

Studies on the Dissociation Constants & Solubilities of Amino Acids in *t*-Butanol + Water Mixtures

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The dissociation constants of a number of amino acids and the solubilities of some of the acids in *t*-butanol + water mixtures have been determined pH-metrically and spectrophotometrically. The free energies of transfer of amino acids from water to mixed solvents thus obtained have been coupled with the free energies of transfer [$\Delta G_i^0(1)$ and $\Delta G_i^0(2)$] for the reactions $\text{RH}_2^+ + \text{H}_2\text{O} \rightleftharpoons \text{H}_3\text{O}^+ + \text{RH}^+ \dots (1)$, and $\text{RH}^+ + \text{H}_2\text{O} \rightleftharpoons \text{H}_3\text{O}^+ + \text{R}^- \dots (2)$; and $\Delta G_i^0(\text{H}^+)$ values determined in our laboratory to get the free energies of transfer of RH_2^+ and R^- in different mixed solvents. These give the quantitative measures of ion-solvent interactions of the zwitterions, cations and anions of the amino acids. The results show that the conversion of zwitterion to neutral form is only marginal in mixed solvents but the amino acids are probably present as equilibrium mixtures of zwitter ions and anions (predominantly) in mixed solvents.

The behaviour of *t*-butanol as a solvent is quite different from that of methanol, ethanol, etc. Therefore, it may be of interest to study the dissociation constants of amino acids in *t*-butanol + water mixtures. Comparison of the dissociation constants in different solvents may throw some light on the ion-solvent interactions of the amino acids in different solvent mixtures. Furthermore, the solvents have profound effect on the solubilities of the amino acids. The solubilities of amino acids in *t*-butanol + H_2O mixtures may throw light on the solute-solvent interactions and the various structural factors responsible for solubility. These studies are of importance in view of the 'zwitter-ionic' nature of the amino acids and their importance in living systems.

These considerations led Lahiri and coworkers¹⁻³ to determine the dissociation constants of amino acids in different mixed solvents. We present in this paper the dissociation constants of some important amino acids in *t*-butanol + water mixtures. We have also determined the solubilities of amino acids in *t*-butanol + water mixtures. The combination of these results has helped us to investigate the effect of solvents on the dissociation constants of the amino acids and the specific solute-solvent interactions.

Materials and Methods

All the amino acids (glycine, α -alanine, β -alanine, L-valine, L-glutamine, L-serine, L-leucine, L-threonine, L-phenylalanine, L-methionine, L-histidine, L-asparagine), except those which were E. Merck's G R Grade reagents, were purified by crystallization from ethanol + water mixtures. The amino acids were analysed titrimetrically in the usual way using acid-free formaldehyde solution. *t*-Butanol (S.D.'s lab. reagent)

was heated with lime, kept overnight and distilled. The distilled alcohol was then frozen below 295 K and the supernatant liquid was rejected. The crystallised alcohol was melted and was finally distilled. The middle-fraction was utilized for preparation of solutions. Perchloric acid and caustic soda (G.R.E. Merck) were standardized in the usual way. Other chemicals were of guaranteed quality. All solutions were made in doubly distilled water. Methylcellosolve (E. Merck, A.G.) was distilled and the middle fraction was utilised. The absence of H_2O_2 in the solvent was properly tested.

The reagent solution (for the calorimetric estimation of amino acids) was prepared by dissolving 1.6 g of ninhydrin (G.R.E. Merck) and 0.24 g of hydrindantin (Puriss, AR) in 60 ml of distilled methylcellosolve and 20 ml of freshly prepared 4 M (sodium acetate + acetic acid) buffer (pH 5.5) was added. The solution was stored in a brown bottle.

The estimation of amino acids was made in the way suggested by Moore and Stein⁴. The reagent solution (2 ml) was added to amino acid (2 ml) of known concentration (concentration range 0.01 mM-0.1 M) taken in capped tubes. The mixture was then heated on a boiling water-bath for 15 min. The resultant solution was cooled to room temperature and 3 ml of 50% ethanol was then added. The optical density of the solution was noted against the appropriate blank solution at 570 nm after 10 min. The calibration curves were thus obtained by plotting optical density against concentration (mM).

For the determination of the solubility of the amino acid, the saturated solution of amino acid was prepared at a higher temperature (about 3°) than the experimental temperature. The clear solution, taken in

Table 1—Solubilities (mol dm⁻³) of Amino Acids in *t*-Butanol-Water Mixtures at 298 K

Wt% of <i>t</i> -BuOH	Glycine	α -Alanine	L-Valine	L-Leucine	L-Histidine	L-Serine	L-phenyl-alanine	L-Asparagine
0	3.325 (3.333)	1.87 (1.868)	0.755 (0.755)	0.186 (0.185)	0.2715 (0.27)	0.47 (0.478)	0.180 (0.179)	0.22625 (0.233 at 301 K)
8.0	2.625	1.45	0.640	0.158	0.2355	0.40	0.155	0.156
16.4	2.35	1.25	0.575	0.136	0.1500	0.156	0.0745	0.070
25.0	1.0125	0.81	0.400	0.112	0.1300	0.133	0.0630	0.032
34.2	0.8875	0.365	0.180	0.053	0.1030	0.060	0.0260	0.0119
43.8	0.65	0.335	0.175	0.051	0.0405	0.050	0.0215	0.00525
54.0	0.105	0.150	0.1575	0.047	0.0150	0.022	0.0170	0.00257
64.5	0.0575	0.130	0.0245	0.020	0.0125	0.01875	0.0056	0.0013
75.8	0.0425	0.017	0.0135	0.0145	0.0014	0.00335	0.0044	0.0008
87.5	0.004	0.0085	0.0003125	0.0048	0.00085	0.00080	0.0032	0.00045
100.0	0.000013	0.0025	0.0002	0.0042	0.00045	0.000085	0.00085	0.00035

a Campbell solubility apparatus⁵, was allowed to equilibrate in a thermostat maintained at $298 \pm (0.01)$ K. After equilibration, the apparatus was inverted in the thermostat and a definite amount of the filtered solution was taken out from the apparatus kept in the thermostat. The solution was diluted appropriately with water and the amino acid was estimated from the optical density readings obtained in the way described earlier. Generally, triplicate readings were taken. The process was repeated for different amino acids in water and mixed solvents. The results are given in Table 1.

For the determination of the acid dissociation constants 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 *t*-butanol + water mixtures.

For the determination of the second dissociation constants of amino acids, the process was the same, only HClO₄ was replaced by caustic soda.

The *pH*-meter readings were taken at 298 K using a Systronics digital *pH*-meter with combined glass and calomel electrodes maintained at 298 K. The instrument was first standardized with potassium hydrogen phthalate and then with 10^{-4} mol dm⁻³ HClO₄ solution. The glass electrode was then kept immersed in the appropriate solvent-medium for equilibration before the measurements were carried out. The colorimetric estimations were made with a Spekol instrument (Carl Zeiss, Germany).

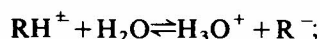
Results

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

$$\text{RH}_2^+ + \text{H}_2\text{O} \rightleftharpoons \text{H}_3\text{O}^+ + \text{RH}^+; \quad \text{and}$$

$$K_1 = \frac{C_{\text{H}^+} \times C_{\text{RH}^+}}{C_{\text{RH}_2^+}} \times \frac{f_{\text{H}^+} \times f_{\text{RH}^+}}{f_{\text{RH}_2^+}} \quad \dots(1)$$

and



$$K_2 = \frac{C_{\text{H}^+} \times C_{\text{R}^-}}{C_{\text{RH}^+}} \times \frac{f_{\text{H}^+} \times f_{\text{R}^-}}{f_{\text{RH}^+}} \quad \dots(2)$$

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 has been scrupulously avoided for reasons given earlier^{3,6,7}. It is also known that the activity coefficients in mixed solvents change due to (i) salt effect (f_i) and (ii) medium effect (m_i)⁸.

The solute-solvent interactions of unknown magnitudes arising from the use of neutral electrolytes are likely to mask the 'medium effects' of the reactions. Thus, we worked in dilute solutions, so that $f_i \rightarrow 1$, and 'medium effects' could be properly ascertained.

The Eqs (1) and (2) can be written as,

$$\begin{aligned} pK_1 &= \text{pH} - \log \frac{C_{\text{RH}^+}}{C_{\text{RH}_2^+}} - \log \frac{f_{\text{RH}^+}}{f_{\text{RH}_2^+}} \\ &= \text{pH} - \log \left(\frac{c}{a - C_{\text{H}^+}} - 1 \right) - \log \frac{f_{\text{RH}^+}}{f_{\text{RH}_2^+}} \quad \dots(3a) \end{aligned}$$

$$\begin{aligned} &= B + \log [U_{\text{H}}] - \log \left(\frac{c}{a - C_{\text{H}^+}} - 1 \right) - \log \frac{f_{\text{H}^+} \times f_{\text{RH}^+}}{f_{\text{RH}_2^+}} \quad \dots(3b) \end{aligned}$$

and

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

$$= B' + \log U_{\text{H}} + \log \left(\frac{c}{b - c_{\text{OH}^-}} - 1 \right) - \log \frac{f_{\text{H}^+} \times f_{\text{R}^-}}{f_{\text{RH}^+}} \quad \dots(4b)$$

Here c = concentration of neutral amino acid, a or b = concentration of acid or base respectively, c_{H^+} or c_{OH^-} = concentration of H^+ ion or OH^- ion in the experimental solution (determined pH-metrically) and B or B' are the appropriate pH-meter readings in acid or alkali.

Appropriate corrections have been made for the measurements of H^+ ions in mixed solvents in the way described earlier⁸⁻¹².

The values of autoprotolysis constants of H_2O in t -butanol + H_2O were taken into consideration¹³ in calculating C_{OH} in mixed solvents. The autoprotolysis constants were determined using glass-Ag/AgCl(3) electrodes described by Wolley *et al.*¹³

Moreover the values of the autoprotolysis constants are limited to 44 wt% of t -butanol. This makes the accurate determination of C_{OH} at higher percentages of t -butanol difficult. Thus, the error in the determination of pK_2 may be considerably greater.

Equations (3a) and (4a)¹⁴ have been used for pK -determination in water where C_{H^+} values have been determined from pH values and the calculated values of the activity coefficients of H^+ ions. But in mixed solvents, $[H^+]$ ion concentrations have been determined rather than the activity of H^+ ions, and therefore Eqs (3b) and (4b) have been used for the calculation of pK_1 and pK_2 .

The highest concentrations of amino acids and $HClO_4$ or $NaOH$ used are $1.0 \times 10^{-2} M$ and $3.0-7.0 \times 10^{-3} M$. The approximation that the activity coefficients of amino acids are unity in the concentration ranges studied may involve slight error but it is unavoidable in view of lack of data on activity coefficients of the amino acids in mixed solvents. In the case of pK_1 , the effect would be only marginal as f_{H^+} and $f_{RH_2^+}$ cancel each other. But the differences would be considerable in the case of pK_2 . f_{H^+} and f_R can be calculated with the help of the equation,

$$-\log f_i = Az_i^2 \sqrt{\mu}$$

using the appropriate values of A in mixed solvents. The values of pK_2 obtained taking the activity coefficients into consideration are given in brackets (Table 2). However, no correction has been made for the activity coefficients of the amino acids using the relation¹⁴,

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

where ϵ is the dielectric constant of the medium of ionic strength 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.

The free energy of transfer of amino acids from water to mixed solvents has been calculated using the relation,

$$\Delta G_t^\circ(\text{amino acid}) = -2.303 RT \log \frac{c_s}{c_w} \quad \dots(6)$$

where c_s and c_w represent the molar concentrations of amino acids in the mixed solvents and water respectively. The activity coefficients of the amino acids in water as well as in mixed solvents can be taken to be unity since the solutes are in their saturated (standard) states in the respective solvents.

The free energies of transfer for the first and second dissociations of amino acids have been calculated in the usual way (Eqs 7, 8).

$$\Delta G_t^\circ(1) = 2.303 RT [pK_s(1) - pK_w(1)] \quad \dots(7)$$

and

$$\Delta G_t^\circ(2) = 2.303 RT [pK_s(2) - pK_w(2)] \quad \dots(8)$$

Discussion

The pK_1 and pK_2 values in t -butanol + water mixtures at 298 K are presented in Table 2. The literature values (starred) of pK_1 and pK_2 are taken from Greenstein and Winitz⁶. It is to be noted that most of the values of the dissociation constants of amino acids in water are from measurements using cells with liquid junction potential though a few determinations using cells without liquid junctions have also been made. It is well known that the method involves approximations regarding the activity coefficients of ions and some other approximations. The extrapolations are usually non-linear. The pK_1 values determined using cells with liquid junction and without liquid junction are almost the same, though the differences in pK_2 values are considerable.

The pK_1 values of the amino acids in water determined by us agree well with the values given in the literature though slight discrepancies exist in some cases. These have been reported in our previous communication³.

The pK_2 values determined by us are generally within ± 0.01 to $\pm 0.05 pK$ 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 Blanchard¹⁵ also noted that the measurements made in alkaline solutions gave unstable e.m.f. readings. Comparison of pK_1 and pK_2 values in mixed solvents is not possible due to lack of data. The usual method of determination of pK_1 and pK_2 using the hydrogen electrode is laborious. In addition to the usual assumptions, the data on accurate autoprotolysis constants and activity

Table 2 - Dissociation Constants of Amino Acids in *t*-Butanol-Water Mixtures at 298 K

Wt % of <i>t</i> -BuOH	$\frac{1}{\epsilon} \times 10^2$	α -Alanine		β -Alanine		L-Valine		L-Glutamine		L-Serine		L-Threonine		L-Phenylalanine		L-Methionine	
		pK_1	pK_2	pK_1	pK_2	pK_1	pK_2	pK_1	pK_2	pK_1	pK_2	pK_1	pK_2	pK_1	pK_2	pK_1	pK_2
0	1.27	2.36	9.64	3.59	10.18	2.32	9.71	2.16	9.08	2.22	8.97	2.21	9.00	2.27	9.23	2.29	9.20
		*2.34	*9.69	*3.55	*10.24	*2.32	*9.62	*2.17	*9.13	*2.21	*9.15	*2.15	*9.12	*1.83	*9.13	*2.28	*9.21
		(9.71)	(9.71)	(10.25)	(10.25)	(9.78)	(9.78)	(9.15)	(9.15)	(9.04)	(9.04)	(9.07)	(9.07)	(9.30)	(9.30)	(9.27)	(9.27)
8.0	1.43	2.48	9.84	3.68	10.23	2.53	9.77	2.28	9.11	2.40	9.16	2.26	9.16	2.39	9.23	2.43	9.18
		(9.92)	(9.92)	(10.31)	(10.31)	(9.85)	(9.85)	(9.19)	(9.19)	(9.24)	(9.24)	(9.24)	(9.24)	(9.31)	(9.31)	(9.26)	(9.26)
16.4	1.58	2.60	9.86	3.79	10.20	2.55	9.71	2.40	9.10	2.52	9.20	2.40	9.21	2.53	9.31	2.57	9.17
		(10.30)	(10.30)	(10.30)	(10.30)	(9.81)	(9.81)	(9.20)	(9.20)	(9.30)	(9.30)	(9.31)	(9.31)	(9.41)	(9.41)	(9.27)	(9.27)
25.0	1.80	2.66	9.88	3.83	10.22	2.64	9.73	2.44	9.13	2.57	9.22	2.44	9.27	2.61	9.40	2.64	9.16
		(10.34)	(10.34)	(10.34)	(10.34)	(9.85)	(9.85)	(9.25)	(9.25)	(9.34)	(9.34)	(9.39)	(9.39)	(9.52)	(9.52)	(9.28)	(9.28)
34.2	2.08	2.73	9.91	3.88	10.24	2.73	9.74	2.52	9.16	2.61	9.24	2.51	9.31	2.73	9.51	2.73	9.19
		(10.38)	(10.38)	(10.38)	(10.38)	(9.88)	(9.88)	(9.30)	(9.30)	(9.38)	(9.38)	(9.45)	(9.45)	(9.65)	(9.65)	(9.33)	(9.33)
43.8	2.50	2.78	9.96	3.95	10.28	3.01	9.77	2.56	9.21	2.63	9.28	2.57	9.34	2.77	9.62	2.77	9.26
		(10.48)	(10.48)	(10.48)	(10.48)	(9.97)	(9.97)	(9.41)	(9.41)	(9.48)	(9.48)	(9.54)	(9.54)	(9.82)	(9.82)	(9.46)	(9.46)
54.0	3.18	2.92	10.04	4.07	10.31	3.16	9.81	2.65	9.28	2.73	9.32	2.62	9.40	2.89	9.70	2.89	9.34
		(10.49)	(10.49)	(10.49)	(10.49)	(10.09)	(10.09)	(9.56)	(9.56)	(9.60)	(9.60)	(9.68)	(9.68)	(9.98)	(9.98)	(9.62)	(9.62)
64.5	4.44	3.07	10.12	4.21	10.36	3.32	9.90	2.78	9.40	2.87	9.38	2.79	9.46	3.03	9.71	3.02	9.44
		(10.83)	(10.83)	(10.83)	(10.83)	(10.37)	(10.37)	(9.87)	(9.87)	(9.85)	(9.85)	(9.93)	(9.93)	(10.18)	(10.18)	(9.91)	(9.91)
75.8	5.63	3.39	10.18	4.56	10.40	3.65	9.94	3.00	9.48	3.18	9.38	3.08	9.47	3.31	9.75	3.39	9.53
		(11.06)	(11.06)	(11.06)	(11.06)	(10.60)	(10.60)	(10.14)	(10.14)	(10.04)	(10.04)	(10.13)	(10.13)	(10.41)	(10.41)	(10.19)	(10.19)

Standard deviations $\pm(0.01-0.02)$ for pK_1 upto 43.8 wt% and $\pm(0.02-0.03)$ above 43.8 wt%; Standard deviations $\pm(0.02-0.03)$ for pK_2 upto 34.2 wt% and $\pm(0.03-0.05)$ above 34.2 wt%.

coefficients are needed to evaluate pK_1 and pK_2 in the different percentages of mixed solvents. However, the method is not without defect. The proponents of the hydrogen electrode method overlook some salient features: (a) E° of hydrogen electrode $[H^+ + e \rightarrow \frac{1}{2}H_2(g)]$ is assumed to be zero in all solvents i.e. the free energy of transfer of H^+ ion is zero irrespective of solvent. This is untenable particularly in view of the differences in ion-solvent interactions due to differences in acid-base and other fundamental properties of the solvents.

(b) The method, as pointed out by Popovych¹⁶, generates quite a large number of thermodynamically unrelated pH or e.m.f. scales and the comparison of results having different thermodynamic standard states involves uncertainties. There is actually no method which can give unambiguous values of pK_1 and pK_2 in mixed solvents without any approximation. However, it is desirable to determine the thermodynamic pK_1 and pK_2 values of the amino acids using different techniques to have consistent set of values as far as practicable.

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 in the order: methanol + water mixtures > ethanol + water mixtures > isopropanol + water mixtures > *t*-butanol + water mixtures, though the decrease in dielectric constants is in the opposite direction. This may be due to decrease in solubilities and ionisations of amino acids as the hydrophobic character of the solvent mixtures decreases.

As expected, pK_2 -values are rather insensitive to solvent changes, though a slight increase in the pK -values is observed with the increase in [*t*-butanol] contrary to the decrease in the pK values in the case of isoelectric reactions.

The pK_1 -values are found to be a linear function of $\frac{1}{\epsilon}$ and mole-fraction of *t*-butanol at low percentages of alcohol. But the interactions of amino acids having two charged sites are found to be different for different amino acids.

Moreover, the ratio of 'zwitterion' to neutral acid may well change in mixed solvents leading to the change in interactions. The ratio of zwitterion to neutral acids has been calculated using the pK_1 and pK_2 values in the present determination and pK_E values of ethyl esters of amino acids like glycine, *L*-leucine hydrochlorides in mixed solvents determined earlier in our laboratory¹⁷ (K_E = dissociation constant of hydrochlorides of amino acid esters).

Table 3—Dissociation Constants of Amino Acids in *t*-Butanol-Water Mixtures at 298 K

Wt% of <i>t</i> -BuOH	$\frac{1}{\epsilon} \times 10^2$	Glycine				Leucine			
		pK_1	pK_2	pK_E	$\log K_z$	pK_1	pK_2	pK_E	$\log K_z$
0	1.27	2.33 *2.34	9.59 *9.60 (9.66)	7.70 *7.73	5.37	2.44 *2.36	9.59 *9.60 (9.66)	7.62 *7.63	5.18
8.0	1.43	2.40	9.55 (9.63)	7.83	5.43	2.53	9.62 (9.70)	7.93	5.40
16.4	1.58	2.50	9.58 (9.68)	8.05	5.55	2.69	9.64 (9.74)	8.15	5.46
25.0	1.80	2.62	9.60 (9.72)	8.15	5.53	2.80	9.62 (9.74)	8.10	5.30
34.2	2.08	2.71	9.61 (9.75)	8.15	5.44	2.91	9.65 (9.79)	8.25	5.34
43.8	2.50	2.73	9.63 (9.83)	7.95	5.22	2.99	9.71 (9.91)	8.15	5.16
54.0	3.18	2.84	9.68 (9.96)	8.05	5.21	3.13	9.75 (10.03)	8.20	5.07
64.5	4.44	2.97	9.78 (10.25)	8.00	5.03	3.28	9.79 (10.26)	8.10	4.82
75.8	5.63	3.27	9.81 (10.47)	7.95	4.68	3.74	9.82 (10.48)	8.15	4.41

†Standard deviations $\pm(0.01-0.02)$ for pK_1 upto 43.8 wt%, and $\pm(0.02-0.03)$ above 43.8 wt%

Standard deviations $\pm(0.02-0.03)$ for pK_2 upto 34.2 wt%, and $\pm(0.03-0.05)$ above 34.2 wt%

Standard deviations $\pm(0.01-0.02)$ for pK_1 upto 54.0 wt%, and $\pm(0.02-0.03)$ above 54.0 wt%

The K_z values in different percentages of mixed solvents have been calculated using the procedure described by Ebert¹⁸, Edsall and Blanchard¹⁵,

$$K_z = \frac{[N^+H_3RCO_2^-]}{[NH_2R.COOH]} \quad \dots(9)$$

The results are given in Table 3. It is clear that the ratio of 'zwitterion' to neutral molecules decreases slightly with increasing percentages of *t*-butanol.

The solubilities of the amino acids in water are high. But the solubility is found to decrease with the introduction of hydrophobic groups in glycine which make $\Delta G^\circ(RH^+)$ ($= -2.303 RT \log C_s$ in saturated solutions) progressively positive. The free energy of transfer for the dissolution of amino acids from water to mixed solvents has been found to be positive i.e. transfer process is increasingly unfavourable. The results suggest that the amino acid is present predominantly in 'zwitterionic' form even in mixed or non-aqueous solvents though a slight conversion to neutral form may take place. The changes in the solubilities of amino acids in different solvents obviously arise from the presence of different hydrophobic or hydrophilic groups in the amino acids.

The free energy changes (of dissociation) of amino acids, as manifested in $\Delta G_i^\circ(1)$ and $\Delta G_i^\circ(2)$, have been found to be predominantly positive or unfavourable in going from water to mixed solvents. The changes may arise due to the change from 'zwitterion' to neutral form but the contribution due to this factor should be almost negligible. The main contributory factors are the structural changes, the structure formation of water molecules due to the addition of alcohol with bulky *t*-butyl group on one hand and hydrophobic interactions of amino acids and alcohols on the other hand. In order to get better insight into the nature of solute-solvent interactions, it is desirable to analyse the results in terms of single ion values. For the reaction (1), we have

$$\Delta G_i^\circ(1) = \Delta G_i^\circ(H^+) + \Delta G_i^\circ(RH^+) - \Delta G_i^\circ(RH_2^+) \quad \dots(10)$$

or

$$-\Delta G_i^\circ(RH_2^+) = \Delta G_i^\circ(1) - \Delta G_i^\circ(H^+) - \Delta G_i^\circ(RH^+) \quad \dots(11)$$

Similarly for reaction (2), we have,

$$\Delta G_i^\circ(R^-) = \Delta G_i^\circ(2) - \Delta G_i^\circ(H^+) + \Delta G_i^\circ(RH^+) \quad \dots(12)$$

The values of $\Delta G_i^\circ(RH_2^+)$ and $\Delta G_i^\circ(R^-)$ have been calculated using the $\Delta G_i^\circ(1)$, $\Delta G_i^\circ(2)$ and $\Delta G_i^\circ(RH^+)$ values from the present investigation and $\Delta G_i^\circ(H^+)$ determined earlier¹⁹. The values of $\Delta G_i^\circ(RH_2^+)$ and $\Delta G_i^\circ(R^-)$ in water have also been calculated from the Eqs (11) and (12) omitting the subscript *i* and using the solubility values determined by us and $\Delta G^\circ(H^+)$ value in water ($-1087.4 \text{ kJ mol}^{-1}$)²⁰. The values are

recorded in Tables 4 and 5.

Comparison of the values of free energy of transfer of ions is not possible as most of the $\Delta G_i^\circ(\text{ion})$ values (mostly of inorganic ions) are limited to low organic solvent region and the values are based on different extra-thermodynamic assumptions with their inherent limitations. Moreover, it is well known that the structure of water is modified not only by *t*-butanol but also by 'zwitterionic' amino acids with different hydrophilic and hydrophobic groups. It is to be noted that ΔG^E for *t*-butanol + water mixtures is positive and a nearly symmetrical function of X_2 . ΔS^E is negative and is minimum at about $X_2 = 0.25$. Plot of ΔH^M vs X_2 shows a maximum (mixing endothermic) at about $X_2 \sim 0.8$ and a sharp minimum (mixing exothermic) at about $X_2 \sim 0.08$ (ref. 21); $-\Delta G_i^\circ(H^+)$ ^{19,22} and ultrasonic absorption^{23,24} also show maxima in the region 16-25 wt% of *t*-butanol. These are naturally reflected in the $\Delta G_i^\circ(\text{ion})$ values. Thus, it has been observed that $\Delta G_i^\circ(RH_2^+)$ values are predominantly negative, show a hump near 16-25 wt% of *t*-butanol and pass through a minimum around 44 wt% of *t*-butanol. The $\Delta G_i^\circ(RH_2^+)$ values become positive around 60 wt%. The $\Delta G_i^\circ(R^-)$ values are predominantly positive, become maximum around 64 wt% and then decrease. The results are consistent with the observations that the free energies of transfer of cations and anions from water to aquo organic solvents are usually negative and positive respectively^{22,25,26}. We could not report the dissociation constant values at higher percentages where errors are high (more than ± 0.05), but from the trend it is apparent that the reverse behaviour may be expected at higher percentages. It is well known that the structural features are not clearly manifested in the free energy of transfer values whereas enthalpy or entropy of transfer values may be more conclusive²⁵. Furthermore, it is difficult to predict the error range of $\Delta G_i^\circ(RH_2^+)$ and $\Delta G_i^\circ(R^-)$ where errors are compounded from $\Delta G_i^\circ(RH^+)$, $\Delta G_i^\circ(1)$ or $\Delta G_i^\circ(2)$ and particularly from $\Delta G_i^\circ(H^+)$. Considering the limitations, the results can be regarded as fairly consistent. It is apparent that the transfer of cations is favourable though the negative maximum depends on the nature of the amino acid i.e. different hydrophobic groups and their interactions with the solvent mixtures. The free energies of transfer of anions are increasingly unfavourable with the increase in [organic solvent]. From these results, it can be inferred that the amino acids are probably present in solution as an equilibrium mixture of zwitterions and ions (cations and anions). The addition of *t*-butanol to water increases the basicity of the solvent mixtures. This results in the conversion of zwitter ions into anions according to the following scheme (Scheme 1),

Table 4—Free Energies of Transfer ($\Delta G_i^0(\text{RH}^+)$) of Amino Acids in kJ/mol at 298 K

Wt% of <i>t</i> -BuOH	Glycine	α -Alanine	L-Valine	L-Leucine	L-Histidine	L-Serine	L-Phenylalanine	L-Asparagine
8.0	0.59	0.63	0.40	0.40	0.35	0.40	0.37	0.92
16.4	0.86	1.00	0.67	0.77	1.47	2.73	2.18	2.91
25.0	2.67	2.07	1.57	1.25	1.82	3.13	2.60	4.85
34.2	3.27	4.05	3.55	3.11	2.40	5.10	4.79	7.30
43.8	4.05	4.26	3.62	3.20	4.71	5.55	5.26	9.32
54.0	8.56	6.25	3.88	3.41	7.18	7.59	5.85	11.09
64.5	10.06	6.60	8.49	5.52	7.63	7.98	8.60	12.79
75.8	10.80	11.65	9.97	6.32	13.05	12.25	9.20	13.99
87.5	16.67	13.36	19.30	9.06	14.29	15.80	9.98	15.41
100.00	30.86	16.40	20.40	9.39	15.86	21.35	13.27	16.04

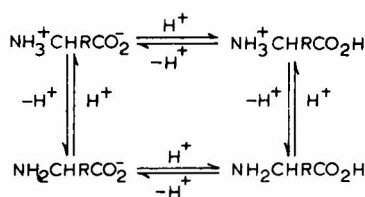
[$-\Delta G_i^0(\text{s})$ in water: Glycine = 2.98 kJ/mol L-Histidine = -3.23 kJ/mol
 L-Alanine = 1.55 kJ/mol L-Serine = -1.87 kJ/mol
 L-Valine = -0.70 kJ/mol L-Phenylalanine = -4.25 kJ/mol
 L-Leucine = -4.17 kJ/mol L-Asparagine = -3.68 kJ/mol]

Table 5—Free Energy of Transfer for Amino Acids in *t*-Butanol-Water Mixtures at 298 K

Wt% of <i>t</i> -BuOH	$-\Delta G_i^0(\text{RH}_2^+)$ in kJ/mol						$\Delta G_i^0(\text{R}^-)$ in kJ/mol					
	Glycine	α -Alanine	L-Valine	L-Leucine	L-Serine	L-Asparagine	Glycine	α -Alanine	L-Valine	L-Leucine	L-Serine	L-Asparagine
8.0	2.38	2.59	3.48	3.20	3.25	3.01	2.99	4.40	3.37	3.20	4.11	3.00
16.4	3.70	3.02	4.35	4.77	2.63	3.01	4.48	5.91	4.32	4.70	7.69	6.29
25.0	2.59	3.31	4.97	5.93	3.52	4.06	7.38	8.10	6.34	6.08	9.21	8.23
34.2	3.68	2.91	3.69	4.87	1.96	2.74	8.22	10.43	8.56	8.29	11.48	11.23
43.8	3.57	3.53	5.76	5.79	2.18	3.04	9.66	11.48	9.35	9.27	12.71	12.88
54.0	-0.81	1.85	5.86	5.89	0.22	2.65	13.97	13.43	9.35	9.22	14.49	14.43
64.5	-2.91	1.01	0.82	3.28	-0.73	-0.65	14.69	12.89	13.12	10.21	13.87	14.89
75.8	-2.94	-3.22	0.23	2.97	-4.28	-0.65	14.60	17.28	13.83	10.18	17.14	14.72

$\Delta G_i^0(\text{RH}_2^+)$ in water: Glycine = -1103.7 kJ/mol
 α -Alanine = -1102.4 kJ/mol
 L-Valine = -1099.6 kJ/mol
 L-Leucine = -1096.8 kJ/mol
 L-Serine = -1098.2 kJ/mol
 L-Asparagine = -1096.7 kJ/mol

$\Delta G_i^0(\text{R}^-)$ in water: Glycine = 1139.1 kJ/mol
 α -Alanine = 1140.9 kJ/mol
 L-Valine = 1143.5 kJ/mol
 L-Leucine = 1146.3 kJ/mol
 L-Serine = 1138.9 kJ/mol
 L-Asparagine = 1140.7 kJ/mol



Scheme 1

It is apparent that the formation of cations or conversion of zwitterions to neutral form is not favoured in mixed solvents but the formation of anions is favoured; the reverse behaviour may be expected at higher percentages where the basicity decrease (as is apparent from $-\Delta G_i^0(\text{H}^+)$ values) results in the conversion of anions to zwitterions and cations. The result indicates that specific solute-solvent interactions are of great importance, but it is inopportune to derive more inferences regarding the structure of the solvent

mixtures from the $\Delta G_i^0(\text{RH}_2^+)$ and $\Delta G_i^0(\text{R}^-)$ values without other experimental evidences.

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References

- 1 Chattopadhyay A K & Lahiri S C, *Indian J Chem*, **15A** (1977) 930.
- 2 Chakravorty S K & Lahiri S C, (unpublished data).
- 3 Dey B P, Dutta S & Lahiri S C, *Indian J Chem*, **21A** (1982) 886.
- 4 Moore S & Stein W H, *J Biol Chem*, **176** (1948) 367; 211 (1954) 907.
- 5 Campbell A N, *J chem Soc*, (1930) 179.
- 6 Greenstein J P & Winitz M, *Chemistry of the amino acids*, Vol. I (John Wiley, New York, London), 1961, Chapter 2.
- 7 Bhattacharyya A & Lahiri S C, *J Indian chem Soc*, **57** (1980) 971.
- 8 Bates R G, *Determination of pH* (Wiley-Interscience, New York, London) 1973, Chapter 8.

- 9 Van Uitert L G & Haas C G, *J Am chem Soc*, **75** (1953) 451.
- 10 Gentile P S, Cefola M & Celiano A V, *J phys Chem*, **67** (1963) 1083.
- 11 Bhattacharyya U G & Lahiri S C, *Z phys Chem, (N.F.)*, **50** (1966) 451.
- 12 Lahiri S C & Aditya S, *J Indian chem Soc*, **51** (1974) 319.
- 13 Wooley E M, Hurkot D G & Hepler L G, *J phys Chem*, **70** (1966) 3908.
- 14 Glasstone S, *An introduction to electrochemistry* (D. Van Nostrand Company, New York) 1942, 423-24, 432.
- 15 Edsall J J & Blanchard M H, *J Am chem Soc*, **55** (1933) 2337.
- 16 Popovych O, *Analyt Chem*, **46** (1974) 2009.
- 17 Dey B P & Lahiri S C (unpublished data).
- 18 Ebert L, *Z phys Chem, (Leipzig)* **121** (1926) 385.
- 19 Chakravorty S K, Sengupta D & Lahiri S C, *Z phys Chem (Leipzig)* (in press).
- 20 Criss C M & Salomon M, *Physical chemistry of organic solvent systems*, edited by A K Covington & T Dickinson (Plenum, London and New York) 1973, 323-326.
- 21 Franks F & Ives D J G, *Quart Rev (London)*, **20** (1966) 1.
- 22 Wells C F, *J chem Soc, Faraday I*, **72** (1976) 601.
- 23 Blandamer M J, Clarke D E, Hidden N J & Symons M C R, *Trans Faraday Soc*, **64** (1968) 2691.
- 24 Pal A, Ph.D. Thesis, University of Kalyani (1983).
- 25 Wells C F, *J chem Soc, Faraday I*, **69** (1973) 984.
- 26 Feakins D, in *Physico-chemical process and mixed aqueous solvents*, edited by F Franks (Heinemann Educational Books, London) 1967, 71-90.