

## Shift in pKa of of L-Aspartic Acid and Ethylenediamine in Low dielectric media: Biomimetic Study

Hadgu Hailekiros Belay

Department of Applied Chemistry, Adama Science and Technology University, Adama, Oromia, Ethiopia  
Corresponding author: Hadgu Hailekiros Belay

**Abstract:** Bioavailability and toxicity of an element depends on its speciation. Hence the protonation constants of L-Aspartic acid and Ethylenediamine have been studied pH-metrically in various concentrations (0–60%, v/v) of Dimethylformamide-water mixtures maintaining an ionic strength of  $0.16 \text{ mol L}^{-1}$  at 310K to mimic the different species formed in physiological. The protonation constants have been calculated with the computer program MINQUAD75 and the best-fit models arrived based on statistical grounds employing crystallographic R factor,  $\chi^2$ , skewness and kurtosis were obtained. The effect of experimental errors on the protonation constants has also been presented. L-Aspartic acid exists as  $\text{LH}_3^+$  at low pH and gets deprotonated with the formation of  $\text{LH}_2$ ,  $\text{LH}^+$  and  $\text{L}^{2-}$  successively with increasing pH. Ethylenediamine has two amino groups with the formation of  $\text{XH}_2^{2+}$ ,  $\text{XH}^+$  and  $\text{X}$ .  $\text{XH}_2^{2+}$  exists up to a pH of 5.1–11.2. The monoprotonated species  $\text{XH}^+$  exists in the pH range 5.1–11.1. Successive deprotonation of  $\text{XH}_2^{2+}$  with increasing pH forms ultimately  $\text{X}$  above a pH of 8.0 in low dielectric media. The variation of protonation constants with dielectric constant of the medium is attributed to the electrostatic and non-electrostatic forces.

**Keywords:** Protonation constants, MINQUAD75, L-Aspartic acid, Ethylenediamine, Low dielectric media

Date of Submission: 15-08-2019

Date of Acceptance: 30-08-2019

### I. Introduction

L-Aspartic acid (Asp) is a non-essential amino acid with the molecular formula  $\text{C}_4\text{H}_7\text{NO}_4$ . It is primarily used in the body's metabolic processes; it is also key for a variety of other biological processes such as production of immunoglobulin, cell functioning, and the movement of minerals across intestinal linings into blood. Asp has also been used in various clinical applications, showing promise in persons suffering from opiate addiction[1].

Asp is also significant in the removal of excess ammonia and other toxins from the blood stream. It also assists in the proper functioning of carriers for genetic information-RNA and DNA. Depletion of serotonin can lead to conditions associated with such a disproportionate change in brain serotonin levels, including fatigue, headache, sleep, eating disorders and in extreme cases, depression. The conversion of Asp to these amino acids begins with the reduction of Asp to its semi aldehyde[2].

Ethylenediamine (en) is a colorless to yellowish hygroscopic liquid with an ammonia-like odor. It is strongly alkaline (pH of 25% en in water is 11.9), highly volatile, pungent material, which fumes profusely in air. It has a melting point of  $8.5^\circ\text{C}$ , a boiling point of  $116^\circ\text{C}$  (at 101.3 KPa) and a vapor pressure of 1.7 KPa at  $25^\circ\text{C}$ . It is involved in the synthesis of seven membered ring components with  $\beta$ -ketoesters resulting secondary amines and  $\beta$ -enaminoesters[3]. En plays an important role in the synthesis of Schiff base compounds[4].

N, N-Dimethylformamide (DMF) is an organic compound and a common solvent for chemical reactions that is miscible with water and the majority of organic liquids. It is a polar aprotic solvent with a high boiling point, which facilitates reactions that follow polar mechanisms. It can be hydrolyzed by strong acids and bases, especially at elevated temperatures[5]. In the present study, protonation/deprotonation of L-Aspartic acid and Ethylenediamine were reported in the presence of DMF as a co-solvent

### II. Experimental

Solutions ( $0.05 \text{ mol L}^{-1}$ ) of L-Aspartic acid (Asp) GR grade (E-Merck, Germany) and Ethylenediamine (en) AR grade (Qualigen, India) were prepared in triple distilled water by maintaining  $0.05 \text{ mol L}^{-1}$  acid (HCl) concentration to increase the solubility. The titrations were carried out in media containing varying amounts of co-solvent (0.0–60.0%, v/v) maintaining an ionic strength of  $0.16 \text{ mol L}^{-1}$  with NaOH at  $310.0 \pm 0.0 \text{ K}$ . To increase the solubility of the ligands,  $0.05 \text{ mol L}^{-1}$  mineral acid concentrations were maintained in their solutions. The computer program COSWT [6] determined the probable errors that might have crept into the concentrations of the stock solutions of the ligands. The strengths of acid and alkali were determined using Gran plot method[7].

To assess the errors that might have crept in to the determination of the concentrations, the data were subjected to analysis of variance of one-way classification (ANOVA)[8].

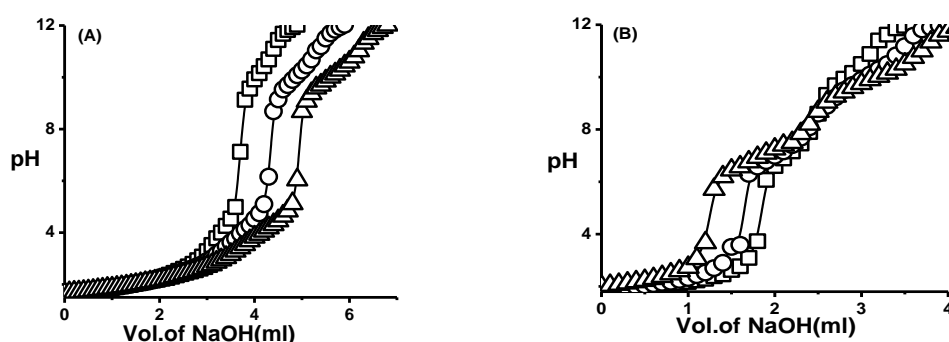
### Modeling Strategy

The approximate protonation constants of *L*-Aspartic acid and Ethylenediamine were calculated with the computer program SCPHD[9] and they were refined using the non-linear least square computer program MINQUAD75[10].

## III. Results And Discussion

### Alkalimetric titrations

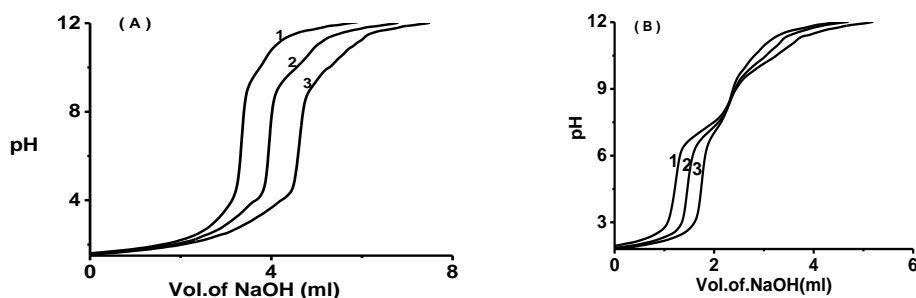
The alkalimetric titration data are simulated using the model parameters given in Tables 1 and 2. These data are compared with the experimental alkalimetric titration data, to verify the sufficiency of the models[11]. The overlap of the typical experimental and simulated titrations data given in (Figure 1) indicates that the proposed models represent the experimental data.



**Figure 1.** Simulated (point) and experimental (solid line) alkalimetric titration curves in 30.0 % (v/v) DMF - water mixture(A) Asp and (B)en; (□) 0.25, (○) 0.375 and (Δ) 0.50 mmol

### Preprocessing of data

The typical alkalimetric titration curves for Asp and en in DMF- water mixtures are shown in Figure 2. A perusal of the titration curves reveals that the acido-basic equilibria are active in the pH ranges 2.0-10.4 in Asp and 5.4-10.4 in en. The computer program SCPHD[9] was used to prune the data obtained in different experiments so that it contains more information than noise and the log  $\beta$ s obtained from the program are used as initial values for final refinement.

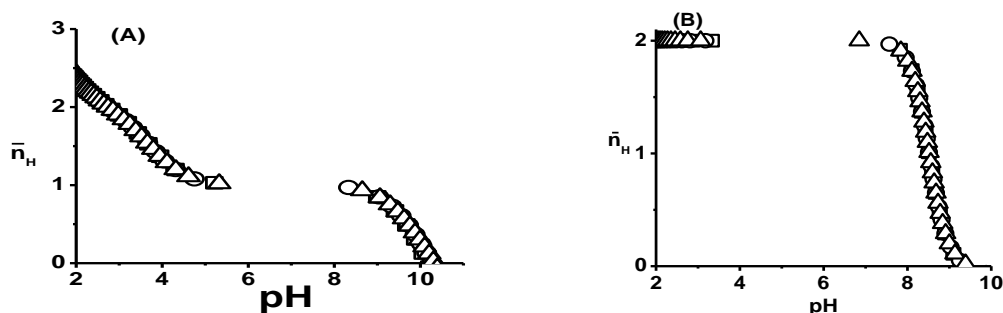


**Figure 2.** Alkalimetric titration curves for (A) Asp, (B) en in aqueous medium. Amounts of ligands, in mmols, are (1) 0.25, (2) 0.375 and (3) 0.500.

### Formation Functions

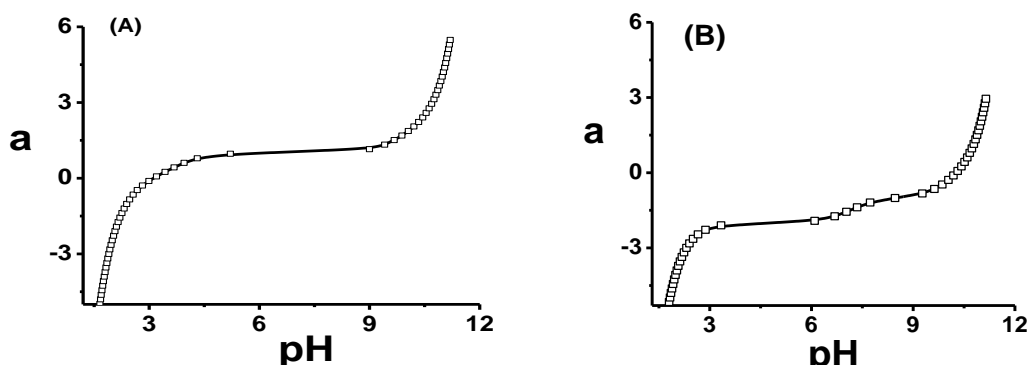
The Secondary formation functions like average number of protons bound per mole of ligand ( $\bar{nH}$ ) and number of moles of alkali consumed per mole of ligand ( $\alpha$ ) are useful to detect the number of equilibria. Plots of  $\bar{nH}$  versus pH (formation curves) for different concentrations of the ligand should overlap if polymeric species are not formed. The present study the polymerization of *L*-aspartic acid and ethylenediamine

ruled out (Figure. 3). The pH values at half-integral values of  $\bar{n}_H$  correspond to the protonation constants of the ligands. Three half-integrals in the case of *L*-aspartic acid and two in the case of ethylenediamine emphasize the presence of three and two protonation, deprotonation equilibria, respectively, in the pH range of present study.



**Figure 3A.** The formation curves of (A) Asp, (B) en and number of moles of alkalia versus pH curves in aqueous medium. Concentration of ligand in mmols are 0.25 ( $\square$ - $\square$ ), 0.375 ( $\bigcirc$ - $\bigcirc$ ), and 0.50 ( $\Delta$ - $\Delta$ )

The number of moles of alkali consumed per mole of the ligand, denoted by  $\bar{a}$ , is another secondary function like  $\bar{n}_H$ . A curve of  $\bar{a}$  versus pH gives the number of equivalents of alkali consumed per mole of ligand. The negative values of  $\bar{a}$  correspond to the excess mineral acid present in the titrand and the number of associable protons whereas the positive values correspond to the dissociable protons. (Figure. 3.) Indicates the deprotonation of the two carboxylic groups below a pH of 7 and the deprotonation of the amino group above pH 7 of Asp. In the case of en, the highest value of  $\bar{a}$  is 0.0, which indicates that it has no dissociable protons.



**Figure 3B.** The formation curves of (A) Asp, (B) en and number of moles of alkali  $\bar{a}$  versus pH curves in aqueous medium. Concentration of ligand in mmols are 0.25 ( $\square$ - $\square$ ), 0.375 ( $\bigcirc$ - $\bigcirc$ ), and 0.50 ( $\Delta$ - $\Delta$ )

### Mathematical models for acido-basic equilibria

The best fit models containing the type of species and overall protonation constants ( $\log\beta$ ) along with some of the important statistical parameters are given in Tables 1 and 2. Very low standard deviations (SD) in  $\log\beta$  values indicate their precision. Small values of Ucorr (sum of squares of deviations in concentrations of the ligand and hydrogen ion at all experimental points corrected for degrees of freedom) indicate that the experimental data can be represented by the models [12]. In data analysis with least squares methods, the residuals are assumed to follow Gaussian or normal distribution. When the data are fit into the models, the residuals should be ideally equal to zero. These statistical parameters show that the best-fit models portray the acido-basic equilibria of Asp and en in DMF-water mixtures, as discussed below.

### $\chi^2$ test

$\chi^2$  is a special case of gamma distribution whose probability density function is an unsymmetrical function [13]. This distribution measures the probability of residuals forming a part of standard normal distribution with zero mean and unit standard deviation. If the  $\chi^2$  calculated is less than the table value, the model is accepted.

### Crystallographic R-test

Hamilton's R factor ratio [14] 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 Rlimit, which represents the upper boundary of R beyond which the model bears no significance. When different values

are obtained for models containing different numbers of species, models whose values are greater than R-table are rejected.

**Table 1:** Best fit chemical models of acido-basic equilibria of L-aspartic acid in DMF- water mixtures. Temperature = 310.0 K, Ionic strength = 0.16 mol L<sup>-1</sup>

| Solvent % v/v | log β <sub>min</sub> (SD) |          |          | NP  | U <sub>corr</sub> × 10 <sup>8</sup> | Skewness | Kurtosis | χ <sup>2</sup> | R-factor | pH-range  |
|---------------|---------------------------|----------|----------|-----|-------------------------------------|----------|----------|----------------|----------|-----------|
|               | 011                       | 012      | 013      |     |                                     |          |          |                |          |           |
| DMF           |                           |          |          |     |                                     |          |          |                |          |           |
| 0.0           | 9.86(1)                   | 13.55(0) | 15.35(1) | 100 | 1.4                                 | 0.01     | 4.30     | 6.17           | 0.0041   | 1.80-10.4 |
| 10.0          | 9.93(2)                   | 13.70(1) | 15.75(0) | 112 | 3.8                                 | -1.77    | 3.22     | 4.16           | 0.00865  | 1.80-10.2 |
| 20.0          | 9.90(3)                   | 13.91(4) | 16.30(3) | 130 | 1.8                                 | -1.28    | 1.33     | 4.08           | 0.00583  | 1.80-10.2 |
| 30.0          | 10.18(2)                  | 14.33(1) | 16.72(3) | 120 | 3.0                                 | -1.07    | 9.00     | 1.02           | 0.00752  | 1.80-10.2 |
| 40.0          | 10.30(1)                  | 14.66(5) | 17.39(1) | 119 | 2.5                                 | -0.71    | 1.30     | 1.91           | 0.00249  | 1.95-10.2 |
| 50.0          | 10.219(1)                 | 14.77(1) | 17.45(1) | 100 | 2.5                                 | -0.67    | 5.91     | 3.72           | 0.00344  | 1.95-10.2 |
| 60.0          | 10.20(1)                  | 15.20(3) | 18.23(5) | 100 | 1.2                                 | 0.07     | 6.54     | 3.26           | 0.00226  | 2.0-10.1  |

U<sub>corr</sub> = U/ (NP-m); where m = number of species; NP = number of experimental points; 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 Tables 1 and 2 are between -1.77 - 0.07. These data evince 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 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 and very few are platykurtic pattern.

**Table 2:** Best fit chemical models of acido-basic equilibria of ethylenediamine in DMF- water mixtures. Temperature = 310.0 K, Ionic strength = 0.16 mol L<sup>-1</sup>

| Solvent % v/v | log β <sub>min</sub> (SD) |          | NP  | U <sub>corr</sub> × 10 <sup>8</sup> | Skewness | Kurtosis | χ <sup>2</sup> | R-factor | pH-range |
|---------------|---------------------------|----------|-----|-------------------------------------|----------|----------|----------------|----------|----------|
|               | 011                       | 012      |     |                                     |          |          |                |          |          |
| DMF           |                           |          |     |                                     |          |          |                |          |          |
| 0.0           | 9.99(1)                   | 17.22(1) | 100 | 2.66                                | -0.25    | 3.00     | 7.70           | 0.0081   | 5.0-10.1 |
| 10.0          | 9.95(1)                   | 16.97(1) | 102 | 1.17                                | -1.17    | 9.30     | 8.00           | 0.0081   | 5.0-10.1 |
| 20.0          | 9.82(0)                   | 16.59(2) | 103 | 6.59                                | -0.39    | 2.08     | 2.03           | 0.0022   | 5.0-10.1 |
| 30.0          | 9.83(2)                   | 15.79(1) | 100 | 4.50                                | -0.22    | 4.22     | 1.22           | 0.0032   | 5.0-10.1 |
| 40.0          | 9.74(3)                   | 16.54(0) | 109 | 2.44                                | -0.35    | 3.17     | 1.17           | 0.0012   | 5.0-10.1 |
| 50.0          | 9.50(6)                   | 16.17(0) | 111 | 7.86                                | -0.44    | 3.03     | 3.08           | 0.0014   | 5.0-10.1 |
| 60.0          | 9.56(7)                   | 16.33(2) | 103 | 1.07                                | -0.66    | 4.22     | 1.34           | 0.0009   | 5.0-10.1 |

U<sub>corr</sub> = U/ (NP-m); where m = number of species; NP = number of experimental points; SD=standard deviation

### Effect of systematic errors

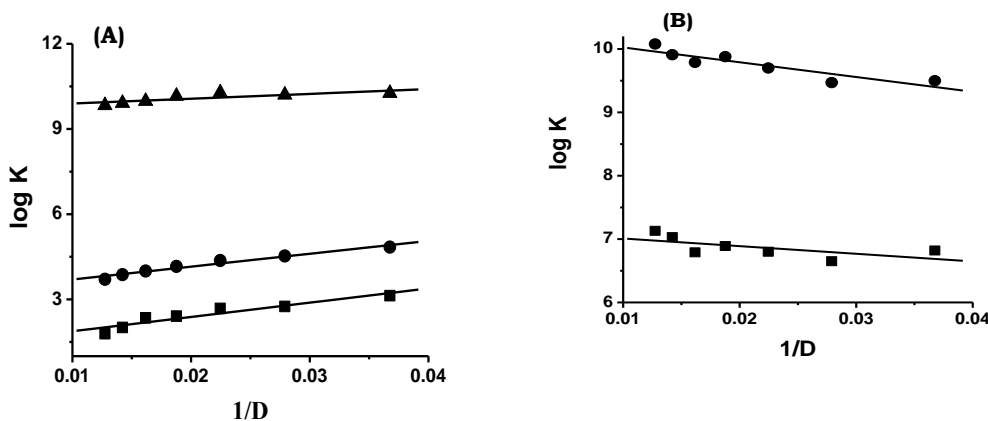
MINIQUAD75 does not have an in-built provision to study the effect of systematic errors in the influential parameters like concentrations of ingredients affect the magnitudes of equilibrium constants. In order to rely upon the best-fit chemical model for critical evaluation and application, an investigation was made by introducing pessimistic errors in the concentrations of alkali, mineral acid and ligand. This type of investigation is useful because the data acquisition was done under varied experimental conditions with different accuracies. Results of typical systems, given in Table 3 emphasize that protonation constants associated with carboxyl proton are more affected than the amino protons. This is probably due to lower magnitude of the former than those for amino protons. Similarly, errors in concentrations of alkali and acid, affect the log β's more than that of the ligand. With the introduction of errors, the SD's are found to increase inferring the appropriateness of experimental conditions and correctness of analytical concentrations.

**Table 3:** Effect of errors in influential parameters on protonation constants of L-aspartic acid and ethylenediamine in 10.0% v/v of DMF- water mixture.

| Ingredient | %Error | log β <sub>mlh</sub> (SD) |                 |                 |           |                 |
|------------|--------|---------------------------|-----------------|-----------------|-----------|-----------------|
|            |        | Asp                       |                 |                 | en        |                 |
|            |        | LH                        | LH <sub>2</sub> | LH <sub>3</sub> | XH        | XH <sub>2</sub> |
| Alkali     | 0      | 9.93(2)                   | 13.70(0)        | 15.75(0)        | 9.95(1)   | 16.97(1)        |
|            | -5     | 10.99(27)                 | 15.79(18)       | 18.69(18)       | 9.99(20)  | 17.01(30)       |
|            | -2     | Rejected                  | 15.07(12)       | Rejected        | 9.83(11)  | 16.70(22)       |
|            | +2     | 10.09(2)                  | 14.29(23)       | 16.89(13)       | 9.59(12)  | 16.30(23)       |
|            | +5     | 09.79(43)                 | 13.78(41)       | 16.27(50)       | Rejected  | Rejected        |
| Acid       | -5     | 09.93(12)                 | 13.99(44)       | 16.36(25)       | 9.37(23)  | 15.70(15)       |
|            | -2     | 10.24(12)                 | 14.48(23)       | 16.94(13)       | 9.58(12)  | 16.20(23)       |
|            | +2     | 10.45(42)                 | 14.96(12)       | 17.75(13)       | 9.85(10)  | 16.78(42)       |
|            | +5     | Rejected                  | 15.43(15)       | 18.43(25)       | 10.15(13) | Rejected        |
|            |        |                           |                 |                 |           |                 |
| Ligand     | -5     | 10.13(12)                 | 14.43(13)       | 17.17(23)       | 9.77(10)  | 16.74(22)       |
|            | -2     | 10.22(12)                 | 14.56(12)       | 17.27(13)       | 9.73(10)  | 16.59(22)       |
|            | +2     | 10.34(10)                 | 14.74(12)       | 17.39(12)       | 9.68(11)  | 16.40(21)       |
|            | +5     | 10.43(10)                 | 14.86(12)       | 17.48(12)       | 9.64(20)  | 16.27(31)       |
|            |        |                           |                 |                 |           |                 |

**Effect of Dielectric Constant of Solvent on Protonation Equilibria**

Co-solvent influences the protonation-deprotonation equilibria in solution by changing the dielectric constant of the medium, which varies the relative contributions of electrostatic and non-electrostatic interactions. Born’s classical treatment[15] holds good in accounting for the electrostatic contribution to the free energy change (ΔG<sub>e</sub>). According to this treatment, the energy of electrostatic interaction or 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. In the present study, it is observed that the linear increase of protonation constants (log K values) in case of Asp and linear decrease in case of en in DMF with reciprocal of dielectric constant as shown in (Figure 4.) The linear variation shows the dominance of electrostatic interactions in both the cases. One dominates the other depending upon the nature of solute and solvent.



**Figure 4:** Variation of stepwise protonation constants (log K) with reciprocal of dielectric constant (1/D) in DMF-water mixture: (A) Asp and (B) en (■) log K<sub>1</sub> (●) log K<sub>2</sub> and (▲) log K<sub>3</sub>.

The logarithm of stepwise protonation constants (logK) was calculated. The calculated stepwise protonation constants are listed in Table 4.

**Table 4:** Stepwise protonation constants of Asp and en in DMF- water mixtures

| Solvent<br>% v/v | L-Aspartic acid    |                    |                    | Ethylenediamine    |                    |
|------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
|                  | log K <sub>1</sub> | log K <sub>2</sub> | log K <sub>3</sub> | log K <sub>1</sub> | log K <sub>2</sub> |
| DMF              |                    |                    |                    |                    |                    |
| 0.0              | 0.054              | 0.138              | 0.993              | 0.236              | 0.999              |
| 10.0             | 0.060              | 0.139              | 0.996              | 0.231              | 0.997              |
| 20.0             | 0.069              | 0.147              | 0.995              | 0.227              | 0.992              |
| 30.0             | 0.067              | 0.148              | 1.007              | 0.205              | 0.992              |
| 40.0             | 0.074              | 0.153              | 1.012              | 0.229              | 0.998              |
| 50.0             | 0.072              | 0.160              | 1.009              | 0.230              | 0.977              |
| 60.0             | 0.079              | 0.173              | 1.008              | 0.232              | 0.980              |

The cation stabilizing nature of co-solvents, specific solvent-water interactions [16] charge dispersion and specific interactions with solute indicated by the changes in the solubility of different species in aqua-organic mixtures account for the deviation of classical linear relationship of log K with 1/D. Though the above demarcation is not rigid, it is instrumental in rationalizing data obtained in a specific solvent range. L-aspartic acid exists in anionic, zwitterionic and cationic forms. Whereas en can exist only in cationic or neutral form at different pH ranges (Figure 5).

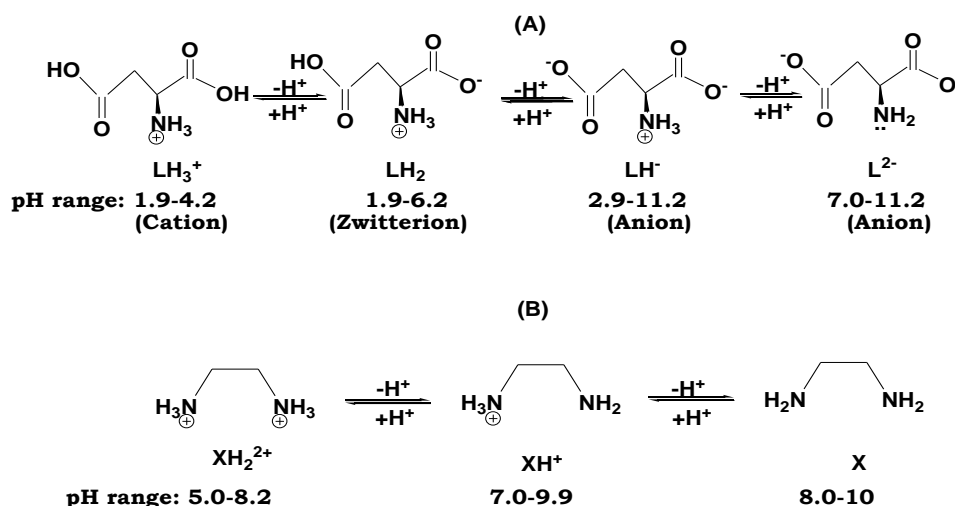
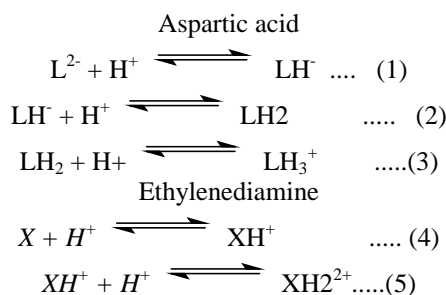


Figure 5: Protonation-deprotonation equilibria of (A) Asp and (B) En.

When the anions of Asp (Equilibria 1-3) are successively protonated, the charge of the species is decreased and low dielectric medium favors the protonation reaction, due to dominant electrostatic interactions. Thus, decrease in dielectric constant of the medium should increase the protonation constant and while the increase in dielectric constant should decrease the protonation constant. The dielectric constant values of DMF-media decrease with increase in the percentage of DMF. The linear trend was observed in Asp in DMF-media. This stabilizes uncharged species (free en) than charged species (protonated en). DMF is protophilic aprotic solvent. DMF stabilizes uncharged species due to its low dielectric constant.



### Distribution Diagrams

Asp has three functional groups all of which participate in protonation equilibria. Typical distribution plots in DMF-water mixture given in (Figure 6) show the existence of LH<sub>3</sub><sup>+</sup>, LH<sub>2</sub>, LH<sup>-</sup> and L<sup>2-</sup> for Asp, the LH<sup>-</sup> is present to an extent of 90% in the pH range 1.9-11.2 Successive deprotonation takes place with an increase of the pH.

Ethylenediamine has two functional (amino) groups. Hence, its various forms are XH<sub>2</sub><sup>2+</sup>, XH<sup>+</sup> and X in the pH range 5.-10.2 The XH<sub>2</sub><sup>2+</sup> exists up to a pH of 8.2. The monoprotonated species (XH<sup>+</sup>) exists in the pH range 5-9.9. Successive deprotonation of XH<sub>2</sub><sup>2+</sup> with increasing pH forms ultimately X above a pH of 8.0.

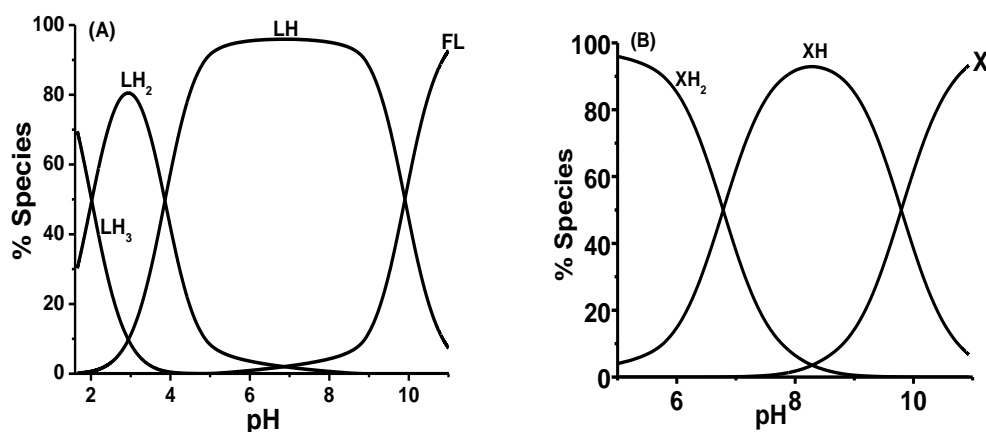


Figure 6: Species distribution diagrams of (A) Asp and (B) en in 10% v/v DMF-water mixture

#### IV. Conclusion

The investigation by the biomimetic studies indicates the pH ranges of protonation equilibria of Asp and en in low dielectric media as 1.9-11.2, 5.0-10. Asp has two dissociable carboxyl protons and one amino group, which can associate with a proton. It exists as  $LH_3^+$  at low pH and gets deprotonated with the formation of  $LH_2$ ,  $LH$  and  $L^-$  successively with increasing pH. En has two amino groups with the formation of  $XH_2^{2+}$ ,  $XH^+$  and  $X$ .  $XH_2^{2+}$  exists up to a pH of 5.0-10. The monoprotonated species  $XH^+$  exists in the pH range 5.0-9.9. Successive deprotonation of  $XH_2^{2+}$  with increasing pH forms ultimately  $X$  above a pH of 8.0 in DMF-water mixtures. The linear increase of log values of protonation constants of Asp with decrease in dielectric constant of DMF-water media indicates the dominance of electrostatic forces on the protonation-deprotonation equilibria. The opposite trend in case of en is due to the dominance of non-electrostatic forces. The negative and positive values of 'a' correspond to the excess mineral acid present in the titrand and the number of dissociable and associable protons of the ligands. The effect of systematic errors in the influential parameters shows that the errors in the concentrations of alkali and mineral acids will affect the protonation constants more than that of the concentration of the ligand.

#### References

- [1]. H. Tapiero, G. Mathe, P. Couvreur, K. D. Tew., Biomed Pharmacother, vol. 56, p. 446-457, 2002.
- [2]. P. Paoletti, Pure Appl. Chem., vol. 56, p. 491, 1984.
- [3]. Hiromichi, F., Kenichi, M., Kita, Y., Ozora, K. and Yuruke, O., Org. Lett., vol. 9, p. 1687, 2007.
- [4]. Arash, L., Habibi, M. H., Harrington, R. W., Morteza, M. and William, C., J. Fluorine Chem, vol. 127, p. 769, 2006.
- [5]. R.J. Sengwa., V.Khatari, S.Sankhala, J.Molecular Liquids, vol. 144, pp. 89-96, 2009.
- [6]. Krishna Prasad, K. M. M., Satyanarayana, A., Kamala Sastry, C. and Sambasiva Rao, R., Proc, Indian Council of Chemists, 1985.
- [7]. Gran G., Anal Chim Acta, vol. 206, p. 111, 1988.
- [8]. Rao R S and Rao G N., in Computer Applications in Chemistry, Mumbai, Himalaya Publishing House, 2005, p. 302.
- [9]. G. N. Rao., Ph. D. Thesis in Chemistry, India.: Visakhapatnam, 1989.
- [10]. P. Gans, A. Sabatini, A., Vacca, Inorg. Chim. Acta, vol. 18, p. 237-239, 1976.
- [11]. M. P. Latha, V. M. Rao, T. S. Rao and G. N. Rao., Bull. Chem. Soc. Ethiop, vol. 21, p. 363-372, 2007.
- [12]. R. B. Jarzeboska, A. Gasowska and L. Lomozik., Bioinorg. Chem. Appl, vol. 10, p. 1155-1159, 2008.
- [13]. Rao, R. S. and Rao, G. N., in 2005, Mumbai, Himalaya publishing house, Computer applications in Chemistry, p. 302-309.
- [14]. Hamilton, W. C., Acta Crystallogr., vol. 18, p. 502, 1965.
- [15]. Born, M., Z Phys., vol. 1, p. 45, 1920.
- [16]. Rao, G. N. and Rao, R. S., J. Teach. Res. Chem., vol. 2, p. 15, 1995.

IOSR Journal of Applied Chemistry (IOSR-JAC) is UGC approved Journal with Sl. No. 4031, Journal no. 44190.

Hadgu Hailekiros Belay. " Shift in pKa of of L-Aspartic Acid and Ethylenediamine in Low dielectric media: Biomimetic Study." IOSR Journal of Applied Chemistry (IOSR-JAC) 12.8 (2019): 55-61.