Effect of Dielectric Constant on Protonation Equlibria of L-Dopa and 1, 10 –Phenanthroline in Dioxan-Water Mixtures

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Abstract

Solute-solvent interactions of L-dopa and 1, 10-phenanthroline have been studied in 0–60% v/v dioxan–water media using pH-metric method. The protonation constants have been calculated with the computer program MINIQUAD75. 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, 1, 10-Phenanthroline, dioxan.

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.

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[3–9] 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 $H_2(phen)^{2+}$ 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[10]. Hence, the authors have studied the effect of dielectric constant of the medium on the protonation equilibria of dopa and phenanthroline. This type of study throws light on the role of amino acid residues and substrates at the active site cavities of enzymes and proteins.

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, 4-dioxan (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. 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)[11]. The strengths of alkali and mineral acid were determined using the Gran plot method[12].

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2. 2. Alkalimetric Titrations

Alkalimetric titrations were carried out in media containing varying compositions of dioxan (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 consisted of approximately 1 mmol of hydrochloric acid. The amounts of the ligands in the titrands ranged between 0.25 and 0.50 mmols. The glass electrode was equilibrated in a well stirred dioxan-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 dioxan-water mixture of equivalent composition as that of the titrand. The details of experimental procedure and titration assembly have been detailed elsewhere[13].

2. 3. Modeling Strategy

The approximate protonation constants of dopa and phen were calculated with the computer program SCPHD[14]. The best fit chemical model for each system investigated was arrived at using non-linear least-squares computer program, MINIQUAD75[15], which exploits the advantage of constrained least-squares method in the initial refinement and reliable convergence of Marquardt algorithm.

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 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 (2.5, 1.5 and 0.5) in the case of dopa and one half integral (0.5) 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.

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 3B is zero, which clearly infers that phen has no dissociable protons. The corresponding value for dopa (Figure 3A) is +3, which indicates that dopa has three dissociable (one carboxyl and two phenolic) protons.

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^+ form. 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[16,17] and Gergely et al[18] concluded that the amine group has higher affinity for protons than the second phenolate oxygen which was criticized by Jameson[19]. Basing on this
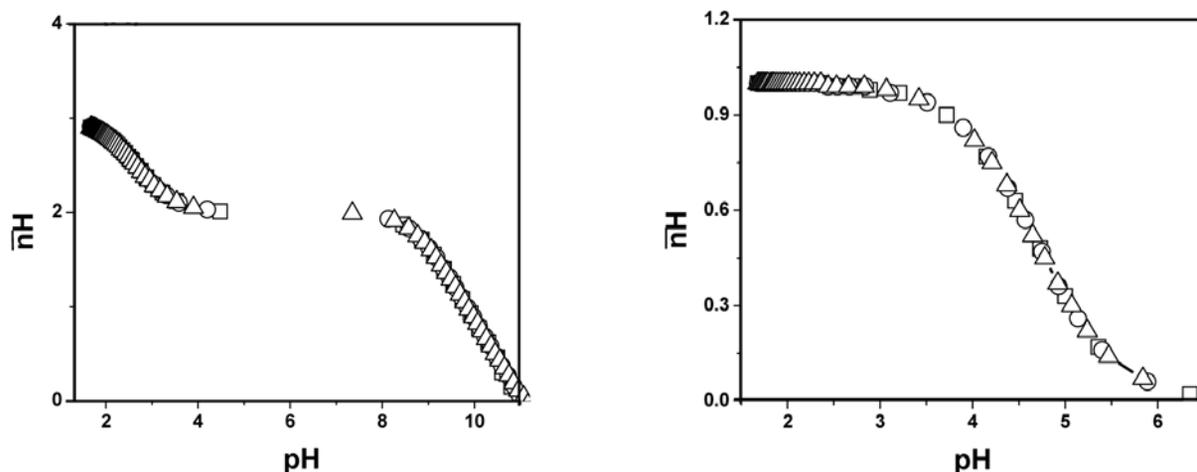

Figure 1: Plots of $\bar{n}H$ versus pH in 20 % v/v dioxan-water mixture; (A) dopa and (B) phen, (□) 0.25, (○) 0.38, and (△) 0.50 mmol, respectively.
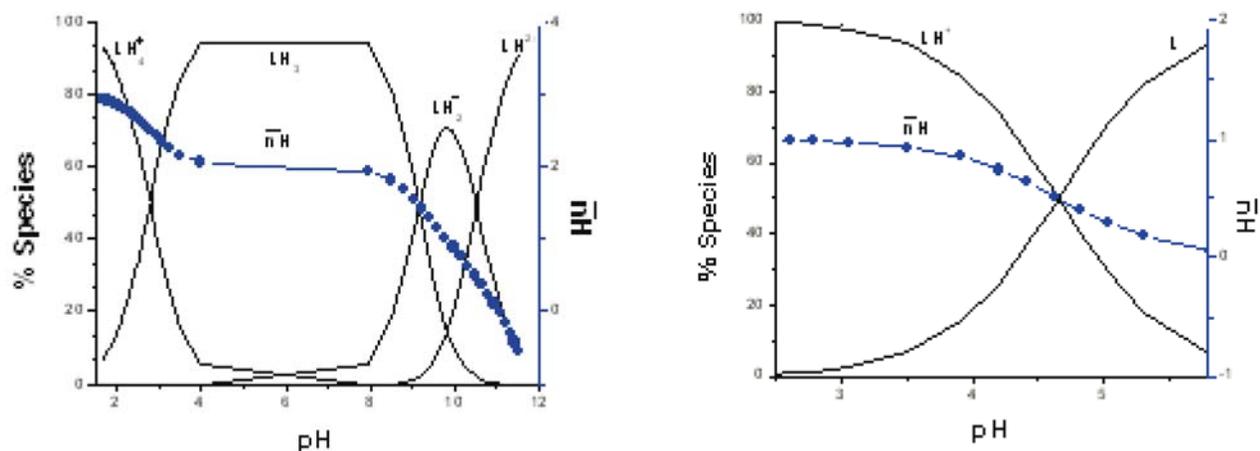
Figure 2: Formation functions (●) and species distribution diagrams of (A) dopa and (B) phen in 30% v/v dioxan-water mixture.

interpretation the phenolate oxygen protonates first (log K_{OH} = 9.76) followed by the amine nitrogen (log $K_{NH3}+$ = 8.93). The uncertainty has been resolved by a proton NMR study in D_2O solution.[20] This study identified the

Figure 3: Variation of **a** with pH in 30 % v/v dioxan-water mixture: (A) dopa and (B) phen

second phenolic group of dopa is more acidic (log K_{OH} = 8.97) than the amino group (log $K_{NH3}+$ = 9.20). Other literature values reported[21–27] allow the calculation of recommended protonation constants at 25 °C and I = 0.1 to 0.2 M to be LogK_{HL} = 13.4, LogK_{H2L} = 9.84, LogK_{H3L} = 8.77 and LogK_{H4L} = 2.2.

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 of the present study are given in Table 1. A very low standard deviation (SD) in log β values, U_{corr} (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.
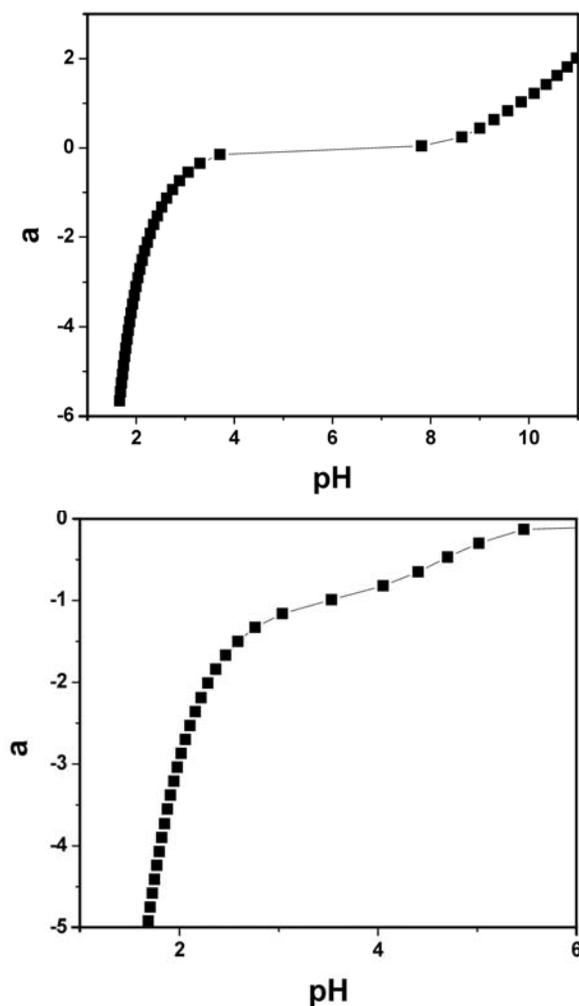
3. 2. Residual Analysis[28]

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 and phen in dioxan-water mixtures, as discussed below.

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

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

% v/v. dioxan	Log β_1(SD)	Log β_2(SD)	Log β_3(SD)	NP	U_{corr}	Skewness	Kurtosis	χ^2	R
			Dopa (pH ranges 2.0–4.8 & 8.0–11.0)						
0	10.11(11)	19.03(9)	21.37(15)	69	1.080	–0.18	3.50	6.93	0.0054
10	9.90 (7)	18.87(7)	21.21(11)	65	0.265	–0.24	3.44	6.00	0.0023
20	10.45 (6)	19.72(6)	22.37(9)	58	0.231	0.41	4.75	14.76	0.0025
30	10.52(8)	19.67(12)	22.46(18)	67	1.176	–0.05	3.53	2.60	0.0051
40	10.84 (11)	20.30(11)	23.24(18)	74	1.362	–0.93	4.62	10.05	0.0051
50	10.87(8)	20.22(9)	23.42(15)	48	0.581	–0.40	5.06	10.00	0.0040
60	11.06(14)	20.37(18)	23.78(30)	49	2.137	–0.74	5.47	5.84	0.0079
			Phen (pH range 2.5–7.0)						
0	5.09 (17)	–	–	49	4.272	–0.10	2.66	10.24	0.0102
10	5.00 (29)	–	–	35	12.57	–0.50	2.55	5.66	0.0295
20	4.69 (8)	–	–	20	1.358	–0.57	2.70	2.40	0.0118
30	4.65 (14)	–	–	20	5.063	0.24	2.83	2.00	0.0235
40	4.47 (8)	–	–	27	1.965	–0.93	3.78	1.11	0.0122
50	4.41 (15)	–	–	37	6.252	0.34	2.81	13.35	0.0231
60	4.28 (14)	–	–	14	2.891	–0.01	2.67	3.43	0.0135

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

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 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.

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 –0.50 and 0.89. 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 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 in-

dicate 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 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. 3. Effect of Systematic Errors in Best Fit Model

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

3. 4. 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 the logarithm of step-wise protanation constant

Table 2: Effect of errors in influential parameters on the protonation constants in 50% v/v dioxan-water mixture.

| Ingredient | % Error | Log β_{mlh} (SD) | | | |
| | | Dopa | | | Phen |
		LH	LH$_2$	LH$_3$	LH
	0	10.87(8)	20.22(9)	23.42(15)	4.41(15)
Alkali	−5	11.29(34)	20.99(35)	24.50(53)	4.74(34)
	−2	11.03(15)	20.52(15)	23.84(25)	4.54(20)
	+2	10.72(8)	19.94(9)	23.03(15)	4.29(18)
	+5	10.48(15)	19.54(22)	22.45(36)	4.10(30)
Acid	−5	10.60(16)	19.67(23)	22.50(37)	4.02(35)
	−2	10.76(9)	20.00(11)	23.05(19)	4.26(18)
	+2	10.98(14)	20.44(14)	23.80(22)	4.56(23)
	+5	11.14(34)	20.78(32)	24.37(57)	4.79(44)
Ligand	−5	10.75(8)	20.08(10)	23.33(18)	4.47(21)
	−2	10.82(8)	20.16(9)	23.39(16)	4.44(17)
	+2	10.92(8)	20.29(9)	23.46(16)	4.39(14)
	+5	10.99(9)	20.38(10)	23.52(17)	4.36(15)
log F	−5	10.87(8)	20.22(9)	23.42(16)	4.40(14)
	−2	10.87(8)	20.22(9)	23.42(16)	4.41(15)
	+2	10.87(8)	20.23(9)	23.43(16)	4.42(15)
	+5	10.87(8)	20.23(9)	23.43(16)	4.42(15)
Volume	−5	10.87(8)	20.22(9)	23.44(16)	4.41(16)
	−2	10.87(8)	20.22(9)	23.43(16)	4.41(15)
	+2	10.88(8)	20.23(9)	23.42(16)	4.41(14)
	+5	10.88(8)	20.23(10)	23.41(17)	4.41(14)
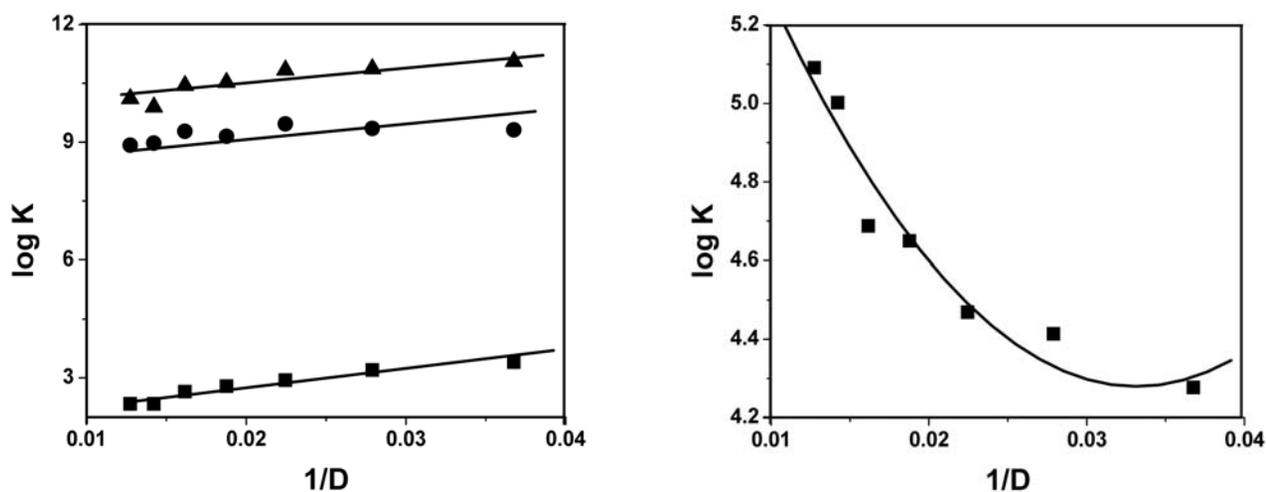
Figure 4: Variation of step-wise protonation constant (log K) with reciprocal of dielectric constant (1/D) in dioxan-water mixture (A) dopa and (B) phen (■) log K$_1$ (●) log K$_2$ (▲) log K$_3$

(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 4) in dioxan-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[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] The logK value of phen varies non-linearly with 1/D (Figure 4B). This may be because phen has no dissociable protons. Since it has no polar groups, specific solvent-water interaction, charge dispersion, and specific interaction of co-solvent with solute (indicated by the changes in the solubility of different species in the aqua-organic mixtures) account for the deviation of classical linear relationship of log K with 1/D.

3. 5. Distribution Diagrams

The distribution plots (Figure 2) produced using the protonation constants from the best fit models (Table 1) 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-deprotonation equilibria are shown in Figure 5. As the alkali is added to the titrand containing the ligands, the protonated forms of the ligands lose their protons. 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 LH^{2-}.

Similarly phen is diprotonated below a pH of 1.0 in the present study, the pH range is 2.5–7.0, where phen is mono protonated (LH^+) and so only one protonated constant is reported.

4. Conclusions

1. Dopa has three dissociable protons and one amino group 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^- and LH^{2-} successively with increase in pH.
2. Phen forms LH_2^{2+} at low pH and gets deprotonated with the formation of LH^+ and L with increase in pH.
3. Secondary formation functions–number of moles of alkali per mole of the ligand and average number of moles of protons bound per mole of the ligand–are useful in detecting the number of protonation equilibria and in guessing the approximate protonation constants.
4. The log values of protonation constants of dopa increase linearly with decreasing dielectric constant of dioxan-water mixtures. This indicates the dominance of electrostatic forces in the protonation-deprotonation equlibria. Phen exhibits non-linear trend indicating the dominance of non-electrostatic forces.
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 the ligand.

5. References

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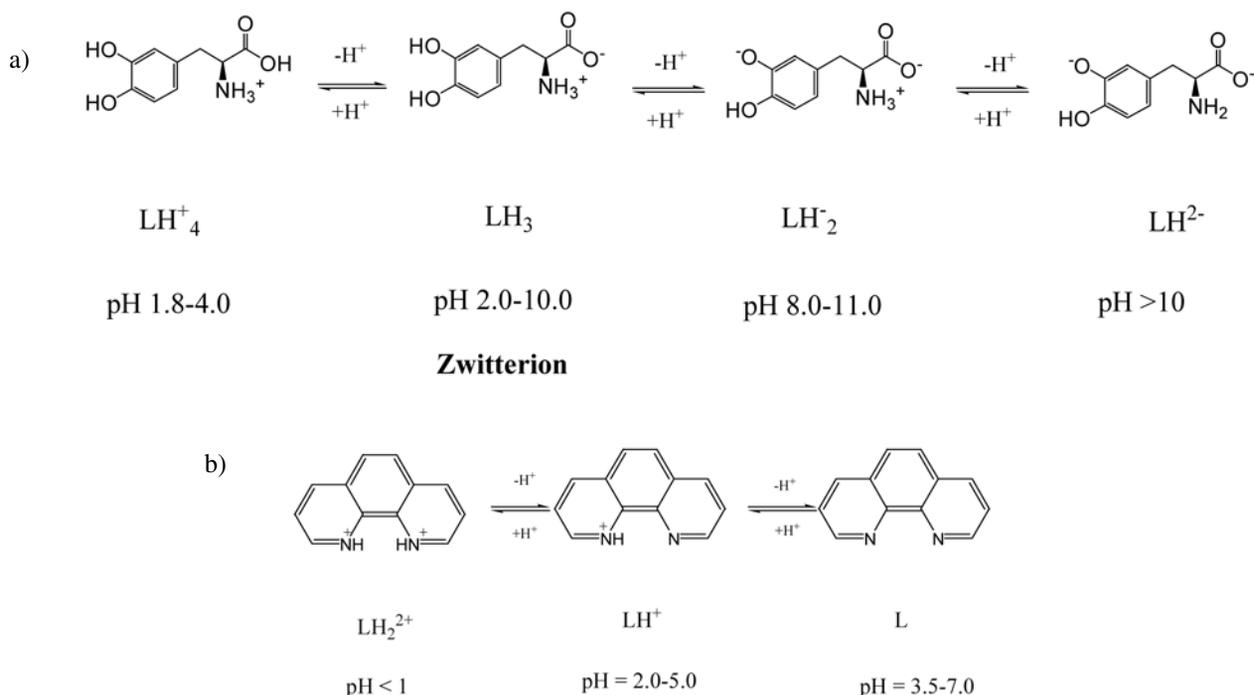
Figure 5: Protonation-deprotonation equilibria of (a) dopa and (b) phen.

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Povzetek

Z merjenjem pH vrednosti smo raziskovali interakcije med L-3,4-dihidroksifenilamaninom (L-dopa) in 1, 10-fenantrilinom (phen) v različnim mešanicah vode in 1,4-dioksana v koncentracijskem območju med 0 in 60 v/v % 1,4-dioksana. S pomočjo MINIQUAD75 progama smo izračunali protonacijske konstante za H(phen)$^+$ in H(phen)$_2^+$ v območju pH med 2.0 in 7.0 ter H$_2$(phen)$^{2+}$ pri [H$^+$] > 1 mol dm^{-3}. Ugotovili smo linearno odvisnost protonacijskih konstant od recipročne vrednosti dielektrične konstante topila. Prikazali smo tudi protonacijska ravnotežja, porazdelitve prisotnih speciesov in vpliv različnih parametrov na konstante protonacije.