Exercise 22

Investigation of acid-base homeostasis in amphoteric electrolyte

Theory:

Activity and activity coefficient of the component of the solution. Ion activity and electrolyte activity. Acid-base homeostasis. Dissociation constant and hydrolysis constant. Ion product of water. pH. Half-cell potential and electromotive force of the cell. Construction of the glass electrode. Silver chloride electrode. Buffer solutions - a qualitative description of their mechanism of action.

Amphoteric electrolytes or ampholytes are substances that can have both acidic and basic properties. The best-known ampholytes, containing both acidic and basic groups in one molecule, are amino acids. Their structure can be represented by the general formula NH_2RCOOH. In these compounds, intramolecular proton exchange may occur, resulting in the formation of zwitterionic ions, the so-called zwitterions:

$$H_2NRCOOH \rightleftarrows H_3N^+RCOO^- \tag{1}$$

The concentration of these ions depends on the relative proton donor capacity of the two groups, and is also a function of the pH of the solution. This is illustrated by the following diagram:

$$\mathrm{H_2NRCOO^-} \overset{+OH^-}{\rightleftarrows} \mathrm{H_3N^+RCOO^-} \overset{+H^+}{\rightleftarrows} \mathrm{H_3N^+RCOOH} \tag{2}$$

In acidic solutions of ampholytes, apart from the zwitterion, there are positive ions H_3N^+RCOOH, while in alkaline solutions negatively charged ions H_2NRCOO^- are present. At a certain pH characteristic for a given compound, the ampholyte reaches the so-called isoelectric state, i.e. a state in which the concentration of positive and negative ions is the same, while the concentration of zwitterionic ions reaches a maximum. The isoelectric point, pJ, is the pH at which ampholyte is in an isoelectric state.

Let denote the bipolar ion $H_3N^+RCOO^-$ by $AH^\pm$, the positive ion by $\mathrm{AH_2^+}$, the negative ion by A^- and the undissociated amino acid molecules by AH. The ion can be considered as a dibasic acid, the dissociation of which proceeds in two steps:

$$\mathrm{AH_2^+} \rightleftarrows \mathrm{AH^\pm} + \mathrm{H^+} \tag{3}$$

$$\mathrm{AH^\pm} \rightleftarrows \mathrm{A^-} + \mathrm{H^+} \tag{4}$$

However it should be noted, that:

$$\mathrm{AH} \rightleftarrows \mathrm{AH^\pm} \tag{5}$$

The value of the first dissociation constant K_1 is described by the equation:

$$K_1 = \frac{a_{\mathrm{H^+}} a_{\mathrm{AH^\pm}}}{a_{\mathrm{AH_2^+}}} \tag{6}$$

while the second dissociation constant K_2 is described by the equation:

$$K_2 = \frac{a_{\mathrm{H^+}}\, a_{\mathrm{A^-}}}{a_{\mathrm{AH^\pm}}} \tag{7}$$

Additionally, for the reaction (5):

$$K_d = \frac{a_{\mathrm{AH}^\pm}}{a_{\mathrm{AH}}} \tag{8}$$

At the isoelectric point, the activities of positive and negative ions (*a*) are equal to:

$$a_{\mathrm{AH}_2^+} = a_{\mathrm{A}^-} \tag{9}$$

Using equations (6) and (7) to calculate $a_{\mathrm{AH}_2^+}$ and a_{A^-}, respectively, and by comparing the two values the formula for the activity of hydrogen ions at the isoelectric point of the ampholyte is obtained:

$$a_{\mathrm{H}^+} = \sqrt{K_1 K_2} \tag{10}$$

from which its isoelectric point can be determined:

$$\mathrm{pJ} = \mathrm{pH}_{\mathrm{izoel}} = -\frac{1}{2}\log(K_1 K_2) \tag{11}$$

If the first and second dissociation constants differ significantly ($K_1/K_2 \geq 10^3$), they can be determined by measuring the pH of the ampholyte in an acidic and alkaline solution.

So far our derivation has been strict. Now assume that the concentration of the reactants in the solution is so small that we can replace their activities with concentrations. If a small amount of a strong acid is added to an amino acid solution, the activity of the A^- form will be practically zero, and almost all AH_2^+ ions present in the solution will be formed by the addition of a proton from the strong acid to the $AH^\pm$ or AH forms of the amino acid (see equations 4 and 5).

Let the total ampholyte concentration be c_{Amf}, and the strong acid concentration c_{Acid}. Therefore, the following relationships are true:

$$c_{\mathrm{Amf}} \cong a_{\mathrm{AH}^\pm} + a_{\mathrm{AH}} + a_{\mathrm{AH}_2^+} = a_{\mathrm{AH}^\pm}\left(1 + \frac{1}{K_d}\right) + a_{\mathrm{AH}_2^+} \tag{12}$$

$$c_{\mathrm{Acid}} \cong a_{\mathrm{H}^+} + a_{\mathrm{AH}_2^+} \tag{13}$$

From these equations you can calculate the $a_{\mathrm{AH}^\pm}$ and $a_{\mathrm{AH}_2^+}$ values required to calculate K_1:

$$a_{\mathrm{AH}_2^+} \cong c_{\mathrm{Acid}} - a_{\mathrm{H}^+} \tag{14}$$

$$a_{\mathrm{AH}^\pm} \cong \frac{c_{\mathrm{Amf}} - c_{\mathrm{Acid}} + a_{\mathrm{H}^+}}{1 - \frac{1}{K_d}} \tag{15}$$

Substituting these values into equation (6) we get:

$$K_1 = \frac{a_{\mathrm{H}^+}\left(c_{\mathrm{Amf}} - c_{\mathrm{Acid}} + a_{\mathrm{H}^+}\right)}{\left(1 - \frac{1}{K_d}\right)\left(c_{\mathrm{Acid}} - a_{\mathrm{H}^+}\right)} \tag{16}$$

In a basic solution of ampholyte, e.g. in the presence of NaOH with a concentration of c_{Base}, the activity of the protonated form will be practically zero, and almost all A^- anions present in the solution will be formed as a result of the reaction:

$$\mathrm{AH}^\pm(\mathrm{AH}) + \mathrm{OH}^- \rightleftarrows \mathrm{A}^- + \mathrm{H_2O} \tag{17}$$

Therefore the following relationships are satisfied:

$$c_{\mathrm{Amf}} \cong a_{\mathrm{AH}^\pm} + a_{\mathrm{AH}} + a_{\mathrm{A}^-} = a_{\mathrm{AH}^\pm}\left(1 + \frac{1}{K_d}\right) + a_{\mathrm{A}^-} \tag{18}$$

$$c_{\mathrm{Base}} \cong a_{\mathrm{OH}^-} + a_{\mathrm{A}^-} \tag{19}$$

Where $a_{\mathrm{OH^-}}$ denotes the activity of hydroxide ions. After taking into account the ionic product of water:

$$K_{\mathrm{W}} = a_{\mathrm{H^+}}\, a_{\mathrm{OH^-}} \tag{20}$$

as well as calculating $a_{\mathrm{AH^\pm}}$ i $a_{\mathrm{A^-}}$ from equations (18) i (19) and including them in equation (7) the expression for the second dissociation constant is obtained:

$$K_2 = \frac{K_{\mathrm{W}}\left(1-\frac{1}{K_d}\right)\cdot(c_{\mathrm{Base}}-a_{\mathrm{OH^-}})}{a_{\mathrm{OH^-}}\cdot(c_{\mathrm{Amf}}-c_{\mathrm{Base}}+a_{\mathrm{OH^-}})} \tag{21}$$

Equations (16) and (21) show that the dissociation constants cannot be determined without knowing the K_d constant. However, by measuring the pH of the ampholyte solution with known c_{Amf}, c_{Acid} i c_{Base} values, the value of the isoelectric point of the ampholyte can be calculated by combining equations (9), (16) and (21):

$$\mathrm{pJ} = -\frac{1}{2}\log\left(\frac{a_{\mathrm{H^+}}\cdot(c_{\mathrm{Amf}}-c_{\mathrm{Acid}}+a_{\mathrm{H^+}})\cdot(c_{\mathrm{Base}}-a_{\mathrm{OH^-}})\cdot K_{\mathrm{W}}}{a_{\mathrm{OH^-}}\cdot(c_{\mathrm{Acid}}-a_{\mathrm{H^+}})\cdot(c_{\mathrm{Amf}}-c_{\mathrm{Base}}+a_{\mathrm{OH^-}})}\right) \tag{22}$$

The aim of the exercise:

The aim of the exercise is to determine the isoelectric point of a selected amino acid based on the measurements of the pH value of its solutions containing a strong acid or strong base.

Experimental procedure:

1. Prepare 5 acidic solutions (of 30 $\mathrm{cm^3}$ volume) of the amino acid, by mixing its 0.10 M solution with 0.10 M HCl solution in volume ratios listed in Tabel 1.
2. Prepare 5 alkaline solutions (of 30 $\mathrm{cm^3}$ volume) of the amino acid, by mixing its 0.10 M solution with 0.10 M NaOH solution in volume ratios listed in Tabel 1.
3. Calibrate the pH-meter (the manual for using and calibrating the pH-meter is provided at the laboratory station).
4. Measure the pH of the prepared solutions and list the results in Tabel 1.
5. Calculate the concentration of acid (c_{Acid}), base (c_{Base}) and ampholyte (c_{Amf}) and write the values down in Tabel 1.
6. Calculate the activities of hydrogen and hydroxide ions; put the results in Table 1.
7. Calculate the isoelectric point of the amino acid for each pair of solutions with the same concentrations of HCl and NaOH; put the results in Table 1.
8. Plot the pJ(c) relationship, where c is the concentration of HCl or NaOH.
9. In order to determine the most accurate value of the isoelectric point, use the least squares method to determine the initial ordinate of the fitted line $\mathrm{pJ} = f(c)$ and its uncertainty.

Tabel 1.

V_{Acid} [cm^3]	V_{Base} [cm^3]	V_A [cm^3]	c_{Acid} [M]	c_{Base} [M]	c_{Amf} [M]	pH	a_{H^+} [M]	a_{OH^-} [M]	pJ
1	0	29		–				–	–
0	1	29	–				–		
2	0	28		–				–	–
0	2	28	–				–		
3	0	27		–				–	–
0	3	27	–				–		
4	0	26		–				–	–
0	4	26	–				–		
5	0	25		–				–	–
0	5	25	–				–		

V_{Acid}, V_{Base}, V_A- volumes of acid, base and amino acid solutions used to prepare the test solutions.

Tips for the calculations:

1. Calculate the isoelectric point of an amino acid from the formula (22), using the activities of hydrogen and hydroxide ions obtained during pH measurements in solutions containing hydrochloric acid and sodium hydroxide of the same concentration, respectively.
2. Puse ionic product of water (20) to calculate a_{OH^-}. At 25°C the ionic product of water is equal to $1\cdot10^{-14}$ ($c^{\ominus}$=1 [M]).

Principles of pH measurements

The potential of the glass electrode is determined by the ratio of the activity of the hydrogen ions in the solutions on both sides of the glass membrane. Since the activity of the hydrogen ions inside the bubble is constant, the potential of the glass electrode, E, can be formally expressed by the equation:

$$E = E_0 + k \cdot \mathrm{pH} \tag{23}$$

where E_0 is the potential of a glass electrode immersed in a pH=0 solution measured against a specific reference electrode. E_0 takes different values for different types of electrodes, and for a given electrode, it changes over time. The proportionality coefficient k depends on the temperature and gives the so-called slope of the electrode characteristics and for a good glass electrode:

$$k = \frac{RT}{F} ln10 \tag{24}$$

where R is the gas constant, T is the absolute temperature and F is Faraday's constant.

The pH measurement using a glass electrode is carried out using a comparative method, i.e. by comparing the potential of the electrode immersed in the test solution with the potential of this electrode placed in a standard buffer solution with a known pH value. The pH standards are buffer solutions which, were assigned $\mathrm{pH_{WZ}}$ values corresponding, within the error limits of the method, to the definition of $\mathrm{pH_{WZ}} = -log a_{\mathrm{H}^+}$. The relationship between the pH of the test solution, $\mathrm{pH_{WZ}}$ and the potentials of the glass electrode in the test and standard solutions, E i E_{WZ}, respectively, is then given by equation:

$$\mathrm{pH} = \mathrm{pH}_{\mathrm{WZ}} + \frac{E - E_{\mathrm{WZ}}}{k} \tag{25}$$

This equation is an instrumental definition of the practical, conventional pH scale. The conventional pH scale, whose points of reference are the pH values of aqueous buffer solutions, applies only to the aquatic environment.

If it cannot be assumed that the value of the slope of the characteristic, k, is known, then equation (25) contains two unknowns. Therefore, in order to unambiguously determine the pH value of the test solution, a system of two equations should be built, so it is necessary to use a second standard solution. In practice, the pH values of the test solution are obtained after prior agreement of the pH meter readings with the known pH values of two standard buffer solutions in accordance with the instruction manual of a specific type of instrument.