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Speciation of Glutathione Complexes of Pb(II), Cd(II) and Hg(II) in Propylene Glycol-Water Mixtures

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Abstract: *Speciation study on complex formation of Glutathione with Pb(II), Cd(II) and Hg(II) has been investigated pH metrically in propylene glycol-water mixtures 0.0-60.0 v/v at 303.0 K and 0.16 M ionic strength. The predominant species detected for Pb(II), Cd(II) and Hg(II) are MLH, ML_2H and ML_2H_2. 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 complex stability constants with change in the dielectric constant of the medium is explained on the basis of electrostatic and non-electrostatic forces.*

Keywords: *Complex equilibria, Chemical speciation, Glutathione, Propylene glycol, Metals.*

1. 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 fluids. Due to its numerous uses and high persistence, lead is a major environmental contaminant[1]. Lead is toxic even at low concentrations for living organisms, which can absorb it in various ways[2]. Lead intake by humans can be due to the consumption of crop plants grown on soils with high plant-available metal concentrations[3]. Cadmium causes iron deficiency by binding to cysteine, glutamate, aspartate, and histidine ligands[4]. Cadmium inhibits enzymes that participate in bilirubine conjunction[5]. It increases urine Ca^{2+} excretions which can cause severe bone pathology[6]. Mercury is one of the most toxic elements and has negative health effects in human populations, highly dependent on fish consumption[7]. Recent research concluded that neither vitamin B12 nor the acetyl-CoA pathways are required for bacterial methylation of mercury[8]. Glutathione (GSH) is an important antioxidant in plants, animals, fungi, and some bacteria and archaic, preventing damage to important cellular components caused by reactive oxygen species such as free radicals and peroxides[9]. It is a tripeptide with a gamma peptide linkage between the carboxyl group of the glutamate side-chain and the amine group of cysteine.

1, 2-Propanediol, also called propylene glycol (PG), is a clear, viscous, colorless and odorless liquid with a dielectric constant of 30.2[10]. The dielectric constant of the medium decreases with increasing mole fraction of the PG. Hence, PG is chosen to mimic the physiological conditions where the concept of equivalent solution dielectric constant for protein cavities is applicable[11]. GSH is selected for speciation studies of its complexes with Pb(II), Cd(II) and Hg(II) in propylene glycol (PG)-water mixtures. The protonation constants of GSH in PG-water mixtures were reported earlier[12].

2. EXPERIMENTAL

2.1. Materials

Propylene glycol (Merck, Mumbai) was used as received. Aqueous solutions of Glutathione and sodium nitrate (E-Merck, Germany) were prepared. Metal solutions of Pb(II), Cd(II), Hg(II) nitrates were prepared. To increase the solubility of GSH and to suppress the hydrolysis of metal salts, the mineral acid concentration in the above solutions was maintained at 0.05M. 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 (concentration) of alkali has been determined using the Gran plot method[13, 14].

2.2. 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 PG-water mixture containing inert electrolyte. All the titrations were carried out in the medium containing varying concentrations of PG (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[15].

2.3. 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 PG-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[16].

2.4. Modeling Strategy

The computer program SCPHD[17] 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[18], which exploits 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 Glutathione 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.

3. RESULTS AND DISCUSSION

Alkalimetric titration curves in PG-water mixtures revealed that the acido-basic equilibria of Glutathione (L^{3-}, LH^{2-}, LH_2^-, LH_3 and LH_4^+) were active in the pH range 2.0-12.0. Based on the active forms of the ligands in this pH range, models containing various numbers and combination of complex species were fed to MINIQUAD75 along with the alkalimetric titration data. Exhaustive modeling was performed for Pb (II)-GSH in 20% v/v PG-water mixture and the results are given in Table 1.

Table1. *Exhaustive modeling of Pb (II)-Glutathione complexes in 20% v/v PG-water mixture. pH range = 2.0-4.0; Number of points = 72*

Model no.	log β_{mlh} (SD)			U_{corr}	Skew-ness	Kur-tosis	χ^2	R-Factor
	111	121	122					
1	20.46(22)	---	---	2.01	7.45	8.85	10.25	0.0307
2	---	30.82(30)	---	4.08	6.21	7.60	12.23	0.0371
3	------	------	32.47(23)	4.21	8.65	5.32	16.25	0.0306
4	-----	32.95(14)	33.85(24)	3.26	9.50	4.71	14.73	0.0332
5	21.45(18)	------	32.24(30)	3.8	10.55	5.32	16.19	0.0406
6	20.86(19)	32.85(20)	-------	2.85	16.43	7.32	12.99	0.0307
7	19.92(1)	31.8(2)	35.78(1)	1.09	28.07	5.22	-0.13	0.0045

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 PbLH, PbL_2H and PbL_2H_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[19] 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 that contain the

type of species and overall formation constants along with some of the important statistical parameters are given in Table 2. A very low standard deviation in log β values indicates the precision of these parameters. The small values of U_{corr} (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 a zero mean with little dispersion.

Table2. *Best fit chemical models of Pb (II), Cd (II) and Hg (II)-GSH complexes in PG-water mixture.*

% v/v PG	log β_{mlh}(SD)			NP	U_{corr}	χ^2	Kur-tosis	Skew-Ness	R-Factor	pH-range
	111	121	122							
Pb(II)										
0.0	19.01(1)	30.39(5)	35.03(6)	114	1.74	77.18	4.40	-0.44	0.0073	2.0-4.0
10.0	19.30(1)	31.02(1)	35.72(2)	79	2.66	61.03	2.98	-0.11	0.0043	2.0-4.0
20.0	19.92(1)	31.8(2)	35.78(1)	72	1.09	28.07	5.22	-0.13	0.0045	2.0-4.0
30.0	20.04(1)	33.23(4)	36.98(6)	60	2.38	42.58	3.94	-0.30	0.0184	2.0-4.0
40.0	20.76(1)	34.03(4)	37.99(6)	80	1.86	36.13	3.88	-0.32	0.0068	2.0-4.0
50.0	20.77(8)	34.84(7)	38.03(2)	62	1.22	28.74	4.81	0.01	0.0181	2.0-4.0
60.0	20.91(1)	36.12(1)	38.94(3)	88	2.04	15.43	4.38	0.79	0.0075	2.0-4.0
Cd(II)										
0.0	18.66(1)	26.91(2)	33.56(1)	156	1.34	52.04	3.14	-0.51	0.0082	1.7-4.0
10.0	18.79(1)	27.31(1)	33.96(1)	87	1.34	29.48	2.87	-0 .59	0.0065	1.9-4.0
20.0	19.01(1)	28.94(1)	35.02(5)	91	2.03	87.29	2.40	-0.72	0.0096	1.9-4.0
30.0	19.35(1)	31.08(1)	36.36(3)	74	1.48	25.78	2.58	-0.81	0.0057	1.9-4.0
40.0	19.37(2)	32.29(7)	36.56(6)	88	2.32	11.56	3.47	0.36	0.0094	1.9-4.0
50.0	19.52(1)	33.49(2)	37.0(2)	90	1.24	10.03	5.10	0.01	0.0182	1.9-4.0
60.0	19.78(1)	34.71(2)	37.2(5)	84	1.89	30.10	4.91	0.77	0.0153	1.9-4.0
Hg(II)										
0.0	19.34(1)	24.63(1)	34.23(1)	82	1.43	21.25	3.12	0.43	0.0090	2.3-9.0
10.0	19.86(1)	25.31(2)	35.42(1)	70	2.21	13.54	4.06	-0.12	0.0082	2.3-9.0
20.0	20.12(1)	27.67(1)	36.02(1)	85	2.85	18.41	5.52	-0.49	0.0072	2.3-9.0
30.0	20.24(1)	29.89(2)	37.22(1)	72	1.99	16.30	4.39	-0.11	0.0083	2.3-9.0
40.0	20.31(1)	31.54(3)	37.54(2)	71	2.63	34.02	5.52	0.44	0.0015	2.3-9.0
50.0	20.56(1)	32.75(1)	37.59(1)	77	2.40	7.30	3.78	0.31	0.0113	2.3-9.0
60.0	20.72(1)	34.06(1)	37.66(1)	83	2.73	42.31	4.68	0.67	0.0179	2.3-9.0
U_{corr} = U/(NP-m) x 10^8, where, m = number of species; NP = number of experimental points.										

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 -0.81 and 0.79. 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 PG-water mixture.

4. 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[20] 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.

Table3. *Effect of errors in influential parameters on the Pb(II) Glutathione complex stability constants in 20% v/v PG-water mixture.*

Ingredient	% Error	log β (SD)		
		111	121	122
Alkali	0	19.21(1)	31.8(2)	35.78(1)
	-5	21.32(85)	Rejected	Rejected
	-2	20.42(76)	Rejected	36.32(84)
	+2	20.48(78)	30.64(73)	36.64(88)
	+5	22.42(82)	31.21(86)	36.96(98)
Acid	-5	Rejected	Rejected	Rejected
	-2	21.10(23)	32.29(16)	36.86(20)
	+2	22.08(30)	Rejected	35.45(19)
	+5	22.21(42)	Rejected	36.12(32)
Ligand	-5	19.13(19)	31.92(32)	36.79(15)
	-2	19.14(16)	31.94(22)	36.82(57)
	+2	19.13(32)	31.95(25)	36.84(48)
	+5	19.12(52)	31.98(35)	36.83(49)
Metal	-5	19.13(26)	31.95(18)	36.83(18)
	-2	19.14(23)	31.98(18)	36.84(19)
	+2	19.14(22)	31.99(19)	36.85(20)
	+5	19.12(21)	32.97(20)	36.86(21)

5. EFFECT OF SOLVENT

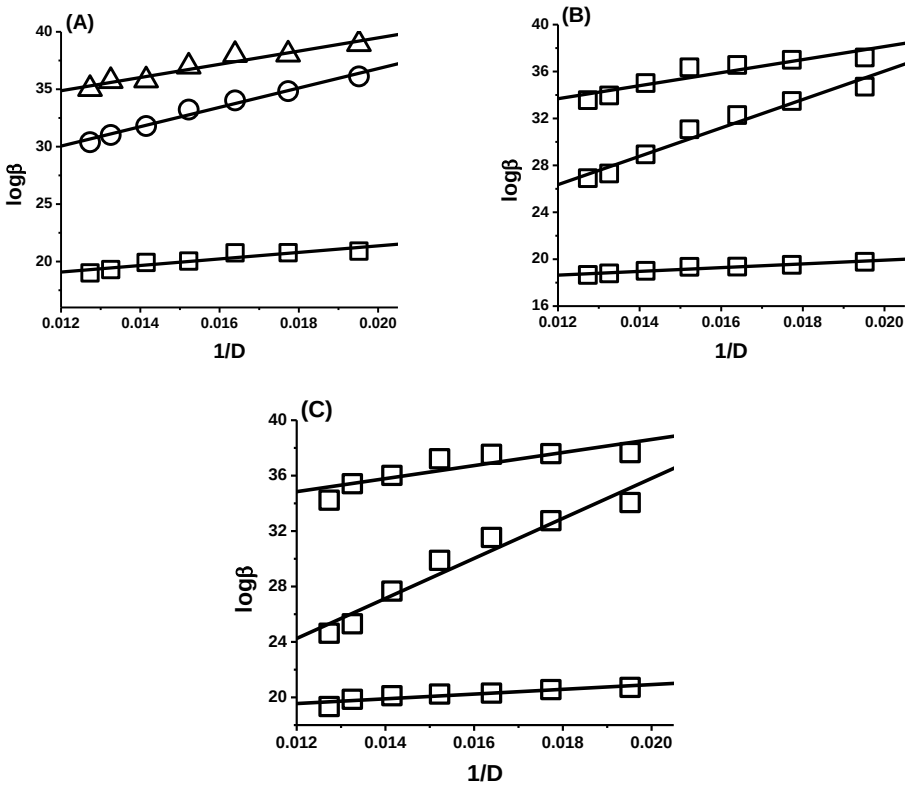

Fig1. *Variation of stability constant values of metal-Glutathione complexes with reciprocal of dielectric constants (1/D) in PG-water mixtures at temperature = 303 K and ionic strength = 0.16 M. (A) Pb(II), (B) Cd(II) and (C) Hg(II); (□) log 111, (O) log 121, (Δ)log 122.*

Variation of logarithmic values of stability constants (log β) with reciprocal of dielectric constant (1/D) are shown in Figure 1. PG is an amphiprotic and coordinating solvent. It is a structure former and it enhances the water structure in PG-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[21].

Variation of logarithmic values of stability constants (log β) or change in free energy with co-solvent content depends upon two factors, viz., electrostatic and non-electrostatic. Born's classical treatment[22] holds good in accounting 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 1/D of the medium. The linear trend observed in the present study (Fig.1) indicates that electrostatic forces are dominating the equilibrium process under the present experimental conditions.

6. DISTRIBUTION DIAGRAMS

Glutathione is an essential nutrient and an antioxidant ligand that has one sulfhydryl group, two carboxylic acid groups and one amino group. The different forms of GSH are LH_4^+, LH_3, LH_2^-, LH^{2-}, and L^{3-} in the pH ranges 2.0-4.0, 4.0-6.0, 6.0-10.0, 10.0-11.0 and 11.0-12.0 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 ML_2H_2 for Pb(II), Cd(II) and Hg(II). The formation of various GSH is shown in the following equilibria. The charges of the species are omitted for simplicity. The species distribution diagrams are shown in Fig. 2.

$M(II) + LH_4$	⇌	$MLH + 4H^+$	--------- (1)
$M(II) + LH_3$	⇌	$MLH + 3H^+$	--------- (2)
$M(II) + LH_2$	⇌	$MLH + H^+$	--------- (3)
$M(II) + 2LH_4$	⇌	$ML_2H_2 + 6H^+$	--------- (4)
$M(II) + 2LH_3$	⇌	$ML_2H_2 + 4H^+$	--------- (5)
$M(II) + 2LH_2$	⇌	$ML_2H_2 + 2H^+$	--------- (6)
$MLH + LH_2$	⇌	$ML_2H_2 + H^+$	--------- (7)
$M(II) + 2LH_3$	⇌	$ML_2H + 5H^+$	--------- (8)
$MLH + LH_2$	⇌	$ML_2H + 2H^+$	--------- (9)
ML_2H_2	⇌	$ML_2H + H^+$	--------- (10)

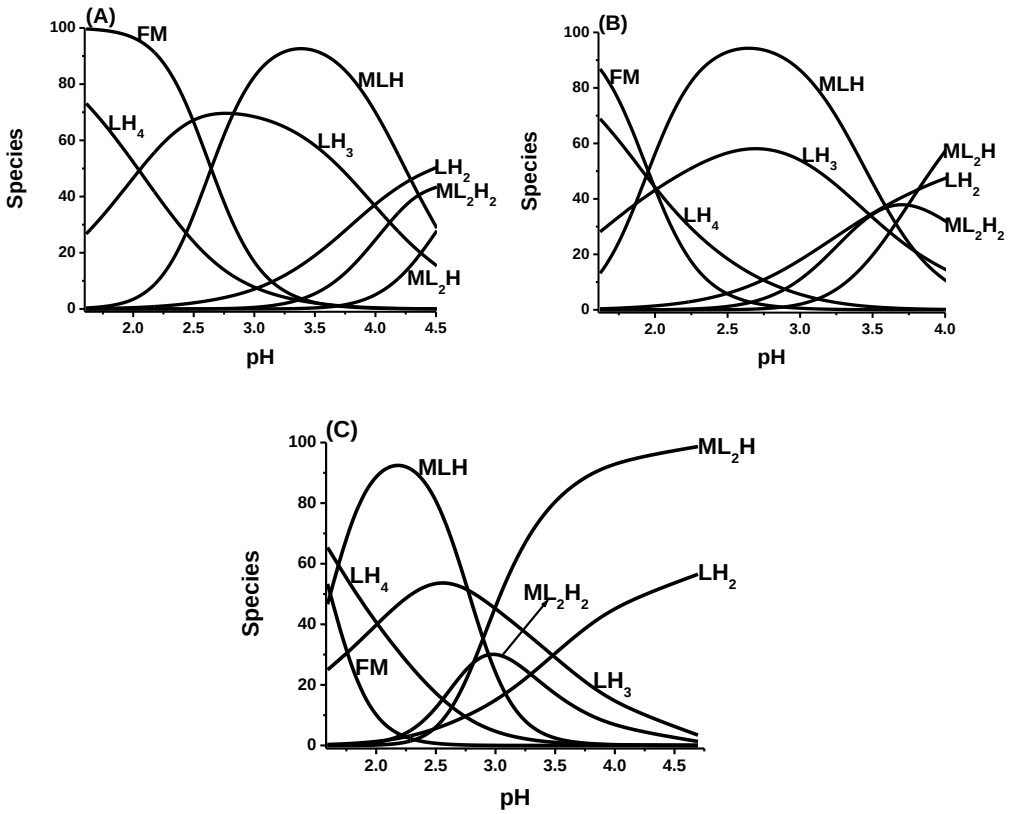

Fig2. *Distribution diagrams of Glutathione complexes in 30% v/v PG-water mixture. Temperature = 303 K, ionic strength = 0.16 M. (A) Pb (II), (B) Cd(II) and (C) Hg(II).*

At lower pH, MLH species is formed by the interaction of free metal ion with LH_4, LH_3 and LH_2 form of the ligand (Equilibria 1, 2 and 3). ML_2H_2 may be formed from free metal ion and LH_4, LH_3 and LH_2 (Equilibria 4, 5 and 6) or from MLH interaction with LH_2 (Equilibrium 7). At higher pH, ML_2H

species is formed from the interaction of free metal ion with LH_3 (Equilibrium (8)) or from MLH interaction with LH_2 (Equilibrium 9) or by the deprotonation of ML_2H_2 (Equilibrium 10).

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 Fig. 3.

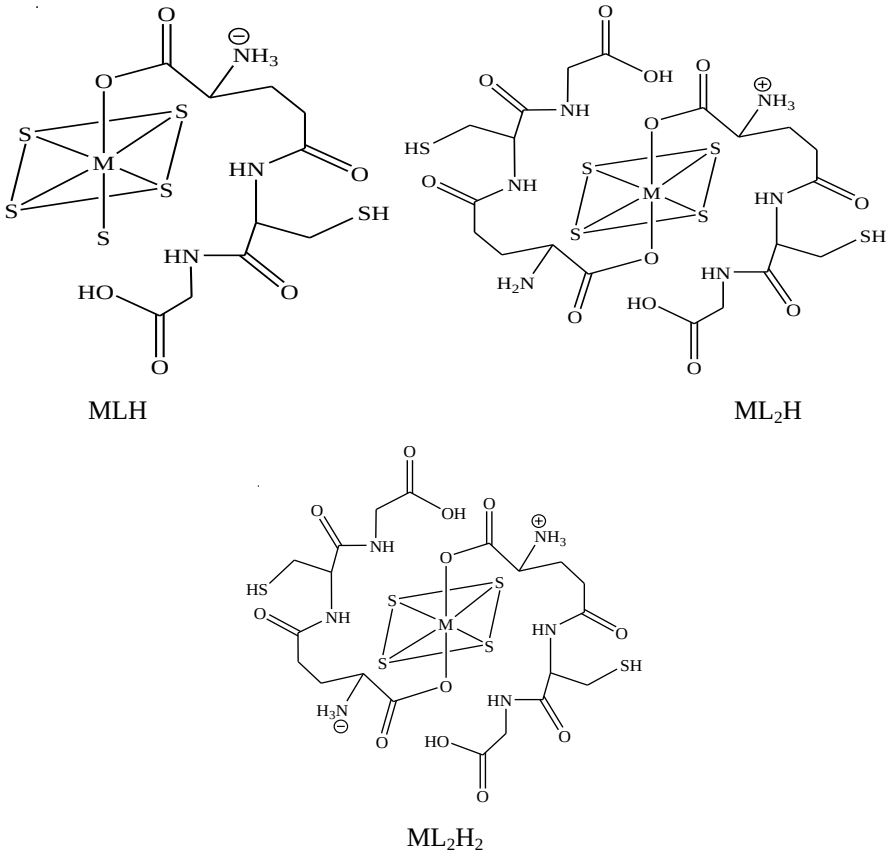

Fig3. *Structures of binary complexes of Pd (II), Cd (II) and Hg (II) with GSH.*

7. CONCLUSIONS

1. The present biomimetic studies of metal ion complexes with Glutathione in PG-water mixtures indicated that the complexes were protonated in acidic pH values. The species detected were MLH, ML_2H and ML_2H_2.
2. The log β values linearly increased with 1/D of the medium, indicating the dominance of electrostatic forces over non-electrostatic forces.
3. The order of ingredients influencing the magnitudes of stability constants due to incorporation of errors in their concentrations was alkali > acid > ligand > metal.
4. The stability constants of binary complexes were found to follow the trend Cd(II) < Pb(II) < Hg(II).

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