International Journal of Chemical Science
Online ISSN: 2523-2843, Print ISSN: 2523-6075
www.chemicaljournals.com
Volume 1; Issue 1; September 2017; Page No. 23-28

Speciation Studies of Some Toxic Metal Complexes of 1, 10-Phenanthroline in Dimethylformamide - Water Mixtures

Aditya Deepthi D, Prasanthi J, * Nageswara Rao G
School of chemistry, Andhra University, Visakhapatnam, India

Abstract
The formation equilibria of complexes of Pb (II), Cd (II), and Hg (II) with 1,10-Phenanthroline were investigated pH metrically in Dimethyl formamide-water mixtures (0-60%v/v) at 303 K and ionic strength of 0.16 mol L^{-1}. The dominant species detected were ML and ML_3 for Pb (II), Cd (II) and Hg (II). The appropriateness of the experimental conditions were verified by introducing errors deliberately. The models containing different numbers of species were refined by using the computer program MINIQUAD75. Selection of the best fit models was based on the statistical parameters. The trend in variation of stability constants of the complexes with dielectric constant of the medium was attributed for the formation and possible structures of the complex species presented.

Keywords: speciation, complex equilibria, Dimethyl formamide, 1, 10-Phenanthroline, stability constants

Introduction

Heavy metals are common in our environment and diet and many of them are essential to living organisms but some of them are highly toxic or become toxic given sufficient exposure and accumulation in the body. Metals such as Hg, Cd, Pb, Sn, Cr, and As are generally not required for metabolic activity and are toxic to living organisms even at low concentrations.[1, 2] The mechanism of the toxicity of metals is very complicated. Generally toxicity of metals results from blocking the essential biological functional groups (-OH, -SH, and –N) or modifying the active conformation of bio-molecules (enzyme, DNA, etc.,) through binding and displacing the essential metal ions from their natural binding sites of the bio-molecules with a foreign metal ion. Human civilization and the increase in industrial activity has gradually redistributed many toxic metals from the earth's crust to the environment and increased the possibility of exposure. Lead, cadmium, and mercury are among the various toxic metals especially prevalent in nature due to their high industrial use. These metals serve no biological function and their presence in tissues reflects contact of the organism with its environment [3]. The main sources of lead pollution specially the bones and teeth, the kidneys, and the nervous, cardiovascular, immune and reproductive systems [4, 6]. Lead also interferes with the normal metabolism of calcium in cells and causes it to build up within them [7]. The sources of Cd pollution in urban areas are metallurgical plants, Cd plating and battery fabricators. It can also enter the environment through natural causes, such as volcanic activity and forest fires [8]. Human exposure to cadmium mainly occurs through cigarette smoking [9]. but exposure can also occur through contaminated food, [10]. water or air [11]. Cadmium is a known carcinogen to mammals [12]. Cadmium interacts with calcium in the skeletal system to produce osteodystropies [13]. Due to their similarilty in properties, cadmium displaces zinc in many metallo-enzymes and many of the symptoms of cadmium toxicity can be traced to a cadmium-induced zinc deficiency [14, 15] Cd (II) binds strongly with the -SH groups of cysteine residues of enzymes [16], e.g. carbonic anhydrase, dipeptidase, carboxy peptidase etc., and effects the active conformation of bio-molecules due to this strong binding. Mercury exposure is related to the release of mercury forms (Hg^0, inorganic Hg (II), and organic Hg (II) into the environment by both natural and man-made activities [17]. Mercury is a highly toxic element because of its accumulative and persistent character in the environment and living organisms [18]. It affects the immune system, alters genetics and enzyme systems, damages the nervous affinity for the protonated forms of thiol ligands such as cysteine [20]. So Hg (II) binds strongly with the thiol group of proteins, enzymes and other bio-molecules in which this binding changes the conformation of bio-molecules in their active site [16, 21]. Speciation studies of toxic and essential metal ion complexes are useful in order to understand the role played by the active site cavities possible ligand groups in proteins are the aminoacid side chains, the terminal amino, carboxyl and thiol groups and, in some cases, the amide group is the peptide backbone [23]. However the study of metal-protein system may be difficult to construct in simple - like aminoacids and peptides but such models may give a tremendous amount of information about the structure of proteins and function of bio-molecules in biological systems [24]. The interaction of metals with amino acids and peptides has been the subject of much research [25, 29], due to the importance of metals in many biochemical processes [30], such as respiration, metabolism and nerve transmission [31]. Investigations of acid-base equilibria of aminoacids and peptides and their interaction with metal ions at varying ionic strengths, temperatures and dielectric constant media throw light on the mechanism of enzyme-catalysed reactions. It is known that the polarity of the active site cavities in proteins is lower than that of the bulk but direct

measurements of the dielectric constant is not possible. Comparing the formation constants of acid-base equilibria and/or metal complex equilibria with those at biological centres offers a way to estimate the effective dielectric constant or equivalent solution dielectric constant for the active site cavity [32]. This has brought an important new approach to the study of complex equilibria in aqua-organic mixtures apart from its established utility in understanding solute-solvent interactions, increasing sensitivity of reactions of analytical and industrial importance, and solubilising ligands of their metal complexes. Chemical speciation of metals is important for an understanding of their distribution, mobility, toxicity, and for setting environmental quality standards [33]. Bioavailability of a particular metal depends on its complex chemical reactions of dissolution, binding and complexation with the constituents of the environmental aquatic media [34]. To reveal the solvent effects on equilibrium processes involving charged species, we have studied the complex formation of 1,10-phenanthroline (Phen) with Pb (II), Cd (II) and Hg (II) as a good example in modelling of the bonding modes of peptides to toxic metal ions in mixtures containing Dimethyl formamide (DMF) and water.

Experimental

Chemicals and standard solution

All the chemicals used in this experiment were of analytical reagent grade purity. Triple distilled deionized water was used for the prepararion of all the solutions. Solutions of 0.1 mol L^{-1} of Pb(II), Cd(II), Hg(II) nitrates (Himedia, India) were prepared maintaining 0.05 mol L^{-1} nitric acid to suppress the hydrolysis of metal salts. A solution (0.05mol L^{-1}) of 1, 10-phenanthroline (Himedia, India) was prepared by maintaining a 0.05mol L^{-1} nitric acid concentration to increase its solubility. Dimethylformamide (Merck, India) was used as received. Solutions of 0.2 mol L^{-1} nitric acid (Merck, India) and 0.4 mol L^{-1} sodium hydroxide (Merck, India) were prepared. A solution of 2.0 mol L^{-1} sodium nitrate (Merck, India) was prepared to maintain the ionic strength in the titrand. All the solutions were standardized by the usual oxalic acid and potassium hydrogen phthalate solutions, while the normality of nitric acid was determined by using the standardized sodium hydroxide and the primary borax solutions [35]. The concentration of the metal ions were determined complex metrically by titrating against a standard solution of EDTA using Xylenol orange indicator and hexamine powder as buffer to maintain the pH at 5.0-6.0 [36]. So as to assess the errors that might have crept into the determination of the concentrations, the data were subjected to one-way analysis of variance (ANOVA) by using the computer program, COST [37]. The carbonate contamination in the sodium hydroxide solutions was determined by using the Gran Plot method [38].

Alkalimetric Titrations

The pH measurements of metal-ligand binary systems were carried out in aqueous media containing varying compositions of Dimethylformamide in the range of 0-60% (v/v) maintaining an ionic strength of 0.16 mol L^{-1} with sodium nitrate at 303±0.1K by using a digital pH meter ELICO type readability of 0.01 (0-14). The electrode of the cell was calibrated with 0.05 mol L^{-1} potassium hydrogen pthalate solution in the acidic region and with 0.01 mol L^{-1} borax solution in the alkaline region to measure the response in the pH range 2.0-11.0. The effect of variations in asymmetry potential, liquid junction potential, activity coefficient, sodium ion error and dissolved carbon dioxide on the response of the glass electrode were accounted for in the form of correction factor [39, 40]. Mechanical stirring of the solution was carried out by means of a Teflon stirrer. The pH metric titration assembly consisted of a double-walled spotless pyrex glass vessel of 100 mL capacity fitted with a Perspex lid through which the glass combination pH electrode, gas inlet-outlet tubes and burette tip were admitted. Water from thermostat was pumped at a constant rate through the annular space between the walls of the titration vessel. The titrand in the double-walled spoutless pyrex glass vessel was maintained inert by bubbling dried and pure nitrogen gas throughout the course of the titration to purge carbon dioxide and oxygen. The electrode was kept, usually for three to four days, in the required solvent system in order to equilibrate. To verify whether the electrode was equilibrated, a strong acid was titrated with an alkali every day until no appreciable differences were observed between the pH values of two titrations at the corresponding volumes of titrant. A colomel electrode was refilled with DMF-water mixture of the equivalent composition to that of the titrand. Free acid titrations were performed before the metal-ligand titrations to calculate the correction factor. In each of the titrations, the titrand consisted of a mineral acid of approximately 1 mmol in a total volume of 5ml. titrations with different ratios (1:2.5, 1:3.75 and 1:5) of metal (Pb, Cd)-ligand (1:5, 1:7.5 and 1:10) of Hg ligand were performed with 0.5 mol L^{-1} sodium hydroxide solution. The mixtures obtained from DMF and water are non-ideal due to increased interactions between unlike molecules and large differences in molar volumes of pure components, which leads to non-additive volumes on mixing. The excess volume (V^E) obtained from mixing of the two solvents at a given condition is calculated by the equation

$$V^E = \frac{X1\,M_1 + x_2M_2}{\rho} - \frac{x_1M_1}{\rho_1} + \frac{x_2M_2}{\rho_2}$$

Where x_1 and x_2 are the mole fractions, M_1 and M_2 are the molar masses, p_1 and p_2 are the densities of the pure components, and p is the mixture density. In this experiment, the volume correction was not taken into consideration because the change in volume was insignificant (its maximum is –0.67 mL mol^{-1} if mixing is carried at 303 K) even though the mixtures obtained from DMF and water show non-ideal behavior. Other experimental details are given elsewhere [41].

Modeling Strategy

The correction factor to be applied to the pH-meter dial readings was calculated with the computer program SCPHD [42]. By using the pH –metric titration data, the binary stability constants were calculated with the computer program MINIQUAD75, which exploits the advantage of the constrained least squares method in the initial refinement and reliable convergence of the Marquardt algorithm [43]. During

the refinement of binary stability constants, pKw and the protonation constants [44] of Phen were fixed.

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 Table 1. The low standard deviation in log β values indicates the adequacy of the models. The small values of U_{corr} (sum of deviation in concentrations of the metal, ligand and hydrogen ions at all represented by the model. Small values of mean, standard deviation and mean deviation for the systems corroborate that the residuals are around zero mean with little dispersion. For an ideal normal distribution, the values of kurtosis and skewness should be three and zero, respectively. The kurtosis values in the present study indicate that the residuals form leptokurtic pattern and a few form a platykurtic pattern. The values of the skewness given in Table 1 are between -2.92 and 2.84. These data evince that the residuals form a part of normal distribution; hence, least squares method can be applied to the present data. The sufficiency of the model is further evident from the low crystallographic R-value[45] recorded. Thus these statistical parameters support the best fit models which portray the metal-ligand species in DMF-water mixtures.

Table 1: Parameters of best fit chemical models, of **Phen** complexes of Pb(II),Cd(II) and Hg(II) in DMF-water mixtures. Temperature =303K, Ionic strength =0.16 mol dm^{-3}

%v/v DMF	$Log\beta_{mlh}$ (SD 1 1 0	1 3 0	NP	U_{corr}	χ^2	Skewness	R factor	kurtosis	pH range
Pb (II)									
0	3.67(05)		41	7.17	25.10	-2.06	0.029	9.94	2.6-6.0
10	3.47(39)	8.76(41)	31	18.96	123.86	0.29	0.132	37.97	2.6-6.0
20	3.60(24)	10.92(10)	19	10.0	4.05	-0.24	0.295	2.34	2.8-4.0
30	3.77(34)	11.33(19)	45	41.95	81.95	0.00	0.052	8.63	2.0-4.0
40		11.48(09)	39	21.72	21.60	-0.16	0.044	4.71	2.5-4.0
50	3.78(34)	11.35(19)	45	41.95	81.95	0.00	0.052	8.63	2.0-4.0
60	3.83(34)	11.56(19)	46	41.25	81.71	0.00	0.052	8.75	2.0-4.2
Cd (II)									
0	7.01(32)	15.77(40)	33	1.74	46.86	-2.92	0.016	32.99	1.9-4.5
10	5.09(12)	11.94(31)	78	35.35	29.93	0.15	0.038	5.12	1.8-5.0
20	3.51(15)	10.66(06)	34	3.81	13.29	0.11	0.017	7.67	2.3-4.5
30	3.84(19)	10.79(16)	44	20.16	73.58	0.02	0.039	8.55	2.2-4.5
40	3.61(11)	10.97(06)	34	3.61	13.29	-0.08	0.017	8.36	2.3-4.5
50	3.97(17)	11.34(12)	42	13.82	55.33	-0.01	0.032	9.33	2.3-4.5
60	4.01(18)	11.57(12)	5	13.86	16.47	2.34	0.006	8.95	2.2-4.2
Hg (II)									
0	5.02(48)		22	14.4	49.15	-2.04	0.124	21.54	2.0-6.0
10	3.77(34)	9.12(37)	23	9.64	4.16	-1.42	0.030	4.16	3.0-5.5
20	3.33(08)	10.91(08)	29	1.50	15.71	0.64	0.011	4.59	2.0-6.5
30	3.77(17)	11.27(10)	30	1.84	10.80	2.09	0.012	9.63	2.5-4.5
40	3.81(20)	11.47(11)	27	2.24	13.72	2.32	0.013	10.38	2.6-4.0
50	3.95(20)	11.86(11)	30	2.58	26.80	2.67	0.014	12.73	2.5-4.2
60	4.05(19)	12.09(12)	33	2.65	28.92	2.84	0.014	14.14	2.4-4.2

U_{corr} =U/(NP-m)*10^8, m –number of species: NP= number of experimental points

Effect of systematic errors on best fit model

In order to rely upon the best fit chemical model for critical evaluation and application under varied experimental conditions with different accuracies of data acquisition, an investigation was made by introducing pessimistic errors in the concentrations of alkali, mineral acid, ligand and metal(Table 2). Errors were introduced in the concentrations of the ingredients intentionally to find their effect on the perturbation of stability constants. If the concentrations determined and the experimental conditions maintained by the researcher were appropriate, any variations in the concentrations of the ingredients will affect the magnitudes and statistical parameters of the stability constants. Sometimes even the species shall be rejected. Hence, we have studied the effect of errors in the concentrations of the ingredients on the stability constants. The data show that the order of affecting the magnitudes of the stability constants is alkali>acid>ligand>metal. Some species, were even rejected when the errors were introduced in the concentrations of the components. This shows that any deviation in the stability constants (log β) and also results in the rejection of the species.

Table 2: Effect of errors in concentrations of ingredients on stability constants of Cd (II) – Phen complexes in 30% v/v DMF-water mixtures

Ingredient	% of error	Log β	
		1 1 0	1 3 0
Alkali	0	3.84(19)	10.79(16)
	-5	2.62(74)	9.57(15)

	-2	3.38(25)	10.29(14)
	+5	Rejected	Rejected
	+2	4.97(18)	11.89(23)
Acid	-5	Rejected	Rejected
	-2	5.79(34)	12.74(12)
	+5	Rejected	Rejected
	+2	3.12(34)	10.19(12)
Ligand	-5	3.34(27)	10.97(18)
	-2	3.65(21)	10.66(14)
	+5	5.00(19)	11.76(24)
	+2	4.09(18)	10.97(18)
Metal	-5	3.86(21)	10.88(15)
	-2	3.85(20)	10.82(16)
	+5	3.83(18)	10.71(15)
	+2	3.84(21)	10.88(16)

Effect of Solvent

DMF is a polar aprotic solvent. The dielectric constant of DMF-water mixture decreases with increasing concentration of DMF and these solutions are expected to mimic physiological conditions where the concept of the equivalent solution dielectric constant for protein cavities is applicable [46]. The dielectric constants of DMF at different percentages (0.0-60.0 v/v %) of water were taken from literature [47]. The linear variation (Fig 1) of stability constants of Phen compexes of Pb(II), Cd(II) and Hg(II) in DMF-water mixtures with 1/D (where D is dielectric constant of the medium) indicates that electrostatic forces dominate the equilibrium process under the present experimental conditions. It is observed that the stability constant of ML species of Hg(II) with Phen decreases with the decrease of dielectric constant.

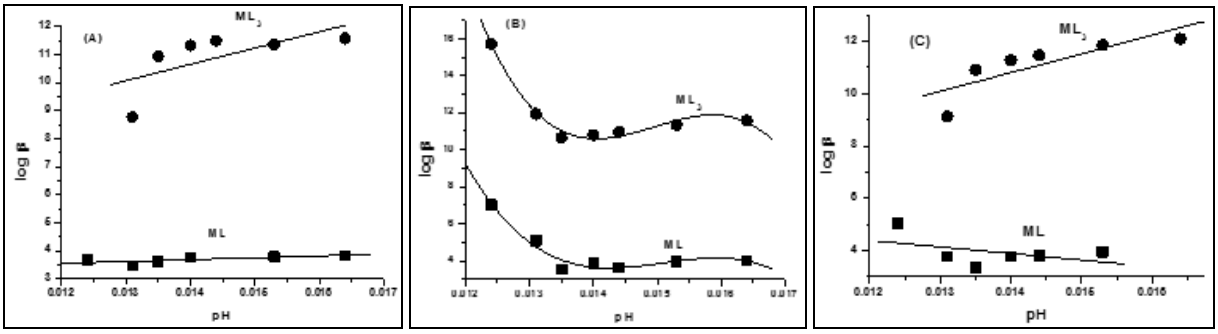

Fig. 1: Variation of log β with reciprocal of dielectric constant of DMF–water mixtures (A) Pb (II), (B) Cd (II) and (C) Hg (II), filled square log β 110, filled circle log β 130

Distribution diagrams

Phen, A neutral base, has only one proton attached to the nitrogen atom under the present experimental conditions. The form of Phen that exist in the pH region of 2.0-6.0 is LH^+. Hence, there is no possibility for the existence of protonated species [48]. The possible species are ML and ML_3 for Pb(II), Cd(II) and Hg(II). The formation equilibria based on the distribution of species with pH are represented below (Fig 2).

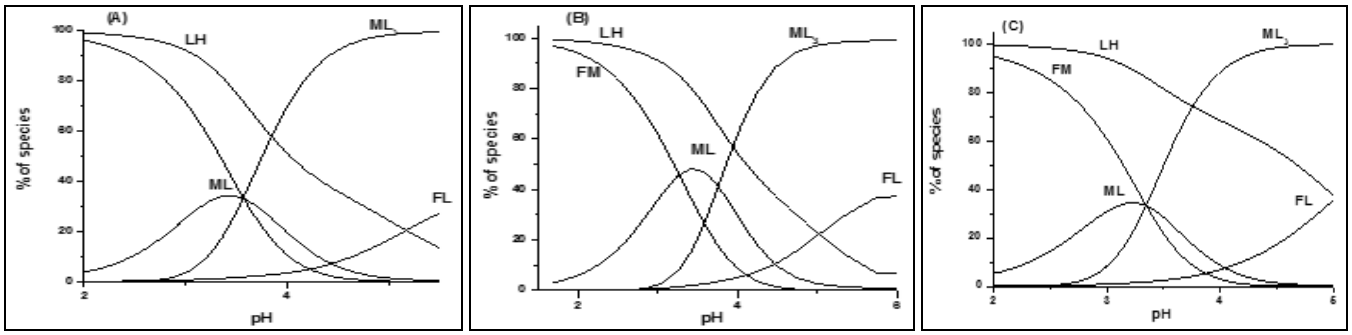

Fig. 2: Distribution diagrams of 1, 10-phenanthroline complexes in 30 % v/v DMF–water mixture. (A) Pb(II), (B) Cd(II) and (C) Hg(II)

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

$$M(II) + 3LH \rightleftharpoons ML_3 + H^+ \quad (2)$$

$$ML + 2LH \rightleftharpoons ML_3 + H^+ \quad (3)$$

Distribution diagrams were drawn for various complex species using the formation constants of the best-fit models as shown in Fig 2. These diagrams indicate that the percentage of ML species of Pb(II),Cd(II) and Hg(II) increases and then

decreases with the increase of pH. The percentage species of ML_3 increases in the present experimental conditions.

Depending upon the active sites in the ligand and the nature of the metal ions, the structures were proposed for the species detected as shown in Fig 3.

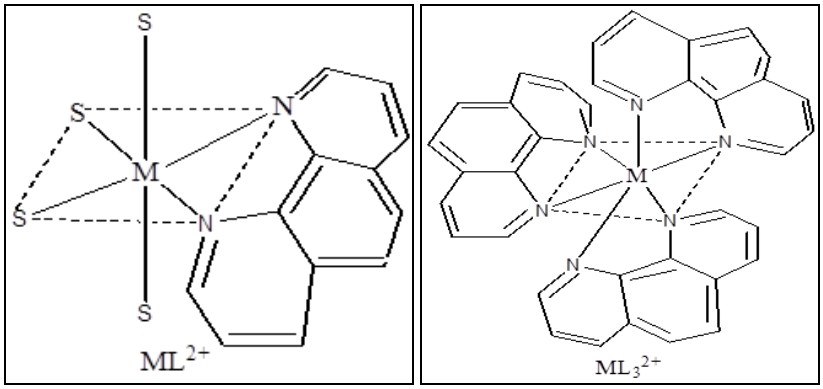

Fig 3: Structures of Metal – Phen complexes where M= Pb, Cd and Hg; L= 1, 10-phenanthroline and S is either solvent or water molecule.

Conclusions

1. The common species formed due to interaction of Phen with the toxic metal ions are ML and ML_3.
2. The linear variation of stability constants of Phen complexes with the reciprocal of dielectric constant of DMF-water mixtures indicates the dominance of electrostatic forces over non-electrostatic forces in case of Pb(II) & Hg(II) and non linear trend in case of Cd(II). A linear increasing trend with DMF content supports the predominance of the structure forming nature of DMF over its complexing ability in case of Pb(II) and Hg(II) and non linear trend of Cd(II) indicates the dominance of non electrostatic forces of electrostatic forces.
3. The order of ingredients in influencing the magnitudes of stability constants due to incorporation of errors in their concentrations is alkali>acid>ligand>metal.
4. At higher pH values, the high concentrations of chemical species indicate that the metals are more amenable for transportation at higher pH values.

References

1. Forster U, GTW Whittmann. Metal Pollution in the Aquatic Environment, Springer-Verlag, Berlin, 1983.
2. Meria E, Metals. Their Compounds in the Environment. Occurrence, Analysis and Biological Relevance, Verlag Chemie, New York, 1991.
3. Chowdhury B, Chandra R. Prog. Food Nutr. Sci, 1987; 11:55-113.
4. CM Freitag. Mol. Psychiatry, 2007; 12:2-22.
5. RC Dart, KM Hurlbut, LV Boyer-Hassen. Medical Toxicology, 3rd edn, Lippincott Williams & Wilkins, Philadelphia, 2004.
6. LJ Casarett, Doull J, CD Klaassen, Casarett, Doull's Toxicology: The Basic Science of Poisons, 7th edn., McGraw-Hill Professional, New York, 2007.
7. Crocetti M, MA Barone, FA Oski, Essential Pediatrics, 2nd edn., Lippincott Williams & Wilkins, Philadelphia, 2004.
8. Filipi M, Fatur T, Vudrag M, Hum. Exp. Toxicol, 2006; 25:67-77.
9. Shaham J, Meltzer A, Ashkenzi R, Ribak J, Occup J. & Environ. Med, 1996; 38:1220-1227.
10. World Health Organization WHO, Cadmium. Geneva: Environmental health criteria, WHO, 1992, 134.
11. Jarup L, Br. Med. Bull, 2003; 68:167-182.
12. International Agency for Research on Cancer IARC, Cadmium in the Human Environment: Toxicity and Carcinogenicity, Lyon, IARC Scientific Publications No. 118, IARC, 1992.
13. AL Miller, Altern. Med. Rev, 1998; 3:199-207.
14. Bremner I, JK Campbell, Environ. Health Perspect, 1978; 25:125-128.
15. CR Bunn, Matrone G, Nutr J, 1966; 90:395-399.
16. BL Vallee, DD Ulmer, Annu. Rev. Biochem. 1972; 41:91-128.
17. Gochfeld M, Ecotox. Environ. Safe. 2003; 56:174-179.
18. Z Li, Q Wei, R Yuan, X Zhou, HZ Liu, HX Shan, *et al.* QJ Song, Talanta. 2007; 71:68-72.
19. Ashkenani H, Dadfarnia S, AMH Shabani, AA Jaffari, A Behjot, J Hazard Mater. 2009; 161:276-280.
20. Ryu S, Hemmingsen L, VL Pecoraro, Chem. Eur. J. 2007; 13:9178-9190.
21. Onyido I, AR Noriis, Buncel E, Chem. Rev, 2004; 104:5911-5929.
22. MR Shehata, MM Shoukry, FMH Nasr, van R, Eldik J. Chem. Soc. Dalton Trans, 2008, 779-786.
23. JJ Gooding, DB Hibbert, Yang W. Sensors, 2001; 1:75-90.
24. FRN Guard, PE Wilcox. Adv. Protein Chem. 1956; 11:311-427.
25. HC Freeman, Adv. Protein Chem., 1967; 22:258-424.
26. Dobbie H, WO Kermack, Biochem J. 1955; 59:246-57.
27. SP Datta, BR Rabin, Trans. Faraday Soc., 1956; 52:1123-1130.
28. WS Kittl, BM Rode, Inorg. Chim. Acta, 1982; 66:105-112.
29. Benzakour M, Mcharifi M, Cartier A, Daoudi A, Mol J. Struct. Theochem, 2004; 710:169-174.
30. MT Rodgers, PB Armentrout, Acc. Chem. Res., 2004; 37:989-998.
31. SJ Lippard, JM Berg, Principles of Bioinorganic Chemistry, University Science Books, Mill Valley, California, 1994.
32. Sigel H, RB Martin, Tribolet R, UK Haring, R.M.

Balakrishnan, Eur. J. Biochem., 1985; 152:187-193.
33. Teigen S, Andersen R. Programme on Marine Pollution PMF. Trace Metals in the Marine Environment: State of the Art and Research Needs, Croatian Society of Chemical Engineers, Zagreb, 1992.
34. DM Di Toro, HE Allen, HL Bergman, JS Meyer, PR Paquin, RC Santore, Environ. Toxicol. Chem., 2001; 20:2383-396.
35. GH Jeffery, Bassett J, Mendham J, RC Denney, eds., Vogel's Text Book of Quantitative Chemical Analysis, 5th edn., Longman, London, 1991.
36. FJ Weltcher. The Analytical use of Ethylenediaminetetraacetic Acid, DVan Nostrand Company, New York, 1957.
37. RS Rao, GN Rao. Computer Applications in Chemistry, Himalaya Publishing House, Mumbai, 2005.
38. Gran G, Anal. Chim. Acta, 1988; 206:111-123.
39. Gonzalez G, Rosales D, JLG Ariza, AG Perez, Talanta, 1986; 33:105-106.
40. LG Van Uitert, CG Haas, Am J. Chem. Soc., 1953; 75:451-455.
41. Padmaja N, MS Babu, GN Rao, RS Rao, KV Ramana, Polyhedron, 1990; 9:2497-2506.
42. GN Rao. Complex Equilibria of some Biologically Important Metal Ions in Aqua-Organic Media, Ph.D. thesis, Andhra University, Visakhapatnam, India, 1989.
43. Gans P, Sabatini A, Vacca A, Inorg. Chim.Acta, 1976; 18:237-239.
44. Hamilton WC. Significance tests on the crystallographic R factor. Acta Cryst 1965; 18:502-510.
45. MT Zekarias, BY Hirpaye, GN Rao, Der Pharma Chem., 2011; 3:69-77.
46. Latha MP, Rao VM, Rao TS, Rao GN. Determination of protonation constants of L-glutamic acid and L-methionine in 1,2- propanediol–water mixtures. Acta Chim Slov 2007; 54:160-165.
47. Akhadov YY. Dielectric properties of binary solutions. Pergamon Press, Oxford. 1980.
48. Padmaja N, Babu MS, Rao GN, Rao RS, Ramana KV. Effect of urea on the speciation of 1, 10-phenanthroline complexes of toxic metal ions: computer augmented modeling studies Polyhedron 1990; 9:2497-2506.