Influence of Dielectric Constant on Complex Equilibria of L- dopa Complexes of Toxic Metal ions

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ABSTRACT

Binary complexes of L-dopa with toxic metal ions such as Pb(II), Cd(II) and Hg(II) have been studied in 0.0-60.0% v/v 1,4 dioxan-water media at 303 K and an ionic strength of 0.16 mol dm^{-3}. The active forms of the ligand are LH_3^+, LH_2 and LH. The best fit chemical models are arrived at based on firm statistical grounds employing crystallographic R-factor, χ^2, Skewness and Kurtosis. The predominant species formed are MLH^+, ML_2H_2 and ML_2H. The stability constants of the binary systems decrease in the order Hg(II) > Cd(II) > Pb(II). The trend in variation of complex stability constants with change in the dielectric constant of the medium is explained on the basis of electrostatic and non-electrostatic forces. The species distribution with pH at different compositions of dioxan-water mixtures and plausible equilibria for their formation are also presented. The chemical speciation and bioavailability of the toxic metal ions is also described.

Key Words: 1, 4-dioxan, L-dopa, toxic metal ions, dielectric constant, chemical speciation.

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INTRODUCTION

L-dopa (3, 4-dihydroxy-L-phenylalanine) is a drug related compound, found in certain kinds of food and herbs and is made from L-tyrosine [1], which is an amino acid naturally occurring in the human body. It is the natural precursor of dopamine, and is the most effective and frequently prescribed therapy for controlling the symptoms of Parkinson's disease [2].

Heavy metals such as lead, cadmium and mercury are toxic substances which exert adverse effects on neurological, reproductive, renal and hematological systems in humans and animals. Organo- mercury and lead compounds exhibit toxic effect on the central nervous system [3]. Similarly cadmium exhibits various chronic and acute disorders like testicular atrophy, hypertension, damage to kidneys and bones, anemia and Itai-Itai [4-8]. Hence, the stability constants of the binary complexes of Pb(II), Cd(II) and Hg(II) have been determined using pH metry. These values are potentially useful to environmental and biological problems [9-11].

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In this study water was substituted by 1, 4-dioxan (Dox) that has lower dielectric constant to mimic the permittivity of bio-active sites. Thus the water-Dox mixtures produce the conditions that exist at the active sites of enzymes and side chains of proteins [12-16]. The present paper describes the complexation of Pb(II), Cd(II) and Hg(II) with L-dopa in Dox-water media.

MATERIALS AND METHODS

Reagents and preparation
L-dopa (dopa), nitrates of lead, cadmium and mercury, Dox, mineral acid (HNO_3), sodium hydroxide and sodium nitrate were of analytical grade reagents. Dox was used as received. Aqueous solutions of dopa, metal salt solutions, nitric acid, a carbonate free sodium hydroxide and sodium nitrate were prepared by dissolving samples in triple distilled water. To increase the solubility of dopa and to suppress the hydrolysis of metal salts, the nitric acid concentration was maintained at 0.05 mol dm^{-3}. The solutions were standardized by employing standard methods. The data were subjected to ANOVA [17], to assess the errors that might have crept into the determination of the concentrations. Gran plot [18] method was employed to determine the strength of the alkali.

Apparatus
Elico LI 120 pH meter was used for the pH measurements. Potassium hydrogen phthalate solution (0.05 mol dm^{-3}) in the acidic region and borax solution (0.01 mol dm^{-3}) in the basic region were used to calibrate the pH meter. The pH meter was equilibrated in a well-stirred Dox-water mixture containing inert electrolyte. The effects of variations in the asymmetry potential, liquid junction potential, activity coefficient, sodium ion error and dissolved carbon dioxide on the response of the glass electrode were accounted for in the form of correction factors [19, 20].

Measurements
All measurements were carried out at 303 K and at an ionic strength of 0.16 mol dm^{-3} which was maintained with sodium nitrate. The electrode was kept usually for 2-3 days, in the required solvent system for equilibration. To verify whether the electrode was equilibrated or not, a strong acid was titrated with an alkali every day until no appreciable differences were observed between the pH values of two titrations at the corresponding volumes of the titrant. Then the electrode was said to be equilibrated. Free acid-base titrations were performed to calculate the correction factor. In each of the titrations, the titrand consists of 1-3 mmol of mineral acid in a total volume of 50 cm^3. Titrations with different metal to ligand ratios (1:2.5, 1:3.75, 1:5) were carried out with 0.40 mol dm^{-3} sodium hydroxide. The analytical concentrations of the ingredients are given in Table 1. Other experimental details are given elsewhere [21].

Modeling strategy
The approximate complex stability constants were calculated using the computer program SCPHD [22]. By following some heuristics in the refinement of the stability constants, the best-fit chemical models for each system were arrived at using the computer program MINIQUAD75 [23].

RESULTS AND DISCUSSION

Alkalimetric titration curves in Dox-water mixtures reveal that the acido-basic equilibria of dopa are active in the pH range of 2.00 – 10.25.

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Table 1: Total initial concentrations of ingredients (in mmol) of titrands in Dox-water mixtures [NaOH]= 0.4 mol dm^{-3}; V$_0$=50.0 cm^3; temp=303 K; ionic strength= 0.16 mol dm^{-3}; mineral acid= 1-3 mmol.

% v/v	TM0			TL0	
Dox	Pb(II)	Cd(II)	Hg(II)	(dopa)	TL0:TM0
				0.250	2.50
00.00	0.099	0.098	0.102	0.375	3.75
				0.500	5.00
				0.250	2.50
10.00	0.099	0.098	0.102	0.375	3.75
				0.500	5.00
				0.250	2.50
20.00	0.099	0.098	0.102	0.375	3.75
				0.500	5.00
				0.250	2.50
30.00	0.099	0.098	0.102	0.375	3.75
				0.500	5.00
				0.250	2.50
40.00	0.099	0.098	0.102	0.375	3.75
				0.500	5.00
				0.250	2.50
50.00	0.099	0.098	0.102	0.375	3.75
				0.500	5.00
				0.250	2.50
60.00	0.099	0.098	0.102	0.375	3.75
				0.500	5.00

Table 2: Parameters of best-fit chemical models, of dopa complexes of Pb(II), Cd(II) and Hg(II) in Dox-water mixtures. Temperature= 303 K, Ionic strength= 0.16 mol dm^{-3}

%v/v	log β$_{mlh}$ (SD)			NP	U$_{corr}$	χ²	Skewness	R- factor	Kurtosis
Dox	111	121	122						
		Pb(II) (pH range 5.00-9.00)							
0	14.94(11)	19.37(18)	28.38(19)	31	2.60	7.90	0.30	0.011	5.73
10	15.11(14)	21.07(17)	29.03(18)	32	2.72	7.00	0.45	0.011	4.14
20	15.13(15)	21.13(18)	29.19(18)	35	2.80	3.42	0.43	0.012	3.72
30	15.13(19)	21.38(28)	29.41(28)	25	5.13	7.88	0.71	0.014	5.50
40	15.47(13)	21.91(15)	29.14(36)	26	4.13	7.54	0.56	0.013	5.15
50	15.78(17)	22.29(24)	29.75(33)	26	4.07	5.49	0.23	0.013	3.9
60	16.01(28)	23.04(27)	30.82(23)	27	4.02	5.64	0.14	0.013	3.61
		Cd(II) (pH range 6.30-9.60)							
0	12.87(28)	16.98(19)	26.10(13)	24	2.20	9.56	0.31	0.010	4.07
10	12.95(31)	17.06(06)	26.38(14)	30	2.53	8.67	0.19	0.013	4.52
20	12.86(64)	17.81(12)	26.62(11)	30	5.14	15.60	-0.21	0.016	2.87
30	13.66(20)	19.61(10)	27.40(12)	20	1.98	2.40	-0.33	0.009	4.21
40	14.56(22)	20.97(16)	27.82(21)	18	0.99	1.11	0.05	0.006	3.05
50	14.68(28)	20.94(22)	28.20(21)	24	2.55	4.89	0.09	0.011	2.63
60	rejected	20.98(22)	28.26(30)	19	1.98	1.53	0.61	0.009	3.16
		Hg(II) (pH range 2.00-9.55)							
0	19.66(4)	27.79(5)	36.17(5)	90	1.85	40.58	0.41	0.007	6.35
10	18.93(2)	27.50(11)	35.39(9)	90	0.50	26.30	0.28	0.003	4.87
20	17.89(9)	27.64(26)	34.97(14)	60	3.17	5.60	0.43	0.010	4.03
30	18.11(8)	27.43(28)	34.78(20)	56	3.30	6.29	0.40	0.010	3.60
40	18.21(6)	28.28(24)	35.13(13)	66	2.47	40.63	-0.02	0.008	4.77
50	17.19(26)	27.60(43)	34.37(13)	45	6.97	9.65	0.37	0.017	3.98
60	17.49(23)	27.07(37)	34.22(18)	28	5.04	11.62	-0.29	0.014	5.45

U$_{corr}$ = U/ (NP-m) X10^8, m=number of species; NP=number of experimental points.

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Table 3: Effect of errors in concentrations of ingredients on stability constants of Hg(II)-dopa complexes in 10% v/v Dox-water mixture

Ingredient	% Error	log β		
		111	121	122
Alkali	0	18.93(2)	27.50(11)	35.39(09)
	-5	17.60(7)	rejected	30.84(17)
	-2	18.60(2)	24.57(12)	33.20(12)
	2	19.16(3)	29.54(14)	36.59(06)
	5	rejected	rejected	rejected
Acid	-5	rejected	rejected	rejected
	-2	19.30(3)	29.51(12)	36.68(06)
	2	18.49(2)	24.70(11)	33.19(11)
	5	17.43(5)	21.75(12)	30.98(12)
Ligand	-5	18.84(2)	27.56(14)	35.32(11)
	-2	18.89(2)	27.53(12)	35.36(09)
	2	18.97(1)	27.48(10)	35.42(08)
	5	19.03(1)	27.44(09)	35.48(08)
Metal	-5	18.98(2)	28.06(10)	35.68(07)
	-2	18.95(2)	27.73(10)	35.51(08)
	2	18.91(2)	27.28(11)	35.26(10)
	5	18.88(2)	26.88(13)	34.99(12)

Based on the active forms of the ligand in this pH range, models containing various numbers and combinations of complex species were generated using an expert system package CEES [24]. These models were inputted to MINIQUAD75 along with the alkalimetric titration data. The best-fit model was selected using the statistical parameters of the least square residuals. The final values of the stability constants of the complexes are given in Table 2.

Effect of systematic errors on best-fit model
In order to rely upon the best-fit model for critical evaluation and application under varied experimental conditions with different accuracies of data acquisition, a study was made by employing pessimistic errors into influential parameters, such as the concentrations of alkali, mineral acid, ligand and metal (Table 3). The order of the constituents that influence the magnitudes of the stability constants due to incorporation of errors are alkali > acid > ligand > metal. The rejection of some species and increased standard deviations in the stability constants on introduction of errors confirm the appropriateness of the best-fit models. This study supports that the experimental concentrations are appropriate and the proposed models are adequate for the experimental data.

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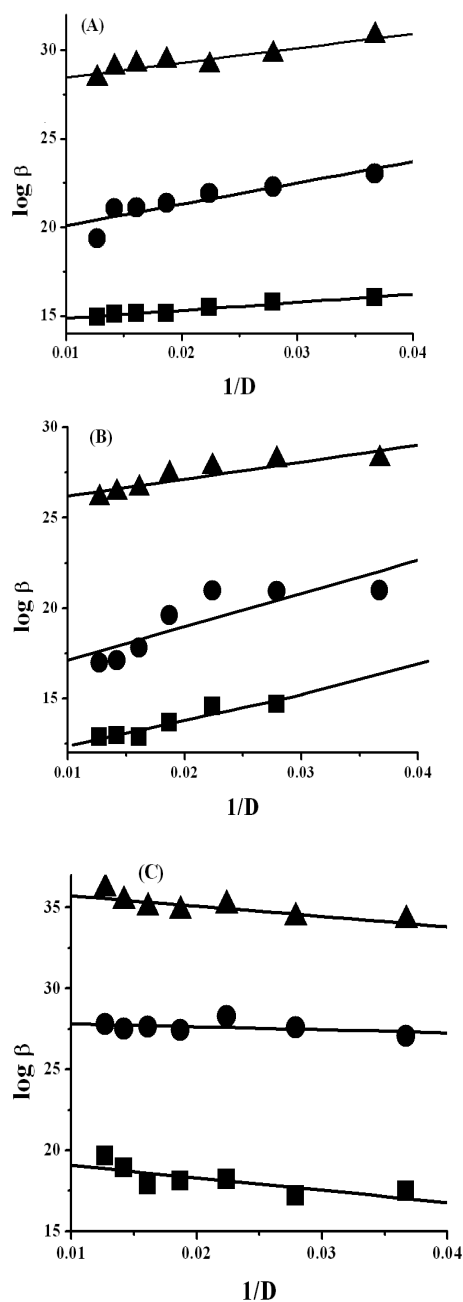
Figure 1: Variation of stability constant values of (A) Pb(II); (B) Cd(II); (C) Hg(II)- dopa complexes with reciprocal of dielectric constant (1/D) in Dox-water mixtures; (■) log β_{111}; (●) log $\beta_{121;}$ (▲) log β_{122}.
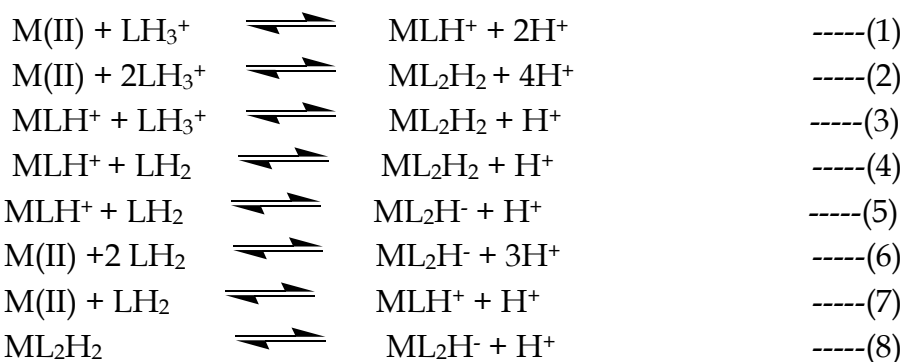
Effect of dielectric constant

Addition of Dox to water decreases the dielectric constant of the medium. The dielectric constants of the medium at different percentages (0-60% v/v) of Dox were taken from literature [25]. The change in overall stability constants or change in free energy with change in co-solvent depends on two factors, viz., electrostatic and non electrostatic. As basicity of Dox is lower than that of water, the non-electrostatic effect seems to decrease proton accepting power of the ligand. Addition of more Dox removes water molecules from hydration sphere of metal ion making it more susceptible to react with the ligand. According to Born's equation [26] the energy of electrostatic interactions is related to dielectric constant of the medium and log β versus 1/D (D is the dielectric constant of the medium) should be linear. So log β should increase/decrease

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linearly with decrease in dielectric constant of medium (with increase in Dox content) if the interaction between metal ion and ligand is electrostatic in nature.

The linear variation of log β values of dopa complexes of Pb(II), Cd(II) and Hg(II) with 1/D (Figure 1) indicates that the electrostatic solute-solvent interactions are predominant. The cation stabilizing nature of co-solvent, specific solvent-water interactions, charge dispersion and specific interactions of co-solvent with the solute may be responsible for small deviation from the linear relationship. This study is helpful to understand the co-solvent effect at a molecular level [27-29] and provide useful information in aiding in the rational drug design, medicinal chemistry, biochemistry and molecular biology.

Distribution diagrams

The species distribution diagrams were obtained using the computer program DISPLOT [30]. Dopa has two ionisable phenolic protons in addition to carboxyl and amino protons and its various forms are LH_3^+, LH_2 and LH^- in the pH ranges 2.0-10.0, 8-11.0 and greater than 10.0, respectively [31]. The plausible refined species are MLH^+, ML_2H^- and ML_2H_2 for Pb(II), Cd(II) and Hg(II) in Dox-water mixture. Typical distribution diagrams of Pb(II), Cd(II) and Hg(II) in Dox-water mixture are shown in Figure 2 (charges of the species are omitted for clarity). These diagrams indicate that ML_2H^- has formed to an extent of 95% at pH values above 6.0. The formation of various binary complexes is shown in the following equilibria:

$$M(II) + LH_3^+ \rightleftharpoons MLH^+ + 2H^+ \qquad -----(1)$$
$$M(II) + 2LH_3^+ \rightleftharpoons ML_2H_2 + 4H^+ \qquad -----(2)$$
$$MLH^+ + LH_3^+ \rightleftharpoons ML_2H_2 + H^+ \qquad -----(3)$$
$$MLH^+ + LH_2 \rightleftharpoons ML_2H_2 + H^+ \qquad -----(4)$$
$$MLH^+ + LH_2 \rightleftharpoons ML_2H^- + H^+ \qquad -----(5)$$
$$M(II) + 2 LH_2 \rightleftharpoons ML_2H^- + 3H^+ \qquad -----(6)$$
$$M(II) + LH_2 \rightleftharpoons MLH^+ + H^+ \qquad -----(7)$$
$$ML_2H_2 \rightleftharpoons ML_2H^- + H^+ \qquad -----(8)$$

In the case of Pb(II) and Hg(II) MLH^+ is formed through Equilibria 1 and 7 where as in the case of Cd(II) it is formed through Equilibrium 1. ML_2H_2 species is formed by the interaction of protonated ligand with the metal ion (Equilibrium 2) and with the MLH^+ species (Equilibria 3 and 4). The ML_2H^- species is formed by the interaction of protonated ligand with the MLH^+ species (Equilibrium 5) and with the metal ion (Equilibrium 6). It is also formed by the deprotonation of ML_2H_2 species (Equilibrium 8). Another notable observation is that the free metal ion concentration of Hg(II) is very low when compared to Cd(II) and Pb(II) at lower pH values, indicating the increasing stability of the metal complexes in the order Hg(II) > Cd(II) > Pb(II).
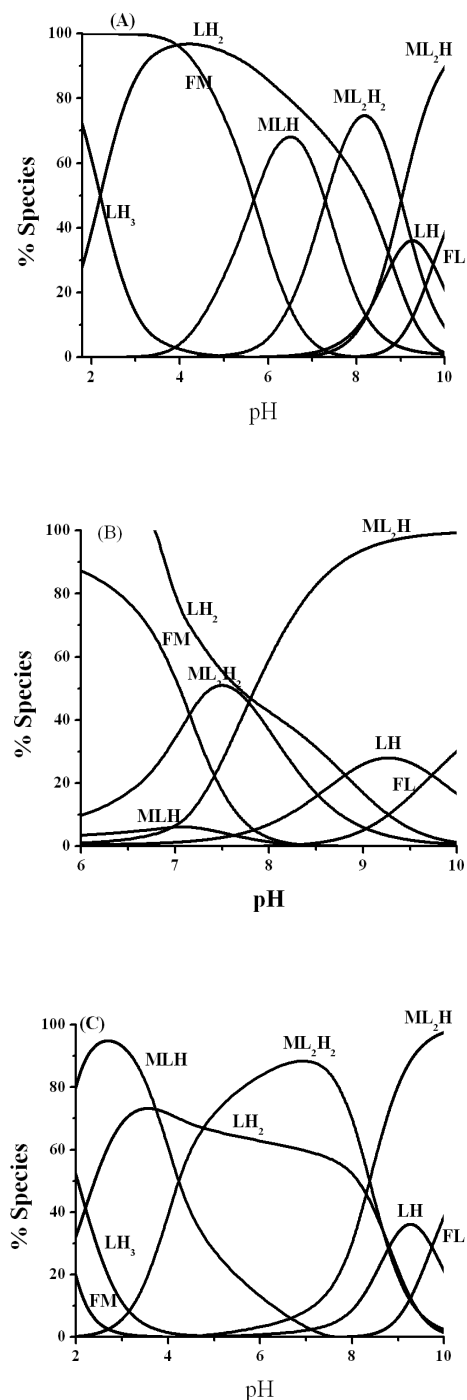
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Figure 2: Distribution diagrams of (A) Pb(II); (B) Cd(II); (C) Hg(II)-dopa complexes in 30% v/v Dox-water mixture

Structures of binary complexes

Dopa has a tendency to chelate with essential or toxic metal ions either to compete with metalloenzymes or to remove toxic ions from living systems. L-dopa has two sets of chelating groups: the amino carboxylate side chain donors (N, O) and the catecholate donors (O, O). The distribution diagrams of dopa with toxic metal ions (Figure 2) demonstrate that these ligands coordinate via the side chain donors at lower pH, but via the catecholate group at higher pH. Of course, there is a possibility of the simultaneous ligand coordination to both bonding sites in the case of ligand excess, which are observed at intermediate pH. Although it is not possible to elucidate or confirm the structures of complexes pH metrically, they can be proposed based on

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the literature reports and chemical knowledge [32]. Amino nitrogen atoms can associate with hydrogen ions in physiological pH range and the amino group is protonated at pH less than 9.0. It is observed that the maximum concentration of the protonated complex is at a pH more than 5 (Figure 2), and is concluded that the amino group is protonated in the protonated complexes but not carboxyl group. The plausible structures of the complexes are shown in Figure 3.

MLH$^+$

ML$_2$H$_2$

ML$_2$H$^-$
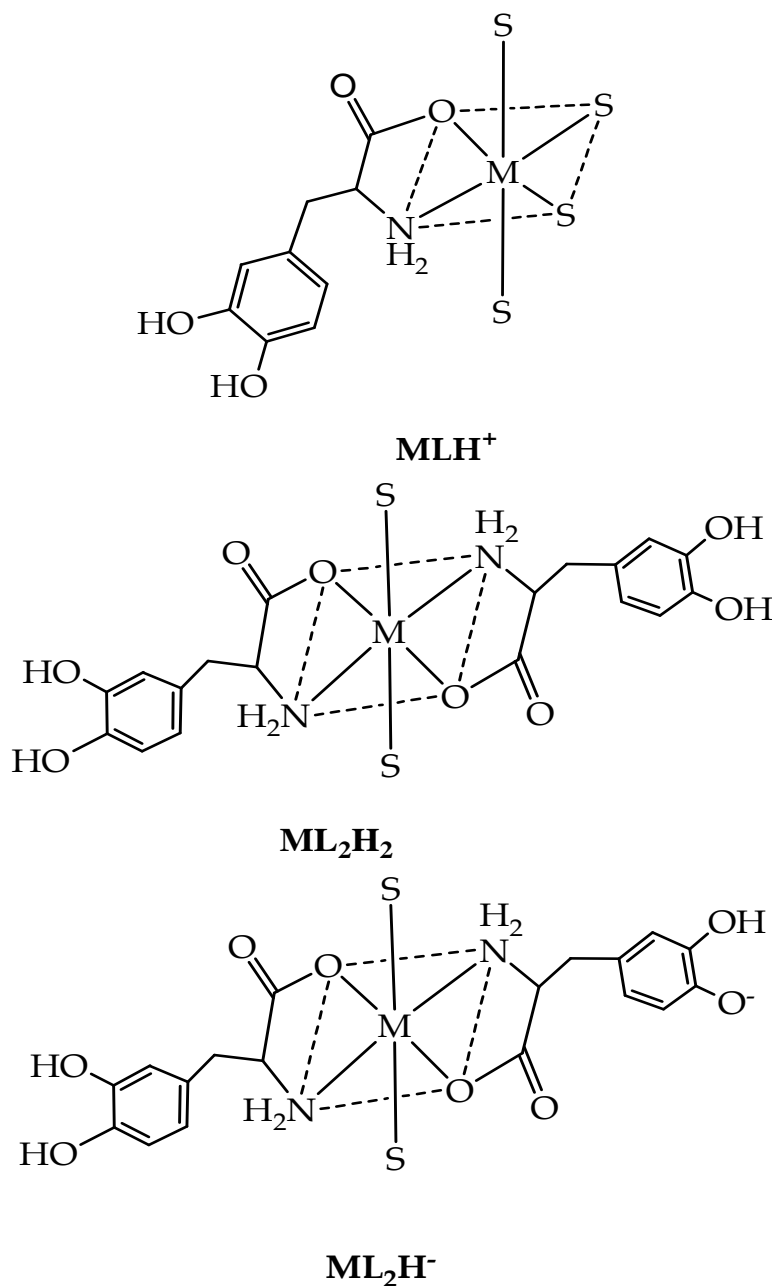
Figure 3: Structures of Metal-dopa complexes where M= Pb, Cd or Hg; L=dopa; S is either solvent or water molecule

CONCLUSION

The following conclusions have been drawn from the modeling studies of L-dopa complexes of Pb(II), Cd(II) and Hg(II) in Dox-water mixtures.

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1. The complex species formed due to interaction of L-dopa with the toxic metals are MLH^+, ML_2H_2 and ML_2H^-.

2. The decreasing of dielectric constant of the medium with increasing in mole fraction of Dox results in larger stability constants. The linear variation of the stability constants with mole fraction of the Dox indicates the dominance of electrostatic forces over non-electrostatic forces.

3. The pH metric studies of all the metal ion complexes with L-dopa indicate that the maximum concentration of protonated complexes is at a pH more than 5. At higher pH hydrolysis of complexes takes place and hence precipitates are formed.

4. The order of the components in influencing the magnitudes of the stability constants due to incorporation of errors is alkali > acid > ligand > metal.

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