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Chelation Study of Captopril with Cd^{2+} and Pb^{2+} Ions

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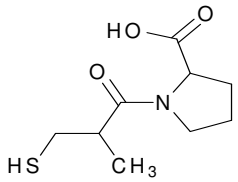
Abstract: The protonation constants of Captopril, (1-(3-mercapto-2-(S)-methyl-1-oxopropyl)-S(L) proline, (CPL) and stabilities of its two divalent metal ions Cd(II) and Pb(II) were determined potentiometrically in two different metal to ligand ratio 1:1 and 1:2 systems and in water and methanol-water binary mixtures using the computer Best program. Two protonation constants were obtained which were assigned to the carboxylic and thiol groups. All protonation and stability constants increased with decreasing the dielectric constant on going from the pure water to the binary mixtures. An excellent similarity in stabilities of studied metal ions strongly suggests that both metal ions are coordinated by CPL in a same manner through the thiol group.

Key words: Potentiometry, stability constant, captopril, binary solvents mixture

INTRODUCTION

Captopril (CPL) is an effective hypertensive agent which was made based on active fragment of nano peptide, one of the most active components of the South American Snake's Venoms[1-4]. It is applied successfully for treatment of high blood pressure disease in many patients whose hypertension could not be controlled by other available drugs[5-9]. It inhibits the active sites of zinc glycoprotein, the angiotension I converting enzyme (ACE), blocking the conversion of angiotension I to angiotension II, whose levels are elevated in patients. The key functional group in the metabolism of CPL is the thiol group[4,7,10,11].



Scheme: Structure of Captopril

Over the last decade, close attention has been paid to the interaction of Captopril with various metal ions.[12-21] CPL has three different functional groups, which can involve in coordination to metal ions. These coordination sites have different hard and soft nature, so have different affinity toward different metal ions and so may be coordinated from different sites. In addition to mode of coordination, different metal-Captopril mole ratio can be obtained depending to the experimental conditions. In acidic pH, the ML complex is more stable, whereas, in the pH range 6.0-8.2, the ML_2 complex is the main species[12]. For example, ZnL has a polymeric structure where the zinc is coordinated by the thiolate and carbonyl oxygen of one ligand and the bidentate carboxylate group of another. In the ZnL_2 complex, two molecules of ligand are bound via sulfur and carbonyl oxygen[13-14]. In copper case, however, EPR spectra indicated that binding to Cu (II) occurs via the ligand oxygen's rather than the thiol moiety[15]

MATERIALS AND METHODS

Subjects and samples: The CPL was purchased from the Merck and was used without any further purification. Metal ions were prepared from analytical grade nitrate salt (Merck) and their stock solutions were standardized complexometrically by EDTA titration[22]. All KOH solutions were prepared with doubly distilled water and standardized against potassium hydrogen phthalate (KHP) with phenol phthalein as an indicator. The HNO_3 solution (0.1 M titrasol) was standardized with standard KOH.

Potentiometric titrations were performed by means of a Hana-pH 300 series Bench -Top pH meter equipped with a Metrohm piston burette (715 Dosimat) with a 5.0 mL exchange unit that was used for precise delivery of the standard KOH. The pH meter was calibrated to read hydrogen ion concentration in aqueous buffer solutions based on Bates *et al.*[23]. The pH read on the pH meter are corrected by introduction

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the parameter δ using the equation pH(C) = pH(R)-δ where pH(C) is the corrected pH and pH(R) is the pH meter reading. The δ is the differences in activity coefficients and liquid junction potential. Samples were titrated in a double-walled glass cell maintained at 25±0.1°C by circulating water and stirred magnetically under a continuous flow of purified nitrogen. The pH range for accurate measurements was considered 2-12. CPL protonation constants and CPL-Cd (II) and CPL-Pb (II) metal complexes in water and binary methanol-water mixtures were calculated using the program BEST described by Martell and Motekaitis[24].

RESULTS AND DISCUSSION

The protonation constants of Captopril has been determined using potentiometric techniques and calculated using the computer Best program. The results are shown in Table 1.

The corresponding dielectric constants of these solutions are also evaluated by interpolation of the data of Akerlof[25-26] and listed for comparison. Two protonation constants were obtained which were assigned to the carboxylic and thiol group, respectively. The obtained values in pure water are in good agreement with the previous values[12,27] which are shown in Table 1 for comparison. It is interesting to note that the composition nature of the binary mixture has a distinct effect on the protonation constants. Both protonation constants increase with increasing the mole fraction of methanol and/or with decreasing the dielectric constant of the binary mixture. Table 1 shows a linear relationship between the Log KH_L and reciprocal of dielectric constant as a typical. This order can be interpreted by solvent effects on proton transfer reactions as Grunwald and Coworker have studied previously[28-29]. It has been shown that the solvating ability[30] (as expressed by the Gutmann donicity scale) and dielectric constant of the solvent play a fundamental role in protonation reactions. It has been reasonably assumed that preferential salvation of the charged particles by water is mainly responsible for such a monotonic dependence of the acidity constants of CPL on the solvent composition. It is clear that the dissociation of an uncharged acid in a solvent requires the separation of two ions of opposite charges. The work required to separate these charges in inversely proportional to the dielectric constant of the solvent. The energy required for dissociation is supplied by salvation of the ions and also the proton transfer from the acid to the solvent molecule supplies an additional energy. If the dielectric constant and the solvating ability of the solvent are decreased, more energy will be required to separate the cation and anion and consequently the extent of dissociation of the acid will be lowered. Water is a solvent of high solvating ability, (i.e. donor number DN = 33[31] and dielectric constants ε = 78) which can dissociate the acid and stabilize the produced anion and hydrogen ion. Thus, it is expected that addition of methanol with lower donor number and dielectric constant (DN = 19 and ε = 32.6) respect to water, decreases the extent of interaction of the solvent and ligand dissociation products and so decreases the acidity constant of acid. The same trend has already been reported for various organic molecules in different solvent mixtures[32-33].

Table 1: Protonation constants of CPL in water and binary mixtures of Methanol-Water at 25°C and ionic strength 0.1 M (KNO_3)

Water	Log ß$_1$	Log ß$_2$
(D = 80) a	(-SH)	(-COOH)
(σfit = 0.018)	9.83	13.64
20%	9.68b,	13.15 b,
(D = 69)	9.88c	13.40 c
(σfit = 0.013)	10.56	14.54
35%	10.7	15.06
(D = 61)	10.82	15.37
(σfit = 0.015)		
50%		
(D = 54)		
(σfit = 0.01)		

[a] D: Dielectric constant, [b] Ref. 12, [c] Ref. 20

The distribution curves of species in binary mixtures are shown in Fig. 1.

The most important features of distribution diagrams are the pH limit of evolving and disappearance of compounds. So according to distribution diagrams at smaller pH than 4, the H_2L form is dominated. The HL^- and L^{2-} forms appeared at pH intervals 6-10 and pH>10, respectively.

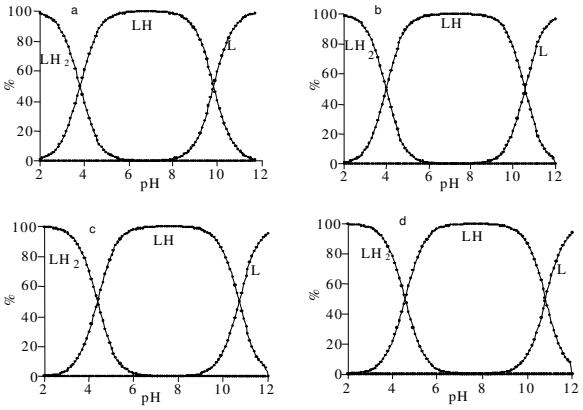

Fig. 1: Species distribution diagram for CPL(L) in (a) water, (b) 20%, (c) in 35% and (d) 50% (Methanol-Water) at 25°C and ionic strength 0.1 M (KNO_3)

Complexation of Cd(II) and Pb(II) with CPL: CPL has three functional groups with different hard-soft nature and so, can be coordinated to soft metal ions through soft thiol donating group. The stabilities of Pb(II) and Cd(II) complexes of CPL were determined by potentiometric technique and are summarized in Table 2. The distribution diagrams of M-CPL are shown in Fig. 2-5.

The resulted stability constants shown in Table 2 show that the stability of all complexes increases gradually with increasing the amount of organic solvent. This trend is the same as protonation constants of the ligand and can be interpreted by the same explanation.

Figure 2 shows the species distribution diagrams for Pb-CPL (1:1) systems in the pure water and methanol-water binary mixtures.

The main metal species in all systems are ML and M_2L_3. In the aqueous system (Fig 2a), in addition to these species, MLH is observed as a main species in acidic region which is gradually converted to ML by increasing the solution pH. This species may be produced by metallation of LH species and/or metallation and subsequently deprotonation of LH_2 species. The concentration of MLH increases while increasing the LH and decreasing the LH_2 concentration. In addition, decrease in metal ion concentration is occurred at lower pH respect to binary mixtures. So, the MLH is probably obtained from LH_2 via metallation-deprotonation path. If the MLH was produced via directly metallation of LH, the MLH might be expected to be present in observable amount in 20 and 35% binary mixtures with relative significant concentration of LH. The absence of MLH species in binary mixtures may be due to relative higher stability of LH_2 in binary mixtures respect to pure water, which prevents the metallation followed by deprotonation of LH_2. The CPL is probably coordinated to Pb(II) as a monodentate ligand in MLH species and may become bidentate while second deprotonation step in ML species. Proton dissociation led some potentially linkable position on the ligand, which initially were occupied by the protons. This feature enforces the system to form multinuclear species such as M_2L_3, which has not significant concentration in pH below 4.

In 2:1 system (Fig. 3), the main species in binary mixture solvents are ML, M_2L_3 and M_2L_4. Again, additional MLH species seems to be important in pure water (Fig 3a). The formation of MLH even before formation of LH strongly supports its formation from LH_2 via metallation-deprotonation path, which takes place at more acidic pH compare to 1:1 system. Contrast to 1:1 system, the M_2L_4 species is observed

Table 2: Stability constants of CPL complexes (2:1) in water and 20%, 35%, and 50% (Methanol-Water) at 25°C and ionic strength 0.1 M (KNO_3)[a]

	Cd				Pb			
	0%	20%	35%	50%	0%	20%	35%	50%
ML	749%	837%	914%	965%	7.81	8.5	9.24	9.62
MLH	-6.98	-8.12	-8.56	-9.08	-7.5	-8.34	-8.91	-9.5
ML2	12.61				12.76			
ML3	-12.57	14.22	14.75	16.49	-12.51			
M2L3	13.62							
M2L4	16.9	26.66	27.62	29.6	16.3	26.85	28.06	29.44
	24.86	-27.69	-28.8	-30.55	24.98	-27.65	-29.1	-30.95
	-24.31				-24.75	30.07	32.03	35.26
					28.69			

[a]Values in parentheses are from 1:1 metal to ligand concentration ratio

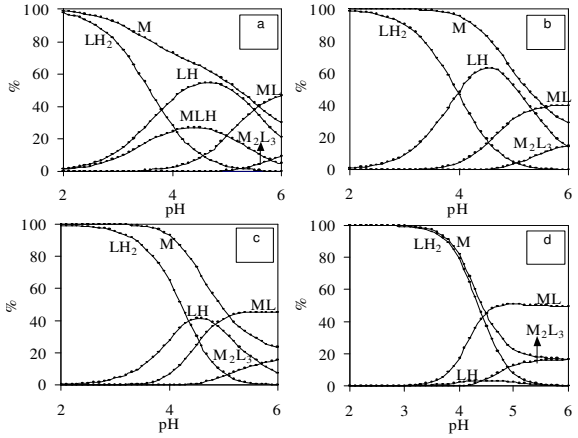

Fig. 2: Species distribution diagram for CPL-Pb (1:1) in (a): water, (b): 20%, (c): 35% and (d): 50% (Methanol-Water) at 25 °C and ionic strength 0.1 M (KNO_3)

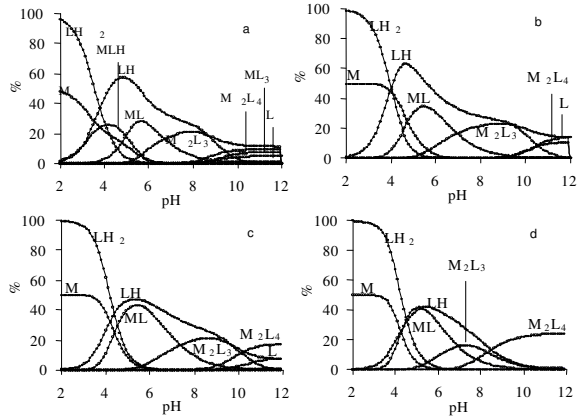

Fig. 3: Species distribution diagram for CPL-Pb (2:1) in (a): water, (b): 20%, (c): 35% and (d): 50% (Methanol-Water) at 25 °C and ionic strength 0.1 M (KNO_3)

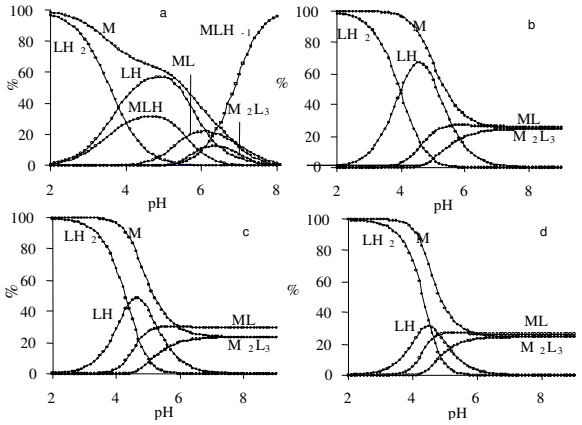

Fig. 4: Species distribution diagram for CPL-Cd (1:1) in a: water, b: 20%, c: 35%, and d: 50% (Methanol-Water) at 25°C and ionic strength 0.1 M (KNO_3)

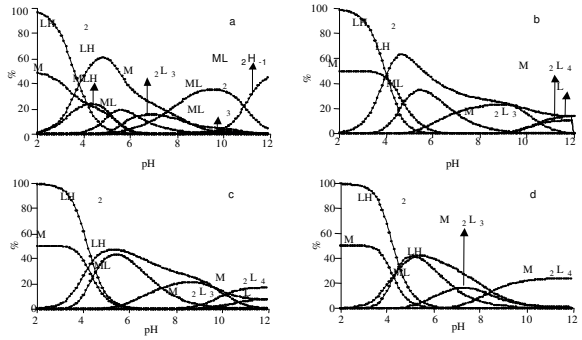

Fig. 5: Species distribution diagram for CPL-Cd (2:1) in (a): water, (b): 20%, (c): 35% and (d): 50% (Mathanol-water) at 25°C and ionic strength 0.1 M (KNO_3)

which becomes more significant on going into binary mixtures. A series of complex equilibria may be responsible for producing the M_2L_3 and M_2L_4 complexes. Simultaneous collision of MLH, ML and LH at pH about 5 may be responsible for formation of M_2L_3 whereas, the M_2L_4 is probably produced by combination of M_2L_3 and LH followed by a fast deprotonation at pH above 8. This suggested formation pattern could be supported from the sharp descending of LH concentration in this pH region.

In Cd-CPL (1:1) system (Fig. 4), the main species in binary mixtures are ML and M_2L_3. Interestingly, species distribution diagrams have distinct difference with that of pure water. Again, additional MLH and also MLH_{-1} species were observed in pure water diagram. The later species was not observed in the Pb(II) case due to limitation in the studied pH range. The absence of MLH in the binary mixtures may be interpreted similar to the Pb(II) case. The metallation and subsequently deprotonation of LH produces the MLH, which converts to MLH_{-1} on going to higher pH Fig 4a. Similarly, multinuclear species such as M_2L_3 is produced by linking the ML and ML_2 monomers via vacant positions on the ligand, which initially were occupied by the protons. In pure water, the concentration of M_2L_3 decrease with increasing pH above 6, whereas in binary mixtures, M_2L_3 along with ML, are dominant species in mentioned pH range. In addition, their concentrations seem to be pH-independent. Since M_2L_3 obtained by aggregation of ML and ML_2 species, the following equilibria can explain the distribution curves at pH above 6

$$M+L \Leftrightarrow ML \qquad K_1$$
$$ML+L \Leftrightarrow ML_2 \qquad K_2$$
$$ML+ ML_2 \Leftrightarrow M_2L_3 \qquad K_3$$

The absence of ML_2 and relatively significant presence of M_2L_3 strongly suggested that the consumption path of ML_2 (K_3) is much greater than its formation (K_2). In the other hand, the ML_2 species is consumed very faster than its production.

A different pattern is observed in 1:2 system wherein, the M_2L_4 (or ML_2) species becomes the main species in basic region (Fig. 5). The formations of multinuclear species are prevented due to the presence of relatively lower amount of the metal ion. The high stability constant of ML_2 led reduction and/or removing of buffered region.

Hughes and Coworker have reported the formation of Cd-CPL complexes such ML, ML_2, ML_3, M_2L_3, M_4L_4 in the presence of NaCl as supporting electrolyte in aqueous solution with relatively large stability constants. The absence of some species in our study may be due to the different supporting electrolyte used in this study and/or the higher concentration of the ligand necessity for the formation of such species.

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