Influence of Dielectric Constant on Protonation Equilibria of L-Aspartic Acid in Acetonitrile- and Ethylene Glycol-Water Mixtures

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Abstract

The protonation constants of L-Aspartic acid have been studied pH-metrically in various concentrations (0–60% v/v) of acetonitrile- and ethylene glycol-water mixtures maintaining an ionic strength of 0.16 mol L^{-1} at 303K. The protonation constants have been calculated with the computer program MINIQUAD75 and are selected based on statistical parameters. Linear variation of step-wise protonation constants (log K) with the reciprocal of the dielectric constant of the solvent mixture has been attributed to the dominance of the electrostatic forces.

Keywords: Protonation constants, L-Aspartic acid, Acetonitrile, Ethylene glycol

1. Introduction

A number of studies has reported on protonation constants of α-amino acids in different media.[1–3] Acidity and basicity of a molecule is governed by its structure and solvent effects.[4–6] L-aspartic acid (Asp) is a non-essential amino acid found in abundance in plant proteins. It plays an important role in maintaining the solubility and ionic character of proteins.[7] It assists the liver in removing excess ammonia and other toxins from the blood stream. It is also very important in the functioning of RNA and DNA, in immunoglobulin and antibody synthesis. Asp is popular as a drug for chronic fatigue as it plays a crucial role in generating cellular energy, moves the coenzyme nicotinamide adenine dinucleotide (NADH) molecules from the main body of the cell to its mitochondria, where it is used to generate adenosine triphosphate (ATP).[8]

Acetonitrile (AN) is a weak base[9] and a much weaker acid[10] 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[11] potential in acetonitrile than in water. It is a protophobic dipolar aprotic solvent and it does not form any hydrogen bond with solute species. The protophobic character of AN may arise from the possible

formation of dimers which are shown to exist from IR studies.[12] Very few studies have been reported in the literature,[13–15] hence the authors are reporting the protonation constants of ethylenediamine and aspartic acid in acetonitrile-water mixtures.

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[16] due to the electron withdrawing effect[17] of the CH_2 group. EG offers several advantages as solvent in titration of weak bases.[18,19]

Ethylene glycol plays an important role in protein conformation studies[20,21] because it is a weak protein denaturant compared to urea or other organic solvents such as ethanol, dioxane etc. The protonation equilibria of L-aspartic acid have been studied in the presence of acetonitrile and ethylene glycol to understand the influence of co-solvent on the chemical speciation.

2. Experimental

2. 1. Materials

A 0.05 mol L^{-1} solution of L-aspartic acid (GR, E-Merck, Germany) was prepared in triple distilled water by maintaining a 0.05 mol L^{-1} acid (HNO_3) concentra-

tion to increase the solubility. Acetonitrile (AR, E-Merck) and ethylene glycol (AR, Qualigen) were used as received. Sodium nitrate was prepared to maintain the ionic strength in the titrand. A sodium hydroxide solution of 0.4 mol L^{-1} was prepared. The strengths of alkali and mineral acid were determined using the Gran plot method.[22, 23]

2. 2. Procedure

The titrimetric data were obtained by using a 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 the glass electrode were accounted for in the form of a correction factor. For the determination of protonation constants of Asp, 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 the titrand. All the titrations have been carried out in a medium containing varying concentrations of solvent (0.0–60.0% v/v) and maintaining an ionic strength of 0.16 mol L^{-1} 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 mL. Titrations with different volumes (2.5, 3.75 and 5.0 mL) of ligand were carried out with 0.4 mol L^{-1} sodium hydroxide. Other experimental details are given elsewhere.[24]

2. 3. Alkalimetric Titration Assembly

The glass electrode was equilibrated in well-stirred acetonitrile- and ethylene glycol-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 titrant. The experimental procedure and titration assembly are given elsewhere.[24] Typical alkalimetric titrations are given in Figure 1.

2. 4. Modeling Strategy

The approximate protonation constants of aspartic acid were calculated with the computer program SCPHD[25] and they were refined using the non-linear least-square computer program MINIQUAD75[26]. The variation in overall protonation constants was analyzed on electrostatic grounds on the basis of solute-solute and solute-solvent interactions.
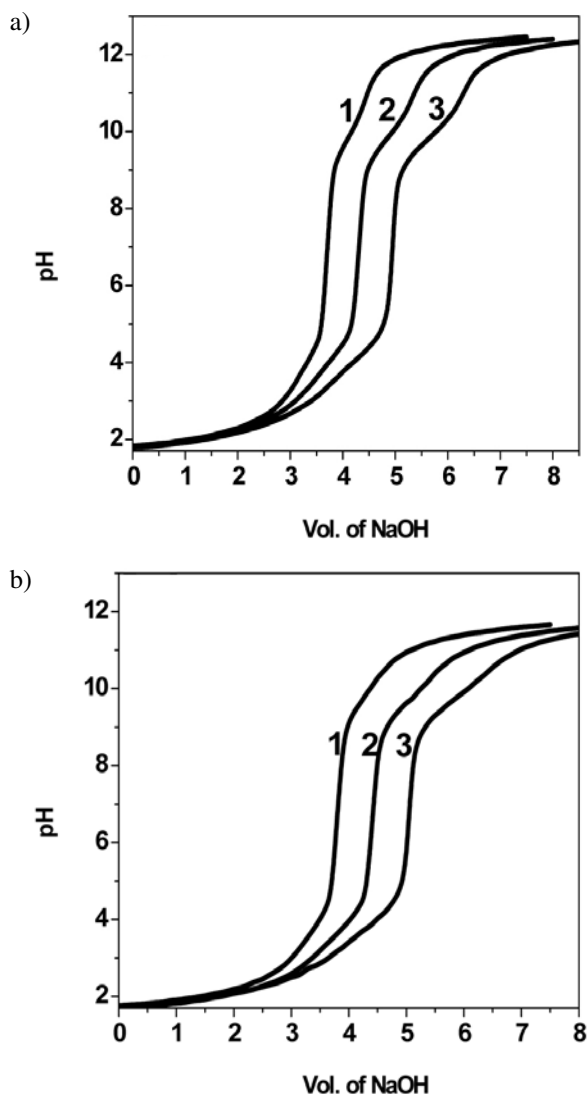

Figure 1: Alkalimetric titration curves of L-aspartic acid in acetonitrile and ethylene glycol–water mixtures; (A) 30% v/v of acetonitrile (B) 20% v/v of ethylene glycol. 1, 2 and 3 indicate 0.25, 0.375, 0.5 mmol of ligand, respectively.

3. Results and Discussion

The best fit models that contain the type of species and overall protonation constants (log β) along with some of the important statistical parameters are given in Table 1 and were correlated with literature values[28–31] of Asp (Table 2) in aqueous medium. Our values are comparable to those reported under the present experimental conditions. A very low standard deviation in log β 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 the 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

Table 1: Best fit chemical models of acido-basic equilibria of L-aspartic acid in acetonitrile and ethylene glycol-water mixtures. Temp = 303 K, Ionic strength = 0.16 mol L^{-1}.

Solvent %v/v	Logβ$_{mlh}$ (SD)			NP	U$_{corr}$	Skewness	Kurtosis	χ^2	R-factor	pH-Range
	011	012	013							
Acetonitrile										
0	9.85(1)	13.56(2)	15.49(9)	61	4.25	0.70	5.87	24.36	0.0157	2.7–10.4
10	9.70(1)	13.71(1)	16.25(2)	82	2.01	1.10	5.42	2.93	0.0090	2.5–10.2
20	9.76(1)	13.86(3)	16.60(3)	104	7.55	0.20	9.84	33.15	0.0142	2.2–10.2
30	9.91(1)	14.20(1)	16.89(2)	88	2.14	0.51	3.82	14.73	0.0085	2.4–10.4
40	9.96(1)	14.38(3)	17.21(3)	102	7.31	0.44	6.78	48.55	0.0141	2.2–10.4
50	9.99(1)	14.58(3)	17.54(3)	79	6.23	1.15	5.67	14.56	0.0152	2.6–10.2
60	10.02(1)	14.77(3)	17.79(4)	82	10.34	0.38	11.01	93.17	0.0208	2.7–10.7
Ethylene glycol										
0	9.85(1)	13.56(2)	15.49(9)	61	4.25	0.70	5.87	24.36	0.0157	2.7–10.4
10	9.63(1)	13.50(3)	15.96(3)	126	6.52	1.76	7.92	33.84	0.0113	1.9– 9.9
20	9.48(1)	13.31(2)	15.68(3)	113	5.92	0.96	5.72	20.16	0.0118	2.0 – 9.9
30	9.47(1)	13.32(1)	15.59(2)	86	3.51	0.87	6.92	15.91	0.0115	2.3–10.0
40	9.57(1)	13.69(3)	16.07(7)	61	6.50	0.08	3.76	7.97	0.0191	2.9–10.0
50	9.53(2)	13.65(4)	16.00(1)	66	10.60	1.01	6.78	30.91	0.0256	2.9–10.3
60	9.34(2)	13.35(3)	15.61(3)	107	8.19	0.05	6.24	32.73	0.0148	2.0– 9.9

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

Table 2: Protonation constants of aspartic acid reported in the literature.

S. No.	Log β			Ionic Strength (M)	Instrumental method	Reference
	011	012	013			
1.	9.63	13.36	15.42	0.1	pH metry	28
2.	9.65	13.63	15.51	0.1	–	28
3.	9.69	13.75	16.02	0.1	–	28
4.	9.52	13.45	15.12	0.1	–	28
5.	9.42	12.84	16.77	0.6	–	29
6.	7.42	10.41	12.88	0.2	–	30
7.	9.60	13.25	15.13	0.1	–	31

Table 3: Effect of errors on the critical parameters on protonation constants of L–aspartic acid in 30% v/v of acetonitrile and ethylene glycol–water mixture.

Ingredient	% Error	Logβ$_{mlh}$(SD)		
		011	012	013
Acetonitrile				
Alkali	0	9.91(1)	14.20(1)	16.89(2)
	–5	10.42(4)	15.14(6)	18.04(7)
	–2	10.10(1)	14.56(3)	17.33(3)
	+2	9.71(1)	13.85(1)	16.47(1)
	+5	9.42(1)	13.34(3)	15.86(4)
Acid	–5	9.57(1)	13.53(2)	15.97(3)
	–2	9.96(1)	13.93(1)	16.52(1)
	+2	10.04(1)	14.47(3)	17.27(3)
	+5	10.25(3)	14.88(5)	17.85(6)
Ligand	–5	9.76(1)	13.99(1)	16.73(1)
	–2	9.85(1)	14.12(1)	16.83(1)
	+2	9.96(1)	14.28(1)	16.95(2)
	+5	10.04(1)	14.40(2)	17.04(2)
Ethylene glycol				
Alkali	0	9.47(1)	13.32(1)	15.59(2)
	–5	9.95(2)	14.21(4)	16.68(4)
	–2	9.66(1)	13.67(1)	16.01(2)
	+2	9.27(2)	12.97(3)	15.18(4)
	+5	8.96(3)	12.44(6)	14.57(8)
Acid	–5	9.12(3)	12.66(5)	14.67(7)
	–2	9.33(1)	13.06(3)	15.23(4)
	+2	9.60(1)	13.58(1)	15.95(2)
	+5	9.79(1)	13.97(3)	16.51(3)
Ligand	–5	9.31(1)	13.10(2)	15.42(3)
	–2	9.41(1)	13.23(2)	15.52(3)
	+2	9.53(1)	13.40(2)	15.65(2)
	+5	9.61(1)	13.52(1)	15.75(2)

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 few platykurtic patterns. The values of skewness are between 0.05–1.76. These data evidence that the residuals form a part of a normal distribution, hence, the 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 L-aspartic acid in acetonitrile and ethylene glycol-water mixtures.

3. 1. Effect of Systematic Errors

In order to rely upon the best chemical model for critical evaluation and application under varied experi-

mental 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 3 emphasize that the errors in the concentrations of alkali and mineral acid affect the protonation constants more than those in the ligand.

3. 2. Effect of Solvent

The effect of the type of solvent on the protonation constant depends on electrostatic and non-electrostatic factors. Born's classical treatment holds good in accounting for the electrostatic contribution[27] which is related to the dielectric constant. Hence, the logarithm of the overall protonation constant (log β) should vary linearly as a function of the reciprocal of the dielectric constant (1/D) of the medium (Figure 2). The log β values are linearly increasing with a decreasing dielectric constant of the medium in both media (acetonitrile/ethylene glycol).

L-aspartic acid exists as anion, zwitterion and cation (Figure 3) at different pH values. The cation stabilizing
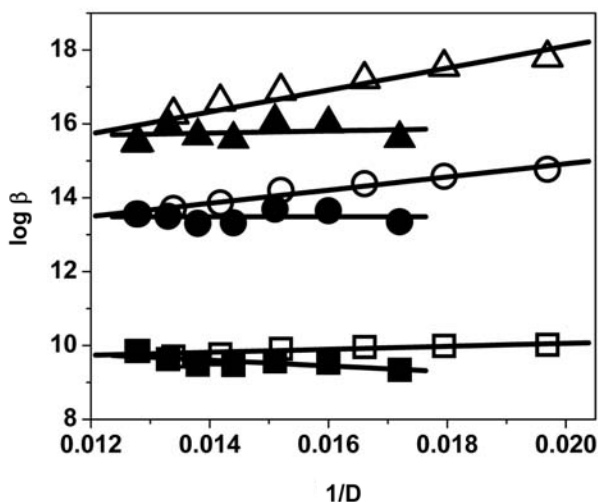
nature of co-solvent, specific solvent-water interactions, charge dispersion and specific interactions of co-solvent with solute account for the linear relationship of log β with 1/D.

3. 3. Distribution Diagrams

The secondary formation function (n_H) averages the number of moles of protons bound per mole of ligand and is useful to detect the number of protonation equilibria. The formation functions (Figure 4) indicate that Asp has three equilibria. The pH values at half integrals of n_H correspond to the log K values of the ligands.

Figure 4: Species distribution diagrams of L-Aspartic acid in (4A) 30% v/v acetonitrile- and (4B) 10% v/v Ethylene glycol-water mixture.

Figure 2: Variation of overall protonation constants (log β) with the reciprocal of the dielectric constants (1/D) of L-Aspartic acid in acetonitrile (open symbols) and ethylene glycol (solid symbols)-water mixtures. (□) logK$_1$, (○) logK$_2$, (△) logK$_3$.

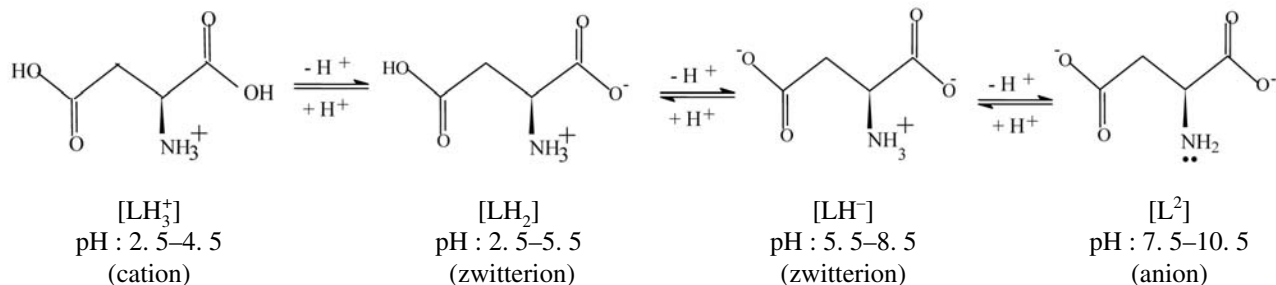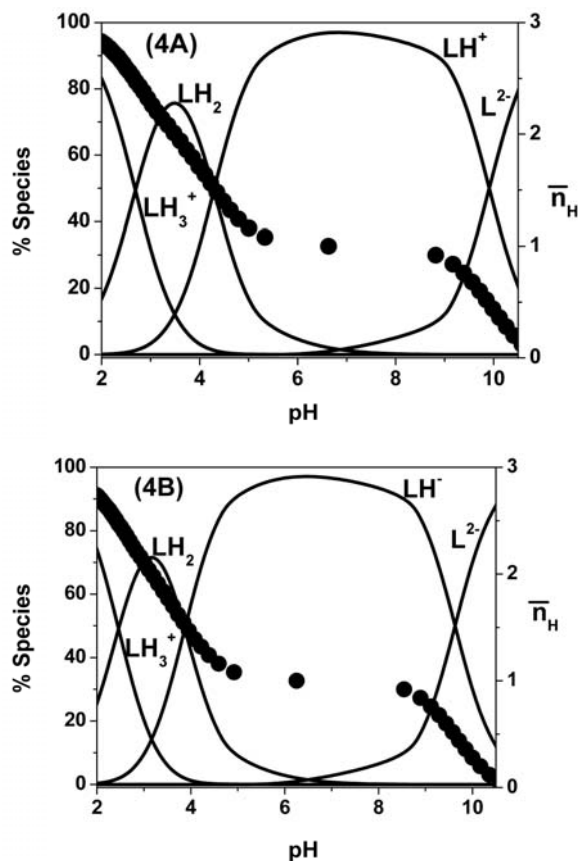| [LH$_3^+$]	[LH$_2$]	[LH$^-$]	[L^2]
pH : 2. 5–4. 5	pH : 2. 5–5. 5	pH : 5. 5–8. 5	pH : 7. 5–10. 5
(cation)	(zwitterion)	(zwitterion)	(anion)

Figure 3: Protonation-deprotonation equilibria of L-aspartic acid

The distribution plots (Figure 4) of L-aspartic acid indicate that LH is present to an extent of 90% in the pH range 4.0–10.0 and successive deprotonation takes place with an increase of the pH.

4. Conclusions

1. L-Aspartic acid forms LH_3^+ species at low pH values and gets deprotonated to LH_2, LH^- and L^{2-} successively with an increase in the pH.
2. The log values of the protonation constants increase linearly with a decreasing dielectric constant of the acetonitrile and ethylene glycol-water mixtures. This trend indicates the dominance of electrostatic forces in the protonation-deprotonation equilibria.
3. 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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6. References

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Povzetek

Za L-asparaginsko kislino je bila pH-metrično določena konstanta protolize v mešanicah acetonitril – voda in etilenglikol – voda (0–60 %v/v) pri ionski moči 0,16 mol L^{-1} in temperaturi 303 K. Konstanta protolize je bila določena z računalniškim programom MINIQUAD75 na osnovi statističnih podatkov. Odstopanja vrednosti protolizne konstante (log K) v odvisnosti od obratne vrednosti dielektrične konstante mešanice topil lahko pripišemo elektrostatskim interakcijam.