

## CHEMICAL SPECIATION OF Pb(II), Cd(II), Hg(II), Co(II), Ni(II), Cu(II) AND Zn(II) BINARY COMPLEXES OF L-METHIONINE IN 1,2-PROPANEDIOL-WATER MIXTURES

M. Padma Latha, V. Maheswara Rao, T. Siva Rao and G. Nageswara Rao\*

School of Chemistry, Andhra University, Visakhapatnam-530 003, India

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**ABSTRACT.** Chemical speciation of Pb(II), Cd(II), Hg(II), Co(II), Ni(II), Cu(II) and Zn(II) complexes of L-methionine in 0.0-60 % v/v 1,2-propanediol-water mixtures maintaining an ionic strength of 0.16 M at 303 K has been studied pH metrically. The active forms of ligand are  $\text{LH}_2^+$ , LH and L<sup>-</sup>. The predominant species detected are ML, MLH,  $\text{ML}_2$ ,  $\text{ML}_2\text{H}$ ,  $\text{ML}_2\text{H}_2$  and MLOH. Models containing different numbers of species were refined by using the computer program MINIQUAD 75. 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.

**KEY WORDS:** Complex equilibria, Chemical speciation, L-Methionine, 1,2-Propanediol, Metals

### INTRODUCTION

Speciation analysis, the determination of the concentrations of separate and unique atomic and molecular forms of an element instead of its total concentration in a sample is important in human biology, nutrition, toxicology and in clinical practice. However, concentrations of essential, endogenous elements are frequently controlled homeostatically, and instances of pathology arising from altered speciation of these elements are relatively rare. When a major ligand is deficient it is the total concentration, rather than its distribution among species, that is of most diagnostic significance. On the other hand, speciation profoundly influences both the toxicity and bioavailability of an element.

The speciation study of toxic and essential 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.

The protonation constants of L-methionine in urea-water [1] and 1,2-propanediol (PG)-water [2] media and stability constants of Pb(II), Cd(II), Hg(II), Co(II), Ni(II), Cu(II) and Zn(II) complexes of L-methionine in urea-water mixtures were reported earlier [3]. The ternary complexes of Pb(II), Cd(II), Hg(II) with L-glutamic acid and L-methionine in urea-water mixtures were also reported [4]. The mixed ligand complexes of  $\beta$ -picoline, lysine and methionine with lead have been studied at constant ionic strength [5]. Based on the importance of methionine (Met) and its involvement in various physiological reactions, speciation studies of Met with different metals have been undertaken.

### EXPERIMENTAL

#### Materials

1,2-Propanediol (Merck, Mumbai) was used as received. Aqueous solutions of L-methionine and sodium nitrate (E-Merck, Germany) were prepared. Metal solutions of Pb(II), Cd(II), Hg(II)

\*Corresponding author. E-mail: gollapallinr@yahoo.com

nitrate and Co(II), Ni(II), Cu(II), Zn(II) chlorides were prepared. To increase the solubility of Met and to suppress the hydrolysis of metal salts, the mineral acid concentration in the above solutions was maintained at 0.05 M. To assess the errors that might have crept into the determination of the concentrations, the data have been subjected to analysis of variance of one way classification (ANOVA). The strength (concentration) of alkali has been determined using the Gran plot [6, 7] method.

### *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 % v/v) maintaining an ionic strength of 0.16 M with sodium nitrate at  $303.0 \pm 0.1$  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 [8].

The emf of the cell may be expressed by the equation  $E = K + (RT/F) \ln a_{H^+}$  or  $E = K + 0.0591pH$  at 25 °C, where K is a constant partly dependent upon the nature of the glass used for making the membrane. The value of K may vary slightly with time, and it is related to the existence of an asymmetry potential [9] in a glass electrode. Owing to the asymmetry potential, if a glass electrode is inserted into a test solution which is identical with the internal hydrochloric acid solution, the electrode shall have a small potential which is found to vary with time. Hence, glass electrode is standardized frequently using a buffer of known hydrogen activity.

### *Procedure*

For the determination of stability constants of metal-ligand binary species, initially titrations of strong acid with alkali were carried out at regular intervals to check whether complete equilibration was achieved. Then the calomel electrode was refilled with 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, 1:3.5, 1:5) of metal-to-ligand were carried out with 0.4 M sodium hydroxide. Other experimental details are given elsewhere [10].

### *Modeling strategy*

The computer program SCPHD [11] were used to calculate the correction factor. By using the pH-metric titration data, the binary stability constants were calculated with the computer program MINQUAD75 [12], 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 methionine are fixed. The variation of stability constants with the dielectric constant of the medium was analyzed on electrostatic grounds on the basis of solute-solute and solute-solvent interactions.

## RESULTS AND DISCUSSION

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 Tables 1 and 2. A very low standard deviation in  $\log \beta$  values indicates the precision of these parameters. The small values of  $U_{\text{corr}}$  (the sum of squares of deviations in 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. 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 and very few are nearer to mesokurtic patterns. The values of skewness recorded in the tables are between -1.14 and 2.26. 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 mixtures.

Table 1. Best fit chemical models of Pb(II), Cd(II) and Hg(II) - Methionine complexes in PG - water mixtures (temperature = 303 K, ionic strength = 0.16 M).

% v/v PG	$\log \beta$			NP	$U_{\text{corr}}$	Skewness	$\chi^2$	R-Factor	Kurtosis	pH-Range
	ML	ML <sub>2</sub>	MLOH							
Pb(II)										
00.00	7.79(8)	-	-	47	0.97	-0.43	25.5	0.005	4.3	1.9-2.5
10.00	7.76(23)	-	-	46	3.12	0.88	39.9	0.008	5.9	1.9-2.5
20.00	8.91(8)	-	-	134	0.02	-0.61	84.5	0.015	3.0	1.6-2.2
30.00	8.97(5)	-	-	129	5.61	-0.66	82.3	0.008	3.8	1.6-2.2
40.00	9.95(5)	-	-	128	16.47	-0.19	21.3	0.014	2.8	1.6-2.2
50.00	9.07(4)	-	-	121	2.06	-0.44	106.4	0.005	3.9	1.6-2.2
60.00	9.59(3)	-	-	78	1.52	-0.45	68.0	0.004	5.0	1.6-1.9
Cd(II)										
00.00	3.47(8)	6.73(5)	-	26	0.98	1.66	3.6	0.010	6.7	6.0-9.2
10.00	3.55(8)	7.13(4)	-	21	0.61	1.13	6.0	0.008	4.5	5.0-9.0
20.00	3.74(7)	7.01(5)	-	21	0.79	0.14	17.6	0.009	1.6	5.0-9.0
30.00	3.82(4)	7.22(3)	-	20	0.26	0.01	8.3	0.005	2.6	5.0-9.0
40.00	3.94(4)	7.48(3)	-	21	0.23	1.33	15.9	0.005	4.4	5.0-9.0
50.00	4.02(9)	7.67(6)	-	21	1.37	0.76	6.5	0.012	3.0	5.0-9.0
60.00	4.01(9)	7.48(7)	-	23	1.72	-0.15	13.2	0.014	8.5	5.0-9.0
Hg(II)										
00.00	8.61(6)	14.30(18)	2.40(64)	41	2.68	-0.38	8.5	0.016	2.1	3.3-6.70
10.00	8.36(6)	13.92(12)	1.55(13)	70	1.66	-0.42	25.1	0.015	2.2	3.0-10.0
20.00	8.08(8)	13.63(18)	1.39(20)	36	2.29	-0.53	9.6	0.016	3.4	3.0-10.0
30.00	8.26(7)	13.84(17)	1.26(19)	34	1.63	-0.60	7.5	0.013	2.3	3.0-10.0
40.00	.42(11)	14.00(23)	1.32(27)	35	3.12	-0.66	11.7	0.018	2.3	3.0-10.0
50.00	8.58(9)	14.19(18)	1.76(19)	38	1.98	-0.41	6.2	0.015	3.2	3.0-10.0
60.00	.75(14)	14.31(29)	1.68(34)	31	4.20	-0.62	17.7	0.019	2.9	3.0-10.0

$U_{\text{corr}} = U/(NP-m) \times 10^8$ , where, m = number of species; NP = number of experimental points.

Table 2. Best-fit chemical models of Co(II), Ni(II), Cu(II) and Zn(II)-methionine complexes in PG-water mixtures (temperature = 303 K, ionic strength = 0.16 M).

% v/v PG	Log $\beta_{mlh}$ (SD)					NP	U <sub>corr</sub>	Skew-ness	$\chi^2$	R-Factor	Kurtosis	pH-Range
	ML	MLH	ML <sub>2</sub>	ML <sub>2</sub> H	ML <sub>2</sub> H <sub>2</sub>							
Co(II)												
00.00	3.77(19)	-	7.36(5)	14.52(50)	-	25	0.67	1.82	16.4	0.008	6.8	4.5-9.5
10.00	3.95(15)	-	7.56(6)	14.79(58)	-	21	0.40	1.55	9.3	0.007	6.2	7.0-9.5
20.00	3.92(36)	-	7.73(11)	14.89(78)	-	23	1.69	2.26	11.1	0.014	9.7	6.5-9.5
30.00	4.13(14)	-	7.99(4)	15.03(35)	-	12	0.19	0.59	9.3	0.004	3.9	6.0-8.0
40.00	4.17(28)	-	8.21(9)	15.44(44)	-	21	0.75	1.39	4.9	0.009	5.0	6.7-9.5
50.00	4.35(27)	-	8.38(9)	15.17(86)	-	23	1.42	1.88	13.0	0.013	6.4	6.0-9.3
60.00	4.36(36)	-	8.54(9)	15.60(46)	-	12	0.86	-0.17	9.3	0.008	1.8	6.0-7.9
Ni(II)												
00.00	5.03(63)	-	9.72(29)	15.94(91)	-	22	3.98	2.02	26.7	0.022	5.6	6.0-9.5
10.00	5.49(74)	-	10.56(13)	17.23(43)	23.20(7)	67	3.25	-0.32	28.1	0.012	4.4	2.0-8.4
20.00	5.25(49)	-	9.99(16)	15.95(57)	21.33(31)	66	4.42	-0.98	68.8	0.014	6.0	2.0-8.4
30.00	5.54(11)	-	10.70(21)	17.20(68)	22.85(10)	69	7.19	-0.48	23.8	0.017	4.1	2.0-8.4
40.00	5.92(90)	-	11.15(21)	17.41(74)	22.99(9)	69	7.10	-0.68	46.9	0.017	5.4	2.0-8.4
50.00	5.66(58)	-	10.76(15)	16.90(69)	22.25(11)	74	3.80	-1.13	47.0	0.012	5.1	2.0-8.4
60.00	5.66(11)	-	10.43(12)	-	-	78	2.79	-0.80	73.3	0.010	5.2	2.0-8.4
Cu(II)												
00.00	7.71(3)	-	13.98(7)	-	-	68	0.68	-1.05	61.0	0.005	5.8	2.0-6.0
10.00	8.06(2)	10.89(6)	14.48(8)	-	-	64	0.43	0.09	35.3	0.004	4.0	2.0-4.7
20.00	7.62(9)	10.76(14)	14.47(14)	-	-	83	3.49	-0.56	34.2	0.010	3.8	1.8-4.7
30.00	8.03(4)	11.06(9)	14.65(11)	-	-	64	1.22	-0.53	56.3	0.007	6.2	2.0-4.7
40.00	8.90(6)	12.0(9)	15.79(12)	-	-	65	1.90	-0.02	4.9	0.008	3.3	2.0-4.7
50.00	8.93(4)	11.85(6)	15.93(8)	-	-	67	1.14	-0.28	5.4	0.006	3.0	2.0-4.7
60.00	9.05(5)	12.0(8)	16.11(12)	-	-	67	2.10	-1.14	6.1	0.009	2.6	2.0-4.7
Zn(II)												
00.00	4.26(14)	-	8.12(8)	-	-	13	2.24	1.03	20.3	0.015	4.7	6.0-8.0
10.00	4.28(5)	-	8.35(3)	-	-	18	0.35	0.47	1.4	0.006	3.2	5.0-8.5
20.00	4.36(6)	-	8.39(4)	-	-	18	0.43	0.19	3.5	0.007	2.8	5.0-8.5
30.00	4.47(9)	-	8.53(6)	-	-	18	1.18	-0.05	14.4	0.011	10.6	5.0-8.5
40.00	4.62(4)	-	8.84(3)	-	-	18	0.25	0.11	0.8	0.005	2.8	5.0-8.5
50.00	4.84(6)	-	9.19(4)	-	-	18	0.54	0.55	2.9	0.008	3.4	5.0-8.5
60.00	4.74(8)	-	8.80(7)	-	-	18	1.13	-0.22	9.7	0.011	5.6	5.0-8.5

The present results are comparable with those reported in the literature. Some typical results reported in the literature for Met are compared with the present values in Table 3.

Table 3. Comparison of the results of present study with the binary stability constants of Met complexes reported in the literature.

Metal ion	Species	Present value	Literature value	Reference
Cd(II)	110	4.33	4.30	13
Ni(II)	110	5.54	5.60	13
	120	10.70	9.71	14
Cu(II)	110	8.03	8.23	13
	120	14.65	14.29	14

*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 [2] like concentrations of alkali, mineral acid, ligand and metal (Table 4). 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.

Table 4. Effect of errors in influential parameters on the metal-methionine complex stability constants in 30 % (v/v) PG-water mixture.

Ingredient	% Error	Log $\beta$ (SD)		Ingredient	% Error	Log $\beta$ (SD)	
		110	120			110	120
Cd(II)				Zn(II)			
	0	3.82(4)	7.22(3)		0	4.47(9)	8.53(6)
Alkali	-5	2.71(20)	Rejected	Alkali	-5	3.55(36)	5.77(12)
	-2	3.52(11)	6.26(11)		-2	4.17(17)	7.63(17)
	+2	4.08(16)	8.03(9)		+2	4.79(12)	9.30(7)
	+5	3.46(486)	9.04(22)		+5	5.18(61)	10.39(25)
Acid	-5	4.17(92)	9.01(21)	Acid	-5	5.43(43)	10.42(26)
	-2	4.13(13)	7.99(8)		-2	4.82(9)	9.27(7)
	+2	3.51(11)	6.38(9)		+2	4.16(18)	7.72(16)
	+5	2.94(19)	Rejected		+5	3.61(37)	6.26(62)
Ligand	-5	3.78(5)	7.34(3)	Ligand	-5	4.47(9)	8.61(6)
	-2	3.80(4)	7.27(3)		-2	4.47(8)	8.56(6)
	+2	3.83(38)	7.17(3)		+2	4.47(8)	8.50(6)
	+5	3.85(4)	7.11(3)		+5	4.47(8)	8.45(6)
Metal	-5	3.79(5)	7.33(3)	Metal	-5	4.44(9)	8.63(6)
	-2	3.81(4)	7.27(3)		-2	4.46(9)	8.57(6)
	+2	3.83(4)	7.18(3)		+2	4.49(8)	8.48(6)
	+5	3.84(3)	7.12(3)		+5	4.50(8)	8.41(7)

*Effect of solvent*

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 variation of overall stability constant values or change in free energy with co-solvent content depends upon two factors, viz., electrostatic and non-electrostatic. Born's [15] 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  $\beta$  values should vary linearly as a function of the reciprocal of the dielectric constant of the medium [15], which is observed in the present study (Figures 1 and 2). The linear variation indicates that electrostatic forces dominate the equilibrium process under the present experimental conditions. The non-linearity observed in Figure 1(A) may be due to considerable contribution from non-electrostatic forces.

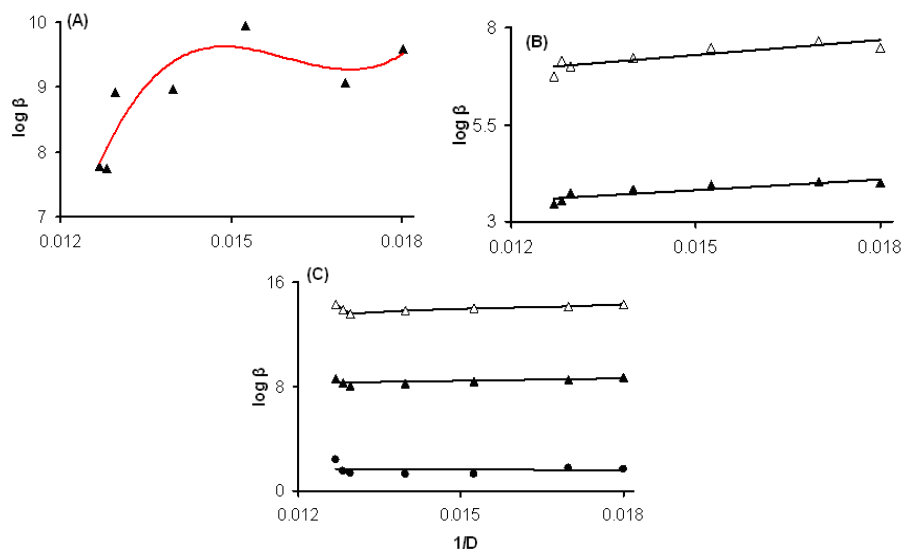


Figure 1. Variation of stability constant values of metal-methionine complexes with reciprocal of dielectric constants ( $1/D$ ) in PG-water mixtures at temperature = 303 K, ionic strength = 0.16 M. (A) Pb(II), (B) Cd(II) and (C) Hg(II); ( $\blacktriangle$ )  $\log \beta_{110}$ , ( $\triangle$ )  $\log \beta_{120}$ , ( $\bullet$ )  $\log \beta_{11-1}$ .

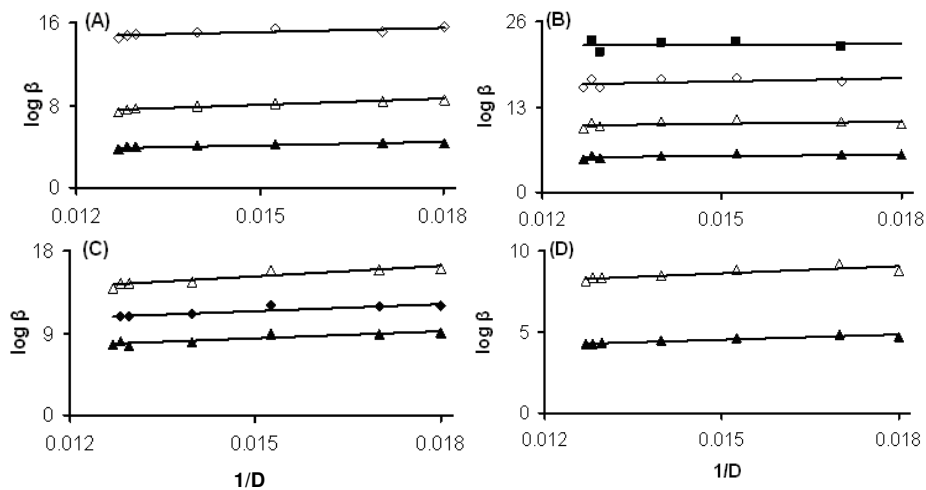


Figure 2. Variation of stability constant values of metal-methionine complexes with reciprocal of dielectric constant in PG-water mixtures at temperature = 303 K, ionic strength = 0.16 M. (A) Co(II), (B) Ni(II), (C) Cu(II) and (D) Zn(II); ( $\blacktriangle$ )  $\log \beta_{110}$ , ( $\blacklozenge$ )  $\log \beta_{111}$ , ( $\triangle$ )  $\log \beta_{120}$ , ( $\diamond$ )  $\log \beta_{121}$  ( $\blacksquare$ )  $\log \beta_{122}$ .

The linear increase indicates the dominance of the structure-forming nature of PG over complexing ability. The cation stabilizing nature of co-solvents, specific solvent-water interactions, charge dispersion and specific interactions of co-solvent with solute (indicated by the changes in the solubility of different species in the aquo-organic mixtures) account for little deviation from the linear relationship.

### Distribution diagrams

Met has one dissociable carboxyl proton and an amino group that can associate with a proton. The different forms of Met are  $\text{LH}_2^+$ , LH and  $\text{L}^-$  in the pH-regions 2.0-3.0, 3.0-8.0 and 8.0-10.0, respectively. Hence, the plausible species in different systems can be predicted from these data. The species refined and determined are ML for Pb(II); ML,  $\text{ML}_2$  for Cd(II) and Zn(II); ML,  $\text{ML}_2$  and MLOH for Hg(II); ML,  $\text{ML}_2$  and  $\text{ML}_2\text{H}$  for Co(II); ML,  $\text{ML}_2$ ,  $\text{ML}_2\text{H}$  and  $\text{ML}_2\text{H}_2$  for Ni(II); ML, MLH and  $\text{ML}_2$  for Cu(II).

The species distribution diagrams of various systems are shown in Figures 3 and 4. They indicate that the percentage of the ML species of Pb(II) increases with increasing PG content.  $\text{ML}_2$  is formed at higher pH with higher percentage than ML in the case of Cd(II) and Zn(II). For Hg(II), ML exists at low pH and  $\text{ML}_2$  and MLOH are successively formed with the increasing pH.

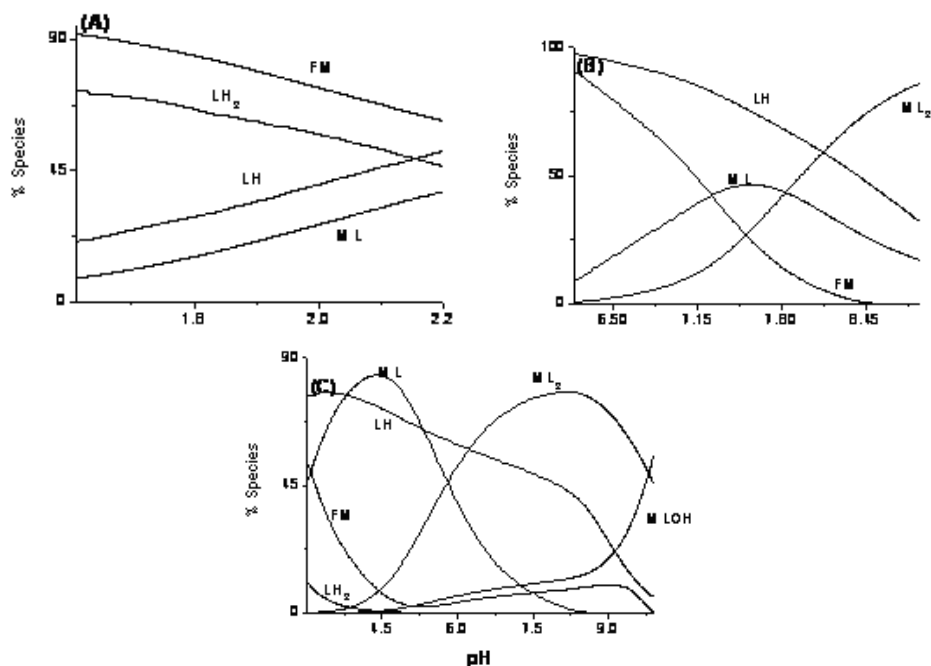


Figure 3. Distribution diagrams of methionine complexes in 20 % v/v PG-water mixture at temperature = 303 K, ionic strength = 0.16 M. (A) Lead(II) = 0.1 mmol, (B) cadmium(II) = 0.099 mmol and (C) mercury(II) = 0.102 mmol. In all the systems the number of mmols of Met are 0.25, 0.375 and 0.50.

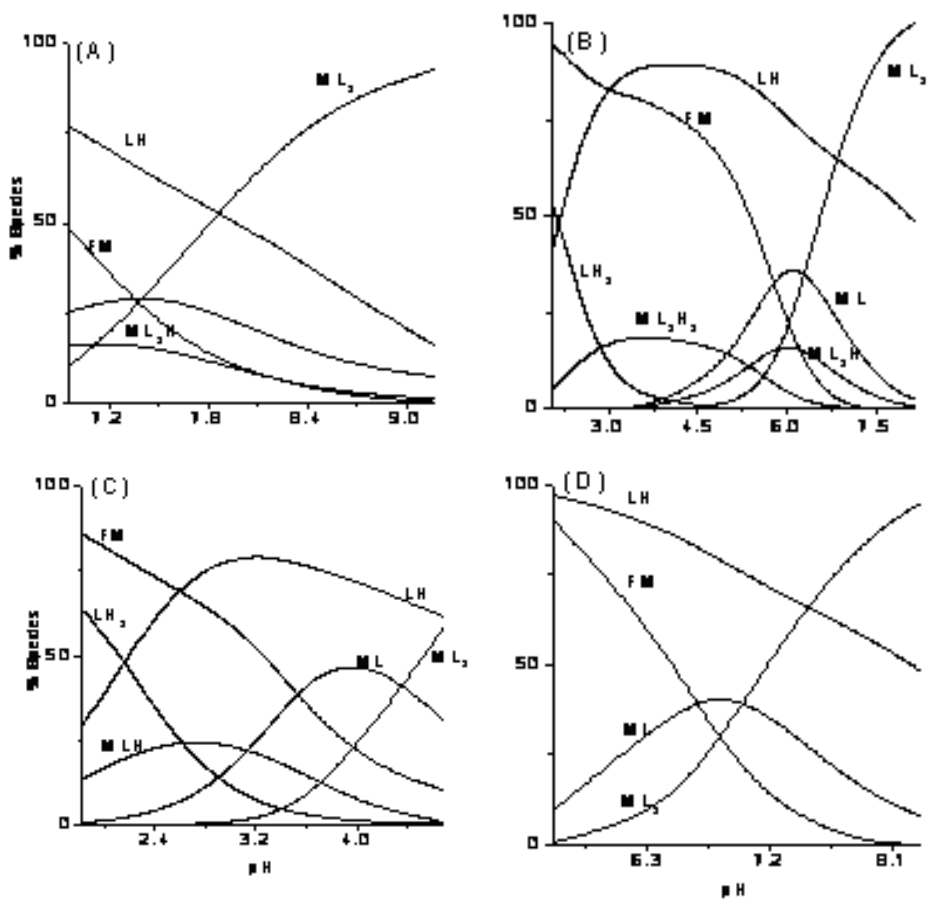
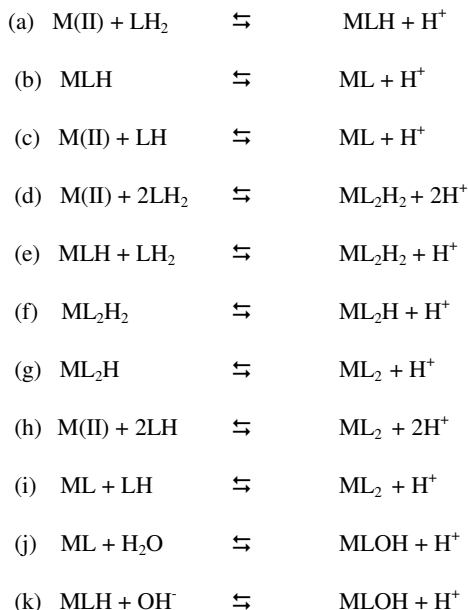


Figure 4. Distribution diagrams of methionine complexes in 20 % v/v PG-water mixture at temperature = 303 K, ionic strength = 0.16 M. (A) Cobalt(II) = 0.106 mmol, (B) nickel(II) = 0.099 mmol, (C) copper(II) = 0.101 mmol and (D) zinc(II) = 0.098 mmol. In all the systems the number of mmols of Met are 0.25, 0.375 and 0.50.

For Co(II),  $ML_2H$ ,  $ML$  and  $ML_2$  are formed simultaneously and their percentages increase in the same order. With increase in pH, deprotonation of  $ML_2H_2$  takes place to form  $ML_2H$ , and at higher pH,  $ML$  and  $ML_2$  are formed for Ni(II). With the increase in solvent content the percentage of  $MLH$  increases and unprotonated forms  $ML$  and  $ML_2$  are formed at higher pH for Cu(II). The protonated species are formed at lower pH than the unprotonated species. The formations of various binary complex species are shown in the following equilibria:





Two possible equilibria (b) and (c) are proposed for ML species. Between these two, (c) appears to be more appropriate, since no metal except Cu(II) formed MLH (Tables 1 and 2). But in the case of Cu(II), equilibrium (b) is more appropriate, because ML is formed with decreasing concentration of MLH (Figure 4C). The equilibrium (d), between (d) and (e) is more appropriate for the formation of  $NiL_2H_2$ , because free metal ion concentration is decreasing (Figure 4B) and no MLH is formed (Table 2). For Co(II) and Ni(II), the equilibrium (g) is more predominant among (g), (h) and (i), because a decrease in the percentage of  $ML_2H$  and an increase in the percentage of  $ML_2$  were observed at the same pH. For Cd(II), Hg(II), Cu(II) and Zn(II) the equilibrium (i) will be more appropriate because during the formation of  $ML_2$  there is a decrease in ML. Formation of HgOH is observed at the pH where the decrease in the distribution of ML has started; hence (j) is a more probable equilibrium than (h). Although different formulations of the same equilibria cannot be distinguished by this method, they are presented above to represent the various possible equilibria that may exist simultaneously in the solution.

## CONCLUSIONS

(1) The common species formed due to the interaction of L-methionine with the metals are ML, MLH,  $ML_2$ ,  $ML_2H$ ,  $ML_2H_2$  and MLOH. (2) The linear variation of stability constants with dielectric constants of the medium indicates the dominance of electrostatic forces over non-electrostatic forces. A linear increasing trend with 1,2-propanediol content supports the predominance of the structure forming nature of PG over its complexing ability. (3) The order of ingredients in influencing the magnitudes of stability constants due to incorporation of errors is alkali > acid > ligand > metal. (4) The study suggests the formation of ML by direct interaction of the ligand with Pb(II), Cd(II), Hg(II), Co(II), Ni(II) or Zn(II), where as it is formed by the deprotonation of MLH in the case of Cu(II). (5) The formation of  $ML_2H_2$  is found to be due to the direct interaction of the protonated ligand with the metal than with the protonated complex (MLH). (6) MLOH is proposed to be formed by the hydrolysis of ML but not due to the hydrolysis of MLH, since MLH is not formed for that metal ion.

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