

## Temperature dependence studies and microscopic protonation constants of L-alanine and $\beta$ -alanine in acetonitrile – water mixtures

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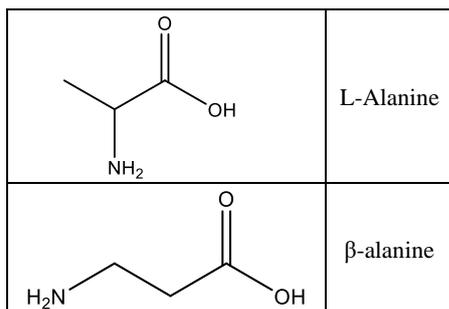
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**Abstract.** L-alanine and  $\beta$ -alanine are important biological molecules and have zwitterion structure. In this study, L-alanine and  $\beta$ -alanine's microscopic protonation constants and thermodynamic parameters (enthalpy, entropy, and free energy changes) for the proton–ligand systems of L-alanine methyl ester,  $\beta$ -alanine and  $\beta$ -alanine methyl ester have been determined at 5; 20; 35 °C, in ACN-water (25% ACN and 50% ACN (v/v)) mixtures at constant ionic strength of 0.1000 mol L<sup>-1</sup> NaClO<sub>4</sub> by potentiometric method. The results shown that, L-alanine and  $\beta$ -alanine's microscopic protonation constants generally tend to decrease with temperature rise and their protonation reactions in ACN-water mixtures generally favor enthalpy-driven.

**Keywords:** L-alanine,  $\beta$ -alanine; microscopic protonation constants; thermodynamics parameters.

### 1. Introduction

Amino acids are an elementary unit of biological molecules and important for living systems [1, 2]. As zwitterionic molecules, amino acids physicochemical properties such as macroscopic and microscopic protonation constants, free energies, entropies, enthalpies of amino acids in water and water-solvent mixtures are significant for various processes in biological systems, chemical and pharmaceutical [3-5]. Alanine is one of the non-essential amino acids and has a significant role in the diet and metabolic activities [2, 6].  $\beta$ -alanine is a non-proteinogenic amino acid and have role in carnosine metabolism [7].



**Figure 1.** Structure of L- alanine and  $\beta$ -alanine

Amino acids' zwitterionic structure reveal macroscopic ( $K_1$ ,  $K_2$ ) and microscopic ( $k_{11}$ ,  $k_{12}$ ,  $k_{21}$ ,  $k_{22}$ ) constants as acid-base interactions [8, 9]. Macroscopic and microscopic protonation constants of amino acids have fundamental importance; besides they are beneficial to explain the biological activity of proteins as a function of pH [2, 10, 11]. In individual pH, protonated and unprotonated forms of molecules determined microscopic protonation constant values are useful for calculating the pH-dependent distribution of

forms and demonstrating and understanding communication between the extracellular and intracellular media in numberless biological systems [8, 12]. Thermodynamic descriptions including enthalpic and entropic contributions of systems reveal us compounds physicochemical parameters such as solubility, stability, or ionization at various temperatures [13-16].

As a general principle, water as a solvent has been considered it reflects biological conditions. Nevertheless, recent works argue that solvents–water mixtures is fairly suitable [2, 17]. Nonaqueous media and water - solvent mixtures are recommended because of express better model for in vivo reactions [8]. Generally, only water and methanol, ethanol, dioxane, acetonitrile and tetrahydrofuran–water mixtures have examined [2, 5, 8, 10, 13, 17, 18]. We intend to contribute to the literature with an acetonitrile-water mixture that has been underutilized. Acetonitrile (ACN) is a dipolar aprotic solvent its hydrogen bonding capability is weaker than water, as all biological systems, show a low polar character and a partially aqueous content, which is also relevant for acid-base studies. One of the most preferred mediums for the determinations of macroscopic and microscopic protonation constants is water - ACN mixture [2, 11, 17, 19-21]. Potentiometric method, which is preferred for a long time by researchers, is the traditional, most typical, simple, and low-cost method for determining the protonation constants and the findings of this method have guided many studies [4, 17, 21, 22]

In the present study, L-alanine and  $\beta$ -alanine's microscopic protonation constants and L-alanine, L-alanine methyl ester,  $\beta$ -alanine and  $\beta$ -alanine methyl ester's entropy and free energy changes in water and ACN-water mixtures (25% ACN and 50% ACN (v/v))

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are determined. The potentiometric pH titration procedure studied these systems at 5, 20, and 35 °C at constant ionic strength (0.1000 mol L<sup>-1</sup> NaClO<sub>4</sub>). Irving and Rossotti method have been used for preparation of the solutions used in the titrations [23, 24]. This study would like to discuss the biological important substances L-alanine and β-alanine's microscopic protonation constants and thermodynamics behaviors and contribute to the literature.

## 2. Experimental

### 2.1. Chemicals and reagents

L-alanine (Ala), L-alanine methyl ester hydrochloride (AlaMe), β-alanine (β-Ala) and β-alanine methyl ester hydrochloride (β-AlaMe) were obtained from Merck as an analytical reagent. Acetonitrile (ACN; HPLC grade) was supplied by Merck. Sodium hydroxide (Titrisol, Merck), perchloric acid (HClO<sub>4</sub>, Riedel-de Haen, 60%) and sodium perchlorate (NaClO<sub>4</sub>, Sigma-Aldrich) were used. Deionized water was obtained by a Milli-Q-water purification system (Millipore).

### 2.2. Potentiometric measurements

Potentiometric titrations were measured on Radiometer Analytical, TIM 860 Titration Manager with the combined glass-pH electrode supplied with a temperature probe. The electrode was calibrated with buffer solutions at pH 4.00 and 7.00. Potentiometric titrations were performed at 5; 20; 35 °C in water and ACN-water (25% ACN and 50% ACN (v/v)) mixtures.

The experimental procedure was prepared according to Irving and Rossotti's method. Solutions used at potentiometric titration are 0.0100 mol L<sup>-1</sup> HClO<sub>4</sub> and 0.0100 mol L<sup>-1</sup> HClO<sub>4</sub> + 0.0200 mol L<sup>-1</sup> ligand ( as ligand: L-alanine, L-alanine methyl ester, β-alanine, or β-alanine methyl ester) with 0.100 mol L<sup>-1</sup> NaOH solution. The ionic strength of solutions was maintained constant with NaClO<sub>4</sub> at 0.1000 mol L<sup>-1</sup>. In the first step, only 0.0100 mol L<sup>-1</sup> HClO<sub>4</sub> titration, then 0.0100 mol L<sup>-1</sup> HClO<sub>4</sub> + 0.02 mol L<sup>-1</sup> ligand (L-alanine, L-alanine methyl ester, β-alanine, or β-alanine methyl ester) was performed and 0.100 mol L<sup>-1</sup> NaOH was dosed and stirred automatically by titrator. Titration mediums were prepared as 0, 25 and 50% water – acetonitrile solutions (volume/volume). All titrations were repeated for 5, 20 and 35 °C and at least three times. Temperature was maintained with water-jacket system (ThermoHaake DC10) with thermostat. All calculations of macroscopic and microscopic protonation constants were calculated by a computer (using an electronic spreadsheet software, e.g., MS Excel 2016) [3, 23].

Using the potentiometric pH titrations data of the solutions, the average proton-ligand formation number,  $n_A$ , at various pHs were determined by following equation:

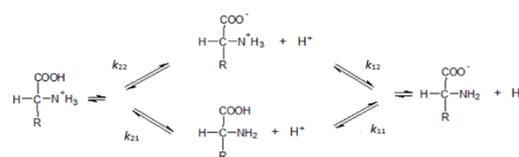
$$n_A = p + [(v_1 - v_2)([B] + [A]_0) / (V_0 + v_1)[L]_0] \quad (1)$$

where; [B] concentration of NaOH titrisol 0.1000 mol L<sup>-1</sup>, [A]<sub>0</sub> initial concentration of HClO<sub>4</sub> solution 0.01 mol L<sup>-1</sup>, [L]<sub>0</sub> total ligand concentration 0.001 mol L<sup>-1</sup>, V<sub>0</sub> volume of solution to be titrated 50.0 mL, v<sub>1</sub> and v<sub>2</sub>

(mL) volumes of NaOH for the same pH value,  $p$ : number of dissociated hydrogen of ligand = 1 for L-alanine, L-alanine methyl ester, β-alanine, or β-alanine methyl ester. Then the values of the macroscopic protonation constants, pK<sub>a</sub>, were calculated at  $n_A = 0.5$  and 1.5 [3, 23, 25].

### 2.3. Microscopic protonation constants calculations

Amino acids could represent four different microforms; besides they are cationic (N<sup>+</sup>H<sub>3</sub>RCOOH), zwitterionic (N<sup>+</sup>H<sub>3</sub>RCOO<sup>-</sup>), neutral (NH<sub>2</sub>RCOOH), and anionic (NH<sub>2</sub>RCOO<sup>-</sup>) forms which are shown in Scheme 1.



**Scheme 1.** General demonstration of amino acids' microscopic equilibria.

Microscopic protonation constants ( $pk_{11}$ ,  $pk_{12}$ ,  $pk_{21}$ ,  $pk_{22}$ ) may be written as below:

$$k_{11} = [\text{NH}_2\text{RCOOH}]/[\text{NH}_2\text{RCOO}^-][\text{H}^+] \quad (2)$$

$$k_{12} = [\text{N}^+\text{H}_3\text{COO}^-]/[\text{NH}_2\text{RCOO}^-][\text{H}^+] \quad (3)$$

$$k_{21} = [\text{N}^+\text{H}_3\text{RCOO}^-]/[\text{NH}_2\text{RCOOH}][\text{H}^+] \quad (4)$$

$$k_{22} = [\text{N}^+\text{H}_3\text{RCOOH}]/[\text{N}^+\text{H}_3\text{RCOO}^-][\text{H}^+] \quad (5)$$

Microscopic protonation constants couldn't observe by potentiometric methods, to resolve this issue macroscopic protonation constants ( $pK_1$ ,  $pK_2$ ) are needed to determine microscopic protonation ( $pk_{11}$ ,  $pk_{12}$ ,  $pk_{21}$ ,  $pk_{22}$ ) constants. [2, 9].  $[\text{NH}_2\text{RCOOH}] + [\text{N}^+\text{H}_3\text{RCOO}^-]$  defines total concentration of zwitterionic and neutral forms. Macroscopic protonation constants ( $pK_1$ ,  $pK_2$ ) of amino acids may be written as:

$$K_2 = [\text{N}^+\text{H}_3\text{RCOOH}]/([\text{NH}_2\text{RCOOH}] + [\text{N}^+\text{H}_3\text{RCOO}^-])[\text{H}^+] \quad (6)$$

$$K_1 = ([\text{NH}_2\text{RCOOH}] + [\text{N}^+\text{H}_3\text{RCOO}^-])/[\text{NH}_2\text{RCOO}^-][\text{H}^+] \quad (7)$$

Amino acids methyl esters have alkylated cite and according to literature, amino acids methyl esters  $pka$  values assumed to be equal  $pk_{21}$  values because of the alkylated cite of the molecule has relatively same influence on molecule [2, 3, 8, 9]. Micro protonation constants  $pk_{11}$ ,  $pk_{12}$ , and  $pk_{22}$  values are calculated with potentiometrically observed macro protonation constants with and below mentioned expressions:

$$K_2 = k_{21} + k_{22} \quad (8)$$

$$1/K_1 = 1/k_{11} + 1/k_{12} \quad (9)$$

## 3. Results and discussion

Macroscopic and microscopic protonation constants have a special relationship for amino acids because of their zwitterionic molecule behavior [9]. Macroscopic and microscopic protonation constants could be determined by various methods, this potentiometric method's advantage is values of  $pka$  have high precision [17, 22]. Microscopic acidity constants couldn't observe by potentiometric methods directly therefore

microscopic acidity constants are calculated by equations 7 and 8 with values macro protonation constants obtained from potentiometric method [2, 8]. Microscopic protonation constant ( $pK_{11}$ ,  $pK_{12}$ ,  $pK_{21}$ ,  $pK_{22}$ ) values of L-alanine and  $\beta$ -alanine are at 5; 20; 35 °C in water and ACN-water (25% ACN and 50% ACN (v/v)) mixtures are shown in Table 1 and Table 2. Microscopic

protonation constants of L-alanine and  $\beta$ -alanine in ACN-water (25% ACN and 50% ACN (v/v)) mixtures generally contribute to increase with the percentage of acetonitrile which might explain with solvation by water is preferred, acetonitrile's dipolar aprotic character, a weaker solvent than water [26]. Protonation constants generally tend to decrease with temperature rise [3].

**Table 1.** Microscopic protonation constants of L-alanine used in this study at 5, 20 and 35 °C and I=0.1000 mol L<sup>-1</sup> (NaClO<sub>4</sub>) in water and ACN-water (25% ACN and 50% ACN (v/v)) mixtures.

	ACN %	t (°C)	$pK_{22}$	$pK_{12}$	$pK_{21}$	$pK_{11}$
Ala	0% ACN*	5.0 °C	2.36 ± 0.04	8.81 ± 0.05	10.69 ± 0.02	4.24 ± 0.06
		20.0 °C	2.19 ± 0.02	8.25 ± 0.04	10.43 ± 0.04	4.37 ± 0.05
		35.0 °C	1.77 ± 0.02	7.66 ± 0.02	10.33 ± 0.04	4.44 ± 0.03
	25% ACN*	5.0 °C	2.56 ± 0.04	9.03 ± 0.02	11.31 ± 0.03	4.84 ± 0.04
		20.0 °C	2.22 ± 0.04	8.10 ± 0.04	10.78 ± 0.04	4.90 ± 0.05
		35.0 °C	1.79 ± 0.04	7.66 ± 0.03	10.13 ± 0.03	4.26 ± 0.05
	50% ACN*	5.0 °C	3.46 ± 0.01	8.48 ± 0.02	10.88 ± 0.01	5.86 ± 0.02
		20.0 °C	2.61 ± 0.03	8.01 ± 0.02	10.63 ± 0.01	5.23 ± 0.04
		35.0 °C	2.45 ± 0.02	7.43 ± 0.02	10.22 ± 0.04	5.24 ± 0.03

± standard deviation values

\*(volume / volume)

**Table 2.** Microscopic protonation constants of  $\beta$ -alanine used in this study at 5, 20 and 35 °C and I=0.1000 mol L<sup>-1</sup> (NaClO<sub>4</sub>) in water and ACN-water (25% ACN and 50% ACN (v/v)) mixtures.

	ACN %	t (°C)	$pK_{22}$	$pK_{12}$	$pK_{21}$	$pK_{11}$
$\beta$ -Ala	0% ACN*	5.0 °C	3.19 ± 0.03	10.38 ± 0.02	10.61 ± 0.03	2.96 ± 0.04
		20.0 °C	3.09 ± 0.03	9.80 ± 0.04	10.51 ± 0.04	2.38 ± 0.05
		35.0 °C	2.84 ± 0.01	9.36 ± 0.01	10.08 ± 0.01	2.12 ± 0.01
	25% ACN*	5.0 °C	4.15 ± 0.03	10.28 ± 0.01	10.35 ± 0.01	4.08 ± 0.04
		20.0 °C	3.59 ± 0.04	9.84 ± 0.04	10.60 ± 0.01	3.83 ± 0.06
		35.0 °C	3.20 ± 0.04	9.18 ± 0.05	10.17 ± 0.02	2.21 ± 0.06
	50% ACN*	5.0 °C	4.26 ± 0.04	10.16 ± 0.04	11.15 ± 0.03	3.27 ± 0.04
		20.0 °C	3.83 ± 0.01	9.41 ± 0.01	10.79 ± 0.03	3.20 ± 0.01
		35.0 °C	3.80 ± 0.02	8.95 ± 0.04	10.40 ± 0.03	2.35 ± 0.04

± standard deviation values

\*(volume / volume)

Enthalpy, entropy, and free energy changes were calculated with well-known equations [27]. Thermodynamic parameters (enthalpy, entropy, and free

energy changes) for the proton–ligand systems of L-alanine, L-alanine methyl ester,  $\beta$ -alanine and  $\beta$ -alanine methyl ester are shown in Table 3 and 4.

**Table 3.** Thermodynamic parameters for the proton–ligand systems of L-alanine and L-alanine methyl ester

		$\Delta H^\circ / \text{kJ mol}^{-1}$	$\Delta G^\circ / \text{kJ mol}^{-1}$	$\Delta S^\circ / \text{J K}^{-1} \text{mol}^{-1}$
Ala	0% ACN*	$pK_1$	-32.0	-12.3
		$pK_2$	-19.8	-58.5
AlaMe		$pK_2$	-57.4	-46.3
Ala	25% ACN*	$pK_1$	-42.0	-12.5
		$pK_2$	-64.4	-60.5
AlaMe		$pK_2$	-73.3	-45.5
Ala	50% ACN*	$pK_1$	-56.4	-14.6
		$pK_2$	-35.9	-59.7
AlaMe		$pK_2$	-409.7	-1244

\*(volume / volume)

**Table 4.** Thermodynamic parameters for the proton–ligand systems of  $\beta$ -alanine and  $\beta$ -alanine methyl ester

			$\Delta H^\circ / \text{kJ mol}^{-1}$	$\Delta G^\circ / \text{kJ mol}^{-1}$	$\Delta S^\circ / \text{J K}^{-1} \text{mol}^{-1}$
$\beta$ -Ala	0% ACN*	$pK_1$	-19.0	-17.3	-6
		$pK_2$	-35.8	-59.3	80
$\beta$ -AlaMe		$pK_2$	-55.9	-55.0	-3
$\beta$ -Ala	25% ACN*	$pK_1$	-52.1	-20.1	-109
		$pK_2$	-9.2	-59.5	172
$\beta$ -AlaMe		$pK_2$	-59.9	-55.2	-16
$\beta$ -Ala	50% ACN*	$pK_1$	-25.5	-21.5	-0.14
		$pK_2$	-66.4	-52.8	-46
$\beta$ -AlaMe		$pK_2$	-500.5	-52.8	-1527

\*(volume / volume)

Enthalpy changes ( $\Delta H^\circ$ ) and free energy changes ( $\Delta G^\circ$ ) tend to demonstrate reactions' formation behaviors and driven actors such as spontaneous reactions have negative enthalpy values. L-alanine, L-alanine methyl ester,  $\beta$ -alanine and  $\beta$ -alanine methyl ester in water and ACN-water mixtures generally favor enthalpy-driven reaction [3, 24, 27, 28].

#### 4. Conclusions

Microscopic protonation constants of L-alanine and  $\beta$ -alanine were determined at 5; 20; 35 °C in water and ACN-water (25% ACN and 50% ACN (v/v)) mixtures at constant ionic strength of 0.1000 mol L<sup>-1</sup> NaClO<sub>4</sub> and solutions prepared according to Irving and Rossotti's method. Values of microscopic constants generally tend to decrease with temperature rise. Acetonitrile has low polarity than water and microscopic protonation constants show a falling tendency with the increase polarity. Thermodynamic parameters (enthalpy, entropy, and free energy changes) for the proton–ligand systems of L-alanine,  $\beta$ -alanine and their methyl esters calculated and the experimental data on enthalpies of solution put forward for consideration protonation constant of L-alanine,  $\beta$ -alanine and their methyl ester in water and ACN-water mixtures are exothermic and generally enthalpy-driven reaction. The free energy changes are negative in all situations, demonstrating that protonation reactions are spontaneous. In the literature, only water and methanol, ethanol, dioxane and tetrahydrofuran-water mixtures and 25 °C have been studied, but microscopic protonation constants of alanine and  $\beta$ -alanine in water and water-acetonitrile mixtures have a crucial aspect for biological environmental conditions. Therefore, this study is to contribute to the literature for the determination of protonation constants the different temperature and solvent mixtures.

#### Conflict of interest

The authors declare no conflict of interest.

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