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Speciation studies of L-histidine complexes of Pb(II), Cd(II) and Hg(II) in Dioxan-water mixtures

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ABSTRACT

The speciation of L-histidine complexes of Pb(II), Cd(II) and Hg(II) has been investigated pH-metrically in dioxan-water mixtures (0-60% v/v) at 303K and 0.16 mol L^{-1} *ionic strength. The predominant species detected for Pb(II) and Cd(II) are* ML_2H_4, ML_2H_3, ML_2H_2, ML_2H *and* ML_2 *and those for Hg(II) are* ML_2H_4, ML_2H_3, ML_2 *and ML. The appropriateness of experimental conditions is verified by introducing errors intentionally in the concentrations of ingredients. The models containing different numbers of species were refined by using the computer program MINIQUAD75. The best-fit chemical models were arrived at based on statistical parameters. The trend in variation of stability constants of the complexes with dielectric constant of the medium is attributed to the electrostatic and non-electrostatic forces. The species distribution and the plausible equilibria for the formation of the species are also presented.*

Keyword: Speciation studies, Complex equilibria, L-histidine, dioxan, stability constants.

INTRODUCTON

The speciation study of toxic metal ion complexes is useful to understand the role played by the active site cavities in biological molecules and the bonding behavior of protein residues with the metal ion. The species refined and their relative concentrations under the experimental conditions represent the possible forms of amino acids in biofluid.

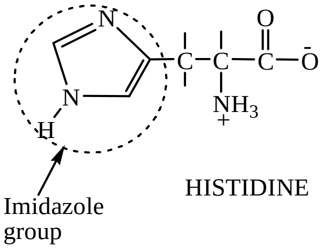
Lead affects every organ of the body, especially the bones and teeth, the kidneys, the nervous, cardiovascular, immune and reproductive systems [1]. Lead has no known physiologically relevant role in the body [2, 3]. Lead and other heavy metals create reactive radicals which damage cell structures including DNA and membranes [4]. Lead also interferes with DNA transcription, enzymes that help in the synthesis of vitamin D, and enzymes that maintain the integrity of the cell membrane [5]. Lead may also be harmful to the developing immune system, causing production of excessive inflammatory proteins; this mechanism may mean that lead exposure is risk factor for asthma in children [6]. Lead also interferes with the normal metabolism of calcium in cells and causes it to build up within them [7].

Cadmium can also enter the environment through natural causes, such as volcanic activity and forest fires [8]. Cadmium affects many different kinds of organisms, ranging from microbes to humans. Human exposure to cadmium mainly occurs through cigarette smoking, but exposure can also occur through contaminated food, water, or air [9]. Cadmium is a known carcinogen to mammals [10]. Cadmium, a potentially toxic metal, has been shown to accumulate in plants, where it is detoxified by binding to phytochelatins [11-13], a family of thiol-rich peptides [14]. Metallothioneins (MT), identified in numerous animals and more recently in plants and bacteria [15], are also compounds (proteins) with heavy metal-binding properties [16].

Mercury is a highly toxic element because of its accumulative and persistent character in the environment and living organisms [17]. It affects the immune system, alters genetics and enzyme systems, and damages the nervous systems, and the senses of touch, taste and vision [18]. It has been introduced into the environment as three major forms, elemental Hg^0, inorganic Hg^{2+} and organic Hg. The inorganic mercury (Hg^{2+}) and monomethylmercury (CH_3Hg^+) are the two major species generally found in various biological samples [19].

Dioxan (DOX) is a versatile polar aprotic solvent capable of acting as hydrogen bond acceptor with random structure [20]. The DOX-water mixtures are the combination of aprotic and protic solvents with wide range of dielectric constants and with good solubility for polar as well as non-polar solutes. The co-solvent induced increased basicity of DOX-water mixtures increases the stabilization of protons.

L-histidine is essential at the active sites of many enzymes and biomolcules like *superoxide dismutase, ferritin, iron uptake regulation protein-FUR, ceruloplasmin, hemoglobin, metallothionein* and *cysteine dioxygenase.* In all these proteins, the major binding group for metal ions is the imidazole group of histidine.



MATERIALS AND METHODS

Materials

L-Histidine (E-Merck, Germany) solution (0.05 mol L^{-1}) was prepared in triple-distilled deionised water by maintaining 0.05 mol L^{-1} nitric acid concentration to increase the solubility. 1, 4-Dioxan (Qualigens, India) was used as received. 0.2 mol L^{-1} Nitric acid (Qualigens, India) was prepared. Sodium nitrate (Qualigens, India) of 2 mol L^{-1} was prepared to maintain the ionic strength in the titrand. Solutions of Pb(II), Cd(II) and Hg(II) nitrates

(0.05 mol L^{-1}) were prepared by dissolving G.R. Grade (E-Merck, Germany) salts in triple distilled water maintaining 0.05 mol L^{-1} acid to suppress the hydrolysis of metal salts. 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 [21]. The strengths of alkali and mineral acid were determined using the Gran plot method [22, 23].

Apparatus

The titrimetric data were obtained with a calibrated ELICO (Model L1-120) pH-meter (readability 0.01) which can monitor the changes in H^+ concentration. The pH meter was calibrated with 0.05 M potassium hydrogen phthalate in acidic region and 0.01 M borax solution in basic region. The glass electrode was equilibrated in a well-stirred dioxan-water mixture containing inert electrolyte. All the titrations were carried out in the medium containing varying concentrations of Dioxan (0-60% v/v) maintaining an ionic strength of 0.16 M with sodium nitrate at 303.0 ± 0.1 K. The effect of variations in asymmetry potential, liquid junction potential, activity coefficient, sodium ion error and dissolved carbon dioxide on the response of glass electrode were accounted for in the form of correction factor [24]. The emf of the cell may be expressed by the equation $E = K + (RT/F) \ln aH^+$ or $E = K + 0.0591\ pH$ at 25^0C, where K is a constant partly dependent upon the nature of the glass used for making the membrane. The value of K may vary slightly with time, and it is related to the existence of an asymmetry potential [25] in a glass electrode. Owing to the asymmetry potential, if a glass electrode is inserted into a test solution which is identical with the internal nitric acid solution, the electrode shall have a small potential which is found to vary with time. Hence, glass electrode is standardized frequently using a buffer of known hydrogen activity.

Procedure

For the determination of stability constants of metal-ligand binary species, initially titrations of strong acid with alkali were carried out at regular intervals to check whether complete equilibration was achieved. Then the calomel electrode was refilled with dioxan-water mixture of equivalent composition as that of titrand. In each of the titrations, the titrand consisted of approximately 1 mmol mineral acid in a total volume of 50 mL. Titrations with different ratios (1: 2.5, 1: 3.75 and 1: 5.0 in the case of Pb(II) and Cd(II) and 1: 7.5, 1: 8.5 and 1: 10.0 in the case of Hg(II)) of metal-to-ligand were carried out with 0.4 mol L^{-1} sodium hydroxide. Other experimental details are given elsewhere [26].

Modeling strategy

The computer program SCPHD [27] was used to calculate the correction factor. By using the pH-metric titration data, the binary stability constants were calculated with the computer program MINIQUAD75 [28], which exploit the advantage of the constrained least-squares method in the initial refinement and reliable convergence of Marquardt algorithm. During the refinement of binary systems, the correction factor and the protonation constants of histidine were fixed. The variation of stability constants with the dielectric constant of the medium was analyzed on electrostatic grounds on the basis of solute-solute and solute-solvent interactions.

RESULTS AND DISCUSSION

The results of exhaustive modeling for the Pb(II)-His system in 50% v/v DOX-water mixture are given Table 1. The models gave better statistics as the number of species was increased, confirming better fit. There was no further improvement in the fit on inclusion of some more species in the model containing PbL_2H_4, PbL_2H_3, PbL_2H_2, PbL_2H and PbL_2. This indicates that the final model appropriately fit the experimental data. Such exhaustive modeling was performed for all the systems. The results of the final best fit models that contain the stoichiometry of the complex species and their overall formation constants along with some of the important statistical parameters are given in Table 2. Very low standard deviation in overall stability constants (log β) signifies the precision of these constants. The small values of U (sum of squares of deviations in concentrations of ingredients at all experimental points) corrected for degrees of freedom, small values of mean, standard deviation and mean deviation for the systems are validated by the residual analysis [29].

Table 1: Exhaustive modeling study performed on Pb(II)-histidine system in 50% v/v Dioxan-water mixture. pH range =3.5-9.0, NP=46

Model No.	Log β_{mlh} (SD)					U_{corr}	Skewness	Kurtosis	χ^2	R – factor
	120	121	122	123	124					
1	12.97(16)	--	--	--	--	31.12	-1.08	2.41	50.99	0.041
2	--	19.08(8)	--	--	--	8.00	0.30	3.81	6.46	0.020
3	--	--	25.20(38)	--	--	83.05	0.24	4.88	82.06	0.067
4	--	--	--	Rejected	--	--	--	--	--	--
5	--	--	--	--	Rejected	--	--	--	--	--
6	10.25(15)	19.08(6)	--	--	--	4.00	-0.66	2.82	18.06	0.014
7	12.29(17)	--	25.14(14)	--	--	11.49	-1.17	3.64	14.58	0.024
8	13.49(34)	--	--	30.75(42)	--	24.97	-1.10	2.76	15.97	0.036
9	12.97(16)	--	--	--	Rejected	31.82	-1.08	2.41	50.99	0.041
10	--	19.01(9)	24.76(12)	--	--	5.19	1.05	8.05	43.80	0.016
11	--	19.39(13)	--	30.34(23)	--	6.03	0.76	5.92	13.65	0.018
12	--	19.08(8)	--	--	Rejected	8.19	0.30	3.81	6.46	0.020
13	--	--	25.20(38)	Rejected	--	84.94	0.24	4.88	82.06	0.067
14	--	--	37.90(**)	--	48.06(**)	81.98	0.25	5.31	106.87	0.066
15	--	--	--	Rejected	Rejected	--	--	--	--	--
16	10.18(7)	19.00(3)	24.77(5)	--	--	0.94	-0.01	3.44	3.80	0.007
17	10.55(13)	19.38(7)	--	30.33(13)	--	1.92	-0.27	2.58	4.14	0.010
18	10.25(15)	19.08(6)	--	--	Rejected	4.09	-0.66	2.82	18.06	0.014
19	12.30(17)	--	25.14(14)	Rejected	--	11.75	-1.17	3.64	14.58	0.024
20	13.49(27)	--	26.55(26)	--	36.68(29)	9.64	-1.41	4.42	28.03	0.022
21	13.49(34)	--	--	30.76(42)	Rejected	25.54	-1.10	2.76	15.97	0.036
22	--	19.15(13)	24.79(15)	29.88(40)	--	5.05	1.12	8.71	64.43	0.016
23	--	19.46(30)	25.43(39)	--	35.54(53)	4.91	1.13	9.14	77.65	0.016
24	--	19.39(13)	--	30.34(23)	Rejected	6.18	0.76	5.92	13.65	0.018
25	--	--	25.20(38)	Rejected	Rejected	86.91	0.24	4.88	82.06	0.067
26	10.31(8)	19.13(5)	24.80(5)	29.83(16)	--	0.74	-0.04	3.40	15.86	0.006
27	10.61(11)	19.42(10)	25.40(13)	--	35.50(18)	0.57	-0.37	3.10	5.30	0.005
28	21.74(**)	--	35.03(**)	Rejected	45.23(**)	10.18	--	--	--	--
29	--	19.82(46)	25.65(51)	30.80(61)	35.87(64)	4.60	1.29	10.65	212.03	0.015
30	10.55(12)	19.39(7)	--	30.33(13)	Rejected	1.97	-0.27	2.58	4.14	0.010
31	10.94(10)	19.76 (9)	25.61(10)	30.72(12)	35.80(13)	0.20	0.66	4.63	23.86	0.003

***: Standard deviation is very high*

Table 2: Parameters of best-fit chemical models of His complexes of Pb(II), Cd(II) and Hg(II) in Dioxan-water mixtures

DIOXAN % v/v	Logβ$_{mlh}$ (SD)						NP	U$_{corr}$	Skewness	Kurtosis	χ^2	R-Factor	pH-Range
	110	120	121	122	123	124							
					Pb(II)								
0.0	-----	9.37(40)	18.36(40)	25.07(41)	31.10(42)	36.90(42)	38	0.13712	0.67	7.15	32.11	0.002	3.5-9.0
10.0	-----	10.12(19)	18.53(19)	24.78(21)	30.38(25)	35.96(25)	51	0.65721	1.84	12.46	52.65	0.006	3.5-9.0
20.0	-----	10.37(15)	19.03(14)	25.12(15)	30.65(17)	36.03(18)	49	0.32306	0.04	2.38	8.37	0.004	3.5-9.0
30.0	-----	10.31(22)	19.66(22)	25.99(23)	31.35(24)	36.93(23)	45	0.24819	0.29	3.76	13.80	0.003	3.5-9.0
40.0	-----	11.61(14)	20.00(11)	25.29(16)	30.69(17)	35.18(31)	47	0.71347	0.06	2.56	8.16	0.006	3.5-8.7
50.0	-----	10.94(10)	19.76 (9)	25.61(10)	30.72(12)	35.80(13)	46	0.20905	0.66	4.63	23.86	0.003	3.5-9.0
60.0	-----	12.38(14)	20.98(13)	26.31(15)	31.79(15)	36.17(23)	48	0.49613	0.14	3.52	8.78	0.005	3.5-9.0
					Cd(II)								
0.0	-----	10.25(14)	17.80(15)	24.35(16)	30.55(17)	36.26(18)	50	0.28284	1.85	12.03	59.28	0.003	3.0-8.0
10.0	-----	10.79(8)	17.93(8)	24.16(10)	30.03(12)	35.37(14)	50	0.22788	1.11	7.28	66.53	0.003	3.0-8.0
20.0	-----	10.93(10)	18.15(10)	24.27(12)	30.04(14)	35.32(16)	49	0.33175	1.99	10.18	37.54	0.003	3.0-8.0
30.0	-----	11.10 (7)	18.23(7)	24.32(9)	29.69(14)	34.67(24)	33	0.08026	1.33	10.13	41.36	0.001	3.5-8.0
40.0	-----	12.32(6)	19.13(6)	24.93(7)	30.23(11)	34.55(33)	56	0.34449	0.26	7.41	14.19	0.004	3.0-8.0
50.0	-----	11.95(6)	18.78(5)	24.68(5)	29.63(18)	Rejected	52	0.61687	0.08	4.98	18.77	0.005	3.0-8.0
60.0	-----	14.19(11)	20.68(10)	26.32(12)	31.62(12)	35.57(28)	57	1.21811	-0.35	3.65	32.93	0.007	3.0-7.5
					Hg(II)								
0.0	10.09(24)	13.18(34)	-----	-----	33.83(23)	35.21(87)	90	0.73810	-0.78	4.70	47.16	0.004	2.0-8.0
10.0	11.77(27)	15.66(28)	-----	-----	34.21(25)	35.40(63)	69	0.22430	-0.51	4.64	6.45	0.002	1.9-8.0
20.0	11.98(31)	15.95(33)	-----	-----	34.29(30)	36.02(45)	89	0.35691	-0.24	3.23	7.25	0.002	2.0-8.0
30.0	12.13(39)	15.71(40)	-----	-----	34.80(38)	36.55(49)	91	0.24577	-0.07	3.51	4.89	0.002	2.0-8.0
40.0	12.22(7)	16.64(9)	-----	-----	34.34(4)	36.13(12)	102	0.12146	-0.39	3.80	6.73	0.001	2.0-8.0
50.0	14.99(60)	20.42(60)	-----	-----	34.98(55)	37.51(62)	66	0.15430	0.20	4.48	34.73	0.001	2.0-8.0
60.0	14.18(10)	19.99(12)	-----	-----	34.87(11)	37.64(11)	105	0.30570	-0.34	3.87	6.85	0.002	2.0-8.0

U_{corr}=U/ (NP-m) x10^8; where m= Number of species, NP=Number of experimental points, SD= Standard deviation.

Residual analysis

In data analysis with least squares methods, the residuals (the differences between the experimental data and the data simulated based on model parameters) are assumed to follow Gaussian or normal distribution. When the data are fit into the models, the residuals should ideally be equal to zero. If statistical measures of the residuals and the errors assumed in the models are not significantly different from each other, the model is said to be adequate. Further, a model is considered adequate only if the residuals do not show any trend. Respecting the hypothesis that the errors are random, the residuals are tested for normal distribution. Such tests are χ^2, skewness, kurtosis and R factor. These statistical parameters show that the best fit models portray the metal-ligand species in DOX-water mixtures, as discussed below.

Table 3: Effect of errors in influential parameters on Pb(II)-Histidine complex stability constants in 50% v/v Dioxan-water mixture

Ingredient	% of error	Log β_{mlh} (SD)				
		ML_2	ML_2H	$ML_2 H_2$	ML_2H_3	ML_2H_4
	0	10.94(10)	19.76(9)	25.61(10)	30.72(12)	35.80(13)
Alkali	-5	Rejected	17.29(69)	25.62(70)	Rejected	36.49(76)
	-2	9.32(21)	19.00(17)	25.54(19)	Rejected	35.97(23)
	+2	12.61(15)	20.27(14)	25.50(17)	31.09(17)	35.49(25)
	+5	14.23(12)	20.79(9)	Rejected	31.38(11)	Rejected
Acid	-5	11.55(13)	20.02(12)	25.53(14)	30.93(16)	35.54(20)
	-2	12.46(16)	20.24(14)	25.41(18)	31.05(18)	35.11(35)
	+2	9.64(25)	19.16(23)	25.68(25)	Rejected	36.19(29)
	+5	Rejected	20.30(77)	28.36(75)	Rejected	39.41(75)
Ligand	-5	11.28(26)	20.03(25)	26.05(26)	30.98(33)	36.54(29)
	-2	11.05(13)	19.85(13)	25.76(14)	30.80(18)	36.09(17)
	+2	10.86(9)	19.71(8)	25.49(9)	30.66(11)	35.51(13)
	+5	10.78(9)	19.67(8)	25.33(9)	30.58(12)	34.97(21)
Metal	-5	11.06(11)	19.75(10)	25.48(11)	30.62(14)	35.62(15)
	-2	10.98(10)	19.75(9)	25.56(10)	30.68(13)	35.73(14)
	+2	10.90(10)	19.77(9)	25.67(10)	30.76(12)	35.88(12)
	+5	10.85(10)	19.79(9)	25.75(10)	30.84(12)	35.99(12)
LogF	-5	10.96(10)	19.77(9)	25.59(10)	30.69(12)	35.76(13)
	-2	10.95(10)	19.77(9)	25.60(10)	30.71(12)	35.79(13)
	+2	10.93(10)	19.76(9)	25.62(10)	30.73(13)	35.82(13)
	+5	10.92(8)	19.76(7)	25.63(8)	30.74(10)	35.85(10)
Volume	-5	10.89(10)	19.72(10)	25.57(10)	30.67(13)	35.76(14)
	-2	10.92(10)	19.74(9)	25.59(10)	30.70(13)	35.79(13)
	+2	10.95(10)	25.63(9)	25.63(10)	30.73(13)	35.82(13)
	+5	10.98(10)	19.81(9)	25.65(10)	30.77(12)	35.85(13)

In the present study, the χ^2 values are less than the table values, and so the models are accepted. The kurtosis values in this study indicate that the residuals form platykurtic patterns. The values of skewness recorded in Table 2 are between 0.04 and 1.84 for Pb(II), -0.35 and 1.99 for Cd(II) and -0.07 and -0.78 for Hg(II). These data evince that the residuals form part of a normal distribution. Hence, least square method can be applied to the present data. The sufficiency of the model is further evident from crystallographic R-values. These statistical parameters thus show that the best-fit models portray the metal-ligand species in dioxan media.

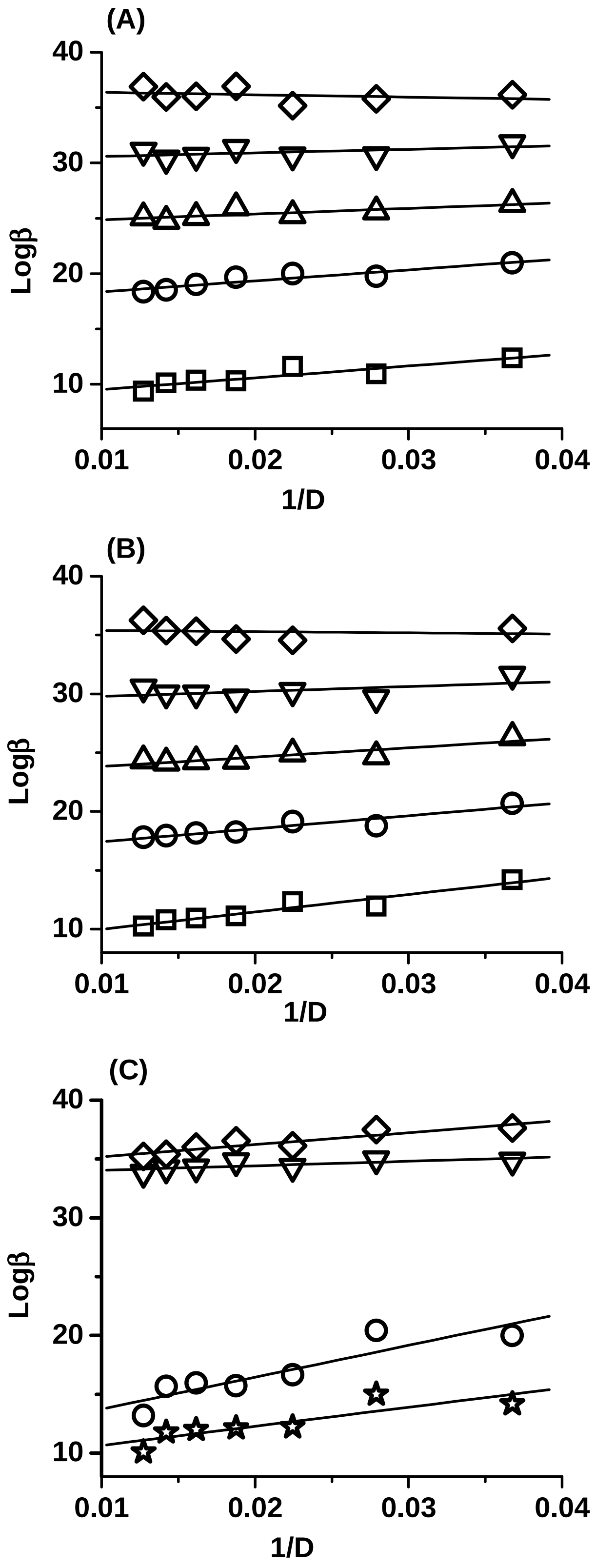


Figure 1: Variation of stability constant values of His complexes with reciprocal of dielectric constant (1/D) of dioxan-water mixtures (A) Pb(II); (B) Cd(II); (C) Hg(II); (☆) logβ_{ML}; (□) logβ_{ML2}; (○)logβ_{ML2H}; (Δ) logβ_{ML2H2}; (∇) logβ_{ML2H3}; (◇) logβ_{ML2H4}.

Effect of systematic errors on best fit model

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 undertaken by introducing pessimistic errors in the influential parameters like concentrations of alkali, mineral acid, ligand, metal, logF and volume (Table 3). The order of the ingredients that influence the magnitudes of stability constants due to incorporation of errors is alkali> acid> ligand> logF> metal> volume. Some species were even rejected when errors were introduced in the concentrations. The rejection of some species and increased standard deviations in the stability constants on introduction of errors confirm the suitability of the experimental conditions (concentrations of ingredients) and choice of the best fit models.

Effect of solvent

The linear variation of stability constants (log β) of histidine complexes with Pb(II), Cd(II) and Hg(II) with variation of 1/D (D is the dielectric constant) of DOX-water mixtures are given in Figure 1. DOX is an aprotic and coordinating solvent. It is a structure former and it enhances the water structure in DOX-water mixtures; hence, it removes water from coordination sphere of metal ions, making them more reactive towards the ligands. As a result, the stability of the complexes is expected to increase. At the same time, it is a coordinating solvent and competes with the ligands for coordinating the metals. This decreases the stability of the complexes. Hence, the stability of the complexes is expected to either increase or decrease. The linear increasing trend indicates the dominance of structure forming nature of the cosolvent.

Distribution diagrams

Histidine is a tridentate ligand that has one dissociable (carboxylate group) and two associable (amino, imidazole) protons. The different forms of His are LH_3^{+2} LH_2^{+} LH and L^- in the pH ranges <4.0, 2.0-6.0, 6.0-10.0 and > 9.0, respectively. Hence, the plausible binary metal-ligand complexes can be predicted from these data. The present investigation reveals the existence of ML_2H_4, ML_2H_3, ML_2H_2, ML_2H and ML_2 for Pb(II) and Cd(II) and Hg(II) forms ML_2H_4, ML_2H_3, ML_2 and ML. The formation of various L-histidine complex species is shown in the following equilibria.

$$\mathrm{M(II)}+2LH_3^{2+} \rightleftharpoons ML_2H_4^{4+}+2H^{+} \tag{1}$$

$$ML_2H_4^{4+} \rightleftharpoons ML_2H_3^{3+}+H^{+} \tag{2}$$

$$ML_2H_3^{3+} \rightleftharpoons ML_2H_2^{2+}+H^{+} \tag{3}$$

$$ML_2H_2^{2+} \rightleftharpoons ML_2H^{+}+H^{+} \tag{4}$$

$$\mathrm{M(II)}+2LH_3^{2+} \rightleftharpoons ML_2H_3^{3+}+3H^{+} \tag{5}$$

$$\mathrm{M(II)}+2LH_2^{+} \rightleftharpoons ML_2H^{+}+3H^{+} \tag{6}$$

$$\mathrm{M(II)}+2LH_2+ \rightleftharpoons ML_2H_2^{2+}+2H^{+} \tag{7}$$

$$\mathrm{M(II)}+LH_3^{2+} \rightleftharpoons MLH_2^{3+}+H^{+} \tag{8}$$

$$MLH_2^{3+} \rightleftharpoons MLH^{2+}+H^{+} \tag{9}$$

$$MLH^{2+} \rightleftharpoons ML^{+}+H^{+} \tag{10}$$

$$\mathrm{M(II)}+2LH_3^{2+} \rightleftharpoons ML_2+6H^{+} \tag{11}$$

$$\mathrm{M(II)}+2LH_2^{+} \rightleftharpoons ML_2+4H^{+} \tag{12}$$

$$\mathrm{M(II)}+2LH \rightleftharpoons ML_2+2H^{+} \tag{13}$$

$$ML_2H^{+} \rightleftharpoons ML_2+H^{+} \tag{14}$$

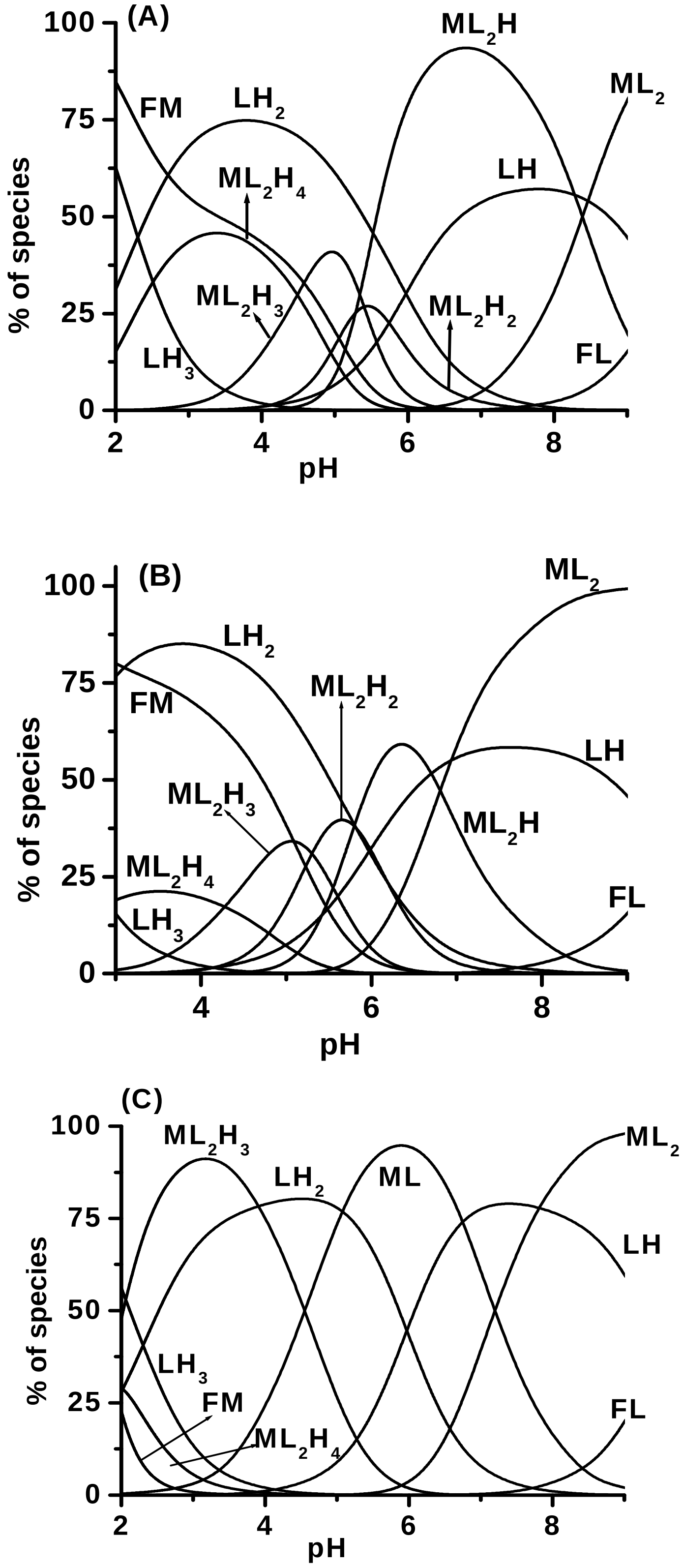


Figure 2: Distribution diagrams of binary complexes of His in 40% v/v dioxan-water mixture: (A) Pb(II), (B) Cd(II) and (C) Hg(II).

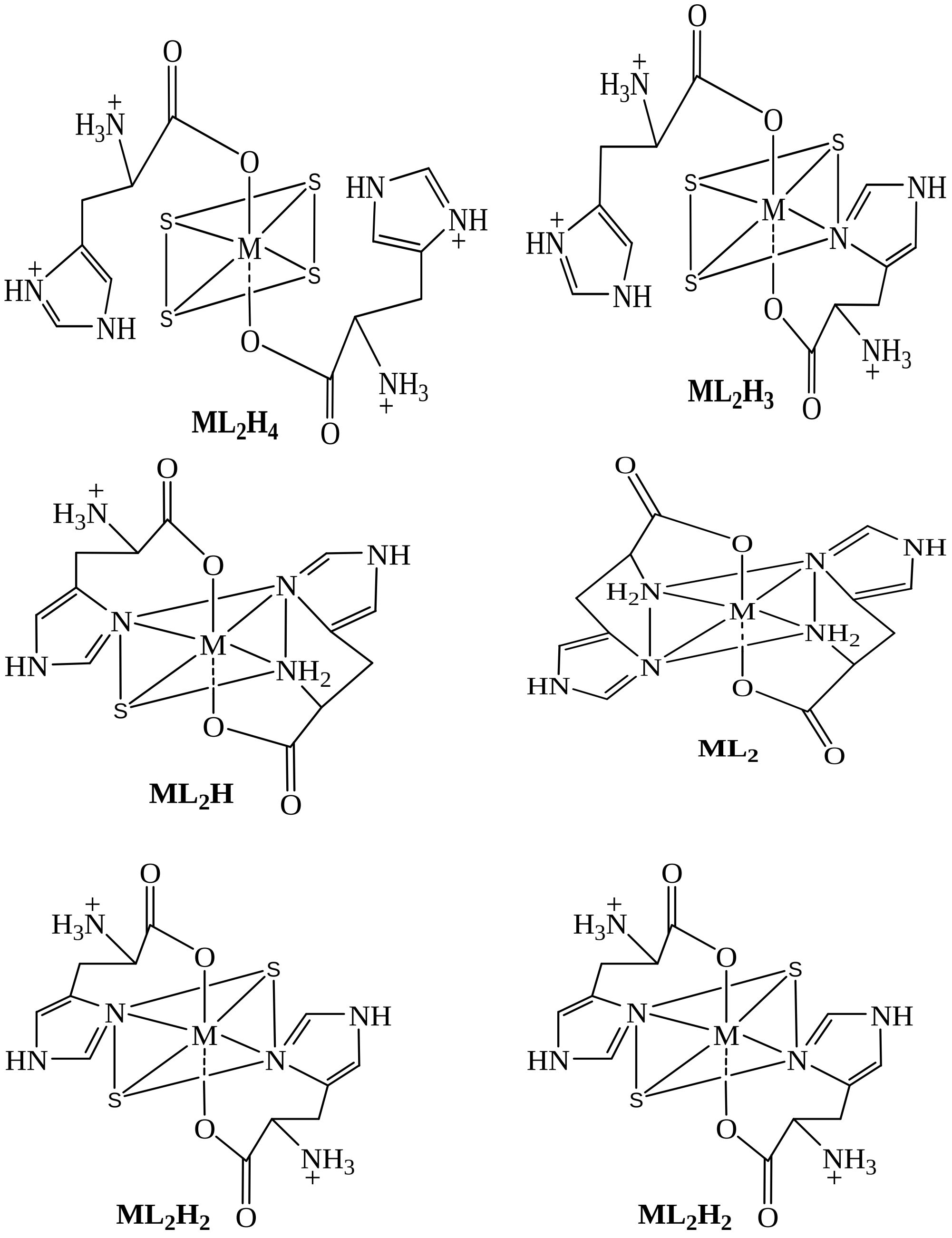


Figure 3: Structures of Metal-His complexes, where S is either solvent or water molecule

The species distribution diagrams of various systems are shown in Figure 2 which indicates the formation of His complexes of Pb(II), Cd(II) and Hg(II) in the pH ranges 3.5-9.0, 3.0-8.0 and 1.9-8.0 respectively. MLH_2 and MLH species could not be detected in the present study. At pH below 4.0, ML_2H_4 species is formed for Pb(II) and Cd(II) (Figure 2A & B) [Equilibrium 1]. With increasing pH, deprotonation of ML_2H_4 takes place to form ML_2H_3 [Equilibria 2 & 5]. Simultaneous increase in the concentrations of ML_2H_2 and ML_2H supports Equilibria 3, 4, 6 & 7. With increasing pH, ML_2H_2 is deprotonated to ML_2H [Equilibria 4 & 6]. ML_2 is formed in the order of Equilibria 11-14 with increasing pH. In the case of Hg(II)-His (Figure 2C) concentration of LH_3 and free metal ion decrease with increasing concentrations of ML_2H_4, ML_2H_3 and ML species [Equilibria 1,2,4,5, and 10].

Structures of complexes

In DOX-water mixtures, Pb(II)-histidine system assumes various coordination modes depending on the pH value. Both *mono*- and *bis*- histidine complexes have been identified. When the second donor site of His is a nitrogen atom, marked bidentate behaviour is frequently found, more so when the additional chelation results in a six-membered ring (Figure 3). Octahedral structures are proposed to the complexes of all the metal ions. The VSEPR theory suggests that Pb(II), Cd(II) and Hg(II) complexes shall be octahedral because there are six outer electron pairs. Amino nitrogen atoms can associate with hydrogen ions in physiological pH ranges. Hence, there is often significant competition between hydrogen and metal ion for this second donor site. This situation results in the simultaneous existence of a number of equilibria producing an array of successively protonated complexes. Hence, protonated complex species are detected in the present study. Imidazole nitrogen and carboxyl oxygen of histidine participate in bonding with metal ions [30].

CONCLUSION

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

1. L-Histidine forms both protonated and unprotonated complexes in the pH range 1.9-9.0.
2. The common species of ML_2H_4, ML_2H_3, ML_2H_2, ML_2H and ML_2 formed due to the interaction of His with the toxic metals of Pb(II) and Cd(II). ML, ML_2H_4, ML_2H_3, and ML_2 species are detected in the case of Hg(II). These models are validated by statistical treatment of data.
3. The linear variation of stability constants as a function of 1/D of the medium indicates the dominance of electrostatic forces over non-electrostatic forces. The linear increasing trend indicates the dominance of structure forming nature of co-solvent over its coordinating power.
4. The order of ingredients influencing the magnitudes of stability constants due to incorporation of errors in their concentrations is alkali> acid> ligand> logF> metal> volume.

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