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# Solvent Effect on Protonation Constants of L-alanine and Alanine Methyl ester in Different Aqueous Solutions of 1,4-Dioxane

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## Abstract

The protonation constants of alanine and alanine methyl ester were determined in different aqueous solutions of 1,4-dioxane containing (0-50% v/v), using a potentiometric method at 25°C and constant ionic strength 0.1 mol.L$^{-1}$ (NaCl). The protonation constants were analyzed in terms of Kamlet, Abboud and Taft (KAT) parameters. The results show that the effect of β is higher in comparison with the other parameters (α and π*). Single-parameter correlation of the constants versus α, β, and π* are poor in all solutions. But multi-parameter correlation represents significant improvements with regard to the single- parameter models.

***Keywords***: *Solvent effect; L-Alanine; Protonation constants; Potentiometry; Kamlet-Taft.*

## Introduction

Acid ionization constants are important for the qualitative and quantitative treatment of systems involving acid-base equilibria in solution and knowledge of these values is essential in a wide range of research areas in chemistry, biochemistry, and pharmacology [1]. Many chemical reactions of experimental and practical processes occur in solution. Aqueous organic solvent, mixtures have been widely used due to the sparingly or insolubility of many compounds in pure water as solvent [2]. Alanine (2-aminopropanoic acid) (Figure 1) is an α-amino acid with the chemical formula $CH_3CH\ (NH_2)\ COOH$. The L-isomer is one of the 22 proteinogenic amino acids, i.e., the building blocks of proteins. The α-carbon atom of alanine is bound with a methyl group ($-CH_3$), making it one of the simplest α-amino acids with respect to molecular structure and

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also resulting in alanine's being classified as an aliphatic amino acid. The methyl group of alanine is non-reactive and is thus almost never directly involved in protein function. Further, the solute-solvent and solvent-solvent interactions in mixed solvents are more complex than in a pure solvent due to the preferential solvation [3]. Chemists have usually attempted to understand solvent effects in terms of polarity, defined as overall solution capabilities that depend on all possible (specific and non-specific) intermolecular interactions between the solute and solvent molecules [4,5]. In this paper, the protonation constants of L-alanine and its methyl ester have been determined in different water-1,4-dioxane mixtures to examine the dependence of acid-base equilibria on solvent composition.

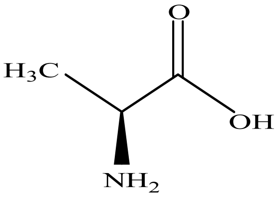

**Figure 1.** Chemical structure of alanine.

## Experimental

### *Materials*

All materials were analytical reagent grade. L-alanine, ($C_3H_7O_2N$ 99%) was obtained from Merck as an analytical reagent grade material and was used without further purification. L-alanine methyl ester ($C_4H_9O_2N$, 99%) was obtained from Aldrich and used as received. The NaOH solution was prepared from titrisol solution (Merck). Hydrochloric acid (70-72%) was supplied from Merck and was used without further purification. Sodium chloride (Merck, 99.5 %) was kept at room temperature in vacuum at least 72 hours before use. 1,4-Dioxane was obtained from Merck and was used without further purification. All dilute solutions were prepared from double-distilled water with a specific conductance equal to 1.3 ± 0.1 $\mu S\ cm^{-1}$.

### *Instruments*

The electromotive force, E, was measured using a pH meter Precisa model 960. The combined glass-pH electrode (model 6.0258.000) was modified by replacing its aqueous KCl solution with 0.01 $mol.L^{-1}$ NaCl + 0.09 $mol.L^{-1}$ $NaClO_4$ saturated with AgCl. The electrode was soaked for (15 to 20) minutes in a water-1,4-dioxane mixture before the potentiometric measurements. All titrations were carried out in a 80-ml thermostated double-walled glass vessel. To exclude carbon dioxide from the system, a stream of purified nitrogen was passed through a sodium hydroxide solution and then bubbled slowly through the reaction solution.

*Methods*

All measurements were performed at 25 °C and constant ionic strength 0.1 mol.L$^{-1}$ (sodium chloride). Potentiometric measurements to determine the protonation constants of l-alanine and its methyl ester were performed in an 80-ml jacketed titration cell thermostated at 25.0 ± 0.1 °C under a nitrogen atmosphere. The potentiometric cell was calibrated before each experiment so that the hydrogen ion concentration, rather than the activity, was measured. The protonation constants were evaluated from measurements of the emf by titration of a 25 ml L-alanine or L-alanine methyl ester [(5.0-8.0)x10$^{-3}$ mol.L$^{-1}$] solution with a 0.1 mol.L-1 sodium hydroxide solution, both at the same ionic strength and mole fraction of organic solvent, 1,4-dioxane [(0-50) %v/v]. The protonation constants of L-alanine and its methylester were calculated by analyzing the titration data using the computer program developed by Microsoft Excel SOLVER and LINEST.

## Results and discussion

The protonation constants of L-alanine and its methylester were determined potentiometrically by titration of appropriate solutions of L-alanine and alanine methyl ester in the water-1,4-dioxane mixtures. In this way, L-alanine was fully protonated at the beginning of a titration by adding a certain amount of hydrochloric acid at first and then using sodium hydroxide solution (0.1 mol.L$^{-1}$) as titrant. The protonation constants were obtained from systematic emf measurements of the following cell: GE/HCl-NaCl, $H_2L^+$+HL +$L^-$ in water-1,4-dioxane /HCl-NaOH/Ag-AgCl, where GE is the glass electrode and $H_2L^+$, HL and L- are the different species of the L-alanine involved in the protonation equilibria studied. The fraction of protons still bound to the L-alanine, $\overline{n}$, can be written as:

$$n_{cal} = (CH - [H^+]) / CL \qquad (1)$$

where $C_H$ and $C_L$ are the total concentrations of protons and the L-alanine, respectively. Substituting $C_L = [H_2L^+] + [HL] + [L^-]$ and $C_H = [H^+] + [HL] + 2[H_2L^+]$ in Eq. 1, leads to

$$n_{cal} = ([HL] + 2[H_2L^+]) / ([L^-] + [HL] + [H_2L^+]) \quad (2)$$

substituting the protonation constants instead of concentration of the different species of L-alanine in Eq. 2, rearranging and cancelling like terms, it gives:

$$\overline{n}_{cal} = (K_1[H^+] + 2K_1K_2[H^+]_2) / (1 + K_1[H^+] + K_1K_2[H^+]^2) \quad (3)$$

where $K_1$ and $K_2$ represent the protonation constants of the L-alanin. On the other hand, electrical neutrality demands that the concentration of the cations should equal the concentration of the anions at all times during a titration, and hence:

$$n_{exp} = (C_L+[Cl^-] - [Na^+] - [H^+]+[OH^-])/C_L \quad (4)$$

In Eqs. 4, $[Na^+]$ originates from the titrant used (NaOH), $[Cl^-]$ is introduced from the hydrochloric acid and NaCl added, and $[H^+] = 10^{(E_{cell} - E'_a)/k}$ and $[OH^-] = K_{ap}/[H^+]$ ($K_{ap}$ is the autoprotolysis constant). Using a suitable computer program (Microsoft Excel Solver), the data from Eqs. 3-4 were fitted for estimating the protonation constant values of L-alanine in different aqueous solutions of the 1,4-dioxane. We used the Gauss-Newton nonlinear least-squares method in the computer program to refine the $\bar{n}$ values by minimizing the error squares sum from Eq. 5.

$$U = \Sigma(\bar{n}_{nexp} - \bar{n}_{ncal})^2 \quad (5)$$

where $\bar{n}_{nexp}$ is an experimental value of $\bar{n}$ and $\bar{n}_{ncal}$ is the corresponding calculated one. The determined values of the protonation constants of alanine in different water-1,4-dioxane mixtures are listed in Tables 1 and 2. In Figure 2 the equilibrium distribution of different species of the alanine in water or 0% 1,4-dioxane (v/v) is shown as a function of $p_cH$ [4].

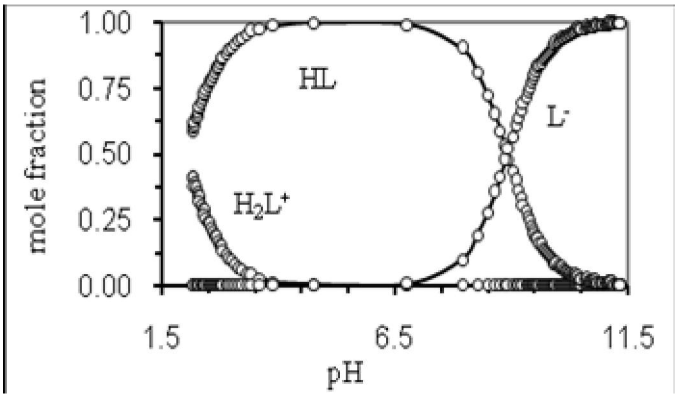

**Figure 2.** Distribution diagram of the different species of alanine in water at 25.0±0.1 °C and an ionic strength of 0.1 mol.L$^{-1}$ (NaCl).

Using the solvatochromic solvent parameters α, β, and π* , listed in Table 3, the following multiparametric equation, Eq. 6, has been proposed for use in the so-called Linear Solvation Energy Relationship:

$$\log_{10} K = A_0 + a\alpha + b\beta + s\pi^* \quad (6)$$

**Table 1.** Protonation constants of the carboxylic, $K_2$, and the amino, $K_1$, groups of alanine and $K_{21}$, alanine methylester at 25.0±0.1°C, different aqueous solutions of 1,4-dioxane, and an ionic strength of 0.1 mol.L$^{-1}$ (NaCl).

| **1,4-dioxane %** | **logK$_1$** | **logK$_2$** | **log K$_{21}$ methyl ester** |
|---|---|---|---|
| 0 | 2.01 | 9.04 | 8.38 |
| 10 | 2.15 | 8.91 | 8.33 |
| 20 | 2.28 | 8.79 | 8.24 |
| 30 | 2.48 | 8.67 | 8.18 |
| 40 | 2.61 | 8.54 | 8.12 |
| 50 | 2.87 | 8.43 | 8.05 |

**Table 2.** The microscopic protonation and tautomeric constant values of alanine at 25°C, different aqueous solutions of 1,4-dioxane, and an ionic strength 0.1 mol dm$^{-3}$ (NaCl).

| **1,4-dioxane % (v/v)** | **log $k_{22}$** | **log $k_{12}$** | **log $k_{12}$** | **log $k_{11}$** | **log $k_T$** |
|---|---|---|---|---|---|
| 0 | 2.01 | 8.38 | 9.04 | 2.67 | 4.17 |
| 10 | 2.15 | 8.33 | 8.91 | 2.73 | 3.87 |
| 20 | 2.28 | 8.24 | 8.79 | 2.83 | 3.61 |
| 30 | 2.50 | 8.18 | 8.67 | 2.97 | 3.27 |
| 40 | 2.61 | 8.12 | 8.54 | 3.02 | 3.11 |
| 50 | 2.87 | 8.05 | 8.43 | 3.25 | 2.80 |

In order to explain the obtained log10 K values through the KAT solvent parameters, the protonation constants were correlated with solvent properties by means of single-, dual- and multi-parameter regression analysis using a suitable computer program (Microsoft Excel SOLVER and LINEST) [6]. The computer program used can determine the values of A$_0$, a, b, s and some statistical parameters which are listed in Table 3. The expressions of the KAT equation thus obtained for each property are given in Table 4 only for the single- parameter models. In general solute-solvent and solvent-solvent interactions in mixed solvents are more complex than in a pure solvent due to the preferential solvation. These interactions can create new mixed solvent entities in the solvation shell of the solute molecules whose properties and their structures are distinct and different from those in pure solvent.

**Table 3.** KAT solvatochromic parameters and the dielectric constants of different 1,4-dioxane-water solvent mixtures at 25.0 ± 0.1 °C.

| **1,4-dioxane % (v/v)** | **$\alpha$** | **$\beta$** | **$\pi^*$** | **$\varepsilon$** |
|---|---|---|---|---|
| 0 | 1.23 | 0.49 | 1.14 | 78.30 |
| 10 | 1.06 | 0.51 | 1.12 | 69.23 |
| 20 | 0.92 | 0.54 | 1.09 | 60.19 |
| 30 | 0.82 | 0.56 | 1.05 | 51.14 |
| 40 | 0.77 | 0.59 | 0.99 | 42.14 |
| 50 | 0.73 | 0.61 | 0.92 | 33.32 |

**Table 4.** Regression coefficients of the KAT equation (single- parameters) for the protonation constants of alanine and alanine methylester in different water-1,4-dioxane mixtures at 25.0 (±0.1) ℃.

| **KAT equation** [a] | **$r^2$** [b] | **rss** [c] | **ose** [d] | **f** [e] | **N** [f] |
|---|---|---|---|---|---|
| $\log_{10} K_1(Ala) = 3.84(\pm0.26) - 1.56(\pm0.28)\ \alpha$ | 0.89 | 0.06 | 0.12 | 30.88 | 6 |
| $\log_{10} K_1(Ala) = -1.34(\pm0.30) + 6.81(\pm0.54)\ \beta$ | 0.98 | 0.01 | 0.06 | 161.82 | 6 |
| $\log_{10} K_1(Ala) = 6.33(\pm0.33) - 3.74(\pm0.31)\ \pi^*$ | 0.97 | 0.01 | 0.06 | 142.20 | 6 |
| $\log_{10} K_2(Ala) = 7.67(\pm0.14) + 1.15(\pm0.15)\ \alpha$ | 0.94 | 0.02 | 0.06 | 61.16 | 6 |
| $\log_{10} K_2(Ala) = 11.46(\pm0.08) - 4.96(\pm0.14)\ \beta$ | 1.00 | 0.00 | 0.01 | 1330.17 | 6 |
| $\log_{10} K_2(Ala) = 5.93(\pm0.33) + 2.66(\pm0.31)\ \pi^*$ | 0.95 | 0.01 | 0.06 | 72.55 | 6 |
| $\log_{10} K_{21}(Ala) = 7.63(\pm0.08) + 0.63(\pm0.08)\ \alpha$ | 0.94 | 0.01 | 0.04 | 57.65 | 6 |
| $\log_{10} K_{21}(Ala) = 9.71(\pm0.05) - 2.72(\pm0.08)\ \beta$ | 1.00 | 0.00 | 0.01 | 1101.03 | 6 |
| $\log_{10} K_{21}(Ala) = 6.69(\pm0.18) + 1.45(\pm0.18)\ \pi^*$ | 0.95 | 0.00 | 0.03 | 69.08 | 6 |

[a] Values in parentheses are the standard error for that coefficient
[b] Regression coefficient
[c] Residual sum of the squares errors
[d] Overall standard error
[e] f-test
[f] Number of the mixed solvents

## Conclusion

Due to the fact that amino acids are widely applied in many chemical and biochemical fields, it is essential to characterize their properties in terms of the microconstants. These give useful information about elucidation of numerous biological compounds like proteins, enzymes, etc. The protonation constants of L-alanine and alanine methylester in water-1,4-dioxane mixed solvents have different behavior (Table 1). The protonation constant of the amino group of the L-alanine, $K_1$, increased as the solvent became enriched in the organic component, but the protonation constant of the carboxylic acid group, $K_2$, and $K_1$ decreased as the organic solvents increased in the mixture. The results show any single-parameter correlations of $\log_{10} K_1$ , $\log_{10} K_2$ and $\log_{10} K_2$ values individually with $\pi^*$, $\alpha$, and $\beta$ did not give good results in all cases. However, the correlation analysis of $\log_{10} K_1$, $\log_{10} K_2$ and log10 $K_{21}$ values with multi-parameter equations indicate significant improvement with regard to the single-models. In this work, the protonation constants of l-alanine and alanine methylester were analyzed in different aqueous 1,4-dioxane mixtures using Kamlet, Abboud and Taft parameters.

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