

# Speciation Studies of Some Toxic Metal Complexes of Glycylglycine in Propylene Glycol–Water Mixtures

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## ABSTRACT

The formation equilibria of complexes of Pb(II), Cd(II) and Hg(II) with glycylglycine were investigated pH-metrically in propylene glycol-water mixtures (0–60 % v/v) at 303 K and an ionic strength of 0.16 mol L<sup>-1</sup>. The dominant species detected were ML<sup>+</sup> and ML<sub>2</sub>H<sub>2</sub><sup>2+</sup> for Pb(II); MLH<sup>2+</sup> and ML<sub>2</sub>H<sup>+</sup> for Cd(II) and ML<sup>+</sup>, ML<sub>2</sub> and ML<sub>2</sub>H<sup>+</sup> are for Hg(II). The appropriateness of the experimental conditions was verified by introducing errors deliberately in the concentrations of ingredients and volume of the solution. The models containing different numbers of species were refined by using the computer program MINQUAD75. Selection of the best fit chemical models was based on statistical parameters. The trend in variation of stability constants of the complexes with dielectric constant of the medium was attributed to electrostatic and non-electrostatic forces. Distribution diagrams and plausible equilibria for the formation and possible structures of the complex species are presented.

## KEYWORDS

Speciation, complex equilibria, glycylglycine, propylene glycol, stability constants.

## 1. Introduction

Heavy metals are common in our environment and diet and many of them are essential to living organisms but some of them are highly toxic or become toxic given sufficient exposure and accumulation in the body. Metals such as Hg, Cd, Pb, Sn, Cr and As are generally not required for metabolic activity and are toxic to living organisms even at low concentrations.<sup>1,2</sup> The mechanism of the toxicity of metals is very complicated. Generally toxicity of metals results from blocking the essential biological functional groups (–OH, –SH and –N) or modifying the active conformation of biomolecules (enzyme, DNA, etc.) through binding and displacing the essential metal ions from their natural binding sites of the biomolecules with a foreign metal ion.

Human civilization and the increase in industrial activity has gradually redistributed many toxic metals from the earth's crust to the environment and increased the possibility of exposure. Lead, cadmium and mercury are among the various toxic heavy metals especially prevalent in nature due to their high industrial use. These metals serve no biological function and their presence in tissues reflects contact of the organism with its environment.<sup>3</sup> The main sources of lead pollution are the battery industry, leaded petrol and gasoline exhaust. Lead affects every organ of the body, specially the bones and teeth, the kidneys, and the nervous, cardiovascular, immune and reproductive systems.<sup>4–6</sup> Lead also interferes with the normal metabolism of calcium in cells and causes it to build up within them.<sup>7</sup>

The sources of Cd pollution in urban areas are metallurgical plants, Cd plating and battery fabricators. It can also enter the environment through natural causes, such as volcanic activity and forest fires.<sup>8</sup> Human exposure to cadmium mainly occurs through cigarette smoking,<sup>9</sup> but exposure can also occur through contaminated food,<sup>10</sup> water or air.<sup>11</sup> Cadmium is a known carcinogen to mammals.<sup>12</sup> Cadmium interacts with calcium in the skeletal system to produce osteodystrophies.<sup>13</sup> Due to their

similarity in properties, cadmium displaces zinc in many metallo-enzymes and many of the symptoms of cadmium toxicity can be traced to a cadmium-induced zinc deficiency.<sup>14,15</sup> Cd(II) binds strongly with the –SH groups of cysteine residues of enzymes<sup>16</sup>, e.g. carbonic anhydrase, dipeptidase, carboxy peptidase, etc., and affects the active conformation of biomolecules due to this strong binding.

Mercury exposure is related to the release of mercury forms (elemental Hg<sup>0</sup>, inorganic Hg(II) and organic Hg) into the environment by both natural and man-made activities.<sup>17</sup> Mercury is a highly toxic element because of its accumulative and persistent character in the environment and living organisms.<sup>18</sup> It affects the immune system, alters genetics and enzyme systems, and damages the nervous system, and the senses of touch, taste and vision.<sup>19</sup> The toxicity of mercury is based on the strong affinity for the deprotonated forms of thiol ligands such as cysteine.<sup>20</sup> So Hg(II) binds strongly with the thiol group of proteins, enzymes and other biomolecules in which this binding changes the conformation of biomolecules in their active site.<sup>16,21</sup>

Speciation studies of toxic and essential metal ion complexes are useful in order to understand the role played by the active site cavities in biological molecules and the bonding behaviour of their residues with metal ions.<sup>22</sup> The possible ligand groups in proteins are the amino acid side chains, the terminal amino, carboxyl and thiol groups and, in some cases, the amide group in the peptide backbone.<sup>23</sup> However, the study of metal-protein interactions involves complications due to the complex nature of proteins. An exact model of the metal-protein system may be difficult to construct in simple biomolecules like amino acids and peptides but such models may give a tremendous amount of information about the structure of proteins and function of biomolecules in biological systems.<sup>24</sup>

The interaction of metals with amino acids and peptides has been the subject of much research,<sup>25–29</sup> due to the importance of metals in many biochemical processes,<sup>30</sup> such as

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respiration, metabolism and nerve transmission.<sup>31</sup>

Investigations of acid–base equilibria of amino acids and peptides and their interaction with metal ions at varying ionic strengths, temperatures and dielectric constant media throw light on the mechanism of enzyme-catalyzed reactions. It is known that the polarity of the active site cavities in proteins is lower than that of the bulk but direct measurement of the dielectric constant is not possible. Comparing the formation constants of acid–base equilibria and/or metal complex equilibria with those at biological centres offers a way to estimate the effective dielectric constant or equivalent solution dielectric constant for the active site cavity.<sup>32</sup> This has brought an important new approach to the study of complex equilibria in aqua-organic mixtures apart from its established utility in understanding solute–solvent interactions, increasing sensitivity of reactions of analytical and industrial importance, and solubilising ligands or their metal complexes.

Chemical speciation of metals is important for an understanding of their distribution, mobility, bioavailability, toxicity and for setting environmental quality standards.<sup>33</sup> Bioavailability of a particular metal depends on its complex chemical reactions of dissolution, binding and complexation with the constituents of the environmental aquatic media.<sup>34</sup> To reveal the solvent effects on equilibrium processes involving charged species, we have studied the complex formation of glycylglycine (GG) with Pb(II), Cd(II) and Hg(II) as a good example in modelling of the bonding modes of peptides to toxic metal ions in mixtures containing propylene glycol (PG) and water.

## 2. Experimental

### 2.1. Chemicals and Standard Solutions

All the chemicals used in this investigation were of Analytical reagent grade purity. Triple-distilled deionized water was used for the preparation of all the solutions. Solutions of 0.1 mol L<sup>-1</sup> of Pb(II), Cd(II) and Hg(II) nitrates (Himedia, India) were prepared maintaining 0.05 mol L<sup>-1</sup> nitric acid to suppress the hydrolysis of metal salts. A solution (0.05 mol L<sup>-1</sup>) of glycylglycine (Acros Organic, USA) was prepared by maintaining a 0.05 mol L<sup>-1</sup> nitric acid concentration to increase its solubility. 1,2-Propanediol (Merck, India) was used as received. Solutions of 0.2 mol L<sup>-1</sup> nitric acid (Merck, India) and 0.4 mol L<sup>-1</sup> sodium hydroxide (Merck, India) were prepared. A solution of 2.0 mol L<sup>-1</sup> sodium nitrate (Merck, India) was prepared to maintain the ionic strength in the titrand.

All the solutions were standardized by the usual standard methods. The concentration of the alkali was determined by titrating it with standard oxalic acid and potassium hydrogen phthalate solutions, while the normality of nitric acid was determined by using the standardized sodium hydroxide and the primary standard borax solutions.<sup>35</sup> The concentrations of the metal ions were determined complexometrically by titrating against a standard solution of EDTA using xylenol orange as indicator and hexamine powder as buffer to maintain the pH at 5.0–6.0.<sup>36</sup> So as to assess the errors that might have crept into the determination of the concentrations, the data were subjected to one-way analysis of variance (ANOVA) by using the computer program COST.<sup>37</sup> The carbonate contamination in the sodium hydroxide solutions was determined by using the Gran plot method.<sup>38</sup>

### 2.2. Alkalimetric Titrations

The pH measurements of metal–ligand binary systems were carried out in aqueous media containing varying compositions

of propylene glycol in the range of 0–60% (v/v) maintaining an ionic strength of 0.16 mol L<sup>-1</sup> with sodium nitrate at 303.0 ± 0.1 K by using a digital pH meter MK-VI type with a readability of 0.01 (0–14). The electrode of the cell was calibrated with 0.05 mol L<sup>-1</sup> potassium hydrogen phthalate solution in the acidic region and with 0.01 mol L<sup>-1</sup> borax solution in the alkaline region to measure the response in the pH range 2.0–11.0. The effect of variations in 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 a correction factor.<sup>39,40</sup> Mechanical stirring of the solution was carried out by means of a teflon stirrer. The pH-metric titration assembly consisted of a double-walled spoutless Pyrex glass vessel of 100 mL capacity fitted with a Perspex lid through which the glass combination pH electrode, gas inlet–outlet tubes and burette tip were admitted. Water from a thermostat was pumped at a constant rate through the annular space between the walls of the titration vessel. The titrand in the double-walled spoutless Pyrex glass vessel was maintained inert by bubbling dried and pure nitrogen gas throughout the course of the titration to purge carbon dioxide and oxygen.

The electrode was kept, usually for three to four days, in the required solvent system in order to equilibrate. To verify whether the electrode was equilibrated, 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 titrant. A calomel electrode was refilled with PG–water mixture of the equivalent composition to that of the titrand. Free acid titrations were performed before the metal–ligand titrations to calculate the correction factor. In each of the titrations, the titrand consisted of a mineral acid of approximately 1 mmol in a total volume of 50 mL. Titrations with different ratios (1:2.5, 1:3.5 and 1:5) of metal–ligand were performed with 0.4 mol L<sup>-1</sup> sodium hydroxide solution.

The mixtures obtained from PG and water are non-ideal due to increased interactions between unlike molecules and large differences in molar volumes of pure components, which leads to non-additive volumes on mixing. The excess volume ( $V^E$ ) obtained from mixing of the two solvents at a given condition is calculated by the equation

$$V^E = \frac{x_1 M_1 + x_2 M_2}{\rho} - \left( \frac{x_1 M_1}{\rho_1} + \frac{x_2 M_2}{\rho_2} \right)$$

where  $x_1$  and  $x_2$  are the mole fractions,  $M_1$  and  $M_2$  are the molar masses,  $\rho_1$  and  $\rho_2$  are the densities of the pure components, and  $\rho$  is the mixture density. In this experiment, the volume correction was not taken into consideration because the change in volume was insignificant (its maximum is –0.67 mL mol<sup>-1</sup> if mixing is carried at 303 K) even though the mixtures obtained from PG and water show non-ideal behaviour.<sup>41</sup> Other experimental details are given elsewhere.<sup>42</sup>

### 2.3. Modelling Strategy

The correction factor to be applied to the pH-meter dial readings was calculated with the computer program SCPHD.<sup>43</sup> By using the pH-metric titration data, the binary stability constants were calculated with the computer program MINQUAD75, which exploits the advantage of the constrained least-squares method in the initial refinement and reliable convergence of the Marquardt algorithm.<sup>44</sup> During the refinement of binary stability constants,  $pK_w$  and the protonation constants<sup>45</sup> of GG were fixed (Table 1). 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.

**Table 1** Protonation constants of GG in PG-water mixtures.<sup>45</sup> (Auto-protolytic constant,  $pK_w = 13.9$ )

% v/v PG	D*	log $\beta$ (S.D.)	
		011	012
00.0	78.48	7.89 (16)	11.12 (22)
10.0	75.45	7.84 (33)	11.20 (34)
20.0	71.19	7.83 (22)	11.14 (29)
30.0	65.70	7.98 (9)	11.33 (15)
40.0	61.01	7.95 (7)	11.42 (11)
50.0	56.40	7.96 (9)	11.48 (15)
60.0	51.24	7.9 9(9)	11.66 (16)

\*Dielectric constants (D) calculated from orthogonal fit.

MINIQUAD75 does not have a provision to study the effect of systematic errors in the influential parameters on the magnitude of the stability constant. Any variation in these parameters like concentrations of ingredients (alkali, mineral acid, metal and the ligand), volume and electrode calibration affects the magnitudes of stability constants. In order to rely upon the best fit 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 concentrations of mineral acid, alkali, ligand, metal and volume.

### 3. Results and Discussion

The results of the best fit models that contain the type of species and formation constants along with some of the important statistical parameters were given in Table 2. Statistically the best chemical models that represent acid–base equilibria under study should have a low standard deviation in their stability constant ( $\log \beta$ ) values that indicates the precision of the parameters. The small values of  $U_{\text{corr}}$  (sum of the squares of the deviations in the concentrations of ligand and hydrogen ions at all experimental points corrected for degrees of freedom) indicate that the experimental data can be represented by the model. Small values of the mean, standard deviation and mean deviation for the systems corroborate that the residuals are around a zero mean with little dispersion.<sup>46</sup> For an ideal normal distribution, the values of the kurtosis and skewness should be three and zero, respectively. The kurtosis values in the present study indicate that the residuals form largely a leptokurtic pattern. The values of the skewness given in Table 2 are between  $-2.54$  and  $1.66$ . 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. Thus, these statistical parameters show that the best fit models portray the metal–ligand species in PG–water mixtures.

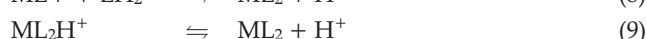
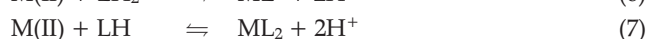
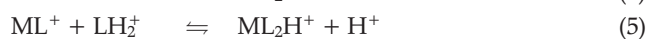
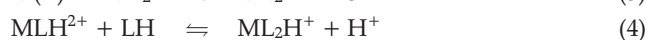
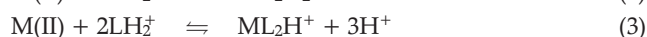
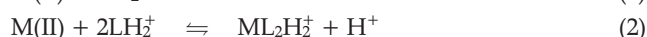
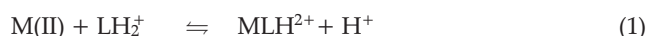
Sensitivity of the measured stability constants to the incorporated errors of the ingredients was in the order: alkali > acid > ligand > metal > volume. Species are even rejected when errors were introduced in the concentrations of the alkali and acid. The rejection of these species and increased standard deviations in the stability constants on introduction of errors confirm the appropriateness of the experimental conditions and the choice of  $H_2^{2+}$  the best fit models. This also indicates the relative sensitivities of model parameters.

The formation of  $ML_2H_2^{2+}$  by Pb(II) probably indicates that the side-chain amino group can still protonate in the presence of Pb(II). But in the presence of the other two metals, this side-chain tends to coordinate to the metal by losing a proton at

lower pHs than its  $pK_a$  value. Hence,  $ML_2H^+$  but not  $ML_2H_2^{2+}$  is detected in the case of Cd(II) and Hg(II). These observations are in agreement with the general concept that Cd(II) and Hg(II) form stronger complexes than Pb(II). The formation of  $ML_2$  by Hg(II) but not by Pb(II) and Cd(II) indicates that Hg(II) forms the strongest complex (even the higher magnitudes of stability constants of Hg(II) complexes than those of other metals given in Table 2). The absence of  $ML_2$  in case of Pb(II) and Cd(II) may be due to decomposition or hydrolysis of the complex species.

### 3.1. Distribution Diagrams

Glycylglycine has one dissociable (carboxylate) and one associable (amino) proton. It exists as  $LH_2^+$ ,  $LH$  and  $L^-$  in the pH ranges below 5.0, 2.0–8.0 and above 3.0, respectively.<sup>45</sup> Hence, plausible binary metal–ligand complexes could be predicted from these data. The present investigation reveals the existence of  $ML_2H_2^{2+}$  and  $ML^+$  for Pb(II);  $ML_2H^+$  and  $MLH^{2+}$  for Cd(II) and  $ML_2H^+$ ,  $ML_2$  and  $ML_2^+$  for Hg(II). The typical species distribution diagrams of various systems are shown in Fig. 1 which indicate the formation of GG complexes of Pb(II), Cd(II) and Hg(II) in the pH ranges 2.5–7.0, 1.6–6.2 and 1.6–6.0 respectively.  $MLH^{2+}$  is formed at low pH and  $ML_2H^+$  is formed at higher pH for Cd(II).  $ML_2H_2^{2+}$  for Pb(II) and  $ML_2H^+$  for Hg(II) species are formed at low pH.  $ML^+$  of Pb(II) and  $ML_2$  of Hg(II) are formed at higher pH. From the distribution curve of the species, formation of various M(II)–GG complex are shown in the following equilibria.



In case of Pb(II),  $ML_2H_2^{2+}$  is formed as the concentration of free metal ion and  $LH_2^+$  decreased as seen in Equilibrium (2). Equilibria (6) and (7) are proposed for the formation of  $ML^+$  but the latter is more appropriate because of the low concentration of  $LH_2^+$  and the concentration of  $ML^+$  is increased with decreasing concentration of  $LH$  (Fig. 1A).

Equilibrium (1) is proposed for the formation of  $MLH^{2+}$  by Cd(II). Equilibria (3) and (4) are proposed for the formation of  $MLH^{2+}$  but the latter is more probable than the former because  $MLH^{2+}$  is formed with decreasing concentration of  $MLH^{2+}$  (Fig. 1B). In the case of Hg(II), Equilibria (3) and (5) are the possible ways for the formation of  $ML_2H^+$  but (5) is more appropriate because during its formation there is a decrease in  $ML^+$  concentration and the percentage of  $ML_2$  is increased as the concentration of  $ML^+$  and  $ML_2H^+$  are decreased at the same pH range (Fig. 1C) which is described by Equilibria (8) and (9).

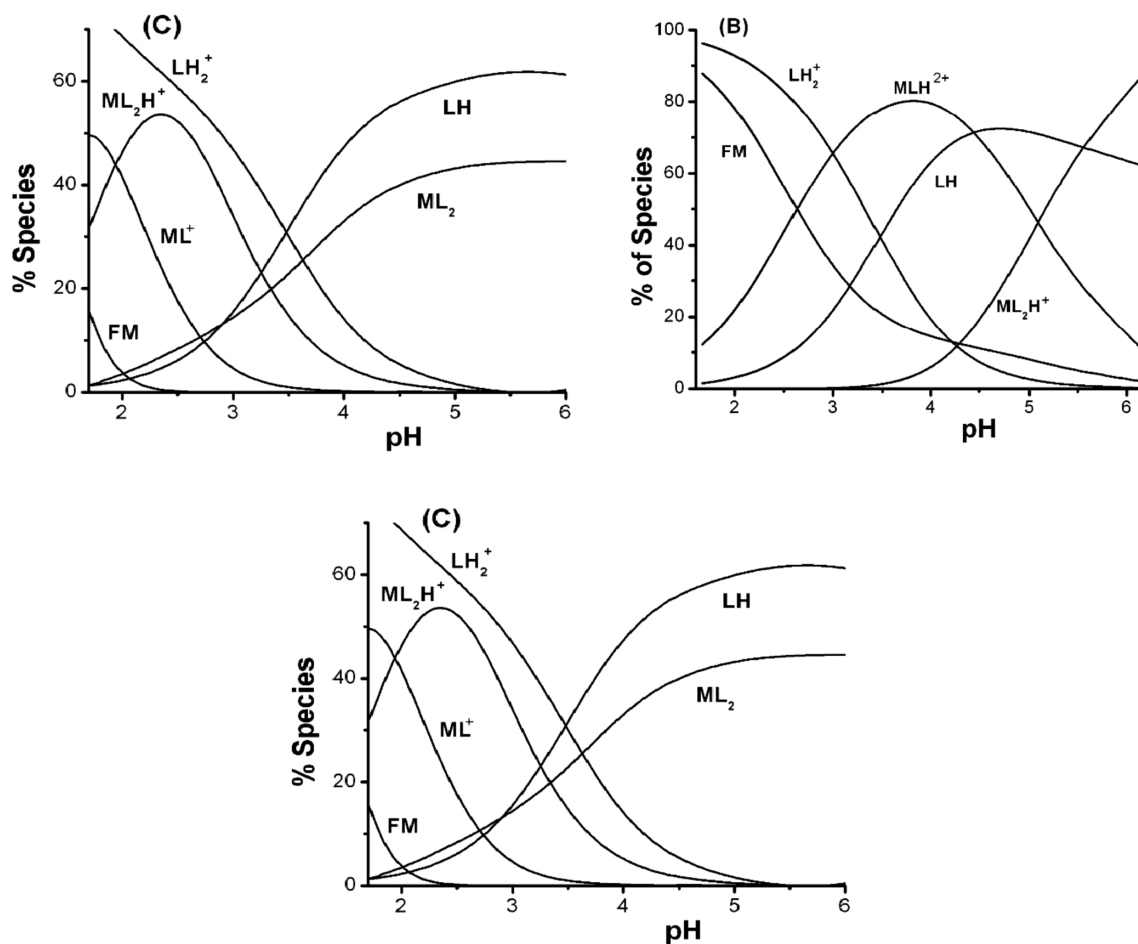
### 3.2. Effect of Solvent

The presence of PG in aqueous solution considerably decreases the dielectric constant of the medium and these solutions are expected to mimic physiological conditions where the concept of equivalent solution dielectric constant for protein cavities is applicable. Hence, PG was selected for the biomimetic study by considering its dielectric constants at different percentages (0–60% v/v) in water. PG is an amphiprotic and coordinating solvent. It is a structure forming solvent (enhances the struc-

**Table 2** Parameters of best-fit chemical models of GG complexes of Pb(II), Cd(II) and Hg(II) in PG-water mixtures (temperature = 303 K, ionic strength = 0.16 mol L<sup>-1</sup>).

% v/v PG	Log β <sub>mth</sub> (S.D.)			NP	U <sub>corr</sub>	χ <sup>2</sup>	Skewness	Kurtosis	R-factor	pH-range	
	110	111	120								121
	Pb(II)										
0.00	3.69 (21)	-	-	19.31 (69)	14.11	69.80	0.93	6.07	0.0226	2.0–8.2	
10.00	4.00 (27)	-	-	20.18 (26)	19.34	31.59	0.38	6.80	0.0256	2.0–8.1	
20.00	4.19 (58)	-	-	20.27 (28)	9.511	74.78	-0.69	7.52	0.0431	2.0–8.2	
30.00	3.84 (17)	-	-	19.84 (47)	6.787	47.51	-0.01	4.05	0.0152	2.0–8.2	
40.00	4.03 (20)	-	-	19.87 (21)	1.367	62.92	-0.83	5.52	0.0231	2.0–8.4	
50.00	4.43 (27)	-	-	19.41 (46)	13.44	48.70	0.61	6.06	0.0234	2.0–8.4	
60.00	4.57 (53)	-	-	20.22 (43)	5.621	132.1	-2.02	6.27	0.0490	2.0–8.2	
	Cd(II)										
0.00	-	9.81 (81)	-	14.05 (24)	6.692	48.33	0.50	5.14	0.0139	2.0–6.0	
10.00	-	10.15 (12)	-	13.91 (49)	5.061	73.10	0.37	3.91	0.0120	2.0–6.3	
20.00	-	10.02 (11)	-	13.85 (29)	2.811	69.38	-0.29	8.07	0.0104	2.2–6.4	
30.00	-	10.21 (20)	-	14.22 (28)	7.961	58.16	-0.87	4.47	0.0155	2.0–6.4	
40.00	-	10.51 (23)	-	14.53 (27)	8.065	16.80	-0.28	2.61	0.0203	2.8–6.4	
50.00	-	10.43 (9)	-	14.61 (14)	1.778	28.09	-0.44	4.09	0.0090	2.5–6.3	
60.00	-	10.23 (8)	-	14.41 (13)	0.828	29.04	-0.19	3.42	0.0063	2.7–6.4	
	Hg(II)										
0.00	9.51 (8)	-	18.00 (10)	20.13 (19)	1.528	36.64	1.50	7.89	0.0205	1.8–6.2	
10.00	9.78 (25)	-	17.61 (29)	20.31 (39)	2.428	36.84	-2.54	8.13	0.0314	2.0–6.4	
20.00	9.77 (8)	-	17.31 (10)	20.33 (11)	4.453	15.42	1.54	3.68	0.0113	1.8–6.2	
30.00	9.58 (11)	-	17.04 (11)	20.14 (14)	3.896	17.91	1.66	6.67	0.0131	2.1–6.5	
40.00	10.11 (88)	-	18.03 (71)	20.87 (56)	1.565	32.74	0.57	11.01	0.0271	2.0–6.0	
50.00	10.23 (24)	-	17.34 (24)	20.79 (20)	3.860	11.25	0.08	3.40	0.0136	2.1–6.0	
60.00	10.80 (34)	-	18.45 (31)	22.18 (28)	2.550	70.67	1.28	8.63	0.0107	2.0–6.4	

U<sub>corr</sub> = U/(NP-n) × 10<sup>6</sup>; NP = number of points; n = number of species; S.D. = standard deviation.  
m, l, h : stoichiometric coefficients of metal, ligand and hydrogen, respectively.



**Figure 1** Distribution diagrams of binary complexes of GG in 40 % (v/v) PG-water mixture: (A) Pb(II), (B) Cd(II) and (C) Hg(II).

ture of water) in PG-water mixtures. It also removes water from the coordination sphere of metal ions, making them more reactive towards ligands. As a result, the stability of the complexes is expected to increase with increasing concentration of PG. On the other hand, PG is a coordinating solvent and competes with ligands for coordination with the metal ions. This decreases the stability of the complexes. Hence, variation in the stability of complexes with solvent is a result of both the opposing behaviours.

The variation of the values of the overall stability constants or change in Gibbs energy with content of co-solvent depends upon two factors, *viz.* electrostatic and non-electrostatic. Born's classical treatment accounts well for the electrostatic contribution to the Gibbs energy change.<sup>47</sup> According to this treatment; the energy of electrostatic interaction is related to the dielectric constant of the medium. Hence, the  $\log \beta$  values should vary linearly with the reciprocal of the dielectric constant of the medium. The linear variation of stability constants ( $\log \beta$ ) of GG complexes of Pb(II), Cd(II) and Hg(II) with  $1/D$  ( $D$  is the dielectric constant) of PG-water mixtures (Fig. 2) indicates the dominance of the electrostatic interactions. Solute-solvent interactions, relative thermodynamic stabilities and kinetic labilities also play important roles.<sup>48,49</sup>

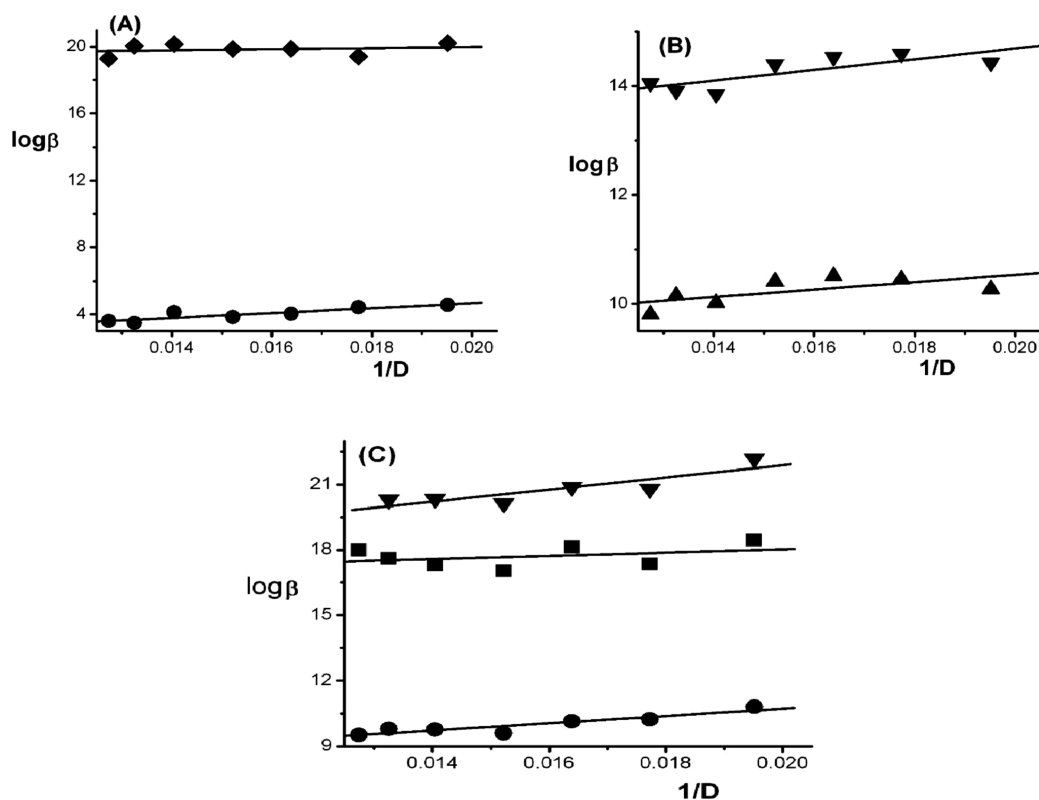
### 3.3. Structures of Complexes

Both *mono*- and *bis*-glycylglycine complexes have been identified for the M(II)-GG systems of the toxic metals. The first donor is the oxygen in the carboxyl group and the second donor site is amine nitrogen atom of GG. The oxygen or nitrogen in the amide group could also act as third donor sites of GG. This additional chelation results in a five-membered ring (Fig. 3). In

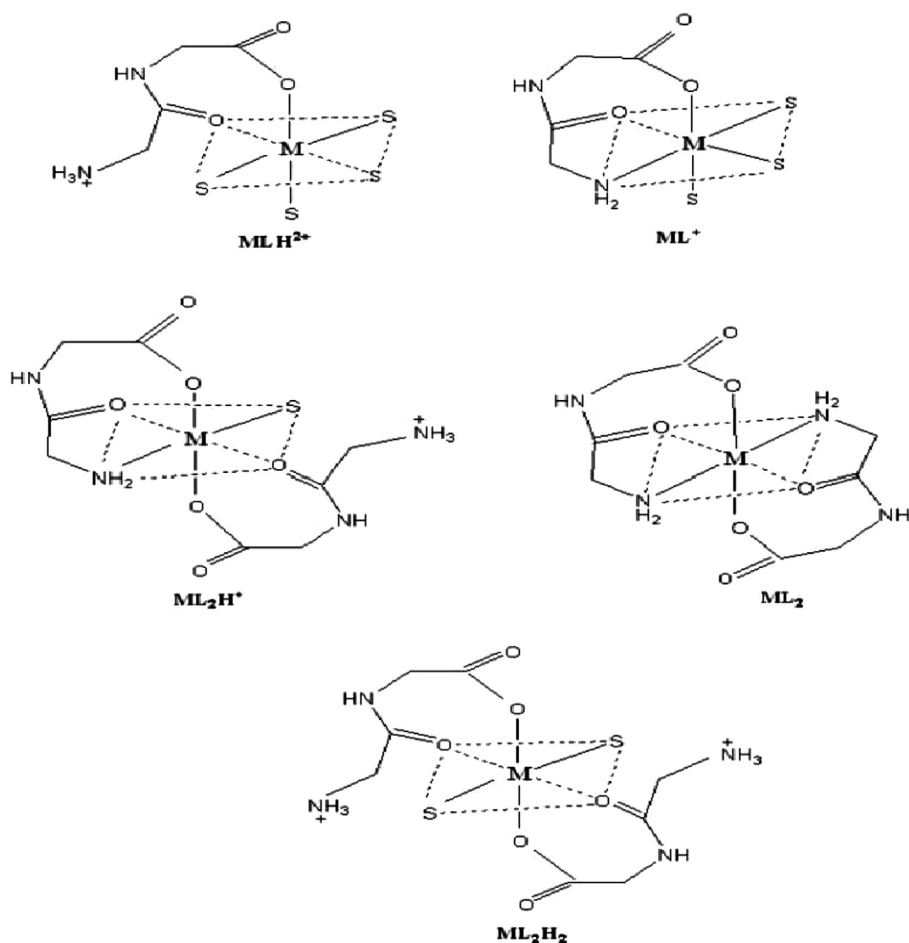
view of the principle of Hard and Soft Acid and Bases (HSAB), the very soft character of Cd(II), Hg(II) and Pb(II) shows the preference for the amine nitrogen site over the harder carboxylate or amide oxygen. Probst and Rode<sup>50</sup> observed that below pH 8, the terminal carboxyl group and the terminal amino group are simultaneously coordinated to the cadmium ion and the amide might also be simultaneously coordinated through the carbonyl oxygen atom to form a five-membered chelate ring. This formation of a five-membered chelate ring from the terminal amino group and the amide oxygen is proved from the X-ray diffraction analysis of GG complexes of the cadmium ion<sup>51</sup> at pH 6.0 and the mercury ion.<sup>52</sup> Octahedral structures are proposed for the complexes of Pb(II), Cd(II) and Hg(II). Amine nitrogen atoms can associate with hydrogen ions in the lower pH ranges. Hence, there is often significant competition between hydrogen and metal ions for this second donor site. This situation results in the simultaneous existence of equilibria producing protonated complexes species.

### 4. Conclusions

Stability constants and different species were determined in the modelling study of the interaction of glycylglycine with the toxic metals ions Pb(II), Cd(II) and Hg(II) in PG-water mixtures. The common species identified in this investigation were  $ML^+$  and  $ML_2H_2^{2+}$  for Pb(II),  $MLH^{2+}$  and  $MLH_2^{2+}$  for Cd(II), and  $ML^+$ ,  $ML_2$  and  $ML_2H^+$  for Hg(II). The linear variation of the stability constants as a function of  $1/D$  of the medium indicates the dominance of electrostatic forces over non-electrostatic forces and the dominance of the structure forming nature of the co-solvent over its coordinating power.



**Figure 2** Variation of the stability constants of metal-GG complexes with the reciprocal of the dielectric constant ( $1/D$ ) in PG-water mixtures. (A) Pb(II); (B) Cd(II); (C) Hg(II): ( $\blacktriangledown$ )  $\log \beta_{121}$ , ( $\blacktriangle$ )  $\log \beta_{111}$ , ( $\blacksquare$ )  $\log \beta_{120}$ , ( $\bullet$ )  $\log \beta_{110}$ , ( $\blacklozenge$ )  $\log \beta_{122}$ .



**Figure 3** Proposed structures of M(II)-GG complexes, where S is either a solvent or water molecule.

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