Final Report on the Minor Research Project in Chemistry

PREDICTION OF CHEMICAL SPECIATION OF TOXIC METAL ION COMPLEXES OF L-PROLINE AND L-VALINE IN LOW DIELECTRIC MEDIA

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By

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Final Project Report

1. Introduction

Chemical speciation may be defined as the existence of an element in various chemical and physical forms which together makeup the total concentration of that element in a sample. Speciation analysis is the determination of the concentrations of separate and unique atomic and molecular forms of an element instead of its total concentration in a sample. Reliable speciation knowledge can be used, to optimize drug efficacy, in discovering antidotes and to identify those metal species that are likely to have adverse effects on biota. The formation and distribution of each species relies on the binding strength of the metal-ligand complexes which can be quantitatively expressed as the stability constant.

Heavy metals are commonly defined as those having a specific density of more than 5 g/mL such as lead, mercury, aluminum, arsenic, cadmium, nickel. They are widely distributed in the earth's crust. Their presence in the atmosphere, soil and water, even in traces, can cause serious problems to all organisms. Their main impact on human health is principally through occupational exposure, environmental contamination, and accumulation in food, mainly in vegetables grown on contaminated soil. Heavy metal ions like Lead (Pb(II)), Cadmium (Cd(II)) and Mercury (Hg(II)) enters the human body through multiple routes and gets distributed and stored in almost every organ resulting in the defective function of the organ[1-3].

Absorption, accumulation, storage and transport of toxic metals in living organisms depends on bonding strength with biological ligands in biofluids surrounding the metal ion. One goal of modern chemical research is to elucidate in detail the toxicity of heavy metal ions in human metabolism. The toxicity is due to the substitution of essential metal ion from the corresponding enzymes in biological systems by them. Due to difficulties in investigating the complex biological macromolecules in presence of a variety of chemical compounds, use of model compounds which have similar characteristics is of vital importance.

Lead is a toxic element which affects every organ of the body, especially the bones, teeth, the kidneys, the nervous, cardiovascular, immune and reproductive systems[4] and damage cell structures including DNA and membranes[5,6]. Lead exposure is a risk factor for children with asthma[7]. It also interferes with the normal metabolism of calcium in cells and

causes it to build up within them[8]. Findings of the studies on biochemical and toxicological effects of lead in detail by many workers have indicated deleterious effects to hematopoietic, renal, neurological, reproductive and skeletal systems[9-11].

Cadmium is one of the most deleterious trace heavy metal in both plants and animals. This ion interferes with biological and biochemical processes in both prokaryotic and eukaryotic organisms[12-14]. Cadmium causes iron deficiency by binding to cysteine, glutamate, aspartate, and histidine ligands[15]. It inhibits enzymes that participate in bilirubin conjunction[16]. Cadmium modes of exposure are either through intake of contaminated food (e.g., leafy vegetables, grains, organ meats and crustaceans), drinking contaminated water or by inhalation of polluted air.

Mercury is one of the most toxic heavy metal and its level of contamination in the environment has increased over a thousand fold as a consequence of anthropogenic activities, such as the discharge of wastewaters from chlor-alkali plants, the incineration of coal and metal mining[17]. The toxicity associated with different forms of mercury has been recognized since the description of Minamata disease in Japan[18]. It has negative health effects in human population, highly dependent on fish consumption[19,20].

The reaction of any metal with ligands in the concentrated bioligands background gives binary complexes. Obviously, studies on formation of binary complexes under physiological conditions are important. Hence in recent years researchers show interest in the study of metal ions interaction with various ligands[21-24]. Amino acids are important low molecular weight ligands in humans[25-28] and other biosystems[29,30].

L-proline is an imino acid containing pyrrole type nitrogen rather than the amino nitrogen of the amino acids[31]. It has several significant applications in biological systems[32-34] and it may be used to reduce the level of toxicity of metals like Co(II) in the biological systems[35]. Formation of binary complexes of L-proline with Pb(II), Cd(II) and Hg(II) were reported earlier, using paper electrophoretic technique[36], Ionophoretic technique[37] and PMR[38], pH metry[39] and polarography studies[40].

L-Valine is a nonpolar essential amino acid. It has wide applications in pharmaceutical and food industries[41]. It is a bidentate ligand and formation of its binary complexes of with several metals was reported earlier, using polarographic study[42], batch equilibrium method with cation exchange resin[43], potentiometric titration method[44] and pH metric titration method[45]. Stability constants were evaluated with the computer program SCOGS[46], CHEMEQ[47] and SUPERQUAD[48]. Norvaline (an isomer of L-valine) binary complexes with Co(II) and Cu(II) were also reported using paper electrophoretic[49] and

ionophoretic[50] techniques. The principle investigator of this project selected these ligands to study the effect of imino group and cyclic side chain of proline on the stability of complexes with toxic metal ions Pb(II), Cd(II) and Hg(II).

l, 2–Propanediol (PG) ($C_3H_8O_2$) is a small, hydroxyl substituted hydrocarbon and is also known as propylene glycol. It is a clear, viscous, colourless, odourless liquid with a dielectric constant of 30.2[51]. It is an amphiprotic liquid fully miscible with water. PG is a widely used compound with diverse applications. PG is a structure former and removes water from coordination sphere. The dielectric constant of the medium decreases with increase in mole fraction of PG.

Acetonitrile (AN) is colourless liquid with chemical formula CH_3CN. Other names are cyanomethane, ethane nitrile and methyl cyanide. AN is a polar aprotic[52] solvent with a dipole moment of 3.84 D. AN behaves as a weaker base[53] and as a much weaker acid[54] than water. When a small amount of this cosolvent is added to water, water structure breaks down and results in more basic monomeric water molecules and increased solvation of H^+. Thus AN acts as structure breaker of the solvent due to the formation of water-AN molecular species. PG and AN solvents are selected in this study to maintain the dielectric properties of the medium comparable to those of the physiological fluids and to study the effect of solvent on stability of complexes with Pb(II), Cd(II) and Hg(II).

With the knowledge of preceding discussion, the chemical speciation of L-proline and L-valine with toxic metal ions Pb(II), Cd(II) and Hg(II) in PG- and AN-water mixtures of varying compositions was investigated.

These solutions are expected to mimic the physiological conditions where the concept of the equivalent solution dielectric constant[55] for protein cavities is applicable. The studies carried out on these systems under the present experimental conditions are 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, (iii) the bonding behavior of the protein residues with the metal ion, (iv) the selectivity and sensitivity of various protonated complexes and the relative abundance of free components, (v) specific solute-solvent interactions, and (vi) the effect of electrostatic and non-electrostatic interactions (denaturation) on complex formation in vitro. The species refined and their relative concentrations under the present experimental conditions represent the possible forms of metal ions in the biological fluids.

Further, computer augmented modeling studies were carried out to arrive at the best fit chemical models and to check their validity. The results of these investigations are presented hereunder.

2. Experimental

'A' grade glassware was used throughout the experimentation and standardized as per standard procedure[56].

2.1 Materials

Solutions 0.05 mol L^{-1} of L-proline (SRL, India), L-valine (SRL, India), 0.10 mol L^{-1} Pb(II), Cd(II) and Hg(II) nitrates (Qualigens, India) (all A.R. grade) were prepared in triple-distilled water by maintaining 0.05 mol L^{-1} nitric acid concentration to increase the solubility. Acetonitrile (Qualigens, India) and 1,2-Propanediol (Qualigens, India) were used without further purification. Carbonate free sodium hydroxide (Qualigens, India) pellets were used for the preparation of 0.40 mol L^{-1} solution. Nitric acid (Qualigens, India) of 0.20 mol L^{-1} was prepared. Sodium nitrate (Qualigens, India) of 2.0 mol L^{-1} was prepared to maintain 0.16 mol L^{-1} ionic strength in the titrand. Triple distilled water was used throughout the work. The strengths of acid and alkali were determined using Gran plot method[57]. To assess the errors that might have crept in to the determination of the concentrations, the data were subjected to analysis of variance of one way classification (ANOVA)[58].

2.2 Alkalimetric titrations

Alkalimetric titrations were carried out using Systronics MK-VI digital pH meter with varying composition of PG (0.0-60.0% v/v) and AN (0.0-60.0% v/v) by maintaining an ionic strength of 0.16 mol L^{-1} with sodium nitrate at 303.0±0.1 K. 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. Potassium hydrogen phthalate (0.05 mol L^{-1}) and borax (0.01 mol L^{-1}) solutions were used to calibrate the pH meter. The glass electrode was equilibrated in a well stirred PG-water and AN-water mixtures containing inert electrolyte for several days. At regular intervals strong acid was titrated against alkali to check the complete equilibration of the glass electrode. 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[59]. The calomel electrode was refilled with PG-water and AN-water mixtures of equivalent composition of the titrand[60].

2.3 Modeling Strategy

The computer program SCPHD[61] was used to calculate the correction factor. The best fit chemical model for each system investigated was arrived at using non-linear least-squares computer program, MINIQUAD75[62], which exploits the advantage of constrained least-squares method in the initial refinement and reliable convergence of Marquardt algorithm. For many of the binary metal complexes, the approximate stability constants were obtained by graphical interpolation[63] of formation curve for ML type complexes. In the present investigation, the data was obtained from linear least squares analysis of the pruned formation data and graphical method. During the refinement of binary systems, the correction factor and protonation constants of L-proline and L-valine were fixed.

3. Results and Discussion

3.1 Protonation constants of ligands

Complex formation of metal ions with ligands depends on the existence of different forms of ligands in the solution. To know the different forms of ligands approximate protonation constants of L-proline and L-valine were calculated with the computer program MINIQUAD75. The best fit chemical model for each system investigated was arrived at using non-linear least-squares method. Amino acids present in bio molecules can gain or lose protons depending on the availability of hydrogen ions in the solution. This situation results in the simultaneous existence of a number of protonation-deprotonation equilibria of ligands in solution.

Alkalimetric titration curves (Figure 1) of ligands in PG-water and AN- water mixtures revealed that the acido-basic equilibria of L-proline (LH_2^+ and LH) were active in the pH range 2.0-10.0 and L-valine (LH_2^+ and LH) were active in the pH range 1.5-11.0. L-proline and L-valine have one dissociable proton and one amino group which can associate with a proton. Both the ligands exist as LH_2^+ at low pH and get deprotonated with the formation of LH and L^- successively with increase in pH. The protonation constants values that are calculated are close to the reported values[64-68], after allowing for changes in experimental conditions as well as methods of calculation.

The presence of co-solvent in the medium influences the protonation-deprotonation equilibria in solution due to change in the dielectric constant of the medium. The change in dielectric constant varies the relative contributions of electrostatic and non-electrostatic interactions. When the ionization of an acid gives a net increase of ions, a decrease in the dielectric constant of the solvent should be accompanied by an increase in the protonation constant of a weak acid dissolved in it. The logarithm of step-wise protonation constants (log K) can be calculated by

$$\log K_1 = \log \beta_2 - \log \beta_1$$
$$\log K_2 = \log \beta_1 \qquad \text{for proline}$$
$$\log K_1 = \log \beta_2 - \log \beta_1$$
$$\log K_2 = \log \beta_1 \qquad \text{for valine}$$

where β_1 and β_2, are overall protonation constants. Proline contains carboxylic and imino protons and exists as LH_2^+, LH and L^- in the pH ranges 1.5-3.5, 3.0-10.0 and 9.0-11.0 respectively (Table1). Valine is a bidentate ligand that has one carboxylic and one amino proton. The different forms of valine are XH_2^+, XH and X^- in the pH ranges 1.5-4.0, 3.0-9.5 and 9.0-11.0, respectively (Table 1).

The protonation-deprotonation equilibria of proline and valine shows that they exist in anionic, zwitterionic and cationic forms. These observations can be explained as follows. When proline (Eqs. 1 and 2) and valine (Eqs. 3 and 4) cations are successively deprotonated, the charge of the species is decreased and low dielectric medium favors the protonation reaction, due to dominant electrostatic interactions. Thus, decrease in dielectric constant of the medium should increase the protonation constants.

$$LH_2^+ \rightleftharpoons LH + H^+ \quad (1)$$
$$LH \rightleftharpoons L^- + H^+ \quad (2)$$
$$XH_2^+ \rightleftharpoons XH + H^+ \quad (3)$$
$$XH \rightleftharpoons X^- + H^+ \quad (4)$$

In PG- and AN-water mixtures, the dielectric constant of the medium decreases as the percentage of co-solvent increases from 0.0 to 60.0% v/v. Hence, the stability of protonated species increases with increase in co-solvent content.

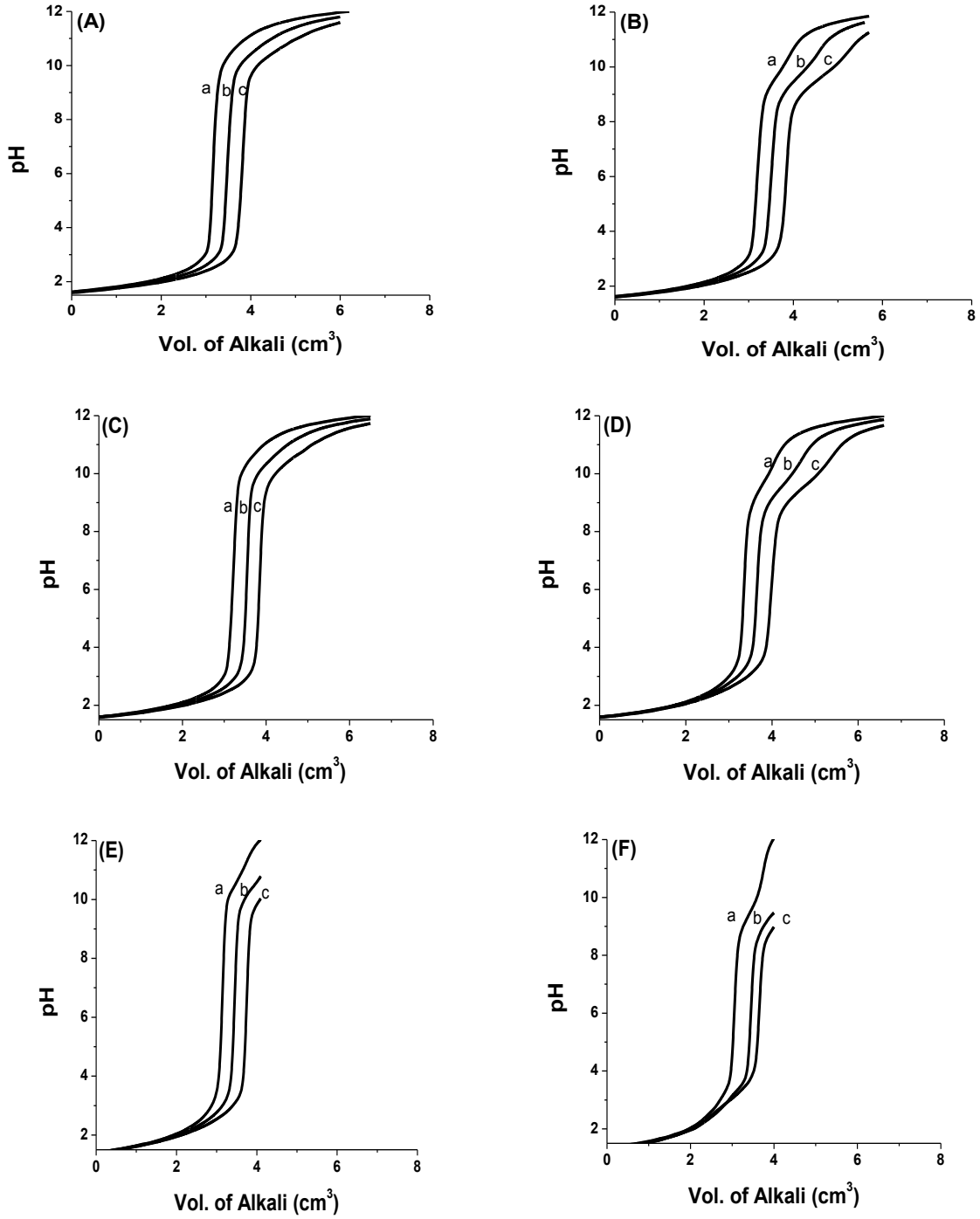

Figure 1: Alkalimetric titration curves for proline and valine in aqueous (A&B), PG-water (C&D) and AN-water (E&F) mixtures respectively.

Table 1: Protonation constants of acido-basic equilibria of L-proline and L-valine in PG-water & AN-water mixtures.

L-Proline			L-Valine		
%v/v PG	**Logβ_1 (SD)**	**Logβ_2 (SD)**	**%v/v PG**	**Logβ_1 (SD)**	**Logβ_2 (SD)**
0.0	10.46(6)	12.41(7)	0.0	9.41(6)	11.70(8)
10.0	10.48(14)	12.48(18)	10.0	9.36(7)	11.84(9)
20.0	10.45(6)	12.69(8)	20.0	9.46(8)	12.13(12)
30.0	10.60(5)	12.88(7)	30.0	9.56(5)	12.25(8)
40.0	10.49(5)	12.86(7)	40.0	9.46(6)	12.22(9)
50.0	10.48(5)	12.97(7)	50.0	9.54(7)	12.44(10)
60.0	10.58(6)	13.27(9)	60.0	9.50(8)	12.66(13)

L-Proline			L-Valine		
%v/v AN	**Logβ_1 (SD)**	**Logβ_2 (SD)**	**%v/v AN**	**Logβ_1 (SD)**	**Logβ_2 (SD)**
0.0	10.46(6)	12.41(7)	0.0	9.41(6)	11.70(8)
10.0	10.72(9)	12.75(12)	10.0	9.71(9)	12.19(13)
20.0	10.77(15)	13.01(19)	20.0	9.72(12)	12.32(19)
30.0	10.88(9)	13.33(12)	30.0	9.74(4)	12.63(7)
40.0	10.92(17)	13.43(20)	40.0	9.78(12)	12.79(19)
50.0	11.04(10)	13.85(14)	50.0	9.91(9)	13.20(18)
60.0	11.13(23)	14.12(29)	60.0	10.02(18)	13.58(32)

3.2 Binary stability constants

Stability constants are well known tools for solution chemists, biochemists and chemists in general to help determine the properties of metal-ligand reactions in water and biological systems. They have important medicinal implication to measure the metal ligand selectivity in terms of relative strength of metal-ligand bonds[69]. Metal coordination complexes have been extensively used in clinical applications as enzyme inhibitors[70], and anti-bacterial[71,72], antiviral[73-75] and as anti-cancerous[76-78] agents. Transition metal ion chelate complexes are also exploited by industry in the large-scale purification of amino acids and a wide range of drug and drug precursors containing an amino carboxylic acid moiety[79,80].

3.3 Binary stability constants of L-proline in PG-water and AN-water mixtures

Alkalimetric titration curves in PG-water and AN-water mixtures revealed that the acido-basic equilibria of L-proline (LH_2^+ and LH) were active in the pH range 2.0-10.0. Based on the active forms of the ligands in this pH range, models containing various numbers and combination of complex species are fed to the MINIQUAD75 along with the alkalimetric titration data. Exhaustive modeling was performed for Cd(II)-L-proline in 30% v/v PG-water mixture and Cd(II)-L-proline in 50% v/v AN-water mixture and the results are given in Table 2 & 3. 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 CdL, CdL_2 and CdLH. This indicates that the final model appropriately fits the experimental data. Such exhaustive modeling was performed for all the systems. The best-fit model is selected using the statistical parameters of the least squares residuals. The final models along with the statistical parameters are given in Table 4 & 5. The closeness of skewness to zero and kurtosis to three indicates that the residuals follow Gaussian distribution and so least squares technique can be applied. The low standard deviation in the model parameters illustrates the adequacy of the models. Perusal of the models indicates that protonated metal complex species are present at lower pH than the unprotonated species.

Table 2. Exhaustive modeling of Cd(II)-proline complexes in 30% v/v PG-water mixture. pH range = 1.8-8.5; Number of points = 89.

S.No.	log β_{mlh} (SD)			$U_{corr}*10^8$	χ^2	Skew ness	Kurtosis	R-factor
	ML	ML_2	MLH					
1	5.01(45)	9.26(41)	---	9.87	132.79	-1.10	3.52	0.0161
2	6.30(13)	---	13.75(07)	1.34	45.96	0.09	4.25	0.0059
3	---	10.48(11)	13.74(08)	1.60	51.05	-0.31	5.14	0.0064
4	5.31(30)	---	---	10.01	123.74	-1.08	3.44	0.0162
5	---	9.43(21)	---	10.14	132.01	-1.06	3.37	0.0163
6	---	---	13.75(16)	10.14	220.70	0.03	20.94	0.0124
7	6.02(16)	10.24(16)	13.75(07)	1.22	42.12	0.00	4.13	0.0056

U_{corr} = U/ (NP-m); m = number of species; NP = number of experimental points;
SD= Standard deviation

Table 3. Exhaustive modeling of Cd(II)-proline complexes in 50% v/v AN-water mixture. pH range = 2.0-9.5; Number of points = 75.

S.No.	log β_{mlh} (SD)			$U_{corr}*10^8$	χ^2	Skew ness	Kurtosis	R-factor
	ML	ML_2	MLH					
1	6.05(59)	11.26(50)		19.55	122.06	-1.04	2.96	0.0264
2	8.44(64)		15.01(51)	9.25	124.55	0.84	8.56	0.0181
3		12.97(30)	15.00(29)	2.98	40.56	-0.08	3.43	0.0103
4	6.65(65)			26.01	134.29	-0.49	2.66	0.0306
5		11.12(300		19.70	112.53	-1.01	2.86	0.0266
6			14.99(78)	46.11	316.26	0.06	12.18	0.0408
7	7.86(35)	13.04(33)	15.00(27)	2.58	42.77	0.20	2.96	0.0095

U_{corr} = U/ (NP-m); m = number of species; NP = number of experimental points;
SD= Standard deviation

Table 4. Parameters of the best-fit chemical models of L-proline complexes of Pb(II), Cd(II) and Hg(II) in PG-water mixtures. (temperature = 303K, ionic strength = 0.16 mol L^{-1})

PG (% V/V)	log β_{mlh} (SD)			NP	$U_{corr}*10^8$	χ^2	Skewness	Kurtosis	R-factor	pH
	ML	ML_2	MLH							
					Pb(II)					
0.0	6.15(15)	10.9(17)	12.07(59)	36	2.16	11.70	-0.09	3.29	0.0130	2.5-9.5
10.0	6.17(10)	10.56(15)	rejected	26	2.02	6.51	0.01	2.43	0.0142	3.3-10.1
20.0	6.24(12)	10.7(15)	11.85(23)	76	1.79	26.67	0.05	3.02	0.0083	2.0-9.8
30.0	6.4(09)	11.07(12)	12.17(14)	77	1.36	36.19	-0.26	3.52	0.0072	2.0-9.8
40.0	6.35(61)	11.01(61)	13.04(94)	26	2.10	5.69	0.12	3.88	0.0146	3.8-10.15
50.0	6.65(14)	11.67(16)	12.99(16)	45	2.04	6.81	0.07	3.37	0.0116	2.5-9.8
60.0	7.45(29)	12.85(45)	rejected	23	15.8	29.67	0.33	5.47	0.0347	3.5-7.8
					Cd(II)					
0.0	5.05(25)	9.36(15)	13.38(12)	95	2.43	26.55	0.02	3.95	0.0084	1.8-9.8
10.0	5.26(19)	9.34(14)	13.44(09)	101	2.34	42.69	0.54	6.05	0.0062	1.5-2.5 & 6.0-9.5
20.0	5.59(79)	9.75(77)	13.56(86)	34	4.32	14.71	-0.23	3.14	0.0177	2.5-9.5
30.0	6.02(16)	10.24(16)	13.75(07)	89	1.22	42.12	0.00	4.13	0.0056	1.8-8.5
40.0	6.88(66)	10.87(87)	13.84(50)	67	16.71	16.19	0.04	2.70	0.0248	2.0-8.5
50.0	7.17(28)	11.72(27)	14.63(25)	94	1.20	20.18	0.48	3.91	0.0056	1.8-8.9
60.0	7.82(70)	13.01(66)	14.84(63)	76	3.34	27.65	-0.07	3.81	0.0110	2.0-9.5
					Hg(II)					
0.0	10.31(03)	18.36(06)	11.57(58)	70	5.04	22.91	0.57	5.06	0.0153	2.0-9.5
10.0	10.16(03)	18.96(04)	12.29(10)	71	1.60	43.49	-0.30	5.43	.0085	2.0-9.5
20.0	10.94(03)	18.82(07)	13.01(07)	72	3.14	10.89	0.35	3.88	0.0117	2.0-9.5
30.0	11.26(04)	18.2(10)	13.41(08)	71	4.25	6.76	0.42	4.19	0.0138	2.0-9.5
40.0	11.36(03)	19.09(06)	13.81(03)	72	1.97	11.78	0.21	5.88	0.0090	2.0-9.5
50.0	11.65(03)	20.59(05)	13.84(05)	86	1.50	21.01	-0.31	4.43	0.0072	1.9-9.5
60.0	12.09(36)	22.77(47)	14.6(51)	82	6.52	41.61	0.32	3.19	0.0139	1.9-3.5 & 6.5-8.5

U_{corr} = U/ (NP-m); m = number of species; NP = number of experimental points;

SD= Standard deviation

Table 5. Parameters of the best-fit chemical models of L-proline complexes of Pb(II), Cd(II) and Hg(II) in AN-water mixtures. (temperature = 303K, ionic strength = 0.16 mol L^{-1})

AN (% V/V)	log β_{mlh} (SD)			NP	$U_{corr}*10^8$	χ^2	Skewness	Kurtosis	R-factor	pH
	ML	ML_2	MLH							
					Pb(II)					
0.0	6.15(15)	10.9(17)	12.07(59)	36	2.16	11.70	-0.09	3.29	0.0130	2.5-9.5
10.0	7.58(15)	12.91(18)	13.46(12)	68	2.33	31.76	-0.15	3.41	0.0098	2.0-9.5
20.0	7.81(56)	13.72(36)	14.16(15)	105	10.96	43.93	0.84	6.66	0.0126	1.5-2.5&6.5-9.5
30.0	8.13(17)	13.74(15)	14.5(07)	101	2.14	33.71	0.65	6.21	0.0055	1.5-2.5&6.5-9.5
40.0	8.31(49)	14.09(49)	14.56(50)	39	1.62	19.55	0.31	3.79	0.0104	2.5-9.5
50.0	8.83(78)	14.81(78)	15.24(78)	44	2.37	10.06	-0.32	3.94	0.0199	2.5-9.6
60.0	9.74(24)	Rejected	15.79(43)	82	16.95	58.39	-0.05	3.21	0.0239	2.0-9.8
					Cd(II)					
0.0	5.05(25)	9.36(15)	13.38(12)	95	2.43	26.55	0.02	3.95	0.0084	1.8-9.8
10.0	6.24(82)	11.19(70)	12.82(42)	68	14.67	33.02	0.71	4.46	0.0266	2.0-9.5
20.0	6.45(88)	11.05(90)	13.04(73)	10	4.00	11.60	0.00	8.02	0.0172	3.8-9.0
30.0	6.31(27)	11.31(27)	Rejected	09	4.28	8.85	-0.01	8.38	0.0178	3.8-8.5
40.0	6.02(73)	11.08(68)	12.83(99)	12	2.07	12.0	0.18	4.51	0.0132	4.0-8.8
50.0	7.86(35)	13.04(33)	15.00(27)	75	2.58	42.77	0.20	2.96	0.0095	2.0-9.5
60.0	8.45(74)	14.07(72)	15.59(69)	73	4.69	17.16	0.00	3.34	0.0130	2.0-9.5
					Hg(II)					
0.0	10.31(03)	18.36(06)	11.57(58)	70	5.04	22.91	0.57	5.06	0.0153	2.0-9.5
10.0	10.71(05)	20.89(08)	13.19(14)	69	1.18	42.0	0.32	4.07	0.0071	2.0-6.5
20.0	11.33(03)	21.06(06)	13.14(13)	95	1.21	15.27	-0.13	3.18	0.0058	1.8-6.5
30.0	11.64(06)	21.44(09)	13.47(21)	69	1.42	12.40	-0.17	3.52	0.0076	2.0-6.5
40.0	12.5(22)	21.96(25)	14.94(25)	91	1.78	4.25	-0.18	3.15	0.0071	1.8-6.5
50.0	13.17(13)	22.87(18)	15.05(20)	132	4.84	7.64	0.00	3.21	0.0088	1.5-5.5
60.0	13.54(04)	24.63(07)	Rejected	120	3.48	6.18	-0.05	3.29	0.0077	1.37-3.5

U_{corr} = U/ (NP-m); m = number of species; NP = number of experimental points;

SD= Standard deviation

3.4 Effect of influential parameters on stability constants

Any variation in the parameters (the concentrations of ingredients) affects the magnitudes of stability constants. Such parameters are called influential parameters. 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, Cd(II)-proline system in 30% v/v PG-water mixture and Cd(II)-proline system in 50% v/v AN-water mixture were studied by introducing errors in the concentrations of ingredients (mineral acid, ligand, metal and alkali). These results (Table 6 & 7) emphasize that errors in the concentrations of acid and alkali affect more than other factors. Increase in the standard deviations with the introduction of errors infers the appropriateness of experimental conditions and correctness of analytical concentrations.

3.5 Effect of solvent

Variation of logarithmic values of stability constants (log β) of metal ions with reciprocal of dielectric constant (1/D) are shown in Figure 2 & 3. The variation of overall stability constant values or change in free energy with co-solvent content depends upon two factors, viz., electrostatic and nonelectrostatic forces. Born's[81] classical treatment 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 reciprocal of the dielectric constant of the medium, which is observed in the present study (Fig. 2).

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. The linear increase indicates the dominance of the structure-forming nature of PG over complexing ability.

Table 6. Effect of errors in influential parameters on the stability constants of Cd(II)-proline binary complexes in 30% v/v PG-water mixture.

Reagent	% Error	log β_{mlh} (SD)		
		ML	ML_2	MLH
	0	6.02(16)	10.24(16)	13.75(07)
Alkali	-5	Rejected	Rejected	Rejected
	-2	4.47(104)	9.14(34)	13.02(13)
	+2	8.81(157)	13.29(157)	15.69(156)
	+5	Rejected	Rejected	Rejected
Acid	-5	Rejected	Rejected	Rejected
	-2	16.80(537)	21.24(537)	23.68(537)
	+2	3.97(112)	8.75(27)	11.53(106)
	+5	Rejected	Rejected	Rejected
Ligand	-5	5.63(14)	9.88(14)	13.26(05)
	-2	5.85(15)	10.08(15)	13.54(06)
	+2	6.24(18)	10.44(19)	13.98(11)
	+5	6.82(37)	10.99(38)	14.58(34)
Metal	-5	6.12(17)	10.42(16)	13.83(09)
	-2	6.06(16)	10.31(16)	13.78(08)
	+2	5.99(15)	10.17(17)	13.71(07)
	+5	5.94(15)	10.07(17)	13.67(07)
Log F	-5	6.22(17)	10.44(18)	13.96(10)
	-2	6.09(16)	10.30(17)	13.81(08)
	+2	5.97(16)	10.18(16)	13.68(07)
	+5	5.86(15)	10.07(15)	13.56(06)

Table 7. Effect of errors in influential parameters on the stability constants of Cd(II)-proline binary complexes in 50% v/v AN-water mixture.

Reagent	% Error	log β_{mlh} (SD)		
		ML	ML_2	MLH
	0	7.86(35)	13.04(33)	15.00(27)
Alkali	-5	4.51(36)	8.52(33)	Rejected
	-2	6.17(78)	10.97(76)	14.03(86)
	+2	Rejected	Rejected	Rejected
	+5	Rejected	Rejected	Rejected
Acid	-5	Rejected	Rejected	Rejected
	-2	Rejected	Rejected	Rejected
	+2	5.85(67)	10.72(64)	13.41(80)
	+5	4.55(44)	8.79(33)	Rejected
Ligand	-5	7.16(21)	12.39(18)	14.30(91)
	-2	7.51(25)	12.71(22)	14.26(14)
	+2	8.88(248)	14.04(248)	16.02(247)
	+5	Rejected	Rejected	Rejected
Metal	-5	8.24(56)	13.50(55)	15.28(50)
	-2	7.99(40)	13.20(38)	15.10(33)
	+2	7.75(31)	12.90(29)	14.93(23)
	+5	7.62(27)	12.72(25)	14.83(18)
Log F	-5	Rejected	Rejected	Rejected
	-2	8.86(224)	14.05(223)	16.01(223)
	+2	7.49(26)	12.66(23)	14.63(9)
	+5	7.13(22)	12.30(19)	14.27(9)

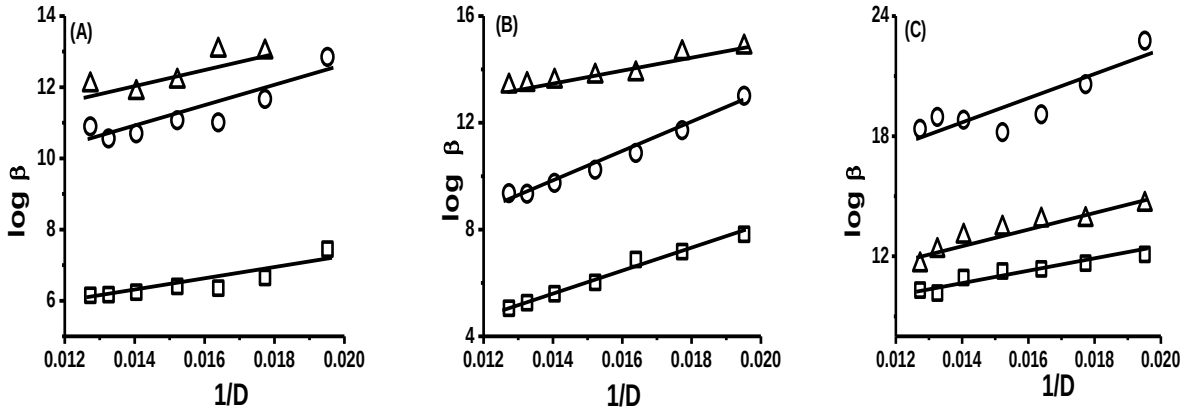

Figure 2. Variation of stability constant values of metal-proline complexes with reciprocal of dielectric constant (1/D) of PG-water mixtures: (A) Pb(II), (B) Cd(II), (C) Hg(II); (□) log β_{ML}, (O) log β_{ML2}, (Δ) log β_{MLH} .

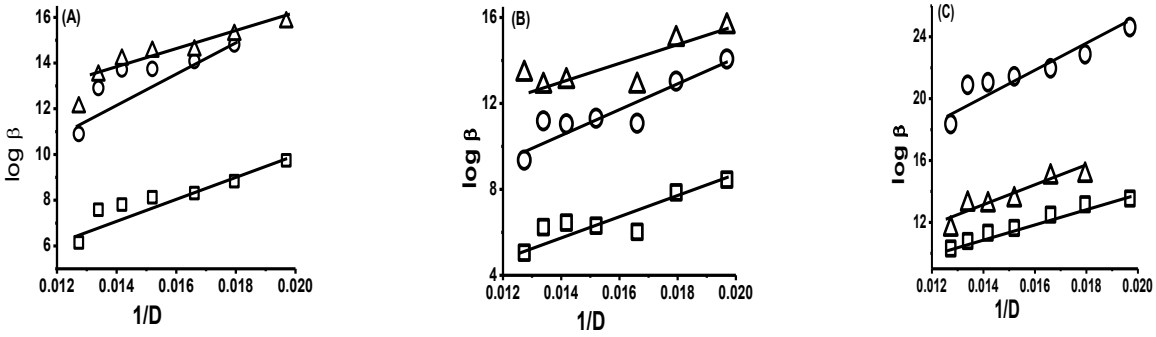

Figure 3. Variation of stability constant values of metal-proline complexes with reciprocal of dielectric constant (1/D) of AN-water mixtures: (A) Pb(II), (B) Cd(II), (C) Hg(II); (□) log β_{ML}, (O) log β_{ML2}, (Δ) log β_{MLH} .

AN is a protophobic, dipolar aprotic and coordinating solvent. It is a structure breaker of water and disrupts the water structure to form AN-water complex of the formula $AN.H_2O$. When small amount of AN is added to water, the water structure breaks down resulting in more basic monomeric water molecules. Hence water molecules compete with the ligands for coordination with metal ions, decreasing the stability of the complexes. But the formation of solvent-water complex decreases the coordinating power of water thereby increases the stability of the complex. The linear increase in the stabilities of the complexes (Fig. 3) with 1/D confirms the dominance of complexing ability of AN with water over its coordinating nature.

3.6 Distribution diagrams

L-proline has one dissociable carboxyl proton and one amino group which can associate with a proton. It exists as LH_2^+, LH and L^- in the pH ranges 1.5-3.5, 3.0-10.0 and 9.0-11.0 respectively. Therefore, the stability constants of metal-ligand complexes of L-proline will depend on the pH range of study. Under the present experimental conditions, the predominant forms of the ligand are LH_2^+ and LH, which limit the probable metal-ligand species to be ML, ML_2, MLH, ML_2H_2 and ML_2H. The species that are refined are ML, MLH and ML_2 for Pb(II), Cd(II) and Hg(II). The formation of various binary complex species in PG-water and AN-water mixtures is represented in the following equilibria:

$$M(II) + LH_2 \rightleftharpoons MLH + H^+ \quad (1)$$

$$MLH \rightleftharpoons ML + H^+ \quad (2)$$

$$M(II) + LH_2 \rightleftharpoons ML + 2H^+ \quad (3) \quad \text{(minor process)}$$

$$M(II) + LH \rightleftharpoons ML + H^+ \quad (4)$$

$$M(II) + 2LH \rightleftharpoons ML_2 + 2H^+ \quad (5)$$

$$MLH + LH \rightleftharpoons ML_2 + 2H^+ \quad (6)$$

$$ML + LH \rightleftharpoons ML_2 + H^+ \quad (7)$$

MLH, ML and ML_2 species are formed in the pH range 5.0-9.5 for Pb(II) and Cd(II) and in the pH range 3.0-7.0 for Hg(II). Their percentages also increase in the same order (Figures 4&5). For all metal-ligand systems, MLH species is formed by the interaction of metal ion with LH_2 (Equilibrium 1), because the percentages of metal ion and LH_2 are decreasing with increasing percentage of MLH. ML species can be formed by the deprotonation of MLH

(Equilibrium 2), the interaction of metal ion with LH_2 (Equilibrium 3) and the interaction of metal ion with LH (Equilibrium 4). Equilibria 2 and 4 are more predominant than equilibrium 3 because the concentrations of metal ion, MLH and LH are decreasing with increasing concentration of ML. Equilibrium 3 is responsible for the initial formation of ML. The simultaneous formation of ML and ML_2 suggests the existence of both the equilibria 4 and 5. ML_2 is formed by the interaction of metal ion with LH (Equilibrium 5), MLH with LH (Equilibrium 6) and ML with LH (Equilibrium 7). The first two equilibria are more appropriate because the concentrations of metal ion, MLH and LH are decreasing where ML_2 species is increasing.

An observation made from the distribution diagrams is that the free metal ion concentration is more in the case of proline complexes of Pb(II) and Cd(II) in PG-water mixtures than in AN-water mixtures. This infers the stronger complexing ability of proline with Hg(II) than Pb(II) and Cd(II) in PG-water mixtures.

3.7 Structures of complexes

Although it is not possible to elucidate or confirm the structures of complex species pH metrically, they can be proposed based on the literature reports and chemical knowledge. In aqueous solutions metal ions are coordinated by six water molecules. Amino acids replace water molecules and form metal-amino acid complexes. Depending upon the nature of the ligands and metal ions and based on the basic chemical knowledge tentative structures of the complexes are proposed as shown in Figure 6 for proline metal complexes. Hence octahedral structures are proposed to the complexes of all the metal ions. Carboxyl oxygen and amino nitrogen of L-proline participate in bonding with metal ions. Amino nitrogen atoms can associate with hydrogen ions in physiological pH ranges. There is often significant competition between hydrogen and metal ion for this second donor site. Hence protonated species are detected in the present study. Proposed structures of ML, MLH and ML_2 are shown in Figure 6.

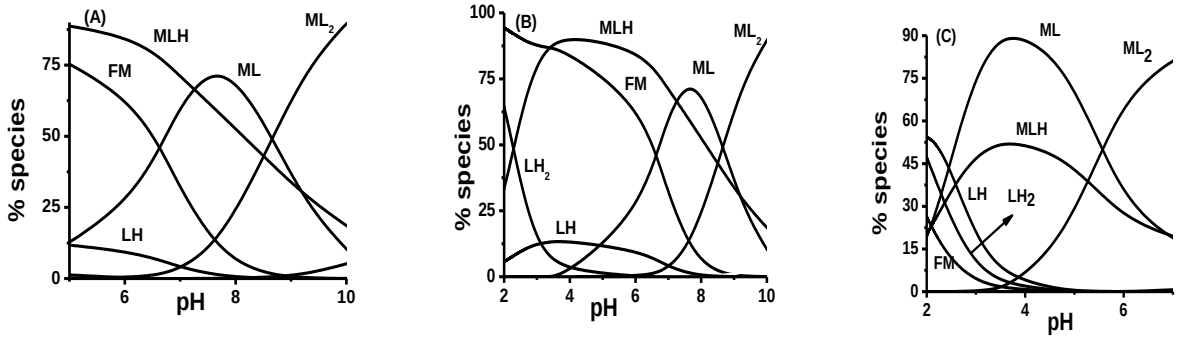

Figure 4. Distribution diagrams of binary complexes of proline in 30% v/v PG-water mixture: (A) Pb(II), (B) Cd(II) and (C) Hg(II).

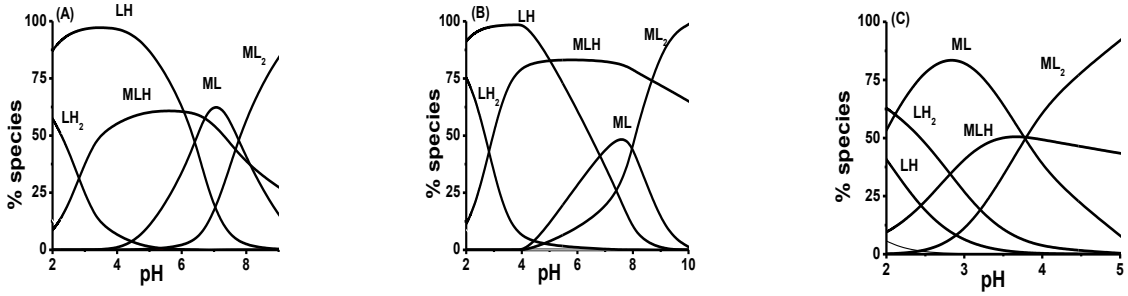

Figure 5. Distribution diagrams of binary complexes of proline in 50% v/v AN-water mixture: (A) Pb(II), (B) Cd(II) and (C) Hg(II).

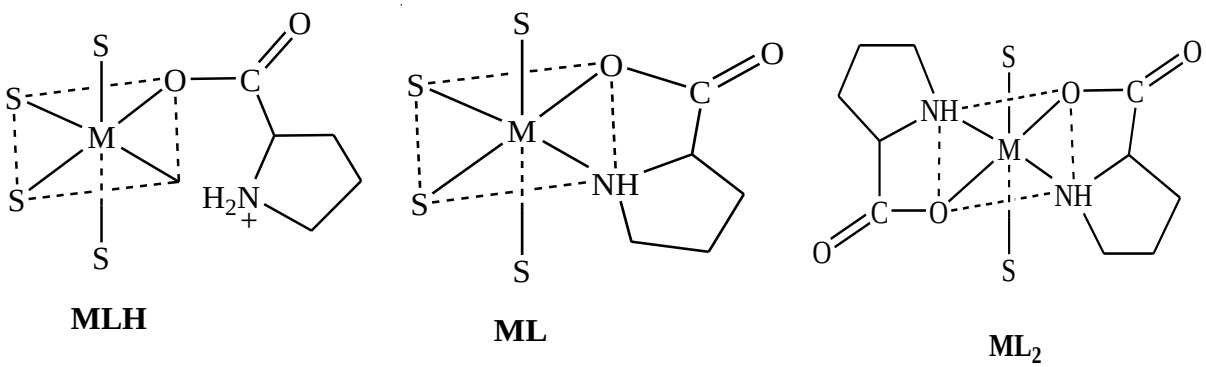

Figure 6. Proposed structures of L-proline complexes, where S is either solvent or water molecules.

4. Binary stability constants of L-valine in PG-water and AN-water mixtures

L-Valine is a bidentate ligand that has one dissociable and one associable protons. The different forms of L-valine are LH_2^+, LH and L^- in the pH ranges 1.5-4.0, 4.0-9.0 and 9.0-11.0, respectively. Based on the active forms of the ligands in this pH range, models containing various numbers and combination of complex species are fed to the MINIQUAD75 along with the alkalimetric titration data. The best-fit model was selected using the statistical parameters of the least squares residuals. The final models along with the statistical parameters are given in Tables 8 & 9. The low standard deviation in the model parameters illustrates the adequacy of the models.

4.1 Effect of influential parameters on stability constants

Any variation in the parameters (the concentrations of ingredients) affects the magnitudes of stability constants. Such parameters are called influential parameters. 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, Cd(II)-valine system in 30% v/v PG-water mixture and Hg(II)-valine system in 40% v/v AN-water mixture was studied by introducing errors in the concentrations of ingredients (mineral acid, ligand, metal and alkali). These results (Tables 10 & 11) emphasize that errors in the concentrations of acid and alkali affect more than other factors. Increase in the standard deviations with the introduction of errors infers the appropriateness of experimental conditions and correctness of analytical concentrations.

4.2 Effect of solvent

Since complex formation can be viewed as a competition between the pure and solvated forms of ligand and the metal ion, the solute-solvent interactions, relative thermodynamic stabilities and kinetic labilities are also expected to play an important role. Different types of electrostatic forces dominate in different ranges of the composition of PG- and AN-water mixtures. With the increase in the percentage of PG and AN from 0.0-60.0% v/v, the dielectric constant of the medium decreases from 78.5 to 51.2 and 50.8, respectively. Thus the variation of stability constants was studied over a range of the dielectric constant from 78.5 to 50.8. It is concluded from these studies that there is considerable increase in the stabilities of proline metal complexes in AN than in PG. But the increase in the case of valine

metal complexes is negligible. This is attributed to the dominanace of complex forming ability of AN with water than the structure forming nature of PG.

The linear trend observed in the present study of valine metal complexes (Figures 7 & 8) in PG-water and AN-water mixtures indicates that electrostatic forces are dominating the equilibrium process under the present experimental conditions.

4.3 Distribution diagrams

The present investigation reveals the existence of ML and ML_2 species for Pb(II), Cd(II) and Hg(II) in PG-water and AN-water mixtures.
The formation of various L-valine complex species are shown in the following equilibria.

$$M(II) + LH_2 \rightleftharpoons ML + 2H^+ \quad (1)$$

$$M(II) + LH \rightleftharpoons ML + H^+ \quad (2)$$

$$M(II) + 2LH \rightleftharpoons ML_2 + 2H^+ \quad (3)$$

$$ML + LH \rightleftharpoons ML_2 + H^+ \quad (4)$$

ML and ML_2 species are formed in the pH range 6.0-9.0 for Pb(II) and Cd(II), 2.0-5.0 for Hg(II) and their percentages increase in the same order (Figures 9 & 10). ML species can be formed by the interaction of metal ion with LH_2 (Equilibrium 1) and the interaction of metal ion with LH (Equilibrium 2). ML_2 species is formed by the interaction of metal ion with LH (Equilibrium 3), and ML with LH (Equilibrium 4).

An observation made from the distribution diagrams is that the free metal ion concentration is more in the case of valine than proline for all the metals in PG- and AN-water media. This infers the stronger complexing ability of proline than valine, eventhough the distinctive cyclic structure of proline's side chain gives proline an exceptional conformational rigidity compared to other amino acids.

4.4 Structures of complexes

Depending upon the nature of the ligands and metal ions and based on the basic chemical knowledge tentative structures of the complexes are proposed as shown in Figure 11. Carboxyl oxygen and amino nitrogen of L-valine are bonded to the metal ions. Octahedral structures are proposed to the complexes of all the metal ions.

Table 8. Parameters of the best-fit chemical models of L-valine complexes of Pb(II), Cd(II) and Hg(II) in PG-water mixtures. (temperature = 303K, ionic strength = 0.16 mol L^{-1})

PG (% V/V)	log β_{mlh} (SD)		NP	$U_{corr}*10^8$	χ^2	Skewness	Kurtosis	R-factor	pH
	ML	ML_2							
				Pb(II)					
0.0	4.65(24)	7.69(33)	37	17.23	34.37	0.24	4.18	0.0407	3.0-9.5
10.0	4.67(13)	7.94(16)	52	4.56	35.08	0.93	6.81	0.0179	2.5-9.5
20.0	4.91(16)	8.73(17)	55	4.89	28.60	-0.01	4.38	0.0181	2.5-9.5
30.0	5.28(19)	9.1(23)	27	7.08	7.99	0.10	5.60	0.0261	3.5-9.2
40.0	5.01(10)	8.81(12)	34	2.42	29.76	0.04	5.31	0.0141	3.0-9.0
50.0	5.68(15)	10.11(18)	51	4.82	28.70	-0.67	3.96	0.0176	2.5-9.0
60.0	6.19(82)	10.93(99)	75	11.97	74.47	1.42	4.96	0.0699	2.5-9.0
				Cd(II)					
0.0	3.38(43)	6.36(26)	84	15.73	13.11	0.80	2.74	0.0249	2.0-9.5
10.0	3.59(15)	6.79(09)	33	1.74	8.56	0.67	4.41	0.0129	3.0-9.5
20.0	3.78(46)	7.33(28)	84	15.36	84.63	-1.08	3.49	0.0243	2.0-9.5
30.0	3.87(39)	7.88(17)	23	6.86	55.17	0.09	6.88	0.0247	3.5-9.0
40.0	4.18(32)	7.88(22)	31	7.86	11.34	0.27	3.33	0.0237	3.0-8.9
50.0	4.54(13)	8.56(09)	52	1.14	40.51	0.80	3.04	0.0078	2.5-8.9
60.0	5.83(84)	9.76(99)	49	4.0	75.86	0.99	4.33	0.0496	3.0-9.5
				Hg(II)					
0.0	8.18(16)	13.68(37)	55	4.53	16.77	0.57	3.42	0.0554	2.5-9.5
10.0	8.77(07)	13.37(15)	52	14.4	6.97	0.09	2.87	0.0311	2.5-9.5
20.0	8.22(14)	16.52(10)	55	8.91	9.88	0.61	4.30	0.0226	2.5-9.0
30.0	8.69(14)	16.44(14)	21	6.42	5.95	0.17	3.30	0.0244	3.0-8.6
40.0	9.46(03)	16.82(08)	50	1.61	8.72	-0.37	2.43	0.0105	2.5-9.5
50.0	9.93(03)	17.96(09)	114	3.76	17.44	2.26	4.54	0.0095	1.8-8.8
60.0	10.39(04)	19.64(06)	86	4.80	13.26	1.08	5.18	0.0135	2.0-8.0

U_{corr} = U/ (NP-m); m = number of species; NP = number of experimental points; SD= Standard deviation

Table 9. Parameters of the best-fit chemical models of L-valine complexes of Pb(II), Cd(II) and Hg(II) in AN-water mixtures. (temperature = 303K, ionic strength = 0.16 mol L^{-1})

AN (% V/V)	log β_{mlh} (SD)		NP	$U_{corr}*10^8$	χ^2	Skewness	Kurtosis	R-factor	pH
	ML	ML_2							
				Pb(II)					
0.0	4.65(24)	7.69(33)	37	17.23	34.37	0.24	4.18	0.0407	3.0-9.5
10.0	5.51(42)	9.57(45)	39	24.27	112.4	0.57	6.07	0.0438	3.0-9.5
20.0	5.8(54)	10.33(57)	42	40.40	58.63	0.66	5.42	0.0546	3.0-9.5
30.0	5.96(67)	11.04(61)	49	65.36	54.74	-0.72	3.83	0.0712	2.5-9.5
40.0	6.15(67)	11.11(74)	45	80.53	102.57	-1.13	4.04	0.0756	2.5-9.0
50.0	6.77(77)	11.97(101)	22	115.7	92.79	-1.30	6.27	0.0987	3.0-8.0
60.0	8.45(16)	13.0(13)	58	37.46	39.43	-0.74	2.49	0.0478	2.5-9.5
				Cd(II)					
0.0	3.38(43)	6.36(26)	84	15.73	13.11	0.80	2.74	0.0249	2.0-9.5
10.0	4.7(30)	7.77(31)	24	4.84	16.67	0.15	6.88	0.0206	3.5-9.5
20.0	5.14(31)	8.95(29)	21	3.88	18.90	-0.11	3.04	0.0167	3.0-8.5
30.0	5.08(124)	9.65(87)	34	82.38	74.31	-1.0	3.45	0.0743	2.5-8.5
40.0	5.332(86)	9.92(72)	22	52.70	72.67	-0.90	4.21	0.0628	3.0-8.0
50.0	5.86(84)	11.17(61)	15	40.35	58.28	-1.02	9.15	0.0587	3.5-7.9
60.0	8.46(11)	12.99(28)	48	4.88	38.78	-0.54	2.99	0.0154	2.5-8.0
				Hg(II)					
0.0	8.18(16)	13.68(37)	55	4.53	16.77	0.57	3.42	0.0554	2.5-9.5
10.0	8.12(46)	17.89(05)	46	4.85	11.10	0.23	2.78	0.0174	2.5-6.5
20.0	9.2(11)	18.31(09)	85	11.57	40.06	1.46	7.26	0.0195	2.0-6.5
30.0	10.98(09)	19.91(08)	34	0.54	4.67	-1.11	5.08	0.0061	2.5-6.5
40.0	11.52(05)	20.37(07)	68	1.83	29.57	-0.13	2.80	0.0087	2.0-6.5
50.0	12.28(08)	21.22(09)	66	1.24	6.69	-0.33	2.95	0.0071	2.0-6.5
60.0	13.47(23)	23.39(23)	40	0.79	19.07	1.69	6.62	0.0073	2.5-6.5

U_{corr} = U/ (NP-m); m = number of species; NP = number of experimental points; SD= Standard deviation

Table 10. Effect of errors in influential parameters on the stability constants of Cd(II)-valine binary complexes in 30% v/v PG-water mixture.

Reagent	% Error	log β_{mlh} (SD)	
		ML	ML_2
	0	3.87(39)	7.88(17)
Alkali	-5	7.67(36)	12.46(43)
	-2	4.40(31)	8.74(16)
	+2	3.43(69)	7.12(28)
	+5	2.36(394)	6.07(51)
Acid	-5	2.44(286)	5.83(63)
	-2	3.47(62)	7.05(28)
	+2	4.35(36)	8.77(17)
	+5	Rejected	9.90(37)
Ligand	-5	3.84(46)	7.96(17)
	-2	3.86(41)	7.91(18)
	+2	3.89(38)	7.85(18)
	+5	3.90(36)	7.81(18)
Metal	-5	3.81(50)	7.97(18)
	-2	3.85(43)	7.92(18)
	+2	3.89(36)	7.85(17)
	+5	3.92(33)	7.80(17)
Log F	-5	3.87(39)	7.88(18)
	-2	3.87(39)	7.88(17)
	+2	3.87(39)	7.88(17)
	+5	3.87(39)	7.88(18)

Table 11. Effect of errors in influential parameters on the stability constants of Hg(II)-valine binary complexes in 40% v/v AN-water mixture

Reagent	% Error	log β_{mlh} (SD)	
		ML	ML_2
	0	11.52(05)	20.37(07)
Alkali	-5	10.89(5)	Rejected
	-2	11.27(4)	19.42(10)
	+2	11.77(7)	21.15(8)
	+5	12.02(18)	22.04(16)
Acid	-5	14.49(57)	24.64(57)
	-2	12.01(12)	21.41(12)
	+2	11.17(4)	19.45(10)
	+5	10.60(4)	17.26(50)
Ligand	-5	11.34(3)	20.09(6)
	-2	11.45(4)	20.25(6)
	+2	11.61(6)	20.50(8)
	+5	11.75(9)	20.73(10)
Metal	-5	11.78(9)	21.01(9)
	-2	11.57(6)	20.49(7)
	+2	11.48(5)	20.26(7)
	+5	11.42(4)	20.10(7)
Log F	-5	11.70(7)	20.61(9)
	-2	11.59(6)	20.46(7)
	+2	11.46(5)	20.29(7)
	+5	11.37(4)	20.17(6)

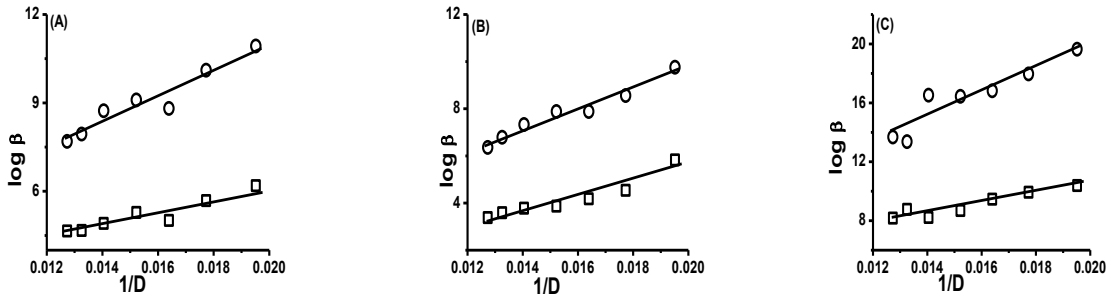

Figure 7. Variation of stability constant values of metal-valine complexes with reciprocal of dielectric constant (1/D) of PG-water mixtures: (A) Pb(II), (B) Cd(II), (C) Hg(II); (□) log β_{ML}, (O) log β_{ML2} .

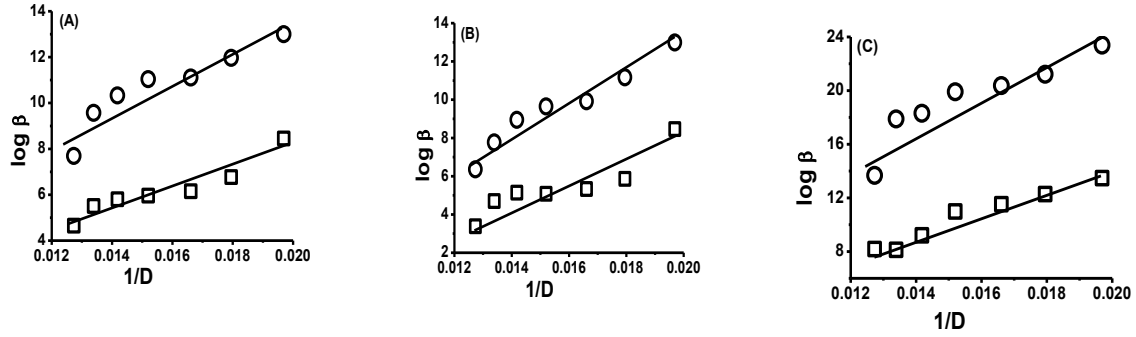

Figure 8. Variation of stability constant values of metal-valine complexes with reciprocal of dielectric constant (1/D) of AN-water mixtures: (A) Pb(II), (B) Cd(II), (C) Hg(II); (□) log β_{ML}, (O) log β_{ML2} .

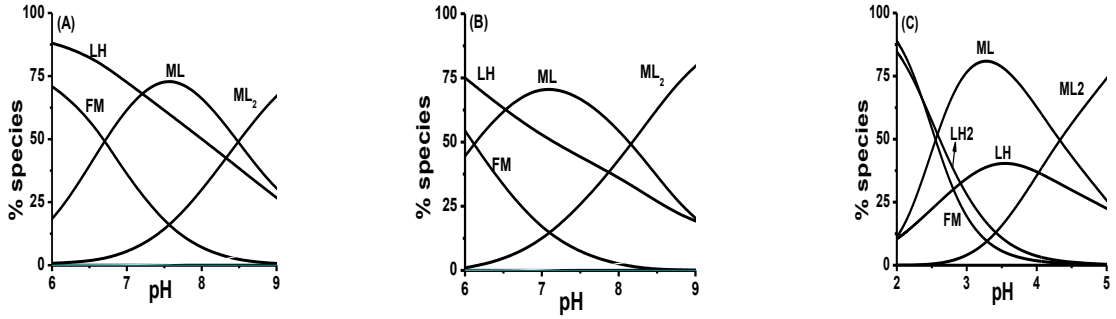

Figure 9. Distribution diagrams of binary complexes of valine in 50% v/v PG-water mixture: (A) Pb(II), (B) Cd(II) and (C) Hg(II).

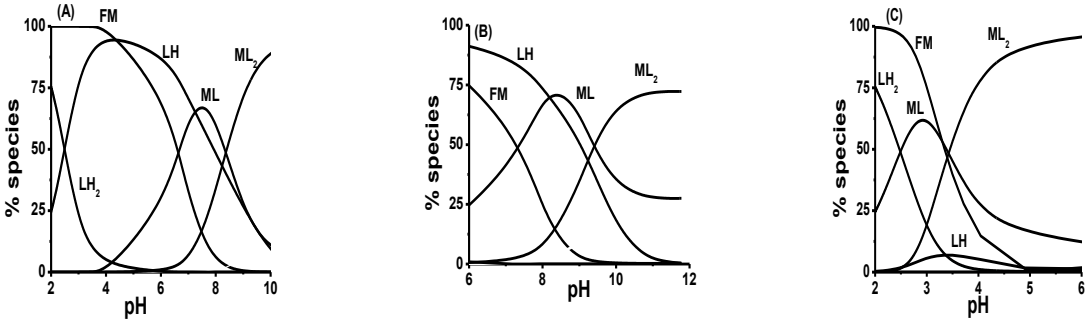

Figure 10. Distribution diagrams of binary complexes of valine in 10% v/v AN-water mixture: (A) Pb(II), (B) Cd(II) and (C) Hg(II).

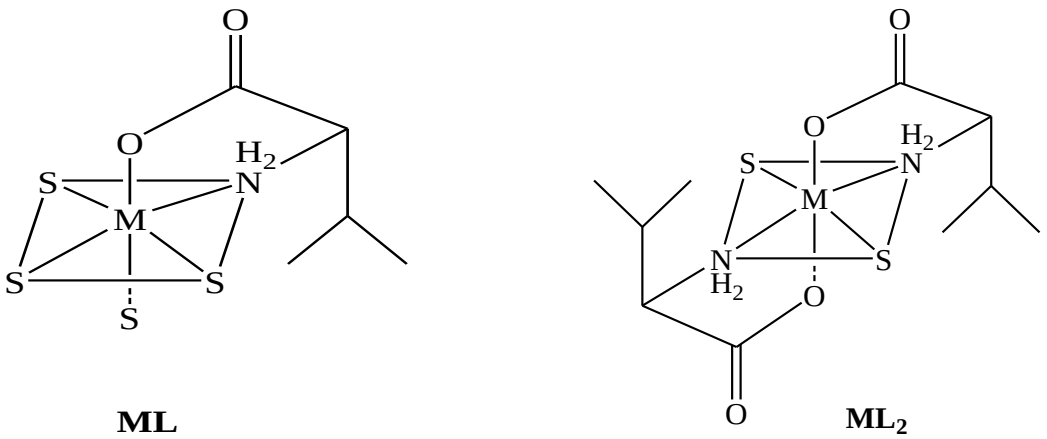

Figure 11. Proposed structures of L-valine complexes, where S is either solvent or water molecule.

5. Conclusions

Pollution and toxicity of heavy metal ions are of latest concern. The toxic action of most of the heavy metals stems from the fact that they are capable of forming strong bonds with metabolically active groups in a living system. The toxic effect of the heavy metal ions is due to the replacement of essential metal ion from the corresponding enzyme in living systems. Toxic heavy metal ions inhibit the metabolic processes, sometimes being fatal to the organism. Studies of the interaction of the heavy metal ions with the bioligands and others as models in vitro studies throw light on the factors contributing to adverse effects of the metal ions in metabolic processes and also help in discovering antidotes. These studies are involved in determining different forms of the toxic metal ions and their speciation.

Speciation has implications for various aspects of medicine and toxicology like dental care, total parenteral nutrition. Knowledge of speciation 1) saves a great deal of time, money and usage of equipment, 2) helps in understanding the complex interactions between dissolved, particulate, sedimentary and biological components, and 3) enhances understanding on the absorption of metal ions from dietary materials which is greatly influenced by the presence of other metal ions and complexing agents.

Better computing and analytical methods led to an understanding of the speciation underlying many chemical reactions, with interesting conclusions in medicine, industry and in environmental studies. Hence, toxic metal ions Pb(II), Cd(II) ad Hg(II) were selected for speciation study by considering L-proline and L-valine as bioligands. The study is carried out in mixtures of i) propylene glycol (PG)-water and ii) Acetonitrile (AN)-water to mimic the nature of the active sites in biological molecules.

The aim of the present study was to probe into the nature of interactions and speciation of L-proline and L-valine complexes with Pb(II), Cd(II) and Hg(II) in PG-water and AN-water mixtures. Based on the experimental results the following conclusions are drawn.

5.1 Protonation equilibria of proline and valine

1. A perusal of the titration curves reveals that the acido-basic equilibria are active in the pH range 1.5-10.5 for proline and 1.5-11.0 for valine.

2. Proline has one dissociable carboxyl proton and one imino group which can associate with a proton. It exists as LH_2^+ at low pH and gets deprotonated with the formation of LH and L^-, successively, with increase in pH.
3. Valine has one dissociable carboxyl proton and one amino group which can associate with a proton. It exists as XH_2^+ at low pH and gets deprotonated with the formation of XH and X^-, successively, with increase in pH.

5.2 Metal-ligand complex equilibria

1. Most of the literature studies reports ML and ML_2 species for proline and valine. But the present study reports protonated species (MLH) also. Protonated complexes are formed at lower pH and unprotonated complexes at higher pH in both PG- and AN-water media for proline.
2. From the exhaustive modeling studies it is concluded that as the number of species is increased, the models give better statistical values which denote the adequacy of the models. This indicates that the final models appropriately portray the experimental data.
3. The binary species refined for Pb(II), Cd(II) and Hg(II) are MLH, ML and ML_2, with proline and ML and ML_2 with valine in both PG- and AN-water media. These models are validated by statistical treatment of the data.
4. An observation made from the distribution diagrams is that the free metal ion concentration is more in the case of valine than proline for Pb(II) and Cd(II) in PG- and AN-water media. This infers the stronger complexing ability of proline than valine, eventhough the distinctive cyclic structure of proline's side chain gives proline an exceptional conformational rigidity compared to other amino acids.
5. The order of the effect of the concentration of the ingredients influencing the magnitude of the stability constants due to the incorporation of errors in their concentrations is, acid > alkali > ligand > metal ions > log F. The introduction of errors adversely affects the statistical parameters like the standard deviation, skewness, kurtosis etc. This indicates that the present experimental data give better models after incorporating the corrections compared with the data with errors, supporting the acceptability of the present experimental results.
6. Formation of the same species in both the media by a ligand indicates that the solvent affected the stability of the complexes only but not the formation of the species. This indicated that the formation of the species depends only on the nature of central ion and the ligand but not on the solvent.

7. The linear variation of stability constants as a function of reciprocal of the dielectric constant of these media indicates the dominance of the electrostatic forces over the non-electrostatic forces.
8. PG is an amphiprotic coordinating solvent with structure forming nature. So it removes water from the coordination sphere of the metal ions making them more reactive towards the approaching ligands. Hence the stability of the complexes is expected to increase.
9. AN is a protophobic, dipolar aprotic and coordinating solvent. It is a structure breaker of water and disrupts the water structure to form AN-water complex of the formula $AN.H_2O$. The formation of solvent-water complex decreases the coordinating power of water thereby increases the stability of the complex.
10. It is concluded from these studies that there is considerable increase in the stabilities of valine metal complexes in AN than in PG. But the increase in the case of proline metal complexes is negligible. This is attributed to the dominanace of complex forming ability of AN with water than the structure forming nature of PG.
11. The stability constants of binary complexes are found to follow the trend Cd(II) < Pb(II) << Hg(II) wih proline and Pb(II) < Cd(II) << Hg(II) with valine in both media.

6. Suggestions for future work

1. Speciation of ternary complexes of Pb(II), Cd(II) and Hg(II) in 0.0-60.0% v/v 1, 2-propanediol-water mixtures with L-proline as primary ligand and L-valine as secondary ligand.

2. Speciation of ternary complexes of Pb(II), Cd(II) and Hg(II) in 0.0-60.0% v/v Acetonitrile-water mixtures with L-proline as primary ligand and L-valine as secondary ligand can be pursued.

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