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Solvent Effects on Protonation Constants of Tryptophan in Some Aqueous Aliphatic Alcohol Solutions

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The protonation constants of tryptophan (K_1 and K_2) were determined in binary mixtures of water with methanol, ethanol, and 1-propanol containing (0, 10, 20, 30, 40, 50, 60, 70, and 80) % (v/v) using a potentiometric method at 25 °C and constant ionic strength (0.1 $\text{mol}\cdot\text{dm}^{-3}$ sodium perchlorate). The autoprotolysis constant values (K_{ap}) of the media were also determined in the same binary mixtures. The protonation constants of tryptophan and the autoprotolysis constant of the medium in different binary mixtures were analyzed in terms of Kamlet, Abboud, and Taft (KAT) parameters. Single-parameter correlations of the constants versus α (hydrogen-bond donor acidity), β (hydrogen-bond acceptor basicity), and π^* (dipolarity/polarizability) are poor in all solutions. Multiparameter correlations show better results, but dual-parameter correlations represent significant improvements with regard to the single- and multiparameter models. Linear correlation is observed when the experimental log K_{ap}, log K_1, and log K_2 values are plotted versus the calculated ones, while the KAT parameters are considered. Finally, the results are discussed in terms of the effect of the solvent on the protonation and autoprotolysis constants.

Introduction

Intermolecular forces play a dominating role in understanding the molecular basis of life and in the development of new chemical technologies. In this way, chemists usually attempt to understand solvent effects in terms of all possible specific and nonspecific intermolecular interactions between solute and solvent molecules. However, solute−solvent interactions are much more complex in mixed solvents than in pure solvents owing to the possibility of preferential solvation by any of the solvents present in the mixture.[1] On the other hand, solvent−solvent interactions can also affect solute−solvent interactions.[2]

Protonation constants have special importance among the physicochemical properties of α-amino acids.[3] Amino acids are found in all naturally occurring proteins, which play a vital role in nearly all biological processes.[4] However, a search in the literature showed few contributions on their acid−base behavior in aqueous−organic solvent mixtures.[5−13] The solvation of amino acids that constitute proteins is closely connected with the stabilizing and destabilizing effects of electrolytes on the protein structure.

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.[14] 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.[15−18] 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.

In the past few years, several experimental and theoretical works dealing with the effect of solvent on intermolecular reactions have been published. Most of these papers are concerned with the effect of solvent on reaction rates. There are surprisingly few articles exclusively dealing with the effect of all properties of the solvent on complexation of some biologically important ligands.[5−7,15−18] The interaction of solvents, with different dielectric constants, with biological molecules is of importance in understanding the chemistry of in vivo processes like enzyme interactions, the assembly of lipids in biomembranes, surfactant aggregation, and so forth.[19]

In previous publications,[5−7,15−18] we have reported that the dielectric constant alone (as believed for many years) cannot serve as a quantitative measure of solvent polarity. This property is often inadequate, since the dielectric constant regards a solvent as a nonstructured continuum, not composed of individual solvent molecules with their own solvent−solvent interaction and does not take into account specific solute−solvent interactions.[20]

In this work the protonation constants of L-tryptophan, one of the eight essential amino acids found in humans, have been determined using a potentiometric method in various aqueous methanol, ethanol, and 1-propanol solvent mixtures to examine the solvent dependence of its acid−base equilibria.

Experimental Section

Chemicals. L-tryptophan, $C_8H_5(NH)-CH_2-CH(NH_2)COOH$ 99 %, (Aldrich, cat. no.: T9, 020-4) was recrystallized from hot water, washed with ethanol, and dried over P_2O_5, and the concentration of its solution was checked by titration against standard alkali. The NaOH solution was prepared from titrisol solution (Merck, cat. no.: 109956). Perchloric acid (70 to 72) % was supplied from Merck (cat. no.: 100519) as an analytical reagent grade material and was used without further purification. Sodium perchlorate 99 % (from Merck, cat. no.: 106564) was kept at room temperature in vacuum at least 72 h before use. Dilute acid solution was standardized against standard NaOH solution. Methanol 99.8 % (cat. no.: 106009), ethanol 99.8 %

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Table 1. Average Values of the Autoprotolysis Constants in Water−Methanol, Water−Ethanol, and Water−1-Propanol Mixtures at 25 °C and Constant Ionic Strength (0.1 mol·dm^{-3} NaClO$_4$), together with the Values Reported in the Literature

alcohol % (v/v)	pK_{ap} water−methanol	pK_{ap} water−ethanol	pK_{ap} water−1-propanol	ref
0	13.71 ± 0.09	13.71 ± 0.05	13.71 ± 0.08	this work
10	13.75 ± 0.10	13.79 ± 0.07	13.82 ± 0.07	″
20	13.86 ± 0.08	13.91 ± 0.01	13.95 ± 0.05	″
30	13.93 ± 0.07	14.05 ± 0.08	14.12 ± 0.08	″
40	14.07 ± 0.11	14.12 ± 0.06	14.24 ± 0.10	″
50	14.19 ± 0.11	14.25 ± 0.07	14.32 ± 0.09	″
60	14.31 ± 0.16	14.47 ± 0.11	14.51 ± 0.10	″
70	14.42 ± 0.15	14.58 ± 0.10	14.68 ± 0.11	″
80	14.58 ± 0.15	14.75 ± 0.11	14.91 ± 0.12	″
0	13.69	13.69	13.69	22
10	13.75	13.82	13.78	″
20	13.73	13.99	13.98	″
30	13.70	14.16	14.04	″
40	13.73	14.24	14.15	″
50	13.71	14.28	14.31	″
60	13.72	14.39	14.46	″
70	13.77	14.59	14.61	″
80	13.94	14.77	14.82	″

(cat. no.: 100983), and 1-propanol 99.8 % (cat. no.: 101024) were obtained from Merck (reagent grade) and were used without further purification. All dilute solutions were prepared from double-distilled water with a specific conductance equal to (1.3 ± 0.1) μS·cm^{-1}.

Apparatus. The electromotive force, E, was measured using a Metrohm model 781 pH ion meter. The combined glass/pH electrode (model 6.0258.000) was modified by replacing its aqueous KCl solution with 0.01 mol·dm^{-3} NaCl + 0.09 mol·dm^{-3} NaClO$_4$ saturated with AgCl. The electrode was soaked for (15 to 20) min in a water−alcohol mixture before the potentiometric measurements. All titrations were carried out in a 80 mL thermostatted 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.

Procedure. All measurements were performed at 25 °C and constant ionic strength (0.1 mol·dm^{-3} sodium perchlorate). The protonation constants were evaluated from measurements of the electromotive force (emf) by titration of a 25 mL tryptophan (5.0·10^{-3} mol·dm^{-3}) solution with a 0.1 mol·dm^{-3} sodium hydroxide solution both in the same ionic strength and mole fraction of organic solvent of methanol, ethanol, and 1-propanol [(0 to 80) % by v/v].

Results and Discussion

Autoprotolysis Constant. The ionization process of a medium like a water−alcohol mixture is given as

$$\mathrm{RH} + \mathrm{RH} \rightleftharpoons \mathrm{RH_2}^{+} + \mathrm{R}^{-} \qquad (1)$$

where RH, RH_2^+, and R^- denote a water or alcohol molecule, lyonium, and the lyate ions, respectively. The stoichiometric autoprotolysis constants of water−alcohol mixtures can be obtained from the potentiometric titration data of the cell

$$\mathrm{GE/HClO_4 - NaClO_4 \text{ in water−alcohol}/NaCl-NaClO_4/Ag-AgCl} \qquad (2)$$

and are defined as

$$K_{\mathrm{ap}} = [\mathrm{RH_2^+}][\mathrm{R^-}] \qquad (3)$$

The autoprotolysis constant in all aqueous−alcohol mixtures were determined at constant ionic strength (0.1 mol·dm^{-3} NaClO$_4$) from titrations of 25 mL perchloric acid (0.01 mol·dm^{-3}) with sodium hydroxide (0.1 mol·dm^{-3}) solutions. The potential was allowed to stabilize after each addition of the titrant and the emf values, E, of cell 2 were then recorded. In acidic solution, the measured potential of the cell, E_{cell}, can be written as

$$E_{\mathrm{cell}}(\mathrm{mV}) = E_{\mathrm{cell}}^{\circ} + k \log a_{\mathrm{ClO_4^-}} + k \log[\mathrm{H^+}] + k \log \gamma_{\mathrm{H}}^{+} + E_{\mathrm{LJ}} \qquad (4)$$

where $E_{\mathrm{cell}}^{\circ}$ is the standard potential of the cell, E_{LJ} is the liquid junction potential, k, the Nerstian slope that is ln(10)RT/F in which R, T, and F have the usual meaning, and $a_{\mathrm{H}}{}^{+}$, $\gamma_{\mathrm{H}}{}^{+}$, and $a_{\mathrm{ClO_4^-}}$ are the activity, the activity coefficient of the hydrogen ion, and the activity of the perchlorate ion, respectively. Difficulties in computing the activity coefficients of the hydrogen ion in various aqueous mixtures of organic solvents leads to the measurement of emf of the cell versus H^+ concentration in solution. Because the ionic strength of the solution is kept constant, the activity coefficient of hydrogen and the activity of perchlorate ions are also constant. The nonideality of the solutions is then included in E_a' (the specific constant of the potentiometric cell in the acidic region), so

$$E_{\mathrm{cell}} = E_{\mathrm{a}}' + k \log[\mathrm{H^+}] \qquad (5)$$

where E_a' is $E_{\mathrm{cell}}^{\circ} + k \log a_{\mathrm{ClO_4^-}} + k \log \gamma_{\mathrm{H}}{}^{+} + E_{\mathrm{LJ}}$. The use of a glass electrode (with an aqueous inner solution) in nonaqueous media introduces a deviation from ideality. However, it has been shown that the deviation is negligible and the glass electrode is always usable in such media to measure H^+ concentrations with a linear relation between E_{cell} and log[H^+].[21] In our case the calculated value of k differs negligibly from the theoretical one (59.157). In the acidic region the hydrogen ion concentration can be expressed as

Table 2. Average Values of the Protonation Constants of Tryptophan at 25 °C and Constant Ionic Strength (0.1 mol·dm^{-3} NaClO$_4$) and Different Aqueous Alcohol Mixtures, K_1 and K_2 Assigned for the Equilibria $L^- + H^+ \rightleftharpoons HL$ and $HL + H^+ \rightleftharpoons H_2L^+$, Respectively[a]

	water−methanol		water−ethanol		water−1-propanol		
alcohol % (v/v)	log K_1	log K_2	log K_1	log K_2	log K_1	log K_2	ref
0	9.31 ± 0.05	2.25 ± 0.03	9.31 ± 0.05	2.25 ± 0.03	9.31 ± 0.05	2.25 ± 0.03	this work
10	9.28 ± 0.04	2.27 ± 0.03	9.26 ± 0.02	2.28 ± 0.04	9.23 ± 0.04	2.30 ± 0.04	″
20	9.24 ± 0.05	2.32 ± 0.05	9.21 ± 0.04	2.36 ± 0.06	9.15 ± 0.05	2.41 ± 0.04	″
30	9.20 ± 0.03	2.48 ± 0.04	9.15 ± 0.05	2.51 ± 0.05	9.07 ± 0.06	2.55 ± 0.03	″
40	9.15 ± 0.06	2.62 ± 0.05	9.09 ± 0.04	2.68 ± 0.03	8.98 ± 0.07	2.76 ± 0.05	″
50	9.09 ± 0.05	2.74 ± 0.05	9.04 ± 0.05	2.81 ± 0.04	8.88 ± 0.04	2.91 ± 0.05	″
60	9.12 ± 0.08	2.83 ± 0.06	9.10 ± 0.07	2.92 ± 0.07	9.02 ± 0.06	3.04 ± 0.07	″
70	9.17 ± 0.09	2.92 ± 0.08	9.22 ± 0.09	3.05 ± 0.08	9.24 ± 0.09	3.18 ± 0.08	″
80	9.25 ± 0.10	3.01 ± 0.09	9.37 ± 0.10	3.17 ± 0.08	9.51 ± 0.09	3.39 ± 0.09	″
0.0	9.39						4
0.0	9.33	2.35					24

[a] The values reported in the literature are also listed.

$$[\mathrm{H}^+] = (M_{\mathrm{HClO}_4}V_0 - M_{\mathrm{NaOH}}V_1)/(V_0 + V_1) \qquad (6)$$

where M_{HClO_4} and M_{NaOH} are the molarities of perchloric acid and sodium hydroxide and V_0 and V_1 are the initial volume of perchloric acid and the added volume of sodium hydroxide solution, respectively.

In a basic solution, the measured potential of the cell can be written as

$$E_{\mathrm{cell}}(\mathrm{mV}) = E_{\mathrm{cell}}^\circ + k\log a_{\mathrm{ClO}_4^-} - k\log[\mathrm{OH}^-] - k\log\gamma_{\mathrm{OH}^-} + E_{\mathrm{LJ}} \qquad (7)$$

so

$$E_{\mathrm{cell}} = E_{\mathrm{b}}' - k\log[\mathrm{OH}^-] \qquad (8)$$

where E_{b}' (the specific constant of the potentiometric cell in basic region) is $E_{\mathrm{cell}}^\circ + k\log a_{\mathrm{ClO}_4^-} - k\log\gamma_{\mathrm{OH}^-} + E_{\mathrm{LJ}}$ and a_{OH^-} and γ_{OH^-} are the activity and the activity coefficient of the hydroxyl ion, respectively. E_{b}' can be calculated from the intercept of the linear plot of E_{cell} versus $-\log[\mathrm{OH}^-]$. In the basic region the hydroxyl ion concentration is expressed as

$$[\mathrm{OH}^-] = (M_{\mathrm{NaOH}}V_1 - M_{\mathrm{HClO}_4}V_0)/(V_0 + V_1) \qquad (9)$$

The autoprotolysis constant of water is then calculated from eq 10, and the values are listed in Table 1 for different aqueous alcohol solutions together with the values reported in the literature for comparison.[22]

$$\mathrm{p}K_{\mathrm{ap}} = (E_{\mathrm{a}}' - E_{\mathrm{b}}')/k \qquad (10)$$

With some differences the autoprotolysis constant values obtained in this work are in agreement with those reported before. The main differences are possibly due to the different experimental method, and some are because of the different background electrolyte used.

Protonation of Tryptophan. The protonation constant values of tryptophan were determined potentiometrically by titration of appropriate solutions of the amino acid in the water−alcohol mixtures studied. In this way, tryptophan was fully protonated at the beginning of a titration by adding a certain amount of perchloric acid at first and then using sodium hydroxide solution (0.1 mol·dm^{-3}) as titrant. The protonation constants were obtained from systematic emf measurements of the following cell:

$$\mathrm{GE/HClO_4{-}NaClO_4,\ H_2L^+ + HL + L^-\ in\ water{-}alcohol/NaCl{-}NaClO_4/Ag{-}AgCl}$$

where $\mathrm{H_2L^+}$, HL, and $\mathrm{L^-}$ are the different species of the amino acid involved in the protonation equilibria studied.

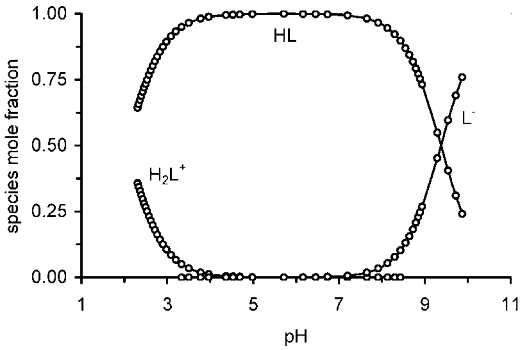

Figure 1. Distribution diagram of the different species of tryptophan in water at 25 °C and an ionic strength of 0.1 mol·dm^{-3} $\mathrm{NaClO_4}$.

The fraction of protons still bound to the amino acid, $\bar{n}$, can be written as

$$\bar{n}_{\mathrm{cal}} = (C_{\mathrm{H}} - [\mathrm{H}^+])/C_{\mathrm{L}} \qquad (11)$$

where C_{H} and C_{L} are the total concentrations of protons and amino acid, respectively. Substituting $C_{\mathrm{L}} = [\mathrm{H_2L^+}] + [\mathrm{HL}] + [\mathrm{L^-}]$ and $C_{\mathrm{H}} = [\mathrm{H^+}] + [\mathrm{HL}] + 2[\mathrm{H_2L^+}]$ in eq 11 leads to

$$\bar{n}_{\mathrm{cal}} = ([\mathrm{HL}] + 2[\mathrm{H_2L^+}])/([\mathrm{L^-}] + [\mathrm{HL}] + [\mathrm{H_2L^+}]) \qquad (12)$$

Substituting the protonation constants instead of concentration of the different species of tryptophan in eq 12, rearranging and canceling like terms, gives

$$\bar{n}_{\mathrm{cal}} = (K_1[\mathrm{H^+}] + 2K_1K_2[\mathrm{H^+}]^2)/(1 + K_1[\mathrm{H^+}] + K_1K_2[\mathrm{H^+}]^2) \qquad (13)$$

where K_1 and K_2 represent the protonation constants of the amino and carboxylic acid groups of the amino acid, respectively. 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

$$[\mathrm{L^-}] + [\mathrm{ClO_4^-}] + [\mathrm{OH^-}] = [\mathrm{Na^+}] + [\mathrm{H^+}] + [\mathrm{H_2L^+}] \qquad (14)$$

substituting $[\mathrm{L^-}]$ from C_{L} in eq 14 and putting the product in eq 11 to simplify lead to

$$\bar{n}_{\mathrm{exp}} = (C_{\mathrm{L}} + [\mathrm{ClO_4^-}] - [\mathrm{Na^+}] - [\mathrm{H^+}] + [\mathrm{OH^-}])/C_{\mathrm{L}} \qquad (15)$$

In eqs 13 and 15, $[\mathrm{Na^+}]$ originates from the titrant used (NaOH) and $\mathrm{NaClO_4}$, $[\mathrm{ClO_4^-}]$ is introduced from the perchloric acid added and $\mathrm{NaClO_4}$, $[\mathrm{H^+}] = 10^{(E_{\mathrm{cell}} - E_{\mathrm{a}}')/k}$ and $[\mathrm{OH^-}] = K_{\mathrm{ap}}/[\mathrm{H^+}]$. Using a suitable computer program (Microsoft Excel Solver),[23] the data from eqs 13 and 15 were fitted for estimating the protonation constant values of tryptophan in different aqueous solutions of alcohols. 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 16.

$$U = \sum(\bar{n}_{\mathrm{exp}}{}^2 - \bar{n}_{\mathrm{cal}}) \qquad (16)$$

where$\bar{n}_{\mathrm{exp}}$ is an experimental $\bar{n}$ and $\bar{n}_{\mathrm{cal}}$ is the calculated one. The calculated protonation constant values of tryptophan in different water−alcohol mixtures are listed in Table 2 together with values reported in the literature for comparison.[4,24] The protonation constant values obtained in this work are in good agreement with those reported before only in water. In Figure 1, the equilibrium distribution of various species of tryptophan in water is shown as a function of pH. The calculations are based on the protonation constant values given in Table 2.

Solvent Effect. The protonation constant values of tryptophan (Table 2) in various aqueous alcohol solutions show different behavior. The protonation constant of the amino group of tryptophan, K_1, first decreases with increasing proportion of each alcohol in the mixed solvents and then increases when the mixtures are richer in the organic solvent. So, the variation of $\log K_1$ values versus the percentage of the organic solvents in different mixtures show a minimum when the amount of each alcohol is about (50 to 60) % (v/v) (Table 2). This can be explained by the fact that in this range there is a change in the behavior of the solvent. It is assumed in the water-rich composition, the alcohol molecules occupy the cavities between

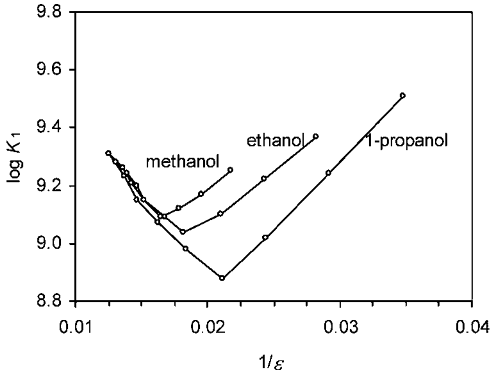

Figure 2. Plots of the experimental values of log K_1 versus the reciprocal of the dielectric constant of different mixed solvents at 25 °C and an ionic strength of 0.1 $\text{mol}\cdot\text{dm}^{-3}$ $NaClO_4$.

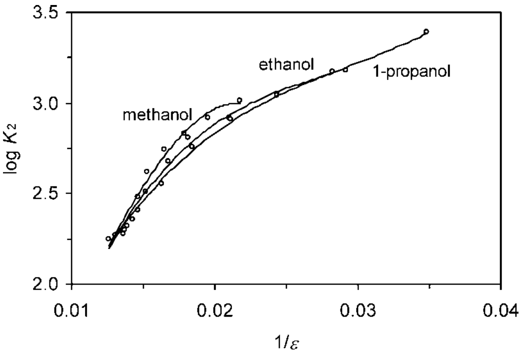

Figure 3. Plots of the experimental values of log K_2 versus the reciprocal of dielectric constant of different mixed solvents at 25 °C and an ionic strength of 0.1 $\text{mol}\cdot\text{dm}^{-3}$ $NaClO_4$.

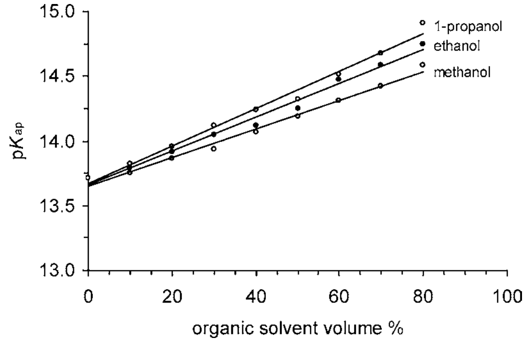

Figure 4. Plots of the experimental values of pK_{ap} versus the percentages of different alcohols (by volume %) in the mixed solvents at 25 °C and an ionic strength of 0.1 $\text{mol}\cdot\text{dm}^{-3}$ $NaClO_4$.

Table 3. KAT Solvatochromic Parameters and the Dielectric Constants of Different Aqueous Alcohol Mixtures

alcohol %	water−methanol				water−ethanol				water−1-propanol			
(v/v)	α	β	π^*	ε	α	β	π^*	ε	α	β	π^*	ε
0	1.23	0.49	1.14	79.50	1.23	0.49	1.14	79.50	1.23	0.49	1.14	79.50
10	1.19	0.51	1.13	76.40	1.13	0.52	1.14	73.70	1.15	0.52	1.13	72.90
20	1.14	0.54	1.10	72.10	1.03	0.57	1.12	70.20	1.04	0.56	1.12	68.30
30	1.08	0.57	1.07	68.30	0.95	0.61	1.09	66.00	0.94	0.60	1.09	61.50
40	1.04	0.60	1.04	65.60	0.89	0.66	1.04	59.60	0.87	0.65	1.04	54.40
50	1.01	0.63	1.00	60.80	0.87	0.70	0.97	55.00	0.86	0.70	0.96	47.40
60	1.01	0.67	0.94	56.10	0.87	0.74	0.89	47.50	0.88	0.75	0.88	41.00
70	1.02	0.70	0.88	51.20	0.89	0.77	0.81	41.10	0.89	0.78	0.81	34.30
80	1.05	0.72	0.80	45.90	0.91	0.80	0.74	35.40	0.90	0.79	0.75	28.70

the water molecules without disrupting the water structure.[25] But, in the alcohol-rich composition, clusters of molecules of either water or alcohol are surrounded by regions containing both kinds of molecules.[25,26] However, the protonation constant of the carboxylic group of the amino acid, K_2, increases with increasing percentages of alcohol in the mixed solvents. The different behavior of K_1 and K_2 observed in this study can possibly be attributed to the fact that in the protonation of the carboxylic acid group of the amino acid, there is no change in the number of charges involved in the process ($HL + H^+ \rightleftharpoons H_2L^+$), and therefore, a change in polarity of the medium has a minor effect on the protonation process. On the contrary, in protonation of the amino group of tryptophan charges are consumed ($L^- + H^+ \rightleftharpoons HL$), and therefore, any variation in polarity of the medium exerts a strong influence on the process. The correlation between log K_1 with the reciprocal of the dielectric constant of the aqueous−alcohol mixtures is linear, but the linear relationship does not hold when the amount of organic solvents exceeds almost 50 % (v/v) in the mixtures. So, the plot of log K_1 versus $1/\varepsilon$ can be accurately depicted by two lines for the amino acid (Figure 2), and the intersection nearly corresponds to about (50 to 60) % (v/v) of each alcohol in the mixtures. This finding may relate to the structural features of the aqueous−organic solvent mixtures described by Marcus and Migron.[27] Furthermore, the correlation between log K_2 with the reciprocal of the dielectric constant of the mixtures is not linear (Figure 3) and deviates from the Born equation.[7] This indicates that the protonation constants not only depend on electrostatic forces but also strongly depend on solute−solvent interactions of the different species in the mixtures.[26] However, pK_{ap} of the media increases with increasing percentages of alcohol in the mixed solvents (Figure 4). Furthermore, pK_{ap} of the media and the protonation constants of the amino acid have different values in the three mixed solvent systems studied in the same percentage of organic solvent in the mixtures. This should be due to the differences in the dielectric constant values of the mixtures which are in the order of water−methanol > water−ethanol > water−1-propanol. These findings necessitate elucidating the nature of solute−solvent interactions for a better understanding of solvent effects.

The protonation constant values of the amino acid show small changes when the mixtures are richer in water and a larger increase when the mixtures are richer in alcohol. This variation with the percentage of the organic solvent is due to solute−solvent and solvent−solvent interaction effects. In fact, solvent−solvent interactions can be influenced by intermolecular forces between the solution components. This effect possibly changes the structure of the mixtures when the percentage of the organic solvents increases to higher values.[26] This discussion is in accordance with previous results for other aqueous−organic solvent mixtures and in agreement with the present results.[28] It is very difficult to interpret the variation of the protonation constant values of tryptophan with respect to the percentage of methanol, ethanol, or propanol in the mixtures using the dielectric constant of the solutions as a single parameter. To obtain a quantitative method for the evaluation of the solute−solvent interaction on protonation or other equilibrium constants, during the last two decades, many empirical solvent scales have been devised.[20] Among these scales (more than 40), the most comprehensive are the solvatochromic ones, but only a few of them have found a wider application in the correlation analysis of solvent effects. A quantitative measurement of the solvent polarity has been introduced by Kamlet, Abboud, and Taft (KAT).[29,30] The KAT equation contains nonspecific as well as specific solute−solvent interactions separately, and the latter should be subdivided into solvent Lewis acidity interactions (hydrogen-bond acceptor, HBA solute, and hydrogen-bond donor, HBD solvent) and solvent Lewis basicity interactions (HBD solute/HBA solvent). In general, all of these parameters

Table 4. Regression Coefficients of the KAT Equation (Dual Parameters) for log K_2 Values of Tryptophan in Different Water−Alcohol Mixtures

species	regression coefficients of the KAT equation[a]	R^2	rss	ose
log K_2 (methanol)	$5.65(0.16) - 1.12(0.21)\alpha - 1.81(0.15)\pi^*$	0.99	0.01	0.03
log K_2 (ethanol)	$5.15(0.07) - 0.70(0.09)\alpha - 1.81(0.08)\pi^*$	1.00	0.00	0.02
log K_2 (1-propanol)	$5.68(0.09) - 0.74(0.12)\alpha - 2.23(0.11)\pi^*$	0.99	0.01	0.03

[a] Values in the parentheses are the standard error for that coefficient.

constitute more comprehensive measures of solvent polarity than the dielectric constant alone, because they reflect more reliably the complete picture of all intermolecular forces acting between solute and solvent molecules. This approach has been widely and successfully applied in the correlation analysis of all kinds of solvent-dependent processes.[19] Using the solvatochromic solvent parameters, α, β, and π^* which have been introduced in previous reports,[5−7,15−17] the multiparametric equation, eq 17, has been proposed for use in the so-called linear solvation energy relationship.

$$\log K = A_0 + a\alpha + b\beta + p\pi^* \qquad (17)$$

where A_0 represents the regression value and π^* is the index of the solvent dipolarity/polarizability, which is a measure of the ability of a solvent to stabilize a charge or a dipole by its own dielectric effects. The π^* scale was selected to run from 0.0 for cyclohexanone to 1.0 for dimethylsulfoxide. The α coefficient represents the solvent HBD acidity; in other words it describes the ability of a solvent to donate a proton in a solvent to a solute hydrogen-bond. The α scale extends from 0.0 for non-HBD solvents to about 1.0 for methanol. The β coefficient is a measure of a solvent HBA basicity and describes the ability of a solvent to accept a proton from a solute molecule. The β scale was selected to extend from 0.0 for non-HBA solvents to about 1.0 for hexamethylphosphoric triamide. In eq 17 the discontinuous polarizability correction term is omitted because of the solvent used in this work containing no chlorine atom.

The regression coefficients *a*, *b*, and *p* measure the relative susceptibilities of the solvent-dependence of log *K* to the indicated solvent parameters. To explain the obtained log *K* values through the KAT solvent parameters, the protonation constants were correlated with solvent properties by means of single-, dual-, and multiparameter regression analysis by a suitable computer program (Microsoft Excel Solver and Linest).[23] We used the Gauss−Newton nonlinear least-squares method in the computer program to refine the log *K* by minimizing the error squares sum from eq 18.

$$S = \sum (\mathrm{p}K_{\mathrm{exp}} - \mathrm{p}K_{\mathrm{cal}})^2 \qquad (18)$$

The procedure used in the regression analysis involves a rigorous statistical treatment to find out which parameter in eq 17 is best suited to the water−organic mixed solvents. The KAT parameters for all of the water−organic solvents mixtures used in this work were obtained from the plots of each property versus the mole fraction of the organic solvent of the values that have been reported in the literature for some other percentages of aqueous solutions of the alcohols used in this study, and the dielectric constant values were obtained from the literature for the different mixed solvents.[31−33] The dielectric constants and the calculated KAT parameter values used are listed in Table 3. A stepwise procedure and least-squares analysis were applied to select the significant solvent properties to be influenced in the model and to obtain the final expression for the protonation constants. Therefore, the KAT equation, eq 17, was reduced to single-, dual-, and finally multiparameters for correlation analysis of log *K* in various solvent mixtures. The computer program used can determine the values of A_0, *a*, *b*, *p*, and some statistical parameters including the R^2 coefficient and standard deviation of any parameter (given in brackets in Tables 4 and 5), the overall standard error (ose), and the residual sum of the squares of errors (rss) of log *K*. The expressions of the KAT equation thus obtained for each property are given in Tables 4 and 5 only as dual parameters.

Although the solvent polarity is identified as the main reason for the variation of log *K* values in water−organic solvent mixtures, the results show that any single-parameter correlation of log K_1 or log K_2 values individually with π^* does not give good results in all cases. However, the correlation analysis of log K_1 and log K_2 values with the dual-parameter equations indicate significant improvement with regard to the single- and multiparameter models, yield the lowest standard deviation, and possess very good coefficients of determination in all cases (Tables 4 and 5).

The coefficients of α and π^* in the correlation analysis of log K_2 with the dual parameters of the KAT equation in all mixed solvents are negative. The negative sign of π^* values indicates that a decrease in the polarity of the mixed solvents increases the log K_2 values. Thus, an increase in the polarity increases the solvation of the species and therefore makes protonation equilibrium more likely. Solvation by decreasing the polarity affects cations and could explain why π^* is the main parameter in the equations given in Table 4. Moreover, the negative sign of α indicates that a decrease in the HBD acidity of the solvent also increases the solvation tendency of the produced cations. Consequently, the protonation constants increase when this parameter decreases. However, the correlation analysis of log K_1 values in the water-rich region with the dual-parameter model of the KAT equation including α and π^* yields the lowest ose and rss terms in all cases. In this case, both of them (α and π^*) have positive signs (Table 5). The positive sign of α and π^* indicates that log K_1 values decrease with decreasing hydrogen-bond acidity as well as with the polarity

Table 5. Regression Coefficients of the KAT Equation (Dual Parameters) for log K_1 Values of Tryptophan, In Water-Rich and in Alcohol-Rich Regions

species	regression coefficients of the KAT equation in water-rich region[a]	R^2	rss	ose
log K_1 (methanol)	$7.74\ (0.08) + 0.21(0.12)\alpha + 1.15(0.18)\pi^*$	1.00	0.00	0.01
log K_1 (ethanol)	$8.07(0.03) + 0.46(0.02)\alpha + 0.60(0.05)\pi^*$	1.00	0.00	0.00
log K_1 (1-propanol)	$7.37(0.10) + 0.63(0.06)\alpha + 1.02(0.14)\pi^*$	1.00	0.00	0.01
	regression coefficients of KAT equation in alcohol rich region			
log K_1 (methanol)	$12.73(0.38) - 2.48(0.35)\beta - 2.08(0.16)\pi^*$	1.00	0.00	0.00
log K_1 (ethanol)	$34.98(4.72) - 21.33(4.11)\beta - 11.44(1.92)\pi^*$	1.00	0.00	0.01
log K_1 (1-propanol)	$18.50(0.48) - 6.05(0.43)\beta - 5.61(0.19)\pi^*$	1.00	0.00	0.01

[a] Values in the parentheses are the standard error for that coefficient.

of the mixed solvent mixtures up to about (50 to 60) % (v/v) of organic solvents. In this case the produced species (HL) has no charge, and the solvation of the L^- species as a reactant is more likely. However, in the alcohol-rich region, the correlation analysis of log K_1 values with β and π^* parameters is the best-fitted model and yields the lowest ose and rss terms (Table 5). The correlation analysis of log K_1 in the alcohol-rich regions indicates that the regression coefficient of β has the major roll [about (55, 65, and 52) % for the different aqueous solutions of methanol, ethanol, and 1-propanol in the mixed solvents, respectively]. So, log K_1 values increase with an increase in the hydrogen-bond basicity parameter.

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