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Influence of Dielectric Constant on Protonation Equilibria of Phenylalanine and Maleic Acid in Ethylene Glycol-Water Mixtures

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Abstract: ***The influence of dielectric constant of medium on protonation equilibria has been studied by determining protonation constants of Phenylalanine and Maleic acid pH metrically in various concentrations (0-60%v/v) of ethylene glycol-water mixtures, at an ionic strength of 0.16 mol L^{-1} and at 303.0 K. MINIQUAD75 computer program has been used for the calculation of protonation constants. Linear and non-linear variations of step-wise protonation constants with reciprocal of dielectric constant of the solvent mixtures have been attributed to the dominance of the electrostatic and non-electrostatic forces, respectively. The trend is explained on the basis of solute-solute and solute-solvent interactions, solvation, proton transfer processes and dielectric constants of the media.***

Keywords: Protonation constants, Phenylalanine, Maleic acid, Ethylene glycol, MINIQUAD75

1. Introduction

Phenylalanine (Phe) is a strong α-amino acid [1]. This essential amino acid is classified as nonpolar because of the hydrophobic 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 neuromodulator 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 this 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 α-amino acids in different media [10]-[13]. Acidity and basicity of a molecule is governed by its structure and solvent effects [14], [15.

Ethylene glycol (EG) is a protophilic dipolar protic solvent and acts as a structure former. EG is more acidic (less basic) than water [16] due to electron withdrawing effect of CH_2 group. It offers several advantages as solvent in titration of weak bases [17], [18]. EG plays an important role in protein conformation studies [19], [20] because it is a weaker protein denaturant compared to urea or other organic solvents such as ethanol, dioxane etc.

The protonation equilibria of Phenylalanine and Maleic acid been studied in the presence of EG to understand the influence of dielectric constant of the medium on the chemical speciation.

2. Experimental

Materials

0.05 mol L^{-1} solutions of Phenylalanine and Maleic acid (AR, Qualigen, India) was prepared in triple-distilled deionized water by maintaining 0.05 mol L^{-1} hydrochloric acid concentration to increase the solubility. Ethylene glycol (AR, Qualigen) were used as received. 2.0 mol L^{-1} sodium chloride was prepared to maintain the ionic strength in the titrand. 0.2 mol L^{-1} hydrochloric acid (Merck, India) and 0.4 mol L^{-1} sodium hydroxide were prepared and 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) [21]. The strengths of alkali and mineral acid were also determined using the Gran plot method [22].

Alkalimetric Titrations

The glass electrode was equilibrated in a well EG-water mixtures containing inert electrolyte for several days. At regular intervals titration of strong acid was titrated with

alkali to check the complete equilibration of the glass electrode. The calomel electrode was refilled with EG-water mixture of equivalent composition as that of the titrand. Alkalimetric titrations were performed in media containing 0-60% v/v EG-water mixtures pH metrically. The details of experimental procedure and titration assembly were detailed elsewhere [23]. Typical alkalimetric titration curves are given in Figure 1.

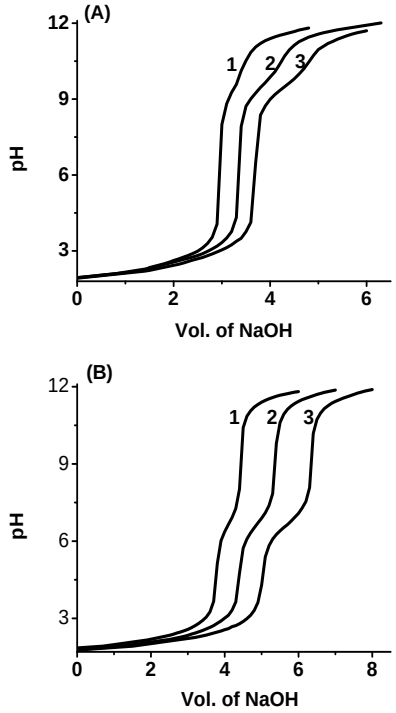


Figure 1: Alkalimetric titration curves of (A) Phenylalanine and (B) Maleic acid in 30% v/v Ethylene glycol-water mixtures. 1, 2 and 3 indicate 0.25, 0.375 and 0.5 mmol of ligand, respectively.

Modeling Strategy

The approximate protonation constants of Phenylalanine and Maleic acid were calculated using the computer program SCPHD [24] and from the secondary formation functions. They are refined using a non-linear least-squares computer program, MINIQUAD75 [25]. The reliability of the protonation constants were verified from the statistical parameters and by introducing errors in the concentrations of the ingredients. The variation of step-wise 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

Secondary formation function (n_H) is the average number of protons bound per mole of ligand. It is useful to detect the number of protonation equilibria. The pH values at half integrals of formation function (n_H) correspond to the log K values of the ligand. The maximum value of n_H is found to be 2.0 (Figure 2) which infers the number of protonation equilibria to be two in both ligands (Phe and Mal). The approximate protonation constants obtained from these formation functions and SCPHD are used for final refinement. The best fit models containing the type of species and log values of overall formation constants (log β) along with some of the important statistical parameters are given in Table 1. A very low standard deviation (SD) in log β values indicates the precision of these parameters. Small values of U_{corr} 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 a zero mean with little dispersion.

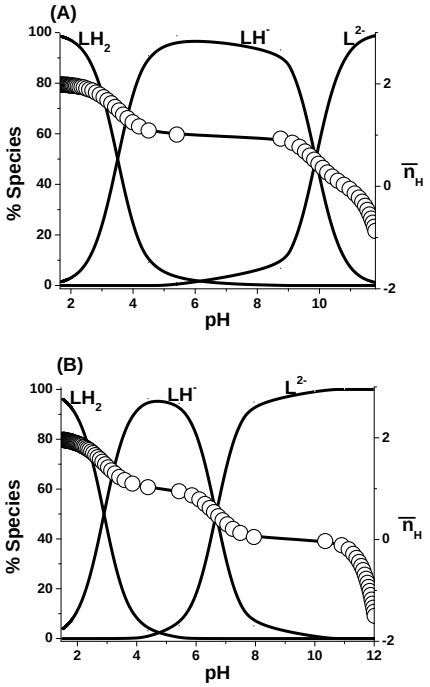


Figure 2: Formation functions (hallow circles) and species distribution diagrams (lines) of (A) Phenylalanine and (B) Maleic acid in 30% v/v Ethylene glycol-water mixtures.

Residual Analysis [26]

In data analysis with least squares methods, residuals (the differences between the experimental data and the data simulated based on 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. If the statistical measures of the residuals and the errors assumed in the model are not significantly different from each other, the model is said to be adequate. Further, a model is considered adequate only if the residuals do not show any trend. Respecting the hypothesis that the errors are random, the residuals are tested for normal distribution. Such tests are χ^2, skewness, kurtosis and R-factor. These statistical parameters show that the best fit models portray the acido-basic equilibria of Phenylalanine and Maleic acid EG-water mixtures, as discussed below.

χ^2 test

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

Crystallographic R-test

Hamilton's R factor ratio test [27] give reference 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. The low crystallographic R-values given in Table 1 indicate the sufficiency of the model.

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 0.39 and 2.89. These data evince that the residuals form a part of normal distribution; hence, least–squares method can be applied to the present data.

Table 1: Best fit chemical models of acido-basic equilibria of Phenylalanine and Maleic acid Ethylene glycol-water mixtures. Temperature = 303.0 K, Ionic strength = 0.16 mol L^{-1}.

%v/v EG	log β_1(SD)	log β_2(SD)	log K_1	log K_2	NP	U_{corr}	Skewness	Kurtosis	χ^2	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	9.48778(1)	12.7423(3)	3.25453	9.48778	62	1.24	0.72	2.81	5.03	0.036229	3.4-10.5
20	9.5646(1)	12.9242(3)	3.35955	9.5646	67	1.61	0.73	3.29	5.82	0.039396	3.4-10.5
30	9.88183(2)	13.3877(3)	3.50584	9.88183	52	2.57	0.39	6.10	15.38	0.040185	3.4-10.5
40	9.72314(2)	13.2658(1)	3.54264	9.72314	61	4.52	0.99	5.54	19.11	0.028822	3.4-10.5
50	9.75452(1)	13.3426(2)	3.58804	9.75452	61	2.31	1.12	5.13	12.95	0.027170	3.4-10.5
60	9.69179(1)	13.3896(2)	3.69782	9.69179	63	3.89	1.02	3.89	19.27	0.026281	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	6.10573(1)	8.72595(2)	2.62022	6.10573	61	1.04	2.89	10.47	73.80	0.103544	2.9-10.8
20	6.49173(2)	9.27566(3)	2.78393	6.49173	60	2.11	2.22	7.78	24.27	0.059912	2.9-10.8
30	6.67482(1)	9.57268(3)	2.89786	6.67482	58	3.22	1.44	5.70	12.97	0.036138	2.9-10.8
40	6.98438(3)	9.95128(1)	2.9669	6.98438	59	1.52	2.69	12.36	33.59	0.041209	2.9-10.8
50	6.87152(2)	9.56068(1)	2.68916	6.87152	59	2.31	2.61	10.01	39.83	0.069411	2.9-10.8
60	7.2289(2)	10.2211(1)	2.9922	7.2289	62	2.89	2.35	8.66	43.10	0.059666	2.9-10.8

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

Kurtosis

It is a measure of the peakedness of the error distribution near a modal 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.

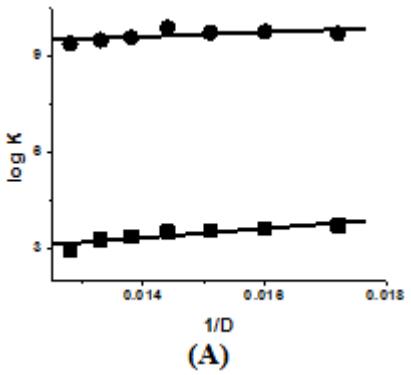
Simulation of titration data

The alkalimetric titration data are simulated using the model parameters given in Table 1. These data are compared with the experimental alkalimetric titration data to verify the sufficiency of the models. The overlap of the experimental and simulated titrations data (figure not given) indicates that the proposed models represent the experimental data.

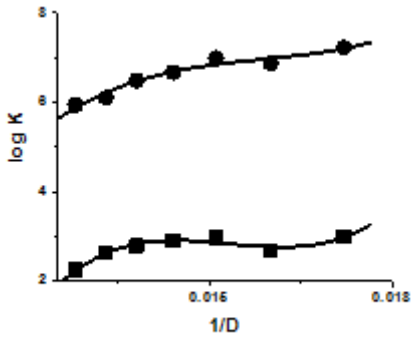
Influence of Solvent

The variation of protonation constant or change in free energy with co-solvent content depends upon two factors, viz., electrostatic and non-electrostatic interactions. 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 constants (log K) should vary linearly as a function of the reciprocal of the dielectric constant (1/D) of the medium.

The linear variation of log K values of Phe in EG-water mixture (Figure 3) indicates the dominance of electrostatic forces over non-electrostatic forces. But the non-linear trend of Mal in EG-water mixture shows the dominance of non-electrostatic forces. These opposite trends are due to the opposite nature of Phe and Mal. Phe is neutral, saturated, stable and aromatic where as Mal is acedic and unsaturated. The cation stabilizing nature of EG, specific solvent-water interactions, charge dispersion, and specific interactions of co-solvent with solute (indicated by the changes in the solubility of different spcies in the aquo-organic mixtures) account for the deviation of classical linear relationship of log K with 1/D.



(A)

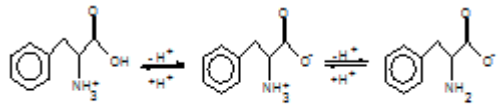


(B)

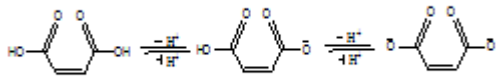
Figure 3: Variation of step-wise protonation constants (log K) of (A) Phenylalanine and (B) Maleic acid with reciprocal of dielectric constant (1/D) Ethylene glycol-water mixtures. (■) logK1, (●) logK2.

Distribution Diagrams

The distribution plots (Figure 2) produced using the protonation constants of Phe and Mal from the best fit models (Table 1) show the formation of LH_2^+, LH and L^- in Phe. The LH_2, LH^-, and L^{2-} in Mal. Successive protonation and deprotonation of the ligands with increasing pH. The species exists in the pH range 2.0–11.0 (Figure 4).



(A) LH_2^+ LH L^-
(cation) (zwitter ion) (anion)



(B) LH_2 LH^- L^{2-}

Figure 4: Protonation-deprotonation equilibria of (A) Phenylalanine and (B) Maleic acid

Influence of Systematic Errors

Any variations in the concentrations of ingredients affect the magnitudes of protonation constants. 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 ligand. The results of a typical system given in Table 2 emphasize that the errors in the concentrations of alkali and mineral acid influence the protonation constants more than that of the ligand.

Table 2: Influencet of errors in concentrations of ingredients on protonation constants of Phenylalanine and Maleic acid in 30% v/v of Ethylene glycol-water mixtures

Ingredient	%Error	logβlh (SD)	
		011	012
Phenylalanine			
	0	9.88(2)	13.38(3)
Alkali	-5	10.16(2)	13.88(4)
	-2	9.99(2)	13.58(3)
	+2	9.77(2)	13.20(3)
	+5	9.60(2)	12.92(5)
Acid	-5	9.54(3)	12.70(5)
	-2	9.74(2)	13.11(3)
	+2	10.01(2)	13.66(3)
	+5	10.20(3)	14.09(5)
Ligand	-5	9.93(2)	13.60(3)
	-2	9.90(2)	13.47(3)
	+2	9.86(2)	13.30(3)
	+5	9.83(2)	13.18(3)
Maleic acid			
Alkali	0	6.67(1)	9.57(3)
	-5	6.92(2)	10.06(4)
	-2	6.75(2)	9.76(4)
	+2	6.52(3)	9.37(6)
	+5	6.34(4)	9.07(7)
Acid	-5	6.27(4)	8.84(9)
	-2	6.50(3)	9.28(6)
	+2	6.77(2)	9.83(4)
	+5	6.96(2)	9.77(4)
Ligand	-5	6.69(2)	9.77(4)
	-2	6.66(2)	9.64(4)
	+2	6.62(2)	9.47(5)
	+5	6.59(3)	9.37(5)

4. Conclusions

1) Secondary formation functions indicated the existence of two protonation equilibria in both ligands. The highest form of ligand is LH_2^+ which successively deprotonates to LH and L^- with increasing pH in Phe and the highest form of ligand is LH_2 which successively deprotonates to LH^- and L^{2-} with increasing pH in Mal.
2) Increased standard deviations in the protonation constants with the introduction of errors in the concentrations of ingredients support the appropriateness of the experimental conditions.
3) The change in the magnitude of protonation constant with co-solvent content indicates the influence of dielectric constant of the medium.
4) The linear and non-linear variation of protonation constants in EG-water mixtures, respectively, infers the dominance of electrostatic and non-electrostatic forces in the equilibria.

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