INTERNATIONAL JOURNAL OF PHARMACEUTICS & DRUG ANALYSIS
VOL.5 ISSUE 5, 2017; 185 – 192 ; http://ijpda.com; ISSN: 2348-8948

Research Article

Chemical Speciation Of Binary Complexes of Pb(II), Cd(II) and Hg(II) With L-Dopa in Dimethylformamide-Water Mixture

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Date Received: 1st May 2017; Date accepted: 9th May 2017; Date Published: 10th May 2017

Abstract

Equilibrium study on complex formation of L-Dopa with Pb(II), Cd(II), and Hg(II) has been investigated pH metrically in dimethylformamide-water mixtures (0–60% v/v) at 303 K and 0.16 mol L^{-1} ionic strength. The predominant species detected for Pb(II) and Hg(II) are MLH_2 and ML_2H and those for Cd(II) are MLH, ML_2H, MLH_2. The appropriateness of experimental conditions was 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.

Keywords: L-dopa, dimethylformamide, MINIQUAD75, electrostatic forces, electrostatic forces

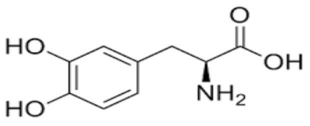
Introduction:

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 bio fluid. Lead affects every organ of the body, especially the bones and teeth, the kidneys, the nervous, cardiovascular, immune and reproductive systems [1]. Lead and other heavy metals create reactive radicals which damage cell structures including DNA and membranes [2-4]. Lead interferes with the normal metabolism of calcium, DNA transcription, enzymes that help in the synthesis of vitamin D and enzymes that maintain the integrity of the cell membrane. Lead may also be harmful to the developing immune system, causing production of excessive inflammatory proteins.

Cadmium enters the environment through volcanic activity and forest fires [5]. Cadmium affects 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 [6]. Cadmium is a known carcinogen to mammals [7]. Cadmium accumulates in plants, where it is detoxified by binding to phytochelatins [8-10], a family of thiol-rich peptides.

Mercury affects the immune system, alters genetics and enzyme systems, and damages the nervous systems, and the senses of touch, taste and vision [11, 12].

L-dopa (L-3,4-dihydroxyphenylalanine)(dopa) 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, [13, 14] since it is able to cross the blood-brain barrier whereas dopamine itself cannot.



Dimethylformamide (dmf) has a dielectric constant[15] of 36.7. The dielectric constant of the medium decreases with increase in the mole fraction of dmf. Hence, this medium is chosen to study the acido-basic equilibria to mimic the physiological conditions where the concept of equivalent solution dielectric constant for protein cavities is applicable [16]. 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 residues with the metal ion.

The species refined and their relative concentrations under the present experimental conditions represent the possible forms of these amino acids in the biological fluids. Hence dopa is selected for speciation studies of its complexes with Pb(II), Cd(II) and Hg(II) in dmf- water mixtures. The protonation constants of dopa in dmf-water mixtures were reported earlier [17].

EXPERIMENTAL

Materials

Dimethyformamide (Merck, Mumbai) was used as received. Aqueous solutions of L-dopa and sodium nitrate (E-Merck, Germany) were prepared. Metal solutions of Pb(II), Cd(II) and Hg(II) nitrates were prepared. To increase the solubility of dopa and to suppress the hydrolysis of metal salts, the mineral acid concentration in the above solutions was maintained at 0.05 M. To assess the errors that might have crept into the determination of the concentrations, the data have been subjected to analysis of variance of one way classification (ANOVA). The strength of alkali has been determined using the Gran plot method [18, 19].

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 dmf-water mixture containing inert electrolyte. All the titrations were carried out in the medium containing varying concentrations of dmf (0-60.0 % 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 [20].

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 dmf-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 : 5.0, 1 : 7.5 and 1 : 10.0 in the case of Hg(II)) of metal-to ligand were carried out with 0.4 M sodium hydroxide. Other experimental details are given elsewhere [21].

Modelling strategy

The computer program SCPHD [22] 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 [23] 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 dopa are 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

Alkalimetric titration curves in dmf-water mixtures revealed that the acido-basic equilibria of L dopa were active in the pH range 2.0-9.0. Based on the active forms of the ligands in this pH range, models containing various numbers and combinations of complex species were fed to MINIQUAD75 along with the alkalimetric titration data. Exhaustive modelling was performed for a typical system (Table1).

Table 1: Exhaustive modeling of Cd(II) – dopa complexes in 30% v/v dmf-water mixtures

Model number	log β_{mlh} 1 1 1	1 1 2	1 2 1	U_{corr}	skewness	χ^2	kurtosis	R factor
1	15.03(3)	-------	-------	33.52	-0.25	29.22	4.64	0.0290
2	-------	23.28(46)	--------	48.87	-0.61	72.99	5.12	0.0350
3	-------	---------	20.67(22)	30.56	0.29	58.55	5.13	0.0027
4	15.93(52)	23.33(42)	--------	29.82	0.81	36.02	4.93	0.0271
5	14.62(50)	--------	20.65(31)	30.00	-0.22	43.47	5.46	0.0272
6	------	23.32(30)	21.59(32)	27.32	0.87	45.68	5.30	0.0260
7	15.80(39)	23.43(25)	21.88(32)	29.14	0.80	42.18	5.91	0.0217

The models indicated 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 MLH, ML_2H and MLH_2. This indicates that the final model appropriately fits the experimental data. Such exhaustive modeling was performed for all the systems. The best-fit model was selected using the statistical parameters [24] of the least squares residuals. The final models along with the statistical parameters are given in Table 2. The results of the best-fit models contain the type of species and overall formation constants. A very low standard deviation in log β values indicates the precision of these parameters. The small values of Ucorr (sum of squares of deviations in the concentrations of ingredients 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. For an ideal normal distribution, the values of kurtosis and skewness should be three and zero, respectively. Kurtosis is a measure of the peakedness of the error distribution near a modal 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 as well as platykurtic patterns. The values of skewness recorded in the tables are between -0.26 and 1.95. These data evince that the residuals form part of a normal distribution. Hence, the least-squares method can be applied to the present data. The sufficiency of the model is further evident from the low crystallographic R-value recorded. These statistical parameters thus show that the best-fit models portray the metal-ligand species in dmf- water mixture.

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 made by introducing pessimistic errors in the influential parameters[25] like concentrations of alkali, mineral acid, ligand and metal (Table 3).

The order of the ingredients that influence the magnitudes of stability constants due to incorporation of errors is alkali > acid > ligand > metal. Some species are even rejected when errors are introduced in the concentrations. This study confirms the appropriateness of the chosen best-fit models. This study also indicates the relative sensitivities of model parameters.

Effect of solvent

Dmf is an amphiprotic and coordinating solvent. It is a structure former and it enhances the water structure in dmf-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 complex is expected to either increase or decrease. The variation of overall stability constant values or change in free energy with co-solvent content depends upon two factors, viz., electrostatic and non-electrostatic. Born's classical treatment [26] accounts for the electrostatic contribution to the free energy change. According to this treatment, the energy of electrostatic interaction is related to dielectric constant. Hence, the log β values should vary linearly as a function of reciprocal of the dielectric constant of the medium [26]which is observed in the present study (Figure 1 A and B) and non linearly in the case of 1C which indicates that the non electrostatic forces dominate over the electrostatic forces.

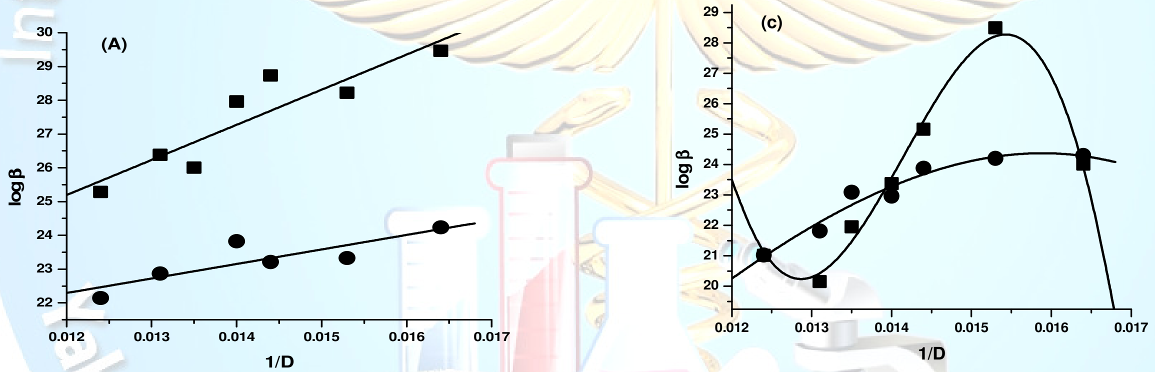
Table 2: Parameters of best fit chemical model of dopa complexes of Pb(II), Cd(II) and Hg(II) in dmf - water mixtures. Temperature =303 K, Ionic strength =0.16 M

%v/v DMF	Logβ_{mlh}(SD) 1 1 1	1 1 2	1 2 1	NP	U_{corr}	χ^2	Skewness	R factor	Kurtosis	pH range
Pb(II)										
0.0		22.14(24)	25.28(26)	113	19.21	153.81	-0.5	0.017	9.12	1.5-7.5
10.0		22.86(06)	26.38(08)	125	1.95	94.91	0.56	0.004	6.54	1.6-6.5
20.0			25.49(08)	18	5.3	12.07	-0.82	0.015	3.64	3.0-6.1
30.0		23.56(23)	27.62(20)	46	10.70	45.42	0.44	0.151	4.44	2.5-6.0
40.0		23.21(38)	28.73(34)	24	46.15	24.0	0.45	0.040	4.77	3.6-5.5
50.0		23.32(36)	22.22(35)	41	14.56	30.17	-0.16	0.018	4.44	3.6-6.0
60.0		24.23(27)	29.46(31)	48	22.30	14.67	-0.47	0.022	4.46	3.0-6.0
Cd(II)										
0.0	16.31(17)		22.48(16)	29	7.30	15.90	-1.23	0.017	5.06	3.0-8.5
10.0	15.45(35)	23.29(25)		108	16.37	76.99	1.28	0.014	4.26	1.7-8.5
20.0	15.15(34)	22.85(25)	21.09(26)	54	10.18	40.77	0.58	0.015	4.22	2.5-8.5
30.0	15.80(39)	23.43(25)	21.88(32)	58	26.45	37.59	1.09	0.025	5.82	2.8-8.5
40.0	15.59(28)		21.81(27)	22	4.74	2.78	-0.4	0.014	2.78	3.0-9.0
50.0	16.35(45)	23.58(43)	23.27(49)	65	25.89	45.62	0.56	0.026	5.45	3.0-8.5
60.0	17.08(35)	23.91(26)	23.30(38)	68	17.96	58.04	0.22	-0.021	7.65	3.1-9.0
Hg(II)										
0.0		21.02(46)	21.01(48)	103	21.20	153.11	1.48	0.017	10.11	1.8-8.5
10.0		21.18(35)	20.15(44)	115	3.54	181.86	1.95	0.006	9.02	1.6-8.0
20.0		22.98(33)	21.86(44)	138	63.25	143.29	0.47	0.027	4.89	1.7-8.5
30.0		22.96(44)	23.37(45)	58	18.73	42.83	-0.08	0.020	4.75	2.5-8.0
40.0		23.88(40)	25.15(40)	88	40.26	40.06	0.15	0.032	3.76	2.5-8.0
50.0		24.19(34)	28.49(37)	89	65.67	76.64	-0.13	0.031	4.53	2.0-8.0
60.0		24.30(45)	24.01(49)	68	36.48	27.92	-0.81	0.032	5.03	3.5-9.0

U_{corr} =U/(NP-m)*10^8, m –number of species : NP= number of experimental point

Table 3: Effect of errors in concentrations of ingredients on stability constants of Cd(II) - dopa complexes in 30% v/v dmf-water mixtures

Ingredient	% of error	Logβ$_{mlh}$		
		1 1 1	1 1 2	1 2 1
alkali	0	15.80(39)	23.43(25)	21.88(32)
	-5	14.02(15)	rejected	18.58(82)
	-2	15.26(50)	23.07(41)	20.91(45)
	+5	17.84(57)	24.34(42)	24.77(57)
	+2	16.48(38)	23.74(25)	22.77(34)
acid	-5	17.53(72)	24.38(44)	24.08(57)
	-2	16.29(53)	23.74(37)	22.46(47)
	+5	13.87(72)	rejected	18.77(57)
	+2	14.88(62)	22.76(61)	20.50(54)
ligand	-5	15.35(55)	23.07(45)	21.37(55)
	-2	15.49(54)	23.23(41)	21.47(45)
	+5	15.77(56)	23.55(38)	21.68(47)
	+2	15.49(56)	23.23(41)	21.47(45)
metal	-5	15.57(53)	23.35(31)	21.64(38)
	-2	15.57(56)	23.33(40)	21.57(46)
	+5	15.56(51)	23.30(38)	21.41(49)
	+2	15.56(53)	23.31(39)	21.48(45)



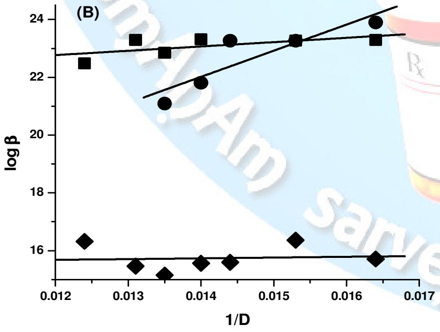
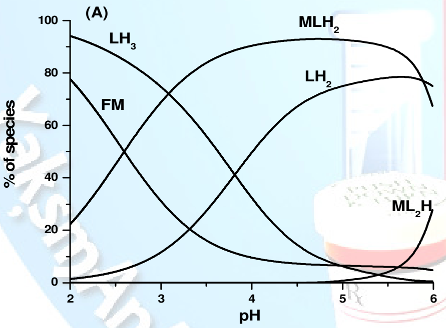


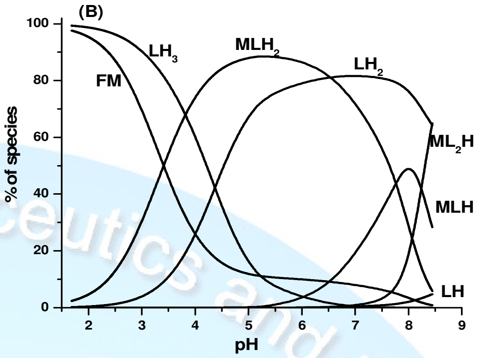
Figure 1: Variation of stability constant values of metal-dopa complexes with reciprocal of dielectric constants (1/D) in dmf-water mixtures at temperature = 303 K, ionic strength = 0.16 M (A)Pb(II), (C) Hg(II), (●) 112 and (■) 121(B) Cd(II) ,(⧫) log 111,(●) 112 and (■) 121

Distribution diagrams: The species distribution diagrams were obtained using the computer program DISPLOT [27]. 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. Hence, the plausible binary metal-ligand complexes can be predicted from these data. The present investigation reveals the existence of MLH, ML_2H, and MLH_2 for Cd(II) and ML_2H, MLH_2 for Pb(II) and Hg(II). The formation of various L dopa complex species is shown in the following equilibria. The charges of the species are omitted for simplicity. The species distribution diagrams are shown in Figure 2.

(a) $M(II)+LH_3 \rightleftharpoons MLH_2+H^+$
(b) $M(II)+2LH_3 \rightleftharpoons ML_2H+5H^+$
(c) $M(II)+LH_3 \rightleftharpoons MLH+2H^+$
(d) $MLH_2+LH_3 \rightleftharpoons ML_2H+4H^+$
(e) $MLH+LH_3 \rightleftharpoons ML_2H+3H^+$
(f) $M(II)+2LH_2 \rightleftharpoons ML_2H+H^+$
(g) $MLH_2+LH_2 \rightleftharpoons ML_2H+3H^+$

MLH_2 and ML_2H species are formed by the interaction of metal with that of the ligand as the concentration of ligand decreases and the concentration of MLH_2 and ML_2H increases in the case of Pb(II) and Hg(II) (equilibria a and b) and equilibria a, g and c may be appropriate for Cd(II) as metal interacts with LH_3 and forms MLH_2 equilibria a and ML_2H may be formed by the interaction of MLH_2 and LH_2 (equilibrium g) and MLH may be formed by the interaction of M(II) and LH_3 (equilibrium c).





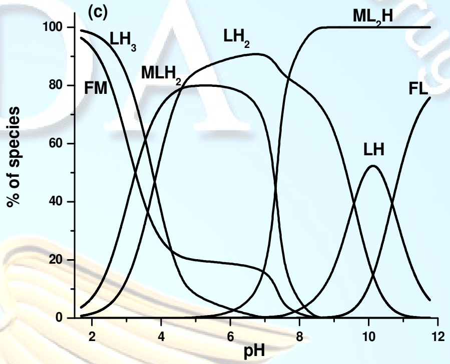
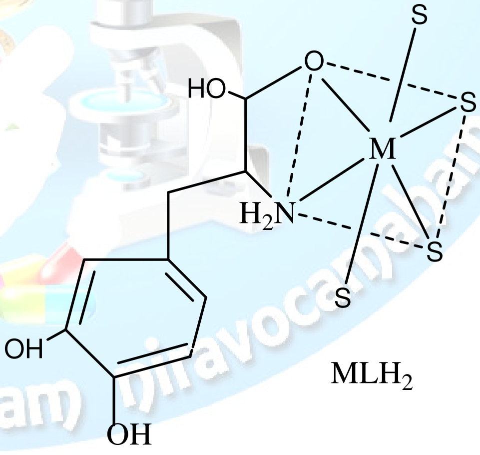


Figure 2: Distribution diagrams of (A) Pb(II) ; (B) Cd(II); (C) Hg(II) –dopa complexes in 30% of dmf-water mixture.

Depending on the active sites in the ligand and the nature of the metal ions, the structures were proposed for the species detected as shown in Figure 3.



MLH_2

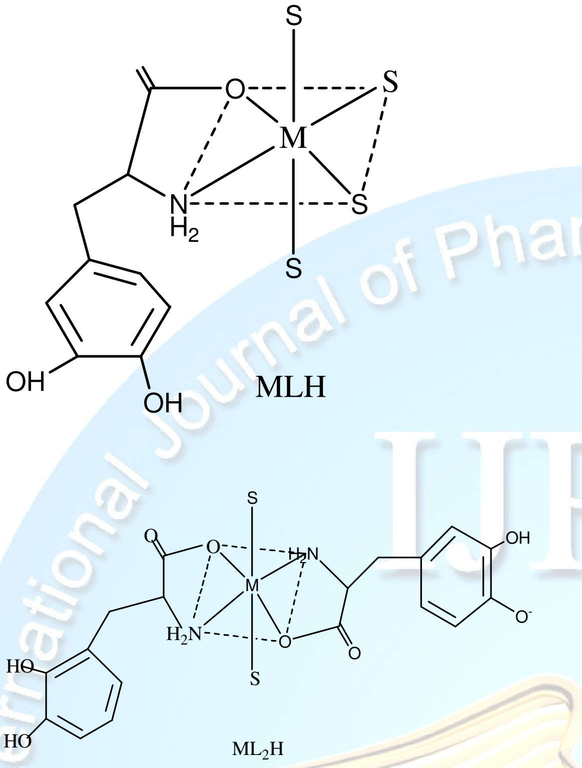


Figure 3: structures of binary complexes of Pb(II), Cd(II) and Hg(II) with dopa

CONCLUSIONS

1. The present biomimetic studies of metal ion complexes with L-dopa in dmf-water mixtures indicate that the complexes are protonated in acidic pH values.
2. The predominant species detected were MLH, ML_2H, and MLH_2 due to the interaction of L dopa with Cd(II) and MLH_2 and ML_2H in the case of Pb(II) and Hg(II).
3. The log β values linearly increased with 1/D of the medium, indicating the dominance of electrostatic forces over non-electrostatic forces in Pb(II) and Cd(II) and vice versa in Hg(II).
4. The order of ingredients influencing the magnitudes of stability constants due to incorporation of errors in their concentrations is alkali > acid > ligand> metal.

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