0,2 mol/L. $pK_{acetic\ acid} = 4,75$; $pK_{H_2PO_4^-} = 6,8$.

2. Proper volumes of acid (base) solutions and their salts should be measured off with a burette, added to a beaker and mixed carefully.

3. Measure pH of the obtained buffer solutions using pH-meter. Use the solutions for the further work.

4. Pipet 5 ml of the buffer solution and transfer into 50 ml volumetric flask. Dilute to the volume with distilled water and mix. Divide the remaining buffer solution into two equal parts, transfer to the beakers. Pipet 1 ml of 0,01M HCl and add to the first beaker. Pipet 1 ml of 0,01M NaOH and add to the second beaker.

5. Measure pH value after dilution, addition of acid and alkali using pH-meter. Record the obtained data into the table:

Solutions	**pH values**	
	calculated	**measured**
Initial buffer solution		
The solution after the HCl addition		
The solution after the NaOH addition		
Diluted solution		

Processing of the experimental data:

1. Calculation of buffer pH after the addition of a strong acid.
2. Calculation of the buffer pH after the addition of alkali.
3. Calculation of buffer pH after dilution.
4. Conclusions.

- List of practical skills:

After studying the subject student should be able to characterize the mechanism of the buffer action of buffer systems of the body and their relationship; calculate buffer systems pH, as well as ΔpH in a buffer system after addition of the strong acid or alkali; calculate buffer capacity of buffer systems; calculate the volume of components needed for the preparation of buffer solutions.

8. **Tasks for knowledge control.**

1. Determine *pH* of the buffer solution, obtained by mixing 400 ml of NH_4Cl with $C(NH_4Cl)$ = 0,1 mol/L and 250 ml of NH_4OH with $C(NH_4OH)$ = 0,2 mol/L, $K(NH_4OH) = 1,8 \cdot 10^{-5}$.

   A: 5,36 B: 9,36 C: 11,23

2. There are two buffer solutions. The *pH* of the first solution is 4,0; *pH* of the second solution is 5,0. Which solution is more resistant to the addition of the same amount of HCl?

   A: first B: the same resistance C: second

3. The following solutions were used for the preparation of the buffer solution: 100 ml of CH_3COOH with $C(CH_3COOH)$ = 0,1 mol/L and 200 ml of CH_3COONa with $C(CH_3COONa)$ = 0,2 mol/L. Calculate the change in the ΔpH value after addition of 5 ml NaOH with C(NaOH) = 0,1mol/L.

   A: ΔpH =0,25 B: ΔpH =0,05 C: ΔpH =0,02

Answers: 1 – B, 2 – C, 3 – C.

9. **Recommendations for the presentation of the results.**

Algorithms for the solving of educational problems of class work and self-work should be recorded in the workbook. Complete a protocol of laboratory work, write down the conclusions about the effect of dilution and addition of a strong acid or alkali for buffers pH.

10. **Suggested readings**

1. V. Kalibabchuk, V. Halinska, L. Hryschenko, S. Hodzynski, T. Ovsyanikova, V. Samarski. Medical chemistry. –Kyiv AUS Medicine Publishing, 2010, −224p.

Навчальне видання

Буферні системи, їх склад, властивості. Буферні системи організму

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

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