

# Effect of Organic Solvent Dielectric Constant on Protonation Equilibria of Phenylalanine and Maleic acid

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## Abstract -

The protonation constants of Phenylalanine and Maleic acid have been studied pH metrically in various concentrations (0-60%v/v) of acetonitrile-water mixtures maintaining an ionic strength of  $0.16 \text{ mol L}^{-1}$  at 303K. The protonation constants have been calculated with the computer program MINQUAD75 and are selected based on statistical parameters. Linear variation and non linear variation of step-wise protonation constants ( $\log K$ ) with reciprocal of dielectric constant of the solvent mixture has been attributed to the dominance of the electrostatic and non-electrostatic forces respectively.

**Keywords -** Protonation constants, Phenylalanine, Maleic acid, Acetonitrile, MINQUAD75.

## I. INTRODUCTION

Phenylalanine (Phe) is a strong  $\alpha$ -amino acid [1]. This essential amino acid is classified as non-polar because of the hydrophilic nature of the benzyl side chain [2]. The first description of phenylalanine was made in 1879, when Schulze and Barbieri identified [3], [4]. Phe is found naturally in the breast milk of mammals. It is used in the manufacture of food and drink products and sold as a nutritional supplement for its reputed analgesic and antidepressant effects. It is a direct precursor to the neuro modulator phenylethylamine, Phe commonly used dietary supplement. Phe is the starting compound used in the flavonoid biosynthesis. Lignan is derived from phenylalanine and from tyrosine. It is converted to cinnamic acid by the enzyme phenylalanine ammonia-lyase. The biological functions of D-amino acids remain unclear, although D-phenylalanine has pharmacological activity at niacin receptor 2 [5]. L-Phenylalanine is an antagonist at higher doses, this may play a role in its analgesic and antidepressant properties [6]. In the brain, L-phenylalanine is a competitive antagonist at the glycine binding site of NMDA receptor [7] and at the glutamate binding site of AMPA receptor [8].

Maleic acid (Mal) is an organic compound. It is an unsaturated dicarboxylic acid, a molecule with two carboxyl groups. Mal is the cis-isomer of butenedioic acid, whereas fumaric acid is the trans-isomer. It is mainly used as a precursor to fumaric acid, and relative to its parent maleic anhydride, Mal is more soluble in water. The solvent effects of phenols, amines and carboxylic acids have been examined [9]. A number of studies have been reported on protonation constants of  $\alpha$ -amino acids in different media [10]-[13]. Acidity and basicity of a molecule is governed by its structure and solvent effects [14], [15].

Acetonitrile (AN) is a weak base [16] and a much weaker acid [17] than water. Therefore cations and especially anions have lower solvation energies in acetonitrile than in water, except in those cases where there is specific interaction with the solvent, thus cations are reduced at considerably more positive [18] potential as in acetonitrile than in water. It is a protophilic dipolar aprotic solvent and it does not form any hydrogen bond with solute species. The protophilic character of AN may arise from the possible formation of dimers which are shown to exist from IR studies [19]. Very few studies have been reported in the literature [20]-[22], hence the author is reporting the protonation constants of phenylalanine and maleic acid in acetonitrile-water mixtures.

The protonation equilibria of Phe and Mal studied in the presence of acetonitrile to understand the influence of co-solvent on the chemical speciation.

## II. EXPERIMENTAL

### A. Materials

0.05 mol L<sup>-1</sup> solutions of Phenylalanine and Maleic acid (GR, E-Merck, Germany) was prepared in triple distilled water by maintaining 0.05 mol L<sup>-1</sup> hydrochloric acid (HCl) concentration to increase the solubility. Acetonitrile (AR, E-Merck) is used as received. Sodium chloride was prepared to maintain the ionic strength in the titrand. Sodium hydroxide of 0.4 mol L<sup>-1</sup> was prepared. The strengths of alkali and mineral acid were determined using the Gran plot method [23], [24].

### B. Procedure

The titrimetric data were obtained by using calibrated ELICO (Model LI-120) pH-meter (readability 0.01). The glass electrode was equilibrated in a well stirred solvent solution containing inert electrolyte. The effects of variations in asymmetry, liquid junction potential, activity coefficient, sodium ion error and dissolved carbon dioxide on the response of glass electrode were accounted for in the form of correction factor. For the determination of protonation constants of Phe and Mal, initially titrations of strong acid with alkali were carried out at regular intervals to check whether complete equilibration was achieved. Then the calomel electrode was refilled with solvent solution of equivalent composition as that of titrand. All the titrations have been carried out in the medium containing varying concentrations of solvent (0.0-60.0% v/v) maintaining an ionic strength of 0.16 mol L<sup>-1</sup> with sodium nitrate at 303.0 ± 0.1K. In each of the titrations, the titrand consisted of approximately 1 mmol mineral acid in a total volume of 50 cm<sup>3</sup>. Titrations with different volumes (2.5, 3.5 and 5.0) of ligand were carried out with 0.4 mol L<sup>-1</sup> sodium hydroxide. Other experimental details are given elsewhere [25].

### C. Alkalimetric Titration Assembly

The glass electrode was equilibrated in a well stirred acetonitrile-water mixtures containing inert electrolyte for several days. At regular intervals titration of acid with alkali was carried out to check whether complete equilibration had been achieved or not. The calomel electrode was refilled with acetonitrile and ethylene glycol-water mixtures of equivalent composition as that of the titrand. Experimental procedure and titration assembly were given elsewhere [26]. Typical alkalimetric titrations are given in Figure 1.

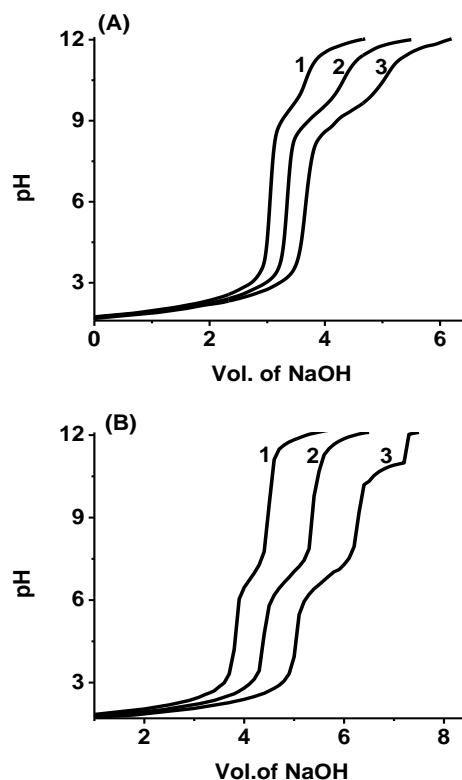


Fig. 1 Alkalimetric Titration Curves of (A) Phenylalanine and (B) Maleic Acid in 20% v/v of Acetonitrile. 1, 2 and 3 indicate 0.25, 0.375, 0.5 mmol of Ligand Respectively.

### D. Modeling Strategy

The approximate protonation constants of Phe and Mal were calculated with the computer program SCPHD [26] and they were refined using non-linear least-square computer program MINQUAD75 [27]. The variation of overall protonation constants were analyzed on electrostatic grounds on the basis of solute-solute and solute-solvent interactions.

## III. RESULTS AND DISCUSSION

The best fit models that contain the type of species and overall protonation constants ( $\log \beta$ ) along with some of the important statistical parameters are given in Table 1 and were correlate with literature values. A very low standard deviation in  $\log \beta$  values indicates the precision of these parameters. The small values of U (sum of the squares of deviations in concentrations of ligand and hydrogen ion at all experimental points) corrected for degree of freedom, indicate that the experimental data can be represented by the models. Small values of mean, standard deviation and mean deviation for the systems corroborate 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 most of the residuals form leptokurtic patterns and very

**Table 1: Best Fit Chemical Models of Acido-Basic Equilibria of Phenylalanine and Maleic Acid in Acetonitrile-Water Mixtures. Temp = 303 K, Ionic Strength = 0.16 Mol Dm<sup>-3</sup>.**

%v/v AN	log β <sub>1</sub> (SD)	log β <sub>2</sub> (SD)	log K <sub>1</sub>	log K <sub>2</sub>	NP	U <sub>corr</sub>	Skewness	Kurtosis	χ <sup>2</sup>	R-factor	pH-Range
PHENYLALANINE											
0	9.37771(1)	12.3263(1)	2.94857	9.37771	41	2.48	2.66	10.65	34.49	0.051977	3.4-10.5
10	9.29062(2)	12.3373(1)	3.04666	9.29062	67	3.64	0.89	3.95	3.07	0.030134	3.4-10.5
20	9.4963(3)	12.7072(1)	3.21088	9.4963	64	2.01	0.89	4.91	6.50	0.036665	3.4-10.5
30	8.98652(2)	11.6175(2)	2.63102	8.98652	61	4.12	0.80	3.90	4.03	0.056502	3.4-10.5
40	9.45737(3)	12.578(2)	3.12065	9.45737	63	2.52	0.84	3.99	4.67	0.027181	3.4-10.5
50	9.46442(3)	12.8621(2)	3.39768	9.46442	63	3.31	0.54	3.19	1.87	0.025592	3.4-10.5
60	9.6714(1)	13.2277(2)	3.5563	9.6714	62	1.89	0.40	4.25	2.84	0.019517	3.4-10.5
MALEIC ACID											
0	5.94589(1)	8.19342(1)	2.24753	5.94589	43	1.48	2.68	8.66	58.37	0.176216	2.9-10.8
10	6.32365(3)	9.08783(2)	2.76418	6.32365	78	1.21	2.21	9.17	21.44	0.063194	2.9-10.8
20	6.83474(2)	9.63299(3)	2.79825	6.83474	78	3.61	0.80	3.47	11.28	0.046738	2.9-10.8
30	6.52651(1)	9.09348(3)	2.56697	6.52651	87	1.57	2.86	10.21	31.01	0.145912	2.9-10.8
40	6.39955(1)	8.92889(2)	2.52934	6.39955	91	2.52	0.94	3.82	16.15	0.050950	2.9-10.8
50	6.42358(1)	8.97786(1)	2.55428	6.42358	66	1.05	2.18	5.48	91.88	0.344524	2.9-10.8
60	6.03007(1)	8.61175(1)	2.58168	6.03007	62	3.89	0.34	2.47	4.13	0.028276	2.9-10.8

$$U_{\text{corr}} = U / (NP - m) \times 10^8; \text{ where } m = \text{number of species}; NP = \text{Number of experimental points}$$

few platykurtic patterns. The values of skewness are between 0.34-2.86. These data evince that the residuals from 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. The statistical parameters thus show that the best fit models portray the acido-basic equilibria of Phenylalanine and Maleic acid in acetonitrile-water mixtures.

#### A. Effect of Systematic Errors

In order to rely upon the best 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 concentrations of alkali, mineral acid and the ligand. The results of a typical system given in Table 2 emphasize that the errors in the concentrations of alkali and mineral acid affect the protonation constants more than those in the ligand.

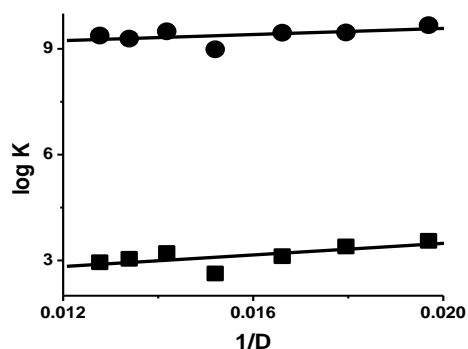
#### B. Effect of Solvent

Effect of solvent on protonation constant depends upon electrostatic and non-electrostatic factors. Born's classical treatment holds good in accounting for the electrostatic contribution [28] which is related to dielectric constant. Hence, the logarithm of overall protonation constants (log β) should vary linearly as a function of the reciprocal of dielectric constant (1/D) of the medium (Figure 2). The log β values are linearly increasing with decreasing dielectric constant of the medium in case of Phe and non-linear with decreasing dielectric constant of the medium in case of Mal in the medium(AN).

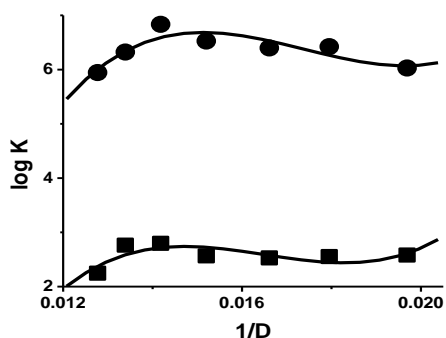
**Table 2: Effect of Errors in Influential Parameters on Protonation Constants of Phenylalanine and Maleic Acid In 30% V/V of Acetonitrile-Water Mixture.**

Ingredient	% Error	logβ <sub>mth</sub> (SD)	
		011	012
Phenylalanine			
Alkali	0	8.98(2)	11.61(2)
	-5	9.49(5)	12.55(7)
	-2	9.17(2)	11.97(4)
	+2	8.78(2)	11.26(2)
	+5	8.49(2)	10.75(4)
Acid	-5	8.64(2)	10.94(3)
	-2	9.03(2)	11.34(2)
	+2	9.11(2)	11.88(4)
Ligand	+5	9.32(4)	12.29(6)
	-5	8.83(2)	11.40(2)
	-2	8.92(2)	11.53(2)
	+2	9.03(2)	11.69(2)
	+5	9.11(2)	11.81(3)
	Maleic acid		
Alkali	0	6.52(1)	9.09(3)
	-5	7.00(2)	9.98(6)
	-2	6.71(1)	9.44(3)
	+2	6.32(2)	8.74(5)
	+5	6.01(3)	8.21(8)
Acid	-5	6.32(3)	8.43(7)
	-2	6.38(1)	8.83(5)
	+2	6.65(1)	9.35(3)
Ligand	+5	6.84(1)	9.74(5)
	-5	6.36(1)	8.87(4)
	-2	6.46(1)	9.00(4)
	+2	6.58(1)	9.17(4)
	+5	6.66(1)	9.29(3)

The linear variation of log β values of Phe in AN-water mixture (Figure 2) indicates the dominance of electrostatic forces over non-electrostatic forces. But the non-linear trend of Mal in AN-water mixture shows the dominance of non-electrostatic forces.



(A)



(B)

Fig. 2: Step-Wise Protonation Constant (Log K) With Reciprocal of Dielectric Constants (1/D) Of (A) Phenylalanine and (B) Maleic Acid in Acetonitrile-Water Mixtures. (■) Logk<sub>1</sub>, (●) Logk<sub>2</sub>.

These opposite trends are due to the opposite nature of Phe and Mal. Phe is amino acid has one amino group and one carboxylic acid group and it is aromatic nature with one phenyl group. Mal is dicarboxylic acid with two carboxylic acid groups both are equal with unsaturation. AN stabilizes uncharged species due to its protophilic nature and low dielectric constant and solvent-water interactions, charge dispersion, and specific interactions of co-solvent with solute (indicated by the changes in the solubility of different species in the aquo-organic mixtures) account for the deviation of classical linear relationship of log K with 1/D Phe exists as anion, zwitter ion and cation and Mal exists as ligand, mono charged anion and divalent anion (Figure 3) 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 β with 1/D.

### C. Distribution Diagrams

Secondary formation function ( $\bar{n}_H$ ) average number of moles of protons bound per mole of ligand is useful to detect the number of

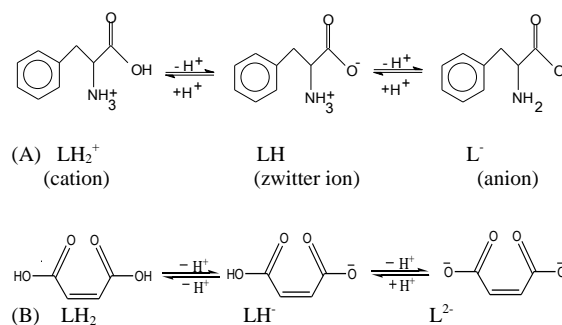


Fig. 3 Protonation-Deprotonation Equilibria Of (A) Phenylalanine and (B) Maleic Acid

protonation equilibria. The formation functions (Figure 4) indicate that Phenylalanine and Maleic acid has two equilibria. The pH values at half integrals of  $\bar{n}_H$  correspond to the log K values of the ligands.

The distribution plots (Figure 4) of (A) Phenylalanine and (B) Maleic acid indicate that  $LH_2^+$ , LH, L<sup>-</sup> and  $LH_2$ , LH<sup>-</sup>, L<sup>2-</sup> ligand forms are present to an extent of 80% in the pH range 2.0-10.0 and successive deprotonation takes place with increase in the pH.

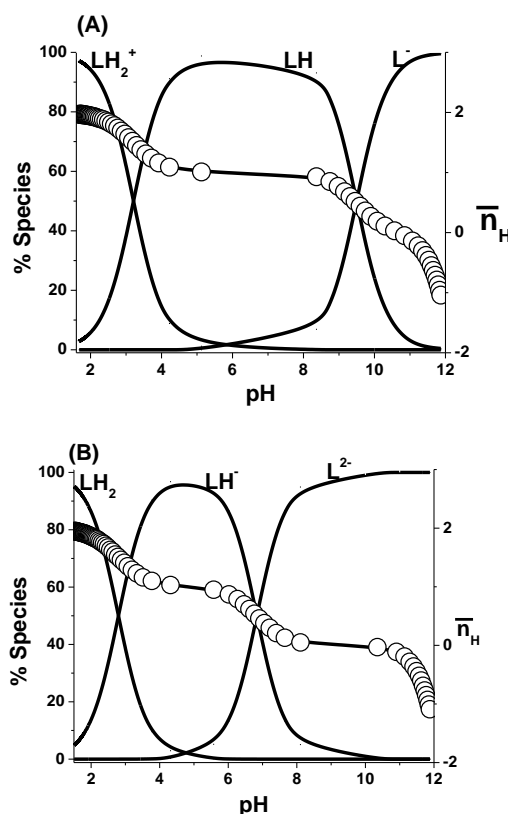


Fig. 4: Species Distribution Diagrams of (A) Phenylalanine and (B) Maleic Acid in 20% V/V Acetonitrile-Water Mixture.

#### IV. CONCLUSIONS

1. Phenylalanine forms  $\text{LH}_2^+$  at low pH and gets deprotonated with the formation of  $\text{LH}$  and  $\text{L}^-$  and Maleic acid in form of  $\text{LH}_2$  at low pH and gets deprotonated with the formation of  $\text{LH}^-$  and  $\text{L}^{2-}$  successively with increase in pH.
2. The log values of protonation constants increase linearly and non-linearly with decreasing dielectric constant of acetonitrile-water mixtures. This trend indicates the dominance of electrostatic and non-electrostatic forces in the protonation -deprotonation equilibria.
3. The effect of systematic errors in 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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