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

Department of General and Bioorganic Chemistry

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

Topic 11: Acid-base equilibrium. Buffer solutions

Time: 2 hours

Approved at the meeting of the Department
of General and Bioorganic Chemistry
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2024

THE TRAINING AND EDUCATIONAL GOAL, TASKS, MOTIVATION TO STUDY THE TOPIC

Training purpose:

– formation of students' basic professional competence for solving diagnostic, research and other tasks of professional activity based on knowledge about acid-base balance in the human body and biological buffer systems that support acid-base homeostasis.

Educational goal:

– to develop your personal, spiritual potential;

– to form the qualities of a patriot and a citizen who is ready to actively participate in the economic, industrial, socio-cultural and social life of the country;

– learn to observe academic and labor discipline, the norms of medical ethics and deontology;

– to realize the social significance of their future professional activities.

Tasks:

As a result of the training session, the student should

know:

– concepts of buffer solutions, acid-base and electrolyte homeostasis;

– classification of buffer systems;

– the mechanism of buffer activity;

– buffer systems of the body;

be able to:

– describe the mechanism of buffer activity using equations of chemical reactions;

– solve computational problems for calculating the pH of buffer solutions;

possess:

– the skills of preparing various types of buffer solutions and calculating the pH value in them.

Motivation to study the topic:

Human biological fluids contain a large number of electrolytes: NaCl, $CaCl_2$, KCl, NaH_2PO_4, Na_2HPO_4, etc. In the process of vital activity, the body receives food from the outside, and as a result of metabolism, products are formed that have both an acidic and basic character. The human body constantly loses water with sweat, urine, through the lungs, and skin. However, the concentration of ions in cells and tissues is normally maintained constant. This property of living systems is called acid-base homeostasis. Maintaining acid-base and electrolyte balance in tissues and biological fluids is extremely important for the normal functioning of the body. A change in the electrolyte composition leads to a change in the reaction of the medium of biological fluids.

There are physiological and physico-chemical mechanisms of regulation of acid-base balance in the body. The physiological mechanisms of regulation are based on the processes of metabolism, respiration, and urinary excretion, which are studied in courses of biochemistry, normal and pathological physiology.

The physico-chemical mechanisms for maintaining constant acid-base and electrolyte homeostasis in blood and tissue fluids include buffer systems, which are represented by buffer systems of blood, cells and intercellular fluids in tissues.

NECESSARY EQUIPMENT

1. Methodological manual for students on the topic "Acid-base equilibrium. Buffer solutions".
2. Training tables:
a) Periodic Table of chemical elements by D.I. Mendeleev;
b) table of solubility of acids, bases and salts.
3. Reference materials of basic physico-chemical constants.
4. Chemical reagents and equipment necessary for laboratory work.

CONTROL QUESTIONS ON THE TOPIC OF THE CLASS

1. Acid-base equilibrium in solutions.
2. Buffer solutions: classification and mechanism of action. Buffer capacity. Calculation of the pH of buffer solutions.
3. Buffer systems of blood.

COURSE OF THE CLASS

The theoretical part

1. ACID-BASE EQUILIBRIUM IN SOLUTIONS

Acid-base equilibrium is a special case of equilibrium in homogeneous systems. Acid-base equilibrium is an equilibrium in which acids and bases participate.

Determination of acid by Arrhenius – it is a substance that dissociates in water to form H^+ ions (protons), and the Arrhenius' base is a substance that dissociates in water to form OH^- (hydroxide) ions. According to this view, the acid-base reaction involves the reaction of a proton with a hydroxide ion to form water.

Water is a weak electrolyte and partially dissociates into ions according to the equation:

$$HOH \rightleftarrows H^+ + OH^-$$

This dissociation can be characterized by ionization equilibrium constant:

$$K_{ion}(H_2O) = \frac{[H^+] \times [OH^-]}{[H_2O]} = 1.8\times10^{-16} \text{ (at 25 °C)}$$

However, the concentration of water is in huge excess and in fact remains constant, since only a tiny fraction of the molecules dissociates. Knowing the molar mass of water (18 g/mol), as well as neglecting the concentration of dissociated molecules and taking a mass of 1 liter of water per 1000 g, it's possible to calculate the concentration of undissociated molecules in 1 liter of water: $[H_2O] = 1000 / 18 = 55.55$ mol /l.

The product of two constant quantities is also a constant quantity:

$$K_{ion} \times [H_2O] = 1.8 \times 10^{-16} \times 55.55 = 1 \times 10^{-14} = K_w$$

K_w is defined as water ionization constant (sometimes called the ionic product of water) – it is a constant value at constant temperature (25 °C) not only for pure water, but also for dilute aqueous solutions of any substances:

$$K_w = [H^+] \times [OH^-] = 1.8 \times 10^{-16} \times 55.55 = 1 \times 10^{-14}$$

The constant K_w is only affected by temperature. It can be used to find the concentration of hydrogen ions by the concentration of hydroxide ions and vice versa.

In pure water, the concentrations of the hydrogen ion and the hydroxide ion are equal, and the solution is therefore neutral: $[H^+] = [OH^-] = \sqrt{10^{-14}} = 10^{-7}$ mol/l.

If $[H^+] > [OH^-]$, however, the solution is acidic, whereas if $[H^+] < [OH^-]$, the solution is basic.

For an aqueous solution, the H^+ concentration is a quantitative measure of acidity: the higher the H^+ concentration, the more acidic the solution. Conversely, the higher the OH^- concentration, the more basic the solution.

Since the numerical value of K_w is very small, it is more conveniently to use the negative base-10 logarithm of the K_w value instead. At 25 ºC temperature:

$$pK_w = -lgK_w = -\lg 10^{-14} = 14$$
$$pK_w = pH + pOH = 14$$

Since for pure liquid water:

$[H^+] = [OH^-] = 10^{-7}$,

$pH = -\lg[H^+] = -\lg 10^{-7} = 7$

$pOH = -\lg[OH^-] = -\lg 10^{-7} = 7$

We can summarize the relationships between acidity, basicity, and pH as follows:

if $pH = 7.0$, the solution is neutral;

if $pH < 7.0$, the solution is acidic;

if $pH > 7.0$, the solution is basic [1-3].

2. BUFFER SOLUTIONS: CLASSIFICATION AND MECHANISM OF ACTION. BUFFER CAPACITY. CALCULATION OF pH OF BUFFER SYSTEMS

Buffer solutions are solutions which have an ability to resist in pH change upon the addition of small quantities of acids and bases.

The mechanism of buffer activity becomes clear from the Brönsted-Lowry's theory point of view (1923). According to the theory, acid is a proton donor; acid loses proton and turns into its conjugate base:

$$\underset{\text{acid}}{A-H} \longrightarrow \underbrace{A^- + H^+}_{\text{conjugate base}}$$

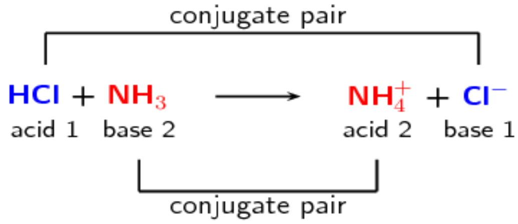
Base is a proton acceptor; base accepts proton and converts into a conjugate acid:

$$\underset{\text{base}}{B:} + H^+ \longrightarrow \underset{\text{conjugate acid}}{BH^+}$$

Acids and bases can be both neutral molecules or ions.

The acid and the base in the acid-base pair are interconnected with each other: the stronger the acid, the weaker the base associated with it, and vice versa.

Each acid-base interaction is an exchange of protons. For example, acid-base interaction from the Brönsted-Lowry's theory point of view:



Each buffer solution contains an acid-base conjugate pair therefore it is able to neutralize both acids and bases added to it.

Classification of buffer solutions:

1) Weak acid/ its salt

Acetate buffer system: CH_3COOH/CH_3COONa (CH_3COOH/CH_3COO^-)

Mechanism of buffer activity:

neutralization of an added acid: $HCl + CH_3COONa \rightleftarrows CH_3COOH + NaCl$

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

neutralization of an added base: $NaOH + CH_3COOH \rightleftarrows CH_3COONa + H_2O$

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

2) Weak base/ its salt

Ammonia buffer system: NH_4Cl / NH_3 (NH_4^+/NH_3)

Mechanism of buffer activity:

neutralization of an added acid: $HCl + NH_3 \rightleftarrows NH_4Cl$

neutralization of an added base: $NaOH + NH_4Cl \rightleftarrows NH_3 + NaCl + H_2O$

3) Two acidic salts

Hydrophosphate buffer system: NaH_2PO_4/Na_2HPO_4 ($H_2PO_4^-/HPO_4^{2-}$)

Mechanism of buffer activity:

neutralization of an added acid: $HCl + Na_2HPO_4 \rightleftarrows NaH_2PO_4 + NaCl$

neutralization of an added base: $NaOH + NaH_2PO_4 \rightleftarrows Na_2HPO_4 + H_2O$

4) Acidic salt/ neutral salt

Carbonate buffer system: $NaHCO_3/Na_2CO_3$ (HCO_3^-/CO_3^{2-})

Mechanism of buffer activity:

neutralization of an added acid: $HCl + Na_2CO_3 \rightleftarrows NaHCO_3 + NaCl$

neutralization of an added base: $NaOH + NaHCO_3 \rightleftarrows Na_2CO_3 + H_2O$

Buffer capacity (B, mmol/l) is a measure of buffer's efficiency in resisting pH changes. Buffer capacity of a solution is expressed as a number of moles of equivalents of strong acid or base that must be added to 1 liter of the solution to change its pH by one unit:

$$B = \frac{C_N \times V}{\Delta pH \times V_{BS}}$$

C_N – normality of added strong acid or strong base, mol/l;

V – volume of an added acid or base, ml;

ΔpH – pH change caused by the addition of strong acid/base;

V_{BS} – volume of a buffer solution, l.

Buffer capacity depends on:

1) concentration: the higher the concentration, the greater the buffer capacity of the solution;

2) the ratio of components' concentration: B_{max}, $when\ \frac{[component1]}{[component2]} = 1$

The greater the buffer capacity of the solution, the higher its activity in supporting acid-base equilibrium.

Biological buffer systems are characterized by:

B_a – buffer capacity by acids,

B_b – buffer capacity by bases.

Usually $B_a > B_b$, because the number of acidic metabolites generated in the human body is much greater than the number of basic metabolites [2, 3].

The *pH* of a buffer solution may be calculated by the **Henderson-Hasselbach's equation:**

$$pH = pK_a - log\frac{[acid]}{[conjugate\ base]}$$

$pK_a = -\log K_a$ (K_a is the acid ionization constant);

[acid], [conjugate base] are the molarities of the acid and its conjugate base in the buffer solution, mol/l.

Problem 1. Calculate the pH of a buffer solution that contains 1 mol of CH_3COOH and 1 mol of CH_3COOK in 1 liter of it. Calculate the pH change followed by the addition of 0.005 mol of KOH to this buffer.

Solution:

According to the Henderson-Hasselbach's equation:

$$pH_{(initial)} = pK_a - log\frac{[acid]}{[conjugate\ base]} = 4.75 - log\frac{1}{1} = 4.75$$

Neutralization of an added base: $CH_3COOH + KOH \rightarrow CH_3COOK + H_2O$

As a result of KOH addition, the number of moles of CH_3COOH decreases as it is consumed in the reaction, the number of moles of CH_3COOK increases as it is formed in the reaction:

$n(CH_3COOH)_{(after\ KOH\ addition)} = 1 - 0.005 = 0.995\ mol$

$n\ (CH_3COOK)_{(after\ KOH\ addition)} = 1 + 0.005 = 1.005\ mol$

$$pH_{(after\ KOH\ addition)} = pK_a - log\frac{[acid]}{[conjugate\ base]} = 4.75 - log\frac{0.995}{1.005} = 4.7543$$

$$\Delta pH = pH_{(after\ KOH\ addition)} - pH_{(initial)} = 4.7543 - 4.75 = 0.0043$$

Answer: $pH_{(initial)} = 4.75$; $\Delta pH = 0.0043$

3. BUFFER SYSTEMS OF BLOOD

The most powerful buffer systems of the human body are concentrated in the blood. They are divided into two categories:

- *buffers of plasma;*
- *buffers of erythrocytes.*

The pH of the blood plasma is maintained at 7.4 by several buffer systems, the most important of which is HCO_3^-/H_2CO_3. In the erythrocyte, where the pH is 7.25, the main buffer systems are HCO_3^-/H_2CO_3 and hemoglobin, oxyhemoglobin (Fig. 1).

Buffer systems of blood

plasma ↙ ↘ erythrocytes

hydrocarbonate,
hydrophosphate

proteins
(albumins, globulins)

hemoglobin,
oxyhemoglobin

Figure 1 – Classification of buffer systems in blood

Let's start the review of buffer systems in blood beginning from the buffers, which are present both on blood plasma and erythrocytes.

1) Hydrocarbonate buffer system: H_2CO_3/HCO_3^-

The formation of the buffer system in biological fluids is represented by:

$$CO_2 + H_2O \overset{\text{carbonic anhydrase}}{\rightleftarrows} H_2CO_3 \rightleftarrows HCO_3^- + H^+$$

Mechanism of buffer activity:

$H^+ + HCO_3^- \rightleftarrows H_2CO_3$

$OH^- + H_2CO_3 \rightleftarrows HCO_3^- + H_2O$

In blood plasma the ratio of H_2CO_3 and HCO_3^- concentrations is:

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

The excess of HCO_3^- creates the *base reservoir of blood*. Its buffer capacity of acids is much greater than buffer capacity of bases: B_a = 20 mmol/l; B_b = 1-2 mmol/l.

Hydrocarbonate buffer system is contained in all biological fluids of a human body.

HCO_3^- analysis in blood is an important diagnostic test which signals about respiratory and metabolic diseases.

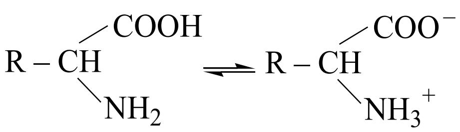
2) Hydrophosphate buffer system: $H_2PO_4^-/HPO_4^{2-}$

The mechanism of its buffer activity is given in above. This buffer exhibits low capacity in blood is due to low concentration of its components: B_a = 1-2 mmol/l; B_b = 0.5 mmol/l.

But hydrophosphate buffer system is most vital in urine, intracellular fluids and other biological liquids.

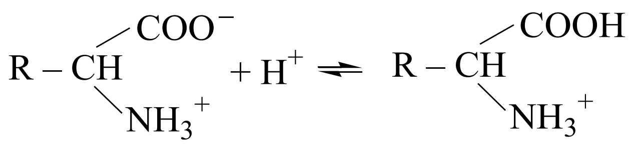
3) Protein buffer system (albumins, globulins) is a strong buffer which is present in blood plasma but not in erythrocytes.

Proteins are amphiprotic polyelectrolytes which exist as bipolar ions:

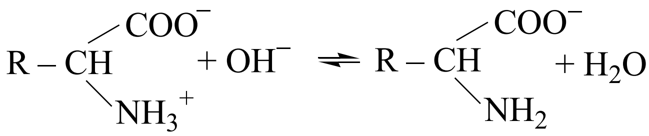


Mechanism of buffer activity:

neutralization of an added acid:



neutralization of an added base:



B_a(albumins) = 10 mmol/l; B_a(globulins) = 3 mmol/l.

Protein buffer system is contained in all biological fluids of a body.

4) Hemoglobin-oxyhemoglobin buffer system is the main buffer system of erythrocytes and has a high buffer capacity.

Hemoglobin buffer is consisting of two forms of hemoglobin: reduced hemoglobin (HHb) and oxidized hemoglobin ($HHbO_2$).

Hemoglobin is converted into oxyhemoglobin by joining oxygen:

$$HHb + O_2 \rightleftarrows HHbO_2$$

Buffer system formed by hemoglobin:

$HHb \rightleftarrows H^+ + Hb^-$ ($K_a = 6.37\times10^{-9}$)

acid *conjugate base*

Buffer system formed by oxyhemoglobin:

$HHbO_2 \rightleftarrows H^+ + HbO_2^-$ ($K_a = 1.17\times10^{-7}$)

acid *conjugate base*

Mechanism of buffer activity:

neutralization of an added acid:

$H^+ + Hb^- \rightleftarrows HHb$ $H^+ + HbO_2^- \rightleftarrows HHbO_2$

neutralization of an added base:

$OH^- + HHb \rightleftarrows Hb^- + H_2O$ $OH^- + HHbO_2 \rightleftarrows HbO_2^- + H_2O$

Hemoglobin and hydrocarbonate buffer systems are working together to deliver oxygen to tissues and to remove carbon dioxide out of tissues. Biological buffer systems maintain acid-base statues of a human body.

All biological fluids are characterized by constant pH values. This phenomenon is defined as acid-base equilibrium or acid-base homeostasis; it is regulated by biological buffer systems.

A disturbance of acid-base status is rather dangerous for people's health since the pH deviation may cause:

– decrease in hormones' and enzymes' activity;

– change in osmotic pressure;

– alteration in rates of biochemical reactions catalyzed by protons.

Even a 0.4 pH unit deviation from the normal pH value in blood may cause coma or even death of a patient. For babies even 0.1 pH deviations are also very dangerous.

The most dangerous types of acid-base disturbance *in vivo* are:

▪ **acidosis** is increase in blood acidity,

▪ **alkalosis** is increase in blood basicity.

Acidosis correction is done by intravenous injection of 4 % $NaHCO_3$ solution:

$$HCO_3^- + H^+ \rightleftarrows H_2CO_3$$

Antacidic (hypocidic) drugs are substances which reduce acidity of biological fluids.

Alkalosis correction is achieved by intravenous injection of ascorbic acid solutions (5 % or 15 %) [2, 3].

The practical part

Safety instructions before laboratory work.

LABORATORY WORK

Preparation of buffer solutions

1. Prepare the acetate buffer solution using a scheme given in the Table 1.

Table 1 – Preparation and determination of pH of buffer solutions

Buffer solution number	1	2	3
Volume of 0.1 N CH_3COOH, ml Volume of 0.1 N CH_3COONa, ml	9.9 0.1	5 5	0.1 9.9
pH (experimental)			
pH (calculated)			

2. For each buffer solution, determine the pH value experimentally and calculate the pH values according to the Henderson-Hasselbach's equation (in this case, the concentration ratio can be replaced by the volume ratio, since the normal concentrations of acid and salt are the same):

$$pH = pK_a - lg\frac{V_{acid}}{V_{salt}}$$

3. Compare the experimental data with the theoretically calculated data. Calculate the absolute and relative errors of the experiments.

Control over the assimilation of the topic

It is conducted in the form of written independent work of students.

METHODOLOGICAL RECOMMENDATIONS FOR THE ORGANIZATION AND PERFORMING OF INDEPENDENT WORK OF STUDENT (IWS)

The time allotted for independent work can be used by students for:

– preparation for laboratory classes;

– taking notes of educational literature;

– performing tasks for self-control of knowledge;

– preparation of thematic reports, abstracts, presentations.

The main methods of organizing independent work:

– studying topics and problems that are not covered in the classroom;
– writing an abstract and making a presentation;
– performing tasks for self-control of knowledge.

List of tasks of IWS:

1. Specify which of the following systems can be classified as buffer systems:

a) HCl / KCl, b) NH_4NO_3 / NH_3, c) NaH_2PO_4 / Na_2HPO_4
d) HNO_2 / KNO_2, e) H_2SO_4 / $KHSO_4$, f) $HCOOH$ / $HCOOK$?

2. Calculate the pH of the buffer solution, which contains 0.50 mol of ammonia (NH_3) and 0.30 mol of ammonium chloride (NH_4Cl) in 1 liter of solution. $pK_a(NH_4^+) = 9.25$. Calculate the pH change as a result of adding 3.65 g HCl to this solution.

Answer: 9.47; 0.22

3. Calculate *pH* of the buffer solution containing 0.1 mol of acetic acid and 0.01 mol of sodium acetate in 1 liter. $K_a(CH_3COOH) = 1.8\times10^{-5}$, $pK_a(CH_3COOH) = 4.75$. How will the *pH* change after adding to 1 liter of the solution:

a) 0.001 mol of HCl; b) 0.001 mol NaOH?

Answer: 3.75; 0.05; 0.05

The control of the IWS is carried out in the form of:

– evaluation of an oral answer to a question, message, report or presentation;
– individual conversation.

LIST OF SOURCES USED

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