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Protonation equilibria studies of the standard α-amino acids in $NaNO_3$ solutions in water and in mixtures of water and dioxane

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Abstract

The protonation equilibria for 20 standard α-amino acids in solutions have been studied pH-potentiometrically. The dissociation constants (pK_a) of 20 amino acids and the thermodynamic functions (ΔG°, ΔH°, ΔS°, and δ) for the successive and overall protonation processes of amino acids have been derived at different temperatures in water and in three different mixtures of water and dioxane (mole fractions of dioxane were 0.083, 0.174, and 0.33). Titrations were also carried out in water ionic strengths of (0.15, 0.20, and 0.25) $mol \cdot dm^{-3}$ $NaNO_3$, and the resulting dissociation constants are reported. A detailed thermodynamic analysis of the effects of organic solvent (dioxane), temperature and ionic strength influencing the protonation processes of amino acids is presented and discussed to determine the factors which control these processes.
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Keywords: Protonation constants; Amino acids; Potentiometric studies

1. Introduction

The standard α-amino acids have special importance among the other chemical groups since they are found in all naturally occurring proteins, which play a vital role in nearly all chemical and biological processes. Despite their recognized importance, there are only a few experimental contributions on their acid–base behaviour in different environments. A search of the literature showed that the studies on the thermodynamic protonation constants of amino acids using a variety of experimental and theoretical tools have been few [1–12]. No work seems to have been done on the determination of the dissociation constants of glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, tyrosine, tryptophan, cysteine, methionine, serine, threonine, aspartate, glutamate, asparagine, glutamine, lysine, arginine, and histidine in different $NaNO_3$ solutions and in various (water + dioxane) mixtures at different temperatures.

The effect of solvents on proteins and model compounds is useful for considering how protein specific structures are stabilized in an aqueous environment. 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 dissociation and solvation processes in solutions of amino acids is important to elucidate the connection of between chemical ability and biological activity. As the polarity and the activity of water are expected to be lower in an active site cavity of an enzyme than in bulk water, the protonation processes of the studied amino acids in this investigation were examined in water containing organic dioxane solvent, from which the thermodynamic data obtained would be useful to research workers in biomedicine. Thus, in light of the above picture of the aqueous solutions of solvents, it is worthwhile to study systematically the amino acids, peptides and proteins in solvents having a different number of hydroxyl groups. These studies may shed some light

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on the mechanism about how the organic solvents affect the stability of proteins.

The stability of the native structure of proteins in aqueous solutions can be ascribed to the weak non-bonding interactions between the groups of amino acids as well as amino acids and the other components in solutions. The majority of proteins exist in aqueous mixed solvents containing many organic substances. Studies on various thermodynamic properties of amino acids and simple peptides in aqueous solutions of organic substances are of current interest due to their importance in the better understanding of the nature and mechanisms taking place in living cells. The simple heterocyclic compounds in pesticides are very important to the living organisms and the environment. The 1,4-dioxane is a type of heterocyclic compound containing an oxygen atom.

Knowledge of thermodynamic properties of amino acids is of great interest for the decoding of the mechanism of bidentate ligand dissociation and for revealing the influence of the nature of the solvent and the hydrophobicity of the alkyl radical of the acid on the energetic of processes involving amino acids. On the other hand, amino acids represent non-cyclic biologically active ligands in processes of complex formation. For the calculations of stability constants of the complex formation of amino acids with metal ions, the dissociation constants of amino acids are used. It is known that the reactions of peptides, proteins, and enzymes with metal ions are of biochemical importance but they are yet to be fully elucidated. The explanation of these phenomena in the biological systems is possible only by determining the protonation constants of the amino acids as well as their stability constants, which are the measure of their tendency to make complexes with other metal ions. The elucidation of the various phenomena in the biological systems requires the determination of the protonation constants of the amino acids and their stability constants with various metal ions in a medium similar to those of biological systems.

Generally, the dissociation constants of acids can be estimated by analysis of acid–base titrations. The methods have been critically reviewed [13–15]. Besides random errors, the systematic errors arise in instrumental measurements and the dissociation constants are obtained with limited precision and accuracy. Systematic errors are caused by limitations of: (i) the apparatus and experimental technique, and (ii) the procedure of data treatment. Both limitations introduce bias into the dissociation constants. Besides ESAB [16,17] which is one powerful programme because it permits refinement of group parameters, another programme PKPOT [18] will be used.

The purpose of this investigation is to determine the dissociation constants of the above amino acids (glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, tyrosine, tryptophan, cysteine, methionine, serine, threonine, aspartate, glutamate, asparagine, glutamine, lysine, arginine, and histidine), and study their equilibria at various temperatures in water and in different aqueous dioxane media at different ionic strengths.

2. Experimental

2.1. Materials and solutions

The standard α-amino acids were commercially available chemicals (ICN Biochemical (USA) and Aldrich, Sigma), and used without further purifications.

The B.D.H. "AnalaR" *p*-dioxane was purified by the procedure of Weissberger and Proskauer [19]. It was refluxed over pellets of KOH for about (8 to 10) h, distilled and the middle fractions of the distillate refluxed over metallic sodium for (5 to 6) h, and distilled. The middle fraction was used. Its purity was established by determining the freezing point which varied from $T = (184.75$ to $184.95)$ K (uncorrected) against the reported range of $T = (184.80$ to $185.15)$ K [20,21].

Carbonate free sodium hydroxide pellets (titrant, prepared in $0.10\ \mathrm{mol \cdot dm^{-3}}$ $NaNO_3$ solution) was standardized potentiometrically with KH-phthalate solution (Merck AG). Nitric acid, sodium hydroxide and sodium nitrate were from Merck P. A. Deionized water was used throughout the experiments.

2.2. Apparatus

The pH-potentiometric titrations were performed using a Metrohm 796 titroprocessor with a 685 dosimate, a 728 magnetic stirrer, coupled with a dosino buret model 700. The pH-titrations were carried out in an $80\ \mathrm{cm^3}$ commercial double-walled glass vessel. The ionic strength of the solutions is maintained at constant level by using the desired concentration of $NaNO_3$ solution as supporting electrolyte, and the temperature was adjusted inside the cell at the desired value by circulating thermostatted water using an oil-thermostatted set-up. During the course of titrations, a stream of oxygen-free nitrogen was passed through the reaction cell to eliminate the adverse effect of the atmospheric carbon dioxide.

2.3. Calibration of glass electrode cell

A computer programme (GLEE, glass electrode evaluation) [22] has been used for the calibration of a glass electrode by means of a strong acid-strong base titration. This programme provided an estimate of the carbonate contamination of the base, the pseudo-Nernstian standard potential and slope of the electrode and, optionally, the concentration of the base and pK_W.

2.4. Procedure for equilibrium titration

To determine the dissociation constants of protonation equilibria of the amino acids, the following solutions were prepared (total volume of $50\ \mathrm{cm^3}$) and titrated

potentiometrically against standard carbon dioxide free NaOH ($0.10\ \mathrm{mol \cdot dm^{-3}}$) solution; (a) HNO_3 ($0.003\ \mathrm{mol \cdot dm^{-3}}$) + $NaNO_3$ ($0.10\ \mathrm{mol \cdot dm^{-3}}$) and (b) solution (a) + amino acid ($0.001\ \mathrm{mol \cdot dm^{-3}}$).

Each of the above solutions was left to stand for about 5 min before titration. Each titration was repeated at least 4 times under carefully controlled experimental conditions.

2.5. Calculations

To account for the differences in acidity, basicity, dielectric constant, and ion activities for partially aqueous solutions relative to the pure aqueous ones, the pH values of the former solutions were corrected by making use of the procedure described by Douheret [23]

$$\mathrm{pH}^* = \mathrm{pH}_{(\mathrm{R})} - \delta, \tag{1}$$

where pH^* = corrected value and $\mathrm{pH}_{(\mathrm{R})}$ = meter readings. The value of δ for the various proportions of solvent was determined as described below.

The pK_a values were calculated adopting the Irving and Rossotti technique as described in our previous work [24–26]. Computations related to the estimation of dissociation constants were performed by regression analysis of titration curves using the least-squares computer ESAB [16,17] and PKPOT programmes [18]. The adequacy of a proposed regression chemical model with experimental data and the reliability of parameter estimates pK_a may be examined by the goodness-of-fit test [15].

The thermodynamic quantities (Gibbs free energy ΔG°, enthalpy ΔH°, and entropy ΔS°, changes) associated with the protonation equilibria of amino acids were calculated by the following equations:

$$\Delta G^\circ = 2.303\,RT\mathrm{p}K_a \tag{2}$$

and

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ. \tag{3}$$

The change in Gibbs free energy from mixed aqueous media, δ, can be calculated as following from the equation

$$\delta = \Delta G^\circ_s - \Delta G^\circ_w, \tag{4}$$

where ΔG°_w and ΔG°_s refer to the standard Gibbs free energy change from pure water as solvent and in non-aqueous mixtures, respectively.

3. Results and discussion

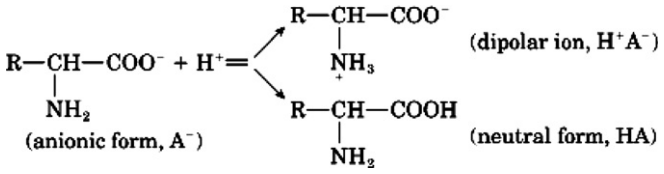
Normal biochemical processes occur in aqueous solution close to neutral pH; typical physiological pH is about 7.2 to 7.4, and pH 7.0 is a close approximation. Certain functional groups found in biological molecules, in particular carboxylic acids or amino groups, can gain or lose H^+ depending on the availability of hydrogen ions (or protons) in the solution.

TABLE 1
Dissociation constants of standard α-amino acids (pK_a) in $0.1\ \mathrm{mol \cdot dm^{-3}}$ $NaNO_3$ solutions in water and (water + dioxane) at different temperatures

Amino acid	Mole fraction of dioxane, x_z (volume fraction, Φ_z)															
	0 (0)				0.083 (0.30)				0.174 (0.50)				0.33 (0.7)			
	T/K															
	298.15	310.15	318.15	328.15	298.15	310.15	318.15	328.15	298.15	310.15	318.15	328.15	298.15	310.15	318.15	328.15
								$\mathrm{p}K_a$								
Glycine	9.80	9.74	9.70	9.64	9.87	9.82	9.77	9.72	9.93	9.89	9.81	9.79	9.98	9.92	9.88	9.82
Alanine	9.90	9.84	9.79	9.71	9.98	9.93	9.87	9.81	10.05	10.00	9.94	9.88	10.11	10.04	10.02	9.95
Valine	9.60	9.53	9.48	9.41	9.67	9.61	9.56	9.50	9.73	9.69	9.63	9.58	9.81	9.74	9.70	9.63
Leucine	9.60	9.52	9.48	9.43	9.68	9.61	9.54	9.50	9.72	9.69	9.62	9.55	9.80	9.70	9.68	9.64
Isoleucine	9.68	9.60	9.56	9.50	9.75	9.69	9.63	9.58	9.80	9.76	9.70	9.64	9.87	9.79	9.76	9.71
Proline	10.60	10.54	10.49	10.44	10.68	10.62	10.57	10.51	10.73	10.68	10.64	10.57	10.79	10.70	10.69	10.63
Phenylalanine	9.10	9.04	8.98	8.93	9.17	9.13	9.07	8.98	9.24	9.18	9.15	9.07	9.30	9.25	9.19	9.14
Tyrosine	9.10	9.03	8.99	8.92	9.18	9.11	9.05	9.02	9.26	9.20	9.10	9.06	9.31	9.26	9.21	9.14
Tryptophan	9.39	9.33	9.28	9.21	9.47	9.40	9.36	9.30	9.52	9.46	9.42	9.37	9.59	9.50	9.45	9.43
Cysteine	10.8	10.75	10.69	10.63	10.86	10.81	10.76	10.72	10.91	10.86	10.82	10.77	10.98	10.93	10.85	10.80
Methionine	9.21	9.16	9.11	9.05	9.27	9.22	9.18	9.14	9.32	9.29	9.23	9.19	9.39	9.33	9.30	9.25
Serine	9.20	9.16	9.10	9.04	9.27	9.23	9.18	9.15	9.34	9.28	9.24	9.18	9.42	9.32	9.29	9.23
Threonine	9.10	9.04	9.00	8.93	9.18	9.12	9.06	9.02	9.22	9.19	9.13	9.05	9.28	9.24	9.18	9.14
Aspartate	10.00	9.96	9.89	9.83	10.06	10.03	9.98	9.91	10.11	10.05	10.02	9.99	10.18	10.10	10.04	10.00
	(3.90)	(3.87)	(3.82)	(3.76)	(3.95)	(3.92)	(3.88)	(3.85)	(3.98)	(3.96)	(3.91)	(3.89)	(3.41)	(3.99)	(3.95)	(3.90)
Glutamate	9.70	9.64	9.58	9.52	9.76	9.73	9.66	9.60	9.81	9.77	9.75	9.68	9.88	9.82	9.77	9.73
	(4.30)	(4.26)	(4.20)	(4.16)	(4.37)	(4.31)	(4.28)	(4.23)	(4.41)	(4.34)	(4.32)	(4.28)	(4.47)	(4.40)	(4.33)	(4.30)
Asparagine	8.84	8.78	8.81	8.76	8.90	8.83	8.79	8.83	8.97	8.93	8.84	8.80	9.03	8.97	8.95	8.86
Glutamine	9.13	9.07	9.02	8.94	9.19	9.14	9.09	9.02	9.24	9.20	9.16	9.08	9.32	9.23	9.21	9.17
Lysine	8.95	8.90	8.84	8.77	9.01	8.93	8.93	8.86	9.08	9.03	8.95	8.94	9.17	9.09	9.04	8.96
Arginine	9.00	8.94	8.88	8.82	9.07	9.01	8.96	8.90	9.14	9.06	9.03	8.94	9.23	9.15	9.05	9.00
Histidine	9.20	9.04	8.99	8.93	9.28	9.21	9.06	9.01	9.34	9.29	9.22	9.05	9.42	9.35	9.30	9.25
	(6.00)	(5.97)	(5.91)	(5.86)	(6.06)	(6.03)	(5.99)	(5.93)	(6.11)	(6.07)	(6.04)	(5.98)	(6.20)	(6.10)	(6.08)	(6.02)

There are 20 amino acids found in all naturally occurring proteins, which can be categorized according to the chemical nature of the R-group, *viz.* amino acids with non-polar R-groups (aliphatic group (glycine, alanine, valine, leucine, isoleucine and proline); aromatic R-group (phenylalanine, tyrosine and tryptophan) and sulfur containing R-group (cysteine and methionine)); amino acids with polar R-groups (hydroxyl R-group (serine and threonine); acidic R-group (aspartate and glutamate); amide R-group (asparagine and glutamine); basic R-group (lysine, arginine, and histidine).

The pK_a values given in tables 1 and 3 refer to the following protonation equilibria shown below:



The protonation constants determined for the amino acids studied at $T = 298.15$ K in 0.1 mol · dm^{-3} $NaNO_3$ aqueous solutions agree fairly well with data reported previously in the literature [1–12], after allowing for changes in experimental conditions as well as methods of calculation. It is worth mentioning that the pK_{a1} values of the amino acids investigated are too low ($\leqslant$2.30) and exist only in strongly acidic solutions. Therefore, these values are not used in our calculations, since the pH-metric data are measured in the range $2 \leqslant$ pH $\leqslant 11$.

When the ionization of an acid gives a net increase of ions, a decrease in the dielectric constant of the solvent should be accompanied by a decrease in the protonation constant (increase of pK_a) of a weak acid dissolved in it. A solvent of low dielectric constant increases the electrostatic forces between the ions and facilitates formation of molecular species, and should increase pK_a, as borne out for amino acids by table 1. The pK_a values increase with increase in dioxane content (mole fraction of dioxane) because of the decrease in the dielectric constant of bulk solvent.

As the dielectric constant decreases, the ion interaction involving the proton and anionic oxygen on the acid decreases to a greater extent than the ion dipole interaction between the proton and the solvent molecule. A plot of pK_a *versus* mole fraction of dioxane, x_z (figure 1) shows a linear relationship of the form $pK_a = mx_z + c$, where m, x_z, and c represent the slope, mole fraction of dioxane and intercept, respectively. A similar behaviour is found for several other acids such as benzoic, acetic, propionic and formic acids [27], and for a number of β-diketones in aqueous dioxane [28]. Similarly linear plots are also obtained for some other mixed water solvents, *e.g.*, acetic, propenoic, butyric and benzoic acids in (methanol + water) mixtures [29].

Regarding the variation of pK_a values with the solvent composition, one can postulate that the zwitterionic to neutral form ratio decreases as the dioxan content increases. This can be inferred from considerations on specific solute–solvent interactions and structural changes in amino acids from water to (dioxin + water) media. The present study supports the idea that the solvent effect on the protonation constants for the amino acids can be utilized to predict whether the zwitterion or the neutral form is the predominant species and how the zwitterionic to neutral form ratio changes with the concentration of organic component in (water + organic) solvent mixtures. The basicity of a compound is a result of various factors such as: (i) the solvent effect: solvation power, the tendency of forming hydrogen bonds, selective solvation, dielectric constant and the composition of the solution in the first solvation layer in the case of mixed solvents and (ii) structural effect, electronic effect, steric effect and the formation of hydrogen bonding.

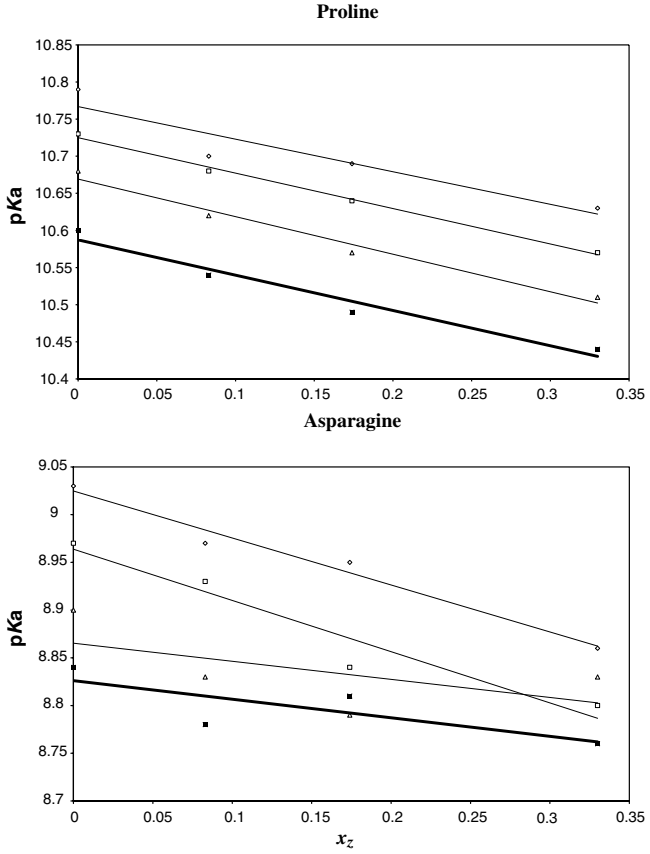


FIGURE 1. Plot of pK_a values of amino acid (Proline) and (Asparagine) *versus* x_z of dioxane solvent in 0.1 mol · dm^{-3} $NaNO_3$ solution at different temperatures. (◇) 298.15 K; (□) 310.15 K; (△) 318.15 K; (■) 328.15 K.

Since equilibrium constants in general are temperature dependent, a variation in temperature during measurements would naturally have a deleterious effect on the quality of recorded data. Of much higher significance, however, is the fact that the measuring electrode itself is highly temperature sensitive, including its response to changes (''slope'') in [H^+]. The sum of these effects is usually of the order of 1 mV · K^{-1}, and it is therefore vital that the

TABLE 2
Thermodynamic quantities for the dissociation processes of standard α-amino acids in 0.1 mol · dm^{-3} $NaNO_3$ solutions in water and in (water + dioxane) mixtures at different temperatures

Amino acid	Mole fraction of dioxane, x_z (volume fraction, Φ_x)															
	0 (0)				0.083 (0.30)				0.174 (0.50)				0.33 (0.70)			
	ΔG°/ (kJ · mol^{-1})	ΔH°/ (kJ · mol^{-1})	$-\Delta S^\circ$/ (J · K^{-1} · mol^{-1})		ΔG°/ (kJ · mol^{-1})	ΔH°/ (kJ · mol^{-1})	$-\Delta S^\circ$/ (J · K^{-1} · mol^{-1})	Δ/ (kJ · mol^{-1})	ΔG°/ (kJ · mol^{-1})	ΔH°/ (kJ · mol^{-1})	$-\Delta S^\circ$/ (J · mol^{-1} · K^{-1})	Δ/ (kJ · mol^{-1})	ΔG°/ (kJ · mol^{-1})	ΔH°/ (kJ · mol^{-1})	$-\Delta S^\circ$/ (J · K^{-1} · mol^{-1})	Δ/ (kJ · mol^{-1})
Glycine	37.2	9.90	154.52		36.19	9.88	157.31	1.1	37.33	9.574	158.73	2.24	37.75	9.574	157.96	2.66
Alanine	38.56	9.60	150.5		33.14	11.73	156.05	−5.42	35.8	10.72	156.62	−2.76	35.97	10.72	161.43	−2.59
Valine	33.88	11.11	144.37		31.25	11.79	137.51	−2.63	30.47	10.53	155.09	−3.41	36.67	9.574	150.87	2.79
Leucine	36.31	9.78	148.2		33.62	10.57	146.48	−2.69	32.13	11.54	150.11	−4.18	33.94	10.82	154.59	−2.37
Isoleucine	36.64	9.84	148.03		33.01	11.12	150.5	−3.63	34.05	10.82	153.83	−2.59	35.62	10.24	155.89	−1.02
Proline	42.48	9.54	169.26		40.37	10.09	168.92	−2.11	39.64	10.72	174.24	−2.84	42.11	9.842	174.46	−0.37
Phenylalanine	32.72	10.2	138.05		30.34	10.82	136.39	−2.38	28.79	11.87	142.65	−3.93	32.19	10.34	143.98	−0.53
Tyrosine	33.26	9.96	136.33		29.56	11.09	141.11	−3.7	31.73	10.34	133.07	−1.53	26.27	13.4	144.94	−6.99
Tryptophan	34.19	10.34	142.45		31.31	11.16	146.09	−2.88	33.03	10.53	150.88	−1.16	35.41	9.574	149.34	1.22
Cysteine	36.5	11.68	170.79		40.16	10.76	178.07	3.66	44.09	8.999	179.98	7.59	44.97	8.693	161.60	8.47
Methionine	36.37	8.62	150.88		36.37	8.616	150.34	0	36.40	8.425	149.73	0.03	36.22	8.425	150.87	−0.15
Serine	30.45	11.68	142.34		32.31	10.13	151.51	1.86	37.32	7.85	145.71	6.87	33.49	9.957	141.30	3.04
Threonine	34.97	8.10	139.39		31.03	10.53	141.34	−3.94	31.88	10.26	141.69	−3.09	31.52	10.72	147.47	−3.45
Aspartate	35.09	11.49	155.28		35.38	10.91	161.54	1.86	38.59	9.574	168.49	3.94	42.58	7.659	156.24	0.28
	(37.3)	(9.574)	(45.38)		(4.00)	(9.60)	(54.00)	(2.8)	(9.60)	(6.51)	(56.30)	(3.3)	(10.81)	(5.97)	(157.2)	(3.3)
Glutamate	37.30	9.574	147.82		32.6	11.37	152.79	1.49	35.22	10.34	161.98	4.24	40.56	7.74	157.2	2.8
	(2.60)	(11.49)	(52.08)		(6.50)	(9.10)	(54.76)	(0.8)	(7.71)	(8.62)	(57.63)	(1.7)	(9.24)	(7.94)	(48.44)	(0.9)
Asparagine	31.41	10.15	155.09		42.03	4.212	153.18	−0.6	40.63	5.036	134.22	9.22	28.53	11.49	139.39	−2.88
Glutamine	34.78	9.191	135.56		28.7	11.72	140.73	10.6	30.47	11.49	144.37	−4.31	33.28	9.765	147.49	−1.5
Lysine	26.43	13.02	133.84		28.61	11.3	143.22	−6.08	33.89	8.808	142.07	7.46	32.79	9.574	132.30	6.36
Arginine	22.76	14.97	134.41		28.72	11.35	138.09	2.18	30.45	10.72	134.8	7.69	28.15	12.04	126.56	5.39
Histidine	32.38	10.72	119.59		18.9	16.75	117.43	5.96	16.82	18.19	120.82	−18.7	18	18.02	144.56	−14.4
	(34.1)	(26.1)	(105.7)		(33.2)	(21.31)	(109.3)	(1)	(36.12)	(24.3)	(104.3)	−2.02	(29.54)	(27.6)	(108.9)	(4.6)

experiments are performed with a temperature control of at least ±0.05 K [30].

In this investigation, the effect of the medium on the protonation processes of amino acids in water solution and in various aqueous dioxane media have been evaluated at $T = (298.15, 310.15, 318.15, \text{and } 328.15)$ K and the ionic strength of the medium was held constant using the desired concentration of $NaNO_3(0.10\ \text{mol} \cdot \text{dm}^{-3})$.

Dissociation constants of a weak acid is a function of temperature and generally it has a maximum value, $K_a(\text{max})$ or $pK_a(\text{min})$, near room temperature. An examination of the data given in table 1 reveals that pK_a of all amino acids studied decreases as the temperature is raised. Their heats of protonation, ΔH°, are positive.

From the data listed in tables 1 and 2, we can conclude that the positive values of ΔH° found for all dioxane media and amino acids studied here indicate that the protonation process of amino acids at temperatures up to 298.15 K is endothermic, and will be exothermic only above T_{max}. It is observed that there is a general tendency for ΔS° to increase with increase in dioxane content of the solvent medium. Further, the change in ΔH° with the change in medium is relatively small and within the experimental error. For this reason, the plots of pK_a *versus* x_z give practically the same slopes at different temperatures (figure 1). It is therefore, justifiable to assume that ΔH° is independent of solvent composition over the range studied. The positive values of ΔG° for the dissociation processes of the amino acids studied denote that the processes are not spontaneous. In addition, the positive values of entropy changes point to increased ordering due to association. The plot of pK_a *versus* $1/T$ gives a straight line (figure 2).

The errors induced in the determination of pK_a are reflected in all the values of all thermodynamic functions. Therefore, an estimate of error is necessary to show how reliable are these result. The values of pK_a were determined generally with a precision of ±0.02 and not exceed ±0.03, and hence the error in ΔG° is estimated to be between (0.03 and 0.04) $\text{kJ} \cdot \text{mol}^{-1}$.

A high electrolyte concentration is used in order to keep variations of the activity coefficients at a minimum. Precise thermodynamic data can only be obtained provided an inert electrolyte of fairly high concentration ($0.10\ \text{mol} \cdot \text{dm}^{-3}$ and above) is used.

The main differences between the different procedures for the study of ionic equilibria in aqueous (water solutions) and in non-aqueous solutions are due to the activity coefficients. As in most equilibria in aqueous medium, a background electrolyte is added to maintain constant ionic strength ranging from about (0.1 to 3.0) $\text{mol} \cdot \text{dm}^{-3}$ $NaNO_3$. This is allowed in some (water + ethanol) or (water + dioxane) mixtures, but not in solvents of low dielectric constants where the solubility of electrolytes is very low.

The dissociation constants of amino acids studied were determined at different ionic strengths ($I = 0.1$, 0.15, 0.2, and 0.25) $\text{mol} \cdot \text{dm}^{-3}$ $NaNO_3$ and listed in table 3. The lin-

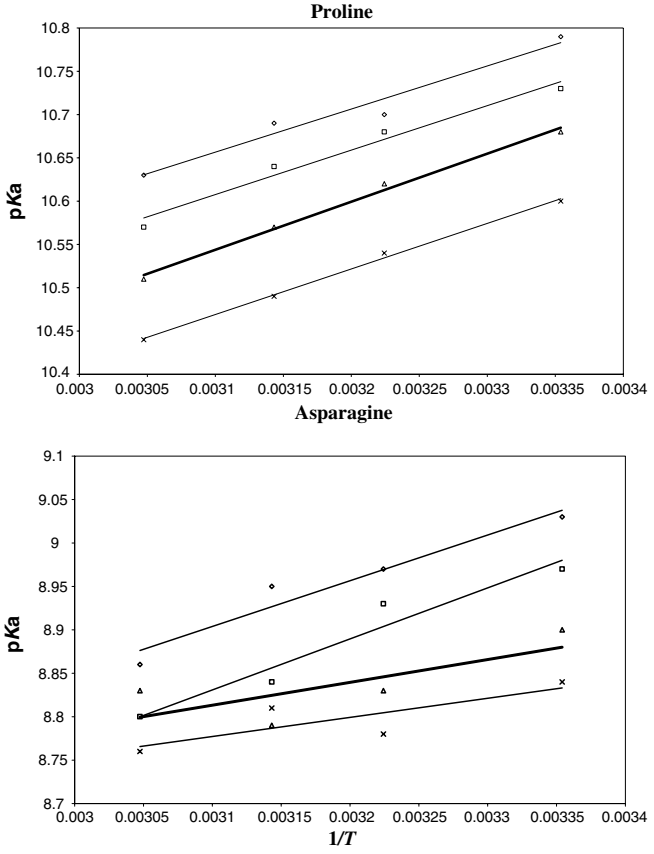


FIGURE 2. Plot of dissociation constants, pK_a, of amino acid (Proline) and (Asparagine) studied *versus* $1/T$ in $0.1\ \text{mol} \cdot \text{dm}^{-3}$ $NaNO_3$ solutions at different (water + dioxane) mixtures. (◇) $x_z = 0.33$, $\Phi_z = 0.70$; (□) $x_z = 0.174$, $\Phi_z = 0.50$; (△) $x_z = 0.083$, $\Phi_z = 030$; (×) $x_z = 0$, $\Phi_z = 0.0$.

TABLE 3
Dissociation constants of standard α-amino acids (pK_a) in water in different ionic strengths of $NaNO_3$ solutions at $T = 298.15$ K

Amino acid	$I/(\text{mol} \cdot \text{dm}^{-3})$			
	0.10	0.15	0.20	0.25
	pK_a			
Glycine	9.80	9.67	9.53	9.48
Alanine	9.90	9.86	9.67	9.51
Valine	9.60	9.43	9.30	9.16
Leucine	9.60	9.49	9.41	9.30
Isoleucine	9.68	9.59	9.50	9.41
Proline	10.60	10.53	10.46	10.37
Phenylalanine	9.10	9.01	8.92	8.81
Tyrosine	9.10	9.03	8.91	8.82
Tryptophan	9.39	9.29	9.16	9.08
Cysteine	10.8	10.69	10.54	10.47
Methionine	9.21	9.13	9.04	8.94
Serine	9.20	9.11	9.06	8.91
Threonine	9.10	9.03	8.91	8.82
Aspartate	10.00 (3.90)	9.91 (3.81)	9.83 (3.71)	9.74 (3.63)
Glutamate	9.70 (4.30)	9.62 (4.23)	9.51 (4.16)	9.42 (4.05)
Asparagine	8.84	8.71	8.59	8.42
Glutamine	9.13	9.04	8.91	8.79
Lysine	8.95	8.86	8.68	8.59
Arginine	9.00	8.91	8.79	8.71
Histidine	9.20 (6.00)	9.10 (5.91)	9.03 (5.89)	8.97 (5.80)

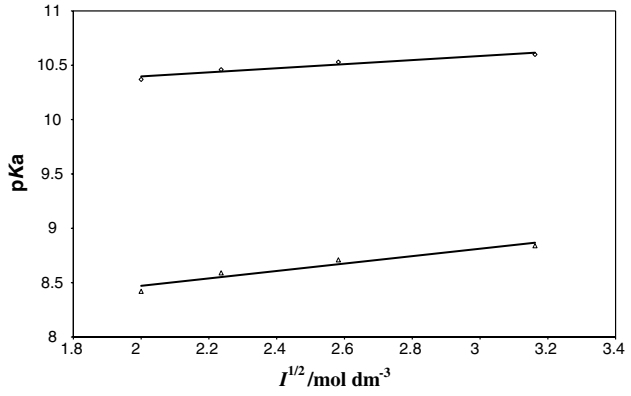


FIGURE 3. Plot of dissociation constants, pK_a, of amino acids studied against $I^{1/2}$ in aqueous water solutions. (△) asparagine, (◇) proline.

ear square-root dependence is observed according to the Debye–Hückel or Davies equations, as shown in figure 3.

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