Buffers, Buffering Capacity

Practical Lesson on

Medical Chemistry and Biochemistry

General Medicine

Jan Ševčík, Libuše Kadlecová

2026/27

Contents

Task 1 – Measurement of the pH of aqueous solutions

Dissociation is the process in which electrically neutral molecules break down into positively and negatively charged ions (cations and anions). The degree of dissociation is characteristic of a given substance and is expressed by the dissociation constant (K), which is the ratio of the product of the concentrations of the dissociated (charged) species to the concentration of the non-dissociated species:

$$\mathbf{K_a = \frac{[H^+].[A^-]}{[HA]}}$$

$$\mathbf{K_b = \frac{[B^+].[OH^-]}{[BOH]}}$$

Often, rather than K, negative decadic logarithm (pK) is used:

$$\mathbf{pK_a = -log\ K_a}$$

Strong acids and bases are 100% dissociated – meaning they exist in solution solely as cations and anions, and their pK_a values approach infinity. Weak acids and bases are partially dissociated, existing in solution in the form of both electrically neutral molecules and ions.

In aqueous salt solutions, the action of water molecules causes the dissolved molecules to break down into charged species; this phenomenon is known as electrolytic dissociation. Furthermore, some of the produced ions can react with water, leaving in the solution some extra protons or hydroxide ions (or both). Consequently, because of this reaction of **hydrolysis**, individual aqueous salt solutions exhibit acidic, neutral, or basic characteristics, depending on their composition.

In this experiment, determine the reaction of a set of aqueous inorganic salt solutions by measuring their pH using a glass electrode (pH meter).

Reagents:

Physiological solution: 0.9% aqueous solution of NaCl

0.1 mol/l hydrochloric acid HCl

0.25 mol/l sodium carbonate Na_2CO_3

0.25 mol/l sodium hydrogen carbonate (bicarbonate) $NaHCO_3$

0.25 mol/l sodium dihydrogen phosphate NaH_2PO_4

0.25 mol/l sodium hydrogen phosphate Na_2HPO_4

0.25 mol/l ammonium sulphate $(NH_4)_2SO_4$

Procedure:

1. Before the experimental phase (pH measurement), record your predicted reaction (acidic, neutral, or basic) and estimated pH value for the given aqueous salt solutions in Table 1 of the report sheet.

2. Turn the pH meter on.
3. Carefully remove the glass electrode from its container and rinse it over a beaker using distilled water from a wash bottle. Carefully blot away any remaining water droplet with a small piece of cellulose tissue before taking the measurement.
4. Immediately immerse the electrode in the container holding the solution to be analysed and measure the pH. Record the result in Table 1.
5. Repeat steps 3 and 4 to measure the entire set of prepared solutions.
6. After completing the measurements, immediately rinse the electrode with water and place it back into the container holding the saturated KCl solution.

Tasks:

1. Estimate the reaction (acidic/neutral/basic) of the particular aqueous solutions.
2. Record the ionic equation for the hydrolysis of the analysed salts in the report.
3. Measure the pH of the aqueous solutions and compare it with the estimated value.

Experiment 2 – Bicarbonate buffer: principle and buffering capacity

A buffer is a multi-component system capable of maintaining a stable pH to a certain extent, even after the addition of an acid or a base. Every buffer system consists of at least three components:

1. water,
2. a weak acid or base,
3. and its salt (i.e., a conjugate pair is present).

The pH of a buffer depends on both its precise composition and the concentration ratio of its individual components – this relationship is expressed by the **Henderson-Hasselbalch equation**:

$$\mathbf{pH = pK_a + \log \frac{[A^-]}{[HA]}}$$

The given mathematical relationship indicates that if the ratio of the concentrations of the salt (A^-) and the acid (HA) is 1:1, then the pH of the buffer is equal to the pKa of the given acid:

$$pH = pKa + \log 1 = pKa + 0$$

Analogously, if the ratio of $[A^-]$ to the acid [HA] is 1:10, the pH of the buffer is equal to the pKa of the given acid minus 1:

$$pH = pKa + \log 0.1 = pKa - 1$$

The bicarbonate buffer is the main human buffering system maintaining blood pH within the physiological range. It consists of bicarbonate and carbonic acid.

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+$$

A major advantage of this **buffering system is its openness**, which allows for dynamic changes in the concentrations of its individual components – and thus their concentration ratios (see the above-mentioned Henderson-Hasselbalch equation). Consequently, the open nature of the bicarbonate buffer system makes it possible to compensate, to a certain extent, for pH imbalances in the internal environment through changes in respiration or increased renal excretion of bicarbonate.

Clinical correlation:
Hyperventilation can be one of the manifestations of kidney failure. Metabolic acidosis develops as a result of the kidney´s impaired ability to maintain a stable internal environment by excreting metabolic waste products. This condition is compensated for by increased exhalation of carbon dioxide, leading to shortness of breath and, in more advanced stages, hyperventilation.

The aim of the task is to understand the principle of the bicarbonate buffer and to verify its buffering capacity in relation to its composition.

Reagents:

0.5 mol/l sodium carbonate Na_2CO_3

0.5 mol/l sodium bicarbonate $NaHCO_3$

1 mol/l hydrochloric acid HCl

20% solution of methylene red in ethanol

Procedure:

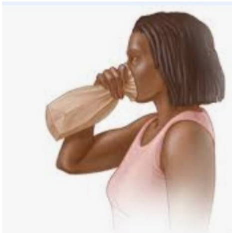
1. Prepare 4 clean glass test tubes and label them A-D.
2. Using an automatic pipette, prepare a set of solutions in the test tubes according to the table:

	A	**B**	**C**	**D**
H_2O	4 ml	0	1.8	1.8
Na_2CO_3	0 ml	2	0.2	2
$NaHCO_3$	0 ml	2	2	0.2
Methylene red	4-5 drops	4-5 drops	4-5 drops	4-5 drops

3. Using an automatic pipette, add 1 ml of HCl to each test tube containing the prepared solutions. After the addition, carefully mix the solution using a vortex mixer. Repeat this step until the indicator changes colour from yellow to **red**. Record the volume of acid added to achieve a permanent colour change.
4. Observe carefully the behaviour of the individual solutions after each addition and record the result in the report.

Tasks:

1. Explain the difference in the volume of added HCl required to cause a colour change in the individual solutions.
2. Explain the principle and the reason for the practice known as "breathing into a paper bag."



Breathing into a bag is one of the methods used to manage hyperventilation, for instance, resulting from a panic attack or a state of shock. In such a situation, the affected individual, under supervision, exhales air into a paper bag and then inhales it back from the bag.

Task 3 – Titration curve of a weak acid, pH measurement, determination of pK_a

Acid-base titration is an analytical method used to determine the concentration of a known substance in a solution based on a neutralization reaction. A fundamental prerequisite is knowledge of the chemical reaction and the correct balancing of its chemical equation.

A graphical representation of the pH changes in the solution being titrated (an acid or a base) throughout the titration process as the titrant (a base or an acid) is gradually added, is referred as a **titration curve**.

Reagents:

0.1 mol/l acetic acid CH_3COOH

0.1 mol/l sodium hydroxide NaOH

Procedure:

1. Pipette 20 ml of the weak acid into a clean titration flask.
2. While stirring continuously, gradually add the prescribed amounts of base from a burette (see table below).
3. After each addition of base, read the pH of the titrated solution using a pH meter.
4. Plot the obtained values on a graph, with the amount of added base (in ml) on the x-axis and the measured pH values on the y-axis.

	20 ml CH_3COOH c = 0.1 mol/l		
	NaOH c = 0.1 mol/l		pH
	Addition in ml	ml	
1	0	0	
2	1	1	
3	2	3	
4	3	6	
5	4	10	
6	5	15	
7	3	18	
8	1	19	
9	1	20	
10	1	21	
11	1	22	
12	3	25	
13	5	30	
14	10	40	

Tasks:

1. Write the ionic equation representing the basis of the titration determination.
2. Plot the titration curve for a weak acid.
3. Calculate the theoretical pH for c(CH_3COOH) = 0.1 mol/l ($K_a = 1.75 \times 10^{-5}$).
4. Locate and mark the pK_a of acetic acid on the graph and compare it with the tabulated value.
5. Determine the pH at the equivalence point of the titrated acid.

Task 4 – Preparation of a buffer with defined properties

Based on an understanding of buffer principles, it is possible to prepare a buffer with defined properties – specifically pH and buffer capacity, as well as other characteristics such as ionic strength.

Reagents:

0.1 mol/l HCl

0.1 mol/l NaOH

0.1 mol/l CH_3COOH; $K_a = 1.75 \times 10^{-5}$

0.1 mol/l NaCl

20 % ethanol solution of methylene red

Task:
Using any of the given reagent prepare 25 ml of buffer with pH = 3.7.