Study on mixed ligand complexes of Pb(II) with EDTA and dietary ligands to investigate the applicability on chelation therapy

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Abstract: Lead is the most distributed toxic metal in the world and chelation therapy is a remedy for removing it from the body, if a person is intoxicated. Stability constants of both binary and mixed ligand complexes of Pb(II) with salicylic acid, ascorbic acid, vitamin B₁(thiamine), vitamin B₁₂(niacin) and Na₂EDTA were potentiometrically studied, in aqueous media at the temperature of 37.0 ± 0.2 °C and the ionic strength of 0.15 mol dm⁻³ in order to identify better mixed ligand systems suitable for chelation therapy of lead. Both metal-ligand and proton-ligand formation constants were calculated using Irving-Rossotti equations. The Formation of ternary complexes was further confirmed theoretically by plotting composite curves. Δ log K values calculated revealed that all mixed complexes were more stable than the complexes of corresponding binary systems. EDTA-Vitamin B₁ and EDTA-Ascorbic mixed complexes with Pb(II) disclosed very high stability constants, which were two times greater than that of EDTA binary complex. Vitamin B₁ revealed a remarkably high ability in forming mixed ligand complexes with all other four ligands. Results of this work clearly indicate that (EDTA-Vitamin B₁), (EDTA-Ascorbic), (Vitamin B₁₂-Ascorbic), (Vitamin B₁₂-Salicyclic) and (Vitamin B₁₂-Vitamin B₁) combinations can be used for detoxification of lead more effectively using dietary ligands.

Keywords: Stability constants, in vitro conditions, chelation therapy, thiamine, niacin, vitamin C, salicylic acid.

INTRODUCTION

Today, lead (Pb) is considered as the most widely distributed toxic metal ion in the world due to many of its industrial applications (Das, 1990; Morose et al., 2008; Turner & Fader, 1962). Meanwhile, a number of serious health problems are associated with lead toxicity (Das, 1990; Branica et al., 2006; Chisolm, 1968). The only way of curing lead toxicity is chelation therapy, which comes in two modes; single ligand and mixed ligand chelation therapy. This involves the formation of chelate complexes of toxic metal ions with administered drugs which can be easily excreted via urine or feces (Chisolm, 1968; Goyer & Cherian, 1978; May and Williams, 1979; Bulman et al., 1979; Das, 1990; Wang et al., 2006). On the other hand, single and mixed ligand chelates of metals with various ligand molecules have been studied extensively related to various aspects quantitatively (Nakayama et al., 1989; Florence 1982; Mildvan et al., 1967; Shelke & Jahagirdar, 1976; Makkell and Carey, 1967; Moustafa, 2005) and many of the mixed chelate systems of bio-molecules with biologically important metals like Cu, Ni and Zn investigated were used as models of biochemistry to understand more complicated biological reactions (Mildvan & Cohn, 1966; El-Haty et al., 1995; Brumas et al., 1995; Kleszczewska, 1999; Bucci, 2000).

Though quantitative studies and stability constants of mixed ligand complexes of Pb(II) with some ligands have been reported (Gupta and Sharma, 1982; Josaghani et al., 2008; Padmaja & Rao, 2012; Thanavelan et al., 2014), the information related to the quantitative investigations on mixed ligand complexes of Pb(II) with biologically important molecules is scarce, especially under the physiological conditions (Chandrathilaka et al., 2013). Thus, the present work was mainly undertaken to study the stability of mixed ligand complexes of EDTA with biologically significant molecules entering into our body via common drugs and food, as an attempt to introduce more effective and less toxic mixed ligand systems for the use in chelation therapy of Pb(II). In order to have more reliable results, physiological conditions were applied during these experiments to study the aqueous

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coordination chemistry of selected ligands; ascorbic acid (AsC), salicylic acid (Sali), vitamin B<sub>1</sub> (Vit B<sub>1</sub>), vitamin B<sub>3</sub> (Vit B<sub>3</sub>) and Na<sub>2</sub>EDTA with Pb(II) using potentiometric pH titration data and UV Visible spectroscopic data.

**MATERIALS AND METHODS**

**Materials**

Following chemicals of analytical grade were used as received for the potentiometric experiments:

HNO<sub>3</sub> (Sigma), NaOH (Sigma), NaNO<sub>3</sub> (Sigma), salicylic acid (Aldrich), vitamin B<sub>1</sub> (Hebei Medipharm Co. Ltd.), Ascorbic acid (Sigma), vitamin B<sub>3</sub> (Western Drugs Pvt. Ltd.), Pb(NO<sub>3</sub>)<sub>2</sub> (Aldrich), Na<sub>2</sub>EDTA (Aldrich).

1×10<sup>-2</sup> mol dm<sup>-3</sup> standard solutions of the metal and all ligands were freshly prepared using double distilled deionized water. Standardizations of NaOH (0.03 mol dm<sup>-3</sup>) and HNO<sub>3</sub> (0.03 mol dm<sup>-3</sup>) solutions prepared were carried out with primary standards, potassium hydrogen phthalate and sodium carbonate respectively.

**Potentiometric studies**

The pH measurements of the potentiometric titrations were carried out with a MARTINI MI 151 pH meter equipped with a MA 917 combination electrode and a temperature probe.

Following mixtures were prepared in a total volume of 40.00 mL by adding required amounts of double distilled deionized water at the ionic strength of 0.15 mol dm<sup>-3</sup> of NaNO<sub>3</sub> for potentiometric titrations. (Note: L, X and M denote ligand 1, ligand 2 and metal ion respectively)

(i) HNO<sub>3</sub> (10.00 mL); (ii) HNO<sub>3</sub> + L (10.00 mL); (iii) HNO<sub>3</sub> + X (10.00 mL); (iv) HNO<sub>3</sub> + L/X (10.00 mL) + M (10.00 mL); (v) HNO<sub>3</sub> + L (10.00 mL) + X (10.00 mL) + M (10.00 mL)

Each solution prepared was titrated against the standardized NaOH solution at 37.0 ± 0.2°C (a YCW – 05 model thermostatic water bath was used to maintain the temperature) and the pH was recorded after the addition of 0.20 mL aliquots of NaOH. Nitrogen was purged through the reaction mixtures to ensure the absence of oxygen and carbon dioxide in reaction mixtures.

**Computation of stability constants**

**Binary systems**

According to the method described by Irving and Rossoitti (1953 and 1954), the degree of formation of proton-ligand complex can be defined by a parameter n<sub>A</sub>, which is the average number of protons associated with a ligand molecule.

\[
 n_A = \frac{\text{total conc. of protons bound to ligand}}{\text{total conc. of ligand not bound to metal}}
\]

Similarly, for the metal ligand complex, average number of ligand molecules attached per metal ion, n can be defined using the relationship,

\[
 n = \frac{\text{total concentration of ligand bound to metal}}{\text{total concentration of metal}}
\]

Values of n<sub>A</sub>, n and the free ligand exponent (pL) can be obtained using following expressions derived by Irving and Rossoitti (equations 1, 2 and 3).

\[
 n = \frac{(V_1 - V_2) \times (N + E + T_L(Y - n_A))}{(V_0 + V_2) n_A T_M} \tag{1}
\]

\[
 n_A = Y + \frac{(V_1 - V_2) \times (N + E)}{(V_0 + V_1) T_L} \tag{2}
\]

\[
 pL = \log \left[ \sum_{\text{diss}} \beta_n \cdot (\text{anti log}(pH))^{-n} \cdot (V_0 + V_1) \right] \tag{3}
\]

The titration curves of the mixtures, (i), (ii) and (iv) and (iv) were used to calculate n<sub>A</sub>, n and pL, V<sub>f</sub>, V<sub>2</sub>. V<sub>2</sub>, V<sub>f</sub> are the volumes of alkali consumed to reach the same pH value in the mixtures (i), (ii) and (iv) respectively and V<sub>0</sub> is the total initial volume of the titration mixture. N, E and T<sub>L</sub> are the total initial concentrations of NaOH, HNO<sub>3</sub> and ligand respectively. Y is the number of dissociable protons attached per molecule of each ligand. Values of Y are 1, 3, 2, 0 and 2 for the ligands: salicylic acid, vitamin B<sub>1</sub>, ascorbic acid, vitamin B<sub>3</sub> and Na<sub>2</sub>EDTA respectively.

From these data, the proton-ligand and metal-ligand stability constants were calculated using Microsoft Excel. Formation curves for the
proton-ligand and metal-ligand complex systems were obtained by plotting $n_A$ Vs pH and $n$ Vs pH respectively. To obtain the value of $K_{H}^j$, all the $K_{H}^j$ values were averaged in the range $0 < n_A < 1$. For $K_{H}^2$ a similar procedure was adopted taking values in the range $1 < n_A < 2$. Calculations of $K_{H}^j$ for each $n_H$ value were carried out using equation 4. Similarly, $K_H^j$ and $K_H^2$ were calculated from equation 5. To ensure accuracy, each titration was carried out in triplicate.

$$K_{H}^j = \frac{(n_H - j + 1)}{[\{j - n_H\} \times [H]]}$$

(4)

$$K_{H}^n = \frac{(n - n + 1)}{[\{n - n\} \times [L]]}$$

(5)

**Ternary systems**

Considering the Irving and Rossotti equations derived for binary complexes, a new set of equations was obtained for the calculation of the stability constants of ternary complexes (Chidambaram & Bhattacharya 1970a; Chidambaram & Bhattacharya 1970b). Expressions for average number of secondary ligand ($X$) molecules attached per complex $ML$, $n_{mix}$ and free secondary ligand exponent, $p^l_{mix}$ are given in equations 6 and 7.

Titrations curves (iii) and (v) were used to calculate $n_{mix}$ and $p^l_{mix}$. $V_x$ and $V_5$ are the volumes of alkali consumed to reach the same pH in mixtures (iii) and (v) respectively. $n_{X}^{5}$ is the average number of protons of the secondary ligand $X$. Stability constant of the ternary complex $MLX$ formed by reacting binary complex $ML$ with free ligand $X$, has been obtained by averaging formation constant values obtained in the range $0 \leq n_{mix} \leq 1$. The ligand that exhibited a higher formation constant in binary complex formation was considered as the primary ligand of the mixed ligand system depending on the ligand combinations.

$$n_{mix} = \frac{(V_4 - V_{X}) \times (N + E + T_X (Y - n_X))}{(V_0 + V_X) n_X^{T_{ML}} T_{ML}}$$

(6)

$$p^l_{mix} = \log \left[ \frac{\sum_{n=1}^{n_{mix}} \beta_n (antilog(pH) - n_{mix}) (V_0 + V_5)}{(T_{L} - n_{mix} T_{ML}) V_0} \right]$$

(7)

By most of the lead complexes found in literature is octahedral in shape (Battistuzzi et al., 1996; Evans et al., 2003; Najafpour et al., 2007; Davidovich et al., 2009). Therefore, assuming that lead complexes formed in aqueous medium under physiological conditions are also in octahedral shape, stabilities of mixed ligand complexes over single ligand complexes were compared by calculating $\Delta \log K$ values according to the literature (Sigel, 1975; Maktell & Carey, 1967; Shelke & Jahagirdar 1976).

**RESULTS AND DISCUSSION**

Variations in the pH values according to the volumes of alkali added are given in Figure 1 for a system which formed a mixed ligand complex and in Figure 2 for a system which did not form a mixed complex, as two representatives. In Figure 1, it can be clearly seen that the ternary system titration curve has deviated from two binary system titration curves significantly, where as in Figure 2 it has superimposed with the titration curve of the primary ligand, salicylic acid. Similarly, titration curves of other systems forming mixed ligand complexes under the physiological conditions, revealed the same behavior providing preliminary information about complexation. As each titration was carried out until a permanent turbidity was observed in the reaction mixture, at that pH value, it was considered that the complex formation was disturbed by the formation of insoluble lead hydroxide.
Protonation constants calculated for five ligands are given in the Table 1 and the overall formation constants of binary and ternary systems and pH values, at which the permanent turbidity was observed are illustrated in Table 2. Accordingly, all ligands have formed ML type binary complexes with Pb(II) within the studied pH ranges before the formation of Pb(OH)$_2$. Observation of permanent turbidity at higher pH values in the mixed ligand systems compared with binary systems implies that, Pb(II) interacts more strongly with ligand mixtures than with a single ligand, by forming more stable mixed ligand complexes. Results reveal that among ten mixed ligand systems investigated, in M:L:X in 1:1:1 ratio, only five ternary complexes were formed. Mixed ligand complex formation was further confirmed by recording UV-Visible spectra for binary and ternary systems at pH 4, 5 and 6 under the physiological conditions.
Table 1: Protonation constants of ligands.

| Ligand       | Log $K_H^1$ | Log $K_H^2$ | Log $K_H^3$ |
|--------------|-------------|-------------|-------------|
| Salicylic acid | 2.76        | -           | -           |
| Vitamin B1   | 9.13        | 8.85        | 4.32        |
| Ascorbic acid | 10.00       | 5.28        | -           |
| Vitamin B3   | -           | -           | -           |
| Na$_2$EDTA   | 9.19        | 5.69        | -           |

Table 2: Overall stability constants ($\log \beta$) for binary (1:1) and mixed ligand systems (1:1:1) at concentration 0.0025 M.

| L            | X            | pH at permanent turbidity | $\log \beta_1^a$ | $\log \beta_2^a$ |
|--------------|--------------|---------------------------|-------------------|-------------------|
| Salicylic acid | -            | 5.80                      | 2.67              | -                 |
| Ascorbic acid | -            | 6.6                       | 3.53              | -                 |
| Vitamin B1   | -            | 6.8                       | 3.83              | -                 |
| Vitamin B3   | -            | 5.64                      | 2.29              | -                 |
| Na$_2$EDTA   | Vitamin B1   | -                         | -                 | 26.47             |
| Na$_2$EDTA   | Ascorbic acid| -                         | -                 | 25.42             |
| Vitamin B1   | Ascorbic acid| 7.12                      | -                 | 7.39              |
| Na$_2$EDTA   | Salicylic acid| -                        | 13.53             | -                 |
| Vitamin B1   | Salicylic acid| 7.74                      | -                 | 7.08              |
| Ascorbic acid | Salicylic acid| 7.04                      | 3.53              | -                 |
| Na$_2$EDTA   | Vitamin B1   | -                         | 13.53             | -                 |
| Vitamin B1   | Vitamin B3   | 7.56                      | -                 | 6.9               |
| Ascorbic acid | Vitamin B3   | 5.81                      | 3.53              | -                 |
| Salicylic acid | Vitamin B1   | 6.13                      | 2.67              | -                 |

$\beta_n = K_1.K_2.K_3.\ldots\ldots\ldots.K_n$

Figure 3: (a) UV absorption spectra for binary complexes; Pb(Vit B1), Pb(Sali) and Pb(Vit B1-Sali) systems at three different pH values; 4, 5 and 6. (b) UV absorption spectra for binary complexes; Pb(AsC), Pb(Sali) and Pb(AsC-Sali) systems at three different pH values; 4, 5 and 6.
According to the Figure 3a, mixed ligand system has a $\lambda_{\text{max}}$ value in between the $\lambda_{\text{max}}$ values of the two binary systems. This indicates that the mixed system contained a species different from either of binary systems. Thus, the new species must be the mixed complex of Pb(II) with two ligands. Similarly, rest of the systems which formed mixed complexes showed the same behavior by proving the formation of ternary complexes under the physiological conditions.

In Figure 3b, a new peak did not appear for a mixed ligand system at a different $\lambda_{\text{max}}$; instead a broader peak area appeared with two maximum regions having their $\lambda_{\text{max}}$ values closer to the $\lambda_{\text{max}}$ values of each parent binary complex. This gave a clue for a system consisting of two different binary complexes. Thus, such a system cannot be expected to form any mixed ligand complex under the experimental constitutions.

On the other hand, theoretically, by plotting composite curves, which were obtained by adding the horizontal distance (volume of alkali consumed) of the secondary ligand curve (X) to the horizontal distance of the Pb(II)-L curve at the same pH (Maktell and Carey, 1967; Shelke and Jahagirdar, 1976) mixed ligand complex formation was proved. Volumes consumed by the ternary system titration curve were compared with the corresponding volumes of composite curve at the same pH by plotting them in a graph. Figure 4a shows a representative composite curve drawn for (EDTA+Vit B$_1$+Pb) system forming a ternary complex, in which the composite curve does not overlap with its titration curve of the mixed ligand system (mixture v) and which proves the formation of a mixed ligand complex in the considered system. Here, the titration curve of the mixed ligand system is on the left hand side of the composite curve within the pH range 2-10. Hence, the complexation can be assumed to take place by removing all possible protons in both ligands as given bellow (eq. 8). Similar types of composite curves were given by the other systems forming ternary complexes.

![Figure 4](image_url)

**Figure 4:** (a): Composite curve (2) and mixed ligand titration curve (1) for Pb(EDTA-Vit B$_1$) ternary system. (b) Composite curve (2) and mixed ligand titration curve (1) for Pb(Sali-Vit B$_3$) ternary system.

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Being a borderline metal, lead is presumed to have strong interactions with the donor atoms S and N preferably and as well as with O (Das, 1990; Chisolm, 1968). Thus, one can expect considerable interactions between Pb(II) and any molecule containing at least one of the above mentioned donor atoms. A chelate ring can be formed, when several donor atoms in the same molecule to bind with lead. According to the aspects in coordination chemistry, five membered chelate rings in complexes are more stable than six membered rings (Chisolm, 1968). When taking into account the geometrical and electronic factors affecting the stabilities of complexes, structures with several chelate rings are the most stable and those with monodentate ligands are the least stable. Thus, it is obvious that hexadentate chelator, EDTA, forms the most stable complex with Pb(II) with the highest formation constant, when compared with other ligands. Vitamin B$_1$, which carries specially S and N atoms as main ligating sites and O atom as the third ligating site, forms the next strongest chelate ring with Pb(II). Ascorbic acid is liable to form a five membered ring with Pb(II) through its two oxygen atoms and salicylic may forms a six membered ring with Pb(II) through its carboxylic and hydroxyl groups. Thus, Asc-Pb(II) complex should be more stable than Sali-Pb(II) complex. Values obtained for these two complexes obey with this theoretical aspect. Vitamin B$_3$ the only monodentate ligand in the set has formed the least stable complex with Pb(II). The structure of vitamin B$_3$ reveals its importance as a chelating agent in medical applications (Sasser et al., 1984; Cheong, 1999). It is interesting to see that only vitamin B$_1$ has formed mixed complexes with all the other ligands under the conditions used. It is clear with regard to stability constants that ternary systems of Pb(II) forming with EDTA-Vit B$_1$ and EDTA-AscC are more than two times stable than EDTA binary system. On the other hand, mixed ligand combinations of Vit B$_1$-AscC, Vit B$_1$-Sali and Vit B$_1$-Vit B$_3$ also have formed fairly stable mixed complexes with Pb(II). Intermolecular interactions arising between two species keep them very closer to each other forming stable molecular combinations in solutions and this phenomenon lead to form more stable mixed ligand complexes in solutions (Sigel, 1975; Cheong et al., 1999; Luth et al., 1999). Yamauchi and Odani (1981) have extensively studied these interactions using acidic and basic amino acids and have proven that two ligands together can contribute remarkable stability for mixed complexes compared with their binary systems. According to the Table 3, it is clearly understood that all the ternary systems formed are much more stable than their parental binary systems attribute to the strong non bonded interactions with in the mixed systems formed. As given in Figure 5, some ligand-ligand interactions due to hydrogen bonding can be expected within each mixed ligand system formed and they can increase the stability of mixed complexes. The most suitable coordinating sites of Vit B$_3$ are its pyridine nitrogen atom and carbonyl oxygen atom (Najafpour et al., 2007). Even though, less stable binary complexes are formed through either of these sites, more stable Pb(Vit B$_3$)(Vit B$_3$) mixed complex can be formed, as a result of some favorable ligand-ligand interactions between them.

Table 3: $\Delta \log K$ values obtained for mixed complexes.

| Mixed complex system          | $\Delta \log K$ |
|-----------------------------|-----------------|
| Pb(EDTA)(Vit B$_1$)          | 9.11            |
| Pb(EDTA)(AscC)               | 8.36            |
| Pb(Vit B$_1$)(Sali)          | 0.58            |
| Pb(Vit B$_1$)(Vit B$_3$)     | 0.78            |
| Pb(Vit B$_1$)(AscC)          | 0.03            |
Figure 5: Proposed structures for mixed ligand complexes in *in-vitro* conditions; (a) Pb(EDTA)(Vit B₁) (b) Pb(EDTA)(AsC) (c) Pb(Vit B₁)(AsC) (d) Pb(Vit B₁)(Sali) (e) Pb(Vit B₁)(Vit B₃)
Since all experiments were carried out at physiological conditions, these results are more applicable for biological systems while enhancing the importance of the combining dietary ligands with EDTA or utilization of dietary ligand mixtures in the Pb(II) chelation therapy for more effective metal detoxification.

Further, parallel to these findings based on stability constants, it was reported that when a combination of Vitamin B1 and ascorbic acid is given to lead poisoned mice their liver damage can be reduced significantly (Wang et al., 2007). It has also been clinically proven with mice that EDTA-Pb-AsC (Goyer & Cherian, 1978) and EDTA-Pb-Vit B1 (Kim, 1992) ternary systems were more effective than EDTA-Pb binary system to reduce and control the organ distribution of lead effectively. Therefore, the findings of this work have quantitatively proved the clinical investigations reported.

**CONCLUSIONS**

The potentiometric data obtained on the lead complexes support that in aqueous medium, Pb(II) can interact with natural compounds like salicylic acid, ascorbic acid, vitamin B1 and vitamin B3 which very often enter into the body through food and nutrient supplements. Remarkable involvement of vitamin B1 in the single and mixed complex formation with Pb(II) observed during the research can contribute to raise its applications in clinical chemistry.

Very stable ternary complexes of EDTA formed with ascorbic acid or vitamin B3 along with the other stable combinations; ascorbic acid-vitamin B1, salicylic acid- vitamin B1 and vitamin B1-vitamin B3 must be able to administer to remove body Pb2+ ions more effectively and this will lead scientists to an interesting path way to find more effective and less harmful ligand combinations to treat metal toxicity.

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**REFERENCES**

Battistuzzi, G., Borsari, M., Menabue, L. and Saladini, M. (1996). Amide Group Coordination to the Pb3+ Ion. *Inorganic Chemistry 35* (14):4239-4247.

Branica, G., Hukovic, M.M. and Omanovic, D. (2006). Voltammetric Determination of Stability Constants of Lead Complexes with Vitamin C. *Croatica Chimica Acta 79*(1): 77-83.

Brumas, V., Brumas, B. and Bethon, G. (1995). Copper(II) interactions with nonsteroidal antiinflammatory agents. I. Salicylic acid and acetylsalicylic acid. *Journal of Inorganic Biochemistry. 57*: 191-207.

Bucci, R., Magri, A.D., Magri, A.L. and Napoli, A. (2000). Spectroscopic, characteristics and thermal properties of divalent metal complexes of diclofenac.*Polyhedron.19*: 2515-2520.

Bulman, R.A., Crawley, F.E.H. And Geden, D.A. (1979).Mixed Ligand Chelation Therapy.*Nature 281*: 406-407.

Chandrathilaka, A.M.D.S., Ileperuma, O.A. and Hettiarachchi, C.V.(2013). Spectrophotometric and pH-metric studies on Pb (II), Cu (II), Al (III) and Cu (II) complexes of paracetamol and ascorbic acid. *Journal of National Science Foundation, Sri Lanka 41* (4): 337-344.

Cheong, J.H., Seo, D.O., Ryu, J.R., Shin, C.Y., Kim, Y.T., Kim, H.C., Kim, W. and Ko, K.H. (1999). Lead induced thiamine deficiency in the brain decreased the threshold of electroshock seizure in rat. *Toxicology 133*(2-3): 105-113.

Chidambaram, M.V. and Bhattacharya, P.K. (1970a). Studies in amine-amino acid mixed ligand chelates-I. *Journal of Inorganic Nuclear Chemistry 32*:3271-3275.

Chidambaram, M.V. and Bhattacharya, P.K. (1970b). Studies in some amino acid chelates: Part II-Ni(II), VO (II) & Zn (II) chelates of leucine, isoleucine, norleucine&valine.*Indian Journal of Chemistry 8*: 941-942.

Chisolm, J.J. Jr (1968). The use of chelating agents in the treatment of acute and chronic lead intoxication in childhood. *Journal of Pediatrics 73*: 1-38.

Das, A.K. (1990) A Text Book on Medicinal Aspects of Bio-inorganic Chemistry. New Delhi, India: CBS Publisher and Distributor.

Davidovich R.L., Stavila, V., Marinin, D.V., Voit, E.I. and Whitmire, K.H. (2009). Stereochemistry of lead(II) complexes with oxygen donor ligands. *Coordination Chemistry Reviews 253*:1316-1352.

El-Haty, M.T., Amrallah, A.H., Mohmoud, R.A. and Ibrahim, A.A. (1995). pH-metric studies on ternary metal complexes of some amino acids and benzimidazole. *Talanta 42*: 1711-1717.
Evans, R.N., Mihalcić, D.J., Jones, D.S. and Rabinovich, D. (2003). *cis*-Dichlorotetrakis(2-mercapto-1-tert-butylimidazole)lead(II). *Acta Crystallographica Section E* **59**(6): 370-372.

Florence, T.M. (1982). The speciation of trace elements in waters. *Talanta* **29**: 354-364.

Goyer, R.A. and Cherian, M.G. (1978). Ascorbic acid and EDTA treatment of lead toxicity in rats. *Life Sciences* **24**: 433-438.

Gupta, K.C. and Sharma, K.K. (1982). Polarographic study of mixed ligand (o-mercaptobenzoate-oxalate) complexes of cadmium(II), lead(II) and thallium(I). *Analyt. Chem.* **107**:1512-1515.

Irving, H.and Rossotti, H. (1953). Methods for computing successive stability constants from experimental formation curves. *Journal of Chemical Society* 3397-3405.

Irving, H. and Rossotti, H. (1954). The calculation of formation curves of metal complexes from pH titration curves in mixed solvents. *Journal of Chemical Society* 2904-2910.

Joshtagani, M., Gholivand, M.B and Mosavat, A.R. (2008), Chelation Study of Captopril with Cd²⁺ and Pb²⁺ Ions. *American Journal of Biochemistry and Biotechnology* **4**(3): 245-249.

Kim, J.S., Hamilton, D.L., Blakley, B.R. and Rousseaux, C.G. (1992). The effects of thiamin on lead metabolism: organ distribution of lead 203.*Canadian Journal of Veterinary Research* **56**: 256-259.

Kleszczewska, E. (1999). The spectrophotometry determination of chelate complex: L-ascorbic acid with cuprum (II) and mercury (II) in alkaline solution. *Polish Journal of Environmental Studies* **8**(5): 313-318.

Luth, M.S., Kapinos, L.E., Song, B., Lippert, B. and Sigel, H. (1999). Extent of intramolecular stacking interactions in the mixed-ligand complexes formed in aqueous solutions by copper (II), 2,2’-bipyridine or 1,10-phenanthroline and 2’-deoxyguanosine 5’-monophosphate. *Journal of Chemical Society (Dalton)* 357-365.

Maktell, A.E. and Carey,G.H.(1967). Mixed Ligand Chelates of Uranium (IV). *Journal of American chemical Society* **89**: 2859-2865.

May, P.M. and Williams, D.R., (1979). Synergistic chelation therapy or mixed ligand complexes for plutonium and cadmium poisoning. *Nature* **278**(5704):581-582.

Mildvan, A.S. and Cohn, M. (1966). Kinetic and Magnetic Resonance Studies of The Pyruvate Kinase ReactionII. Complexes of Enzyme, Metal and Substrates. *Journal of Biological Chemistry* **241**: 1178-1193.

Mildvan, A.S., Leigh, J.S. and Cohn, M. (1967). Kinetic and Magnetic Resonance Studies of Pyruvate Kinase. III. The Enzyme-Metal-Phosphoryl Bridge Complex in the Fluorokinase Reaction *Biochemistry* **6**: 1805-1818.

Morose, G., Civie, P., Harriman, E. and Tenny, H. (2008). *Encyclopedia of Earth* [online] Available at: http://www.eoearth.org/article/Industrial_uses_of_lead Accessed on 05 July 2016.

Moustafa M H. (2005). Potentiometric Studies Of The Binary And Ternary Complexes Of Mercury(II) With Acetazolamide And Some Amino Acids. *Assiut University Bulletin Environmental Research* **8**(2):81-88.

Najafpour, M. M., Lis, T. and Holynska, M. (2007) Complexation of lead(II) by nicotinamide (nia): Crystal structure of polymeric Pb(nia)(NO₃)₂. *Inorganica Chimica Acta* **360**(10): 3452-3455.

Nakayama, E., Suzuki, Y., Fujiwara, K. and Kitano, Y.(1989).Chemical Analysis of Seawater for Trace Elements: Recent Progress in Japan on Clean Sampling and Chemical Speciation of Trace Elements. *Analytical Chemistry* **5**:129-140.

Padmaja, N. and Rao, G.N. (2012) Speciation of ternary complexes of lead(II), cadmium(II) and mercury(II) with L-dopa and phenanthrol ine in propanediol-water mixtures. *Chemical Speciation and Bioavailability* **24**(4): 234-240.

Sasser, L.B., Hull, G.G., Bratton, G.R. and Zmudzki, J. (1984). Absorption and tissue distribution of lead in thiamin-replete and thiamin-deficient rats. *Journal of Nutrition* **114**: 1816-1825.

Shelke, D. N. and Jahagirdar, D. V. (1976). Ternary Complexes: Equilibrium Studies of Mixed-ligand Complexes of Neodymium Ion with Carboxylic and Phenolic Acids in Aqueous Medium, *Bulletin of Chemical Society of Japan* **49**(8): 2142-2147.

Thanavelan, R., Ramalingam, G., Manikandan, G. and Thanikachalam,V. (2014). Stability constants of mixed ligand complexes of lead(II) with 1-(aminomethyl) cyclohexane acetic acid and α-amino acids. *Journal of Saudi Chemical Society* **18**(3):227-233.

Turner, S.W. and Fader, B.A. (1962). New Uses For Lead. *Industrial and Engineering Chemistry* **54**(4): 52-55.

Wang, C., Liang, J., Zhang, C., Bi, Y., Shi, X. and Shi, Q.(2007). Effect of Ascorbic Acid and Thiamine Supplementation at Different Concentrations on Lead Toxicity in Liver, *Annals Occupational Hygiene* **51**(6): 563-569.

Yamauchi, O. and Odani, A. (1981). Electrostatic ligand-ligand interactions in ternary amino acid-palladium(II) complexes. Synthetic studies and spectroscopic evidence, *Journal of American Chemical Society* **103**:391-398.