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Solvent effect on protonation equilibria of L-asparagine and maleic acid in dimethyl sulfoxide

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Abstract : A pH-metric study of protonation equilibria of L-asparagine and maleic acid in dimethyl sulfoxide-water media of varying composition (0–50% v/v) has been made at different concentrations of the ligands maintaining an ionic strength of 0.16 mol L^{-1} at 303.0 K. The best fit chemical models are arrived at using MINIQUAD75 based on statistical parameters like crystallographic *R* factor, χ^2, skewness and kurtosis. The variation in the protonation constants with the dielectric constant of the medium is attributed to the electrostatic and non electrostatic forces.

Keywords : Acid-base equilibria, L-asparagine, maleic acid, dimethyl sulfoxide, protonation constant, dielectric constant.

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 dimethyl sulfoxide-water mixture. DMSO is a polar aprotic solvent and is less toxic than other members of this class, such as dimethyl formamide, dimethyl acetamide, *N*-methyl-2-pyrrolidone, and HMPA. It is also extensively used as an extractant in biochemistry and cell biology. It penetrates the skin very readily, giving it the unusual property for many individuals of being secreted onto the surface of the tongue after contact with the skin and causing a garlic-like taste in the mouth[8]. It is industrially produced by oxidation of dimethyl sulfide with oxygen or nitrogen dioxide[9]. In medicine, DMSO is predominantly used as a topical analgesic, a vehicle for topical application of pharmaceuticals, as an anti-inflammatory, and an antioxidant. DMSO is finding increased use in manufacturing processes to produce microelectronic devices[10]. Because of its ability to dissolve many kinds of compounds, DMSO plays a role in sample management and high-throughput screening operations in drug design[11]. In organic synthesis, DMSO is used as a mild oxidant[12].

L-Asparagine is the non essential amino acid. It is stable under recommended storage and use condition. Exposure to temperatures above 30 °C can cause decomposition. L-Asparagine has no known toxicity. These products occur in baked goods such as French fries, potato chips and toasted bread. The precursor to asparagine is oxaloacetate. It is used in the life sciences industry for biochemical research and preparation of culture media. It is biodegradable, unlikely to accumulate in the food chain. It participates in the function of the brain and nervous system. It is required by the nervous system to maintain equilibrium and is also required for amino acid transformation from one form to the other done in the liver.

Maleic acid is an organic compound that is a dicarboxylic acid. It is a colorless crystalline (sugar and sand-like) material with a faint acid odor. The melting point is 135 °C. It is soluble in water and moderately toxic. The heat of combustion is –1355 kJ/mole. Inhalation causes irritation of nose and throat. Contact with eyes or skin causes irritation. Maleic acid is used to make artificial resins and anti-histamins, and to preserve fats and oils. Maleic acid is an industrial raw material for the production of glyoxylic acid by ozonolysis. The major industrial use of maleic acid is its conversion to fumaric acid.

Maleic acid and fumaric acid do not spontaneously interconvert because rotation around a carbon carbon double bond is not energetically favorable. However, conversion of the *cis* isomer into the *trans* isomer is possible by photolysis in the presence of a small amount of bromine.

Materials and methods :

Reagents :

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, dimethyl sulfoxide (Merck, India) was used as 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 :

Alkalimetric titrations were carried out in media containing varying compositions of DMSO (0–50% v/v) maintaining an ionic strength of 0.16 mol dm^{-3} with sodium nitrate at 303 $\pm$ 0.05 K. 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 mmol. The glass electrode was equilibrated in a well stirred DMSO-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 DMSO-water mixture of equivalent composition as that of the titrand. The details of experimental procedure and titration assembly have been detailed elsewhere[16].

Modeling strategy :

The approximate protonation constants of L-asparagine and maleic acid were calculated with the computer program SCPHD[17]. The best fit chemical model for each system investigated was arrived at by using non-linear least-squares computer program, MINIQUAD75[18], 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

Residual analysis[19] :

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. When the data are fit into the models, the residuals should be ideally equal to zero. Further, a model is considered adequate only if the residuals do not show any trend. Respecting the hypothesis of the least squares analysis, the residuals are tested for normal distribution. Such tests are χ^2, skewness, kurtosis and *R*-factor. These statistical parameters of the present data shows that the best fit models portray the acido-basic equilibria of L-asparagine and maleic acid in DMSO-water mixtures, as discussed below.

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.

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 1 indicate the sufficiency of the model.

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

% v/v DMSO	log β_1 (SD)	log β_2 (SD)	NP	U_{corr}	Skewness	Kurtosis	χ^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	8.75(2)	11.31(6)	34	11.19	–0.17	2.36	0.94	0.0305
20.0	8.56(2)	11.28(5)	40	10.09	0.16	2.03	10.00	0.0292
30.0	8.74(1)	11.40(6)	25	2.94	0.23	2.56	5.68	0.0175
40.0	8.74(2)	11.42(3)	36	4.93	0.28	2.81	6.89	0.0199
50.0	8.73(1)	11.48(2)	37	1.87	0.01	7.44	6.65	0.0119
			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	5.68(3)	7.29(5)	80	17.07	1.42	8.88	8.00	0.0307
20.0	5.82(2)	7.41(4)	82	15.30	2.31	11.19	15.71	0.0286
30.0	6.25(3)	7.82(5)	76	20.34	0.02	3.04	6.11	0.0322
40.0	6.62(5)	8.11(8)	67	35.08	–0.01	3.44	3.79	0.0414
50.0	7.20(6)	8.49(9)	55	22.79	–0.08	2.04	9.42	0.0326

U_{corr} = U/(NP – m) × 10^8; NP = number of points; m = number of protonation constants; SD = standard deviation.

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 1 are between –0.17 and 1.97. These data evidence that the residuals form a part of normal distribution; hence, least-squares method can be applied to the present data.

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 platykurtic pattern in the case of L-asparagine and leptokurtic pattern in the case of maleic acid.

The primary alkalimetric titration data in DMSO-water mixture are given in the Table 1 and the typical curves are in Fig. 1.

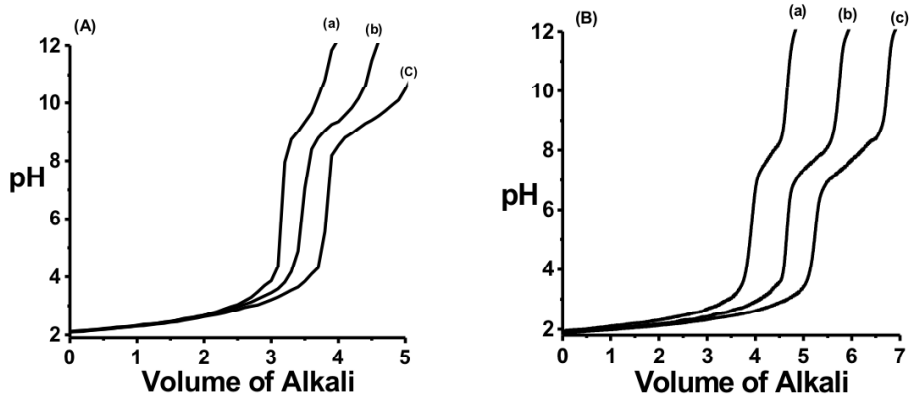

Fig. 1. Alkalimetric titration curves in 40% v/v DMSO-water mixture : (A) L-asparagine, (B) maleic acid; (a) 0.25, (b) 0.375 and (c) 0.50 mmol, respectively.

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 (Fig. 2) 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 1.25 and 0.25 in the case of L-asparagine Fig. 2(A) and maleic acid Fig. 2(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 Fig. 3. 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 Fig. 3(A) is +1, which indicates that L-asparagine has one dissociable (one carboxyl) proton. The corresponding value for **a** in Fig. 3(B) is +2, which clearly that maleic acid has two dissociable (two carboxyl) protons.

Distribution diagrams :

Typical distribution plots produced by DISPLOT[20] using protonation constants from the best fit models are shown in Fig. 6. 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 99% in the pH range 1.5–11.0. LH^- form of

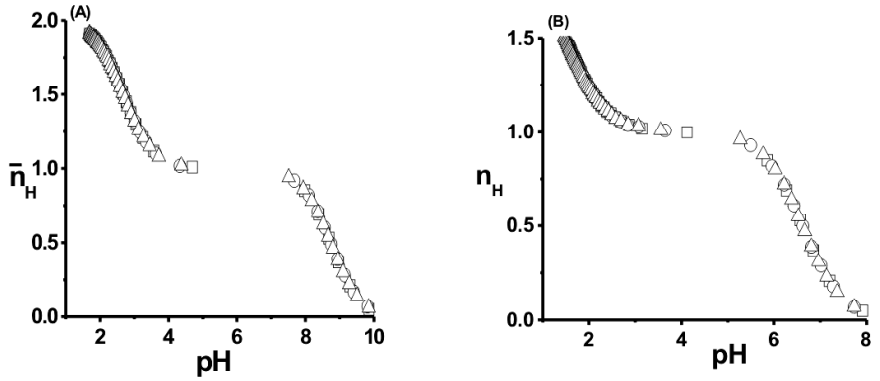

Fig. 2. Plots of $\bar{n}_H$ versus pH in 40% v/v DMSO-water mixture : (A) L-asparagine, (B) maleic acid; (□) 0.25, (○) 0.375 and (Δ) 0.50 mmol, respectively.

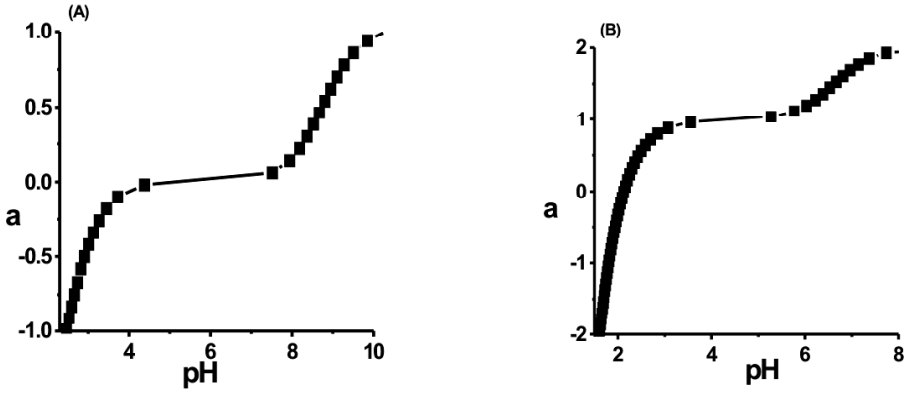

Fig. 3. Variation of **a** with pH in 40% v/v DMSO-water mixture : (A) L-asparagine, (B) maleic acid, respectively.

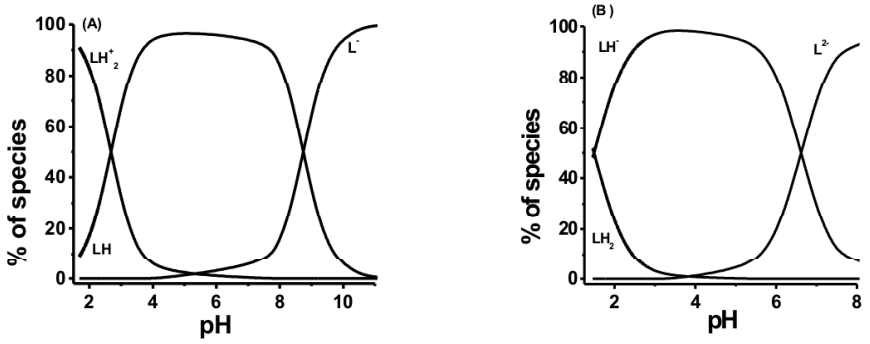

Fig. 4. Species distribution diagrams of (A) L-asparagine, (B) maleic acid in 40% v/v DMSO-water mixture.

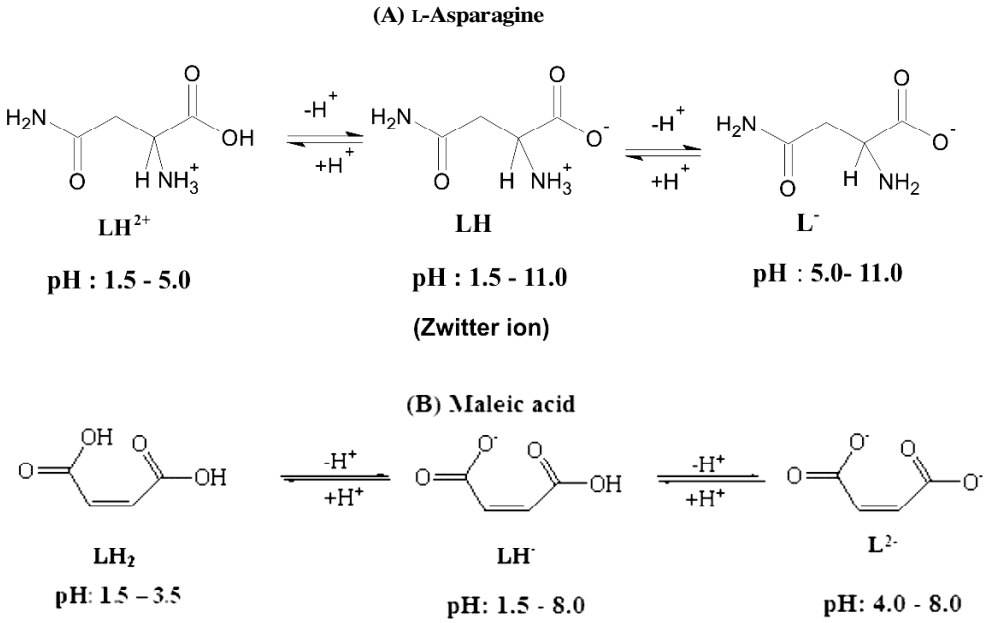

Fig. 5. Protonation-deprotonation equilibria of (A) L-asparagine, (B) maleic acid.

maleic acid is present to an extent of 99% 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 1.5–11.0 and 5.0–11.0 respectively, in the case of L-asparagine. LH_2 is deprotonated with increasing pH to form LH^- and L^{2-} in the pH ranges 1.5–8.0 and 4.0–8.0 respectively, in the case of maleic acid.

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[21]. According to this treatment, the energy of electrostatic interaction is related to dielectric constant. Hence, the logarithm of stepwise protonation constant (log K) should vary linearly as a function of the reciprocal of the dielectric constant ($1/D$) of the medium. These plots (Fig. 6) in DMSO-water mixtures show that the log K values are linearly varies with decreasing dielectric constant values. L-Asparagine exists as anion, zwitterion and cation (Fig. 5A) 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 $1/D$. K_1 and K_2 are stepwise protonation constants for the reactions mentioned in Fig. 6.

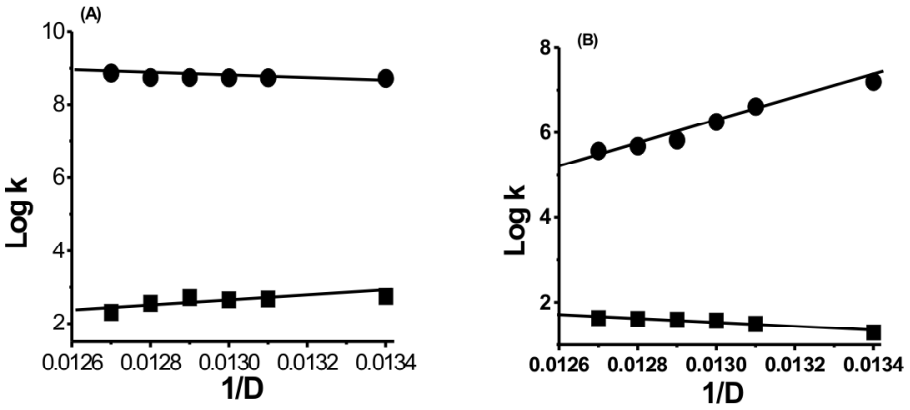

Fig. 6. Variation of stepwise protonation constant (log *K*) with reciprocal of dielectric constant (1/*D*) in DMSO-water mixtures : (A) L-asparagine, (B) maleic acid; (■) log K_1 (●) log K_2.

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 20% v/v DMSO-water mixture

		L-Asparagine		Maleic acid	
Ingredient	% Error	log β_1 (SD)	log β_2 (SD)	log β_1 (SD)	log β_2 (SD)
	0	8.56(2)	11.28(5)	5.82(2)	7.41(4)
Acid	–5	8.32(3)	10.76(8)	5.90(10)	8.83(15)
	–2	8.33(1)	10.74(3)	6.04(10)	9.09(16)
	+2	8.64(3)	11.46(5)	6.23(10)	9.47(13)
	+5	8.67(3)	11.51(7)	6.26(10)	9.56(16)
Alkali	–5	8.95(5)	12.00(8)	6.62(11)	10.11(19)
	–2	8.71(3)	11.56(5)	6.34(10)	9.60(19)
	+2	8.41(2)	10.99(5)	5.94(11)	8.99(17)
	+5	8.18(3)	10.54(9)	3.78(23)	6.73(21)
Ligand	–5	8.51(2)	11.27(5)	5.98(10)	9.12(14)
	–2	8.54(2)	11.27(5)	6.08(11)	9.22(19)
	+2	8.57(2)	11.28(5)	6.20(10)	9.36(17)
	+5	8.60(2)	11.28(4)	6.29(10)	9.45(17)

Conclusions

(i) 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.

(ii) Maleic acid has two dissociable protons. Maleic acid form LH_2 at low pH and gets deprotonated with the formation of LH^- and L^{2-} successively with increase in pH.

(iii) 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.

(iv) The log values of protonation constants of L-asparagine and maleic acid linearly vary with decreasing dielectric constant of DMSO-water mixtures. This trend indicates the dominance of electrostatic forces in the protonation-deprotonation equilibria.

(v) 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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