

Experiment 8A

Electrolytic and buffer properties of water solutions of amino acids

Basic notions: amino acids, hydrolysis, ionisation, zwitterion, amphoteric species, electrolyte, protolysis, pH, dissociation constant, isoelectric point, conjugate base, buffer solution, pK, buffer capacity

Introduction

Amino acids are the carboxylic acids containing an amine group in the molecule. In the amino acids obtained by hydrolysis of proteins, the amine group occurs always at position α , which means it is at the same carbon atom as the carboxylic acid group. General formula for amino acids is $\text{RCH}(\text{NH}_2)\text{COOH}$. The α -carbon atom in amino acids is asymmetric and the vast majority of amino acids present in nature belong to the configuration series L.

Besides the above-mentioned functional groups, the molecules of amino acids can include e.g. $-\text{OH}$, $-\text{SH}$, indole group or an additional carboxylic acid group or additional amine group. According to the types of functional groups in the side chain, amino acids can be divided into four main types 1) amino acids with apolar side chain $-\text{R}$, 2) amino acids with the side chain $-\text{R}$ containing a polar group not undergoing ionisation in a water solution ($-\text{OH}$, $-\text{SH}$), 3) amino acids with an additional carboxylic acid group and 4) amino acids with an additional amine group. The first two types of amino acids are neutral.

The aim of experiment is to establish the buffer properties of a water solution of alanine $\text{CH}_3\text{CH}(\text{NH}_2)\text{COOH}$ which is a neutral amino acid of the first type.

Theory

Carboxylic acid group as well as amine group of amino acids easily undergo ionisation. The carboxylic acid group releases a proton H^+ and becomes a carboxyl anion, while the amine group attracts a proton and becomes an ammonium cation. In this way bipolar zwitterions are formed, containing both a positive and a negative charge:



According to the proton theory, amino acid zwitterions are typical amphoteric species. Their ammonium groups $-\text{NH}_3^+$ are acidic proton donors and their carboxyl groups $-\text{COO}^-$ are basic proton acceptors.

The zwitterion of a monoaminomonocarboxyl amino acid is a weak electrolyte and in water solution it undergoes protolysis to a small degree but nevertheless high enough to make the pH of the solution significantly different from the pH of water. The acidic strength of ammonium group $-\text{NH}_3^+$ is greater ($K_a = \text{close to } 10^{-8}$) than the basic strength of carboxyl group $-\text{COO}^-$ ($K_b = \text{close to } 10^{-11}$), so in the water solution of this amino acid a protolytic equilibrium is reached:

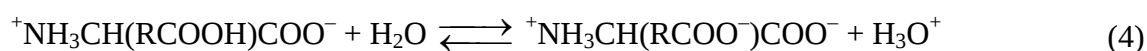


and thanks to the H_3O^+ ions formed, the pH of the solution is lower than 7. In the presence of a strong acid, the protolytic equilibrium of reaction (2) is shifted to the left and the following reaction takes place:

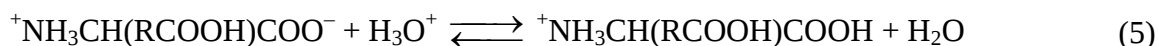


At a certain concentration of the strong acid, the solution contains a large excess of zwitterions and small but equal amounts of the anions $\text{NH}_2\text{CH(R)COO}^-$, and cations $^+\text{NH}_3\text{CH(R)COOH}$. The pH of such a solution is defined as the isoelectric point (pI) of a given amino acid. At this point molecules of amino acids do not migrate in an electric field. In the solution of $\text{pH} < \text{pI}$, the amino acid molecules move towards the cathode, while in the solutions of $\text{pH} > \text{pI}$, they move towards the anode. The pI values of monoaminomonocarboxyl amino acids vary between 5 and 6, so they do not differ much from the pH of pure water and therefore they are called neutral.

The presence of a carboxylic acid group $-\text{COOH}$ ($K_a = \sim 10^{-4}$) in the side chain of monoaminodicarboxyl amino acids means that their water solutions have pH much smaller than 7. The reason for this phenomenon is the protolysis of the zwitterion taking place according to the following equation:

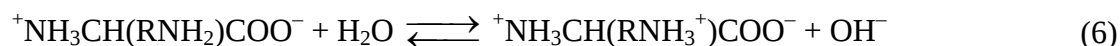


In the presence of a strong acid, the equilibrium of reaction (4) is shifted to the left and the following protolysis takes place:

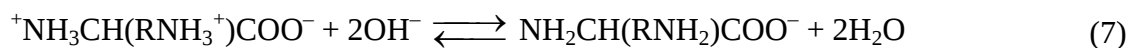


For these amino acids the concentration of the strong acid in the solution must be much higher to achieve $\text{pH} = \text{pI}$ of the amino acid than for monoaminomonocarboxyl amino acids. The pI values for monoaminodicarboxyl amino acids are near 3, so that these amino acids are known as acidic.

Thanks to the presence of $-\text{NH}_2$ group ($K_b = \text{ok. } 10^{-6}$) in the side chain, diaminomonocarboxyl amino acids make with water solutions of $\text{pH} > 7$ according to the protolytic reaction given below:



To shift the pH of the solution to the isoelectric point, the equilibrium of this reaction should be shifted to the left and the amount of the positively charged ammonium groups should be reduced by addition of a strong base:



Therefore, diaminomono-carboxyl amino acids have $pI > 7$ and are called basic amino acids.

The electrolytic properties of neutral amino acids can be analysed on the example of equation of protolysis (3). According to the protonic theory of acids, the amphoteric dipolar ions $^+NH_3CH(R)COO^-$ is a base conjugated with a weak acid of $^+NH_3CH(R)COOH$ cation. Therefore, the solution of amino acid titrated with a strong acid becomes a mixture of a weak acid and a base conjugated with it, showing the properties of a buffer system. The pH value of a buffer system is defined as:

$$pH = pK_{HA} + \log \frac{[A^-]}{[HA]} \quad (8)$$

where pK_{HA} is a negative logarithm of the dissociated constant of the weak acid HA, $[HA]$ – is the molar concentration of this acid, and $[A^-]$ is the molar concentration of the base conjugated with it.

Substituting the appropriate molar concentrations of the amino acid cations (weak acid) and dipolar ions (conjugated base), dependent on the amount of the strong acid added, the following equation describing changes in pH of the amino acid solution titrated is obtained:

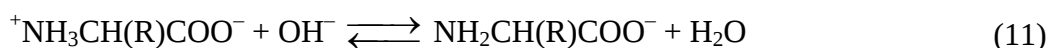
$$pH = pK_1 + \log \frac{[^+NH_3CH(R)COO^-]}{[^+NH_3CH(R)COOH]} \quad (9)$$

where K_1 is the dissociation constant of the carboxylic acid group of this amino acid. As the pK_1 value is constant (depends only on temperature), the changes in pH of the titrated solution depend only on the value of the second term on the right hand side of equation (9). At the beginning of titration the buffering ability of the solution is low and even small amounts of the strong acid added cause considerable changes in pH. It is easy to note that if the molar concentrations of the dipolar ions and cations are the same $[^+NH_3CH(R)COO^-] = [^+NH_3CH(R)COOH]$, then:

$$\log \frac{[^+NH_3CH(R)COO^-]}{[^+NH_3CH(R)COOH]} = \log 1 = 0 \quad (10)$$

and eq. (9) becomes $pH = pK_1$. Under this condition the amino acid solution shows a very high buffer capacity.

If a water solution of the amino acid is titrated with a strong base, the reaction takes place according to the equation:



The dipolar ion having amphoteric character, behaves in the above conditions as a weak acid and transforms into the conjugated base $NH_2CH(R)COO^-$. The buffer solution formed is characterised by the following equation:

$$pH = pK_2 + \log \frac{[NH_2CH(R)COO^-]}{[^+NH_3CH(R)COO^-]} \quad (12)$$

where K_2 is the dissociation constant of the ammonium group $-\text{NH}_3^+$. The buffer solution obtained has the highest capacity near $\text{pH} = \text{p}K_2$.

The titration curves of neutral amino acids of first type (like alanine, valine, leucine, isoleucine, phenylalanine, proline) and those of second type (serine, methionine, tryptophan, hydroxyproline) as well as glutamines and asparagines have similar or almost identical course as these amino acids have very close values of $\text{p}K_1$, $\text{p}K_2$ and pI .

Experiment

Method

Characterisation of buffer properties of a water solution of an amino acid is realised by determination of the titration curve of this amino acid. The values of pH of the amino acid solution are measured as a function of millimoles of the added strong acid and millimoles of the strong base added. In order to find out in which pH ranges the amino acid forms buffer solutions, the logarithms from equation (9) – titration with a strong acid, and equation (12) – titration with a strong base, are calculated. In the pH ranges in which the logarithms take values close to zero, the amino acid shows a high buffer capacity.

The amino acid used in the experiment will be alanine $\text{NH}_2\text{CH}(\text{CH}_3)\text{COOH}$.

Reagents and apparatuses

- alanine
- HCl solution of concentration 1.0 mol/dm^3
- NaOH solution of concentration 1.0 mol/dm^3
- Standard buffer of $\text{pH} = 4$
- Standard buffer of $\text{pH} = 7$
- Standard buffer of $\text{pH} = 9$
- pH-meter with a combined electrode
- Magnetic stirrer with a stirring element
- Analytical balance, spatula, vessel for weighting
- 2 burettes of 10 cm^3 in capacity
- 1 pipette of 20 cm^3
- 4 beakers of 50 cm^3
- Volumetric flask of 50 cm^3
- A wash bottle

Procedure

1. In a volumetric flask of 50 cm^3 in capacity prepare a solution of alanine of a concentration 0.27 mol/dm^3 .
2. Calibrate the H-meter for the reference buffers of $\text{pH} 7$ and 4 .
3. Pour 20 cm^3 of alanine solution to a beaker and measure its pH.
4. Titrate the solution of alanine with a solution of hydrochloric acid in the following way. To the alanine solution add HCl from the burette, at first add portions of one drop. After adding each portion of one drop of HCl solution, carefully stir the contents and measure its pH. Read off the volume of HCl added (V_{HCl}) that corresponds to a given pH value. If pH changes between subsequent readings will not be greater than $0.07 - 0.09$, measure pH values after adding two drops of HCl, then after three, four drops etc. The number of drops is

increased by 1 drop when the pH value differs from the previous pH value by about 0.09. Continue the titration until pH of the solution becomes close to 1.6.

5. Pour 20 cm³ of distilled water to a beaker (the so-called blank sample), measure pH of water and perform titration with HCl in the way described above.

6. Calibrate the pH-meter for the reference buffers of pH 7 and 9.

7. Pour 20 cm³ of alanine solution to a beaker and measure its pH.

8. Titrate the alanine solution with a solution of NaOH until getting pH 11, performing the pH measurements in the same way as described for titration with HCl (point 4.).

9. Pour 20 cm³ of distilled water to a beaker (the so-called blank sample), measure pH of water and perform titration with NaOH solution in the way described above.

10. Put down the results of measurements in Tables 1 and 2.

Table 1

Titration of water		Titration of alanine solution				
V_{HCl} [cm ³]	pH	V_{HCl} [cm ³]	pH	c_2 [mmole]	c_2/c_1	$\log(c_2/c_1)$

V_{HCl} – the volume of added HCl solution, it is equal to the number of millimoles of HCl added and, for titration of alanine solution, it is also equal to the number of millimoles of alanine cation, $^+\text{NH}_3\text{CH}(\text{CH}_3)\text{COOH}$, c_1 (explain the relations);

c_2 – number of millimoles of the dipolar ion of alanine, $^+\text{NH}_3\text{CH}(\text{CH}_3)\text{COO}^-$, the following relation holds $c_2 = c_0 - c_1$ (explain why), where c_0 – is the initial number of millimoles of aniline in the solution from point 3.

Table 2

Titration of water		Titration of alanine solution				
V_{NaOH} [cm ³]	pH	V_{NaOH} [cm ³]	pH	c_2 [mmole]	c_3/c_2	$\log(c_3/c_2)$

V_{NaOH} – is the volume of NaOH added, which is numerically the same as the number of millimoles of NaOH added and for titration of alanine solution it is equal to the number of millimoles of aniline anion $\text{NH}_2\text{CH}(\text{CH}_3)\text{COO}^-$, c_3 (explain the relation);

c_2 – the number of millimoles of a dipole ion of aniline $^+\text{NH}_3\text{CH}(\text{CH}_3)\text{COO}^-$, the following relation holds $c_2 = c_0 - c_3$ (explain why), where c_0 – is the initial number of aniline millimoles in the solution from point 7.

Calculations

1. Calculate the values of c_2 , c_2/c_1 and $\log(c_2/c_1)$ (Table 1) and the values of c_2 , c_3/c_2 and $\log(c_3/c_2)$ (Table 2).

2. Draw the titration curves of alanine so the dependence of pH measured on the number of millimoles of HCl added and NaOH added. The value of zero millimoles of added HCl and added NaOH should be marked in the middle of the abscissa axis; the millimoles of NaOH added should be marked on the right hand side of this point, while the millimoles of

HCl added – on the left hand side. For the sake of comparison, in the same figure draw also the titration curve of water.

3. Plot the dependence of pH on $\log(c_2/c_1)$ for HCl, and the dependence of pH on $\log(c_3/c_2)$ for NaOH. For each plot draw a line through the points near the zero values of the logarithms and find out the parameters of this line. Determine the values of pK_1 and pK_2 for alanine.

Discussion

1. Find out if the titration curves of alanine and water differ from each other. If so, interpret the differences.

2. Find out in which pH ranges alanine makes buffer systems.

3. Calculate pH values of alanine solution ($pK_1 = 2.34$, $pK_2 = 9.69$) of a concentration 0.1 mol/dm^3 , if alanine is present as:

a) hydrochloride (cation),

b) zwitterion.

References

[1] B. Filipowicz, W. Ostrowski, *Ćwiczenia z chemii ogólnej i fizjologicznej*, Państwowy Zakład Wydawnictw Lekarskich, Warszawa, 1976, rozdz. 24.

[2] L. Stryer, *Biochemia*, Wydawnictwo Naukowe PWN, Warszawa, 1999.

Supplementing literature

[3] A. Zgirski, R. Gondko, *Obliczenia biochemiczne*, Wydawnictwo Naukowe PWN, Warszawa, 1998, rozdz. 4.

Related problems

Acid-base equilibria, Brönsted theory of acids and bases. Half-cells, half-cell potential, types of half-cells. Electrochemical cells. Electromotive force. Potentiometric measurements of pH.

EXPERIMENT 8B

Aqueous solutions of amino acids. Calculation of titration curves

Key concepts: microscopic equilibrium, macroscopic equilibrium, conservation of mass, condition of electro-neutrality

Introduction

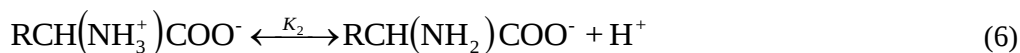
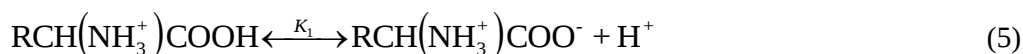
The aim of the second part this experiment is to calculate the titration curve for alanine titrated by a strong acid or a strong base and to compare the calculated curves with those measured in the first part of the experiment.

Theory

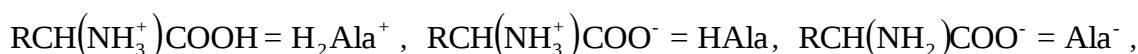
The following reactions take place in an aqueous solution of an amino acid $\text{RCH}(\text{NH}_2)\text{COOH}$ containing one amine group and one carboxylic group:



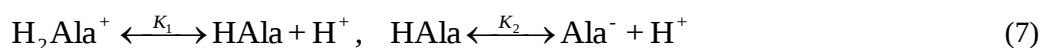
where k_1, k_2, k_3, k_4 are the constants of *microscopic* equilibria. As the concentration of neutral molecules $\text{RCH}(\text{NH}_2)\text{COOH}$ is much lower than the concentration of the zwitterion $\text{RCH}(\text{NH}_3^+)\text{COO}^-$ (see Discussion), only the following two approximate macroscopic equilibria are relevant:



where K_1 and K_2 are the appropriate *macroscopic* equilibrium constants. In an aqueous solution of alanine, $\text{RCH}(\text{NH}_3^+)\text{COOH}$ is a diprotic weak acid. Applying the following notation:



equations (5,6) for the macroscopic equilibria become:



1. Titration with a strong base BOH

Titration of alanine with a strong base BOH is described by several equations: two equations for the dissociation constants K_1 and K_2 , an equation for the conservation of mass of alanine, an equation for the ionic product of water K_w , an equation expressing the condition of electro-neutrality and two equations describing the dilution of alanine and the base upon titration:

$$\frac{[H^+][HAla]}{[H_2Ala^+]} = K_1, \text{ equilibrium constant } K_1 \quad (8)$$

$$\frac{[H^+][Ala^-]}{[HAla]} = K_2, \text{ equilibrium constant } K_2 \quad (9)$$

$$c_{Ala} = [H_2Ala^+] + [HAla] + [Ala^-], \text{ total concentration of different forms of alanine} \quad (10)$$

$$[H^+][OH^-] = K_w, \text{ ionic product of water} \quad (11)$$

$$[Ala^-] + [OH^-] = [H^+] + [B^+] + [H_2Ala^+], \text{ condition of electro-neutrality} \quad (12)$$

$$c_{Ala} = \frac{c_{Ala0}V_{Ala}}{V_{Ala} + V_B}, \text{ concentration of alanine during titration (dilution)} \quad (13)$$

$$[B^+] = \frac{c_{B0}V_B}{V_{Ala} + V_B}, \text{ concentration of the base during titration (dilution)} \quad (14)$$

where c_{Ala0} is the initial concentration of alanine, c_{B0} is the concentration of the base used for titration, V_{Ala} is the volume of alanine solution, and V_B is the volume of the base added.

The set of equations (8-14) describing titration of alanine can be solved with respect to the volume of the strong base, $V_B = V_B([H^+]; c_{Ala0}, c_{B0}, V_{Ala}, V_B, K_1, K_2)$. The concentration of hydrogen ions $[H^+]$ is the independent variable while $c_{Ala0}, c_{B0}, V_{Ala}, V_B, K_1, K_2$ are the known parameters.

One way to solve the set of equations (8-14) is, first, to solve equations (8-10) with respect to $[H_2Ala^+]$, $[HAla]$, $[Ala^-]$, and then to eliminate the variables $[Ala^-]$, $[OH^-]$, $[B^+]$, $[H_2Ala^+]$ from the condition of electro-neutrality (12). Solving equations (8-10) leads to:

$$[H_2Ala^+] = c_{Ala} \alpha_{H_2Ala^+}, [HAla] = c_{Ala} \alpha_{HAla}, [Ala^-] = c_{Ala} \alpha_{Ala^-} \quad (15)$$

where:

$$\alpha_{H_2Ala^+} = \frac{[H^+]^2}{[H^+]^2 + [H^+]K_1 + K_1K_2} \quad (16)$$

$$\alpha_{HAla} = \frac{[H^+]K_1}{[H^+]^2 + [H^+]K_1 + K_1K_2} \quad (17)$$

$$\alpha_{Ala^-} = \frac{K_1K_2}{[H^+]^2 + [H^+]K_1 + K_1K_2} \quad (18)$$

Using equations (15) and (11,13,14), the condition of electro-neutrality (12) becomes:

$$\frac{\alpha_{\text{Ala}^-} c_{\text{Ala}0} V_{\text{Ala}}}{V_{\text{Ala}} + V_{\text{B}}} + \frac{K_{\text{w}}}{[\text{H}^+]} = [\text{H}^+] + \frac{c_{\text{B}0} V_{\text{B}}}{V_{\text{Ala}} + V_{\text{B}}} + \frac{\alpha_{\text{H}_2\text{Ala}^+} c_{\text{Ala}0} V_{\text{Ala}}}{V_{\text{Ala}} + V_{\text{B}}} \quad (19)$$

from which we get :

$$V_{\text{B}} = \frac{c_{\text{Ala}0} (\alpha_{\text{H}_2\text{Ala}^+} - \alpha_{\text{Ala}^-}) + [\text{H}^+] - K_{\text{w}} / [\text{H}^+]}{K_{\text{w}} / [\text{H}^+] - [\text{H}^+] - c_{\text{B}0}} V_{\text{Ala}} \quad (20)$$

Eq. (20) expresses the volume V_{B} of strong base as a function of the concentration $[\text{H}^+]$ of hydrogen ions. Note that for a measured titration curve V_{B} is an independent variable while $\text{pH} = -\log[\text{H}^+]$ is a dependent variable. A calculated titration curve is obtained when V_{B} are placed on the x axis and pH on the y axis. That calculated titration curve will be used for comparison with the measured curve.

2. Titration with a strong acid HAc

Similarly to titration with a strong base, titration of alanine with a strong acid HAc is described by two equations for the dissociation constants K_1 and K_2 , the law of mass conservation for alanine, an equation for ionic product of water K_{w} , the condition of electro-neutrality and two equations describing dilution of alanine and acid upon titration. Equations (8-11) do not change whereas equations (12-14) are replaced by:

$$[\text{Ala}^-] + [\text{OH}^-] + [\text{Ac}^-] = [\text{H}^+] + [\text{H}_2\text{A}^+], \text{ condition of electro-neutrality} \quad (21)$$

$$c_{\text{Ala}} = \frac{c_{\text{Ala}0} V_{\text{Ala}}}{V_{\text{Ala}} + V_{\text{Ac}}}, \text{ concentration of alanine } c_{\text{Ala}} \text{ during titration (dilution)} \quad (22)$$

$$[\text{Ac}^-] = \frac{c_{\text{Ac}0} V_{\text{Ac}}}{V_{\text{Ala}} + V_{\text{Ac}}}, \text{ concentration of acid upon titration (dilution)} \quad (23)$$

where $c_{\text{Ac}0}$ is the concentration of the acid used for titration, and V_{Ac} is the volume of the acid added. The set of equations describing the titration with a strong acid can be solved with respect to the volume of the strong acid $V_{\text{Ac}} = V_{\text{Ac}}([\text{H}^+]; c_{\text{Ala}0}, c_{\text{Ac}0}, V_{\text{Ala}}, V_{\text{Ac}}, K_1, K_2)$. The independent variable is the concentration of hydrogen ions $[\text{H}^+]$, while the parameters are $c_{\text{Ala}0}, c_{\text{Ac}0}, V_{\text{Ala}}, V_{\text{Ac}}, K_1, K_2$.

The electro-neutrality condition becomes:

$$\frac{\alpha_{\text{Ala}^-} c_{\text{Ala}0} V_{\text{Ala}}}{V_{\text{A}} + V_{\text{Ac}}} + \frac{K_{\text{w}}}{[\text{H}^+]} + \frac{c_{\text{Ac}0} V_{\text{Ac}}}{V_{\text{A}} + V_{\text{Ac}}} = [\text{H}^+] + \frac{\alpha_{\text{H}_2\text{A}^+} c_{\text{Ala}0} V_{\text{Ala}}}{V_{\text{Ala}} + V_{\text{Ac}}} \quad (24)$$

from which we get

$$V_{\text{Ac}} = \frac{(\alpha_{\text{H}_2\text{A}^+} - \alpha_{\text{Ala}^-}) c_{\text{Ala}0} + [\text{H}^+] - K_{\text{w}} / [\text{H}^+]}{c_{\text{Ac}0} - [\text{H}^+] + K_{\text{w}} / [\text{H}^+]} V_{\text{Ala}} \quad (25)$$

Calculations

Calculate the titration curve and plot it together with the measured titration curve:

1. Based on the data from the first part of this experiment, determine the pH ranges for the curves of

titration with a strong base and a strong acid.

2. Convert the pH ranges of titration with a strong base or a strong acid into the ranges of the hydrogen ions concentration $[H^+]$.

3. Calculate $V_B = V_B([H^+]; c_{Ala0}, c_{B0}, V_{Ala}, V_B, K_1, K_2)$ and $V_{Ac} = V_{Ac}([H^+]; c_{Ala0}, c_{Ac0}, V_{Ala}, V_{Ac}, K_1, K_2)$ according to equations (20) and (25) for the selected range of $[H^+]$. Use the parameters $c_{Ala0}, c_{B0}, c_{Ac0}, V_{Ala}, V_B, K_1, K_2$ from the first part of this experiment. The calculations can be performed using a spreadsheet. First, enter the selected $[H^+]$ values in a column. Then enter the parameter $c_{Ala0}, c_{B0}, V_{Ala}, V_B, K_1, K_2$ in cells for further use. Enter formulas that evaluate $\alpha_{H_2Ala^+}$ and α_{Ala^-} as functions of $[H^+]$

according to (16) and (18). Evaluate $\alpha_{H_2Ala^+}$ and α_{Ala^-} in separate columns. Next, use formulas to calculate the numerator and denominator values in (20) from the $[H^+]$, $\alpha_{H_2Ala^+}$ and α_{Ala^-} columns.

Finally, use the numerator and denominator columns to calculate a column of V_B values.

4. Plot the theoretical curve $pH = f(V_B)$, $pH = f(-V_A)$ and compare with the one measured in the first part of the experiment.

Discussion

1. Show that for the microscopic equilibria (1-4) the following relation holds $k_1 k_3 = k_2 k_4$.

2. Dissociation of a proton from a molecule of $RCH(NH_3^+)COOH$ is described by the macroscopic equilibrium constant:

$$K_1 = \frac{([RCH(NH_3^+)COO^-] + [RCH(NH_2)COOH])[H^+]}{[RCH(NH_3^+)COOH]} \quad (26)$$

Write an expression for the macroscopic equilibrium constant K_2 describing the formation of $RCH(NH_2)COO^-$.

3. Show that $K_1 = k_1 + k_2$ and $K_2^{-1} = k_3^{-1} + k_4^{-1}$, where K_1 and K_2 are the macroscopic equilibrium constants defined in (5) and (6).

4. Show that

$$\frac{[RCH(NH_3^+)COO^-]}{[RCH(NH_2)COOH]} = \frac{k_1}{k_2} = \frac{k_4}{k_3} \quad (27)$$

Equation (27) means that the ratio of the zwitterion concentration $[RCH(NH_3^+)COO^-]$ and the concentration of molecules $RCH(NH_2)COOH$ is independent of the concentration of hydrogen ions. From the experimental data for k_1, K_1 and K_2 one can estimate

$$[RCH(NH_3^+)COO^-]/[RCH(NH_2)COOH] \approx 2.23 \times 10^5 \quad (28)$$

so the concentration of electrically neutral molecules of $RCH(NH_2)COOH$ is much smaller than that of the zwitterion $RCH(NH_3^+)COO^-$, irrespective of the pH value.