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SHIFT IN PKA OFL-HISTIDINE AND L-GLUTAMIC ACID IN ORGANIC MEDIA: A POTENTIOMETRIC STUDY

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ABSTRACT

Protonation equilibria of L-histidine and in L-glutamic acid have been studied in various concentrations (0.0-50% v/v) in Dimethylformamide-water mixture by maintaining an ionic strength of 0.16 mol dm^{-3} at 310 K. The protonation constants have been calculated using 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 trend of log values of stepwise protonation constants with mole fraction of the medium have been explained based on electrostatic and non-electrostatic forces operating on the protonation equilibria. Distributions of species, protonation equilibria and effect of influential parameters on the protonation constants have also been discussed. The protonation constants from the best fit models show the formation of LH_3^{2+}, LH_2^{+}, LH and L^{-} in the case of L-histidine and XH_3^{+}, XH_2, XH^{-} and X^{2-} in the case of L-glutamic acid. In both the cases, LH/XH is present to an extent of 90% in the pH range 4.0-11.0.The effect of experimental errors on the protonation constants has also been discussed.

Keywords: Protonation equilibria, MINIQUAD75, Formation functions; Distribution diagrams; Co-solvent effect.

1. INTRODUCTION

Knowledge of the acido-basic properties of amino acids is extremely important in understanding and analyzing the properties of proteins. Further, separation, identification, and quantification of different amino acids and determining their sequence in proteins are based on their acido-basic behavior [1].

Histidine (H) has been used in the treatment of rheumatoid arthritis, allergies, ulcers and anemia. L-Histidine and L-Glutamic acid act as tridentate ligands resulting in six membered ring structures. Histidine is a basic amino acid, containing weakly basic imidazole group. It has three potential metal-binding sites, namely, carboxylate oxygen ($O_{caboxyl}$), imidazole imido nitrogen (N_{im}) and amino nitrogen (N_{am}) [2].

Glutamic acid (E) is a non-essential amino acid, and is interconvertible to glutamine, which is known to be a very important in preventing ammonia intoxication. E has three functional groups and all are protonated, in the order of amino followed by two carboxylate groups. Nitrogen donor atoms can associate with hydrogen ions in physiological pH ranges and there is often significant competition between hydrogen and metal ion for the donor sites [3].

This situation results in the simultaneous existence of a number of equilibria, producing an array of successively protonated complexes.

N, N-Dimethylformamide (DMF) is an organic compound and a common solvent for chemical reactions that is miscible with water and the majority of organic liquids. It is a polar aprotic solvent with a high boiling point, which facilitates reactions that follow polar mechanisms. It can be hydrolyzed by strong acids and bases, especially at elevated temperatures [4].

2. EXPERIMENTAL

2.1. Materials

Solutions (0.05 $moldm^{-3}$) of L-Histidine (Kerala, India) and L-Glutamic acid (Kerala, India)) were prepared in triple-distilled water. To increase the solubility of ligands, 0.05 mol dm^{-3} nitric acid concentration was maintained in the solutions. The computer program COSWT determines the probable errors that may creep into the concentrations of the stock solutions of the ligands [5]. 0.2 $moldm^{-3}$ sodium nitrate was prepared to maintain the ionic strength in the titrand. Sodium hydroxide (Merck, India) of 0.4 $moldm^{-3}$ was prepared. All the solutions were standardized by standard methods.

The concentrations of alkali and mineral acid were determined using the Gran plot method [6].

2.2. Alkalimetric titrations

Alkalimetric titrations were carried out in media containing varying compositions of DMF (0-50% v/v) maintaining an ionic strength of 0.16 moldm^{-3} with sodium Nitrate at 310K. An Elico(Ad8000, India) pH meter (readability -2.00-16.00) was used. Potassium hydrogen phthalate (0.05 mol/dm^{-3}) and borax (0.01 moldm^{-3}) solutions were used to calibrate the pH meter. In each titration, the titrand contained approximately 1 mmol of Nitric acid while the concentration of ligand was of the order of 0.25-0.50 mmol in different experiments.

2.3. Modeling strategy

The best-fit chemical model for each system investigated was achieved at using non-linear least-squares computer program, MINIQUAD75 [7], 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.

3. RESULTS AND DISCUSSION

The alkalimetric titration curves for H and E in DMF-water mixtures are shown in Fig. 1. A perusal of the titration curves reveals that the acido-basic equilibria are active in the pH range 2.0-10.0. The computer program SCPHD [8] was used to prune the data obtained in different experiments so that it contains more information than noise and the logβs obtained from the program are used as initial values for final refinement.

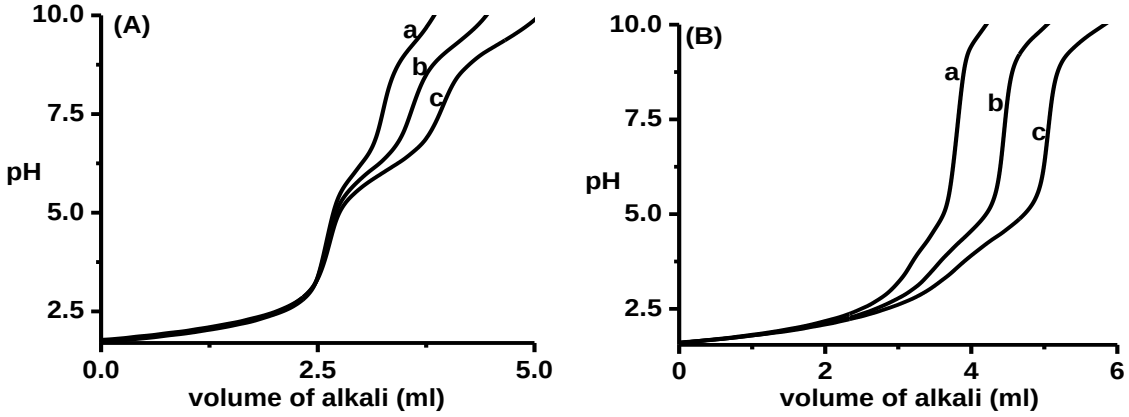

Fig. 1: Alkalimetric titration curves for (A) H and (B) E in 10% and 20% DMF- Water mixture: Number of mmols of H and E are a) 0.250, b) 0.375 and c) 0.500.

3.1. Secondary formation functions

Simultaneous neutralization and proton liberation are due to auto-ionization of ligand and many earlier investigators recognized complexation. Irving and Rossotti [9] systematized the formulae representing these two parallel reactions by introducing a function, formation function, $\bar{n}_H$. It is the average number of moles of protons bound per mole of uncomplexed ligand. It is a useful parameter for the detection of polymeric species (L_2H_x). If there are, no polymeric species a plot of $\bar{n}_H$ versus pH should overlap. Any deviation indicates the presence of polymeric species. Figure 2 shows that there is no polymerization of H and E. Another secondary function relevant to protonation equilibria is the number of moles of alkali consumed per mole of ligand, **a**. We can detect whether the given ligand solution contains acid or not by using **a**. The negative values of **a** correspond to the excess of mineral acid present in the titrand and the number of dissociable protons whereas the positive values correspond to the associable protons. Figure 2C shows that one equivalent of alkali is consumed before attaining pH 4.2.

3.2. Mathematical Models for Proton Ligand Equilibria of L-Histidine and L-Glutamic Acid

The computer program MINIQUAD75 [10] was used to refine protonation constants of ligands with DMF-water mixtures. The results of the best-fit model along with some of the important statistical parameters of H and E are given in Tables1.

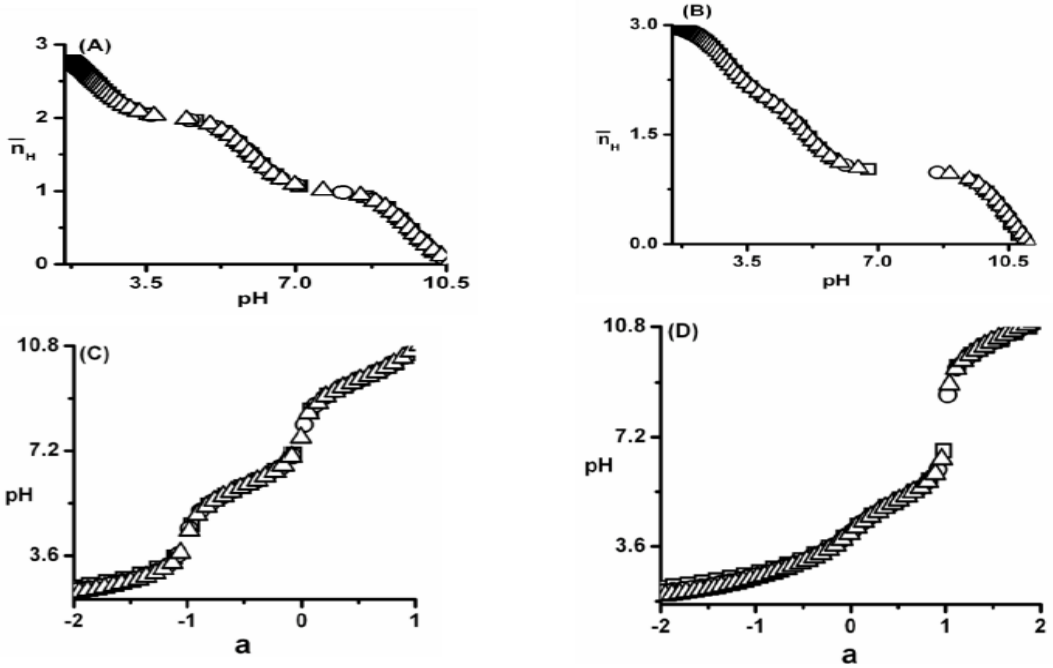

Fig. 2: Formation curves of H and E and number of moles of alkali (a) versus pH curves of (C) H (D) E in 40% v/v DMF-water mixture.Ligand concentrations in mmols are 0.25(□-□), 0.375(O-O) and 0.5(Δ-Δ).

% of DMF	log β_{mlh}(SD)			NP	U_{corr} x10^8	Skewness	Kurtosis	χ^2	R-factor
	LH	LH_2	LH_3						
H (pH range 1.72-10.28)									
0.0	9.24(0)	15.43(4)	17.16(1)	8.5	1.553	-0.32	3.26	12.87	0.0080
10.0	9.23(1)	15.30(1)	17.10(5)	8.5	1.342	-0.35	2.66	12.01	0.0092
20.0	9.27(3)	15.27(1)	17.23(4)	9.2	1.232	0.06	3.43	12.04	0.0034
30.0	9.35(3)	15.39(2)	17.40(4)	8.6	1.92	-0.83	3.31	12.33	0.0097
40.0	9.46(9)	15.40(8)	17.71(0)	9.9	2.44	-0.22	3.87	12.52	0.0042
50.0	9.48(4)	15.36(1)	17.72(1)	10.0	1.34	-0.37	2.99	11.99	0.0187
E (pH ranges 1.60-10.60)									
0.0	9.75(1)	14.00(1)	16.22(1)	80	3.27	-0.09	3.83	12.33	0.0084
10.0	9.83(0)	14.15(8)	16.40(0)	90	1.97	-0.02	3.03	11.97	0.0032
20.0	9.91(0)	14.50(9)	17.19(1)	77	3.24	-0.06	3.10	12.05	0.0036
30.0	10.06(1)	14.91(0)	17.73(3)	75	3.44	0.08	3.81	12.06	0.0045
40.0	10.23(6)	15.33(2)	18.26(5)	66	3.67	0.14	2.93	11.96	0.0072
50.0	10.21(9)	15.50(0)	18.70(6)	72	3.05	0.32	3.06	12.07	0.0067

Table 1: Best-fit chemical models of protonation equilibria of L-Histidine and L- Glutamic acid in DMF-water mixtures

U_{corr} = U/ (NP-m), m = number of species; NP =Number of experimental points; SD= Standard deviation

A very low standard deviation in log β values indicates the precision of these parameters. The small values of U (sum of squares of deviations in the concentrations of ligand and hydrogen ion at all experimental points) corrected for degrees of freedom indicates that the experimental data can be represented by the models [11].

3.3. 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 χ2 calculated is less than the table value, the model is accepted [6].

3.4. Crystallographic R-test

Hamilton's R factor ratio test[12] 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 1 indicate the sufficiency of the model.

3.5. 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 Tables 1 are between -0.83and 0.06 for H and -0.09 and 0.32 for E respectively. These data evince that the residuals form a part of normal distribution; hence, least-squares method can be applied to the present data.

3.6. 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) [11]. The kurtosis values in the present study for both H and E indicate that the residuals form mainly leptokurtic pattern.

3.7. Effect of systematic errors

In order to rely upon the best-fit chemical model for critical evaluation and application, a brief investigation was made by introducing pessimistic errors in the concentrations of alkali, mineral acid, ligand and volume. Results of a typical system are given in Tables 2 emphasize that protonation constants associated with carboxyl proton are more affected. This may be due to their lower magnitude than those for amino protons. Similarly, errors in concentration of alkali and acid, affect more than volume. With the introduction of errors, the SD's are found to increase inferring the appropriateness of experimental conditions and correctness of analytical concentrations.

Table 2: Effect of errors in influential parameters on protonation constants in 50% v/v DMF-water mixture

Ingredient	% Error	log β_{mlh}(SD)					
		Histidine			Glutamic acid		
		LH	LH_2	LH_3	XH	XH_2	XH_3
	0	9.48(4)	15.36(1)	17.72(1)	10.21(9)	15.50(0)	18.70(6)
Alkali	-5	Rejected	16.15(48)	18.36(49)	10.64(35)	15.98(69)	Rejected
	-2	9.55(44)	14.60(77)	16.80(34)	10.33(41)	15.39(91)	18.55(66)
	+2	9.29(98)	14.22(34)	Rejected	Rejected	14.63(44)	17.60(90)
	+5	9.97(99)	Rejected	16.90(39)	Rejected	14.99(23)	17.99(26)
Acid	-5	9.32(88)	14.69(33)	Rejected	9.75(74)	14.29(76)	16.67(34)
	-2	9.27(80)	15.09(85)	16.88(69)	Rejected	14.74(44)	17.41(14)
	+2	9.66(40)	15.68(69)	16.81(99)	9.23(41)	15.29(99)	17.20(94)
	+5	9.72(59)	Rejected	18.57(55)	9.44(56)	15.73(77)	Rejected
Ligand	-5	9.33(28)	15.36(25)	17.59(32)	10.94(15)	15.77(10)	18.62(12)
	-2	9.37(27)	15.32(23)	17.50(36)	10.03(15)	14.90(19)	17.70(11)
	+2	9.41(26)	15.30(22)	17.38(31)	10.15(17)	15.07(12)	18.81(15)
	+5	9.44(27)	15.33(23)	17.30(35)	10.23(39)	15.19(16)	17.88(19)
Volume	-5	9.39(14)	15.38(19)	17.73(10)	10.18(20)	15.47(19)	18.83(23)
	-2	9.39(15)	15.38(11)	17.68(13)	10.19(27)	15.48(12)	18.78(15)
	+2	9.39(29)	15.38(14)	17.70(17)	10.19(26)	15.49(12)	18.72(14)
	+5	9.39(39)	15.38(18)	17.64(21)	10.20(10)	14.99(17)	18.67(21)

3.8. Effect of Dielectric Constant of Solvent on Protonation Equilibria

Ligand protonation constants are strongly affected by the dielectric constant of the medium because of the fact that at least one of the constituents is charged and other either is charged or has a dipole moment. The change in dielectric constant varies the relative contributions of electrostatic and non-electrostatic interactions. The values of stepwise protonation constants are given in Table 3. The different forms of H are LH_3^{2+}, LH_2^{+}, LH and L^{-} in the pH ranges 1.8-4.0, 1.8-6.0, 6.0-7.5 and 6.5-11.0, respectively. In the case of E contains two carboxylic and amino protons. The different forms of E are XH_3^{+}, XH_2, XH^{-} and X^{2-} in the pH ranges 2.0-4.2, 3.5-5.5, 3.0-10.5 and 7.4-11.0, respectively.

Table 3: Stepwise protonation constants of H His and E in aqua-organic mixtures

% v/v solvent	L- Histidine			L- Glutamic acid		
	$logK_1$	$logK_2$	$logK_3$	$logK_1$	$logK_2$	$logK_3$
0.0	0.046	0.222	0.965	0.063	0.157	0.989
10.0	0.048	0.219	0.965	0.064	0.158	0.992
20.0	0.052	0.216	0.967	0.073	0.165	0.996
30.0	0.053	0.216	0.970	0.075	0.170	1.002
40.0	0.060	0.211	0.975	0.075	0.175	1.009
50.0	0.062	0.209	0.0976	0.081	0.181	1.009

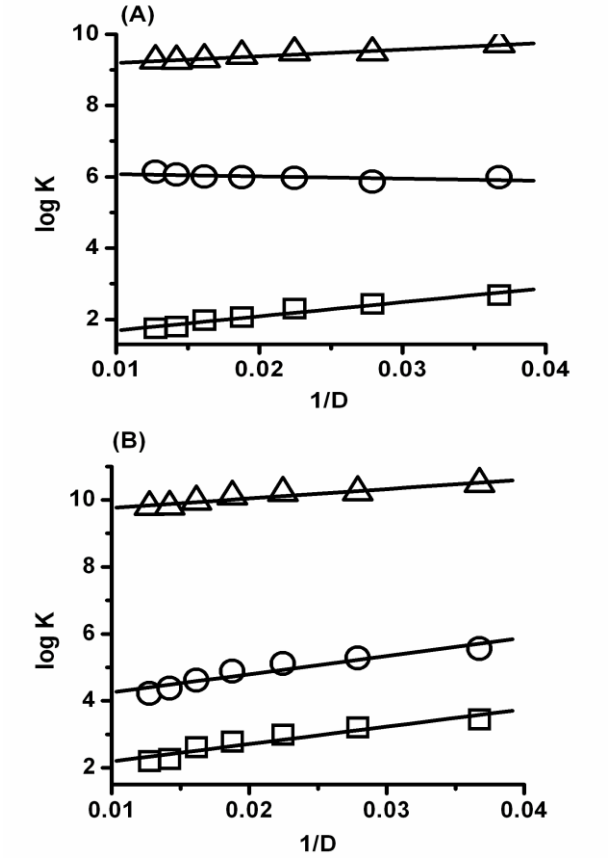

Fig. 3: Variation of stepwise protonation constant (logK) with reciprocal of dielectric constant (1/D) of solvent. (A) H and (B) E in DMF-water mixtures; (□) $logK_1$, (o) $logK_2$ and (Δ) $logK_3$.

In the present study, logKs of H and E increased linearly (Fig. 3) as a function of the reciprocal of the dielectric constant (1/D) of DMF-water mixtures. Since it has no polar groups, specific solvent-water interaction, charge dispersion and specific interaction of co-solvent with solute (indicated by the changes in the solubility of different species in the aqua-organic mixtures) account for the deviation of classical linear relationship of logK with 1/D.

The cation stabilizing nature of co-solvents specific solvent-water interactions [13], charge dispersion and specific interactions with solute indicated by the changes in the solubility of different species in aqua-organic mixtures account for the deviation of classical linear relationship of logK with 1/D.

The protonation-deprotonation equilibria of H and E are shown in Fig. 4. In these equilibria, H and E can exist in anionic, zwitterionic and cationic forms.

These observations can be explained as follows. When H (Equip. 1 -3) and E (Equip. 4 - 6) cations are successively deprotonated, the charge of the species is decreased, and low dielectric medium favors the protonation reaction, due to dominant electrostatic interactions. Thus, decrease in dielectric constant of the medium should increase the protonation constants.

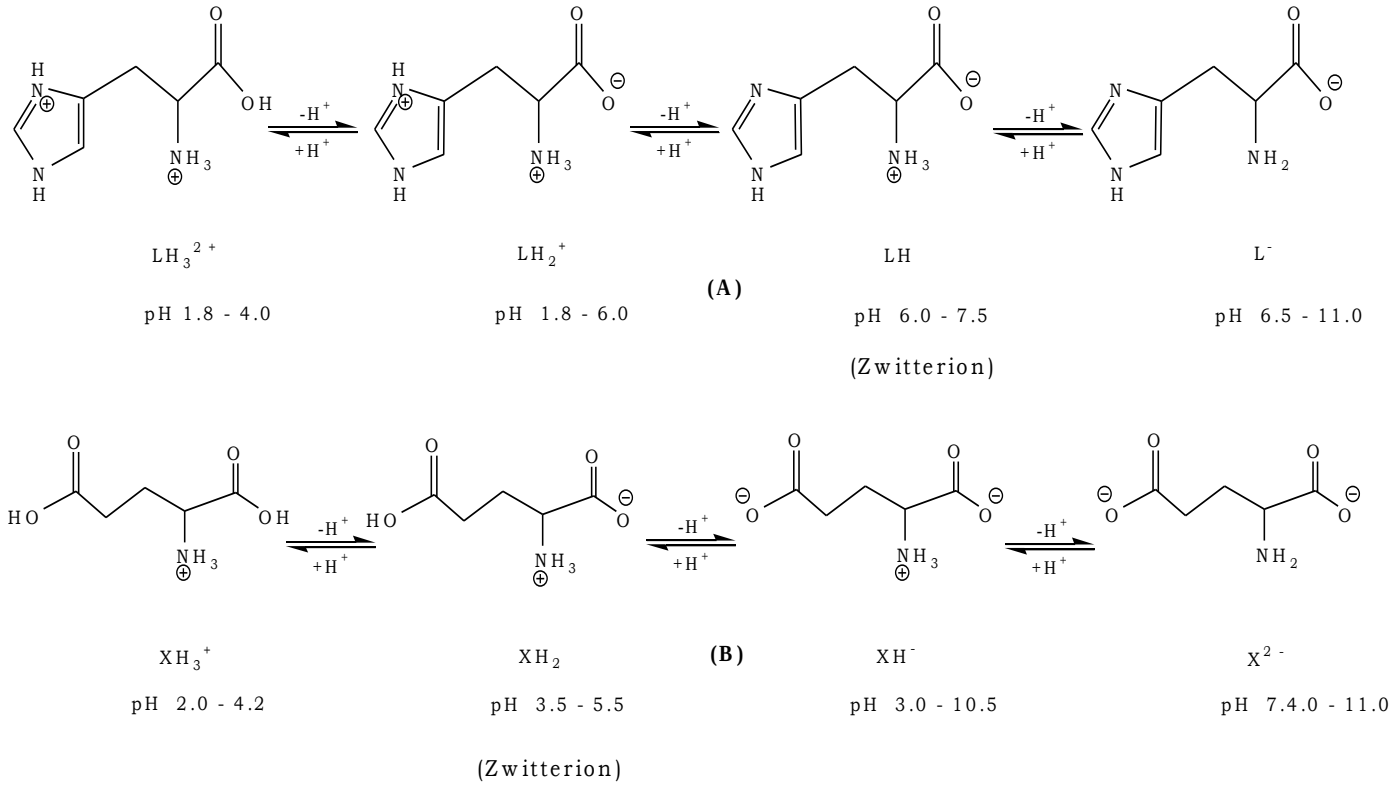

Fig. 4: Protonation-deprotonation equilibria of (A) H, and (B) E.

L-Histidine			
LH_3^{2+}	⇌	$LH_2^{+} + H^{+}$	----------(1)
LH_2^{+}	⇌	$LH + H^{+}$	----------(2)
LH	⇌	$L^{-} + H^{+}$	----------(3)
L-Glutamic acid			
XH_3^{+}	⇌	$XH_2 + H^{+}$	----------(4)
XH_2	⇌	$XH^{-} + H^{+}$	----------(5)
XH^{-}	⇌	$X^{2-} + H^{+}$	----------(6)

3.9. Distribution diagrams

The distribution plots (Fi. 5) produced using the protonation constants from the best fit models (Table 2) show the formation of LH_3^{2+}, LH_2^{+}, LH and L^- in the case of H and XH_3^{+}, XH_2, XH^- and X^{2-} in the case of E. In both the cases, LH/XH is present to an extent of 90% in the pH range 4.0-11.0. The most protonated species (LH_3^{2+} in the case of H and XH_3^{+}in the case E) exist in the pH range 2.0-4.0 and 2.0-5.0, respectively. LH_3^{2+} is deprotonated with increasing pH to form LH_2^{+}, LH and L^- in the pH ranges 2.0-7.0, 5.0-10.0 and above 8.0 respectively, in the case of H and XH_3^{+} is deprotonated with increasing pH to form XH_2, XH^- and X^{2-} in the pH ranges 2.0-6.0, 3.0-11.0 and above 8.0 in the case of E.

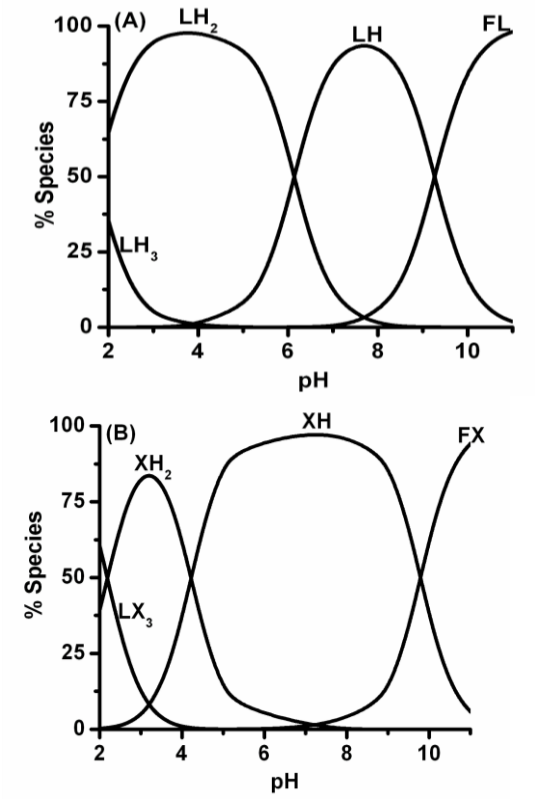

Fig. 5: Distribution diagrams of (A) H and (B) E in aqueous medium.Amount of H and E is 0.375 mmol.

4. CONCLUSION

The investigation of protonation studies indicates the pH ranges of protonation equilibria of H and E in DMF-water media as 1.8 -11.0and 2.0 -11.0 respectively. L-Histidine has three potential metal-binding sites, namely, carboxylate oxygen ($O_{caboxyl}$), imidazole imido nitrogen (N_{im}) and amino nitrogen (N_{am}). It exists as LH_3^{2+} at low pH and gets deprotonated with the formation of LH_2^+, LH and L^- successively with increasing pH.L-glutamic acid has three functional groups and all are deprotonated.E has two carboxylate groups and one amino groups with the formation of XH_3^+, XH_2, XH^- and X^{2-} in the pH ranges 2-11.0. The monoprotonated species XH^- exists in the pH range 3.0-10.5. Successive deprotonation of XH_3^+ withincreasing pH forms ultimately X^{2-} above a pH of 8.0 in DMF- water mixtures. Overlapping of formation curves for various concentrations of the ligands indicate non-polymerization of H and E under the pH conditions employed.The negative values of **a** correspond to the excess of mineral acid present in the titrand and the number of dissociable protons whereas the positive values correspond to the associable protons. The effect of systematic errors in the influential parameters shows thatprotonation constants associated with carboxyl proton are more affected. This may be due to their lower magnitude than those for amino protons. Similarly, errors in concentration of alkali and acid, affect more than volume.

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