LABORATORY 3

Buffers

1. Mechanism of buffer action

A. The influence of sodium acetate (CH_3COONa) addition on pH shift of CH_3COOH (acetic acid) solution.

Weak electrolytes at the same molar concentration as the strong ones, release to the solution considerably lower amount of ions. For example, the molar concentration of hydrogen ions $[H^+]$ in 0.1 M solution of strong hydrochloric acid is 10^{-1} moles/L, but for the same 0.1 M concentration of acetic acid, hydrogen ions concentration reaches value 10^{-3} moles/L. Additionally, hydrogen ions concentration can be diminished after addition of sodium acetate:

$$CH_3COOH \xleftrightarrow{\text{Partial dissociation}} CH_3COO^- + H^+$$

Sodium acetate completely dissociates into acetate anions and sodium cations:

$$CH_3COONa \xrightarrow{\text{Complete dissociation}} CH_3COO^- + Na^+$$

Adding acetate anions from completely dissociated sodium acetate to the solution of partially dissociated acetic acid shifts the equilibrium state (of acetic acid dissociation) to the left. There are less hydrogen ions, more undissociated molecules of acid, pH increases.

Procedure:

Add 0.1 M acetic acid (10 drops) and 1 drop of methyl orange indicator (at pH below 3.1 methyl orange is pink; at pH above 4.4 it is yellow) to two test tubes. Mix until a pink solution is obtained. Add a few crystals of sodium acetate to one of the tubes and mix. The colour change of the solution is the result of a diminution of hydrogen ions concentration.

B. The influence of ammonium chloride (NH_4Cl) addition on pH shift of ammonia water ($NH_3 \bullet H_2O$) solution.

Procedure:

Add 0.1 M ammonia water solution (10 drops) and 1 drop of phenolphthalein indicator (at pH below 8.3 phenolphthalein is colourless and at pH above 8.3 it is pink) to two test tubes. Mix to obtain a pink-coloured solution. To one of the tubes add a few crystals of ammonium chloride. Mix and observe the decolourization of the solution.

Answer the questions:

- what ions from the salt added to the solution of weak acid (point A) or weak base (point B) were responsible for the change of the colour of the solution and why. How pH of the solution of weak electrolytes was changed?
- how equilibrium state in the solution of weak acid/weak base was shifted after addition of sodium acetate/ammonium chloride?
- how dissociation degree of weak electrolyte solutions was changed after addition of the salts?

2. The influence of buffer concentration on pH value and buffer capacity

Procedure:

Prepare buffer's mixtures according to the table.

beaker number	V [cm^3] **phosphate buffer**		V [cm^3] H_2O	pH_1	V [cm^3] added 0.05M HCl	pH_2	ΔpH	n_{HCl}/ 1 dm^3	β
	0.1M	0.01M							
1	40	—	—		2				
2	—	40	—		2				
3	—	—	40		2				

- measure initial pH (pH_1) of the solutions and water
- add 2 ml of 0,05 M HCl to each beaker, mix and again measure pH (pH_2)
- calculate pH difference (ΔpH) and moles of acid (n) added to 1 liter of buffers or water
- calculate buffer capacity for buffers (not for water)

- draw the conclusions from the experiment (determine the relation between concentration of buffer and buffer capacity and compare pH changes in water and buffers occurring after addition of strong acid)

$$\beta = \frac{n}{\Delta pH}$$

n – moles of HCl per 1 dm^3 of the solution

3. The influence of lactic acid on buffer capacity of bicarbonate buffer

Bicarbonate buffer plays a main role in maintaining a constant pH of the blood. Bicarbonate ions constitute an alkali reserve. During intense anaerobic metabolism, lactic acid is formed, and after dissociation H^+ ions are released. In physiological state, alkali reserve easily neutralizes hydrogen ions, whereas, in pathological state (excessive physical effort, shock) HCO_3^- ions are completely used up by the excess of lactic acid. In the living organism, in the life-threatening pH decrease, other buffers (hemoglobin, protein or phosphate buffers) start to act. Additionally, organism initiates respiratory (CO_2 excretion) and lung (H^+ removal) compensation.

Procedure:

Add 10 mL of lactic acid to 2 beakers: 0.002 M (physiological condition) and 0.02 M (pathological condition) and measure pH. Write the results in the table (pH_1). Then, add 1 mL of 0.02 M $NaHCO_3$ do both beakers, mix and again measure pH (pH_2).

Sample number	Lactic acid (10 ml)	Milimoles of lactic acid	pH_1	$NaHCO_3$ (1 ml)	Milimoles of $NaHCO_3$	pH_2	ΔpH	Milimoles of acid/milimoles of base
1	0.002 M			0.02 M				
2	0.02 M			0.02 M				

Write proper reaction of lactic acid neutralization by bicarbonate buffer, calculate number of moles of acid and base, compare pH change in both beakers, and calculate the ration of milimoles of acid to milimoles of base. Answer, how the excess of lactic acid influence buffer capacity of bicarbonate buffer.

Problems

1. What is pH of the mixture composed of 20 g of NaOH (40 g/mol) and 1 liter of 2 M acetic acid? ($K_a = 1.8 \cdot 10^{-5}$)? (Answer: pH = 4,26)
2. Calculate pH of phosphate mixture composed of 0.01 mole KH_2PO_4 and 0.001 mole Na_2HPO_4 in 1 liter of the solution. $K_{H2PO4^-} = 2 \cdot 10^{-7}$. (Answer: pH = 5.7)
3. 1.2 mL of 0.1 M HCl was added to 10 mL of acetate buffer pH 5.4. pH decreased to 4.2. Calculate the buffer's capacity. (Answer: $\beta = 0.01$)
4. Enzymatic reaction was performed in 0.25 M phosphate buffer (0.125 mol/L $H_2PO_4^-$ and 0.125 mol/L HPO_4^{2-}), pH 6.8. As the result of the reaction 0.05 moles of H^+ were produced (per 1 L of the buffer). What was the final pH of the solution? What would be pH in case of a lack of a buffer ? $K_{H2PO4^-} = 1.58 \times 10^{-7}$. (Answer: $pH_1 = 6.43$, $pH_2 = 1.3$)
5. The concentration of H_2CO_3 in the human plasma is 1.25×10^{-3} mol/L. What is concentration of HCO_3^- if pH of the blood is 7.4? $K_{H2CO3} = 8 \times 10^{-7}$. (Answer: 25 mmol/L)
6. Bicarbonate buffer is the main buffer of human plasma responsible for regulation of blood pH. At pH 7.4, HCO_3^- ions concentration is 25 mM, CO_2 – 1.2 mM. What will be pH change after addition 0.005 moles of H^+ to 1 L of the blood? pK = 6.1. In the human body the excess of CO_2 is removed together with exhaled air and CO_2 concentration is not changed. (Answer: 7.3 is the final pH)
7. What is p_{CO2} in the patient blood if pH = 7.25, HCO_3^- = 25 mmol/L, $K_a = 8 \times 10^{-7}$, $\alpha = 0.03$ mmol/L · mmHg ($[CO_2] = \alpha \times p_{CO2}$)? What type of abnormality does the patient have? (Answer: 59 mmHg, acidosis)
8. How many mL of 2 % (m/v) $NaHCO_3$ (84 g/mol) should be given to patient to increase pH of the blood from 7.05 to 7.45 ? P_{CO2} = 35 mmHg, $\alpha = 0.03$ mmol/L · mmHg ($[CO_2] = \alpha \times p_{CO2}$), pK_a 6.1; V_{plasma} = 3 L. (Answer: 178.2 mL)
9. In the patient plasma, after intense physical exercise, the concentration of lactic acid (dissociated in 100 % in the human body) increased by 4 mmol/L. Assuming, that lactic acid completely reacted with alkali reserve of bicarbonate buffer, calculate final pH of the plasma and decide if the acidosis was developed. $pK_a = 6.1$. (Answer: pH 7.3)