

Dissociation Constants of Amino Acids in Isopropanol + Water Mixtures

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The pK values of a number of amino acids have been determined in isopropanol + water mixtures of varying compositions pH -metrically. The results show that the changes in pK_1 values are smaller compared to those in the corresponding carboxylic acids, but the changes in pK_2 values are only marginal. It is apparent that the ratio of zwitterions decreases as the [organic solvent] increases. It has been found that the specific solute-solvent interactions are of importance in explaining the change in pK values but their nature is not well understood.

Studies on the dissociation constants of acids and bases in a number of mixed and non-aqueous solvents have been extensively made but relatively little work has been done to determine the dissociation constants of amino acids in different mixed and non-aqueous solvents. Recently, Lahiri and coworkers^{1,2} determined the dissociation constants of amino acids in methanol + water and ethanol + water mixtures. We present in this paper the pH -metric determination of dissociation constants of some important amino acids, namely glycine, α -alanine, β -alanine, L-threonine, L-proline, L-valine, L-phenylalanine, L-glutamine, L-asparagine, L-serine, L-methionine and L-histidine in different isopropanol + water (0-75.8% by weight of isopropanol) mixtures.

Materials and Methods

The amino acids (except those of G R, E. Merck variety) were purified by crystallization from water-ethanol mixture. The amino acids were analysed titrimetrically in the usual way using acid-free formaldehyde solution. Isopropanol (A R, BDH) was distilled and the middle fraction was utilised. The weight percentages of the organic solvents were determined in the same way as described earlier³ and the dielectric constant values were calculated from the data of Akerlof and Short⁴.

Perchloric acid and sodium hydroxide (G R, E. Merck) were standardized in the usual way. Other chemicals were of G R grade. All solutions were made in doubly distilled water.

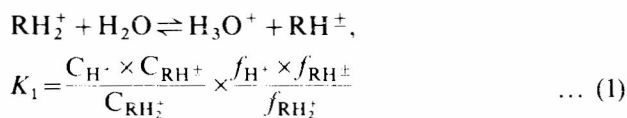
For the determination of the acid dissociation of dipolar ions, a known concentration of amino acid was neutralized to different extents with perchloric acid and the hydrogen ion concentrations were measured pH -metrically. The process was repeated with different concentrations of different dipolar ions in isopropanol + water mixtures.

To determine the pK_1 of histidine, one equivalent of $HClO_4$ was first added to the amino acid, the acidified amino acid solution was further neutralised with $HClO_4$ in the usual way and the pH meter readings were noted. For the determination of the second dissociation constant of amino acids, the process was the same; only $HClO_4$ was replaced by $NaOH$.

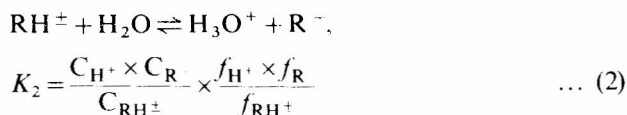
The pH -meter readings were taken at 298 K using a Systronic digital pH -meter with a combined glass and calomel electrode assembly maintained at 298 K. The instrument was first standardized with potassium hydrogen phthalate and then with $10^{-4} M$ $HClO_4$ solution. The glass electrode was kept immersed in the appropriate solvent medium for equilibration before the measurements were carried out.

Results and Discussion

The dissociation equilibria of amino acids in acid and in alkali can be represented by Eqs (1) and (2),



and



In view of non-availability of the activity coefficient data of different ions in mixed solvents, the K_1 and K_2 values are difficult to determine. The addition of neutral electrolytes (to maintain a constant ionic strength) has been avoided^{5,6} as the neutral electrolytes increase the solubility of amino acids and form molecular combinations with amino acids; these effects would be more pronounced in mixed solvents having low dielectric constants. The Eqs (1) and (2) can be written as,

$$pK_1 = pH - \log \frac{C_{RH^+}}{C_{RH_2^+}} - \log \frac{f_{RH^+}}{f_{RH_2^+}}$$

$$= pH - \log \left(\frac{c}{b - c_{H^+}} - 1 \right) - \log \frac{f_{RH^+}}{f_{RH_2^+}} \quad \dots (3)$$

and

$$pK_2 = pH + \log \left(\frac{c}{b - c_{OH^-}} - 1 \right) - \log \frac{f_{RH^+}}{f_{RH_2^+}} \quad \dots (4)$$

where C = concentration of neutral amino acid, a or b = concentration of acid or base respectively, and C_{H^+} or C_{OH^-} = concentration of H^+ ion or OH^- ion in the experimental solution (determined pH -metrically). Appropriate corrections were applied to the pH -meter readings⁸⁻¹¹ to get the true H^+ concentrations in the mixed solvents.

Though the correction factors for the mixed solvents were known, we considered it desirable to find the correction factor with each set of measurement to avoid any error resulting from (i) change in the asymmetry potential of the glass electrode and (ii) liquid-junction potential of uncertain magnitude which may change from solution to solution.

The highest concentrations of amino acids and $HClO_4$ or $NaOH$ used were $1.0 \times 10^{-2} M$ and 3.0 – $7.0 \times 10^{-3} M$ (except in the determination of the first dissociation constant of histidine). The approximation that the activity coefficients are unity in all our measurements may involve a slight error but it is unavoidable in view of lack of data on activity coefficients of amino acids in mixed solvents. In case of pK_1 , the effect would be only marginal as f_{H^+} and $f_{RH_2^+}$ cancel each other.

Though the total charge of zwitter ion is zero, but due to the separation of charges, the activity coefficients of amino acids should have values different from unity. The variation of activity coefficients of the dipolar ion in dilute solutions is given by¹²,

$$\log f = \frac{A}{\epsilon^2 T^2} \cdot \frac{R^2}{a} \mu \quad \dots (5)$$

where ϵ is the dielectric constant of the medium of ionic strengths μ , R and a are the distance between the charges and the mean distance of closest approach of the other ions to zwitter ion respectively. A is a constant derived theoretically. It is apparent that the activity coefficient values would be small.

However, corrections due to activity coefficients are likely to be greater in case of pK_2 values. The lack of data on the autoprotolysis constants in isopropanol + water mixtures makes the accurate determination of H^+ ion in alkaline solutions difficult. The error involved in the determination of pK_2 would thus be considerably greater.

The pK_1 and pK_2 (pK_1 , pK_2 and pK_3 values of histidine) in isopropanol + water mixtures at 298 K are presented in Tables 1 and 2 along with literature values⁵ for pK_1 and pK_2 in water. The pK_1 values of the amino acids in water reported in this paper agree well with the values given in the literature though slight discrepancy exists in some cases. However, the pK_1 values in the cases of asparagine and phenylalanine differ much. The introduction of phenyl group is likely to change the pK value of alanine but the change in pK value from 2.34 for alanine to 1.83 (literature value) for phenylalanine is rather unlikely. The pK_1 value of 2.27 for phenylalanine determined in the present study thus appears reasonable. The pK_1 values in mixed solvents also corroborate this. The pK_1 value of asparagine has been determined by different groups of workers in our laboratory and the results indicate that the literature value is erroneous. The pK_1 value of leucine reported by us is also considerably higher than the literature value.

The pK_2 values determined by us are generally within $\pm 0.1 pK$ units of the literature values. It is to be noted that the pH -measurements in acid solutions are rapid whereas a long time is required for equilibration in alkali solutions, particularly in mixed solvents. Edsall and Blanchard¹³ also noted that the measurements made in alkaline solutions gave unstable e.m.f. readings.

The pK_1 values in mixed solvents of all the amino acids increase with increase in the alcohol content of the solvent mixture but the increase in the values has been found to be much smaller than that in the case of the corresponding carboxylic acids due to dipolar nature of the amino acids. It has been found that the change in pK values is slightly greater in methanol + water mixtures and ethanol + water mixtures² than those in the corresponding isopropanol + water mixtures though the latter solvents have lower dielectric constants. This may be due to difference in solubilities and ionisations of amino acids in different solvent mixtures. It is likely that the solubility of amino acids decreases in the order isopropanol + water < ethanol + water < methanol + water < water i.e. in the order of decreasing hydrophobic character of the solvents. However, for the same percentages of organic solvents, the water content is greatest in isopropanol + water mixtures than those in methanol + water and ethanol + water mixtures causing increased solubility and association (due to lower dielectric constants of amino acids in isopropanol + water mixtures compared to corresponding methanol + water and ethanol + water mixtures)-leading to increased K_1 in isopropanol + water mixtures (vide Eq. 1).

An expected, pK_2 values change very slightly with solvent composition. In the case of proline, a

Table 1—Dissociation Constants of Amino Acids in Isopropanol-Water Mixtures

Alcohol (w/w, %)	$\frac{1}{\epsilon} \times 10^2$	<i>L</i> -Threonine		<i>L</i> -Proline		<i>L</i> -Valine		<i>L</i> -Phenylalanine		<i>L</i> -Glutamine	
		pK_1	pK_2	pK_1	pK_2	pK_1	pK_2	pK_1	pK_2	pK_1	pK_2
0	1.27	2.21 (2.15)	9.00 (9.12)	2.02 (1.99)	10.60 (10.60)	2.32 (2.32)	9.71 (9.62)	2.27 (1.83)	9.23 (9.13)	2.16 (2.17)	9.08 (9.13)
8.0	1.37	2.30	8.98	2.12	10.61	2.50	9.69	2.37	9.18	2.40	9.08
16.3	1.50	2.40	9.02	2.21	10.63	2.58	9.69	2.46	9.18	2.51	9.08
25.1	1.65	2.52	9.02	2.29	10.66	2.74	9.70	2.58	9.18	2.64	9.08
34.3	1.86	2.59	9.03	2.46	10.66	2.89	9.71	2.72	9.18	2.78	9.11
43.9	2.13	2.73	8.95	2.59	10.70	3.03	9.75	2.82	9.18	2.91	9.18
54.0	2.52	2.87	9.02	2.79	10.72	3.36	9.79	2.93	9.23	3.09	9.23
64.6	3.10	3.00	9.04	3.05	10.72	3.66	9.84	3.08	9.38	3.56	9.26
75.8	3.87	3.19	9.04	3.24	10.73	3.92	9.89	3.22	9.46	3.70	9.27
		<i>L</i> -Asparagine		<i>L</i> -Serine		<i>L</i> -Methionine		<i>L</i> -Histidine			
		pK_1	pK_2	pK_1	pK_2	pK_1	pK_2	pK_1	pK_2	pK_3	
0	1.27	2.28 (2.02)	8.69 (8.80)	2.22 (2.21)	8.97 (9.15)	2.29 (2.28)	9.20 (9.21)	1.75 (1.78)	5.96 (5.97)	9.10 (8.97)	
8.0	1.37	2.40	8.68	2.33	8.96	2.37	9.20	1.78	5.79	9.11	
16.3	1.50	2.51	8.68	2.43	8.99	2.46	9.17	1.91	5.72	9.13	
25.1	1.65	2.65	8.67	2.55	8.99	2.61	9.17	2.02	5.69	9.13	
34.3	1.86	2.78	8.69	2.65	8.99	2.76	9.19	2.16	5.53	9.10	
43.9	2.13	2.92	8.76	2.72	9.04	2.95	9.20	2.28	5.60	9.10	
54.0	2.52	3.07	8.78	2.82	9.06	3.06	9.23	2.36	5.58	9.16	
64.6	3.10	3.30	8.85	2.90	9.07	3.29	9.27	2.61	5.54	9.16	
75.8	3.87	3.46	8.91	3.11	9.09	3.47	9.30	2.72	5.46	9.16	

Values in parentheses refer to the literature values; standard deviations: $\pm(0.01-0.02)$ for pK_1 upto 43.9 wt% and $\pm(0.02-0.03)$ above 43.9 wt%; standard deviations: $\pm(0.02-0.03)$ for pK_2 upto 34.3 wt% and $\pm(0.03-0.0)$ above 34.3 wt%.

Table 2—Dissociation Constants of Amino Acids in Isopropanol-Water Mixtures

Alcohol (w/w, %)	$\frac{1}{\epsilon} \times 10^2$	Glycine			α -Alanine			β -Alanine			<i>L</i> -Leucine		
		pK_1	pK_2	$\log K_z$	pK_1	pK_2	$\log K_z$	pK_1	pK_2	$\log K_z$	pK_1	pK_2	$\log K_z$
0	1.27	2.33 (2.34)	9.59 (9.60)	5.40	2.36 (2.34)	9.64 (9.69)	5.44	3.59 (3.55)	10.18 (10.24)	5.54	2.44 (2.36)	9.59 (9.60)	5.19
8.0	1.37	2.40	9.59	5.33	2.55	9.59	5.25	3.71	10.14	5.42	2.53	9.50	5.10
16.3	1.50	2.51	9.58	5.22	2.71	9.59	5.09	3.75	10.14	5.38	2.58	9.50	5.05
25.1	1.65	2.64	9.59	5.09	2.79	9.58	5.01	3.84	10.14	5.29	2.81	9.50	4.82
34.3	1.86	2.81	9.63	4.92	3.00	9.62	4.80	3.93	10.17	5.20	2.95	9.52	4.68
43.9	2.13	3.03	9.68	4.70	3.17	9.62	4.63	4.01	10.20	5.12	3.13	9.52	4.50
54.0	2.52	3.17	9.74	4.56	3.49	9.69	4.31	4.08	10.28	5.05	3.37	9.52	4.26
64.6	3.10	3.49	9.80	4.24	3.65	9.71	4.15	4.20	10.33	4.93	3.57	9.55	4.06
75.8	3.87	3.66	9.81	4.07	3.91	9.77	3.89	4.32	10.39	4.81	3.74	9.58	3.89

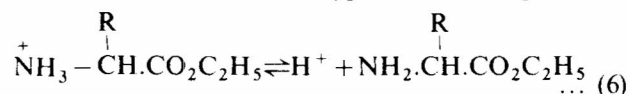
Values in parentheses refer to the literature values; standard deviations: $\pm(0.01-0.02)$ for pK_1 upto 43.9 wt% and $\pm(0.02-0.03)$ above 13.9 wt; standard deviations: $\pm(0.02-0.03)$ for pK_2 upto 34.3 wt% and $\pm(0.03-0.05)$ above 34.3 wt%; pK_E values for ester of glycine (7.73); α -alanine (7.80); β -alanine (9.13); *L*-leucine (7.63)

continuous increase in pK_2 values was observed, but in most cases there is a slight decrease in pK_2 values with addition of isopropanol. But increase was observed above 40-50% (v/v) of alcohol. However, in view of the lack of data for the autoprotolysis constants of H_2O in $Pr^iOH + H_2O$, a slight error is expected. The pK_1 values of histidine increase but the pK_2 values decrease with increase in organic solvent. The pK_3 values are found to increase slightly.

The pK_1 values are found to be a linear function of $1/\epsilon$ and mole fraction of isopropanol at low concentrations of alcohol. But the interactions of amino acids having two charged sites are found to be different for different amino acids. However, the ratio of zwitter ion to neutral acid decreases as the alcohol content increases leading to the change in interactions¹³. An approximate idea of the ratio of zwitter ion to neutral acid can be had by comparing the

pK_E of ethyl esters of amino acids¹³ like glycine, α -alanine, β -alanine and L-leucine in water with the pK_1 values reported here.

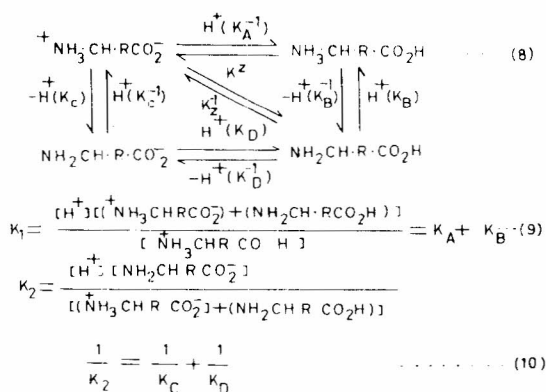
It is well known that the dissociation constants of isoelectric reactions of the type shown in Eq. 6,



are very slightly altered by the change in solvent composition. This is borne out by the pK_2 values of amino acids determined by us. Assuming that $pK_E(\text{ester})$ is almost unaltered on change in solvent composition, we could determine K_z ,

$$K_z = \frac{[\text{NH}_3 \text{R} \text{CO}_2^-]}{[\text{NH}_2 \text{R} \cdot \text{CO}_2 \text{H}]} \quad \dots (7)$$

using the procedure described by Ebert¹⁴, Edsall and Blanchard¹³. The results are given in Table 2. The sequence of reactions in zwitter ions can be represented as,



In spite of limitations, it is clear that the ratio of zwitter ion to neutral molecules decreases with increasing percentage of isopropanol.

Thus, it is apparent from Eqs (8)-(10) that the changes in K_1 and K_2 actually represent the changes in (K_A and K_B) and (K_C and K_D) values together with K_z values. These are reflected in small changes in pK_1 and pK_2 values.

Since the nature of the solute-solvent interactions of amino acids could only be ascertained from the free-energy of transfer (ΔG_t) values, attempts have been made to throw light on this important aspect.

For reaction (1), we have

$$\Delta G_t(1) = \Delta G_{t(\text{H}^+)} + \Delta G_{t(\text{RH}^\pm)} - \Delta G_{t(\text{RH}_2^\pm)} \quad \dots (11)$$

The left hand side can be calculated from $\Delta G_t(1)$ and $\Delta G_{t(\text{H}^+)}$ values reported from our laboratory¹⁵. The results are presented in Table 3. The $\Delta G_t(1)$ values increase sharply with increasing [alcohol]. The change in $\Delta G_t(1)$ may arise due to change from zwitter ion to neutral form but contribution due to this factor is found to be small. The main contributory factors are the structural changes, the structure formation of water molecules due to the addition of alcohol on one hand and hydrophobic interactions of amino acids and alcohols on the other hand. The change of zwitter ions to neutral molecules would be great at higher concentrations of alcohol. The $\text{RH}^\pm$ must be in the lower free-energy state due to solvation of the amino acids by large number of water molecules¹⁶. It is likely that $\Delta G_{t(\text{RH}^\pm)}$ becomes increasingly positive as the concentration of alcohol increases due to decrease in the solubility. But the values of $\Delta G_{t(\text{RH}^\pm)}$ would be very much dependent on the ratio of zwitter ion to neutral form and the presence of various hydrophobic and hydrophilic chemical groupings in the molecules. This leads to the variations of $\Delta G_t(1)$ of amino acids and hence $\Delta G_{t(\text{RH}^\pm)} - \Delta G_{t(\text{RH}_2^\pm)}$. From Eq. (11), it is seen that $\Delta G_{t(\text{RH}_2^\pm)}$ would be dependent on $\Delta G_{t(\text{RH}^\pm)}$ and would probably be negative. Similarly for reaction (2), we have

$$\Delta G_t(2) - \Delta G_{t(\text{H}^+)} = \Delta G_{t(\text{R}^-)} - \Delta G_{t(\text{RH}^\pm)} \quad \dots (12)$$

$\Delta G_t(2)$ changes very slightly and whatever may be the value of $\Delta G_t(2)$, $\Delta G_{t(\text{R}^-)}$ should be increasingly positive as observed for most anions¹⁷, i.e., transfer of R^- should be highly unfavourable as the [organic solvent] increases.

Table 3—Free Energy of Transfer [$G_{t(\text{acid})} - G_{t(\text{H}^+)}$] in kJ/mol of Amino Acids in Isopropanol-Water Mixtures

Isopropanol (w/w, %)	$-\Delta G_t(\text{H}^+)$ (kJ/mol)	L-Proline	L-Threonine	L-Phe-nylalanine	L-Glutamine	L-Asparagine	Glycine	α -Alanine	β -Alanine	L-Leucine	L-Valine	L-Serine	L-Methionine
8.0	0.76	1.33	1.27	1.33	2.13	1.44	1.16	1.84	1.45	1.27	1.75	1.39	1.22
16.3	1.82	2.90	2.90	2.90	3.76	3.13	2.85	2.82	2.73	2.62	3.37	3.01	2.79
25.1	3.35	4.89	5.11	5.12	6.03	5.46	5.12	5.79	4.78	5.46	5.57	5.23	5.18
34.3	4.18	6.69	6.35	6.75	7.72	7.03	6.92	7.83	6.12	7.09	7.40	6.63	6.84
43.9	5.04	8.29	8.01	8.18	9.32	8.69	9.04	9.66	7.46	8.98	9.06	7.89	8.81
54.0	5.33	9.73	9.10	9.09	10.64	9.84	10.12	10.78	8.13	10.64	11.23	8.75	9.73
64.6	5.26	11.24	9.77	9.88	13.25	11.08	11.28	12.62	8.74	11.71	12.87	9.14	10.97
75.8	4.69	11.65	10.25	10.11	13.48	11.43	12.28	13.54	8.86	12.11	13.79	9.77	11.43

The present results indicate that specific solute-solvent interactions are of great importance. However, further work on the determination of the solubility values of amino acids, solvation and activity coefficients, etc. are necessary to explore the role of the solvents and the nature of interactions.

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