

Effect of Dielectric Constant on Protonation Equilibria of L-Dopa, Mercapto Succinic Acid and 1, 10 – Phenanthroline in Acetonitrile-Water Mixture

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Abstract: *Solute-solvent interactions of L-dopa, Mercaptosuccinic acid and 1, 10-phenanthroline have been studied in 0-60% v/v acetonitrile—water media using pH-metric method. The protonation constants have been calculated with the computer program MINQUAD75. Selection of the best fit chemical model of the protonation equilibria is based on standard deviation in protonation constants and residual analysis using crystallographic R-factor and sum of squares of residuals in all mass balance equations. Linear variation of protonation constants with inverse of dielectric constants of the solvent mixture has been attributed to the dominance of the electrostatic forces. Distribution of species, protonation equilibria and effect of influential parameters on the protonation constants have also been presented.*

Keywords: Solute-solvent interactions, L-Dopa, Mercaptosuccinic acid, 1, 10-phenanthroline, Acetonitrile.

1. Introduction

L-dopa (L-3,4 dihydroxyphenylalanine) is a naturally occurring dietary supplement and psychoactive drug found in certain kinds of food and herbs, and is synthesized from the essential amino acids L-phenylalanine and L-tyrosine in the mammalian body and brain. Dopa is the precursor to the neurotransmitters dopamine, norepinephrine (noradrenaline) and epinephrine (adrenaline). Dopa is used as a prodrug to increase dopamine levels in the treatment of Parkinson's disease [1,2], since it is able to cross the blood-brain barrier whereas dopamine itself cannot.

Mercaptosuccinic acid (MSA) or thiomalic acid (HOOC – CH(SH)–CH₂ –COOH) is a dicarboxylic acid containing a thiol functional group (-SH group) instead of an -OH group in malic acid [3]. It is an important organic compound with multifunctional intermediate in organic synthesis. It is widely used in the synthesis of various biologically active sulfur containing compounds such as the antileukemic spiro [indoline- 3, 2' -thiazolidine]-2, 4' -diones[4], and the antimicrobial[5,6] and antiubercular 4-thiazolidinones. It is also used as a building block in the synthesis of novel polyanionic inhibitors of human immunodeficiency virus and other viruses [7], and as a starting material in the synthesis of isocysteine, an important non-proteinogenic amino acid in a potent peptide inhibitor of stromelysin [8]. MSA is a tridentate ligand which has the ability to form strong complexes with many metal ions in natural environment and within cells[9] and it has three replaceable hydrogen ions (two from the carboxylic and one from the sulfhydryl functional groups). The determination of protonation constants of MSA is important in understanding its physico-chemical behavior and its interaction with metals under low dielectric constants in norganic solvent-water mixtures because it is known that such mixed solvents produce a solvent with quite different properties, both, physically (dielectric, density and viscosity and chemically

(acid-base and donor-acceptor properties) and provide a better model for in vivo reactions [10,11].

1,10-Phenanthroline (phen) is an organic compound. As a bidentate ligand in coordination chemistry, it forms strong complexes with many metal ions. Phen, an N-donor ligand with planar aromatic rings, is known [12-18] to form protonated species in acidic solution, *i.e.*, H(phen)⁺ and H(phen)₂⁺ in the pH range of 2.0- 7.0, and H₂(phen)²⁺ at H⁺ > 1 mol dm⁻³. Due to hydrophobicity of aromatic rings of phen, the solubility of the neutral species is low in water which remarkably increases in organic solvents and also in aqua-organic mixtures. The protonation constant of phen has been studied in various aqueous alcohol solutions [19]. Hence, the authors have studied the effect of dielectric constant of the medium on the protonation equilibria of dopa, mercapto succinic acid and phenanthroline. This type of study throws light on the role of amino acid residues and substrates.

Acetonitrile (AN) is a protophobic dipolar aprotic solvent and it does not form any hydrogen bond with solute species. It is a weak base [20] and a much weaker acid [21] than water. Therefore cations and anions have lower solvation energies in acetonitrile than in water [22]. The protophobic character of AN is due to formation of dimers [23].

2. Materials and Methods

2.1 Materials

Solutions (0.05 mol L⁻¹) of L-dopa (Himedia,India), mercaptosuccinic acid (Himedia,India) and 1, 10-phenanthroline mono hydrate (Excelar) were prepared in triple-distilled water by maintaining 0.05 mol L⁻¹ nitric acid concentration to increase the solubility. Acetonitrile(Finar, India) was used as received. Nitric acid (Merck, Germany) of 0.2 mol L⁻¹ was prepared. Sodium nitrate (Merck,

Germany) of 2 mol L^{-1} was prepared to maintain the ionic strength in the titrand. Sodium hydroxide (Merck, Germany) of 0.4 mol L^{-1} was prepared. Acid and alkali 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) [24]. The strengths of alkali and mineral acid were determined using the Gran plot method [25].

2.2 Alkalimetric Titrations

Alkalimetric titrations were carried out in media containing varying compositions of AN (0-60% v/v) maintaining an ionic strength of 0.16 mol L^{-1} with sodium nitrate at $303 \pm 0.05 \text{ K}$. An Elico LI – 120 pH meter was used. Potassium hydrogen phthalate (0.05 mol L^{-1}) and borax (0.01 mol L^{-1}) solutions were used to calibrate the pH meter. In each titration, the titrand consisted of approximately 1 mol of hydrochloric acid. The amounts of the ligands in the titrands ranged between 0.25, 0.375 and 0.5 mmols. The glass electrode was equilibrated in a well stirred AN-water mixture containing inert electrolyte for several days. At regular intervals titration of strong acid was titrated against alkali to check the complete equilibration of the glass electrode. The calomel electrode was refilled with AN-water mixture of equivalent composition as that of the titrand. The details of experimental procedure and titration assembly have been detailed elsewhere [26].

2.3 Modeling Strategy

The approximate protonation constants of dopa, MSA and phen were calculated with the computer program SCPHD [27]. The best fit chemical model for each system investigated was arrived at using non-linear least-squares

computer program, MINQUAD75 [28], which exploits the advantage of constrained least-squares method in the initial refinement and reliable convergence of Marquardt algorithm.

3. Results and Discussions

3.1 Secondary Formation Functions

Secondary formation functions like average number of protons bound per mole of ligand (nH) is useful to detect the number of equilibria. Plots of nH versus pH for different concentrations of the ligand should overlap if there is no formation of polymeric species. Overlapping formation curves for dopa, MSA and phen (Figure 1) rule out the polymerization of the ligand molecules. The pH values at half integral values of nH correspond to the protonation constants of the ligands and the number of half integrals in the pH range of the study corresponds to the number of equilibria. Thus, three half integrals (0.5, 1.5 and 2.5) in case of dopa, MSA and one half integral (0.5) in case of phen (Figure 1) emphasize the presence of three in case of dopa, MSA and phen has one protonation-deprotonation equilibria in the pH range present study. The number of plateaus in the formation curves corresponds to the number of these equilibria. A very low standard deviation (SD) in log β values, Ucorr (sum of the squares of deviations in concentrations of ligand and hydrogen ion at all experimental points corrected for 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 systems corroborate that the residuals are around a zero mean with little dispersion.

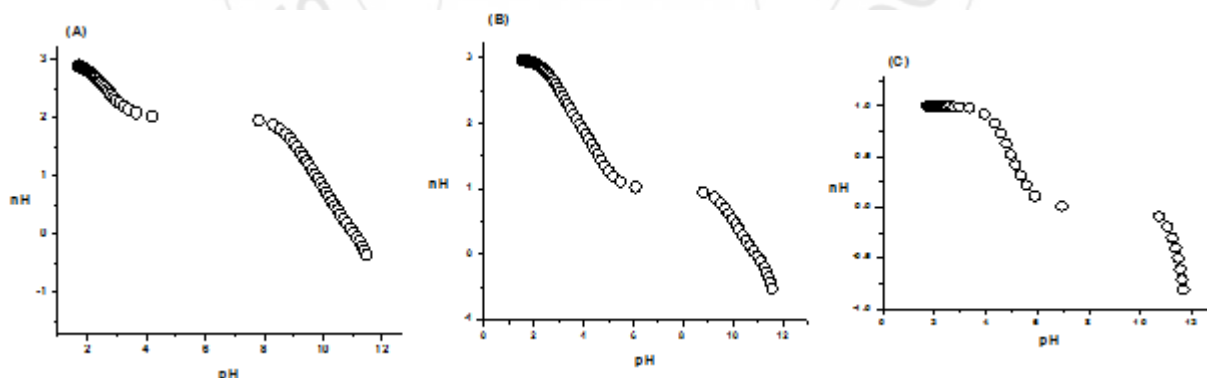


Figure 1: Plots of nH versus pH in 30 % v/v AN-water mixture; (A) dopa (B) MSA and (C) phen, respectively

3.2 Residual Analysis

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 show that the best fit models portray the acido-basic equilibria of dopa,MSA and phen in AN-water mixtures, as discussed below.

Table 1: Best-fit chemical models of acido-basic equilibria of dopa, MSA and phen in AN-water mixtures

%v/v AN	Logβ _{mlxh} (SD)			NP	U _{corr}	Skewness	Kurtosis	χ ²	R-Factor	pH range
	LH ₃	LH ₂	LH							
L -dopa										
0.0	10.18(02)	19.37(02)	21.50(04)	145	38.02	-0.75	4.86	46.77	0.0301	1.6-11.5
10	10.23(02)	19.49(02)	22.47(04)	69	10.87	0.32	2.72	1.48	0.0199	2.5-3.0, 9.5-10.00
20	10.28(05)	19.52(01)	22.36(03)	33	2.56	0.24	2.44	4.79	0.0081	3.0-4.9, 8.9-10.0
30	10.36(07)	19.54(03)	22.17(05)	24	5.38	0.07	3.06	2.00	0.0123	3.0-3.7, 8.4-10.5
40	10.41(04)	19.72(01)	22.57(02)	23	0.965	-0.21	3.64	4.78	0.0048	2.5-3.0 9.5-10.0
50	10.57(03)	19.75(04)	22.43(06)	64	12.73	-1.12	5.36	18.75	0.0187	2.1-4.2, 9.5-10.8
60	10.64(03)	20.00(02)	23.09(04)	70	6.64	0.06	4.01	14.63	0.0149	2.5-4.5, 6.0-10.5
Mercaptosuccinic acid										
0.0	9.97(04)	14.35(05)	17.16(06)	87	43.21	-0.09	4.27	28.11	0.0218	2.5-3.1, 4.0-9.8
10	9.98(03)	14.78(10)	17.54(09)	102	21.11	0.92	4.35	102.59	0.0333	2.5-4.5, 0-11.8
20	10.01(01)	14.37(03)	17.07(05)	61	5.86	-0.03	7.94	42.72	0.0180	3.0-10.5
30	10.06(02)	14.62(05)	17.69(10)	81	15.64	2.09	8.21	117.80	0.0350	3.5-11.5
40	10.09(06)	14.69(08)	17.24(12)	69	35	-1.64	8.53	20.72	0.0362	2.1-10.2
50	10.12(05)	14.89(08)	18.01(10)	73	36	-2.54	13.21	26.93	0.0315	2.1-10.5
60	10.14(04)	15.02(07)	18.47(09)	85	36.7	-2.27	11.52	88.54	0.0384	2.0-11.0
1,10-phenanthroline										
0.0			4.92(02)	21	14.88	-0.39	2.78	3.71	0.0427	3.6-6.0
10			4.95(06)	46	65.33	0.48	3.66	12.00	0.0838	3.0-7.0
20			4.97(01)	14	2.97	-0.62	3.30	2.86	0.0016	3.5-5.0
30			5.00(07)	22	36.85	0.08	2.04	9.82	0.0652	3.5-5.0
40			5.02(02)	15	6.41	-0.04	3.29	5.20	0.0244	1.0-4.5
50			5.05(02)	35	15.35	-0.13	4.10	8.40	0.0401	3.0-6.5
60			5.10(04)	36	49.42	-0.31	3.09	8.00	0.0689	3.0-6.0

U_{corr}=U/(NP-m) X10⁸; NP= Number of points; m= number of protonation constants; SD= Standard deviation

3.3 χ² test

χ² 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 χ² calculated is less than the table value, the model is accepted.

3.4 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 number 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.

3.5 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 -2.54 and 2.09. These data evince that the residuals form a part of normal distribution; hence, least-squares method can be applied to the present data.

3.6 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 leptokurtic pattern in the case of dopa, msa and phen. 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 typical experimental and simulated titrations data indicates that the proposed models represent the experimental data.

3.7 Effect of Systematic Errors

Any variation in the concentrations of ingredients like alkali, mineral acid and ligand affects the magnitudes of protonation constants. Such parameters are called influential or dangerous parameters. 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 influential parameters. 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 that of the ligand.

Table 2: Effect of errors in influential parameters on the protonation constants in 30% AN-water mixture

L-dopa		MSA			Phen		
Ingredient	% of error	LH ₃	LH ₂	LH	LH ₃	LH ₂	LH
	0	10.41(07)	19.55(03)	22.19(05)	10.06(02)	14.62(05)	17.69(10)
Alkali	-5	10.57(22)	19.669(10)	22.34(14)	10.16(04)	14.79(09)	17.87(20)
	-2	10.47(12)	19.59(05)	22.24(08)	10.19(02)	14.69(05)	17.76(12)
	2	10.36(04)	19.52(02)	22.14(03)	10.03(03)	14.56(06)	17.63(13)
Acid	5	10.30(06)	19.48(02)	22.09(05)	9.98(05)	14.48(10)	17.54(22)
	-5	10.32(05)	19.49(02)	22.09(04)	10.01(04)	14.53(07)	17.58(16)
	-2	10.37(05)	19.52(02)	22.15(03)	10.04(02)	14.58(05)	17.64(12)
Ligand	2	10.46(11)	19.58(05)	22.24(08)	10.09(02)	14.66(05)	17.74(11)
	5	10.54(19)	19.64(09)	22.33(13)	10.12(03)	14.73(07)	17.82(14)
	-5	10.38(06)	19.53(02)	22.18(04)	10.03(03)	14.58(06)	17.65(12)
	-2	10.40(07)	19.54(03)	22.18(05)	10.05(02)	14.60(05)	17.67(11)
	2	10.42(08)	19.56(03)	22.20(06)	10.07(02)	14.64(05)	17.71(10)
	5	10.44(09)	19.57(04)	22.20(06)	10.10(02)	14.67(05)	17.73(11)

3.8. Effect of Dielectric Constant of Medium

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 [29]. According to this treatment, the energy of electrostatic interaction or logarithm of step-wise protonation constant ($\log K$) should vary linearly as a function of the reciprocal of the dielectric constant ($1/D$) of the medium. Such linear variation of the protonation constants of dopa, MSA and phen (Figure 2) in acetonitrile-water mixture shows the dominance of electrostatic interactions. In the case of some mono- and dicarboxylic acids and simple phenolic ligands, electrostatic (long-range, non-specific or universal) solute-solvent interactions are predominant in binary mixtures of water with methanol, ethanol, dioxin or acetone as cosolvent [30]. Many workers were of the opinion that both electrostatic and non-electrostatic effects should be considered even in the case of simple acido-basic equilibria; one dominates the other, depending upon the nature of solute and solvent [31-33].

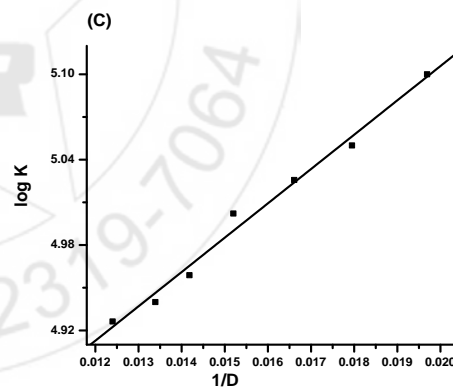
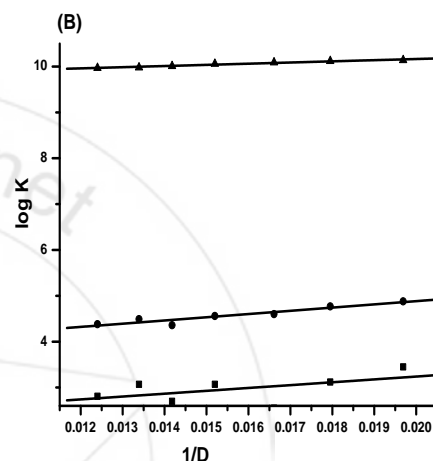
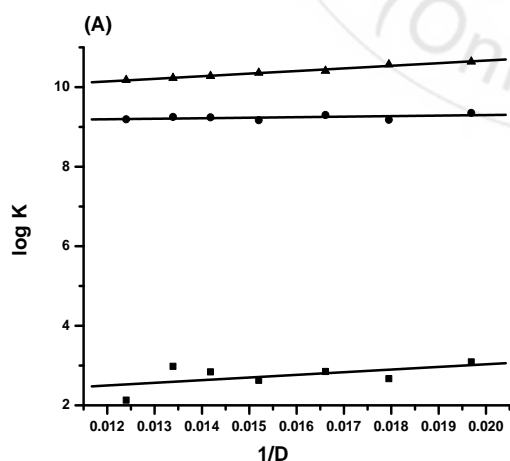


Figure 2: Variation of step-wise protonation constant ($\log K$) with reciprocal of dielectric constant ($1/D$) in AN-water mixture (A) dopa (B) MSA and (C) Phen respectively

3.9 Distribution Diagrams

The distribution plots (Figure 3) produced using the protonation constants from the best fit models (Table 1) show the existence of LH_4^+ , LH_3 , LH_2^- and LH^- in the case of dopa, MSA has LH_3 , LH_2^- , LH^- and L and phen has LH^+ , L in different pH ranges. The corresponding protonation-deprotonation equilibria are shown in (Figure 4). The most predominant species in dopa is LH_3 , in case of MSA is LH_2^- and phen is LH^+ and the corresponding pH range is 2.0-11.0, 3.0-11.8 and 3.0-6.0. As the alkali is added to the titrand containing the ligands, the protonated forms of the ligands lose their protons.

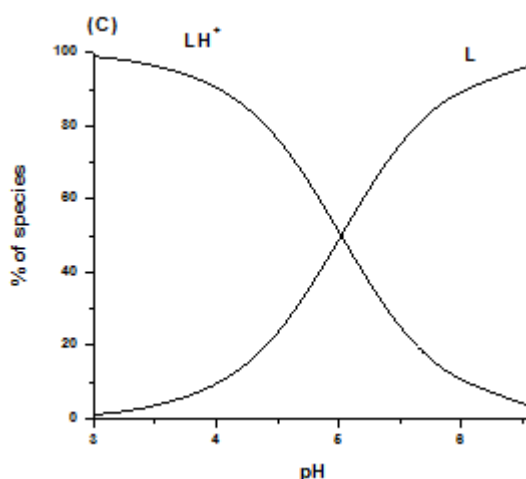
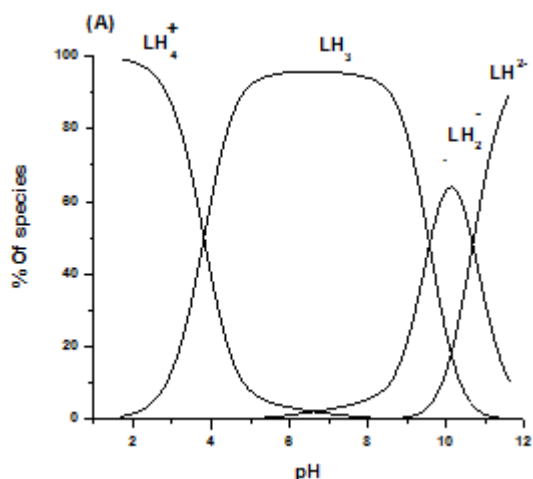
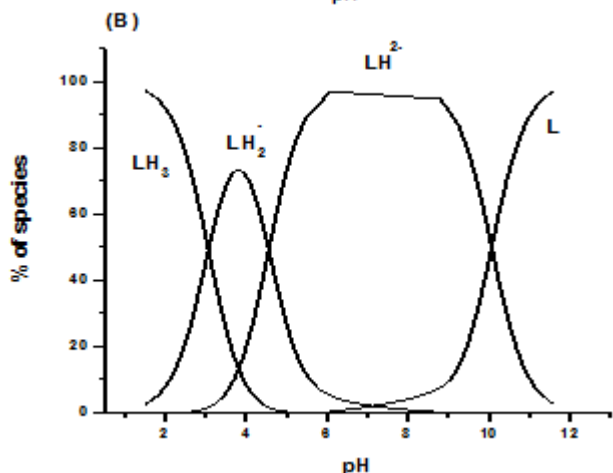
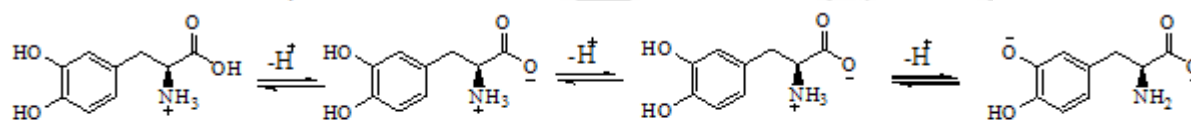


Figure 3: Distribution diagram of (A) dopa (B) MSA and (C) phen in 30% AN-water medium

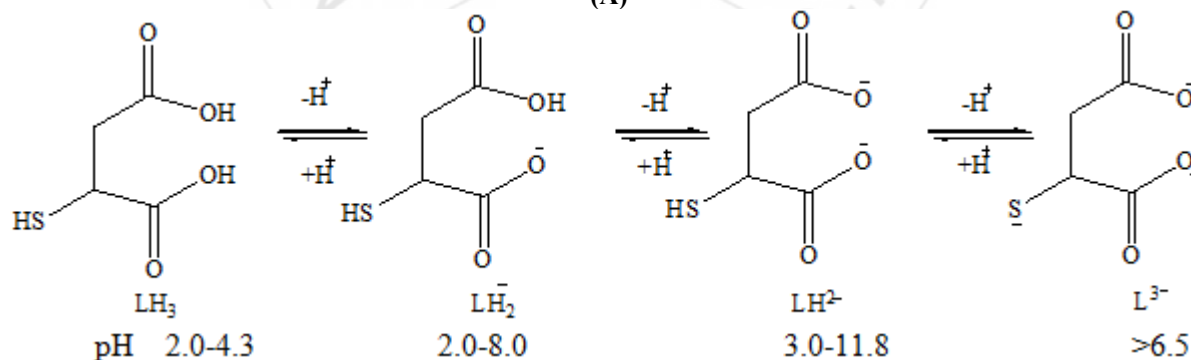


In the pH range of study, dopa loses carboxylic, phenolic and amino protons successively. The second phenolic proton is lost at pH greater than 12.0. Hence, under present experimental conditions the most deprotonated form of dopa is LH²⁻. Mercaptosuccinic acid loses carboxylic and thiol protons and the most deprotonated form is L. Similarly phen is deprotonated below pH 1.0, where phen is mono protonated (LH⁺) at a pH 3.0-7.0 and so only one protonated constant is reported.



LH₄⁺ pH 2.0-8.0 LH₃ 2.0-11.0 LH₂ 5.0-11.8 LH²⁻ >9

(A)



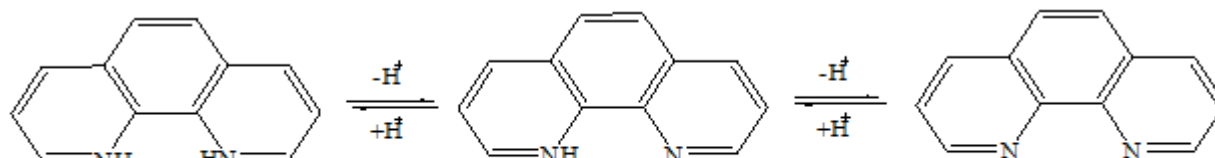
LH₃ pH 2.0-4.3

LH₂ 2.0-8.0

LH²⁻ 3.0-11.8

L³⁻ >6.5

(B)



LH₂⁺ pH <1

LH⁺ 3.0-6.0

L >7.0

(C)

Figure 4: Protonation–deprotonation equilibria of (A) dopa, (B) MSA and (C) phen, respectively.

4. Conclusions

- 1) Dopa has three dissociable protons and one aminogroup which can associate with a proton. It exists as LH_4^+ at low pH and gets deprotonated with the formation of LH_3 , LH_2^- , LH^- successively with increase in pH.
- 2) MSA has three dissociable protons exists in LH_3 form at low pH and deprotonated with the formation of LH_2^- , LH^- and L species, successively with increase in pH.
- 3) Phen forms LH_2^{2+} at low pH and gets deprotonated with the formation of LH^+ and L with increase in pH.
- 4) The log values of protonation constants of dopa, MSA and phen increase linearly with decreasing dielectric constant of AN-water mixtures. This indicates the dominance of electrostatic forces in the protonation-deprotonation equilibria.
- 5) 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 ligand.

References

- [1] W. Birkmayer, O. Horneykiewicz. Wien. Klin. Wschr, 1961, 73, 787-788.
- [2] O. Horneykiewicz, Wien. Klin. Wschr, 1963, 75, 309-312.
- [3] S.Patai, The Chemistry of the Thiol Group, Vol. 15, Wiley-Interscience, London, 1974; pp. 569.
- [4] M.Rajopadhye, F.D.Popp, J. Heterocyclic Chem. 24, 1637, (1987)
- [5] B. S. Vashi, D. S. Mehta, V. H. Shah, Indian J. Chem. 34B, 802, (1995)
- [6] K. Desai, A. J. Baxi, J. Indian Chem. Soc. 69, 212, (1992)
- [7] M. P. Dave, J. M. Pagel, N. A. Langalia, K. A. Thaker, J.Indian Chem. Soc. 61, 891, (1984)
- [8] R. Pires, K. Burger, Tetrahedron Lett. 37, 8159, (1966)
- [9] H. Rozan, M. E. Lassman, D. P. Ridge, G. W. Luther, Nature 406, 879, 2000
- [10] J. Crosby, R. Stone, G.E. Lienhard, J. Am. Chem. Soc. 92, 2891, (1970)
- [11] M. Arroyo, R. Torres-Guzman, I. Mata, M. P. Castillon, C. Acebal, Enzyme Microb. Technol. 27, 122, (2000)
- [12] M. J. Fahsel, C. V. Banks, J. Am. Chem. Soc. ,1966, 88, 878-884.
- [13] P. Paoletti, A. Dei, A. Vacca, J.Chem. Soc., (A),1971 , 2656-2659.
- [14] R. D. Alexander, A. W. L. Dudeney, R. J. Irving, J. Chem Soc. Faraday Trans 1, 1978, 74, 1075-1080.
- [15] P. Mitchell, J. Chem. Soc. Dalton Trans., 1980, 1079-1086.
- [16] P. G. Daniele, C. rigano, S. Sammartano, Talanta, 1985 , 32, 78-80.
- [17] S. Capone, A. D. Robertis, C. D. Stefano, R. Scarcella, Talanta, 1985, 32, 675-677.
- [18] A. D. Robertis, C. Foti, A. Gianguzza, C. Rigano, J. Solution Chem., 1996, 25, 597 -606.
- [19] S. Bandyopadhyay, A. K. Mandal, S. Aditya, J. Indian Chem. Soc., 1981, 58, 467-473.
- [20] Loubengayer AW and Sears DS (1945), J. Am. Chem. Soc. 67: 164- 172.
- [21] Fritz JS (1953) "Differential titration of amines", Anal. Chem., 5:407-411.
- [22] Kolthoff IM and Lingane JJ (1952) "Polarography, Interscience Publishers, New York.
- [23] De AL (1984), Electrochim. Aca 29: 471 - 475.
- [24] R. S. Rao, G. N. Rao, Computer Applications in Chemistry, Himalaya Publishing House, Mumbai 2005, 302 – 309.
- [25] G. Gran, Anal. Chim. Acga. 1988, 206, 111-123.
- [26] N. Padmaja, M. S. Babu, G. N. Rao, R. S. Rao, K. V. Ramana, Polyhedron,1990, 9, 2497 – 2506.
- [27] G. N. Rao Complex equilibria of some biologically important metal ions in aqua-organic media, Ph. D. thesis, Andhra University, Visakhapatnam, India 1989.
- [28] P. Gans, A. Sabatini, A. Vacca, Inorg. Chim. Acta 1976, 18, 237 – 239.
- [29] M. Born, Z. Phys., 1920, 1, 45-57.
- [30] M. Padma Latha, V. Maheswara Rao, T. Siva Rao, G. N. Rao, Acta Chim. Slov., 2007 , 54,160- 165.
- [31] Schneider, Top. Curr. Chem., 1976, 68, 103 – 110.
- [32] M. H. Abraham, J. Liszi, J. Inorg. Nucl. Chem., 1981, 43, 143– 151.
- [33] D. Feakins, D. O' Neille, W. E. Woghnie, J. Chem. Soc. Faraday Trans, 1983, 2289-2297