Evaluation of Protonation Constants of L-Asparagine and Maleic Acid in Aqueous and Aqueous Solutions of Ethylene Glycol

Chemistry

KEYWORDS :Acid-base equilibria, L-asparagine, Maleic acid, Ethylene glycol, Protonation constant.

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ABSTRACT

The solute-solvent interactions of L-asparagine and maleic acid have been studied in 0-50% v/v ethylene glycol-water media using the pH-metric method. The protonation constants have been calculated with the computer program MINIQUAD75. Selection of the best fit chemical model of the acid-base equilibria is based on standard deviation in protonation constants and residual analysis using crystallographic R-factor and sum of squares of residuals in all mass balance equations. Linear variation of protonation constants with inverse of dielectric constant of the solvent mixture has been attributed to the dominance of the electrostatic forces. Distributions of species, protonation equilibria and effect of influential parameters on the protonation constant have also been presented.

INTRODUCTION

The solvent effects of phenols, amines and carboxylic acids have been examined[1]. A number of studies have been reported on protonation constants of α-amino acids in different media[2-5]. Acidity and basicity of a molecule is governed by its structure and solvent effects[6, 7]. The present work is an attempt to study the effects of organic solvent on the dissociation equilibria of the two biologically or industrially useful acids, viz., L-asparagines and maleic acids in the ethylene glycol - water mixture. Ethylene glycol (EG) is a protophilic dipolar protic solvent and acts as a structure former. Ethylene glycol, having two hydroxyl groups, is distinctly different from monohydric alcohols. EG is more acidic (less basic) than water[8] due to the electron withdrawing effect9 of the CH^2 group. EG offers several advantages as solvent in titration of weak bases[10]. Ethylene glycol plays an important role in protein conformation studies[11,12] because it is a weak protein denaturant compared to urea or other organic solvents such as ethanol, dioxane etc. The protonation equilibria of L-asparagine have been studied in the presence of ethylene glycol to understand the influence of solvent on the chemical speciation.

EXPERIMENTAL

Materials

Solutions (0.05 mol dm^{-3}) of maleic acid (Merck, India) and L-asparagine (Qualigens, India) were prepared in triple-distilled water by maintaining 0.05 mol dm^{-3} nitric acid concentration to increase the solubility. Ethylene glycol (Merck, India) was used as a solvent. Nitric acid (Merck, India) of 0.2 mol dm^{-3} was prepared. Sodium nitrate (Merck, India) of 2 mol dm^{-3} was prepared to maintain the ionic strength in the titrand. Sodium hydroxide (Merck, India) of 0.4 mol dm^{-3} 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)[13]. The strengths of alkali and mineral acid were determined using the Gran plot method[14, 15].

Alkalimetric Titrations

Alkali metric titrations were carried out in media containing varying compositions of Ethylene glycol (0-50% v/v) maintaining an ionic strength of 0.16 mol dm-3 with sodium nitrate at 303 ± 0.05K. An Elico LI-120 pH meter was used. Potassium hydrogen phthalate (0.05 mol dm-3) and borax (0.01 mol dm-3) solutions were used to calibrate the pH meter. In each titration, the titrand consisted of approximately 1 mmol of nitric acid. The amounts of the Ligands in the titrands ranged between 0.25 and 0.50 mmols. The glass electrode was equilibrated in a well stirred EG - water mixture containing inert electrolyte for several days. At regular intervals the strong acid was titrated against alkali to check the complete equilibration of the glass electrode. The calomel electrode was refilled with Ethylene glycol -water mixture of equivalent composition as that of the titrand. The details of experimental procedure and titration assembly have been detailed elsewhere[16-19].

Modeling Strategy

The approximate protonation constants of L-asparagine and maleic acid were calculated with the computer program SCPHD[20-23]. The best fit chemical model for each system investigated was arrived at by using non-linear least-squares computer program, MINIQUAD75[24], which exploit 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 mole fraction of the medium was analyzed on electrostatic grounds for the solute-solute and solute-solvent interactions.

RESULTS AND DISCUSSION

The computer program SCPHD[25] was used to calculate the correction factor applied to pH meter dial reading to calculate approximate protonation constants of L-asparagine and maleic acid. The best fit chemical model for each system investigated was arrived at using non-linear least-squares computer program, MINIQUAD75 which exploits the advantage of constrained least-squares method in the initial refinement and reliable convergence of Marquardt algorithm[26.] The variation of stepwise protonation constants was analyzed mainly on electrostatic grounds on the basis of solute-solute and solute-solvent interactions.

Table 1 Best fit chemical model of acido-basic equilibria of L-asparagine and maleic acid in EG -water mixtures. Temp= 303 K, Ionic strength= 0.16 mol $dm^{-3.}$

% v/v EG	$\log \beta_1$ (SD)	$\log \beta_2$ (SD)	NP	U_{corr}	Skewness	Kurtosis	x^2	R-factor
L-asparagine (pH range: 2.80 - 9.00)								
0.0	8.87(2)	11.18(4)	31	4.78	0.03	2.60	0.97	0.0185
10.0	8.84(1)	11.27(2)	31	1.80	0.06	2.96	3.55	0.0122
20.0	8.77(1)	11.28(4)	25	2.63	-0.10	2.40	3.44	0.0169
30.0	8.72(1)	11.41(3)	35	2.62	0.07	2.84	4.29	0.0155
40.0	8.57(2)	11.54(4)	45	7.02	0.18	2.78	7.42	0.0251
50.0	8.47(3)	11.70(5)	29	5.26	0.40	2.90	2.97	0.0266
Maleic acid (pH range: 2.00 -7.00)								
0.0	5.57 (3)	7.20 (5)	87	19.52	1.97	10.48	19.66	0.0331
10.0	5.72 (4)	7.38 (7)	82	43.95	1.47	7.59	25.27	0.0488
20.0	5.91 (3)	7.64 (5)	21	2.03	1.65	8.63	9.05	0.0130

30.0	5.82 (3)	7.85 (5)	93	34.90	1.27	5.36	4.80	0.0404
40.0	5.88 (5)	8.00 (7)	32	26.21	0.09	4.78	16.00	0.0282
50.0	6.39 (1)	8.45 (1)	80	2.81	0.63	4.07	9.00	0.0113

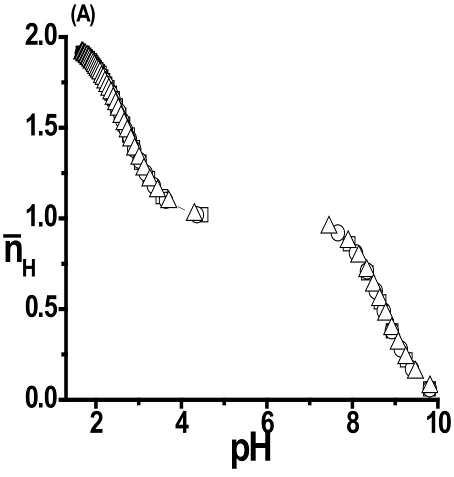
Ucorr = U/ (NP-m) X 108; NP = Number of points; m = number of protonation constants; SD = Standard deviation

The results of best fit models that contain the type of species and overall protonation constants of L-asparagine and maleic acid along with some important statistical parameters are given in Table 1. A low standard deviation in log β values indicates the precision of these parameters. The small values of U_{corr} (the sum of the 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 system confirm that the residuals are around a zero mean with little dispersion. 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 the residuals form mesokurtic pattern in case of L- asparagine and leptokurtic patterns in case of maleic acid. The values of skewness recorded in the table are between -0.10 and 1. 65. These data evince that the residuals form part of a 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. Thus, the statistical parameters show that the best fit models represent the acid-base equilibria of L-asparagine and maleic acid in EG-water mixtures.

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 for different concentrations of the ligand should overlap if there is no formation of polymeric species. Overlapping formation curves for maleic acid and L-asparagine (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. Two half integrals 1.5 and 0.5 in the case of L-asparagine Figure 1(A) and maleic acid Figure 1(B) emphasize the presence of two 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.

The plots of a versus pH are given in Figure 1. The negative values of a corresponds to the number of moles of free acid present in the titrand and the number of associable protons. The positive values of a indicate the number of dissociable protons in the ligand molecules. The maximum value of a in Figure 1(C) is +1, which indicates that L-asparagine has one dissociable (one carboxyl) proton. The corresponding value for a in Figure 1(D) is +2, which clearly that maleic acid has two dissociable (two carboxyl) protons.



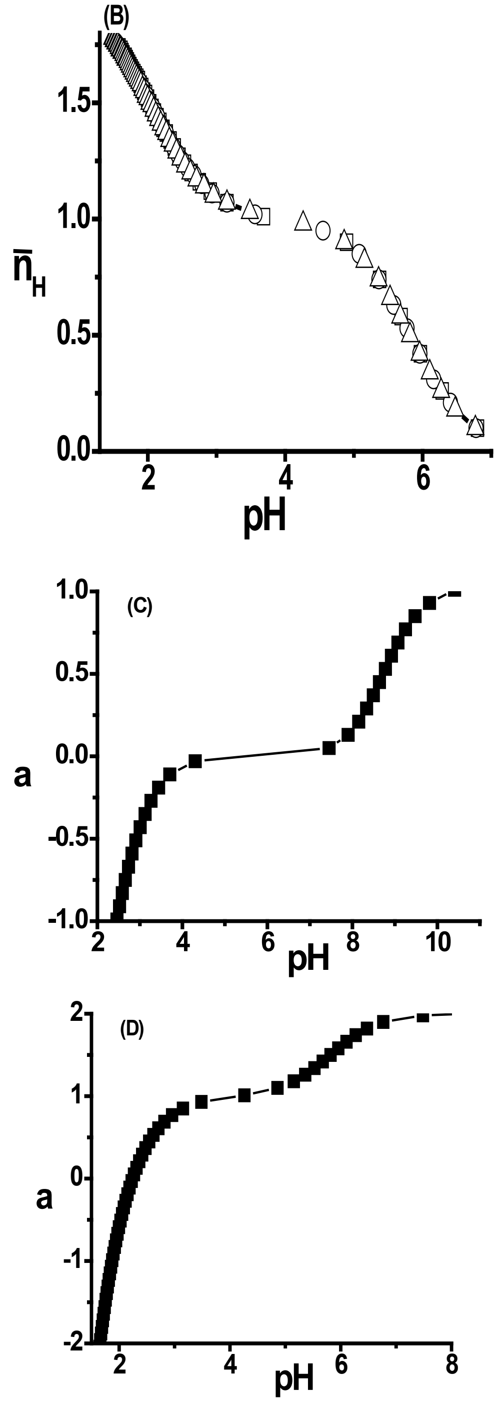


Figure 1 Plots of $\bar{n}_H$ versus pH in 30% v/v EG - water mixture: (A) L-asparagine (B) maleic acid, (□) 0.25, (○) 0.375, and (Δ) 0.50 mmol, respectively. Variation of a with pH in 30 % v/ EG -water mixture: (C) L-asparagine (D) maleic acid, respectively

Distribution Diagrams

Typical distribution plots produced by DISPLOT[27] using protonation constants from the best fit models are shown in Fig. 2. Representative plots show the existence of LH_2^+, LH and L^- in the case of L-asparagine and LH_2, LH- and L^{2-} in the case of maleic acid in different pH ranges. LH form of L-asparagine is present to an extent of 95 % in the pH range 2.0-11.0. LH- form of maleic acid is present to an extent of 95 % in the range 1.5-8.0. The higher protonated species (LH_2^+ in the case of L-asparagine and LH$_2^+$, in the case of maleic acid) exist below a pH of 4.0. LH$_2^+$ is deprotonated with increasing pH to form LH and L- in the pH ranges 2.0-11.0 and 6.0-11.0 respectively, in the case of L$^-$asparagine. LH^2 is deprotonated with increasing pH to form LH$^-$ and L2$^+$ in the pH ranges 1.5-8.0 and 4.0-9.0 respectively, in the case of maleic acid. The corresponding protonation-deprotonation equilibria are shown in Figure 3.

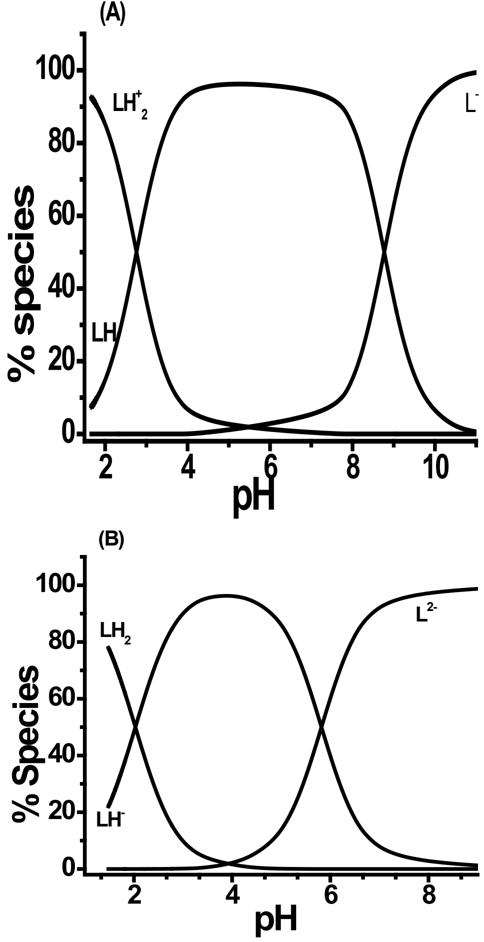


Figure 2 Species distribution diagrams of (A) L-asparagine (B) maleic acid in 30 % v/v EG -water mixture.

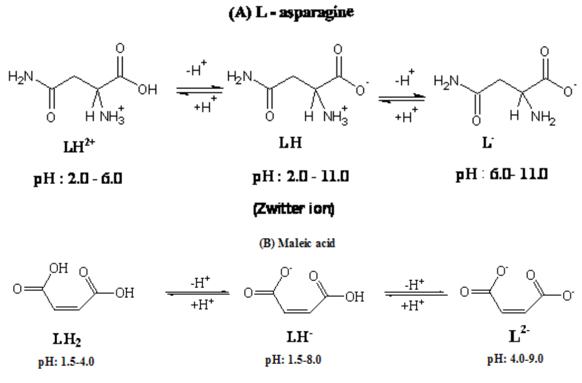


Figure 3 Protonation-deprotonation equilibria of (A) L-asparagine (B) maleic acid

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[28]. According to this treatment, the energy of electrostatic interaction is related to dielectric constant. Hence, the logarithm of step-wise protonation constant (log K) should linearly vary as a function of the reciprocal of the dielectric constant (1/D) of the medium. These plots (Figure 4) in EG-water mixtures show that the log K values are linearly vary with decreasing dielectric constant values. L-asparagine exists as anion, zwitterion and cation (Figure 3A) at different pH values. The cation stabilizing nature of co-solvent, specific solvent-water interactions, charge dispersion and specific interactions of co-solvent with solute account for the linear relationship of log k with 1/D. K1 and K2 are step-wise protonation constants for the reactions mentioned in Figure 3.

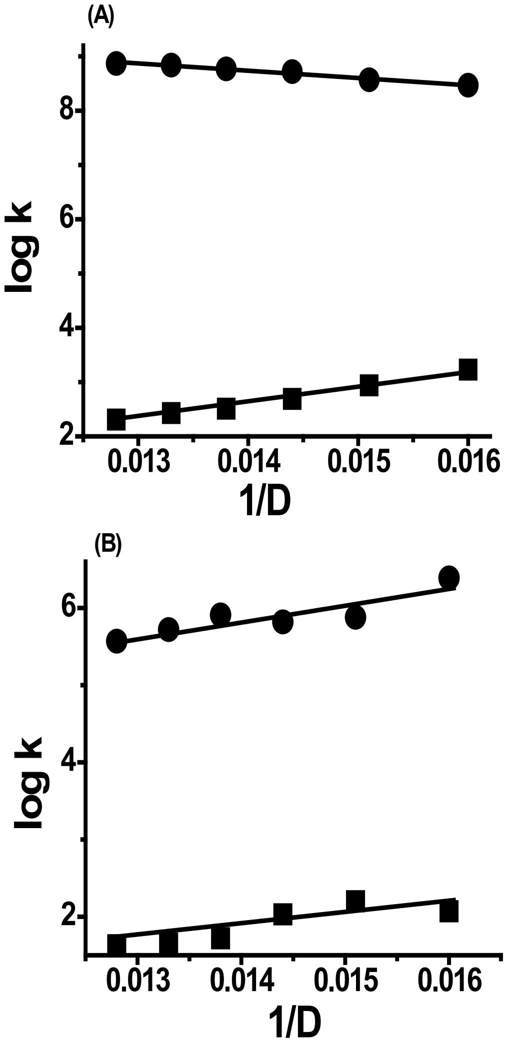


Figure 4 Variation of stepwise protonation constant (log K) with reciprocal of dielectric constant (1/D) in EG-water mixtures (A) L-asparagine (B) maleic acid (■) log K$_1$ (●) log K$_2$.

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 fit chemical model for critical evaluation and application under varied experimental conditions 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 2 emphasize that the errors in the concentrations of alkali and mineral acid affects the protonation constants more than that of the ligand.

Table 2 Effect of errors in influential parameters on the protonation constants in 30 % v/v EG-water mixture.

Ingredient	% Error	L-asparagine		Maleic acid	
		log β_1(SD)	log β_2(SD)	log β_1(SD)	log β_2(SD)
Acid	0	8.72(1)	11.41(3)	5.82(4)	7.85(5)
	-5	8.48(2)	10.43(5)	5.56(6)	7.34(9)
	-2	8.62(1)	11.18(2)	5.72(5)	7.65(6)
	+2	8.81(2)	11.62(4)	5.92(3)	8.04(4)
	+5	8.97(4)	11.95(7)	6.07(3)	8.32(4)
Alkali	-5	9.13(6)	12.20(10)	5.88(4)	7.97(6)
	-2	8.87(3)	11.71(5)	5.85(4)	7.89(5)
	+2	8.57(1)	11.10(2)	5.80(4)	7.80(6)
	+5	8.33(2)	10.56(8)	5.77(6)	7.74(8)
Ligand	-5	8.68(1)	11.40(3)	5.64(5)	7.61(7)
	-2	8.70(1)	11.41(2)	5.75(4)	7.75(6)
	+2	8.73(1)	11.41(2)	5.89(3)	7.93(5)
	+5	8.72(1)	11.41(2)	5.82(4)	7.85(5)

CONCLUSIONS

1. L-asparagine has one dissociable proton and one amino group which can associate with a proton. L-asparagine forms LH_2^+ at low pH and gets deprotonated with the formation of LH and L^- successively with increase in pH.
2. Maleic acid has two dissociable protons. Maleic acid form LH_2 at low pH and gets deprotonated with the formation of LH^- and $L2^-$ successively with increase in pH.
3. Secondary formation functions, number of moles of alkali per mole of the ligand and average number of moles of protons bound per mole of the ligands are useful in detecting the number of protonation equilibria and in guessing the approximate protonation constants.
4. The log values of protonation constants of L-asparagine and maleic acid increased linearly varies with decreasing dielectric constant of EG-water mixtures. This trend indicates the dominance of electrostatic forces in the protonation-deprotonation equilibria.
5. The effect of systematic errors on the influential parameters shows that the errors in the concentrations of alkali and mineral acid affect the protonation constants more than that of the ligand.

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