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RESEARCH ARTICLE

Speciation Studies of L-Methionine complexes of Pb(II), Cd(II) and Hg(II) in acetonitrile-water mixtures

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Abstract:

Equilibrium study on complex formation of L-methionine with Pb(II),Cd(II) and Hg(II) has been investigated pH metrically in acetonitrile-water mixtures 0.0-60% v/v at 303 K and 0.16 M ionic strength. The predominant species detected for Pb(II),Cd(II) and Hg(II) are MLH,ML and ML_2.. Models containing different numbers of species were refined by using the computer program MINIQUAD75. The best-fit chemical models were arrived at based on statistical parameters. The trend in variation of complex stability constants with change in the dielectric constant of the medium is explained on the basis of electrostatic and non-electrostatic forces.

Keywords: Complex equilibria, Chemical speciation, L-Methionine, Acetonitrile, Metals

Introduction

The speciation study of toxic metal ion complexes is useful to understand the role played by the active site cavities in biological molecules and the bonding behavior of protein residues with the metal ion. The species refined and their relative concentrations under the experimental conditions represent the possible forms of amino acids in bio fluids. Due to its numerous uses and high persistence, lead is a major environmental contaminant [1]. Lead is toxic even at low concentrations for living organisms, which can absorb it in various ways [2]. Lead intake by humans can be due to the consumption of crop plants grown on soils with high plant-available metal concentrations [3]. Cadmium causes iron deficiency by binding to cysteine, glutamate, aspartate, and histidine ligands [4]. Cadmium inhibits enzymes that participate in bilirubine conjunction [5]. It increases urine Ca^{2+} excretion which can cause severe bone pathology [6]. Mercury is one of the most toxic elements and has negative health effects in human populations, highly dependent on fish consumption [7]. Recent research concluded that neither vitamin B12 nor the acetyl-CoA pathways are required for bacterial methylation of mercury [8].

L-methionine [Met] is a sulfur-containing amino acid and it forms stable complexes with heavier metals according to Hard and Soft Acid Base theory. It is chiefly available in cheeses, eggs, fish, meat and poultry.

Acetonitrile (AN) is a colorless polar aprotic solvent [9]. It behaves as a weaker base [10] and as a much weaker acid [11] than water. It has a dielectric constant of 36 and autoprotolysis constant of 33.6. AN also acts as a strongly differentiating solvent with a modest solvating power for many polar ionic solutes [12].
Hence Met is selected for speciation studies of its complexes with Pb(II), Cd(II) and Hg(II) in acetonitrile (AN)-water mixtures. The protonation constants of Met in AN-water mixtures were reported earlier [13].

2.1 Materials

Acetonitrle (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) nitrates 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 0.05M. To assess the errors that might have crept into the determination of the concentrations, the data have been subjected to analysis of variance of one way classification (ANOVA). The strength (concentration) of alkali has been determined using the Gran plot method [14,15].

2.2 Apparatus

The titrimetric data were obtained with a calibrated ELICO (Model L1-120) pH-meter (readability 0.01) which can monitor the changes in H^+ concentration. The pH meter was calibrated with 0.05 M potassium hydrogen phthalate in acidic region and 0.01 M borax solution in basic region. The glass electrode was equilibrated in a well-stirred AN-water mixture containing inert electrolyte. All the titrations were carried out in the medium containing varying concentrations of AN (0-60.0 % v/v) maintaining an ionic strength of 0.16 M with sodium nitrate at 303.0 ± 0.1 K. The effect of variations in asymmetry potential, liquid junction potential, activity coefficient, sodium ion error and dissolved carbon dioxide on the response of glass electrode were accounted for in the form of correction factor [16].

2.3 Procedure

For the determination of stability constants of metal-ligand binary species, initially titrations of strong acid with alkali were carried out at regular intervals to check whether complete equilibration was achieved. Then the calomel electrode was refilled with AN-water mixture of equivalent composition as that of titrand. In each of the titrations, the titrand consisted of approximately 1 mmol mineral acid in a total volume of 50 mL. Titrations with different ratios (1 : 2.5, 1 : 3.75 and 1 : 5.0 in the case of Pb(II) and Cd(II) and 1 : 7.5, 1 : 8.5 and 1 : 10.0 in the case of Hg(II)) of metal-to-ligand were carried out with 0.4 mol L^{-1} sodium hydroxide. Other experimental details are given elsewhere [17].

2.4 *Modeling strategy*

The computer program SCPHD [18] was used to calculate the correction factor. By using the pH-metric titration data, the binary stability constants were calculated with the computer program MINIQUAD75 [19], 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

Alkalimetric titration curves in AN-water mixtures revealed that the acido-basic equilibria of L-methionine (LH_2^+, LH and L^-) were active in the pH range 2.0-12.0. Based on the active forms of the ligands in this pH range, models containing various numbers and combination of complex species were fed to MINIQUAD75 along with the alkalimetric titration data. Exhaustive modeling was performed for Pb(II)-Met in 50% v/v AN-water mixture and the results are given in Table 1. The models indicated better statistics as the number of species was increased, confirming better fit. There was no further improvement in the fit on inclusion of some more species in the model containing PbLH, PbL and PbL_2. This indicates that the final model appropriately fits the experimental data. Such exhaustive modeling was performed for all the systems. The best-fit model was selected using the statistical parameters [20] of the least squares residuals.

The final models along with the statistical parameters are given in Table 2. The results of the best-fit models that contain the type of species and overall formation constants along with some of the important statistical parameters are given in Table 2. A very low standard deviation in log β values indicates the precision of these parameters. The small values of U_{corr} (sum of squares of deviations in the concentrations of ingredients at all experimental points corrected for degrees of freedom) indicate that the experimental data can be represented by the model. Small values of mean, standard deviation and mean deviation for the systems corroborate that the residuals are around a zero mean with little dispersion.

For an ideal normal distribution, the values of kurtosis and skewness should be three and zero, respectively. Kurtosis is a measure of the peakedness of the error distribution near a modal value. For an ideal normal distribution kurtosis value should be three (mesokurtic). If the calculated kurtosis is less than three, the peak of the error distribution curve is flat (platykurtic) and if the kurtosis is greater than three, the distribution shall have sharp peak (leptokurtic). The kurtosis values in the present study indicate that the residuals form leptokurtic as well as platykurtic patterns. The values of skewness recorded in the tables are between -0.55 and 0.85. 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 AN-water mixture.

Effect of systematic errors on best-fit model

In order to rely upon the best chemical model for critical evaluation and application under varied experimental conditions with different accuracies of data acquisition, an investigation was made by introducing pessimistic errors in the influential parameters [21] like concentrations of alkali, mineral acid, ligand and metal (Table 3). The order of the ingredients that influence the magnitudes of stability constants due to incorporation of errors is alkali > acid > ligand > metal. Some species are even rejected when errors are introduced in the concentrations. This study confirms the appropriateness of the chosen best-fit models. This study also indicates the relative sensitivities of model parameters.

Effect of solvent

Variation of logarithmic values of stability constants (log β) with reciprocal of dielectric constant (1/D) are shown in Figure 1. 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 [22] 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. Variation of logarithmic values of stability constants (log β) or change in free energy with co-solvent content depends upon two factors, viz., electrostatic and non-electrostatic. Born's classical treatment [23] holds good in accounting for the electrostatic contribution to the free energy change. According to this treatment, the energy of electrostatic interaction is related to dielectric constant. Hence, the log β values should vary linearly as a function of 1/D of the medium. The linear trend observed in the present study (Figure 1) indicates that electrostatic forces are dominating the equilibrium process under the present experimental conditions.

The linear increase indicates the dominance of the structure-forming nature of AN over complexing ability. The cation stabilizing nature of co-solvent, 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 $LH_2^{+,}$ LH and L^- in the pH-regions 2.0-4.0, 4.0-10.0 and 9.0-12.0, respectively. Hence, the plausible species in different systems can be predicted from these data. The species refined and determined are ML, MLH and ML_2 for Pb(II), Cd(II) and Hg(II). The species distribution diagrams of various systems are shown in Figure 2. They indicate that the percentage of the ML and ML_2 species of Pb(II) increases with increase of pH and MLH species decrease with pH. In the case of Cd(II), ML_2 and MLH species increase with the increase of pH and ML species decrease with pH. In the case of Hg(II), ML, ML_2 and MLH species increase with pH.

The formation of various Methionine binary complex species is shown in the following equilibria.

(a) $M(II) + LH \rightleftharpoons MLH$
(b) $M(II) + LH_2 \rightleftharpoons MLH + H^+$
(c) $M(II) + LH_2 \rightleftharpoons ML + 2H^+$
(d) $M(II) + 2LH_2 \rightleftharpoons ML_2 + 4H^+$
(e) $M(II) + 2LH \rightleftharpoons ML_2 + 2H^+$

Two possible Equilibria (a) and (b) are proposed for MLH species. Between these two, Equilibrium (b) appears to be more appropriate, because LH_2 decrease MLH species formed. Equilibrium (c) is proposed for ML species because LH_2 decreases with increasing concentration of ML. Equilibria (d) and (e) represent the formation of ML_2 species. Between these two (d) appears to be more appropriate, because LH_2 decreases as ML_2 species is formed. Depending on the active sites in the ligand and the nature of the metal ions, the structures were proposed for the species detected as shown in Figure 3.

CONCLUSIONS

1. The biomimetic studies of metal ion complexes with L-methionine in AN-water mixtures indicate the formation of MLH, ML and ML_2.
2. The linear increase of log β values with 1/D of the medium indicate the dominance of electrostatic forces over non-electrostatic forces.
3. The order of ingredients influencing the magnitudes of stability constants due to incorporation of errors in their concentrations is alkali > acid > ligand > metal .
4. The stability constants of binary complexes are found to follow the trend Cd(II) < Pb(II) < Hg(II).

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Table 1. Exhaustive modeling of Pb(II)-methionine complexes in 50% v/v AN-water mixture. pH range = 1.8-8.0; Number of points = 80.

Model no	log β_{mlh} (SD)			U_{corr}	Skewness	Kurtosis	χ^2	R-Factor
	110	111	120					
1	9.67(22)	---	---	1.01	1.43	7.85	121	0.0207
2	------	11.43(30)	----	3.08	0.11	4.60	122	0.0271
3	------	------	13.06(21)	3.21	1.55	8.32	136.19	0.0206
4	-----	13.01(15)	12.07(29)	2.26	1.20	6.77	94.73	0.0232
5	9.60(13)	------	12.32(50)	1.8	1.55	8.32	136.19	0.0206
6	9.66(14)	13.85(22)	-------	1.85	1.43	7.32	122.99	0.0207
7	10.93(6)	13.28(8)	17.30(12)	1.66	0.32	4.42	18.43	0.0023

Table 2: Best fit chemical models of Pb(II), Cd(II) and Hg(II) - Methionine complexes in AN-water mixture.

v/v% AN	log β_{mlh} (SD)			NP	U_{corr}	Skewness	Kurtosis	χ^2	pH Range	R-Factor
	110	111	120							
				(Pb(II)						
0.0	8.51(7)	12.21(16)	13.60(4)	78	5.6	-0.35	2.51	18.58	1.9-7.0	0.0134
10.0	8.75(9)	12.21(8)	14.44(10)	69	1.22	-0.25	4.51	27.71	1.6-6.8	0.0023
20.0	9.72(6)	11.71(7)	15.06(9)	102	1.17	0.47	4.24	20.73	1.5-7.4	0.0034
30.0	10.20(4)	12.37(9)	15.54(10)	67	1.14	0.13	3.32	20.17	1.6-7.5	0.0042
40.0	10.29(4)	12.50(8)	17.23(11)	56	-0.4	-1.14	4.37	7.43	1.8-6.4	0.0089
50.0	10.93(6)	13.28(8)	17.30(12)	80	1.66	0.32	4.42	18.43	1.8-8.0	0.0023
60.0	11.30(9)	14.64(9)	17.66(10)	79	1.22	-0.31	3.42	20.38	1.6-8.0	0.0069
				Cd(II)						
0.0	3.34(10)	7.50(6)	11.14(8)	81	1.96	0.38	4.33	56.95	2.0-9.0	0.0085
10.0	5.12(4)	7.82(8)	12.79(10)	63	1.23	-0.03	3.36	20.19	2.0-9.0	0.0023
20.0	5.14(8)	8.96(9)	12.84(11)	62	1.10	-0.25	4.79	26.39	2.0-9.0	0.0045
30.0	5.64(10)	10.08(9)	12.48(10)	50	1.05	0.00	4.15	21.43	2.0-9.0	0.0054
40.0	5.65(9)	9.54(8)	13.20(11)	73	1.15	0.03	4.27	22.50	2.0-9.0	0.0068
50.0	5.94(9)	10.13(9)	13.22(5)	77	1.23	-0.55	4.12	30.92	2.0-9.0	0.0081
60.0	6.34(8)	10.33(11)	13.72(9)	72	1.13	0.41	3.25	8.30	2.0-9.0	0.0092
				Hg(II)						
0.0	9.00(8)	12.56(11)	14.00(6)	49	1.96	0.85	3.91	34.39	1.8-8.0	0.0153
10.0	9.25(6)	11.63(9)	15.43(10)	49	1.12	-0.11	3.42	21.98	2.2-8.0	0.0074
20.0	9.33(4)	12.56(11)	15.90(11)	50	1.04	0.31	2.90	7.23	2.2-8.0	0.0099
30.0	9.05(11)	12.55(9)	15.92(12)	51	1.02	0.23	2.45	8.10	2.2-8.0	0.0098
40.0	10.84(9)	12.68(12)	17.20(10)	53	1.01	-0.16	3.20	6.08	2.2-8.0	0.0097
50.0	10.66(10)	12.99(9)	16.98(9)	58	1.02	0.33	3.72	11.47	2.2-8.0	0.0089
60.0	10.68(9)	14.21(9)	17.18(6)	51	1.04	0.19	3.14	12.80	2.2-8.0	0.0081

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

Table 3: Effect of errors in influential parameters on the Cd(II) L-methionine complex stability constants in 20% v/v AN-water mixture.

Ingredient	% Error	Log β (SD)		
		110	111	120
Alkali	0	5.14(8)	8.96(9)	12.84(11)
	-5	3.84(28)	Rejected	Rejected
	-2	4.32(26)	Rejected	11.33(21)
	+2	4.94(24)	9.97(15)	13.66(18)
	+5	6.52(92)	10.21(86)	14.86(88)
Acid	-5	Rejected	Rejected	Rejected
	-2	5.10(23)	9.29(16)	10.86(20)
	+2	5.08(30)	Rejected	11.45(19)
	+5	5.21(52)	Rejected	13.12(82)
Ligand	-5	5.13(29)	8.92(72)	12.79(15)
	-2	5.14(26)	8.94(22)	12.82(17)
	+2	5.13(22)	8.95(15)	12.84(18)
	+5	5.12(22)	8.92(15)	12.83(19)
Metal	-5	5.13(26)	8.95(18)	12.83(18)
	-2	5.14(23)	8.96(18)	12.84(17)
	+2	5.14(22)	8.96(19)	12.85(17)
	+5	5.12(21)	8.97(20)	12.86(17)

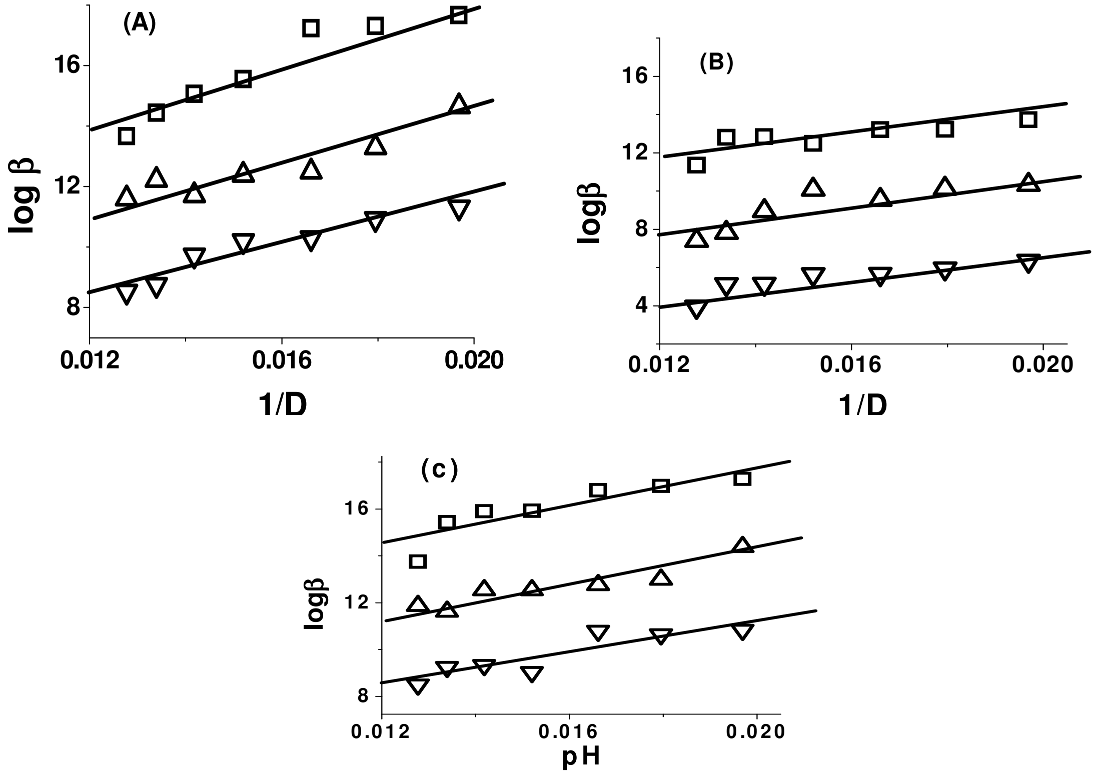


Figure 1. Variation of stability constant values of metal-methionine complexes with reciprocal of dielectric constants (1/D) in AN-water mixtures at temperature = 303 K and ionic strength = 0.16 M. (A) Pb(II), (B) Cd(II) and (C) Hg(II); (▿) log 110, (Δ) log 111, (□) log 120.

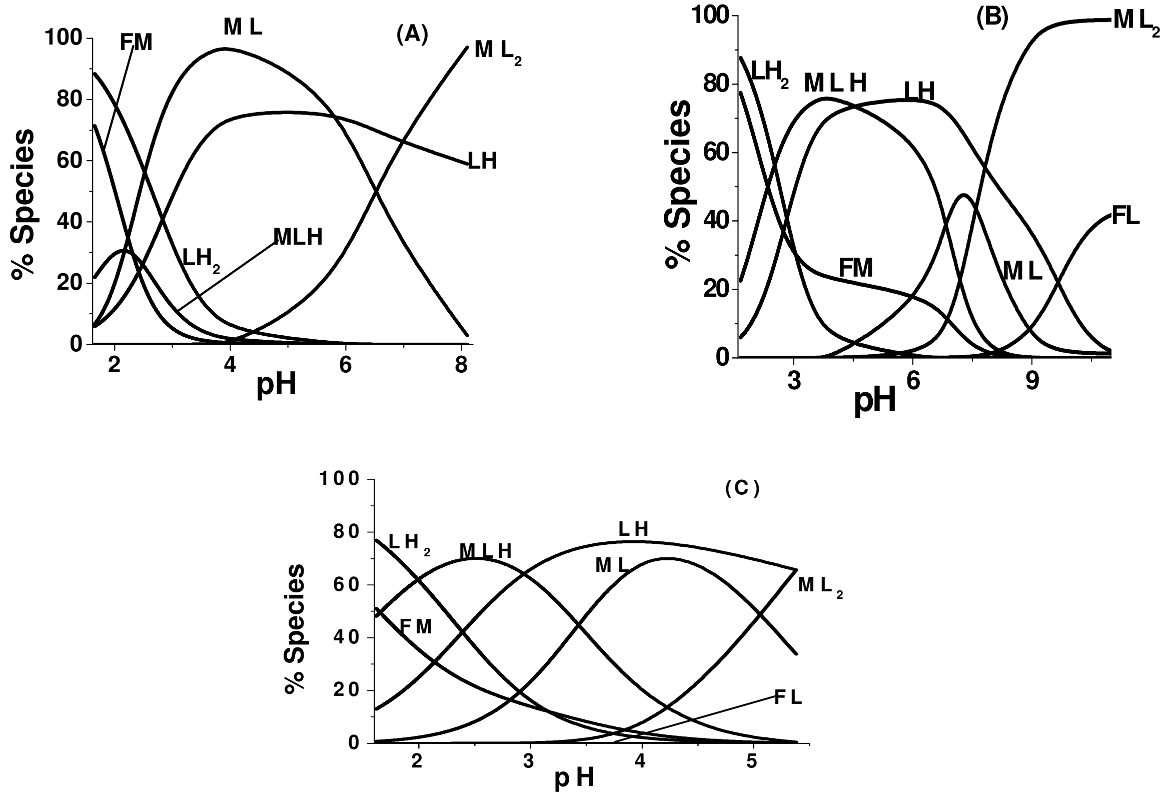
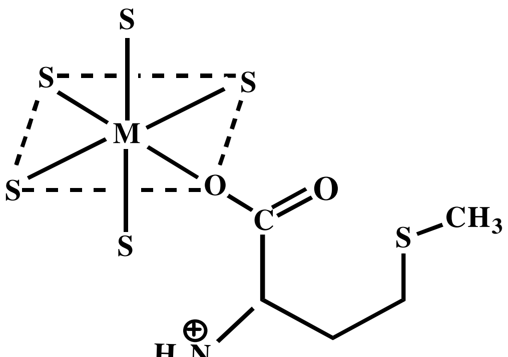


Figure 2. Distribution diagrams of methionine complexes in 30% v/v AN-water mixture. Temperature = 303 K , ionic strength = 0.16 M. (A) Pb(II) = 0.0999 mmol, (B) Cd(II) 0.1057 mmol and (C) Hg(II) = 0.0516 mmol. In all the systems the number of mmols of Met is 0.50.



MLH

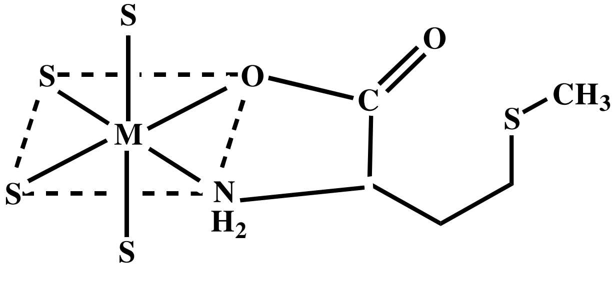


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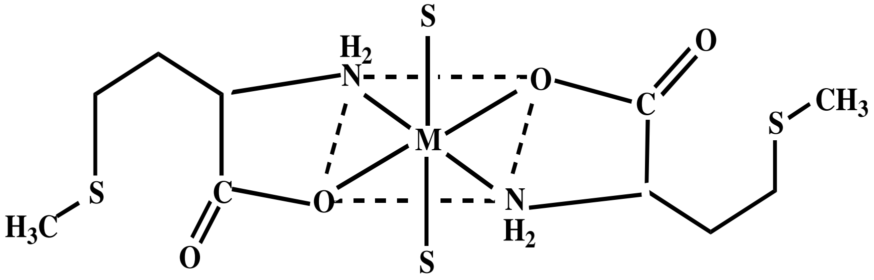
Figure 3. Structures of binary complexes of Pd(II), Cd(II) and Hg(II) with Met.



ML_2

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