Subject 6. Properties of buffer solutions. Role of buffer solutions in biological systems

1. Importance

Constant pH is crucial for proper functioning of the organism. Constant pH is maintained through buffer systems and physiological mechanisms. Buffer systems regulate concentrations of hydrogen and hydroxide ions and pH-dependent reactions. It is important to know properties and composition of buffer systems in studying biochemistry, physiology and clinical sciences.

Competences

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

Ability to apply knowledge in practical situations.

Ability to assess and ensure quality of work.

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

Use English 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 self-control and healthy lifestyle, adaptability and ability to perform in new situations.

Motivation and perseverance in achieving goals.

Skills in using information and communication technologies.

Desire to preserve the environment.

Ability to evaluate results of laboratory and practical studies.

Ability to solve typical problems and practical problems in learning.

2. Concrete aims

Prepare buffer solutions with a needed pH value.

Calculate pH of buffer systems.

Explain the mechanism of action of buffer systems and their role in maintaining acid-base balance in biological systems.

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

Previous subjects	Obtained skills

1. Foreign language for professional purposes	Possess basic knowledge of English language. Be able to communicate in English language. Use English language in professional activity.
2. Latin language and medical therminology	Master medical therminology in Latin language.
3. Medical Biology	Know structure components of cytoplasm and nucleus.
4. 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.
5. Biological and Bioorganic Chemistry	Apply theories of acids and bases, formulas, names and properties of acids and bases. Understand the concepts of acidity and basicity.

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. Buffer solutions	Solutions that are able to maintain constant concentration of hydrogen ions, ie pH value in the medium upon addition of small amounts of a strong acid or an alkali or upon dilution.
2. Power of hydrogen, pH	pH= -lg[H^+] It is negative logarithm of molarity of hydrogen ions.

4.2. Theoretical questions to the lesson:

1. Classification of buffer solutions.
2. Mechanisms of action of buffer systems.
3. pH of buffer solutions (Henderson-Hasselbach equation).
4. Buffer systems of blood:
   a) bicarbonate buffer (hydrogen carbonate buffer system);
   b) phosphate buffer;
   c) protein buffer systems.
5. Buffer capacity and factors that influence it.
6. Acid-base state of blood.

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

1. Calculate pH of a buffer system

Calculate pH of an acetate buffer solution, which was prepared with 80 mL of 0.1 M solution CH_3COOH and 20 mL of 0.1 M solution CH_3COONa; K_d (CH_3COOH) = $1.74 \cdot 10^{-5}$.

Calculate pH of the buffer solution with Henderson-Hasselbach equation:

$$pH = pK_a + \lg \frac{[salt]}{[acid]}$$, where $pK_a = -\lg K_a$;

$$pH = 4.76 + \lg \frac{0.02\ L \cdot 0.1\ mol/L}{0.08\ L \cdot 0.1\ mol/L} = 4.16.$$

2. Prepare a buffer solution with needed pH

First, use a reference book to choose the type of a buffer system that is within the needed pH range. For example, if pH=5 is needed, an acetate buffer solution (pH range 3.7-5.6) can be prepared.

Then calculate the concentration of hydrogen ions for the pH value. For example, at pH = 5 concentration of hydrogen ions is:

$$[H +] = 10^{-5}$$

The ratio of the components of the buffer solution is found from the formula

$$[H^+]=K \frac{[acid]}{[salt]}$$

As the solutions of acid and salt are of same molarity, then the ratio of their concentrations can be replaced by ratio of their volumes. For example, for 10 mL of a buffer solution, denote the volume of the acid solution as x, and the volume of the salt solution as (10-*x*), respectively. Then we obtain:

$$[H^+]=K \frac{x}{10-x}$$

In the formula, substitute $[H^+]$ for the concentration of hydrogen ions corresponding to the needed pH, and K for the dissociation constant of the weak acid (or base) from the reference book. Calculate the volumes of the solutions of the weak acid and its salt that are needed to prepare 10 mL of a buffer solution. Thus, the volume of the acid solution is 3.6 mL, and the volume of the salt solution is 10-3.6 = 6.4 mL.

Potentiometric measurement of buffer capacity of blood plasma is carried out with the ionometer EV-74, calibrated with standard buffer solutions beforehand.

8.1. Preparation.

Press the buttons “t” and “ -1 / 19”. Leave for 30 min.

Compose a galvanic circle with a measurement electrode and a comparison electrode. The measurement electrode is a glass electrode with H^+ function, the comparison electrode is a chlorinesilver electrode with constant potential 0.201 V. Plug the electrodes in the sockets on the back side of the machine. Fix the electrodes over the magnete mixer. Titration is to be carried out in a 50 cm^3 cup with a magnete inside.

Press the buttons ”anions / cations”, “pX” and the pH range button “4 / 9”.

8. 2. Measurement of acid buffer capacity (B_{acid}).

Add 20 mL of blood plasma to a cup with a magnete inside. Fill a burette with titrated solution of HCl. Measure initial pH_0. While mixing, add the acid until pH is changed for approximately 1. Determine exact pH with the machine scale and exact volume of acid with the burette.

Calculate buffer capacity with the formula:

$$B_{acid} = \frac{N_{(HCl)} \cdot V_{(HCl)}}{V_{(plasma)} \cdot |pH_1 - pH_0|}, mol/L$$

$N_{(HCl)}$ - normality of HCl solution (mol/L),
$V_{(HCl)}$ - volume of HCl solution, mL,
$V_{(plasma)}$ – volume of blood plasma, mL,
$(pH_1 – pH_0)$ – difference between pH values.

8. 3. Measurement of alkali buffer capacity (B_{alkali}).

Add 20 mL of blood plasma to a cup with a magnete inside. Fill the burette with titrated solution of NaOH. Titrate as in the previous experiment. Register obtained pH of blood plasma and used volume of NaOH.

Calculate with the formula:

$$B_{alkali} = \frac{N_{(NaOH)} \cdot V_{(NaOH)}}{V_{(plasma)} \cdot |pH_1 - pH_0|}, mol/L$$

$N_{(NaOH)}$ - normality of NaOH solution (mol/L),
$V_{(NaOH)}$ - volume of NaOH solution, mL,
$V_{(plasma)}$ – volume of blood plasma, mL,
$(pH_1 – pH_0)$ – difference between the pH values.

4. Writing a report of the laboratory work:

Calculate the acid and alkali buffer capacities, compare the obtained values and write down the conclusions.

Contents of the subject (abstract):

1. **Classification of buffer systems**

Buffer systems are solutions that are able to maintain constant concentration of hydrogen ions, ie pH value of the solution upon addition of small amounts of a strong acid or an alkali or upon dilution.

The main types of buffer systems.
- Buffer systems containing a weak acid + salt of the weak acid and a strong base. For example:
acetate buffer system:

$CH_3COOH + CH_3COONa$

hydrocarbonate buffer system:

H_2CO_3 + $NaHCO_3$

- Buffer systems containing a weak base + salt of the weak base and a strong acid. For example:
ammonium buffer system:

NH_4OH + NH_4Cl

- Buffer systems containing two salts of polybasic acids.
For example:
phosphate buffer system:

NaH_2PO_4 + Na_2HPO_4

2. **Mechanism of action of buffer systems**

Mechanism of action of acetate buffer system:

CH_3COOH + CH_3COONa

$HCl + CH_3COONa \rightleftarrows CH_3COOH + NaCl$

$NaOH + CH_3COOH \rightleftarrows CH_3COONa + H_2O$

Mechanism of action of ammonium buffer system:

NH_4OH + NH_4Cl

$HCl + NH_4OH \rightleftarrows NH_4Cl + H_2O$

$NaOH + NH_4Cl \rightleftarrows NH_4OH + NaCl$

3. **Henderson - Hasselbach equation for acidic buffer solution**

$$\mathbf{pH = pK_a - lg \frac{[acid]}{[salt]}}$$

or

$$\mathbf{pH = pK_a + lg \frac{[salt]}{[acid]}}$$

4. **Henderson - Hasselbach equation for basic buffer solution**

$$\mathbf{pH = 14 - \left(pKb + \frac{[salt]}{[base]}\right)}$$

5.Buffer systems of blood

Buffer systems maintain constant pH.

a) bicarbonate (hydrogen carbonate) buffer

H_2CO_3 + $NaHCO_3$.

carbonic acid sodium hydrogen carbonate

$H^+ + NaHCO_3 \rightleftarrows Na^+ + H_2CO_3 \nearrow CO_2\uparrow$, $\searrow H_2O$

$NaOH + H_2CO_3 \rightleftarrows NaHCO_3 + H_2O$.

b) phosphate buffer

NaH_2PO_4 + Na_2HPO_4.
sodium dihydrogen phosphate sodium hydrogen phosphate

$H^+ + Na_2HPO_4 \rightleftarrows NaH_2PO_4 + Na^+$.
$NaOH + NaH_2PO_4 \rightleftarrows Na_2HPO_4 + H_2O$.

c) protein buffer systems

It is the hemoglobin buffer system and proteins of blood plasma.

6.. Acid-base state of blood

Normal pH of blood plasma is 7.36 ± 0,04.

Acidosis is a decrease of blood pH.

Alkalosis is an increase of blood pH.

7.. Buffer capacity and factors that influence it

Buffer capacity is the number of mole-equivalents of a strong acid or a strong base that should be added to 1 liter of a buffer solution to change its pH in one unit

Buffer capacity depends on concentration of components of the buffer system and on their ratio.

Materials for self control:

A. Tasks for self control:

1. Why does not pH of the hydrocarbonate buffer system change when a small amount of a strong acid is added to it?

a) dissociation of sodium hydrocarbonate increases;
b) equilibrium state shifts to formation of hydrogen cations:
$CO_2 + H_2O = H^+ + HCO_3^-$;
c) carbonic acid decomposes under influence of strong acids;
d) the strong acid is replaced by an equivalent amount of weak carbonic acid.

2. Which factors effect pH of a buffer system?

a) identity of the weak electrolyte (acid or base);
b) ratio of the buffer system components;
c) concentration of the weak electrolyte (acid or base);
d) concentration of salt.

B. Practical tasks for self control:

1. Choose the correct formula to calculate pH of a HCN – NaCN buffer system:

a) $pH = pK_{HCN} + \lg \frac{[NaCN]}{[HCN]}$; c) $pH = 14 - pK_{HCN} + \lg \frac{[NaCN]}{[HCN]}$;

b) $\mathrm{pH} = \mathrm{p}K_{\mathrm{HCN}} + \lg \frac{[\mathrm{HCN}]}{[\mathrm{NaCN}]}$; d) $\mathrm{pH} = 14 + \mathrm{p}K_{\mathrm{HCN}} - \lg \frac{[\mathrm{HCN}]}{[\mathrm{NaCN}]}$.

2. Calculate pH of a buffer solution, prepared from 0.040 L 0.15*M* ammonia solution and 0.020 L 0.25 *M* ammonia chloride. $\mathrm{p}K_{\mathrm{NH_4OH}} = 4.74$.

a) 4.67; b) 6.42; c) 9.33; d) 10.12.

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. 80 – 86).

Informational resources:

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

(Web page of Poltava State Medical University).