Solvent Effects on Protonation Equilibria of Some Amino Acids and Peptides in Different Aqueous Solutions of Ethanol

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Abstract

The protonation constants of glycine, L-alanine, glycyl-glycine, glycyl-glycyl-glycine and glycyl-L-alanine have been determined in 10–80% (v/v) ethanol-water mixtures at 25 °C and constant ionic strength (0.1 mol L^{-1} sodium perchlorate), by potentiometric technique and calculated using a suitable computer program, which employs a nonlinear least-squares method. The effect of solvent composition on the protonation constants in the mixed solvents were correlated with the Kamlet-Taft solvatochromic parameters (α, β and π^*). It was shown that $\log K_1$ and $\log K_2$ values of glycine, L-alanine and peptides increasing with increase ethanol content up to 50% and then tend to decrease. Further, an overview of the effect of preferential solvation and solvent structure of electrolytes in ethanol-water mixtures on the values of the protonation constants in these media were also discussed.

Keywords: Protonation constants, Peptides, Ethanol-water mixtures, Kamlet-Taft Solvatochromic parameters, Solvent effect

1. Introduction

The importance and vital role of α-amino acids and their peptides in living systems is well known. Nonetheless, there are research subjects in relation to amino acids and peptides that should be investigated. One of these subjects is the study of solvent effect on the protonation constants of these compounds in organic solvent-water mixture for understanding their biological action. Now, it is understood that in proteins, active site cavities of enzymes, and in different complexes of nucleotides and nucleosides the effective dielectric constant is decreased at the ligand-water interface and the activity of water is decreased because of the presence of aliphatic or aromatic side chains of the ligand at the location.[1] So, metal ion interactions with a ligand increase considerably with decreasing solvent polarity of the media. This effect is well-established in most metal ion complexes of biological ligands.[2–5] Hence, knowledge of the physicochemical properties of the solvent, to understand the intermolecular interactions between solute-solvent and also solvent-solvent molecules, is required for proper bench work.

As for, their recognized importance, there are many experimental and theoretical works dealing with the effect of organic solvent on protonation constants of α-amino acids and peptides.[6–13] The protonation constants of α-amino acids in these media are often different from those in water alone, as these media tend to be lipophilic rather than hydrophilic.[14–15] The solvation of amino acids that constitute proteins is closely connected with the stabilizing and destabilizing effects of electrolytes on protein structure. Therefore, the study of protonation and solvation processes of amino acids in various organic media is important to elucidate the connection between their chemical ability and biological activity.

The study of solvent effects particularly solvent-solute and solvent–solvent interactions has been of interest to many researchers.[16–18] These interactions generate new solvent properties that are absent in the pure solvent molecules which leading to phenomena such as preferential solvation that makes the nature of solute–solvents more complex. The influence of solvent on solute molecules has been studied intensively but the problem is far from being completely understood. However, the acceptance of a single solvent polarity scale as the most appropriate for interpreting any solvent effect has not been achieved yet.

Although the exact definition of solvent polarity is still elusive, it seems reasonable to consider that this property related to the overall solvation capability of solvent, encompassing all possible specific and nonspecific intermolecular interactions with solute ions or molecules.[19]

In previous publications,[2–4,20] we have shown that dielectric constant alone (as believed for many years) cannot serve as a quantitative measurement of a solvent polarity. This approach is often inadequate, since dielectric constant regards a solvent as a non-structured continuum, not composed of individual solvent molecules with their own solvent-solvent and does not take into account specific solute-solvent interactions.

In this paper, we have determined the protonation constants of glycine, L-alanine and some glycine peptides potentiometrically in various ethanol–water mixtures to show how the solvents and their mixtures with various polarities affect the protonation of such compounds.

2. Experimental

2. 1. Materials

Glycine, L-alanine, Glycyl-glycine, Glycyl-glycyl-glycine and Glycyl-L-alanine purchased from Fluka (analytical reagent grade) and their purity were assessed by potentiometric titration. Ethanol (G. R. E. Merck) was refluxed with metallic sodium for six hours and then distilled. The NaOH solution was prepared from titrisol solution (Merck), and its concentration determined by several titrations with standard HCl. Perchloric acid and sodium perchlorate supplied from Merck (analytical reagent grade) and used without further purification. Dilute perchloric acid solution was standardized against standard NaOH solution. All dilute solutions were prepared from double-distilled water, whose conductivity did not exceed $0.5\ \mu S\ cm^{-1}$.

2. 2. Apparatus

A Jenway pH-meter, model 3520 (precision of 0.001 units of pH), was used for pH measurements. The hydrogen ion concentration was determined with a combination electrode (Jenway). A thermostat having accuracy of ± 0.1 °C maintained the temperature of the potentiometric cell.

2. 3. Measurements

All pH-metric equilibrium determinations carried out under N_2 atmosphere to exclude carbon dioxide from the system and at 25.0 ± 0.1 °C, which was maintained by circulating water from a constant-temperature thermostat through the double-wall Pyrex titration cell of 80 mL capacity. The pH-meter was calibrated for the relevant H^+ concentration with a solution of 0.01 mol L^{-1} perchloric acid solution containing 0.09 mol L^{-1} sodium perchlorate

(for adjusting the ionic strength to 0.1 mol L^{-1}). For this standard solution, we set $-\log[H^+] = 2.00$.[2] Junction potential corrections have been calculated from eq. (1):

$$-\log[H^+]_{real} = -\log[H^+]_{measured} + a + b[H^+]_{measured} \quad (1)$$

Where a and b were determined by measuring the hydrogen ion concentration of two different solutions of $HClO_4$ with sufficient $NaClO_4$ to adjust the ionic media.

Measurements were carried out by titrating 50 mL of a solution containing the amino acids or peptides (5 × 10^{-3} mol L^{-1}) and $NaClO_4$ (0.1 mol L^{-1}) for adjusting ionic strength, with standard NaOH and $HClO_4$ solutions, separately. For each experiment, at least three replicates were performed.

2. 4. Calibration of the Glass Electrode

To perform reliable pH measurements, the standardization of potentiometric sensors in the solvent mixture is needed and the pH scale in these media must be established. The pH-measured in aqueous solution by the glass electrode refer to the H^+ ion activity as the glass calomel electrode is standardized against suitable buffers of known H^+ ion activities. However, for calibration of the glass-calomel electrodes, suitable buffers are not available for mixed solvents. Thus, the measured meter readings do not give H^+ ion activities in mixed solvents but the measured readings with appropriate corrections give H^+ ion concentrations.

The correction term log U_H in mixed solvents obtained from eq. (2):

$$-\log [H^+] = B + \log U_H \quad (2)$$

$-\log [H^+]$ and B represent the stoichiometric H^+ ion concentration (10^{-4} mol L^{-1} $HClO_4$) in water and mixed solvents respectively. It is observed that U_H is concentration dependent, as one would expect. If this dependence is primarily the result of changing in the activity coefficient of hydrogen ion with total ionic concentration, one should be able to correct for the effect by using known activity coefficients and thus obtain a correction factor U^0_H which is independent of ionic concentration; *i.e.,* U^0_H will correspond to the correction at zero ionic strength in the solvent under consideration. For this purpose, it is defined that $U^0_H = U_H \times 1 / \gamma_\pm$; where $\gamma_\pm$ is the mean activity coefficient for the solvent composition and the ionic concentration for which U_H was determined. If the assumption of equivalence of ions of the same charge type is valid, then one may use for any experimental activity coefficient, provided the coefficient for the appropriate solvent composition and total ionic concentration is selected.

The method was suggested by Van Uitert et al. and extensively used by Lahiri and co-workers as well as by others.[22–28] The reliability of the method was tested expe-

rimentally by determining the H$^+$ ion concentrations of solutions with known concentration of H$^+$ ion.

3. Results and Discussion

The protonation constant values of glycine, L-alanine and some glycine peptides have been determined using potentiometric technique under the same condition of temperature, ionic media and mole fraction of ethanol as mentioned, and calculated using a suitable computer program which employs a nonlinear least-squares method.[29] The numerical values of logK_1 and logK_2 determined in ethanol–water mixtures are given in Table 1 together with the values reported in the literature for comparison. The results are in agreement with those reported before, but the small differences are possibly due to the different experimental procedures and the various solvent mixtures and different background electrolytes used.

$$L^- + H^+ \rightleftarrows HL \qquad K_1 = [HL]/[L^-][H^+] \qquad (3)$$

$$HL + H^+ \rightleftarrows H_2L^+ \qquad K_2 = [H_2L^+]/[HL][H^+] \qquad (4)$$
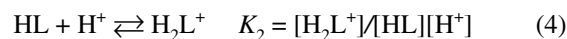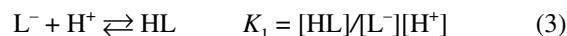
The logK_1 and logK_2 values refer to the protonation of –COO$^-$ and –NH$_2$ groups, respectively.

3. 1. Effect of Solvent

In several studies, many efforts have been performed to provide a possible simple description of the solute-solvent interactions treating with the solvent as a continuum possessing a cavity in which the solute molecule is placed.[30–33] Previously, the solvent effect on the protonation equilibrium was believed to be chiefly guided by electrostatic interactions (Born model). In other words, a corrected Born equation, which describes the solute-dipolar solvent interaction by placing the charges of the atoms of the solute at their position in space inside a spherical cavity of

Table 1. Protonation constants of glycine, L-alanine, glycyl-glycine, glycyl-glycyl-glycine and glycyl-L-alanine at 25 °C, different ethanol- water solvent mixtures and ionic strength 0.1 mol L^{-1} NaClO$_4$ and NaCl together with the values reported in the literature

Species		Ethanol % (v/v)								ref
		10	20	30	40	50	60	70	80	
Glycine	logK_1	2.237±0.02	2.431±0.04	2.499±0.02	2.583±0.03	3.109±0.01	3.078±0.02	3.012±0.04	2.638±0.02	this
	logK_2	9.436±0.03	9.507±0.02	9.69±0.02	9.707±0.04	9.509±0.02	9.454±0.01	9.381±0.02	9.194±0.03	work
	logK_1		2.68	2.81	2.89	2.90	3.05	3.06	3.10	51[*]
	logK_2		9.30	9.54	9.39	9.35	9.20	9.05	9.00	
L-alanine	logK_1	2.501±0.01	2.535±0.03	2.565±0.02	2.676±0.02	2.763±0.03	2.524±0.02	2.425±0.03	2.395±0.01	this
	logK_2	9.456±0.02	9.696±0.01	9.76±0.02	9.945±0.04	9.908±0.02	9.878±0.03	9.856±0.01	9.714±0.02	work
	logK_1		2.79	2.88	2.97	3.05	3.06	3.08	3.10	51[*]
	logK_2		9.41	9.70	9.55	9.45	9.18	9.12	9.15	
Glycyl-alanine	logK_1	2.545±0.03	2.665±0.01	2.839±0.02	3.048±0.02	2.586±0.01	2.471±0.02	2.182±0.03	2.172±0.04	this
	logK_2	8.493±0.02	8.347±0.02	8.261±0.01	8.188±0.03	8.124±0.02	8.001±0.03	7.998±0.04	7.763±0.03	work
	logK_1 = 3.18		logK_2 = 8.28							52[**]
Glycyl-glycine	logK_1	2.436±0.01	2.486±0.02	2.559±0.02	2.684±0.01	3.182±0.02	2.921±0.04	2.652±0.03	2.642±0.02	this
	logK_2	8.13±0.03	8.137±0.03	8.145±0.01	8.176±0.02	8.389±0.01	8.337±0.02	8.260±0.04	8.109±0.03	work
	logK_1		3.33		3.45		3.81			53[*]
	logK_2		7.66		6.92		6.98			
Glycyl-glycyl-glycine	logK_1	2.29±0.02	2.462±0.01	2.515±0.02	2.733±0.04	3.189±0.01	2.915±0.03	2.864±0.01	2.67±0.02	this
	logK_2	8.135±0.03	8.138±0.02	8.145±0.03	8.153±0.04	8.142±0.01	8.086±0.04	8.058±0.03	7.932±0.01	work
	logK_1	3.46				4.42				54[***]
	logK_2	8.09				8.32				

[*] In the water-ethanol mixtures of different compositions (v/v) and I = 0.1 mol L^{-1} NaCl
[**] In aqueous solution and I = 0.1 mol L^{-1} NaClO$_4$
[***] In the water-acetonitrile mixtures of different compositions (w/w)

A maximum number of two protons can be released from all amino acids and peptides examined, in the fully protonated form (H$_2$L$^+$) on titration with strong base in the range 2 ≤ pH ≤ 11. The titration data indicates solely the presence of simple L$^-$, HL and H$_2$L$^+$ species, where L$^-$ denotes the fully dissociated ligand anion. The following equilibria were considered:

the solvent, is used to calculate the solvent effect on systems and is written as

$$\Delta(-\log K) = (121.6 n/a)(1/\varepsilon_r - 0.0128) \qquad (5)$$

where a is the radius of the solvent cavity formed by the introduction of the ion, ε_r is the dielectric constant of the me-

dium and n is the square summation of the charges involved in the protonation equilibria. For the dissociation of the carboxylic group, electrostatic interactions overwhelm the specific solvation because charges are created (L$^-$ + H$^+$ $\rightleftarrows$ HL). Hence, logK_1 increases with ethanol content (decreasing the dielectric constant of the medium, Table 2) up to 50%, but then decreasing that may be due to other solute-solvent interactions. However, in the dissociation of the amino group, there is no change in the number of charges (HL + H$^+$ $\rightleftarrows$ H$_2$L$^+$) and the dissociation depends only on the solvation of the different species by the solvents of the mixture; since, the Born model for logK_2 is insignificant. But recent studies have revealed that any change in macroscopic properties such as the dielectric constant (ε_r) or molar fraction of solvent cannot be the sole factor.[34–36] Thus, it is desirable to develop other empirical functions that account for the complete picture of all intermolecular forces acting between solute and solvent molecules.[19, 37–40]

There are several empirical ways to measure the effects of solvent in organic-water cosolvent mixtures,[19] one of the most ambitious and successful method is the quantitative treatment using a multiparameters equation, that is known as linear solvation energy relationship (LSER). This method explains any solute property varying with solvent composition as a linear combination of the solvatochromic parameters of the solvent, π^* (solvent dipolarity/polarizability), α (solvent hydrogen-bond donating acidity, HBD), and β (solvent hydrogen-bond accepting basicity, HBA). These solvatochromic parameters, together with other macroscopic parameters (molar fraction and dielectric constant) and an independent term tested as targets. The Kamlet-Taft equation deemed the best appropriate method for each substance worked out.[41–43] When the property is the dissociation constant values (log$K$) in mixtures with the same solvents, the appropriate form of the Kamlet-Taft equation would be:

$$\log K = A_o + a\alpha + b\beta + p\pi^* \qquad (6)$$

Where A_o is the regression value of the solute property in cyclohexane as the reference solvent. The regression coefficients a, b, and p measure the relative susceptibilities of the solvent dependent on the solute property to the indicated solvent parameter.

In the present study, since the variation of the obtained experimental protonation constant values in the range 10–80% (v/v) ethanol is non-linear (Figure 1) the constants investigated separately from 10–40% and 50–80% ethanol (v/v). The values of the Kamlet-Taft solvatochromic parameters (α, β, and π^*) were taken from the literature,[44–46] that are in some other percentages of aqueous solution of ethanol used in this study. So, the reported values of α, β and π^* were plotted separately versus mole fraction of ethanol to determine the parameters at desired mole fraction of ethanol used in this work. The calculated values listed in Table 2.

Table 2. Kamlet-Taft solvatochromic parameters and the dielectric constants of different ethanol-water mixtures

%Ethanol (v/v)	α	β	π^*	ε_r^a
0	1.23	0.49	1.14	85.60
10	1.13	0.52	1.12	78.16
20	1.03	0.57	1.12	75.12
30	0.95	0.61	1.09	74.10
40	0.89	0.66	1.04	72.58
50	0.87	0.70	0.97	69.29
60	0.87	0.74	0.89	65.85
70	0.89	0.77	0.81	64.12
80	0.91	0.80	0.84	55.10

^{a} Have taken from Ref. 50.
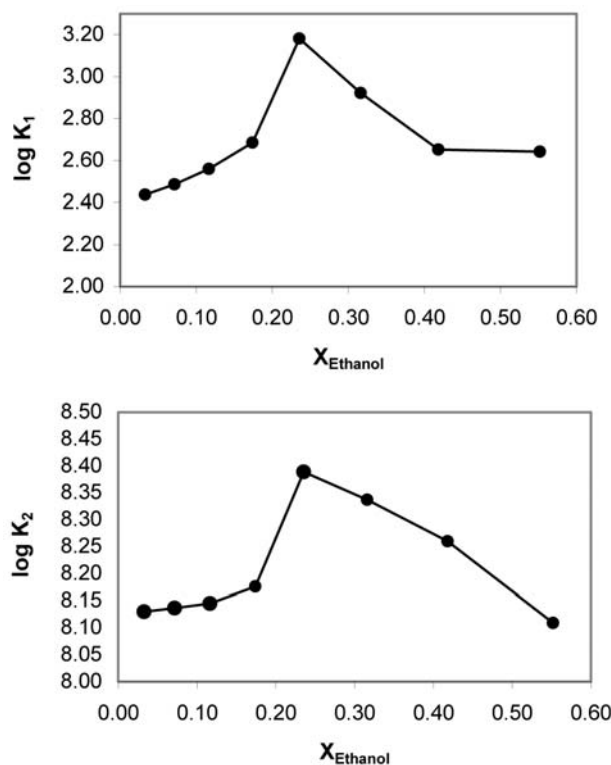
Figure 1. The plots of the experimental values of protonation constants of glycyl-glycine versus mole fraction of ethanol at 25 °C, ionic strength 0.1 mol L^{-1} NaClO$_4$ in different ethanol-water solvent mixtures.

In eq. (6) the discontinuous polarizability correction term is omitted because the solvent used in this work contains no chlorine atom. In order to explain the obtained log$K$ values through Kamlet-Taft solvent parameter, the protonation constants were correlated with solvent properties by means of multiple linear regression analysis using a suitable computer program.[29] We used the Gauss-Newton linear least-squares method in the computer program to refine the log$K$ by minimizing the error squares sum from eq. (7). The results presented in Table 3. In all cases $n = 4$ and $r^2 \cong 0.9999$.

$$S = \Sigma(pK_{exp} - pK_{cal})^2 \qquad (7)$$

In order to show the efficiency of the suggested multi-parameter correlations, experimental values of log K plotted versus their calculated ones from eq. (6) for different aqueous ethanol solutions. It can be seen, Figure 2, that the experimental and calculated values of log K are in good agreement with each other, $r^2 > 0.99$ in both cases. The coefficients of α, β and π^* in Table 3 are different

The multiple regression analysis of the data in different ranges of ethanol shown in Table 3 lead to the following preliminary conclusions: I) The protonation constants are strongly influenced by the specific solute-solvent interactions as indicated by the percentage contribution of β and π^* parameters. II) Among the solvatochromic parameters of the solvent mixtures, the hydrogen-bond accepter basicity parameter of the solvent is the most important, the hydrogen-bond donor acidity parame-

Table 3. Expressions of Kamlet-Taft equations obtained through a suitable computer program applied to data built from log K_1 and log K_2 values in different ethanol-water mixtures

Species	%Ethanol (v/v)	Linear salvation energy relationships	
Glycine	10–40	$\log K_1 = -4.95 + 0.88\alpha + 5.64\beta + 2.90\pi^*$	$\log K_2 = 27.75 - 6.61\alpha - 11.79\beta - 4.21\pi^*$
	50–80	$\log K_1 = 14.58 - 4.94\alpha - 6.37\beta - 2.80\pi^*$	$\log K_2 = 15.21 - 2.62\alpha - 3.45\beta - 1.03\pi^*$
L-alanine	10–40	$\log K_1 = -1.67 + 1.81\alpha + 4.30\beta - 0.09\pi^*$	$\log K_2 = -6.41 + 3.96\alpha + 12.71\beta + 4.26\pi^*$
	50–80	$\log K_1 = 1.42 + 6.64\alpha - 4.72\beta + 0.63\pi^*$	$\log K_2 = 13.94 - 1.06\alpha - 2.93\beta - 1.09\pi^*$
Glycyl-glycine	10–40	$\log K_1 = 2.06 + 0.50\alpha + 0.20\beta - 1.10\pi^*$	$\log K_2 = 7.06 + 0.5\alpha + 1.14\beta - 0.08\pi^*$
	50–80	$\log K_1 = 3.31 - 1.31\alpha - 1.81\beta + 2.35\pi^*$	$\log K_2 = 13.13 - 2.57\alpha - 2.64\beta - 0.67\pi^*$
Glycyl-glycyl-glycine	10–40	$\log K_1 = -12.80 + 4.67\alpha + 12.79\beta + 2.82\pi^*$	$\log K_2 = 8.42 - 0.06\alpha - 0.07\beta - 0.15\pi^*$
	50–80	$\log K_1 = 5.47 + 6.42\alpha - 9.45\beta - 1.30\pi^*$	$\log K_2 = 11.39 - 0.19\alpha - 3.18\beta - 0.89\pi^*$
Glycyl-alanine	10–40	$\log K_1 = 6.43 - 1.02\alpha + 0.35\beta - 2.60\pi^*$	$\log K_2 = 9.97 + 0.54\alpha - 1.83\beta - 1.02\pi^*$
	50–80	$\log K_1 = 5.12 - 7.58\alpha + 2.23\beta + 2.55\pi^*$	$\log K_2 = 13.23 + 2.35\alpha - 7.29\beta - 2.10\pi^*$

with each other and are almost in the order of $\beta > \alpha > \pi^*$ for log K_1 and log K_2 values of the amino acids and peptides in the proposed various aqueous solutions of ethanol.
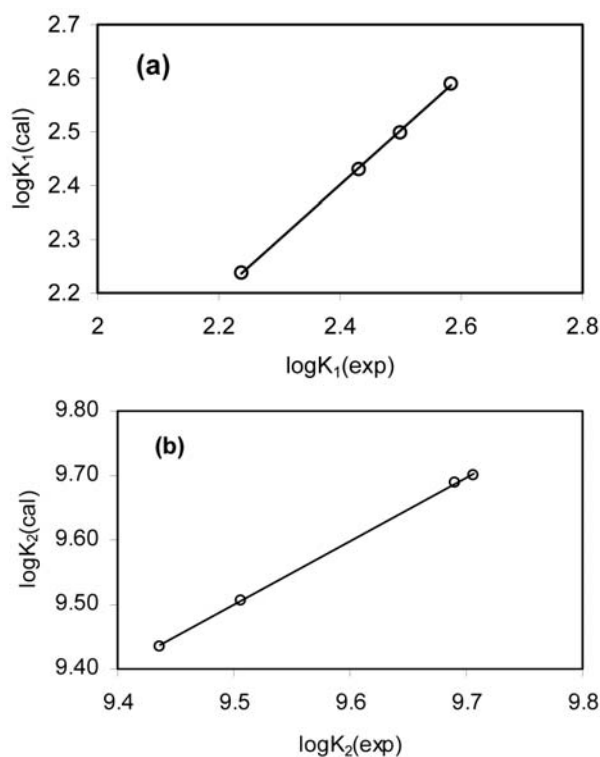
Figure 2. The plots of the experimental values of log K versus the calculated ones for glycine (a) log K_1, (b) log K_2.

ter and the polarity parameter play relatively small roles in variation of protonation constants of the amino acids and the peptides used. III) Further, the negative signs of the coefficients of α, β and π^* terms indicate that a decrease in the HBD, HBA properties and polarity of the medium increases the protonation constants.

If the dielectric constant of the media were the only factor for the solvent effect on the protonation, it would be expected that the log K in a solution with higher dielectric constant should be greater than those of all the other aqueous solution of ethanol. Moreover, the variation in the log K values obtained and the acid-base behavior of the solutes over the whole composition range studied can be explained by taking into account the preferential solvation of ions. Following the model of Frankel et al. for a solute in a binary mixture, the solvent considered to be distributed between two phases, the bulk solvent and the solvation shell of the solute.[47] It assumed that the solvation shell made up of independent sites that are always occupied. If the solvation number considered the same for both solvents, there is a one by one replacement of the solvent molecules. Hence, preferential solvation can influence protonation constants.

In the present study, the inverse variation of log K_1 and log K_2 in 50% ethanol (v/v) may be due to possible changes in the structure of the mixture.[48] In fact, the water structure remains intact in the water rich region and the ethanol molecules occupy the cavities between water molecules without changing the water structure.[51]

4. Conclusions

In this work, the protonation constants of some amino acids and peptides have been determined in ethanol-water mixture of varying compositions (10–80% ethanol by volume). It is very difficult to interpret the log$K$ variations of the amino acids and the peptides studied by only macroscopic parameters of the ethanol-water mixtures. It is known that one of the most important factors determining the equilibrium constants is the reaction medium, so, the solvent effect on protonation constants could be explained on the basis of dielectric constant of the medium, solvent structure, preferential solvation, and microscopic parameters (as Kamlet-Taft solvatochromic parameters).

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Povzetek

S potenciometrično metodo smo določali konstante protonacije glicina, L-alanina, glicil-glicina, glicil-glicil-glicina ter glicil-L-alanina v mešanicah etanola in vode v območju sestave med 10 in 80 v/v % etanola pri 25 °C ter pri konstantni ionski moči (0.1 mol L^{-1} natrijev perklorat). Vpliv topila na konstante protonacije smo korelilali s solvatohromnimi parametri mešanic (α, β and π*) z Kamlet-Taft-ovo metodo. Ugotovili smo, da vrednosti logK_1 in logK_2 za glicin, L-alanin in peptide naraščajo z naraščajočo vsebnostjo etanola v mešanici do 50 % in potem kažejo tendenco padanja. Proučevali smo tudi vpliv preferenčne solvatacije in strukture topila na konstante protonacije.