Protonation Equilibria of L-Glutamic Acid and L-Histidine in Low Dielectric Media

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Abstract

Effect of DMSO on the protonation equilibria of L-Glutamic acid and L-Histidine have been studied in varying concentrations (0-60% v/v) of DMSO–water mixtures maintaining an ionic strength of 0.16 mol/l at 303 K using pH metric method. The protonation constants have been calculated with the computer program MINIQUAD75 and the best fit models are arrived at based on statistical grounds employing crystallographic R factor, $\chi 2$, skewness and kurtosis. The variation of protonation constants with dielectric constant of the medium is attributed to the electrostatic and non-electrostatic forces. The effect of errors on the protonation constants has also been presented.

Keywords- Protonation equilibria, DMSO, Glutamic acid, Histidine, MINIQUAD75

I. INTRODUCTION

Glutamic acid is a non-essential amino acid, and it is interconvertible to glutamine, which is known to be a very important in preventing ammonia intoxication. Adults may ingest 20 to 35 mg per day of this amino acid without any apparent ill-effects. It is an excitatory neurotransmitter for the central nervous system, the brain and spinal cord. It is important in the metabolism of sugars and fats. It aids in the transportation of potassium into the spinal fluid. It acts as fuel for the brain and helps correct personality disorders. It is used in the treatment of epilepsy, mental retardation, muscular dystrophy and ulcers [1-6].

Histidine has been used in the treatment of rheumatoid arthritis, allergies, ulcers and anemia. It is essential for the growth and repair of tissues and important for the maintenance of the myelin sheaths, which protect nerve cells. It is needed for the production of both red and white blood cells and it protects the body from radiation damage. It lowers blood pressure. It aids in the removal of heavy metals from the body and also aids in sexual arousal [7-11].

A number of studies have been reported on protonation constants of α-amino acids in different media [12-14]. Acidity and basicity of a molecule is governed by its structure and solvent effects [15, 16]. A review of literature has revealed that a little is reported for protonation constants of Glutamic acid and Histidine in organic-water solvents. The present study reveals the determination of protonation constants of Glutamic acid and Histidine in DMSO-water compositions.

II. MATERIALS AND METHODS

A. Materials

Solutions (0.05 mol/l) of L-glutamic acid (Merck, Germany) and L-histidine (Merck, Germany) were prepared in triple-distilled water by maintaining 0.05 mol/l hydrochloric acid concentration to increase the solubility. Dimethlysulphoxide (DMSO) (Qualigens, India) was used as received. Hydrochloric acid (Qualigens, India) of 0.2 mol/l was prepared. Sodium chloride (Merck, India) of 2 mol/l was prepared to maintain the ionic strength in the titrand. Sodium hydroxide (Merck, India) of 0.4 mol/l was prepared. All the solutions were standardized by standard methods. To assess the errors that might have crept into the determination of the concentrations, the data were subjected to analysis of variance of one way classification (ANOVA) [17]. The strengths of alkali and mineral acid were determined using the Gran plot method [18, 19].

B. Alkalimetric Titrations

Alkalimetric titrations were carried out in media containing varying compositions of DMSO (0-60% v/v) maintaining an ionic strength of 0.16 mol/l with sodium chloride at 303.00 ± 0.05K. An Elico LI-120 pH meter was used. Potassium hydrogen phthalate (0.05 mol/l) and borax (0.01 mol/l) solutions were used to calibrate the pH meter. In each titration, the titrand contained approximately 1 mmol of hydrochloric acid. The initial concentrations of ingredients are given in Table 1.

The glass electrode was equilibrated in a well stirred DMSO-water mixture containing inert electrolyte for several days. At regular intervals strong acid was titrated against alkali to check the complete equilibration of the glass electrode. The calomel

electrode was refilled with DMSO-water mixture of equivalent composition as that of the titrand. Alkalimetric titrations were performed in media containing 0-60 % v/v DMSO-water mixtures pH metrically. The details of experimental procedure and titration assembly have been detailed elsewhere [20].

Table - 1: Total Initial Concentrations of Ingredients (In Mmol) In Proton-Ligand Titrations

DMSO % (v/v)	*TLO*	
	Glu	*His*
0	*0.249*	*0.251*
	0.374	*0.376*
	0.499	*0.501*
10	*0.250*	*0.248*
	0.375	*0.371*
	0.500	*0.495*
20	*0.250*	*0.249*
	0.375	*0.374*
	0.500	*0.499*
30	*0.250*	*0.250*
	0.375	*0.375*
	0.500	*0.500*
40	*0.250*	*0.250*
	0.374	*0.375*
	0.499	*0.500*
50	*0.250*	*0.250*
	0.375	*0.374*
	0.499	*0.499*
60	*0.249*	*0.250*
	0.374	*0.376*
	0.499	*0.502*

C. Modeling Strategy

The approximate protonation constants of glutamic acid and histidine were calculated with the computer program SCPHD [21]. The best fit chemical model for each system investigated was arrived at using non-linear least-squares computer program, MINIQUAD75 [22], which exploits the advantage of constrained least-squares method in the initial refinement and reliable convergence of Marquardt algorithm. The variation of stepwise protonation constants (log K) with the dielectric constant of the medium was analyzed on electrostatic grounds for the solute-solute and solute-solvent interactions.

III. RESULTS AND DISCUSSION

A. Secondary Formation Functions

Secondary formation functions like average number of protons bound per mole of ligand ($\bar{n}_H$) and number of moles of alkali consumed per mole of ligand (a) are useful to detect the number of equilibria. Plots of $\bar{n}_H$ versus pH (formation curves) for different concentrations of the ligand should overlap if there is no formation of polymeric species. Overlapping formation curves for glutamic acid and histidine (Figure 1) rule out the polymerization of the ligand molecules. The pH values at half integral values of $\bar{n}_H$ correspond to the protonation constants of the ligands. Three half integrals in the case of glutamic acid and histidine (Figure 2) emphasize the presence of three protonation-deprotonation equilibria in the pH range of present study. The number of plateaus in the formation curves corresponds to the number of these equilibria.

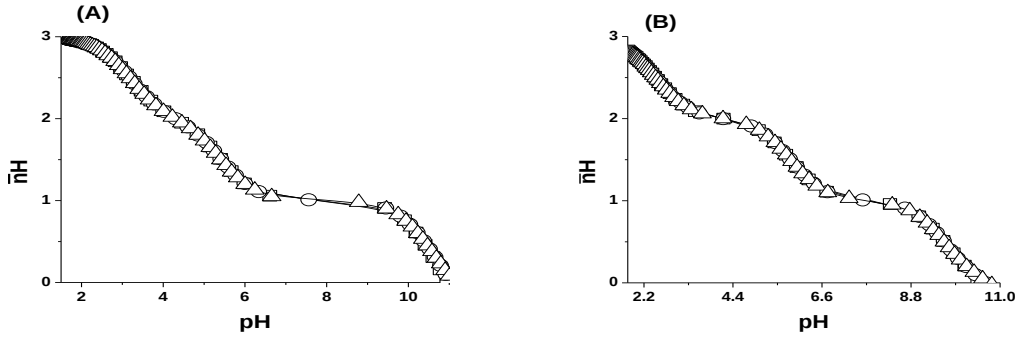


Fig. 1: Plots of $\bar{n}H$ versus pH in 50 % v/v DMSO-water mixture; (A) glutamic acid and (B) histidine; (□) 0.25, (○) 0.375, and (Δ) 0.499mmol.

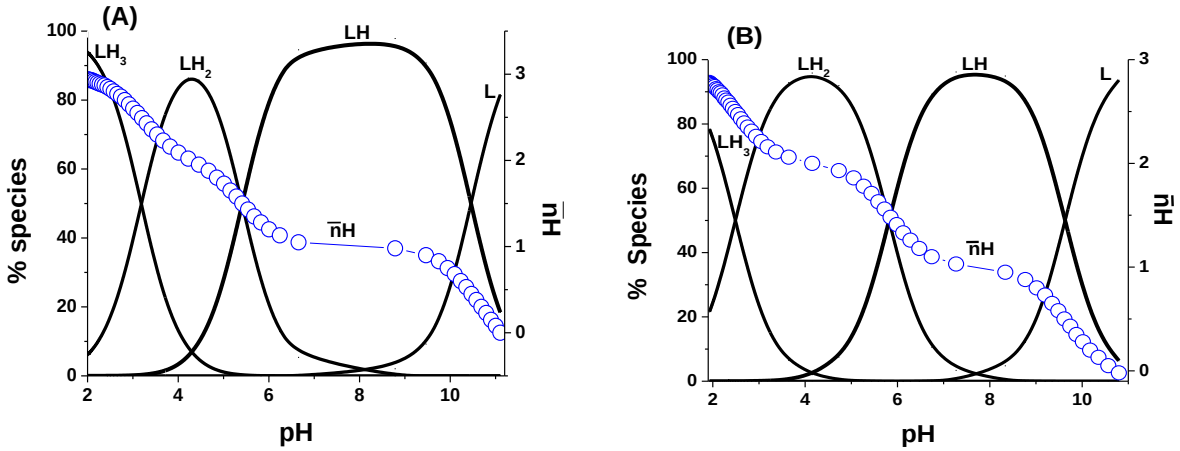


Fig. 2: Formation functions (○) and Species distribution diagrams of (A) glutamic acid and (B) histidine in 50% v/v DMSO-water mixture.

The plots of a versus pH are given in Figure 3. The negative values of correspond to the number of moles of free acid present in the titrand and the number of associable protons. The positive values of indicate the number of dissociable protons in the ligand molecules. The maximum value of a in Figure 3A is 2, which indicates that glutamic acid has two dissociable (two carboxyl) protons. The corresponding value for histidine (Figure 3B) is 1, which clearly shows that L -histidine has only one dissociable (carboxyl) proton.

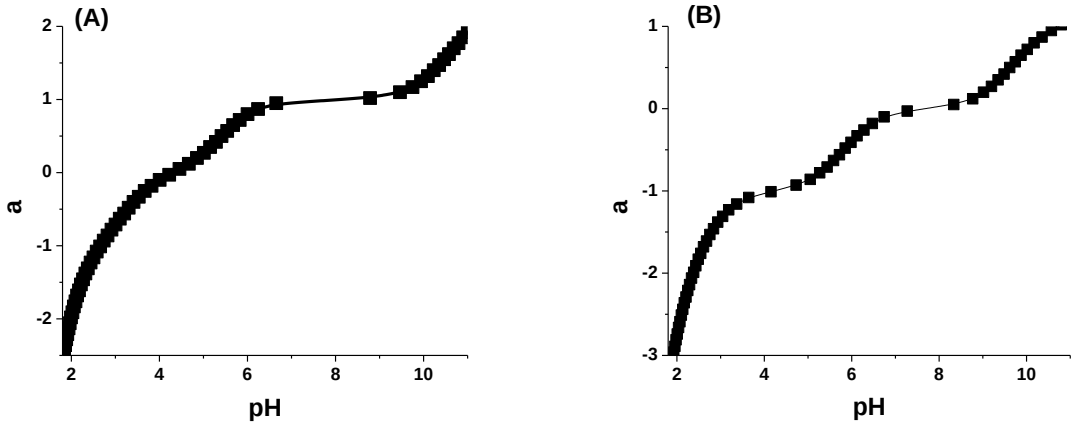


Fig. 3: Variation of a with pH in 50% v/v DMSO-water mixture: (A) Glutamic acid and (B) Histidine.

The best fit models containing the type of species and overall formation constants along with some of the important statistical parameters are given in Table 2. A very low standard deviation (SD) in log β values indicates the precision of these parameters. The small values of U_{corr} (sum of squares of deviations in concentrations of ligand and hydrogen ion at all experimental points corrected for degrees of freedom) indicate that the experimental data can be represented by the model. Small values of mean, standard deviation and mean deviation for the systems corroborate that the residuals are around zero mean with little dispersion.

Table - 2: Best-fit chemical models of acido-basic equilibria of Glu and His in DMSO-water mixtures.

% v/v DMSO	*Log β_1 (SD)*	*Log β_2(SD)*	*Log β_3 (SD)*	*NP*	*U_{corr}*	*Skew ness*	*Kurtosis*	*χ^2*	*R*
Glutamic acid (pH 1.7-10.7)									
0	*9.78(7)*	*14.01(13)*	*16.19(15)*	*162*	*1.35*	*-0.08*	*2.74*	*35.31*	*0.0045*
10	*9.97(7)*	*14.30(11)*	*16.56(12)*	*140*	*1.12*	*10.06*	*-0.17*	*3.84*	*0.0043*
20	*10.21(5)*	*14.79(9)*	*17.34(11)*	*139*	*0.57*	*42.17*	*-0.37*	*7.42*	*0.0031*
30	*10.35(16)*	*15.22(26)*	*17.82(31)*	*121*	*3.62*	*6.40*	*-0.82*	*4.50*	*0.0083*
40	*10.37(9)*	*15.46(14)*	*18.41(18)*	*137*	*1.29*	*9.33*	*0.22*	*3.96*	*0.0048*
50	*10.45 (7)*	*15.86(11)*	*19.05(13)*	*126*	*0.89*	*27.62*	*0.00*	*5.79*	*0.0042*
60	*10.61 (9)*	*16.30(13)*	*19.66(17)*	*111*	*0.91*	*47.55*	*-0.32*	*4.70*	*0.0045*
Histidine (pH 1.7-10.5)									
0	*9.26(5)*	*15.40(9)*	*17.14(11)*	*135*	*0.66*	*0.20*	*0.20*	*5.50*	*0.0036*
10	*9.42(6)*	*15.56(10)*	*17.43(13)*	*135*	*0.81*	*42.71*	*0.17*	*5.52*	*0.0040*
20	*9.58(11)*	*15.70(20)*	*17.68(24)*	*131*	*2.81*	*17.02*	*-1.04*	*5.34*	*0.0075*
30	*9.65(12)*	*15.75 (22)*	*17.82 (26)*	*125*	*3.03*	*43.25*	*-1.63*	*7.39*	*0.0077*

40	*9.63(6)*	*15.60 (12)*	*17.90 (15)*	*137*	*1.06*	*17.85*	*-0.71*	*3.82*	*0.0046*
50	*9.63(6)*	*15.44 (11)*	*17.93 (14)*	*132*	*0.87*	*24.12*	*-0.87*	*4.77*	*0.0042*
60	*9.70(5)*	*15.32 (10)*	*18.07(12)*	*132*	*0.68*	*36.12*	*-0.93*	*4.90*	*0.0037*

U_{corr}= U/ (NP-m) x 10^8; NP = Number of points
m = number of protonation constants, SD = Standard deviation

B. Residual Analysis [23]

In data analysis with least squares methods, the residuals (the differences between the experimental data and the data simulated based on the model parameters) are assumed to follow Gaussian or normal distribution. For an ideal normal distribution, the values of kurtosis and skewness should be three and zero, respectively. The kurtosis values in the present study indicate that residuals form leptokurtic patterns. The values of skewness given in Table 2 are between -0.08 and 43.25. These data evince that the residuals form a part of normal distribution; hence, least squares method can be applied to the present data. The sufficiency of the model is further evident from the low crystallographic R-values. These statistical parameters thus show that the best fit models portray the acido-basic equilibria of Glu and His in DMSO-water mixtures.

C. χ^2 test

χ^2 is a special case of gamma distribution whose probability density function is an asymmetrical function. This distribution measures the probability of residuals forming a part of standard normal distribution with zero mean and unit standard deviation. If the χ^2 calculated is less than the table value, the model is accepted.

D. Crystallographic R-Test

Hamilton's R factor ratio test is applied in complex equilibria to decide whether inclusion of more species in the model is necessary or not. In pH metric method, the readability of pH meter is taken as the R_{limit} which represents the upper boundary of R beyond which the model bears no significance. When these are different numbers of species the models whose values are greater than R-table are rejected. The low crystallographic R-values given in Table 2 indicate the sufficiency of the model.

E. Skewness

It is a dimensionless quantity indicating the shape of the error distribution profile. A value of zero for skewness indicates that the underlying distribution is symmetrical. If the skewness is greater than zero, the peak of the error distribution curve is to the left of the mean and the peak is to the right of the mean if skewness is less than zero. The values of skewness recorded in Table 2 are between -0.08 and 42.71. These data evince that the residuals form a part of normal distribution; hence, least-squares method can be applied to the present data.

F. Kurtosis

It is a measure of the peakedness of the error distribution near a model value. For an ideal normal distribution kurtosis value should be three (mesokurtic). If the calculated kurtosis is less than three, the peak of the error distribution curve is flat (platykurtic) and if the kurtosis is greater than three, the distribution shall have sharp peak (leptokurtic). The kurtosis values in the present study indicate that the residuals form leptokurtic pattern in the case of dopa and platykurtic for phen.

Alkalimetric titration data are simulated using the model parameters given in Table 2. These data are compared with the experimental alkalimetric titration data, to verify the sufficiency of the models. The overlap of the typical experimental and simulated titrations data given in Figure 4 indicates that the proposed models represent the experimental data.

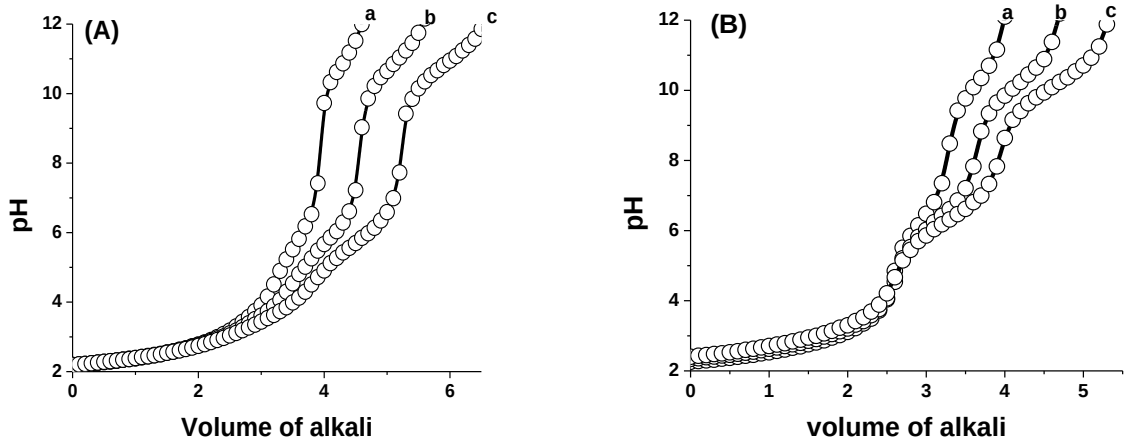


Fig. 4**:** Simulated (o) and experimental (solid line) alkalimetric titration curves in 50% v/v DMSO- water mixture: (A) Glutamic acid and (B) Histidine (a) 0.25, (b) 0.38 and (c) 0.50 mmol.

G. Effect of Systematic Errors in Best Fit Model

MINIQUAD75 does not have provision to study the effect of systematic errors in the influential parameters like the concentration of ingredients and electrode calibration on the magnitude of protonation constant. In order to rely upon the best chemical model for critical evaluation and application under varied experimental conditions with different experimental with different accuracies of data acquisition, an investigation was made by introducing pessimistic errors in the concentration of alkali, mineral acids and the ligands. The results of a typical system given in Table 3 emphasize that the errors in the concentrations of alkali and mineral acid affects the protonation constants more than that of the ligand.

Table - 3: Effect of errors in influential parameters on the protonation constants in 20%v/v DMSO-water mixture.

Ingredient	*% Error*	*Log β_{mlh} (SD)*					
		Glutamic acid			*Histidine*		
		LH	*LH_2*	*LH_3*	*LH*	*LH_2*	*LH_3*
	0	*10.21(5)*	*14.79(9)*	*17.34(11)*	*9.58(11)*	*15.70(20)*	*17.68(24)*
Alkali	*-5*	*10.76(31)*	*15.80(41)*	*18.59(46)*	*9.98(17)*	*16.45(29)*	*18.55(34)*
	-2	*10.41(8)*	*15.17(12)*	*17.81(14)*	*9.74(10)*	*15.99(18)*	*18.02(22)*
	+2	*10.02(11)*	*14.42(19)*	*16.89(22)*	*9.41(15)*	*15.40(28)*	*17.34(33)*
	+5	*9.71(23)*	*13.86(39)*	*16.22(44)*	*9.16(25)*	*14.95(46)*	*16.83(55)*
Acid	*-5*	*9.86(20)*	*14.07(33)*	*16.28(38)*	*9.21(27)*	*14.94(50)*	*16.56(63)*
	-2	*10.08(10)*	*14.51(17)*	*16.92(20)*	*9.44(16)*	*15.40(30)*	*17.24(36)*
	+2	*10.35(7)*	*15.08(12)*	*17.77(14)*	*9.72(9)*	*15.99(16)*	*18.11(19)*
	+5	*10.57(23)*	*15.52(34)*	*18.44(39)*	*9.93(14)*	*16.43(25)*	*18.78(30)*
Ligand	*-5*	*10.06(8)*	*14.57(14)*	*17.19(16)*	*9.52(11)*	*15.69(20)*	*17.82(24)*
	-2	*10.15(6)*	*14.70(11)*	*17.28(12)*	*9.56(11)*	*15.69(20)*	*17.74(24)*
	+2	*10.27(5)*	*14.87(9)*	*17.40(10)*	*9.60(11)*	*15.70(20)*	*17.63(24)*
	+5	*10.35(6)*	*14.99(10)*	*17.48(12)*	*9.63(12)*	*15.70(21)*	*17.55(26)*
log F	*-5*	*10.22(6)*	*14.80(11)*	*17.39(13)*	*9.17(25)*	*14.97(45)*	*16.89(54)*
	-2	*10.22(6)*	*14.80(10)*	*17.36(11)*	*9.17(25)*	*14.96(46)*	*16.86(54)*
	+2	*10.21(5)*	*14.78(9)*	*17.32(10)*	*9.16(25)*	*14.95(46)*	*16.81(55)*
	+5	*10.20(5)*	*14.78(9)*	*17.29(11)*	*9.15(26)*	*14.94(46)*	*16.77(56)*
Volume	*-5*	*10.21(9)*	*14.78(15)*	*17.40(18)*	*9.16(25)*	*14.95(45)*	*16.92(53)*
	-2	*10.21(6)*	*14.79(11)*	*17.37(13)*	*9.16(25)*	*14.95(45)*	*16.87(54)*
	+2	*10.21(5)*	*14.79(9)*	*17.31(11)*	*9.16(26)*	*14.95(46)*	*16.79(56)*
	+5	*10.22(7)*	*14.80(12)*	*17.27(14)*	*9.16(26)*	*14.95(47)*	*16.73(58)*

H. Effect of Solvent

The variation of protonation constant or change in free energy with co-solvent content depends upon two factors, viz., electrostatic and non-electrostatic. Born's classical treatment holds good in accounting for the electrostatic contribution to the free energy change [24]. According to this treatment, the energy of electrostatic interaction or the logarithm of step-wise protonation constant (log K) should vary linearly as a function of the reciprocal of the dielectric constant (1/D) of the medium. Such linear variation of the protonation constants (Figure 5) in DMSO-water mixture shows the dominance of electrostatic interactions. In the case of some mono- and di- carboxylic acids and simple phenolic ligands, electrostatic (long-range, non-specific or universal) solute-solvent interactions are predominant in binary mixtures of water with methanol, ethanol, dioxan or acetone as co-solvent [25].

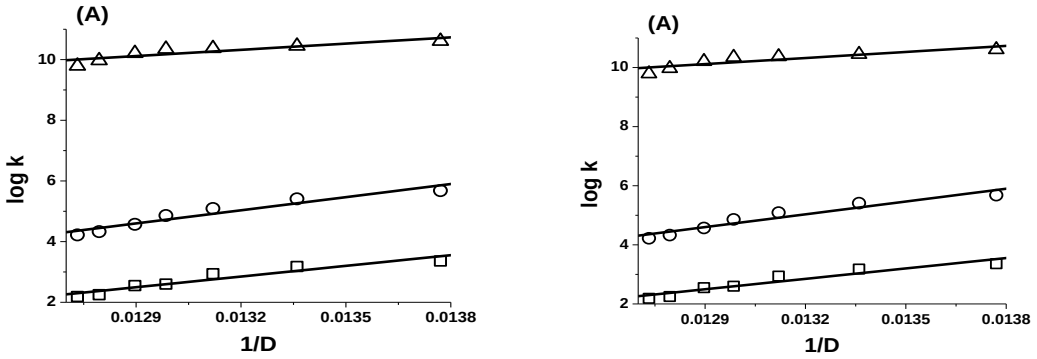


Fig. 5: Variation of step-wise protonation constant (log K) with reciprocal of dielectric constant (1/D) in DMSO-water mixture: (A) Glutamic acid and (B) Histidine (□) log K_1 (○) log K_2 and (Δ) log K_3.

Many workers were of the opinion that both electrostatic and non-electrostatic effects should be considered even in the case of simple acido-basic equilibria; one dominates the other depending upon the nature of solute and solvent [26-28].

I. Distribution Diagrams

Typical distribution plots produced by DISPLOT [29] using protonation constants from the best fit models are shown in Figure 2. Representative plots show the existence of LH_3^+, LH_2, LH^- and L^{2-} in the case of L-glutamic acid and LH_3^{2+}, LH_2^+, LH and L^- in the case of L-histidine in 2.0-11.0 pH ranges. LH form of L-glutamic acid is present to an extent of 90% in the pH range 6.0-10.0 and LH_2 form of L-histidine is present to an extent of 80% in the range 3.0-5.0. The higher protonated species (LH_3^+ in the case of Glu and LH_3^{2+} in the case of His) exist below a pH of 4.0. LH_3 is deprotonated with increasing pH to form LH_2, LH and L^{2-} in the pH ranges 2.0-6.0, 3.0-11.0 and above 8.0, respectively, in the case of L-glutamic acid and 2.0-7.0, 5.0-10.0 and above 8.0 in the case of L-histidine.

The present study is useful to understand (i) the role played by the active site cavities in biological molecules, (ii) the type of complex formed by the metal ion and (iii) the bonding behavior of the protein residue with the metal ion. The species refined and the relative concentrations under the present experimental conditions represent the possible forms of these amino acids in the biological fluids.

IV. CONCLUSIONS

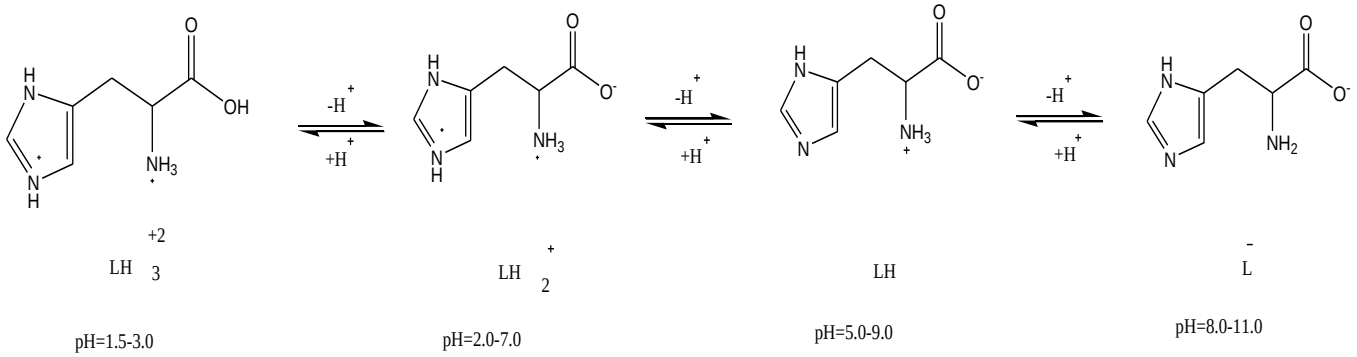
L-Glutamic acid has two dissociable protons and one amino group which can associate with a proton. It exists as LH_3^+ at low pH and gets deprotonated with the formation of LH_2, $LH^-$and L^{2-} successively with increase in pH.

L-Histidine has one dissociable proton and one amino and one imidazole groups which can associate with protons. It exists as LH_3^{2+} at low pH and gets deprotonated with the formation of LH_2^+, LH and L^- successively with increase in pH.

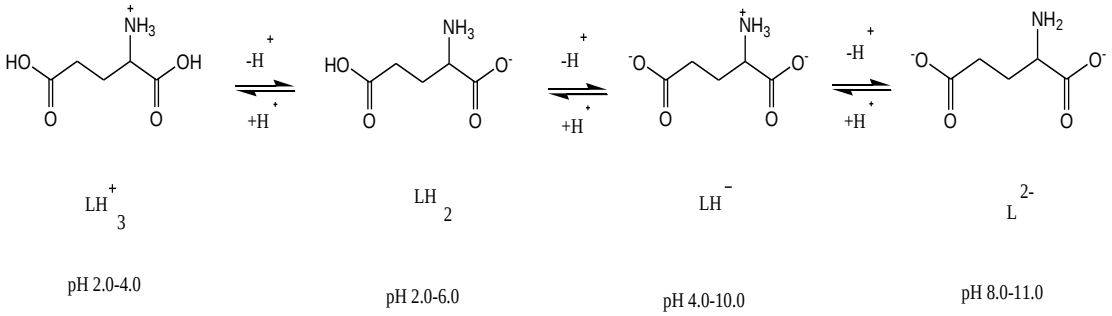
Secondary formation functions are useful in detecting the number of protonation equilibria and in guessing the approximate protonation constants.

The log values of protonation constants increase linearly with decreasing dielectric constant of DMSO-water mixtures. This indicates the dominance of electrostatic forces in the protonation-deprotonation equilibria.

The effect of systematic errors in the influential parameters on protonation constants shows that the errors in the concentrations of alkali and mineral acid will affect the protonation constants more than that of the ligand.



(A)



(B)

Fig. 6: Protonation-deprotonation equilibria of (A) histidine and (B) glutamic acid

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