PROTONATION EQUILIBRIA OF L-DOPA AND 1,10 PHENONTHROLINE IN PROPYLENE GLYCOL-WATER MIXTURES

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ABSTRACT

Protonation equilibria of L-Dopa and 1,10 phenonthroline have been studied in varying concentrations (0-60% v/v) of propylene glycol–water mixtures maintaining an ionic strength of 0.16 mol dm^{-3} at 303 K using pH metric method. The protonation constants have been calculated with the computer program MINIQUAD75 and the best fit models are arrived at based on statistical grounds employing crystallographic R factor, χ^2, skewness and kurtosis. Dopa has three dissociable protons and one amino group which associate with proton. It exists as LH_4^+ at low pH and gets deprotonated with the formation of LH_3, LH^-_2 and LH^{2-} successively with increase in pH. Phen forms LH_2^{2+} at low pH and gets deprotonated with the formation of LH^+ and L with increase in pH. Secondary formation functions confirm the existence of 3 and 2 protonation equilibria for dopa and phen, respectively. The linear increase of log values of protonation constants of Dopa with decreasing dielectric constant of PG-water mixtures indicates the dominance of electrostatic forces in the protonation-deprotonation equilibria. Phen exhibits non-linear trend indicating the dominance of non-electrostatic forces.

Keywords: Protonation equilibria, propylene glycol, L-Dopa, 1,10-Phenanthroline

1. INTRODUCTION

L-Dopa (L-3,4-dihydroxyphenylalanine) is a naturally occurring dietary supplement. Its richest natural source is from plant kingdom like the seeds of Mucuna Pruriens.[1] Dopa is a popular drug in the treatment of manganese poisoning and Parkinson's disease (PD)[2] which are accompanied by neurologically similar sequels[3]. Dopa increases dopamine concentration, since it is capable of crossing the blood brain barrier, where dopamine itself cannot. Once dopa enters the central nervous system (CNS) it is converted in to dopamine by the enzyme aromatic L-amino acid decarboxylase, also known as dopa decarboxylase. However, conversion to dopamine also occurs in the peripheral tissues, causing adverse effects and decreasing the available dopamine to the CNS. So it is the standard practice to co-administer a peripheral dopa decarboxylase inhibitor. Compounds containing Dopa were found to cross-link to proteins[4]. Protonation reactions of dopa were reported[5-13] that $Hdopa^+$ $(H_4L)^+$, dopa (H_3L), $dopa^-$ $(H_2L)^-$ and $dopa^{2-}$ $(HL)^{2-}$ were formed in the pH range of 1.6-11.0 and $dopa^{3-}$ $(L)^{3-}$ above pH 13.0.

1,10 Phenonthroline (phen) or 4,5-diazaphenanthrene is a tricyclic compound. Phen is a metal chelator. As a bidentate ligand in coordination chemistry, it forms strong complexes with many metal ions through N-atoms[14-20]. 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 were reported in various aqueous alcohol solutions[21]. The protonated species $Hphen^+$ and H_2phen^{2+} were reported in the pH range 3.8-5.5 and < 1.0, respectively[14-16,22,23].

1,2-propanediol, also known as propylene glycol (PG) has a dielectric constant[24] of 30.2. The dielectric constant of PG-water mixture decreases with increase in the mole fraction of PG. Hence this medium is chosen to study the acido-basic equilibria to mimic the physiological conditions where the concept of equivalent solution dielectric constant[25] for active site cavities of protein is applicable. The effect of dielectric constant on the protonation equlibria of Dopa and phen in Dioxan-water mixtures has been studied earlier in our laboratory.[26]

2. EXPERIMENTAL

2.1 Materials

Solutions (0.05 mol L^{-1}) of L-Dopa (Loba, India) and 1,10-phenanthroline mono hydrate (Finar, India) were prepared in triple-distilled water by maintaining 0.05 mol L^{-1} hydrochloric acid concentration to increase the solubility. 1,2 Propanediol (Finar, India) was used as received. Hydrochloric acid (Qualigens, India) of 0.2 mol L^{-1} was prepared. Sodium chloride (Qualigens, India) of 2 mol L^{-1} was prepared to maintain the ionic strength in the titrand. Sodium hydroxide (Qualigens, India) of 0.4 mol L^{-1} was prepared. All the 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)[27]. The strengths of alkali and mineral acid were determined using the Gran plot method[28,29].

2.2 Alkalimetric Titrations

Alkalimetric titrations were carried out in media containing varying compositions of PG (0-60% v/v) maintaining an ionic strength of 0.16 mol L^{-1} with sodium chloride at 303±0.05K. 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 contained approximately 1 mmol of hydrochloric acid. The initial concentrations of ingredients are given in Table I.

Table I: Total initial concentrations of ingredients (in mmol) in proton-ligand titrations.

PG % (v/v)	TL0	
	Dopa	Phen
0	0.2484	0.2491
	0.3727	0.3737
	0.4969	0.4983
10	0.2512	0.2535
	0.3768	0.3802
	0.5024	0.5069
20	0.2518	0.2535
	0.3777	0.3802
	0.5036	0.5069
30	0.2961	0.2598
	0.4441	0.3897
	0.5921	0.5195
40	0.2556	0.2494
	0.3834	0.3741
	0.5112	0.4988
50	0.2462	0.2486
	0.3694	0.3728
	0.4925	0.4971
60	0.2501	0.2501
	0.3752	0.3751
	0.5002	0.5002

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The glass electrode was equilibrated in a well stirred PG-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 PG-water mixture of equivalent composition as that of the titrand. Alkalimetric titrations were performed in media containing 0-60 % v/v PG-water mixtures pH metrically. The details of experimental procedure and titration assembly have been detailed elsewhere[30].

2.3 Modeling Strategy

The approximate protonation constants of dopa and phen were calculated with the computer program SCPHD[31]. The best fit chemical model for each system investigated was arrived at using non-linear least-squares computer program, MINIQUAD75[32], which exploits the advantage of constrained least-squares method in the initial refinement and reliable convergence of Marquardt algorithm. The variation of stepwise protonation constants (log K) with the dielectric constant of the medium was analyzed on electrostatic grounds for the solute-solute and solute-solvent interactions.

2.4 Residual Analysis[27]

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. For an ideal normal distribution, the values of kurtosis and skewness should be three and zero, respectively.

χ^2 test

χ^2 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 χ^2 calculated is less than the table value, the model is accepted.

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 numbers of species the models whose values are greater than R-table are rejected.

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.

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).

3. RESULTS AND DISCUSSION

3.1 Secondary formation functions

Secondary formation functions like average number of protons bound per mole of ligand ($\bar{n}H$) and number of moles of alkali consumed per mole of ligand (**a**) are useful to detect the number of equilibria. Plots of $\bar{n}H$ versus pH (formation curves) for different concentrations of the ligand should overlap if there is no formation of polymeric species. Overlapping formation curves for dopa and phen (Figure 1) rule out the polymerization of the ligand molecules. 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 dopa and one half integral in the case of phen (Figure 2) emphasize the presence of three and one protonation-deprotonation equilibria in the pH range of present study. The number of plateaus in the formation curves corresponds to the number of these equilibria.

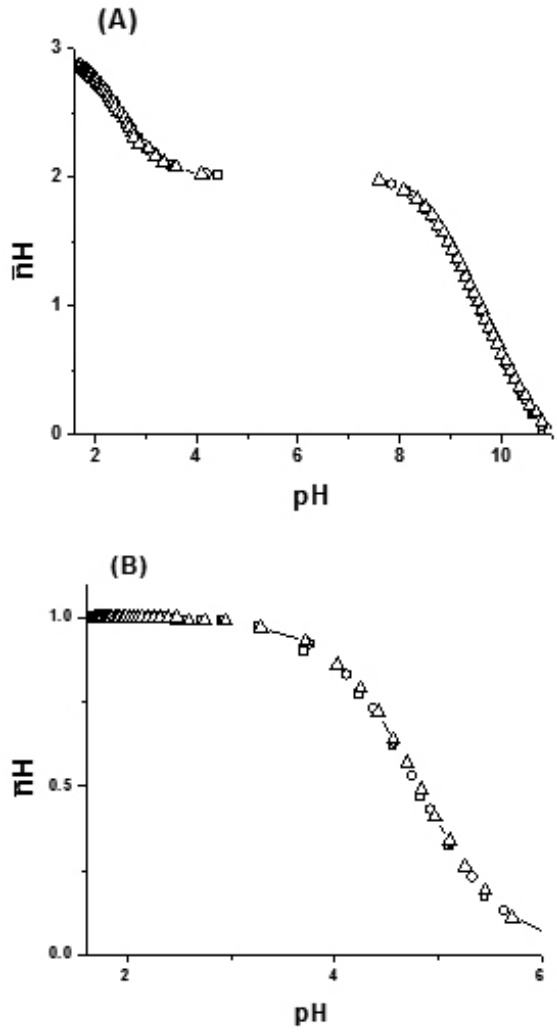


Figure 1: Plots of $\bar{n}H$ versus pH in 30 % v/v PG-water mixture; **(A)** dopa (□) 0.29, (○) 0.44, and (Δ) 0.59mmol and **(B)** phen (□) 0.25, (○) 0.38, and (Δ) 0.51mmol, respectively.

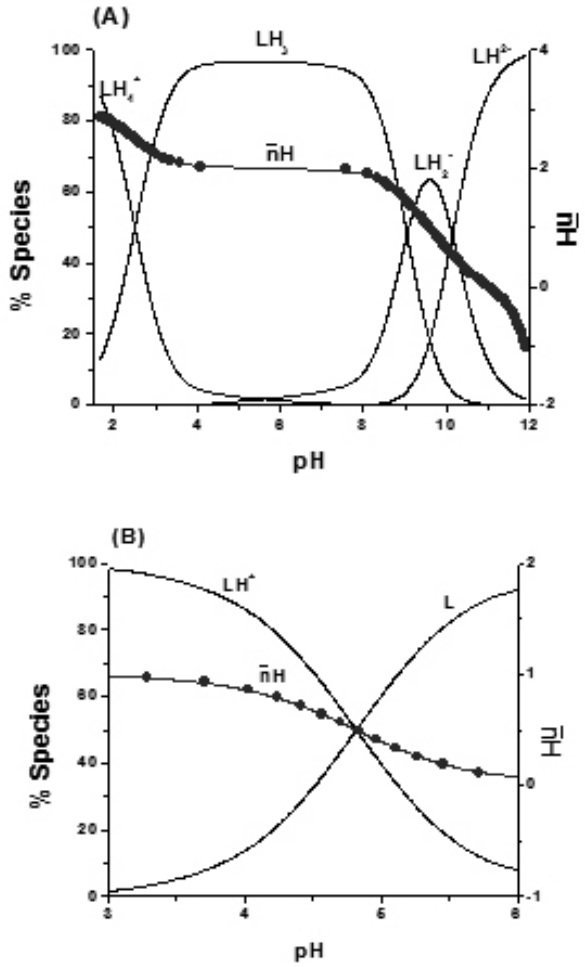


Figure 2: Formation functions (●) and Species distribution diagrams of **(A)** Dopa and **(B)** phen in 30% v/v PG-water mixture.

The plots of **a** versus pH are given in Figure 3. The negative values of **a** correspond to the number of moles of free acid present in the titrand and the number of associable protons. The positive values of **a** indicate the number of dissociable protons in the ligand molecules. The maximum value of **a** in Figure 3(A) is +3, which indicates that dopa has three dissociable (one carboxyl and two phenolic) protons. The corresponding value for phen (Figure 3(B)) is zero, which clearly infers that phen has no dissociable protons.

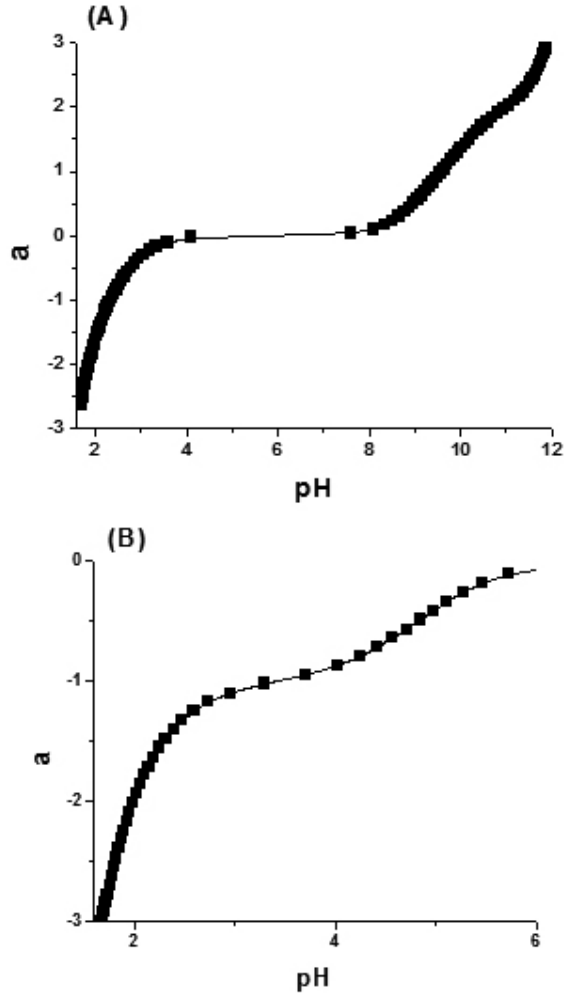


Figure 3: Variation of **a** with pH in 30 % v/v PG-water mixture: **(A)** Dopa and **(B)** phen, respectively.

Dopa contains two ionizable phenolic protons (catecholate) in addition to carboxylic and amino protons. Its neutral ligand form is a tribasic acid, H_3L, with four potential co-ordination centers. So Dopa possesses four protonation constants corresponding to four protons in H_4L^+ from. The first proton (a phenolate proton) to coordinate has a very high affinity for the L^{3-} ion (log K ~13). The next two protons coordinate to the other phenolate oxygen and the amine nitrogen. These two formation reactions overlap. The fourth proton to coordinate is the carboxyl proton (log K ~ 2). From spectroscopic evidence Martin[5,6] and Gergely et al[7] concluded that the amine group has higher affinity (log K_{NH3} = 9.17) for protons than the second phenolate oxygen (log K_{OH} = 8.97). Based on linear free energy relationship and kinetic evidence, Jameson[8] interpreted the phenolate oxygen to protonate first (log K_{OH} = 9.76) followed by the amine nitrogen (log K_{NH3} = 8.93). This ambiguity was resolved by Jameson et al[9], in a proton NMR study which identified the second phenolic group of dopa to be more acidic (log K_{OH} = 8.97) than the amino group (log K_{NH3} = 9.20).

The best fit models containing the type of species and overall formation constants along with some of the important statistical parameters are given in Table II. A very low standard deviation (SD) in log β values indicates the precision of these parameters. The small values of U_{corr} (sum of 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 zero mean with little dispersion.

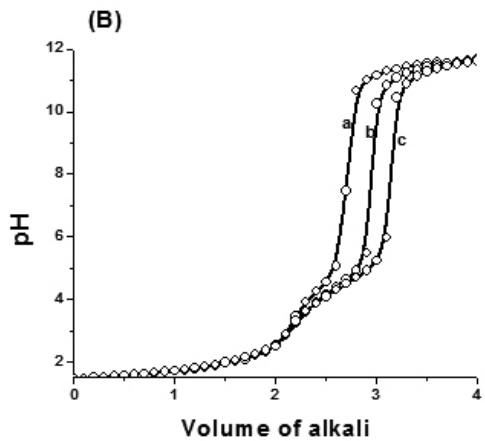
Table II: Best-fit chemical models of acido-basic equilibria of dopa and phen in PG-water mixtures.

% v/v PG	Log β_1 (SD)	Log β_2 (SD)	Log β_3 (SD)	NP	U_{corr}		Kurtosis	χ^2	R
Dopa (pH ranges 1.6-3.5 & 8.0-11.0)									
0	10.11 (14)	19.03 (12)	21.31 (16)	128	1.78	-0.06	3.23	4.69	0.0048
10	10.00 (9)	19.05 (9)	21.51 (14)	150	1.76	0.44	5.71	14.03	0.0048
20	10.16 (10)	19.26 (9)	21.86 (15)	140	1.70	0.14	7.63	46.51	0.0049
30	10.14 (8)	19.19 (7)	21.69 (12)	141	0.30	-0.33	3.69	7.62	0.0032
40	10.39 (10)	19.58 (11)	22.29 (18)	132	0.35	-0.13	3.93	7.09	0.0031
50	10.56 (9)	19.86 (11)	22.89 (21)	152	0.39	0.45	4.36	7.49	0.0038
60	10.48 (9)	19.61 (11)	22.43 (18)	162	0.39	-1.17	4.01	17.14	0.0034
Phen (pH range 2.5-7.0)									
0	5.13 (26)	_	_	23	7.14	-0.76	2.72	2.53	0.0332
10	5.06 (13)	_	_	20	1.14	-0.23	2.95	3.33	0.0130
20	5.09 (14)	_	_	18	3.7	-0.30	2.64	2.67	0.0194
30	4.82 (8)	_	_	17	0.76	-0.24	2.45	6.00	0.0095
40	4.56 (8)	_	_	17	0.11	-0.21	2.60	1.33	0.0066
50	4.50 (17)	_	_	19	1.66	-0.65	3.49	3.43	0.0199
60	4.35 (9)	_	_	21	1.94	-0.16	2.72	10.57	0.0144

U_{corr} = U/ (NP-m) X 10^8; NP = Number of points
m = number of protonation constants, SD = Standard deviation

The kurtosis values in the present study indicate that residuals form leptokurtic patterns. The values of skewness given in Table II are between 2.6 and 7.63. These data evince that the residuals form a part of normal distribution; hence, least square**s** method can be applied to the present data. The sufficiency of the model is further evident from the low crystallographic R-values. These statistical parameters thus show that the best fit models portray the acido-basic equilibria of dopa and phen in PG-water mixtures. The low crystallographic R-values given in Table II indicate the sufficiency of the model. The values of skewness recorded in Table II are between -1.77 and 0.44. These data evince that the residuals form a part of normal distribution; hence, least-squares method can be applied to the present data. The kurtosis values in the present study indicate that the residuals form leptokurtic pattern in the case of dopa and platykurtic for phen.

Alkalimetric titration data are simulated using the model parameters given in Table II. 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 given in Figure 4 indicates that the proposed models represent the experimental data.



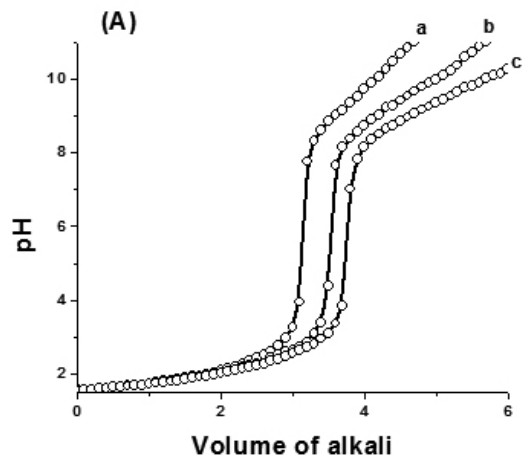


Figure 4: Simulated (o) and experimental (solid line) alkalimetric titration curves in 20% v/v PG water mixture: **(A)** Dopa and **(B)** phen; (a) 0.25, (b) 0.38 and (c) 0.50 mmol, respectively.

3.2 Effect of systematic errors in best fit model

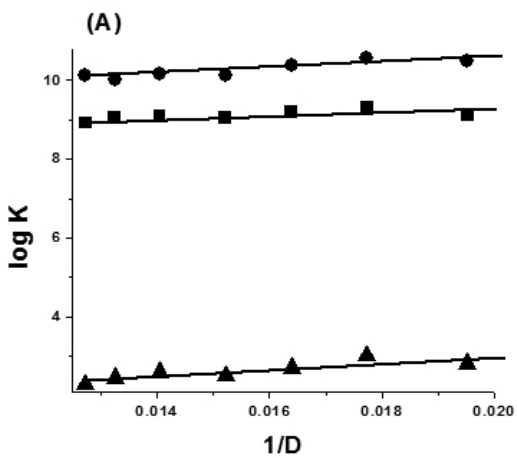
MINIQUAD75 does not have provision to study the effect of systematic errors in the influential parameters like the concentration of ingredients and electrode calibration on the magnitude of protonation constant. In order to rely upon the best chemical model for critical evaluation and application under varied experimental conditions with different experimental with different accuracies of data acquisition, an investigation was made by introducing pessimistic errors in the concentration of alkali, mineral acids and the ligands. The results of a typical system given in Table III emphasize that the errors in the concentrations of alkali and mineral acid affects the protonation constants more than that of the ligand.

Tabla III: Effect of errors in influential parameters on the protonation constants in 30%v/v PG-water mixture.

Ingredient	% Error	Log β_{mlh} (SD)			
		Dopa			Phen
		LH	LH_2	LH_3	LH
	0	10.14 (8)	19.19 (7)	21.69(12)	4.82 (8)
Alkali	-5	10.38 (21)	19.67(16)	22.35 (24)	5.11 (27)
	-2	10.24 (11)	19.38 (9)	21.95 (15)	4.93 (11)
	+2	10.04 (9)	19.00 (8)	21.44 (14)	4.72 (12)
	+5	9.88 (13)	18.73 (13)	21.07 (19)	4.57(18)
Acid	-5	10.25 (12)	19.47 (9)	22.20 (15)	4.50 (21)
	-2	10.08 (10)	19.05 (9)	21.44 (15)	4.69 (13)
	+2	10.19 (8)	19.32 (7)	21.94 (12)	4.65 (15)
	+5	10.27 (14)	19.52 (11)	22.31 (18)	5.18 (44)
Ligand	-5	10.03 (7)	19.06 (6)	21.62 (11)	4.89 (13)
	-2	10.09 (7)	19.14 (7)	21.66 (12)	4.85 (9)
	+2	10.18 (9)	19.24 (8)	21.72 (13)	4.80 (9)
	+5	10.24 (11)	19.31 (9)	21.76 (15)	4.76 (13)
log F	-5	10.13 (10)	19.17 (9)	21.64 (15)	4.81 (9)
	-2	10.13 (9)	19.18 (8)	21.67 (13)	4.82 (8)
	+2	10.14 (7)	19.20 (8)	21.72 (11)	4.83 (8)
	+5	10.15 (7)	19.21 (6)	21.75 (10)	4.84 (8)
Volume	-5	10.14 (6)	19.19 (5)	21.73 (9)	4.83 (8)
	-2	10.14 (7)	19.19 (6)	21.71 (10)	4.82 (8)
	+2	10.14 (9)	19.19 (8)	21.68 (14)	4.82 (8)
	+5	10.14 (12)	19.19 (11)	21.65 (17)	4.82 (9)

3.3 Effect 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. Born's classical treatment holds good in accounting for the electrostatic contribution to the free energy change[33]. According to this treatment, the energy of electrostatic interaction or the logarithm of step-wise protanation 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 (Figure 5) in PG-water mixture shows the dominance of electrostatic interactions. In the case of some mono- and di- carboxylic 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, dioxan or acetone as co-solvent[34].



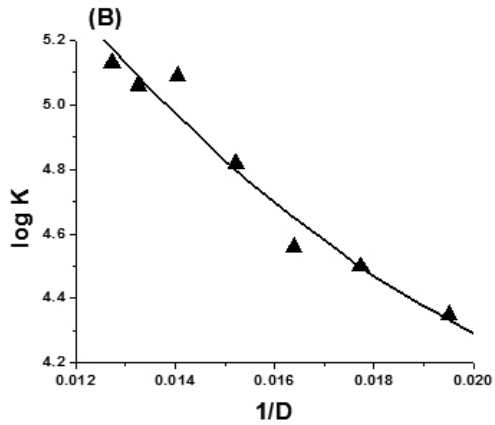


Figure 5: Variation of step-wise protonation constant (log K) with reciprocal of dielectric constant (1/D) in PG-water mixture: **(A)** Dopa and **(B)** phen (▲) log K_1 (■) log K_2 and (●) log K_3.

3.4 Distribution Diagrams

Typical distribution plots produced by DISPLOT[38] using protonation constants from the best fit models are shown in Figure 2. A single representative plot is shown for each system at a particular PG-water concentration. The zwitterion of dopa, LH_3 is present to an extent of 95% in the pH range 2.0-10.0. The distribution plots show the existence of LH_4^+, LH_3, LH_2^- and LH^{2-} in the case of dopa and LH^+ and L in the case of phen in different pH ranges. The corresponding protonation-deproonation equilibria are shown in Figure 6.

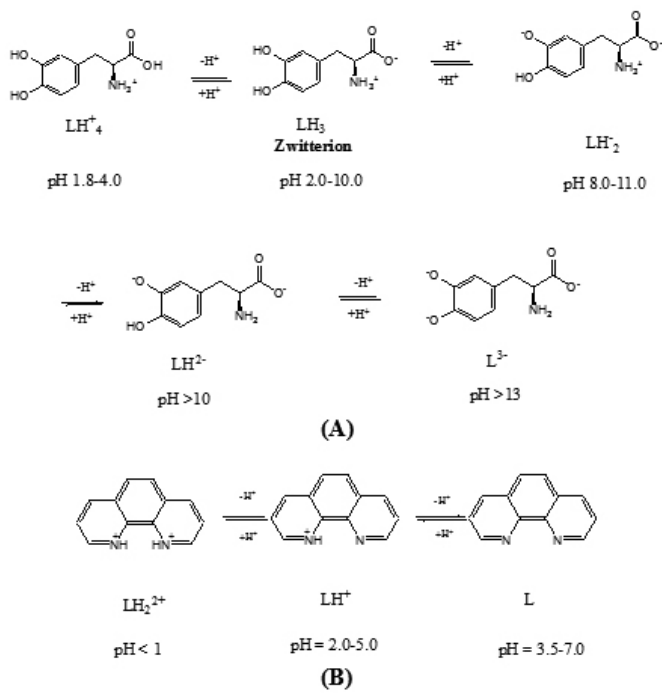


Figure 6: Protonation-deprotonation equilibria of **(A)** dopa, and **(B)** phen

The present study is useful to understand (i) the role played by the active site cavities in biological molecules, (ii) the type of complex formed by the metal ion and (iii) the bonding behavior of the protein residue with the metal ion. The species refined and the relative concentrations under the present experimental conditions represent the possible forms of these amino acids in the biological fluids.

4. CONCLUSIONS

Dopa has three dissociable protons and one amino group which associate with proton. It exists as LH_4^+ at low pH and gets deprotonated with the formation of LH_3, LH^-_2 and LH^{2-} successively with increase in pH. Phen forms LH_2^{2+} at low pH and gets deprotonated with the formation of LH^+ and L with increase in pH. Secondary formation functions confirm the existence of 3 and 2 protonation equilibria for dopa and phen, respectively. The linear increase of log values of protonation constants of Dopa with decreasing dielectric constant of PG-water mixtures indicates the dominance of electrostatic forces in the protonation-deprotonation equilibria. Phen exhibits non-linear trend indicating the dominance of non-electrostatic forces. 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 ligand.

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